EFFECTS OF UNDERWATER SOUND SIMULATING THE INTERMEDIATE SCALE MEASUREMENT SYSTEM ON FISH AND ZOOPLANKTON OF LAKE PEND OREILLE, IDAHO

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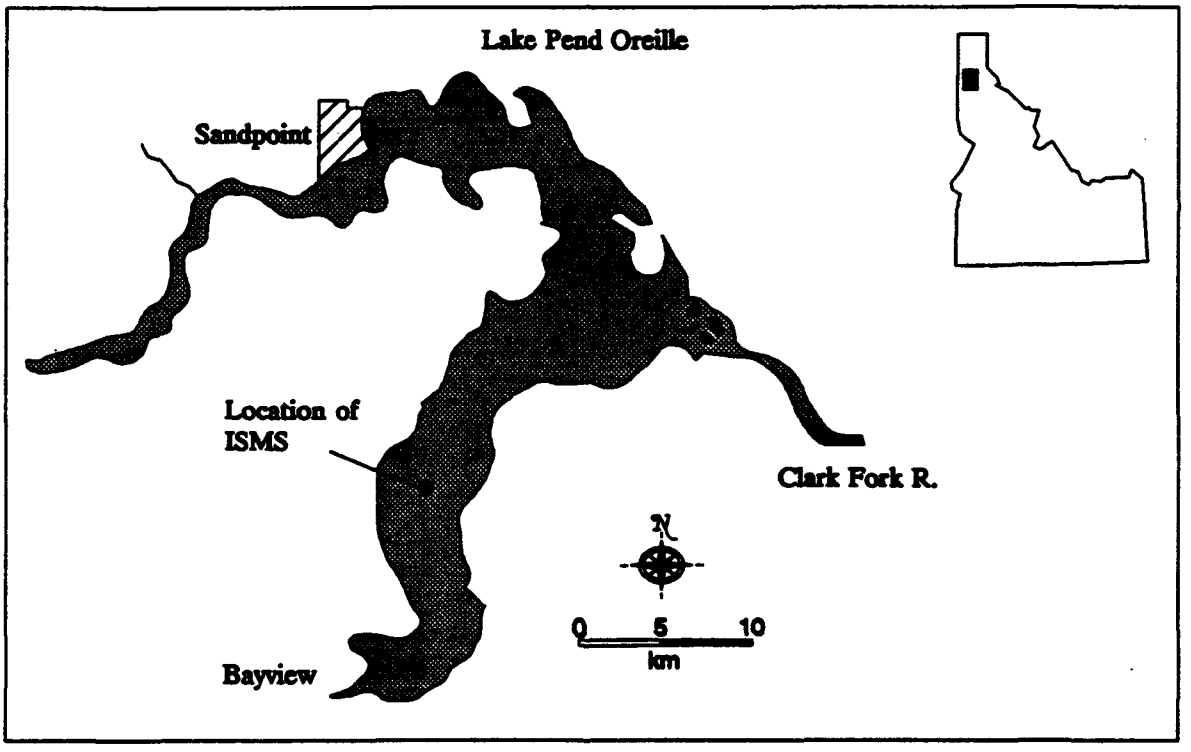

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Effects of Underwater Sound Simulating the Intermediate Scale Measurement System on Fish and Zooplankton of Lake Pend Oreille, Idaho

Final Report

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Preface

This report resulted from work sponsored by the Office of Naval Research (ONR), Arlington, Virginia. Information contained herein does not necessarily infer official endorsement or reflect the position or policy of the ONR.

All data pertaining to this report are available on 90 mm floppy disks and can be obtained by contacting the authors. Data are stored as both Lotus 1-2-3 and ASCII files. Limited hard copies are also available upon request.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

LIST OF FIGURES

LIST OF FIGURES (cont'd)

LIST OF FIGURES (cont'd)

LIST OF FIGURES (cont'd)

LIST OF FIGURES (cont'd)

page

LIST OF TABLES

LIST OF TABLES (cont'd)

LIST OF ABBREVIATIONS

ARD	-- Acoustic Research Detachment
BIZ	-- bioacoustic interaction zone
C	-- degrees Celsius
CV	-- coefficient of variation
dB re 1 μPa@ 1 yard	-- decibel referenced to one microPascal at one yard typically associated with the source level of an acoustic projector
dB re 1 μPa	-- decibel referenced to one microPascal typically associated with a sound pressure level.
DO	-- dissolved oxygen
ETOH	-- ethanol
F	-- test statistic (ANOVA)
h	-- hour
Hz	-- Hertz
ISMS	-- Intermediate Scale Measurement System
ITC	-- International Transducer Corp.
J	-- joule
kHz	-- kilohertz
L	-- liter
log	-- logarithm (base 10)
ln	-- logarithm (natural)
LT	-- long-term exposure cages
m	-- meter
mm	-- millimeter
ms	-- millisecond
n	-- sample size

LIST OF ABBREVIATIONS (cont'd)

NFTA	-- near field transmit array
no.	-- number
NRL	-- Naval Research Laboratory
ONR	-- Office of Naval Research
P	-- probability of type I error (i.e. probability that random variation caused apparent treatment effect)
PVC	-- polyvinyl chloride
s	-- seconds
SD	-- standard deviation
SL	-- source level
SPL	-- sound pressure level
t	-- test statistic (T-test)
μg	-- microgram
z	-- test statistic (Wilcoxon)
ZP	-- zooplankton pens

ABSTRACT

The University of Idaho, with assistance from the Naval Surface Warfare Center, Acoustic Research Detachment, examined effects of proposed operations of the U.S. Navy's Intermediate Scale Measurement System (ISMS) to aquatic resources in Lake Pend Oreille, Idaho. We conducted in-situ experiments to study effects of simulated ISMS sound on zooplankton abundance and biomass, kokanee feeding and growth, predator/prey interactions, kokanee behavior, and kokanee embryo survival. Frequencies of 100, 800 and 5,600 Hz at sound pressure levels ranging from 105-167 dB re 1 μPa were used to ensonify aquatic organisms. Response variables from ensonification experiments were compared to controls for evidence of ISMS effects.

Neither zooplankton abundance and biomass nor growth of age-0 kokanee affected as a result of simulated ISMS ensonification. Similarly, we found no evidence that kokanee embryo survival was reduced as a result of simulated ISMS sound.

Ensonified prey did not exhibit increased or decreased susceptability to predation as a result of simulated ISMS ensonification. Predators ensonified at 100 and 5,600 Hz consumed similar number of prey as did controls. We observed reduced consumption of cutthroat trout by northern squawfish ensonified at 800 Hz ($P=0.058$). A frequency of 800 Hz is within the hearing range of many fishes and northern squawfish may use underwater sound as a cue to detect prey fishes. Effects on squawfish populations in the lake, however, are not anticipated since northern squawfish are littoral predators and would likely not be exposed to sound pressure levels used during conservative experiments.

We found no evidence that behavior of small kokanee was affected by simulated ISMS sound. We did not observe a startle response or avoidance reaction from either large or small kokanee exposed to simulated ISMS sound. We did observe a transient increase in schooling behavior among large kokanee ensonified at 100 Hz. Large kokanee appeared to acclimate quickly to the stimulus and did not exhibit depolarization or disorientation as a result of ISMS ensonification. We do not expect this transient schooling response to result in a significant impact to kokanee in the area ensonified by ISMS projectors.

The general lack of adverse effects associated with the simulated ISMS sounds leads us to conclude that the frequencies and sound pressure levels tested will not affect zooplankton abundance, kokanee growth, and kokanee embryo survival in Lake Pend Oreille. A frequency of 800 Hz may interfere with northern squawfish predation on cutthroat trout and transient changes in schooling by adult kokanee may occur at 100 Hz in the areas ensonified by ISMS projectors.

INTRODUCTION

Lake Pend Oreille, in northern Idaho, contains a valued sport fishery dominated by kokanee *Oncorhynchus nerka* and rainbow trout (Kamloops strain) *O. mykiss*. The lake was renown for the kokanee fishery in the 1940's-early 1950's when kokanee were fished commercially and for sport. Since the early 1950's, the lake has experienced several major disturbances. Completion of the Albeni Falls Dam on the Pend Oreille River in 1952 changed annual water level regimes in the lake. In the same year, completion of the upstream Cabinet Gorge Dam eliminated a major spawning run of kokanee into the Clark Fork River, the major tributary to Lake Pend Oreille (Bowler et al. 1980). Additionally, the introduction of the opossum shrimp *Mysis relicta* in 1966 and subsequent changes in the macrozooplankton community may have been detrimental to the kokanee population (Rieman and Falter 1981). Low kokanee abundance combined with many "unknown effects" of historic changes in Lake Pend Oreille have prompted public interest in environmental issues concerning the lake's aquatic resources.

As part of the Navy's continued efforts in the study of submarine silencing, the Naval Surface Warfare Center Acoustic Research Detachment (ARD), located in Bayview, Idaho, was selected as the site of a new acoustic test system. The new test system, the Intermediate Scale Measurement System (ISMS), will be located between Maiden Rock (west) and Whiskey Point (east) in Lake Pend Oreille and consists of both underwater and shore based facilities (Figure 1). Recent concern for Lake Pend Oreille fisheries has been generated as a result of expanded operations by the U.S. Navy.

ISMS Environmental Assessment

In October 1990, an Aquatic Studies Technical Report examined potential impacts of ISMS to aquatic resources in Lake Pend Oreille (Denny et al. 1990).

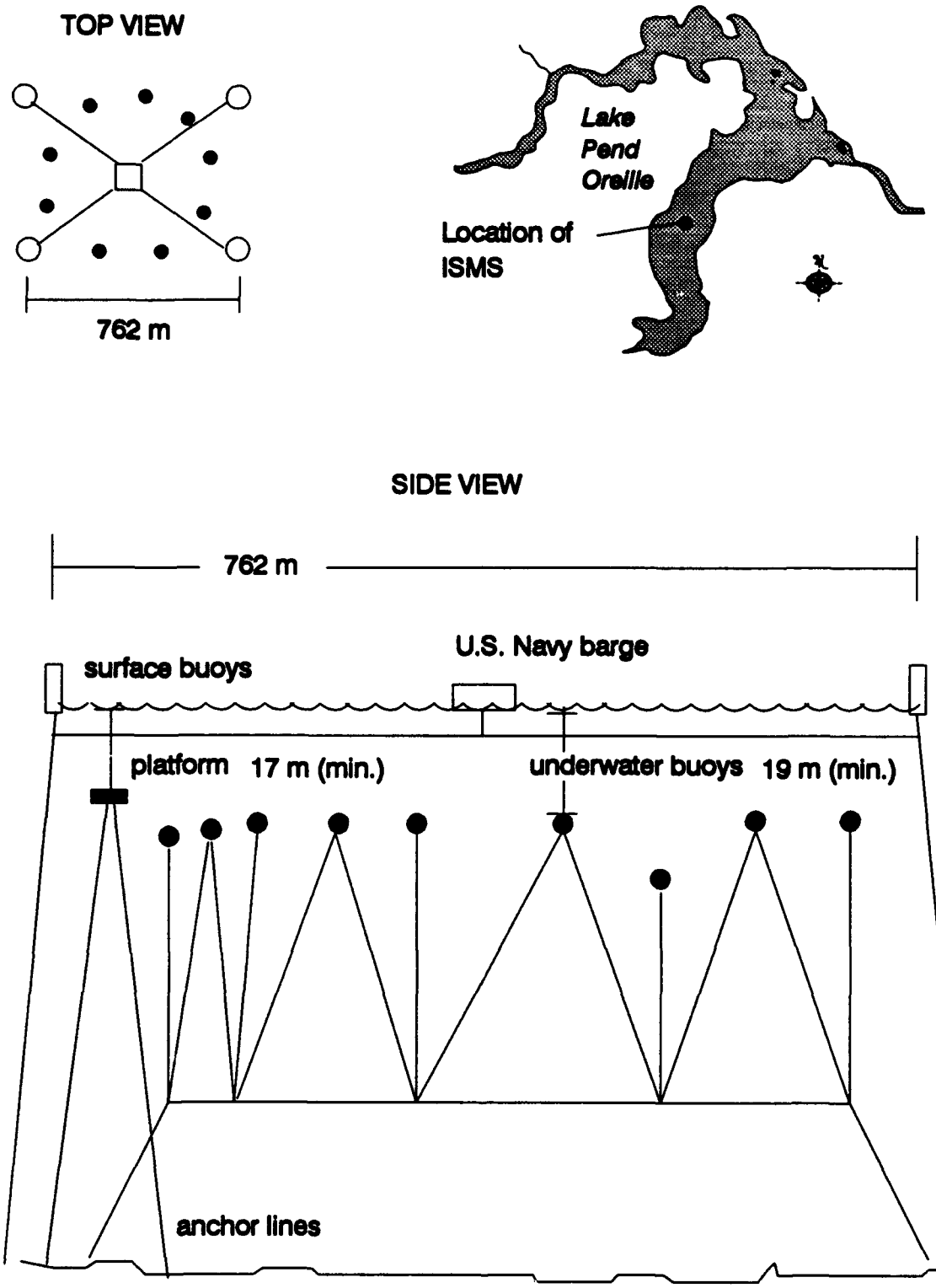


Figure 1. Location and conceptual overview of the ISMS.

Information concerning sound pressure levels and frequency ranges associated with the ISMS projectors was provided by AT&T Bell Laboratories. Based on fish and zooplankton distribution in Lake Pend Oreille, a bioacoustic interaction zone (BIZ) was established in the upper 45 m of the lake. Consultations and examination of available literature indicated that a conservative sound pressure level of 150 dB re 1 μPa, attributable to ISMS operations, would provide a safe operating level in the BIZ (Denny et al. 1990). From this information, the potential impacts from ISMS underwater sound were examined by superimposing the 45 m depth contour (BIZ) on the isoacoustic diagram for the near field transmit array (NFTA) sound projector. Based on the depth at which the NFTA projector will be located (182 m, i.e. 600 ft) and spreading loss associated with underwater sound signals, it was estimated that 0.02% of the BIZ region would be ensonified at sound pressure levels higher than 150 dB re 1 μPa (Figure 2). It was concluded that operations of ISMS frequencies (100-5,600 Hz) at sound pressure levels less than 150 dB would have no significant impact on aquatic resources in the lake. Sound pressure levels higher than 150 dB produced by the ISMS projector are localized and at a depth where few fish are thought to frequent (Denny et al. 1990). Additionally, an Environmental Assessment completed in 1991 (West Sound Associates 1991) addressed environmental issues associated with installation and operation of the ISMS and concluded that there would be no significant impacts associated with implementation of the ISMS.

Public Concern

Although the Environmental Assessment concluded that ISMS frequencies and sound pressure levels pose no threat to aquatic organisms in Lake Pend Oreille, local concern remained because of the high interest in Lake Pend Oreille fisheries. Sportsmen and other citizens voiced concern over the potential adverse effects of ISMS and as a result initiated a study to collect empirical data related to ISMS effects on

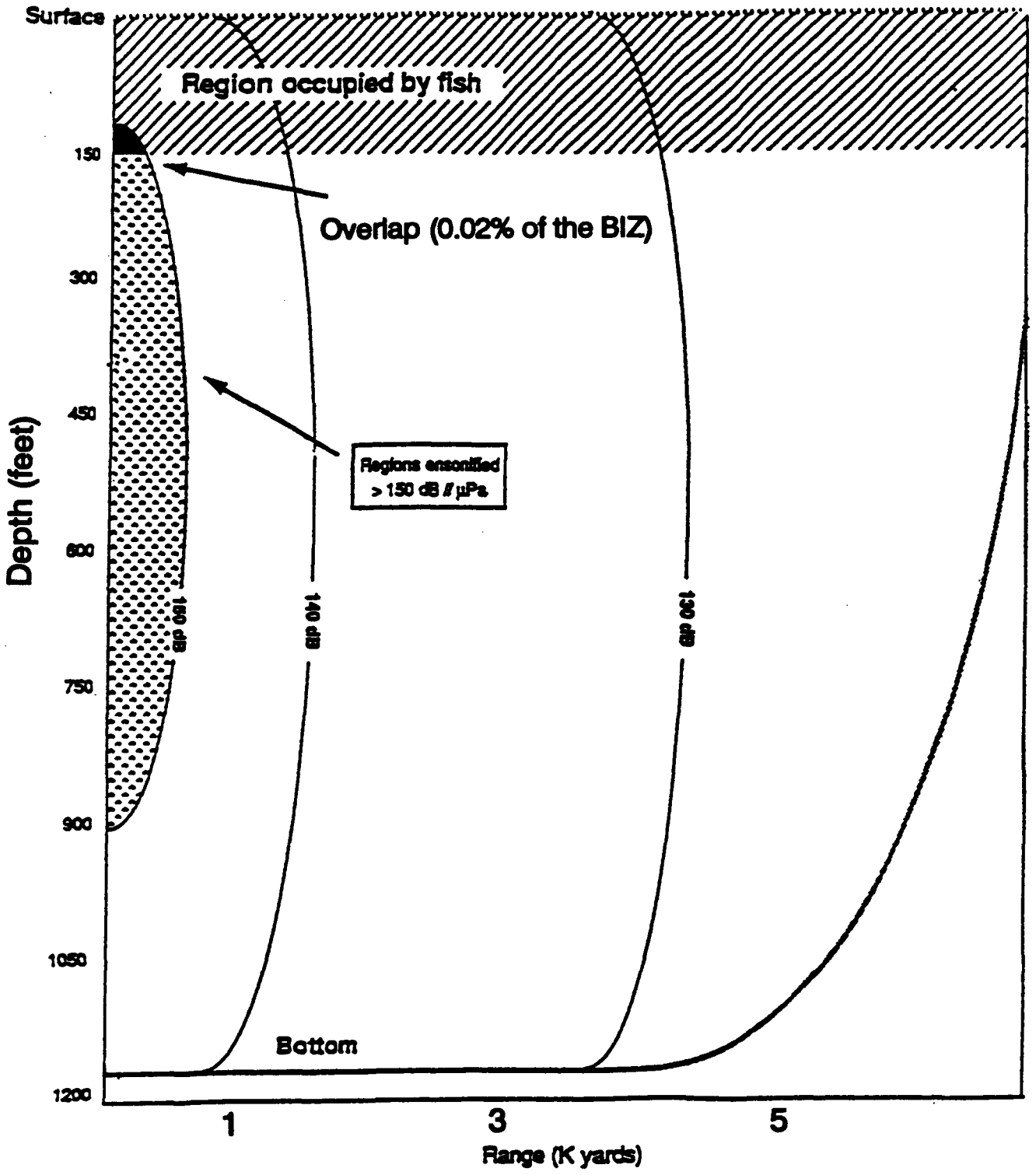


Figure 2. Bioacoustic Interaction Zone for the Near-Field Transmit Array (from Denny et al. 1990).

aquatic organisms in Lake Pend Oreille. In response to these concerns, the Office of Naval Research (ONR) established a Citizens Advisory Committee and a Technical Review Committee to oversee and identify specific areas of investigation. The ONR contracted with the University of Idaho (# N0014-92-J-4106) to investigate effects of simulated ISMS underwater sounds on biological elements important to the ecology of the lake.

This report summarizes results from experiments that assessed simulated ISMS operations on aquatic resources of Lake Pend Oreille. The acoustic level and frequency content of the ISMS was simulated using prototypes of instrumentation which will eventually be installed in the lake. Frequencies of 100, 800, and 5,600 Hz were selected based on the range of frequencies produced by ISMS projectors (i.e. 100-5,600 Hz) and those frequencies (i.e. 100 and 800 Hz) within the hearing range (30-3,000 Hz) of most fishes (Hawkins 1981). Sound pressure levels for the tests were selected based on either the maximum output of the ISMS projector for a particular frequency or a level of approximately 165 dB re 1 μPa. A conservative approach was taken in using a level of 165 dB since this level is 15 dB higher than the projected safe operating level of 150 dB (Denny et al. 1990). Simulated ISMS test protocol (20 ms pulse, 6 s repetition rate, and one hour on/one hour off) was based on anticipated ISMS operations. All sound pressure levels are referenced in dB re 1 μPa.

BACKGROUND--LAKE PEND OREILLE

Fisheries

Kokanee abundance in Lake Pend Oreille has generally declined since the mid-1960's. This decline is believed to be associated with lake level changes related to dam operations (Melo Maiolie, Idaho Fish & Game, Coeur d' Alene, pers. comm.). Present-day significant fisheries consist of kokanee, rainbow trout, and to a lesser extent, lake

trout *Salvelinus namaycush*, bull trout *S. confluentus*, cutthroat trout *O. clarki*, and brown trout *Salmo trutta*.

Kokanee migrated into Lake Pend Oreille in 1933 via the Clark Fork River from Flathead Lake, Montana (Wydoski and Bennett 1981). Kokanee populations were well established by the early 1940's and provided a significant fishery until their decline in the mid-1960's. The decline of kokanee continues to be the focus of much research. Kokanee have been stocked experimentally since 1985 when a kokanee hatchery became operational on the Clark Fork River. Although hatchery supplementation has stabilized the kokanee population, the abundance of wild kokanee continues to decline (M. Maiolie, Idaho Fish & Game, pers. comm.).

The Gerrard stock of rainbow trout (kamloops) was first introduced into Lake Pend Oreille in the early 1940's from the Lardeau River, British Columbia (Goodnight and Reininger 1978). The stock flourished in the lake as a result of abundant forage (kokanee) and is highly sought for its large size (Wydoski and Bennett 1981). Lake Pend Oreille is one of only two lakes in the world that contains a self-sustaining "Kamloops" rainbow trout fishery and produced the world record rainbow trout (16.8 kg/37 lbs) in 1947. The lake also boasts the world record bull trout (14.6 kg/32 lbs) creeled in 1949. Bull trout, native to Lake Pend Oreille, are currently being considered for listing by the U.S. Fish and Wildlife Service pursuant to the Endangered Species Act of 1973.

Other cold water game fishes introduced into Lake Pend Oreille include lake trout, brook trout *S. fontinalis*, and brown trout. A variety of other game and nongame fishes inhabit Lake Pend Oreille (Table 1).

Limnology

Lake Pend Oreille has been characterized as a 'morphometrically oligotrophic' lake because of its extreme depth (mean = 165 m), deep mixing of plankton-containing

Table 1. Fishes identified from Lake Pend Oreille, Idaho.

Species	Scientific name
Percidae	
Yellow perch	*Perca flavescens*
Cyprinidae	
Peamouth	*Mylocheilus caurinus*
Northern squawfish	*Ptychocheilus oregonensis*
Redside shiners	*Richardsonius balteatus*
Tench	*Tinca tinca*
Centrarchidae	
Pumpkinseed	*Lepomis gibbosus*
Smallmouth bass	*Micropterus dolomieu*
Largemouth bass	*Micropterus salmoides*
Black crappie	*Pomoxis nigromaculatus*
Esocidae	
Northern pike	*Esox lucius*
Salmonidae	
Kokanee	*Oncorhynchus nerka*
Rainbow trout	*Oncorhynchus mykiss*
Cutthroat trout	*Oncorhynchus clarki*
Bull trout	*Salvelinus confluentus*
Brook trout	*Salvelinus fontinalis*
Lake trout	*Salvelinus namaycush*
Brown trout	*Salmo trutta*
Mountain whitefish	*Prosopium williamsoni*
Lake whitefish	*Coregonus clupeaformis*
Pygmy whitefish	*Prosopium coulteri*
Catastomidae	
Longnose sucker	*Catostomus catostomus*
Largescale sucker	*Catostomus macrocheilus*
Ictaluridae	
Brown bullhead	*Ameiurus nebulosus*
Cottidae	
Slimy sculpin	*Cottus cognatus*

water, and long residence of water masses (Rieman and Falter 1981). These characteristics combine to suppress primary productivity, though phosphorus loading into the lake is moderately high at 1.37 g P/m^2 (Watson et al. 1987; Beckwith 1989). Early signs of eutrophication have appeared around inshore areas where development along the shoreline and subsequent nutrient inputs have increased (Kann and Falter 1989; Falter and Olson 1990). In 1991, significant nutrient inputs were reduced due to operations of the sewage treatment plant in Bayview, Idaho.

Eleven species of macrozooplankton have been identified from Lake Pend Oreille (Table 2). *Bosmina* is the smallest member of the community (5 μg wet weight) and *M. relicta* is the largest (up to 800 μg wet weight). Establishment of *M. relicta* in Lake Pend Oreille has resulted in temporal shifts in *Bosmina* (Figure 3) and *Daphnia* spp. (Figure 4) abundance from early summer peaks to August-September peaks. Mysid shrimp migrate vertically to surface waters at night and descend to deep, darker cooler strata during the day. Kokanee feed in the upper strata during the day and descend at night and reduce feeding rates. Because of spatial segregation, Mysid production provides little support for kokanee populations and may limit food abundance, particularly for age-0 kokanee (Rieman and Falter 1981).

Acoustic Characteristics

The transmission of sound in water is superior to that of air. Underwater sound waves travel almost five times as fast as sound waves in air with very little loss (Bergmann and Spitzer 1969). Long distance transmission of underwater sound is influenced by reflection from the water surface, bottom characteristics of a body of water, and from boundaries created by water masses of different temperatures (Bergmann and Spitzer 1969).

The acoustic environment of Lake Pend Oreille is produced by a combination of factors both natural and man-made. Wind and waves are major sources of natural

Table 2. Crustacean zooplankton identified from Lake Pend Oreille, Idaho, with approximate lengths and weights (Rieman 1980).

Species	Length (mm)	Wet weight (μg)
Copepoda		
Cyclops biscuspidatus thomasi	0.6	10
Diaptomus ashlandi	0.8	30
Epischura nevadensis	1.6	160
Cladocera		
Bosmina longirostris	0.4	5
Ceriodaphnia sp.	-	-
Chydorus sphaericus	-	-
Daphnia galeata mendotae	1.1	70
Daphnia thorata	1.1	70
Diaphanasoma leuchtenbergianum	1.0	90
Leptodora kindtii	4.0	250
Polyphemus pediculus	-	-
Malacostraca		
Mysis relicta	4-20	20-800

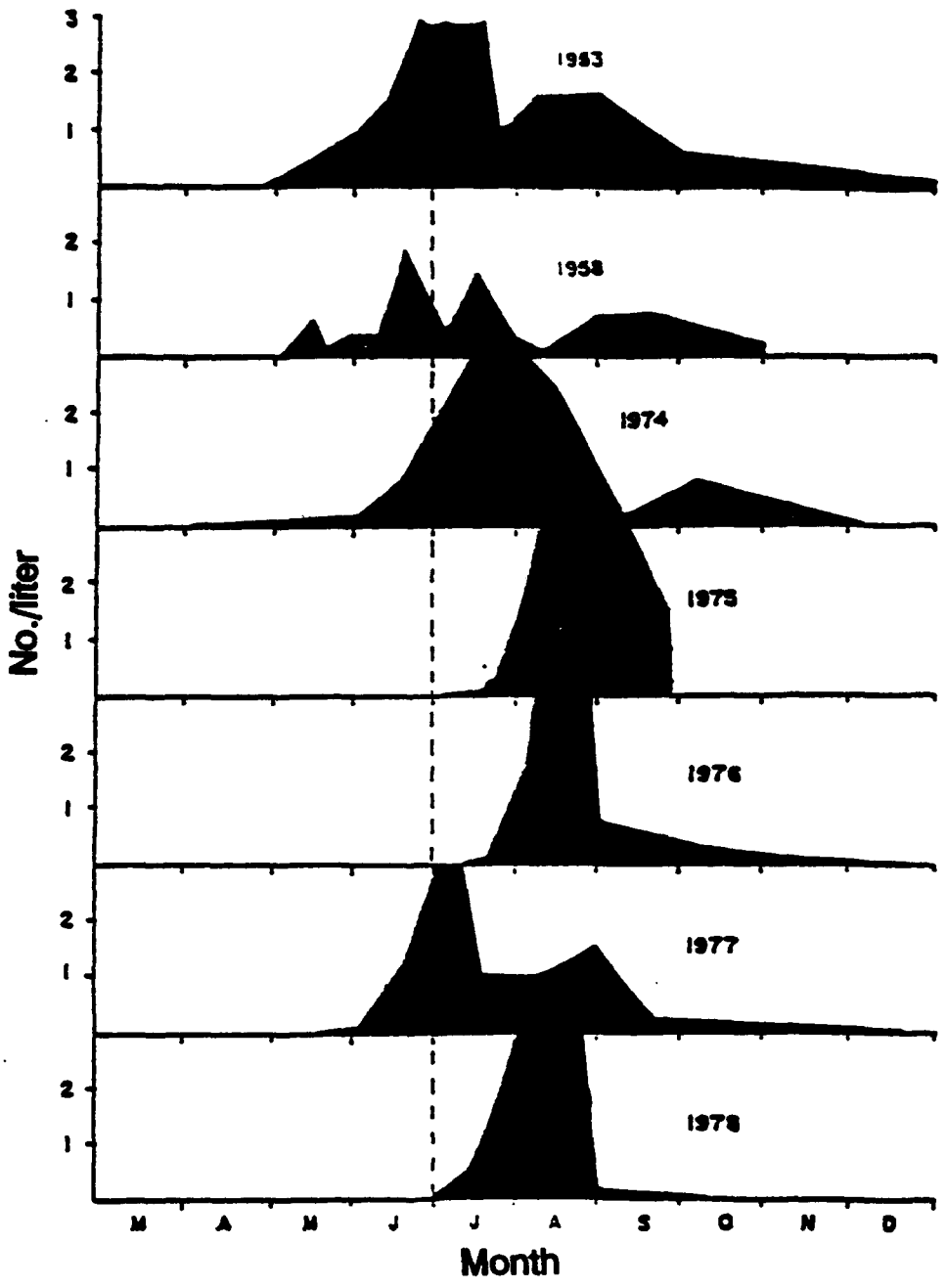


Figure 3. Temporal distribution of Bosmina in Lake Pend Oreille, Idaho (Rieman 1980).

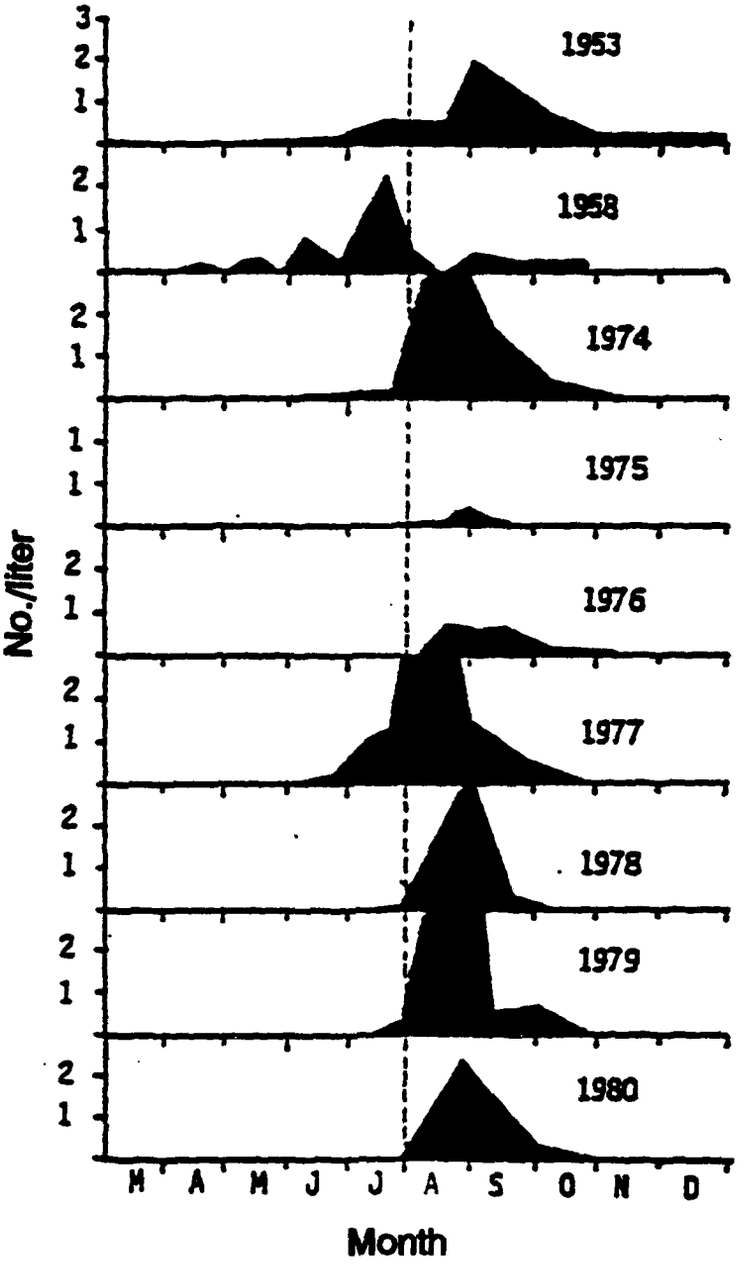


Figure 4. Temporal distribution of Daphnia spp. in Lake Pend Oreille, Idaho (Rieman 1980).

background noise within the lake. A direct correlation exists between measured wind speed and background noise levels; the higher the measured wind speed, the higher the background noise level.

The primary source of man-made acoustic energy in the lake is recreational boat traffic. High speed boats produce a broad band noise spectrum which covers the entire frequency range of interest of this study from 100 Hz to 5,600 Hz. Figure 5 compares the total sound pressure levels versus time for typical 800 Hz ISMS pulses and a high speed boat powered by a 90 HP outboard. The sound pressure level versus time was created from the raw acoustic data by processing the received voltage signal V(t) using $10Log_{10}V^2$ and applying the appropriate hydrophone sensitivity to convert to pressure level. The solid line on the figure is the level due to ISMS transmissions at the maximum transmission power and the dotted line represents a boat as it passes approximately 24 m away from the hydrophone. The sound pressure level for the boat varies with time, with the maximum level occuring at the closest point of approach (24 m) and trailing off in level as the boat moves further away. Since the ISMS signal is present for only a short period of time during each 6 second cycle, an averaging process was performed on both complete sets of data to compare the average power in the water for each signal during the 12 second time period. For the ISMS source, the average power is approximately 131 dB. The average power for the boat during this same 12 second time period is 133 dB, or about 2 dB higher than the ISMS signal.

Since recreational boat traffic is temporal in nature, the average background noise levels within Lake Pend Oreille exhibit temporal variation. During the summer months when boat traffic is at a maximum, background noise tends to be higher than during the winter months.

Natural or "ambient" noise in Lake Pend Oreille excludes man-made noises and represents natural conditions in the lake. Background noise includes both ambient and man-made noises in the lake (i.e. boats, construction, etc.) and is higher than ambient

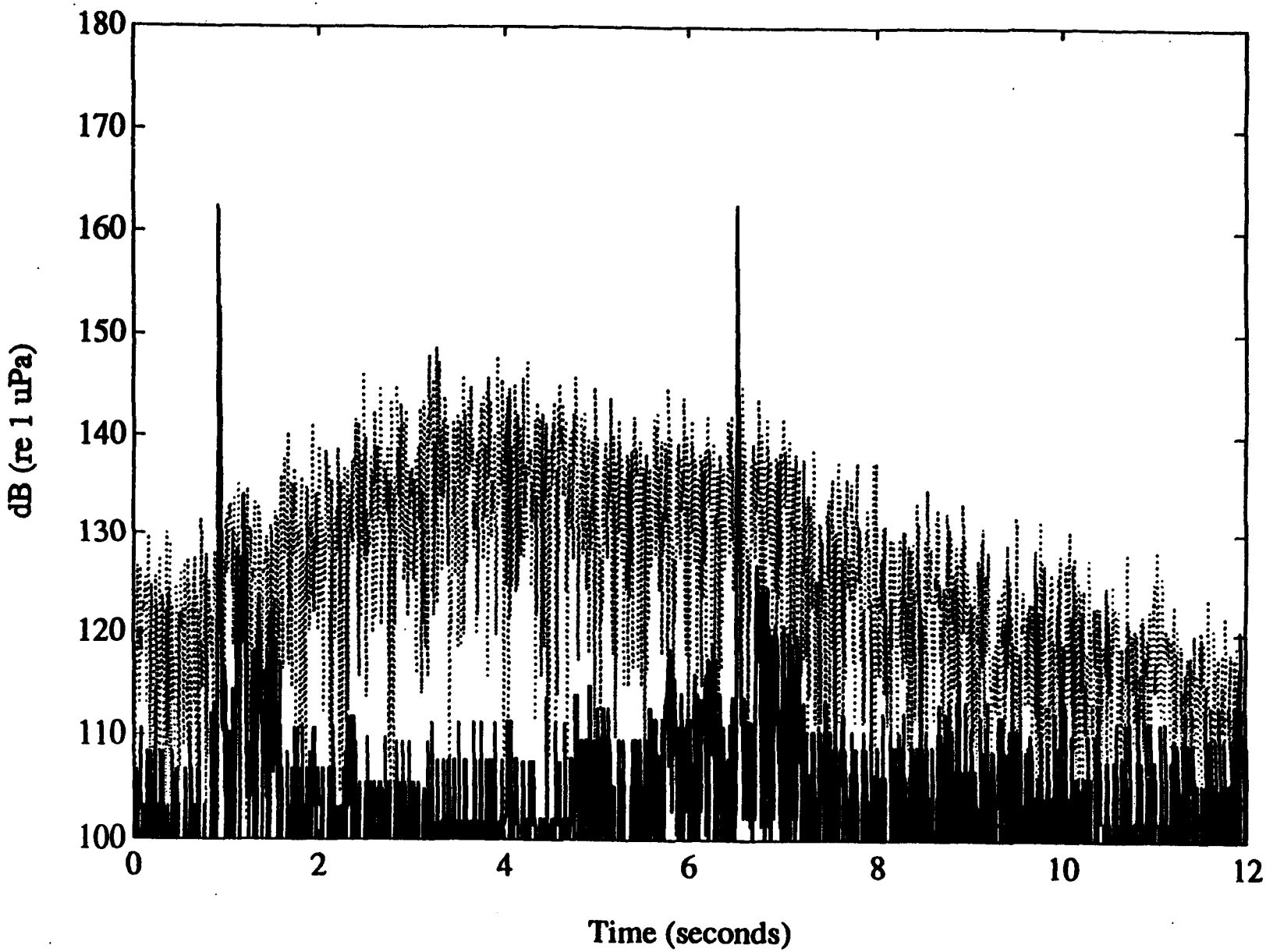


Figure 5. Analysis of time series signal for typical 800 Hz ISMS pulse (solid line) versus a high speed boat powered by a 90 HP motor (dotted line). Time series data were rectified by 20Log(time series) and hydrophone sensitivity was applied. For legibility only one data point of every 32 is displayed.

noise levels. Figure 6 shows a characteristic low ambient noise level (i.e. no man-made noises) and a background noise level that includes boat noise (i.e. a 90 hp boat passing 21 m by a hydrophone located at a depth of 12 m).

FISH BIOACOUSTICS

Available data indicate that fish are aware of their acoustic environment to the extent that underwater sound is within their physiological limits of hearing (Schwarz 1985). Most fishes hear sounds in the frequency range of 30-2,000 Hz depending on sound pressure level and background noise (Hawkins 1981). Considerable variation exists in the morphology of the inner ears of different fish species -- so much in fact that generalizations within taxonomic groups are often not valid (Platt and Popper 1981). Hence, fishes demonstrate great variation in the frequency ranges they can hear and their sensitivities across frequencies (Popper and Fay 1973; Schwarz 1985).

Most research examining effects of underwater sound on aquatic organisms has been conducted on marine mammals, fish, and crustaceans. Much of this research is related to effects of explosive, low frequency sounds associated with seismic surveying. Little information is available concerning effects of sustained underwater sound (i.e. sonar) on freshwater fishes. In light of information that is available, generalizations concerning effects of underwater sound on freshwater fishes are often difficult to make (Platt and Popper 1981; Sand 1981). For example, among clupeids, alewife *Alosa pseudoharengus* avoided pulsed, low frequency sounds in the range of 50-60 Hz at sound pressure levels of 180 dB re 1 μPa (Haymes and Patrick 1986). However, Dunning et al. (1992) found that alewives also avoided pulsed, high frequency sounds in the range of 110 kHz-150 kHz at sound pressure levels of 125-180 dB re 1 μPa. Similarly, blueback herring *A. aestivalis* avoided pulsed, high frequency sounds in the range of 110 kHz-140 kHz at sound pressure levels above 180 db re 1 μPa (Nestler et al. 1992). Schwarz and Greer (1984) found no visible response from Pacific herring *Clupea*

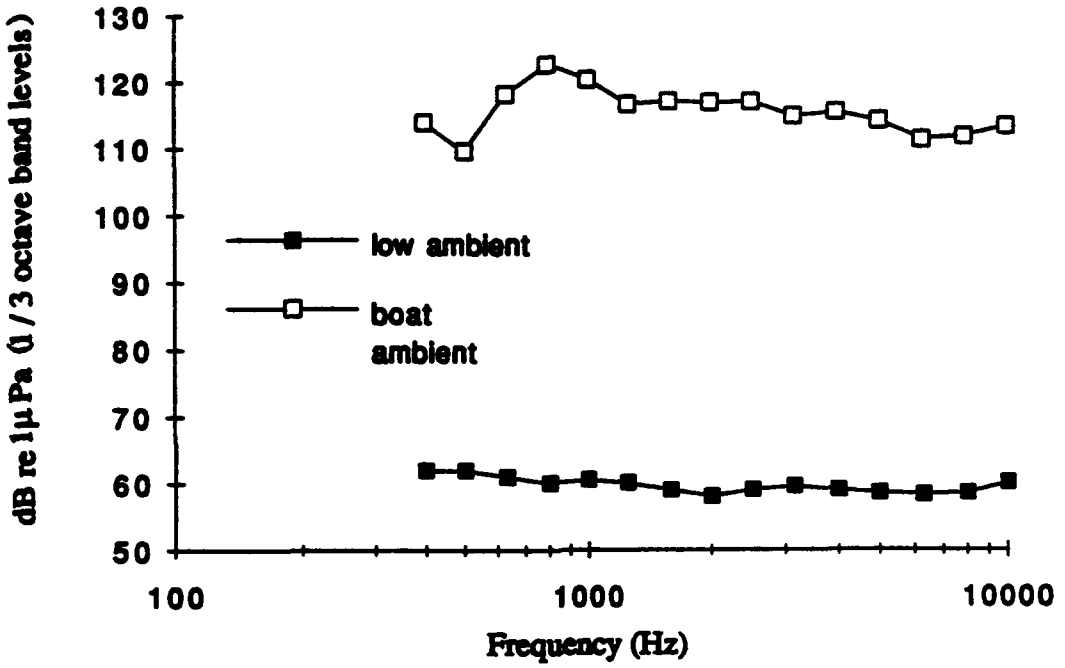


Figure 6. Representative ambient background noise level in Lake Pend Oreille (Cummings 1987) and background noise including the noise from a 90 HP outboard motor at full throttle (21 m from a hydrophone 12 m below water surface).

harengus exposed to high frequency sonar or echo sounder equipment and indicated that herring behavior would not be affected by these sources in the field. To date, mechanisms for detecting high frequency sounds (i.e. >110 kHz) are unknown since they are outside the known normal hearing range of fishes (R.R. Fay, Loyola University, Chicago IL, pers. comm.).

Hearing in Fishes

In many fishes, hearing is accomplished by a combination of the inner ear and the lateral line, collectively termed the octavolateralis system (Platt et al. 1989). The presence of a swimbladder in fishes enhances their sensitivity to underwater sound (Blaxter 1981). The swimbladder serves several functions including buoyancy, sound production, hearing sensitivity, and as a gas reservoir (Blaxter 1981). In the superorder Ostariophi (i.e. goldfish) the swimbladder is connected to the inner ear via a small chain of bones, the Weberian ossicles. Although Ostariophi are believed to have the best hearing sensitivity among fishes (Lowenstein 1971), Platt and Popper (1981) caution that the presence of Weberian ossicles does not necessarily mean an extended hearing range for a particular species.

The inner ear of fishes is similar to the mammalian system and consists of two major end-organs, the semicircular canals and the otolith organs. Functionally, the inner ear is responsive to mechanosensory stimuli such as oscillations at auditory frequencies (Platt and Popper 1981). Physiological responses have also been elicited from gravistatic, acceleratory, and vibrational stimuli (Platt and Popper 1981). The epithelium of the inner ear contain mechanoreceptive hair cells that function to transfer mechanical energy to electrochemical energy. Similar to hair cells in the ears of other vertebrates, hair cells in fish ears are stimulated by sound wave oscillations (Platt and Popper 1981).

The lateral line in fishes serves to detect local water currents and aids fishes in detecting close range obstacles (Sand 1981). In many fishes, the lateral line is believed to be responsible for detection of low frequency underwater sounds (Sand 1981). Recently, Denton and Gray (1988, 1989) have demonstrated that lateral-line organs may be responsible for detecting local water accelerations (or velocity) rather than water displacement as concluded by Harris and van Bergeijk (1962; Karlsen 1992a). Additionally, Karlsen (1992a) demonstrated that the inner ear (and not the lateral line) in perch *Perca fluviatilis* is responsible for detection of infrasound frequencies (i.e. <20 Hz) and suggests the inner ear in other fishes may also be capable of detecting low frequency underwater sounds (Karlsen 1992a, 1992b). Additional research in the area of infrasound detection in fishes is needed before generalizations concerning the mechanisms of detection can be made (Karlsen 1992a).

Hearing in Salmoniformes

Fishes in the order Salmoniformes (i.e. trout and salmon) have poor hearing compared to other fishes and are sensitive to a narrow range of frequencies (Hawkins and Johnstone 1978). Salmonids do not have Weberian ossicles connecting the swimbladder to the inner ear. Hearing studies of the Atlantic salmon *Salmo salar* indicated that the fish responded to sound frequencies below 380 Hz at sound pressures ranging from 93.9 to 112.1 dB re 1 μPa (Hawkins and Johnstone 1978). Atlantic salmon exhibited avoidance responses from 5 Hz to 10 Hz at intensities of 100-150 dB above physiological awareness thresholds (Knudsen et al. 1992). Pulsed sounds generated at 150 Hz did not elicit an avoidance response from Atlantic salmon (Knudsen et al. 1992).

VanDerwalker (1967) observed that salmonids responded to frequencies mainly between 35 and 170 Hz and up to 280 Hz. Stober (1969) observed physiological responses from cutthroat trout to sound frequencies up to 650 Hz. Both studies were

conducted in the near-field where the acoustical and vestibular apparatus as well as the lateral line may be responsible for detection of the stimulus (Harris and van Bergeijk 1962; Stober 1969).

The use of underwater sound as a deterrent to salmonid fishes has generally not been successful (Moore and Newman 1956; Taft 1986), though several studies have produced mixed results. Patrick (1988) reported using underwater sound as an acoustic barrier with some success on wild Pacific salmon smolts (*Oncorhynchus* spp.) but had no success with hatchery-released Atlantic salmon smolts. Studies by Loeffelman et al. (1991) indicated that salmonids respond to species-specific frequencies and sound pressure levels. Loeffelman et al. (1991) used underwater sound as a deterrent with some success on chinook salmon *O. tshawytsha* and steelhead trout. Moreover, recent research indicates that salmonid fishes are sensitive to infrasound frequencies (i.e. less than 20 Hz) (Michael Curtin, SONALYSTS Inc., Waterford, CT, pers. comm.). Research using infrasound is focused on determining frequencies and sound pressures that can be used to elicit avoidance responses from salmonid fishes.

Harmful sound levels

Sound is believed to be the major form of communication for aquatic organisms, hence a properly functioning auditory system is essential for survival of many fishes (Hastings 1990). High intensity underwater sounds may be harmful to fishes but not easily observed (Hastings 1990). Hastings (1990) describes morphological damage as damage to the physical structure of the auditory organs whereas physiological damage is damage of the processes in which signals are transmitted from auditory organs through the nervous system. Hastings (1987) and Enger (1981) observed sound pressure levels greater than 180 dB re 1 μPa can be harmful to goldfish *Carassius auratus* and cod *Gaddus* spp. Goldfish exposed to sound pressure levels of 150 dB re 1 μPa did not exhibit morphological or physiological damage and because of their increased

sensitivity to underwater sound, levels below 150 dB are not believed to be harmful to other fishes (Hastings 1990; Denny et al. 1990).

A biologically meaningful perspective of sound pressure level (dB) can be seen if we consider explosives such as TNT and dynamite which have well-known effects on fishes. For many fishes, lethal thresholds produced by explosives range between 229-234 dB 1 μPa (Norris and Mohl 1983). A difference of 80 dB (i.e. 230 - 150 dB) is equivalent to a 10,000 fold decrease in underwater sound pressure.

Other Responses to Underwater Sound

Pulsed underwater sound is generally more effective in eliciting changes in fish behavior than continuous sound (Chapman 1975; Blaxter et al. 1981). Fishes habituate quickly to underwater sounds, hence the difficulties in developing acoustic barriers that effectively deter fishes (Schwarz 1985; Knudsen et al. 1992).

Sounds produced by fishing vessels and fishing nets affect fish behavior (Chapman 1975; Erikson 1979). Negative correlations between catch rates of Albacore tuna *Thunnus alalunga* and sound frequencies above 1,500 Hz were attributed to noise produced by propeller shaft bearings of fishing vessels (Erickson 1979). Additionally, Schwarz and Greer (1984) indicated that the rate of change in amplitude and the direction of boat noises were most effective in eliciting an avoidance response from Pacific herring.

Dolphins and many other toothed whales emit high-frequency "clicks" or echolocation with peak energy levels at 100 kHz-200 kHz (Norris and Evans 1967; Popper 1980). Hawaiian spinner dolphins *Stenella* spp. have been observed to depolarize prey using high-frequency echolocation making prey more vulnerable to predation (Norris and Mohl 1983).

Prey stunning by the snapping shrimp *Crangon* spp. has been documented on small fish and crustacean and is a response to sharp sound impulses (MacGinitie and

MacGinitie 1968; Zagaeski 1987). Falter (co-author) hypothesized that zooplankton, with their rigid exoskeletons, might register greater force of sound impulses on their bodies than larval fish of similar size. Yelverton et al. (1975) indicated that lethal thresholds of sound to aquatic organisms are inversely proportional to body size; an impulse causing no damage to a 10 g organism could potentially cause 50% mortality among 10 mg organisms. However, no experimental evidence corroborates this relationship with zooplankton, either in laboratory or in-lake situations.

High frequency underwater sound can also cause caviation and resonance within biological systems (Frizzel 1988). For example, in a study by Dunning et al. (1992b) the response of alewives to high frequency underwater sound was believed to be related to cavitation and resonance created by high frequencies (122-128 kHz) and sound pressure levels (SL = 190 dB re 1 μPa) since these sounds were well outside the hearing range for alewives.

To date, most studies in fish bioacoustics have examined responses of fishes to single sound events (Schwarz 1985). Little is known about responses of fishes, particularly salmonids, to sustained underwater sound. Additionally, field studies examining responses of fishes (i.e. clupeids) often produce varied results (Sand 1981; Haymes and Patrick 1986 vs. Nestler et al. 1992; Patrick 1988) indicating that generalizations concerning effects of underwater sound are often not valid.

OBJECTIVES

Overall Objective

To evaluate potential effects of simulated ISMS underwater sound on biological elements and processes important to the ecology of Lake Pend Oreille.

Specific Objectives

Task 1.1

To evaluate effects of simulated ISMS underwater sound on zooplankton population dynamics.

Task 1.2

To evaluate effects of simulated ISMS on growth of age-0 kokanee.

Task 1.3

To evaluate effects of simulated ISMS underwater sound on predator/prey interactions.

Task 2.1

To evaluate effects of simulated ISMS underwater sound on kokanee behavior.

Task 3.1

To evaluate effects of simulated ISMS underwater sound on kokanee egg and embryo survival.

STUDY AREAS

Experimental Approach

In-situ (field) experiments were designed to study effects of simulated ISMS sounds on aquatic organisms in Lake Pend Oreille. In-situ experiments were conducted for two major reasons: 1.) Though experiments in highly controlled laboratory settings are often desirable, for acoustic studies it is often impossible to control oscillatory sound waves in an aquarium or tank so they resemble frequencies and sound pressure levels encountered by fish/zooplankton under natural conditions (i.e. ISMS in Lake Pend Oreille). Parvulescu (1967) describes many problems encountered in conducting acoustic studies in small tanks and aquariums. 2.) Since the ISMS will be an in-lake operation, we wanted to observe natural responses of representative species to ISMS sound regimens. Though kokanee can be reared under artificial conditions, in the wild they migrate vertically in the water column and feed predominantly on zooplankton. In the field, kokanee could be provided with enough space (and naturally occurring zooplankton food) to carry out their natural migratory and feeding behavior. Additionally, zooplankton communities were sampled directly from the lake. Natural zooplankton communities are difficult to rear under laboratory conditions and thus laboratory experiments would be limited by the use of only those species easily reared under artificial conditions.

In field experiments it is difficult (if not impossible) to compensate for factors such as zooplankton distribution and abundance, temperature differences, and weather conditions. For these reasons, we attempted to minimize effects of these factors by choosing a control area as close as possible to the test area, yet at a distance where sound pressure level was significantly reduced (i.e. a 7,000 fold decrease in sound pressure level). We stratified the design into arcs, at varying distances from the sound source, to examine effects of different sound pressure levels for each frequency tested.

Test Facilities

Aquatic studies were conducted in the pelagic zone of Lake Pend Oreille's southern basin (Figure 7). Experiments were based from ARD barges located on the lake. Underwater sound sources, used to ensonify fish and zooplankton, were located on the ARD Yellow barge or the ARD Kamloops barge. All experiments were conducted in the far-field of the simulated ISMS sound source. A minimum distance of 1.5 wave lengths at the lowest frequency tested was used as a definition of the interface between the near- and far-field. The near-field was thus defined as a sphere with a radius of approximately 23 m surrounding the sound source.

Two test/control areas were used during the experiments (Table 3). The test area for fish and zooplankton experiments (Task 1.1 and 1.2) was based from the ARD Kamloops barge. The control area was chosen 6.4 km northwest of the ARD Kamloops barge. The control area was selected to represent lake conditions similar to the test area yet at a distance sufficient for simulated ISMS sound to be considered negligible. Based on the assumption of conservation of sound energy, at 6.4 km the sound pressure level of the simulated ISMS source decreased approximately 77 dB from the original level. This level represented a 7,000 fold decrease in sound pressure compared to sound pressure levels at the ARD Kamloops barge.

Embryo survival experiments (Task 3.1) were based from the ARD Yellow barge (Figure 7). The control area for the embryo survival tests was located along the northwestern shore of Idlewilde Bay (Figure 7). Controlled experiments for predator/prey studies (Task 1.3) were based from the ARD Kamloops barge and ARD Green barge. Fish behavior experiments (Task 2.1) were conducted from the ARD Kamloops barge during late July 1993.

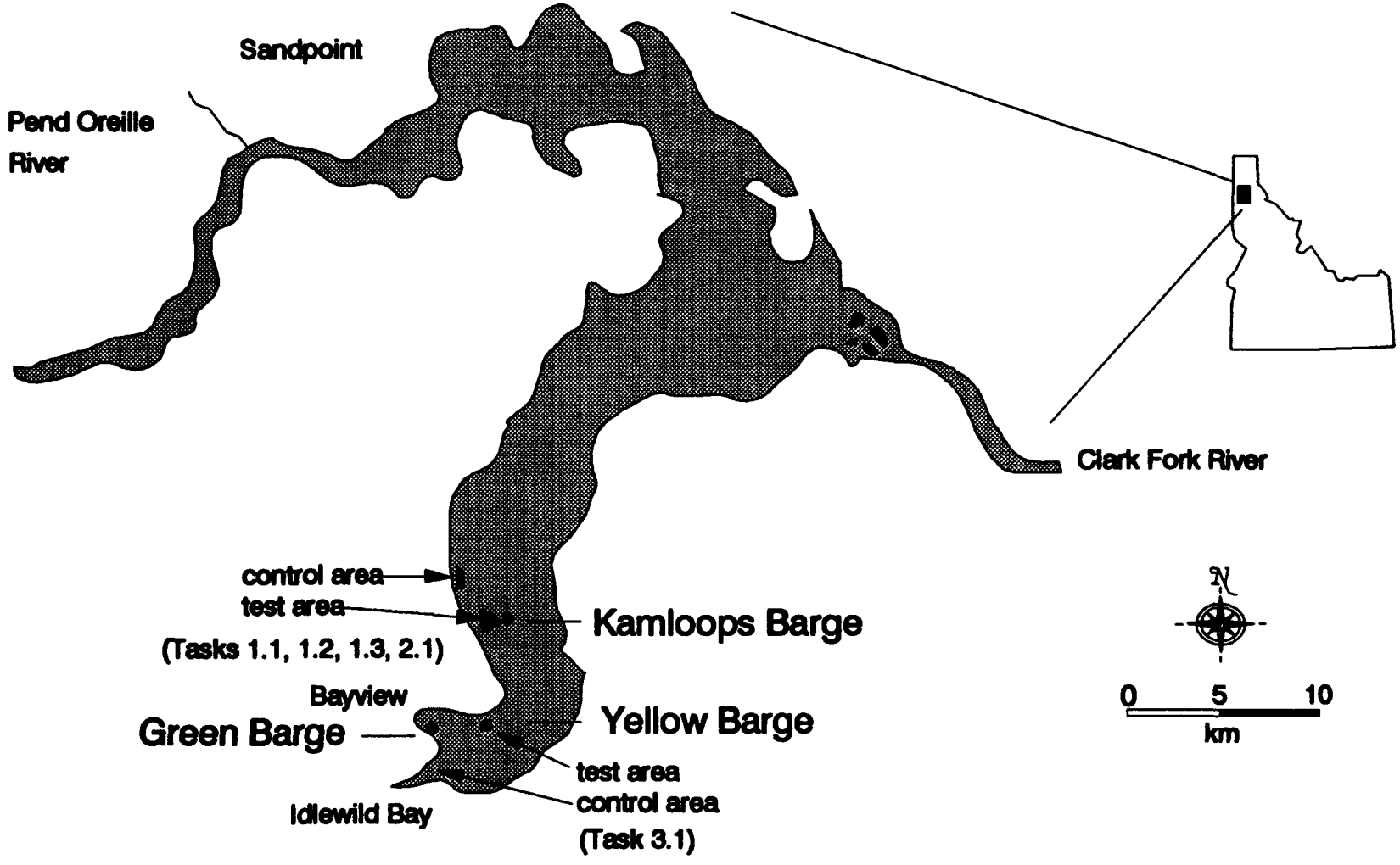


Figure 7. Map of Lake Pend Oreille showing locations of test and control areas and ARD barges. Ensonification studies were conducted from the ARD Kamloops barge and Yellow barge.

Table 3. Locations where test and control areas were based during ISMS ensonification studies.

Task	Area	Location
1.1 Zooplankton	Test	ARD Kamloops barge
	Control	6.4 km NW of ARD Kamloops barge
1.2 Kokanee growth	Test	ARD Kamloops barge and vicinity
	Control	6.4 km NW of ARD Kamloops barge
1.3 Predator/Prey	Test	ARD Kamloops barge
	Control	ARD Kamloops barge/ARD Green barge
2.1 Fish behavior	Test	ARD Kamloops barge
	Control	ARD Kamloops barge
3.1 Embryo survival	Test	ARD Yellow barge
	Control	West shore of Idelwilde Bay

ACOUSTIC METHODS

Experiments examining effects of underwater sound on fish and zooplankton were executed in two stages. Task 3.1 -- kokanee embryo survival -- was performed in December 1992. Tasks 1.1, 1.2, 1.3 and 2.1 were conducted during May through October 1993. To cover the entire frequency range of operation of ISMS, frequencies of 100, 800, and 5,600 Hz were used. A typical ISMS operational scenario was developed for the ensonification tests. A pulsed sound with a 20 ms pulse width and a 6 s repetition rate was used to ensonify fish and zooplankton with a cycle of 1 hour on and 1 hour off.

Acoustic equipment used to generate the simulated ISMS sound was located on the ARD Yellow barge for the kokanee embryo survival experiments (Task 3.1) and on the ARD Kamloops barge for the remainder of the experiments (Tasks 1.1, 1.2, 1.3, 2.1) (Figure 8). All signal generation was controlled by a Macintosh IIfx computer. Waveforms were generated digitally on the Macintosh computer and downloaded as text files to the Tektronix arbitrary waveform generator. The output of the arbitrary waveform generator signal was sent to either an Instruments Incorporated L6 or L40 power amplifier. A Naval Research Laboratory (NRL) F56 source or an International Transducer Company (ITC) 4138 source was then used to generate the acoustic signal. The combination of the L6 amplifier and NRL F56 source was used to generate the 5,600 Hz signal, and the L40 amplifier driving the ITC 4138 source produced the 100 and 800 Hz signals.

May-October Experiments (Tasks 1.1, 1.2, 1.3, 2.1)

Figure 9 shows the deployment of the source and hydrophone for the fish and zooplankton studies that were performed on the ARD Kamloops barge. Fish and zooplankton were ensonified for a total of 7 days during each experiment. Some variation in the ensonification cycles occurred from shutdowns to accommodate other

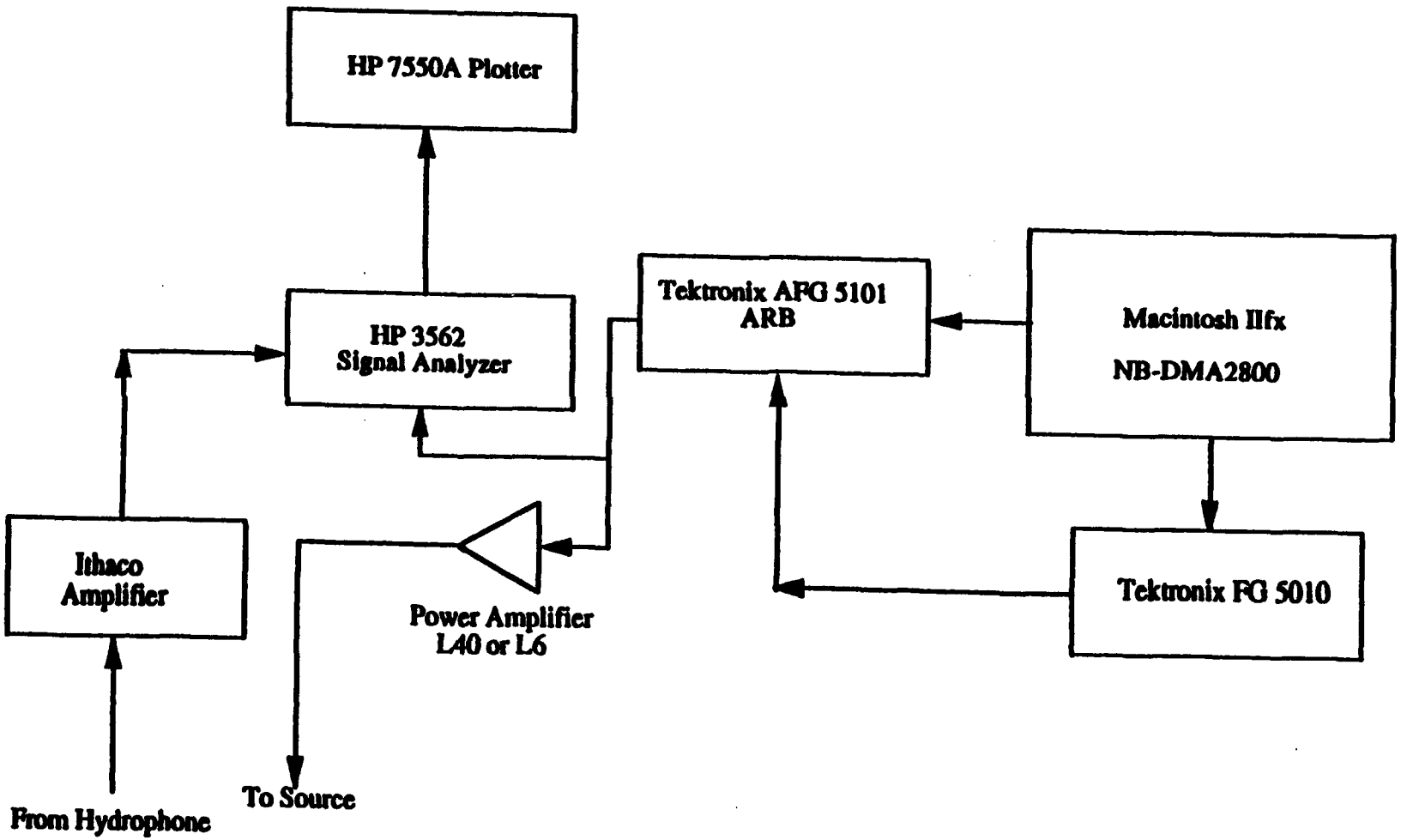


Figure 8. Signal generation/recording equipment.

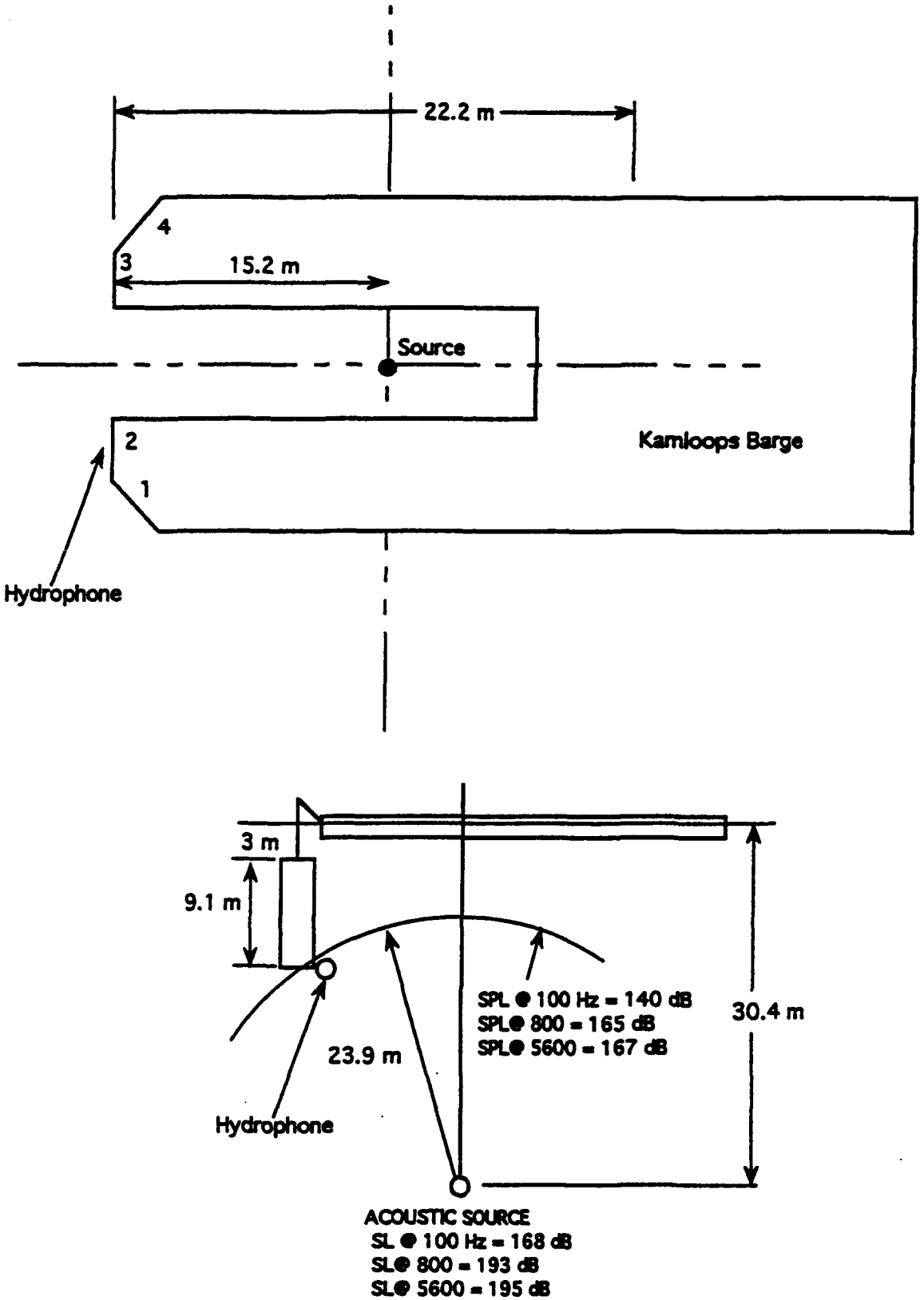


Figure 9. Acoustic source and hydrophone deployment on the Kamloops barge.

acoustic testing during the study period (Table 4). Source levels were verified at the beginning of each ensonification test.

The first test cycle began on May 4, 1993 and was completed on May 11, 1993. The acoustic instrumentation was installed and checked before starting the test. The distance between the source and the hydrophone was calculated to be 23.9 m based on the time delay between signal generation and reception at the hydrophone. A peak pressure level of approximately 847 millivolts was measured at the hydrophone (Figures 10 and 11). The source level for this test at 5,600 Hz, using the calculated range and peak pressure level, was determined to be 195 dB re 1 μPa at 1 yard. This source level resulted in a sound pressure level of 167 dB re 1 μPa at the bottom of the cages along the first arc on the ARD Kamloops barge. The sound pressure level along the second arc of cages (129 m) was 152 dB. The sound pressure level at the third arc of cages (1,292 m) was 132 dB. Sound attenuation between the source and the different cage locations was based on a spherical spreading model which was defined as 20 log (R) where R is the range, in yards, between source and point of interest. Although the spreading loss at the distant cages (1,292 m) may be transitioning to cylindrical spreading, which is defined as 10 times the logarithm of the range in yards, all attenuation values were calculated using spherical spreading. The actual sound pressure level at the distant cages (1,292 m) may be higher than predicted by the spherical spreading model, thus a spherical spreading model provided a conservative estimate of sound pressure level to which fish and zooplankton were exposed.

The second cycle of the fish and zooplankton studies began on June 8, 1993 and was completed on June 15, 1993. Fish and zooplankton were again ensonified for 7 days with a 20 ms, 5,600 Hz pulse. A check of the output of the hydrophone verified that the source level of the F56 was the same as for the first cycle of the ensonification.

The third ensonification cycle began on July 1, 1993 with an ensonification frequency of 100 Hz. The 100 Hz pulse was generated using a ITC 4138 source

Table 4. Ensonification log for monthly test cycles. Data represent time periods that acoustic signals were not generated in order to accomodate other acoustic testing on the lake.

Date	Time off	Time on
May 4 1993	--	1042
May 5 1993	1242	1243
May 5 1993	1255	1307
May 5 1993	1331	1338
May 5 1993	1349	1357
May 5 1993	1420	1428
May 5 1993	1438	1448
May 5 1993	1500	1525
May 5 1993	1533	1540
May 10 1993	1445	1740
May 11 1993	0952	--
June 8 1993	--	0931
June 10 1993	0950	1430
June 14 1993	0945	1326
June 14 1993	2201	0248 (June 15)
June 15 1993	1200	--
August 13 1993	--	1030
August 13 1993	1605	2129
August 17 1993	2015	0120 (Aug 18)
August 19 1993	1743	2218
August 20 1993	1100	--
September 7 1993	--	1045
September 7 1993	2231	0219 (Sept 8)
September 14 1993	1445	--
October 1 1993	--	1615
October 6 1993	1700	1145
October 8 1993	1615	--

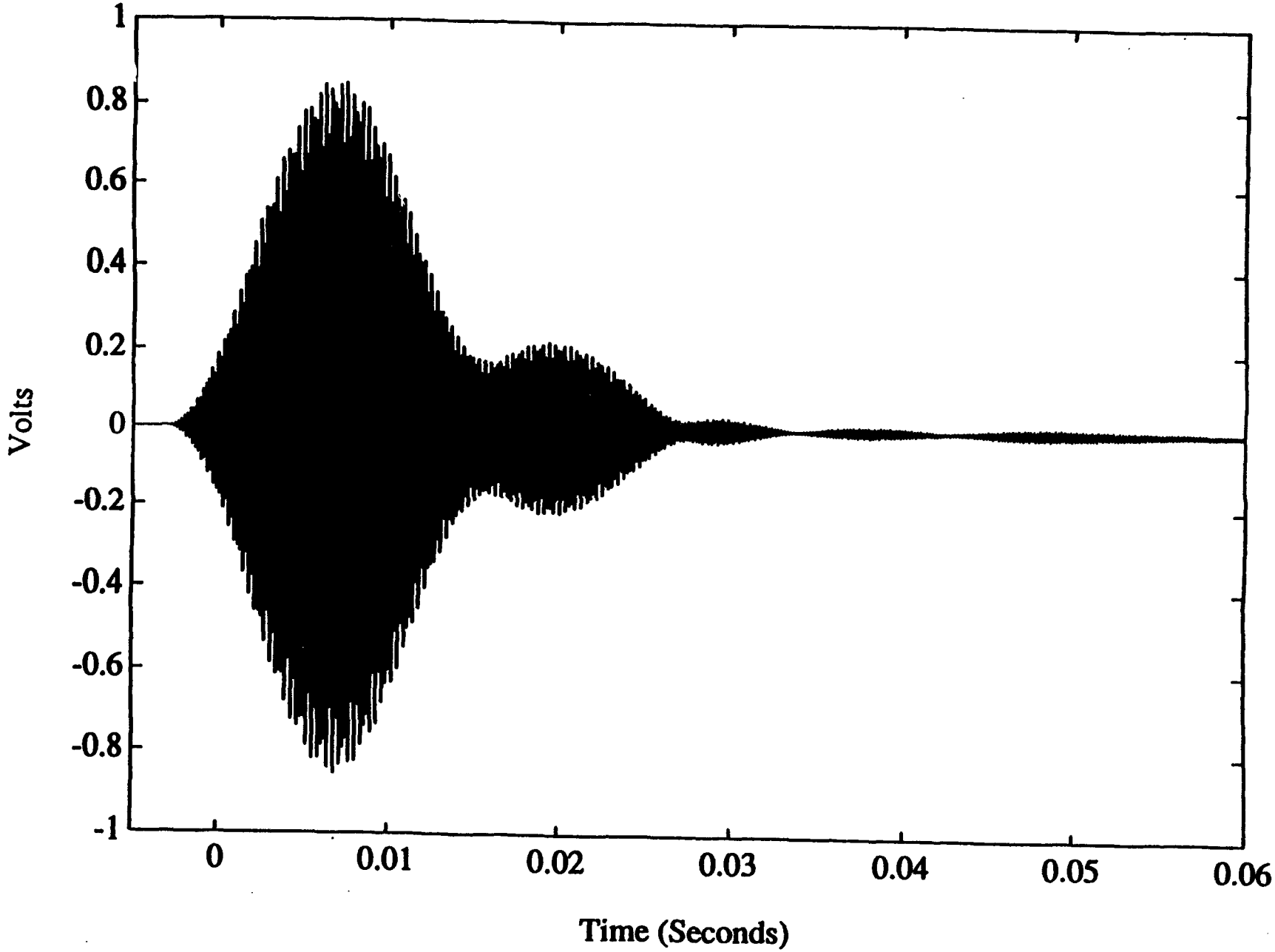


Figure 10. Time series output of the receive hydrophone for 5,600 Hz. The surface reflection begins at approximately 17 ms on the plot.

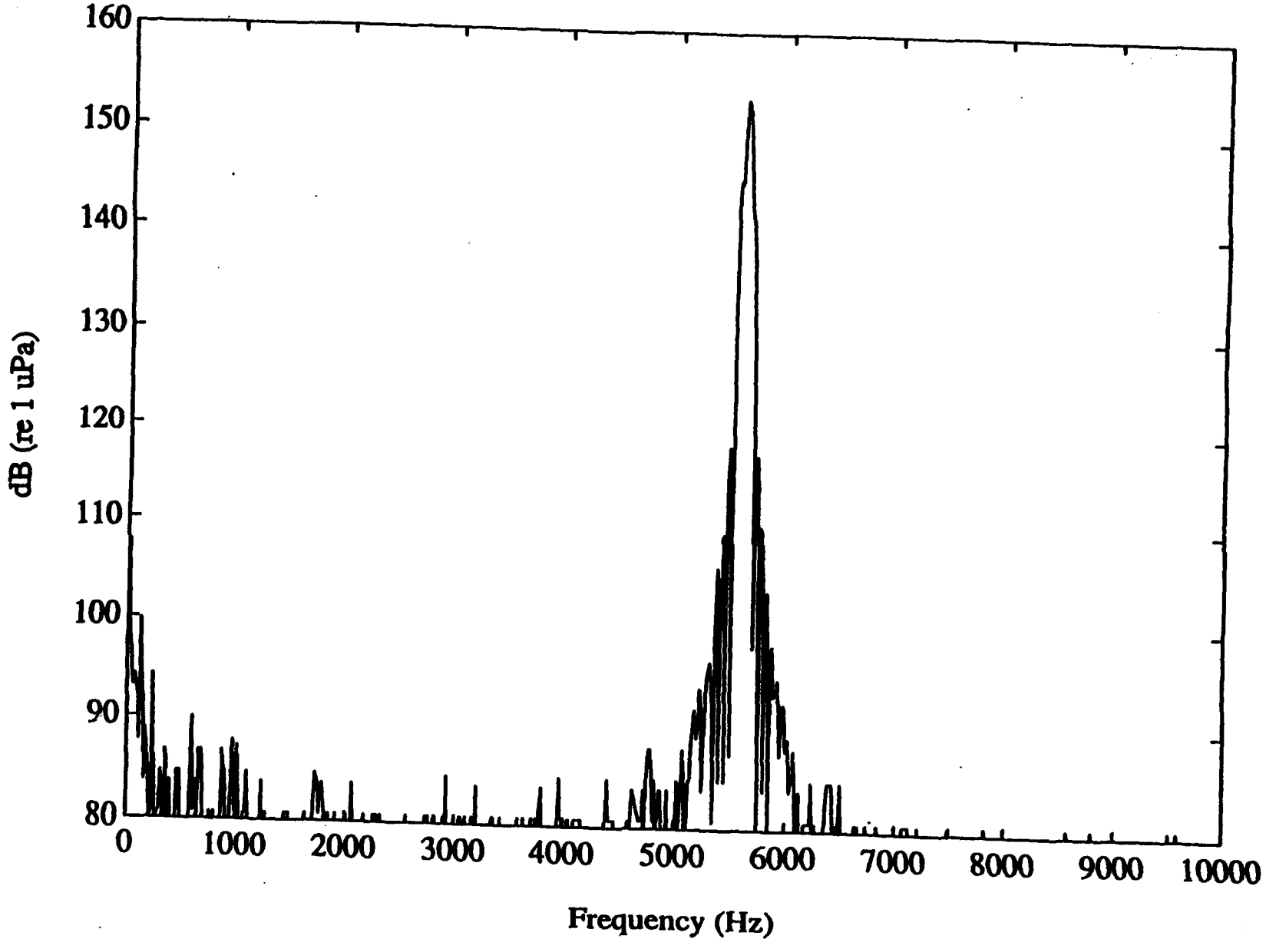


Figure 1[4]. Power spectrum for the 5,600 Hz pulse.

powered by a L40 power amplifier (Figures 12 and 13). The source level of the ITC 4138 at 100 Hz was calculated to be 168 dB[1]. This source level resulted in a sound pressure level of 140 dB at the bottom of the cages along the first arc on the ARD Kamloops barge, a calculated sound pressure level of 125 dB at the second arc of cages (129 m), and a calculated sound pressure level of 105 dB at the third arc of cages (1,292 m).

The third cycle of ensonification was interrupted by a power outage at the Kamloops barge. The sound generating equipment was checked at 1300 h on July 2, 1993 and all was functioning properly. When the equipment was checked again at 0910 h on July 6, 1993, following the long holiday weekend, the power amplifier was in an over-voltage shutdown mode. As a result, the length of time for which sound was not being generated is unknown. It was subsequently determined that if the arbitrary function generator loses power and then powers back on, a transient will be sent to the amplifier causing the over-voltage shutdown. The amplifier was reset and the sound generation began again at 0930 hours on July 6, 1993 and continued without incident until 1130 h on July 8, 1993. After discovering the power shut-down, we decided to complete this test and continue with the other 100 Hz experiment (August). Personnel from the University of Idaho and the ARD agreed that if results from July and August experiments (Tasks 1.1, 1.2, 1.3) indicated any detrimental effects, another experiment at 100 Hz would be warranted and conducted either late October 1993 or early Spring 1994.

The fourth cycle of ensonification began on August 2, 1993, with a frequency of 100 Hz. The output of the ITC 4138 was verified to be a source level of 168 dB @ 1 yard by checking the sound pressure level received at the H56 hydrophone. This

[1] The L40 power amplifier and ITC 4138 acoustic source are prototype ISMS components. The source level of 168 dB is the maximum level the ISMS will produce at 100 Hz.

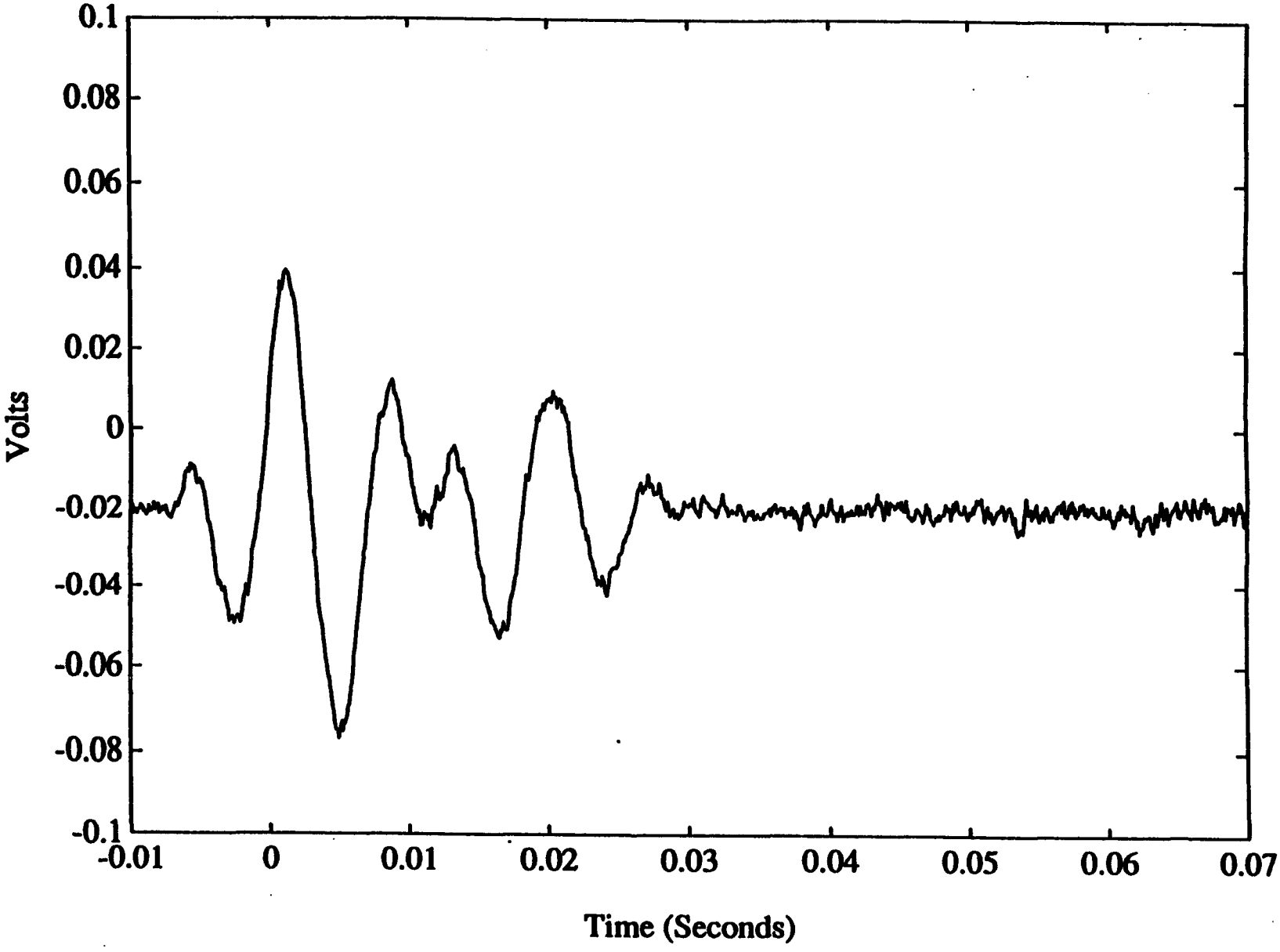


Figure 12. Time series output of the receive hydrophone for 100 Hz.

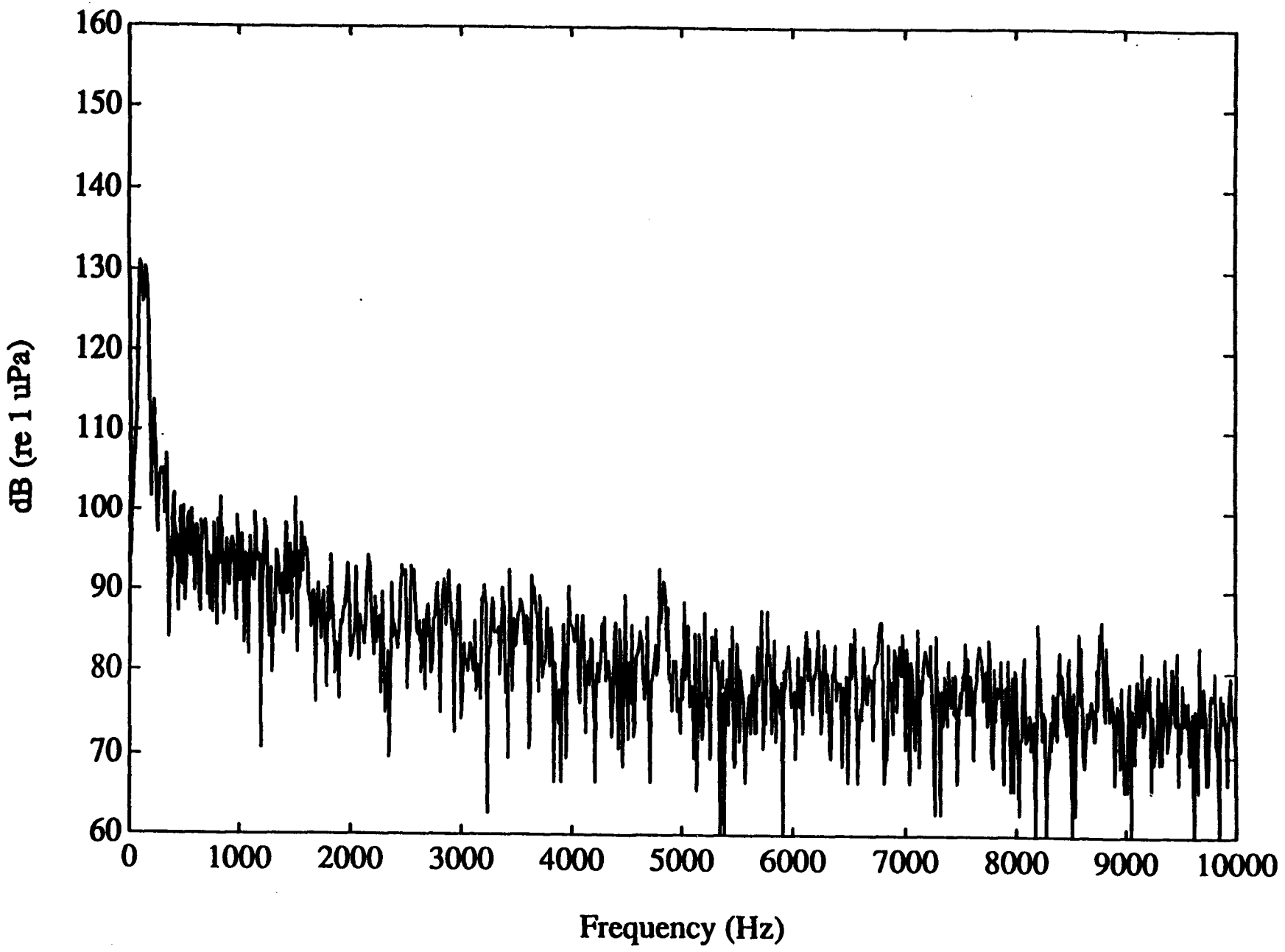


Figure 13. Power spectrum for the 100 Hz pulse.

experiment was repeated on August 12, 1993 because of problems caused by the bacterium *Flexibacter columnaris* -- a epithelial disease affecting fish that have been stressed from handling and high water temperatures. Kokanee were restocked on August 11, 1993 and the repeated test was completed on August 20, 1993.

The fifth cycle of ensonification began on September 7, 1993, with a frequency of 800 Hz and ended on September 14, 1993. The source level of the ITC 4138 at 800 Hz was calculated to be 193 dB (Figures 14 and 15). For this cycle of ensonification, the source at the Kamloops barge was lowered by 10 m to compensate for lowering the fish cages to avoid excessively warm water temperatures. This source level resulted in a sound pressure level at the bottom of the cages on the Kamloops barge of 162 dB, a calculated sound pressure level of 150 dB at the second arc of cages (129 m), and a calculated sound pressure level of 130 dB at the third arc of cages (1,292 m).

The sixth cycle of ensonification began on October 1, 1993 and was completed on October 8, 1993. The source was raised back to its original position for this cycle so the calculated sound pressures were 165 dB at the first arc of cages along the ARD Kamloops barge, 150 dB at the second arc of cages (129 m), and a calculated sound pressure level of 130 dB at the third arc of cages (1,292 m). A check of the output of the hydrophone verified that the source level of the ITC 4138 source was the same as for the September experiment.

Background Noise

To quantify the non-ISMS acoustic energy present in the lake, background noise levels were recorded by ARD from May 4 to September 6, 1993 at the ARD Kamloops barge in the southern basin of Lake Pend Oreille. Background noise sound pressure levels were recorded six times per day for a duration of 2 minutes at 4-hour intervals. The recording times in a 24 hour clock were; 0000, 0400, 0800, 1200, 1600, and 2000 hours. A hydrophone was located at a depth of 12 m below the surface of the water.

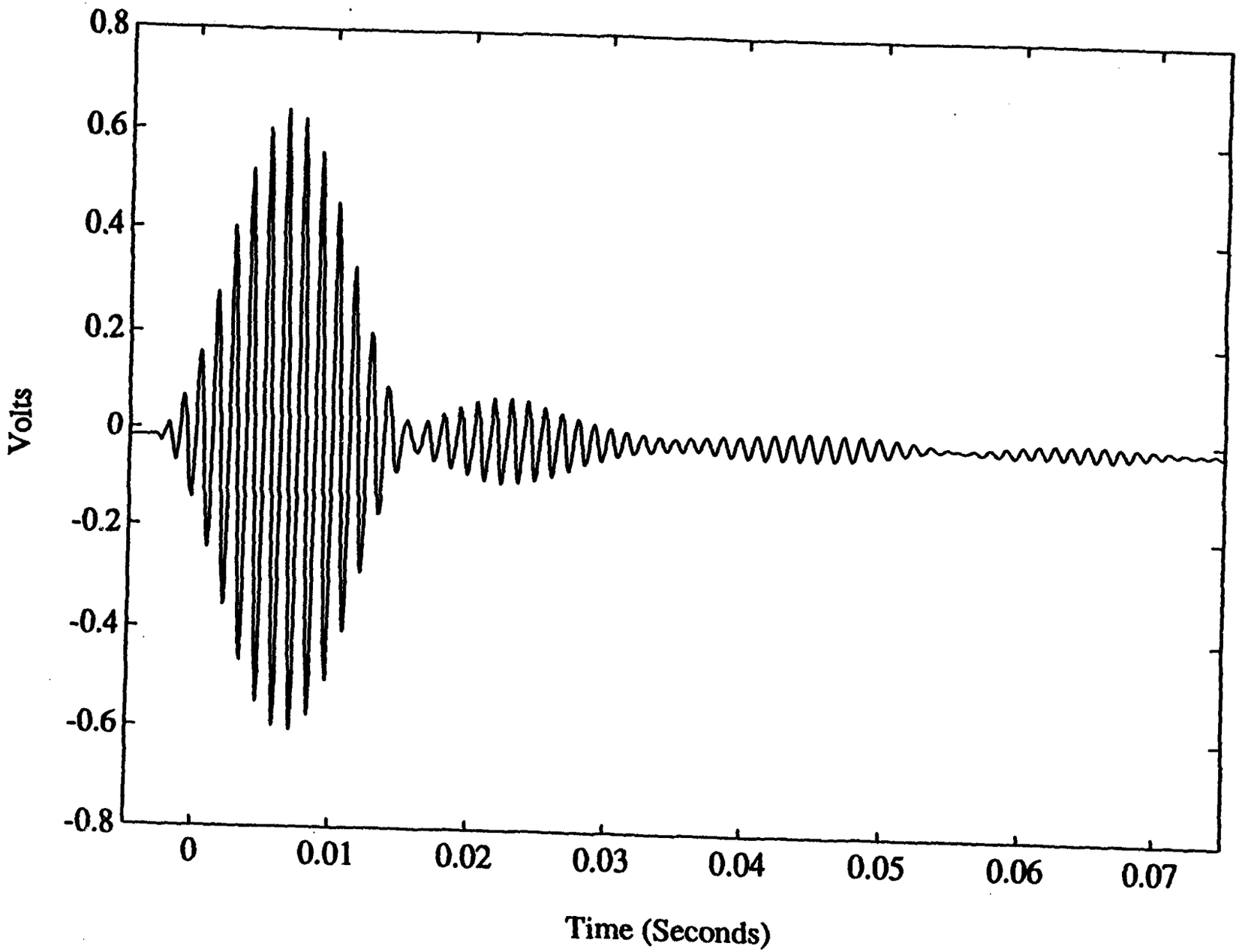


Figure 14. Time series output of the receive hydrophone for 800 Hz.

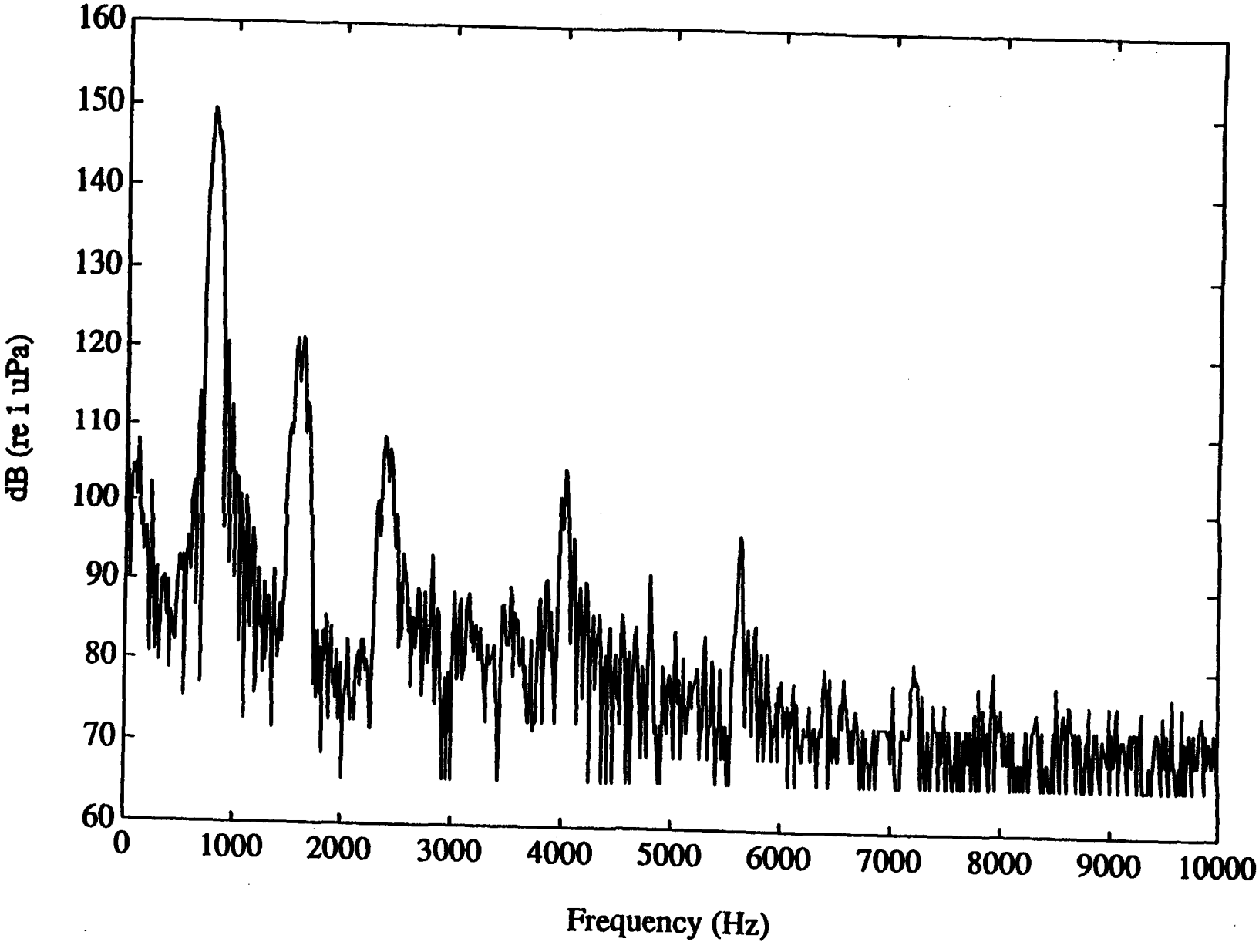


Figure 15. Power spectrum for the 800 Hz pulse.

The hydrophone signal was amplified using an Ithaco type 456 amplifier and recorded on a Panasonic 1500 Super VHS (Figure 16). Recorded data were processed using a Hewlett Packard 3561A signal analyzer. Final data is a plot of averaged one-third-octave band levels, in dB re 1 μPa, versus frequency for frequencies between 100 Hz and 10,000 Hz.

Underwater ambient noise studies in Lake Pend Oreille usually exclude man-made noise sources when presenting data on the acoustic characteristics of the lake. Since the intent of these data were to quantify the non-ISMS related noise to which fish and zooplankton were exposed at the ARD Kamloops barge, both natural and man-made noises were included in the final average background noise results. The only background noise recordings not included in the averages are those contaminated by the acoustic sources used for the test.

The average background noise levels in the south-central portion of the lake for the first ensonification cycle (5,600 Hz) were measured from May 4-June 7 (Figure 17). The average background noise levels during the second cycle of ensonification (5,600 Hz) were measured from June 8-30 (Figure 18). Average background noise levels during cycles three and four (100 Hz) were measured from July 1-August 1 and August 2-September 6, respectively (Figures 19 and 20).

Chronological comparisons of the May-September, 1993 background noise data show that May is the quietest month and August is the noisiest. August is the period of heaviest recreational boat traffic on Lake Pend Oreille resulting in higher overall levels than those in May. Since the data between May and September were averages for the period of interest, and boat noise was included, the background noise levels were dominated by man-made noise. Natural noise from wind and wave action, typically lowest in the summer months, was masked by man-made acoustic energy.

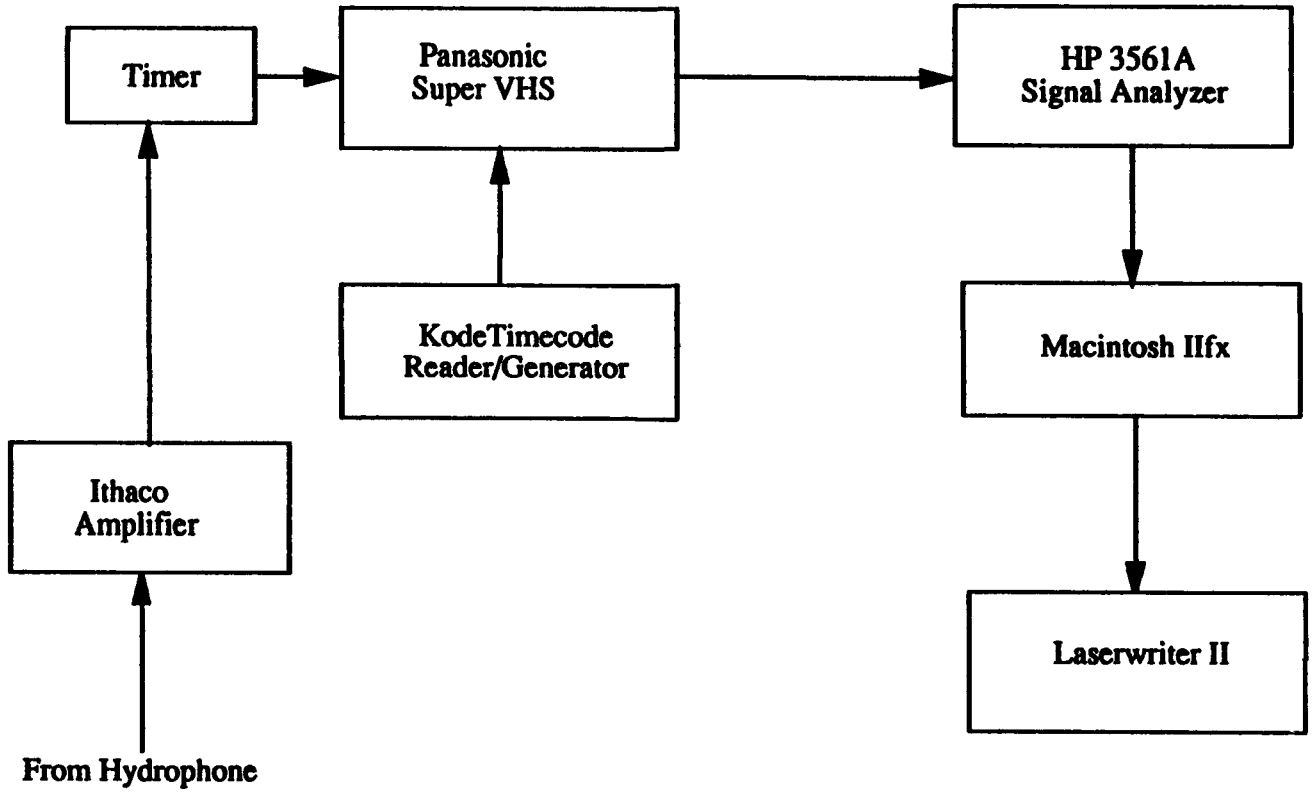


Figure 16. Background noise recording/processing equipment.

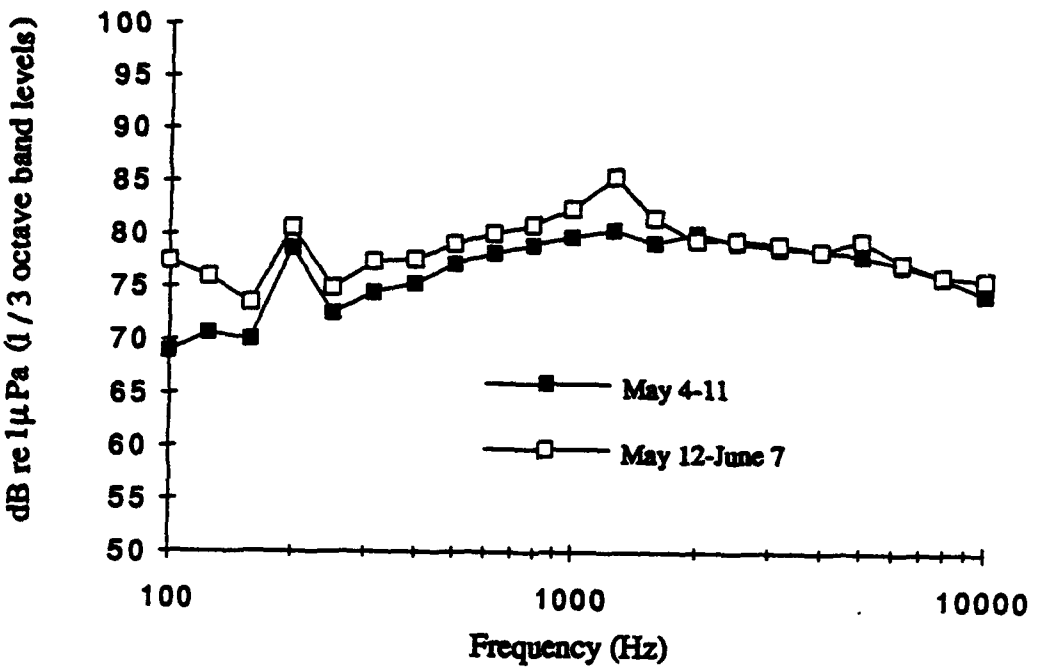


Figure 17. Average background noise level during first 5,600 Hz ensonification (May 4-11) and while no ISMS sound was generated (May 12-June 7).

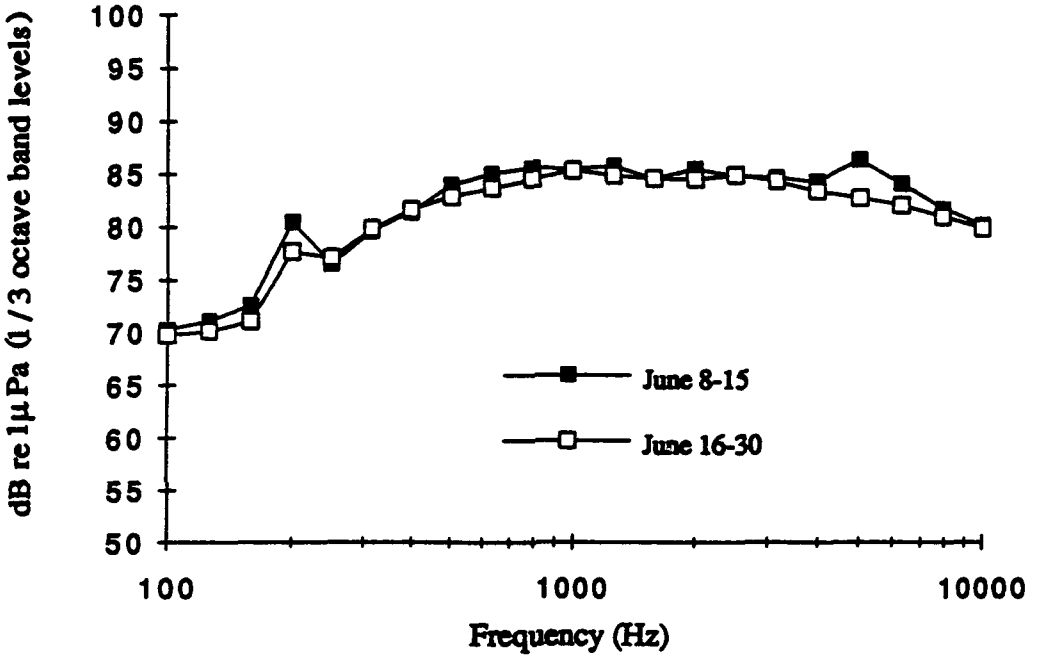


Figure 18. Average background noise level during second 5,600 Hz ensonification (June 8-15) and while no ISMS sound was generated (June 16-30).

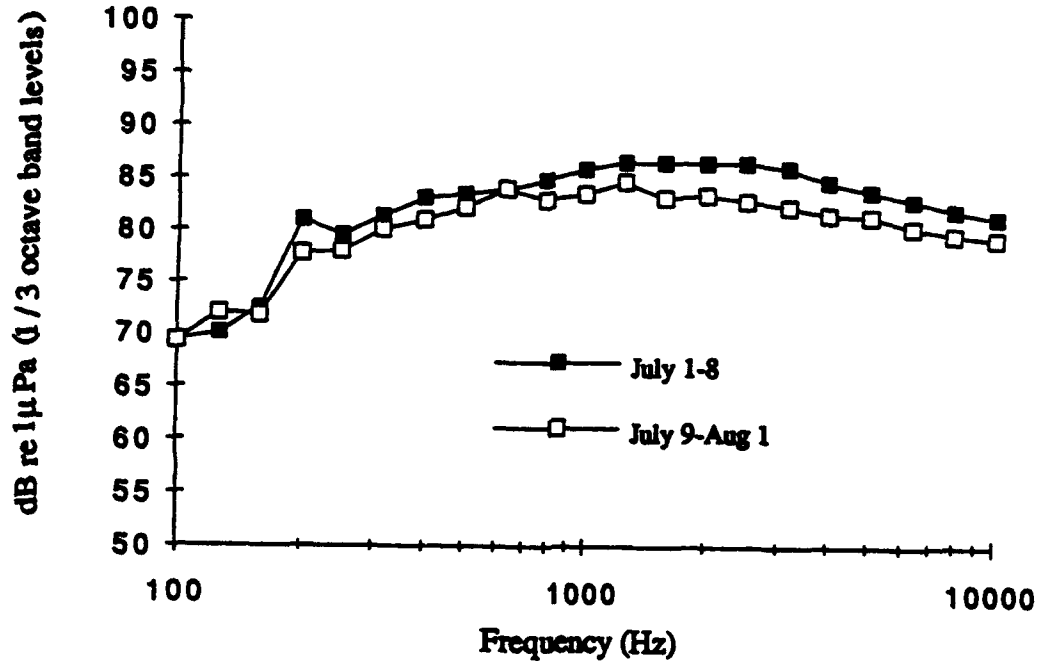


Figure 19. Average background noise level during first 100 Hz ensonification (July 1-8) and while no ISMS sound was generated (July 9-August 1).

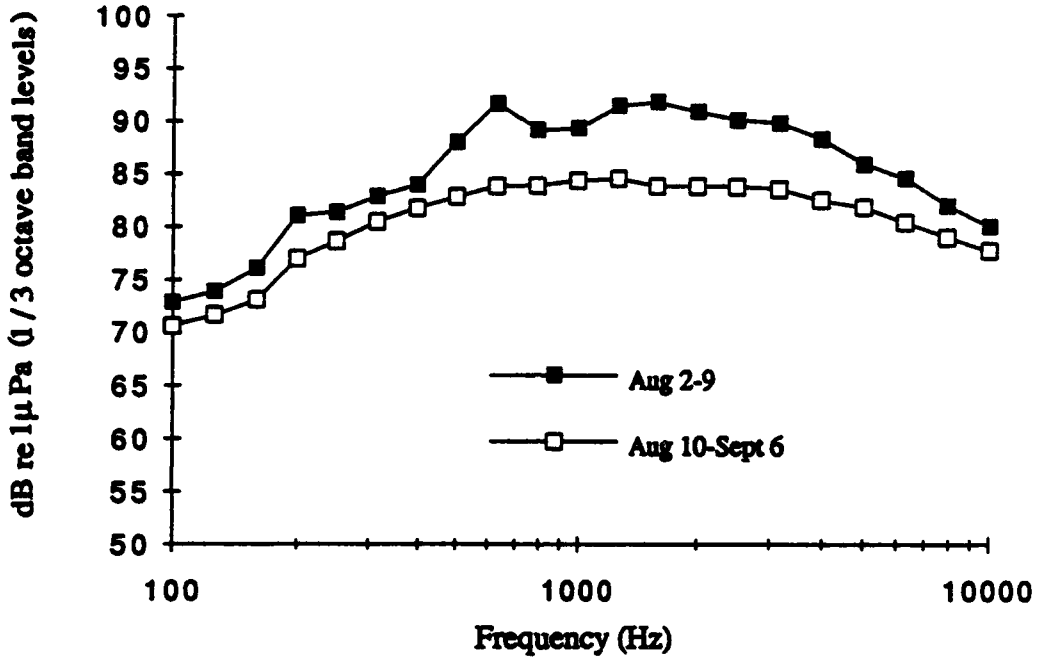


Figure 20. Average background noise level during second 100 Hz ensonification (August 2-9) and (August 11-18). Note: Test was repeated on August 11, 1993).

Task 1.1

Zooplankton Abundance and Biomass in Response to Simulated ISMS Ensonification.

INTRODUCTION

Zooplankton are an important food base for kokanee and other fishes in Lake Pend Oreille. Most of the macrozooplankton occur from the surface to a depth of 46 m (Rieman and Falter 1981) and are within the BIZ region identified by the Aquatic Studies Technical Report (Denny et al. 1990). The dominant zooplankton in Lake Pend Oreille consist of Copepoda with *Diaptomus ashlandi* and *Cyclops bicuspidatus* the predominant copepods. Kokanee utilize *D. ashlandi* and *C. biscuspidatus* during winter and spring and switch to Cladocerans in mid to late summer (Rieman and Falter 1981). *Bosmina longirostris* and *Daphnia* spp. are the predominant Cladocerans in Lake Pend Oreille. Since the introduction of *Mysis relicta*, there has been a temporal shift in *B. longirostris* abundance from early summer peaks to late summer peaks and a concomitant decrease in *Daphnia* spp. densities (Rieman and Falter 1981). These changes in the macrozooplankton community may have been detrimental to kokanee populations, particularly emerging kokanee fry (Rieman and Falter 1981).

Little information has been published on effects of underwater sound on invertebrates. In a study of marine invertebrates, Frings and Frings (1967) noted that because of their small size, invertebrates are stimulated differently and can be bodily transported or grossly vibrated as a result of underwater sound waves. Ensonifying *Paramecium* with ultrasound (frequencies and sound pressures not given), Frings and Frings (1967) noticed that micro-currents that would be unnoticed by an animal the size of a killifish, caused mortality among *Paramecium*. Additionally, other research suggests an inverse relationship between biological impact and body size of fishes (Yelverton et al. 1975) , i.e. the smaller the body size the greater the effect of sound

waves. The inverse relationship between sound wave effects and body size indicates that zooplankton may be more susceptible to underwater sound than larger animals such as fishes.

The limited amount of available data, however, should not indicate that underwater sound would have only gross, physically destructive effects on macrozooplankton. Morphologically, zooplankton exhibit a wide array of appendages and sensory projections (Pennak 1989). The potential role of these receptors and appendages with respect to detection of underwater sound are virtually unknown (Frings and Frings 1967).

Secondary production in Lake Pend Oreille's southern basin is an important element in the Lake's ecology, particularly as a food source for emerging kokanee (Rieman and Falter 1981). The objective of this aspect of the study was to examine effects of simulated ISMS underwater sound on zooplankton densities and biomass in the southern basin of Lake Pend Oreille. We conducted in-situ experiments to examine frequency and sound pressure level effects on zooplankton abundance, biomass, and composition.

METHODS

Zooplankton Ensonification

Zooplankton communities were held in net pens constructed of 160 micron Nitex fabric stretched around 0.76 m diameter hoops. The hoops were made of 0.015 m diameter steel conduit. The pens were 2.1 m in length with a zipper sewn along the inside diameter of the top hoop to allow access into the pen. The volume of each zooplankton pen was 1,000 L.

We deployed a total of 12 zooplankton pens at test and control sites based from the ARD Kamloops barge. The zooplankton pen was attached to a mooring system by a 3 m separation bar and kept parallel to the main mooring line at 4 m in depth by two

surface buoys attached to the end of the separation bar (Figure 21). Three of the four available sites from each sound pressure level (i.e. arcs) were randomly selected during each test cycle for sampling (Figure 22).

Six test cycles were conducted from May through October 1993 (Table 5). We inoculated zooplankton pens at the beginning of each test cycle. Inoculation was accomplished by lowering the pen, with the top panel open, to a depth of 7 m and pulling the pen vertically to the surface. The inoculation technique provided us with high zooplankton densities inside the pens. We attempted, using this technique, to increase density of the zooplankton community in the pens. However, initial variability in zooplankton densities was unavoidable since zooplankton distribution is often "patchy" within a natural body of water and not uniformly distributed. Realizing limitations in conducting these in-situ experiments, our goal was to monitor changes in zooplankton density over time with respect to each sound pressure level and compare these trends to the controls. Differences in individual growth rates of zooplankton, species-specific responses, or reproductive rates would not be detected using our inoculation technique and were neither controlled nor studied during the experiments. Additionally, zooplankton densities (no./L) inside the pens were higher than ambient lake densities and do not reflect actual zooplankton densities in open water of Lake Pend Oreille.

Simulated ISMS sound was initiated at the beginning of each month and zooplankton were ensonified for 7 days (Figure 23). We sampled zooplankton weekly for 3 weeks after ensonification. Sampling occurred at night to take advantage of more even distribution of zooplankton in the pens due to diel vertical migration (Hutchinson 1967; Hall et al. 1970; Wright et al. 1980). Recovery of the zooplankton pens required gaffing the two surface buoys attached to the end of the separation bar and securing them to the research boat (Figure 21). The boat was allowed to stabilize before pulling the zooplankton pen to the surface. The top hoop of the zooplankton pen was raised

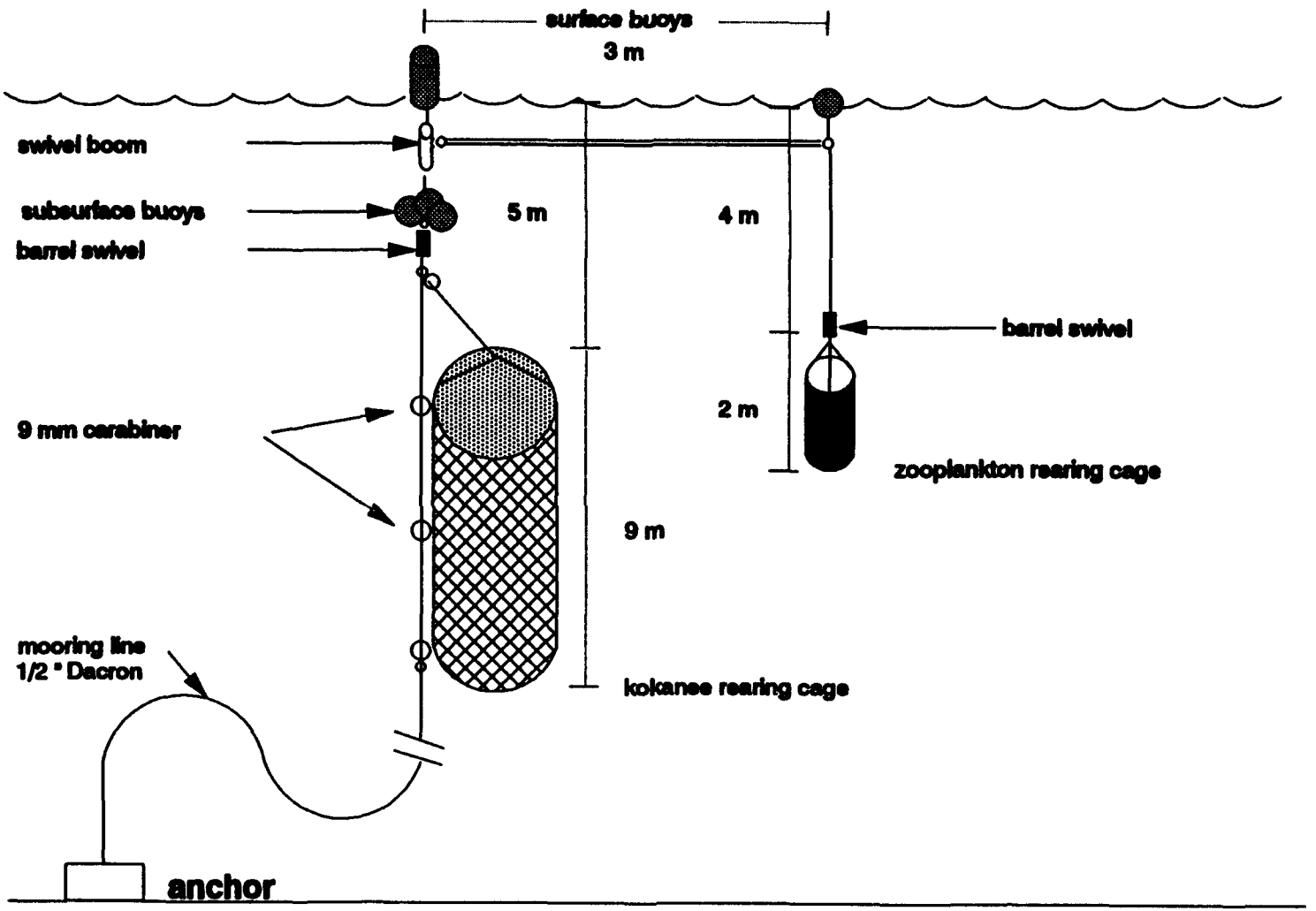


Figure 21. Cage configuration for kokanee and zooplankton rearing cages.

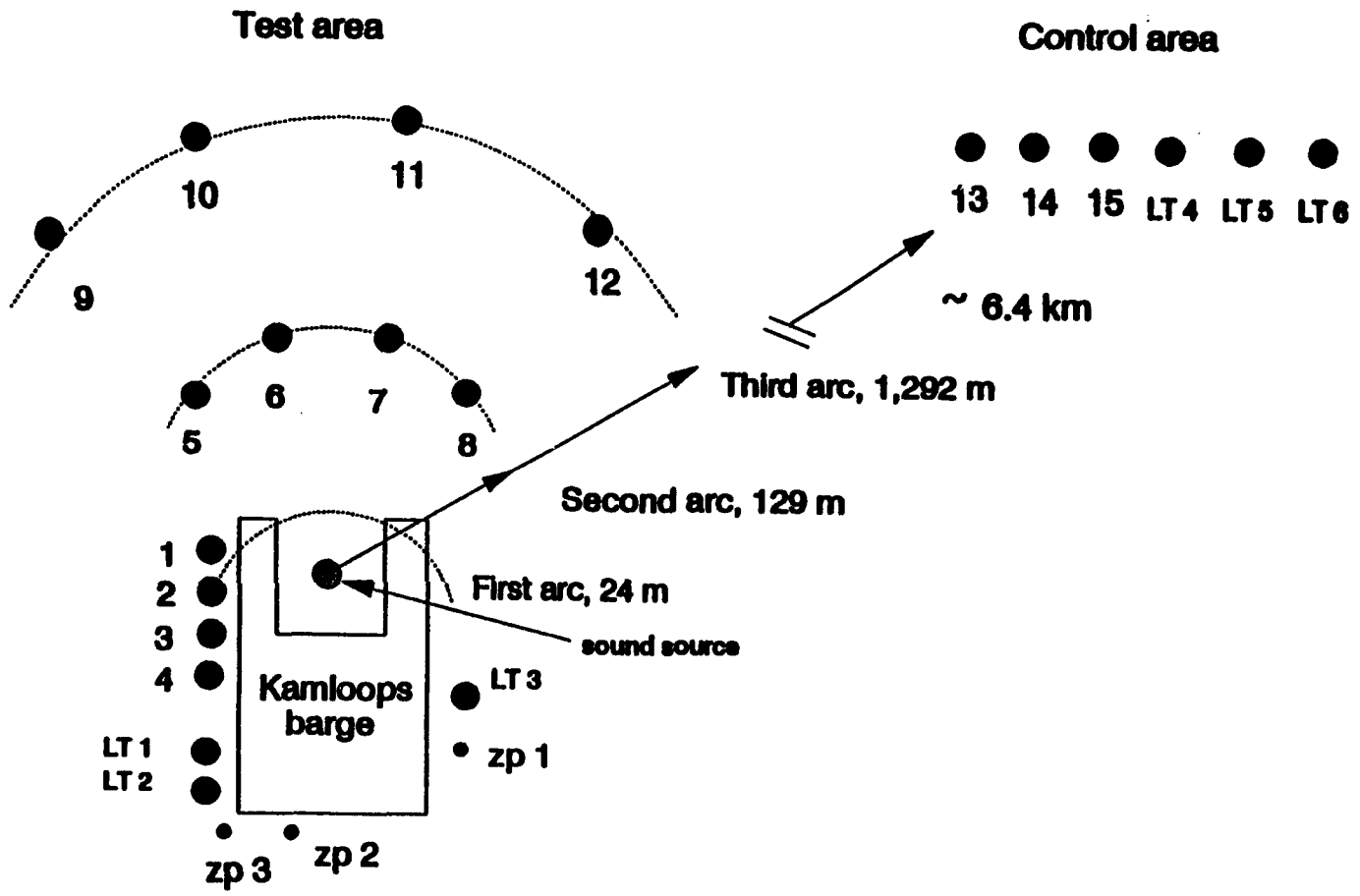
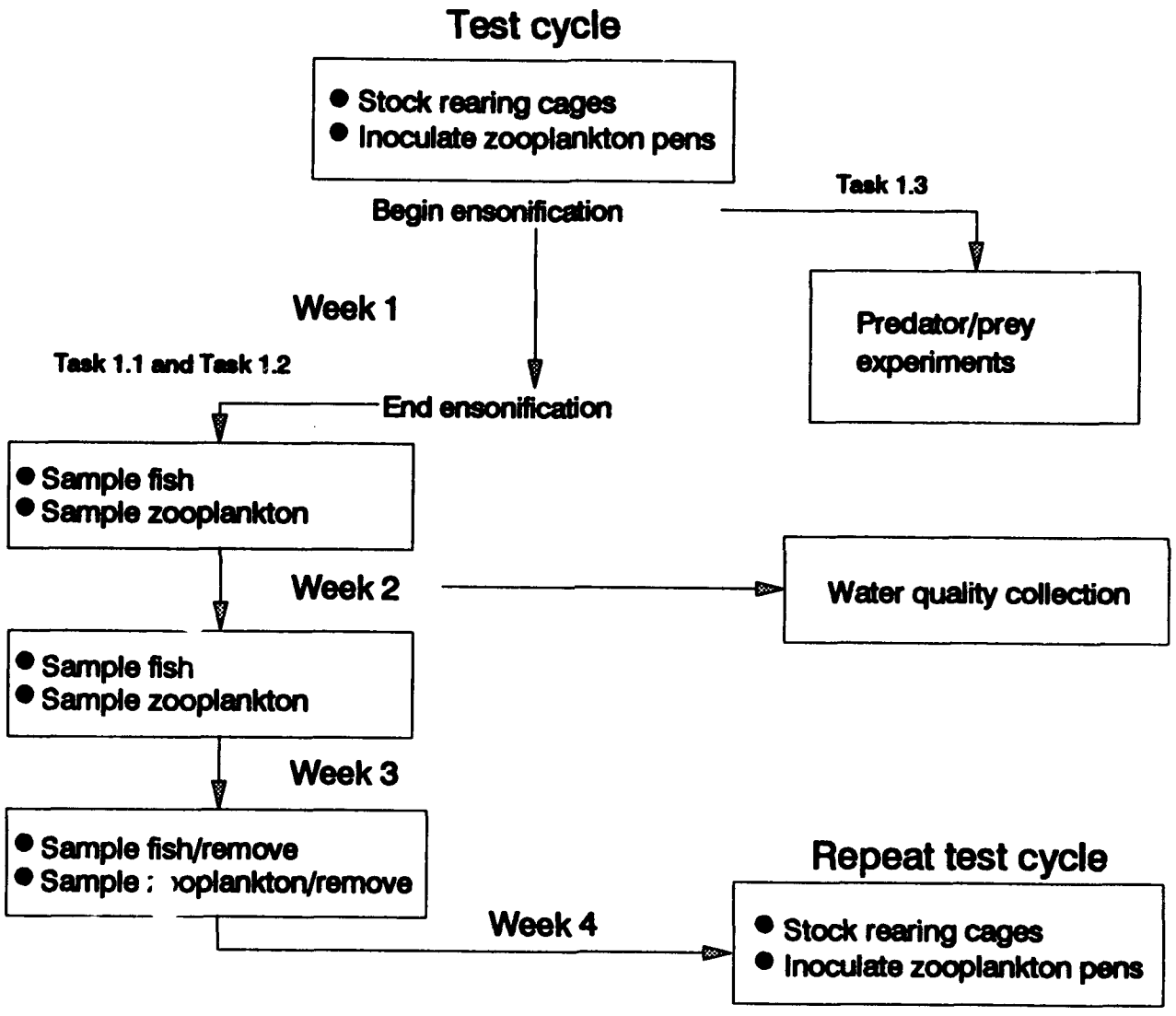


Figure 22. Site diagram of test and control areas for zooplankton and kokanee growth studies (Tasks 1.1 and 1.2). Three of the four sites per arc were randomly selected each month. LT represents long-term exposure cages and ZP represents locations of zooplankton pens on the Kamloops barge.

Table 5. Frequencies and sound pressure levels used to ensonify fish and zooplankton during monthly test cycles. All sound pressure levels measured in dB re 1 μPa.

Month (1993)	Frequency (Hz)	Sound pressure levels (dB)
May	5,600	167 152 132
June	5,600	167 152 132
July	100	140 125 105
August	100	140 125 105
September	800	162[a] 150 130
October	800	165 150 130

[a] Sound source was lowered from 30 m to a depth of 40 m. This resulted in a sound pressure level of 162 dB for cages deployed from the Kamloops barge. SPL at the remaining cages were unchanged by lowering the source. All other cages were lowered 10 m to provide fish with thermal refuge. Cages at the Kamloops barge (162 dB) were not lowered due to obstructions underneath the barge.



ure 23. Flow chart showing monthly sampling schedule for Task 1.1, Task 1.2 and Task 1.3.

approximately 0.3 m above the water surface to prevent introduction of non-captive zooplankton into the pen. The zooplankton community contained inside the pen was allowed 5-minutes to redistribute within the water column before sampling. We used a 10 L Schindler box to sample from the top, mid, and bottom sections of the zooplankton pen. Individual samples were transferred to a 50-ml jar and preserved with a 90% ETOH and 10% Glycerin solution. In addition to the three pen samples, a single lake sample was obtained to monitor ambient pelagic zooplankton densities at each pen site.

At the University of Idaho laboratory, zooplankton samples were analyzed for species composition, species abundance (no./L), and biomass (μg/L). We identified and counted species using a compound dissecting microscope at 160 x (Pennak 1989). For large samples, either two 5-ml or one 10-ml subsamples were obtained (Masundire 1992). A total of 16% or 131 of the 819 pen samples were subsampled. Where subsampled, a representative subsample was ensured by transferring the sample to a 100-ml beaker, adjusting to a known volume, and mixed. The subsample was then extracted from the beaker using a 5-ml pipette withdrawing from the bottom to top and transferred to a counting chamber (Masundire 1992). Subsamples were multiplied by a correction factor to estimate zooplankton densities for each pen.

Zooplankton weights (μg) were determined from length/weight (dry weight) regression equations for individual species (Downing and Rigler 1984). Individual lengths were measured on the first 30 individuals from the five dominant genera collected during May through October 1993, and mean weights were calculated for each species (Table 6). Total zooplankton biomass and biomass of cladocerans and copepods were calculated by multiplying the mean dry weight and abundance data for each species from each pen (Pace et al. 1992).

We compared densities and biomass (total zooplankton, copepods, and cladocerans) of ensonified and non-ensonified zooplankton for each test cycle. We tested for a treatment x date interaction among zooplankton densities and biomass

Table 6. Five dominant zooplankton species collected during May-October 1993.

Species	Copepoda	Cladocera
Cyclops biscuspidatus thomasi	X	
Diaptomus ashlandi	X	
Bosmina longirostris		X
Daphnia galeata mendotae		X
Diaphanasoma leuchtenbergianum		X

using repeated measures analysis of variance (PROC GLM, SAS 1987). We compared densities and biomass of each arc (or sound pressure level) to the control for each experiment. An experiment-wise error rate of 0.016 was used to detect significant differences. The experiment-wise error rate represents an alpha level of 0.05 divided by the number of individual comparisons (i.e. 3). Besides total zooplankton densities, we also analyzed copepod and cladoceran densities and biomass to examine potential responses from the two different taxa.

Water Chemistry

We monitored physical and biological characteristics along each arc throughout the study. Our intent in monitoring physical and biological characteristics was to provide baseline data that could be used to characterize natural conditions at the test and control areas. Water samples for chlorophyll "a" were taken monthly at each site with a 2-liter Kemmerer bottle. Though chlorophyll "a" does not represent a direct measure of primary productivity, it is often used as an indicator of primary production (Carlson 1977). All samples for chlorophyll "a" were stored in ice while in the field and transferred to refrigerators upon return to the University of Idaho laboratory. Within 24 hours, all samples were filtered through 0.45-μm glass fiber filters and frozen. The frozen filters were later ground in iced acetone with hand tissue grinders and the supernatant analyzed with a Beckman DU-8 Spectrophotometer (American Public Health Association 1992). Chlorophyll "a" was calculated by the trichromatic method and expressed as μg/L.

Temperature (C) and dissolved oxygen (mg/L) data were collected monthly at all test and control sites with a YSI Model 57 Temperature and Dissolved Oxygen meter. Temperature and dissolved oxygen were obtained at a depth of 9 m. Mean monthly water temperatures and dissolved oxygen were calculated for each arc.

RESULTS

Zooplankton Ensonification

We ensonified captive zooplankton at a frequency of 5,600 Hz during May and June 1993. Sound pressure levels at 5,600 Hz were 167 dB at the first arc, 152 dB at the second arc, and 132 dB at the third arc. We did not observe significant treatment x date interactions ($P > 0.016$) among zooplankton densities (Table 7) or biomass (Table 8) during experiments at 5,600 Hz. Zooplankton abundance was low in May, though trends in abundance were generally similar among arcs (Figure 24). Abundance of captive zooplankton was generally highest during May 18 and declined by May 25. Lake samples showed a similar trend during May with densities generally decreasing by late May at all test and control sites (Figure 25). Cladocerans were extremely rare in the captive zooplankton communities during May. Thus, total zooplankton densities (no./L) and biomass (μg/L) estimates reflect mostly copepod abundance during May 1993. Zooplankton abundance during June was similar among arcs though we observed more variability in zooplankton densities at the 132 dB sites (Figure 26). Densities at these sites decreased more dramatically after the first week of captivity compared to zooplankton densities at other arcs. Additionally, densities at the 132 dB sites increased after week two (June 15) and three (June 28). Lake samples (Figure 25) at the 132 dB arc showed a similar peak in abundance during week two of the experiment (June 15) whereas we did not observe higher abundance in lake samples at other locations during June 15. Cladoceran abundance was again low during June though trends were generally similar among arcs (Figure 27).

During July and August 1993, we ensonified captive zooplankton at 100 Hz. Sound pressure levels at 100 Hz were 140 dB at the first arc, 125 dB at the second arc, and 105 dB at the third arc. We observed more variability among zooplankton densities during July at 100 Hz ($P = 0.04$) (Figure 28) than we did during May or June experiments at 5,600 Hz. Initial zooplankton densities at the control sites (June 29)

Table 7. Summary statistics comparing zooplankton densities at test and control sites. Differences considered significant at an experiment-wise error rate of 0.016.

Month	Sound pressure level (dB)	Total zooplankton	Cladocerans	Copepods
		P>F	P>F	P>F
May	167	0.64	--	0.64
	152	0.16	--	0.16
	132	0.08	--	0.08
June	167	0.96	0.93	0.96
	152	0.05	0.13	0.05
	132	0.14	0.69	0.14
July	140	0.42	0.08	0.40
	125	0.04	0.23	0.04
	105	0.02	0.91	0.02
August	140	0.51	0.36	0.62
	125	0.21	0.09	0.30
	105	0.11	0.05	0.19
September	162	0.67	0.50	0.91
	150	0.26	0.40	0.16
	130	0.43	0.54	0.34
October	165	0.90	0.70	0.75
	150	0.47	0.18	0.73
	130	0.84	0.66	0.94

Table 8. Summary statistics comparing zooplankton biomass at test and control sites. Differences considered significant at an experiment-wise error rate of 0.016.

Month	Sound pressure level (dB)	Total zooplankton	Cladocerans	Copepods
		P>F	P>F	P>F
May	167	0.17	--	0.17
	152	0.17	--	0.17
	132	0.17	--	0.17
June	167	0.78	0.60	0.36
	152	0.14	--	0.07
	132	0.45	0.82	0.20
July	140	0.88	--	0.88
	125	0.65	0.97	0.65
	105	0.52	0.36	0.52
August	140	0.25	0.36	0.20
	125	0.41	0.32	0.50
	105	0.36	0.28	0.44
September	162	0.84	0.57	0.99
	150	0.40	0.45	0.31
	130	0.51	0.47	0.45
October	165	0.82	0.89	0.76
	150	0.55	0.22	0.75
	130	0.82	0.49	0.96

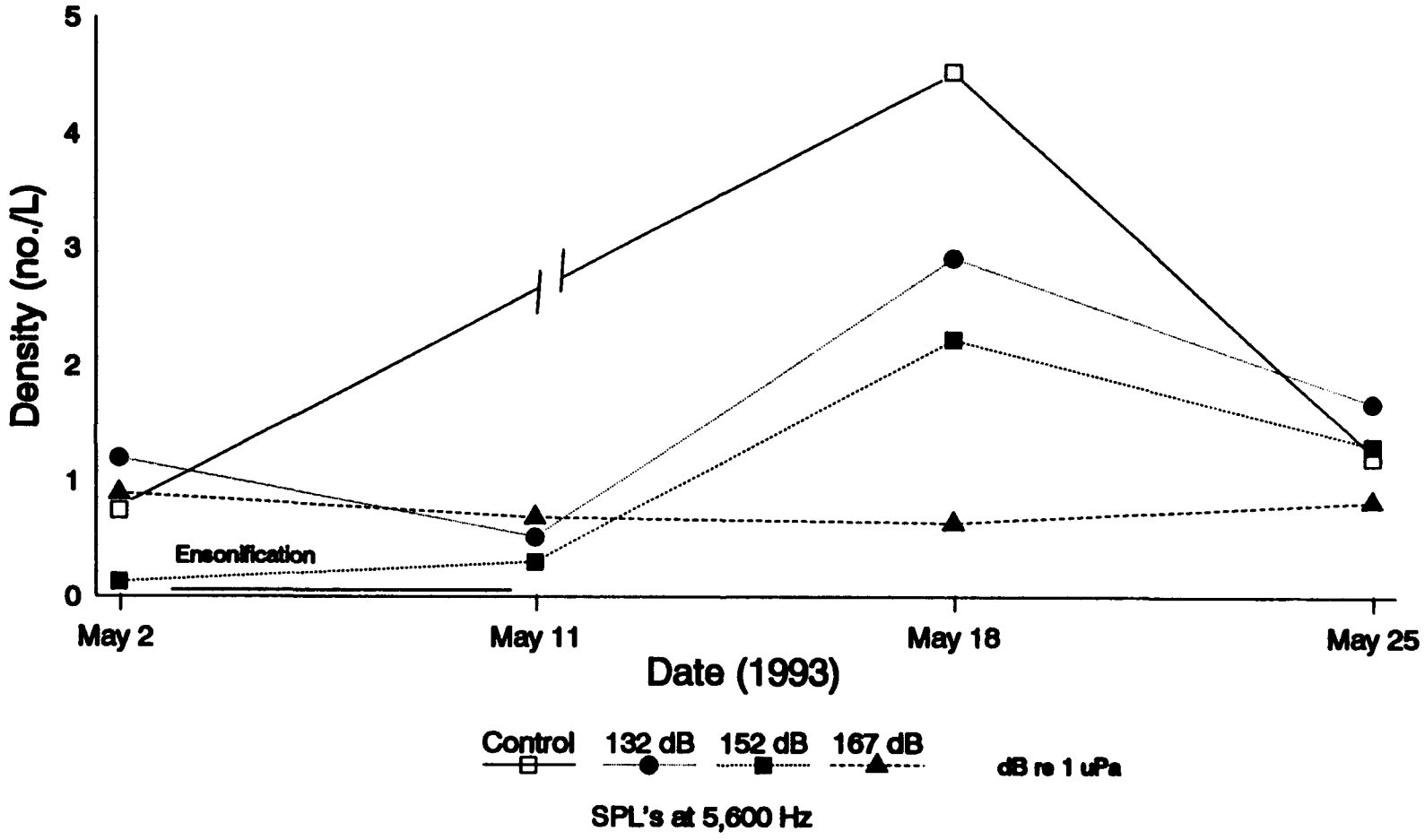


Figure 24. Total zooplankton densities inside net pens for May 1993. Control sites were not sampled on May 11, 1993 due to submerged mooring systems.

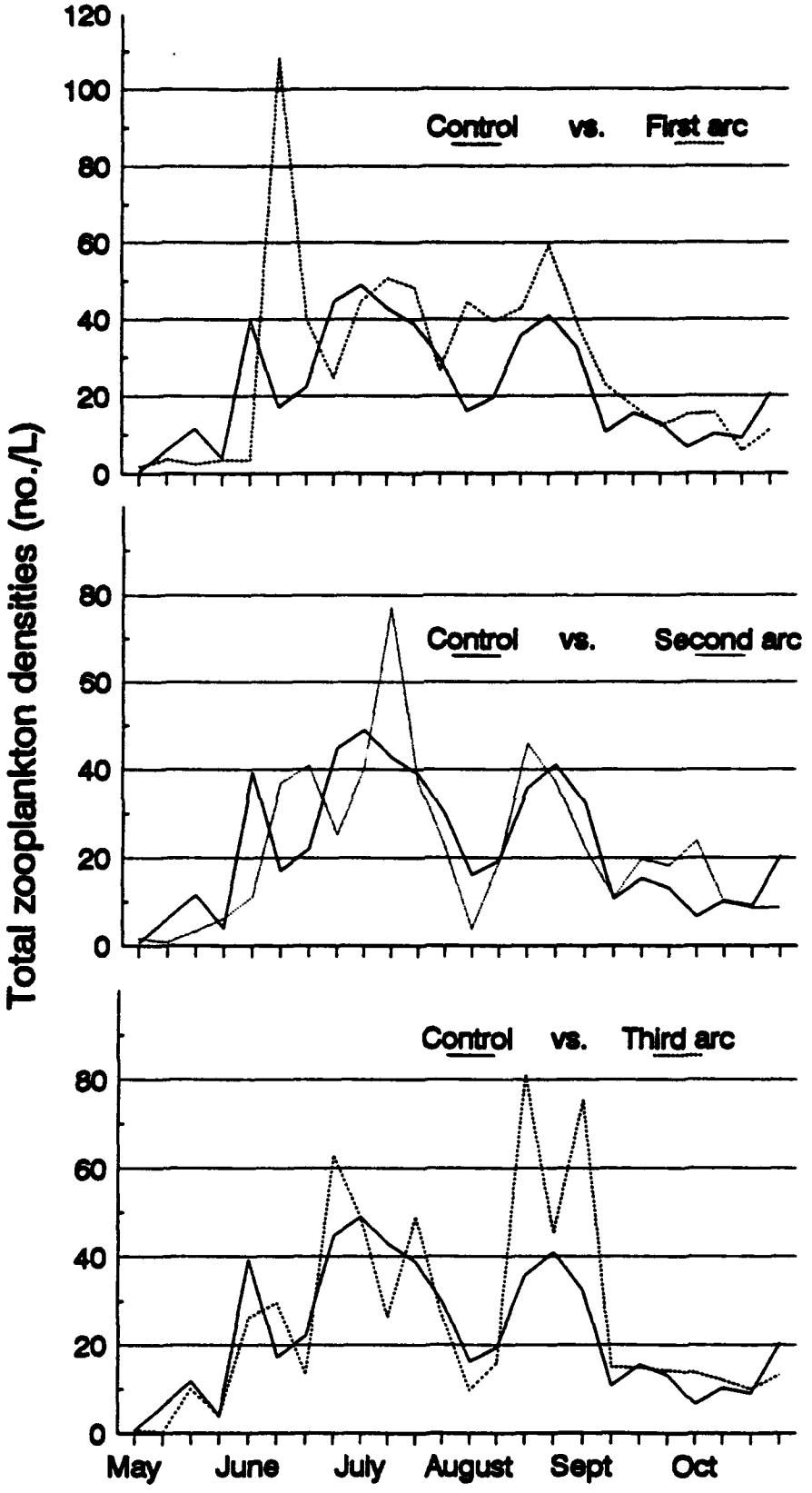


Figure 25. Lake sample estimates of zooplankton abundance at each arc (test vs. control) during summer 1993.

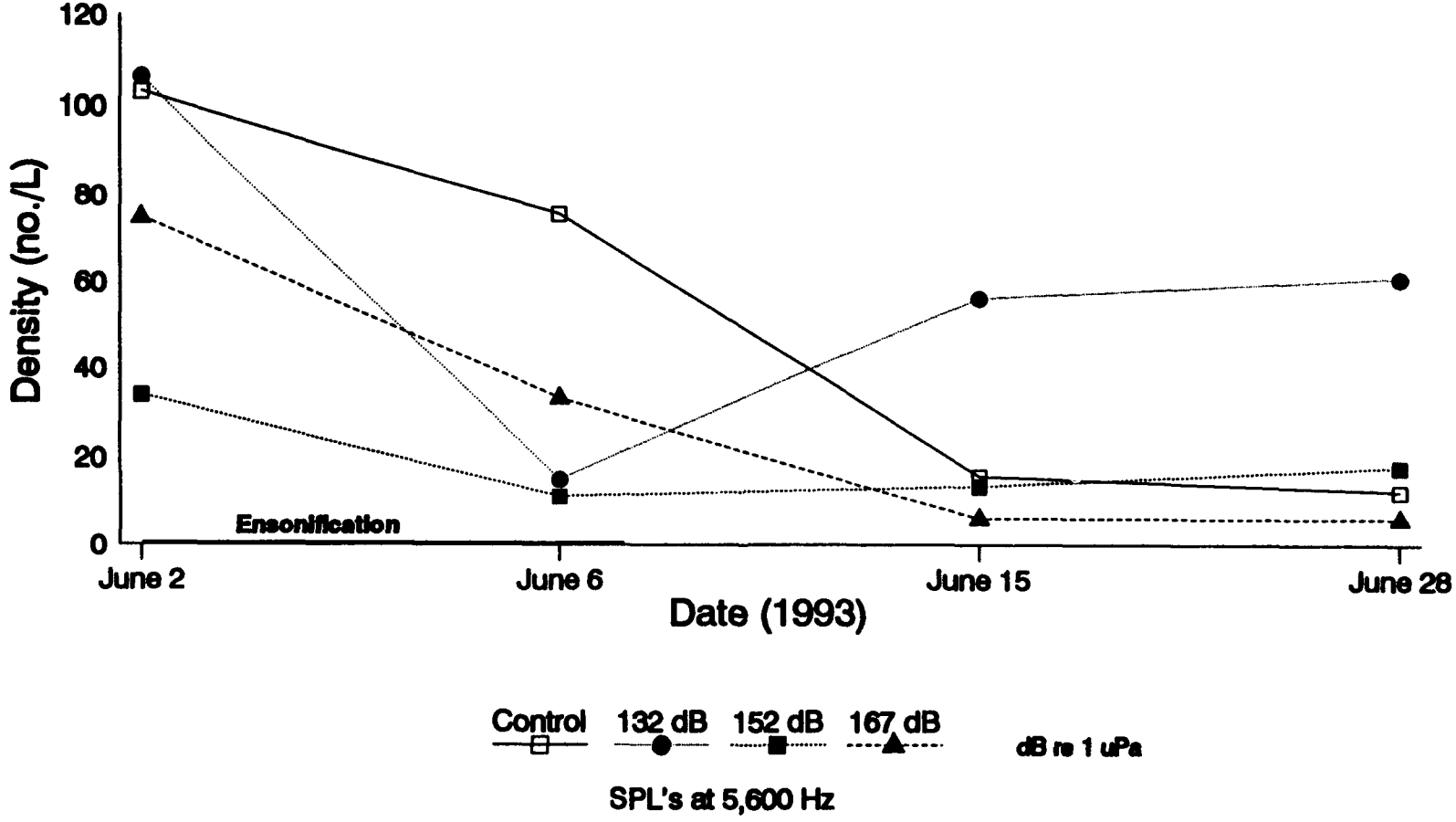


Figure 26. Total zooplankton densities inside net pens for June 1993.

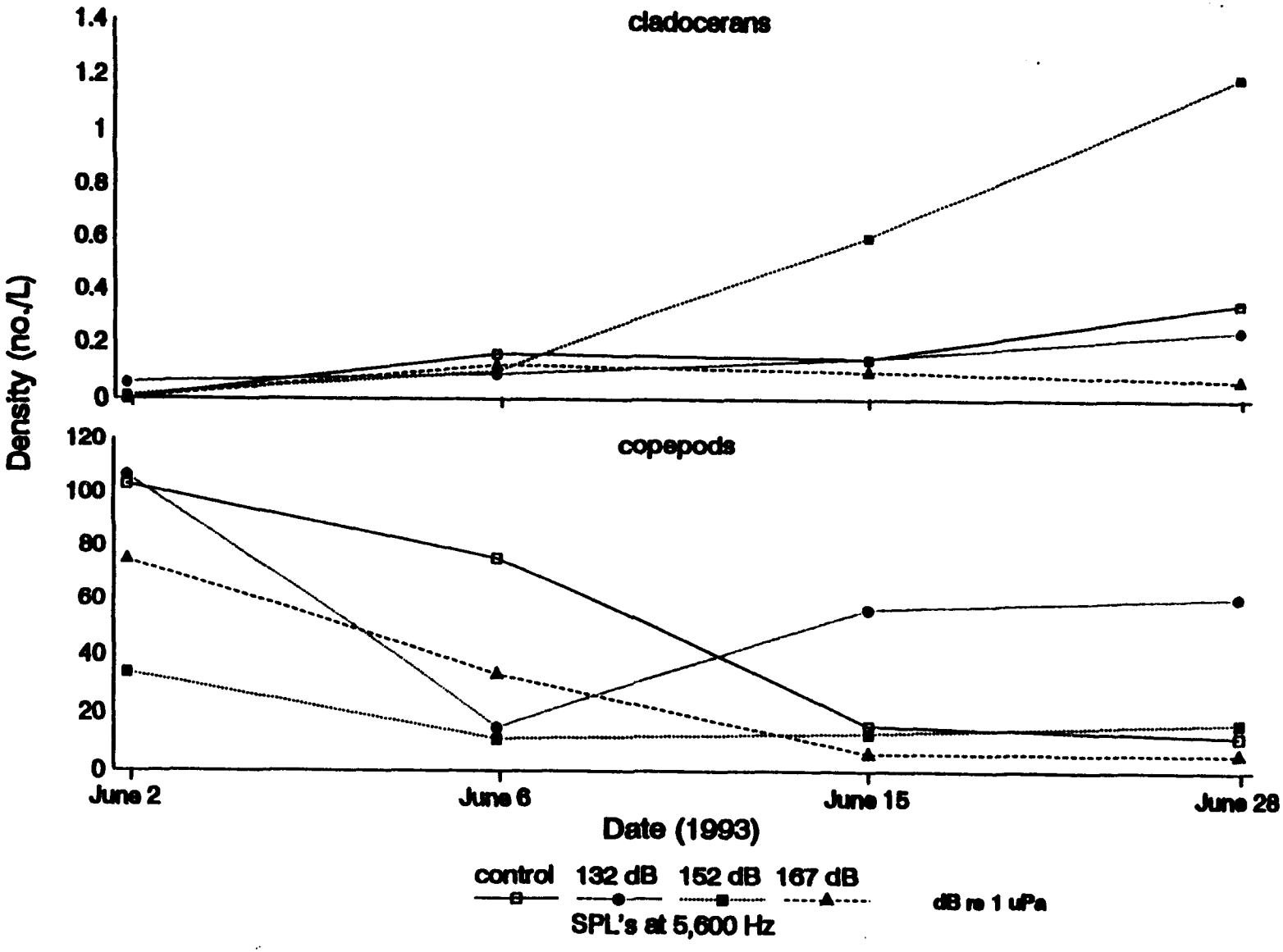


Figure 27. Zooplankton densities inside net pens for cladocerans (top) and copepods (bottom) during June 1993.

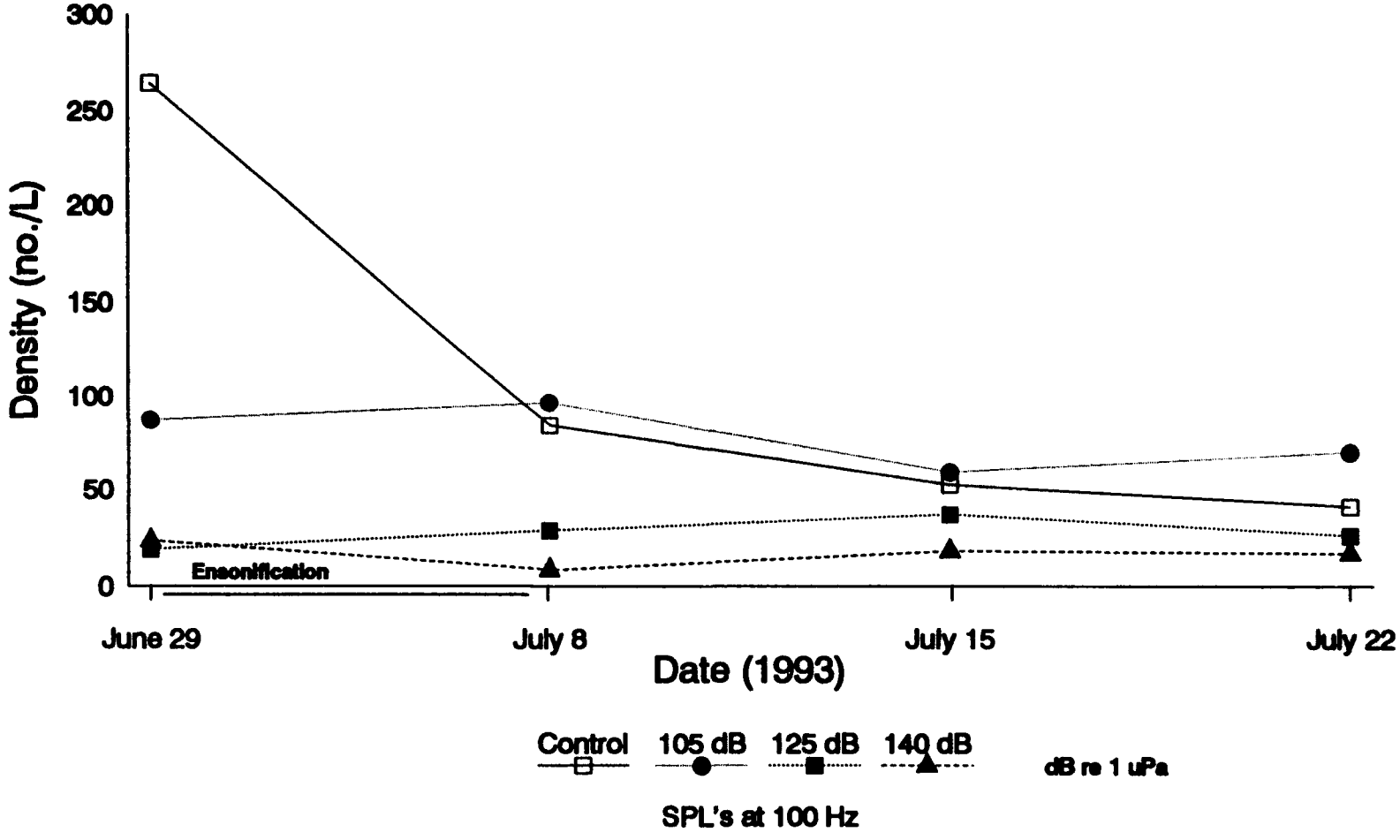


Figure 28. Total zooplankton densities inside net pens for July 1993.

were high compared to other arcs and decreased sharply after the first week of the July experiment. These observations were consistent with lake samples where we observed highest summer zooplankton abundance at the control sites in early July and a decrease by August 1, 1993 (Figure 25). Cladoceran abundance was low during early July and increased in all pens by the end of the experiment (Figure 29). Cladoceran densities were similar among arcs during the July experiment ($P > 0.016$). Additionally, we found no significant differences ($P > 0.016$) among biomass estimates during July (Table 8).

We did not observe significant differences in zooplankton densities and biomass ($P > 0.016$) during August 1993 (Tables 7 and 8). Trends in captive zooplankton densities were generally similar among arcs though we observed increased abundance during August 24 at the 105 dB sites, whereas abundance at other sites decreased by week 2 (August 24) (Figure 30). Lake samples at the 105 dB sites showed considerable variation in pelagic zooplankton abundance during the August experiment compared to other arcs (Figure 25). We observed high abundance of cladoceran zooplankton during August 24 in pens deployed at the 105 dB sites (Figure 31) indicating that a surge in cladoceran abundance contributed to the increase in total zooplankton abundance observed on August 24.

During September and October 1993, we ensonified captive zooplankton at a frequency of 800 Hz. Sound pressure levels during September were 162 dB at the first arc, 150 dB at the second arc, 130 dB at the third arc. Sound pressure levels in October were 165 dB at the first arc, 150 dB at the second arc, and 130 dB at the third arc. The 2 dB difference in sound pressure levels at the ARD Kamloops barge was due to lowering the sound source in September to accommodate kokanee thermal requirements for the feeding and growth experiments (see Task 1.2). We did not observe significant differences ($P > 0.016$) in zooplankton densities and biomass during September or October 1993 (Tables 7 and 8). Initial zooplankton abundance was high at the 130 dB sites during September and we observed reduced zooplankton abundance

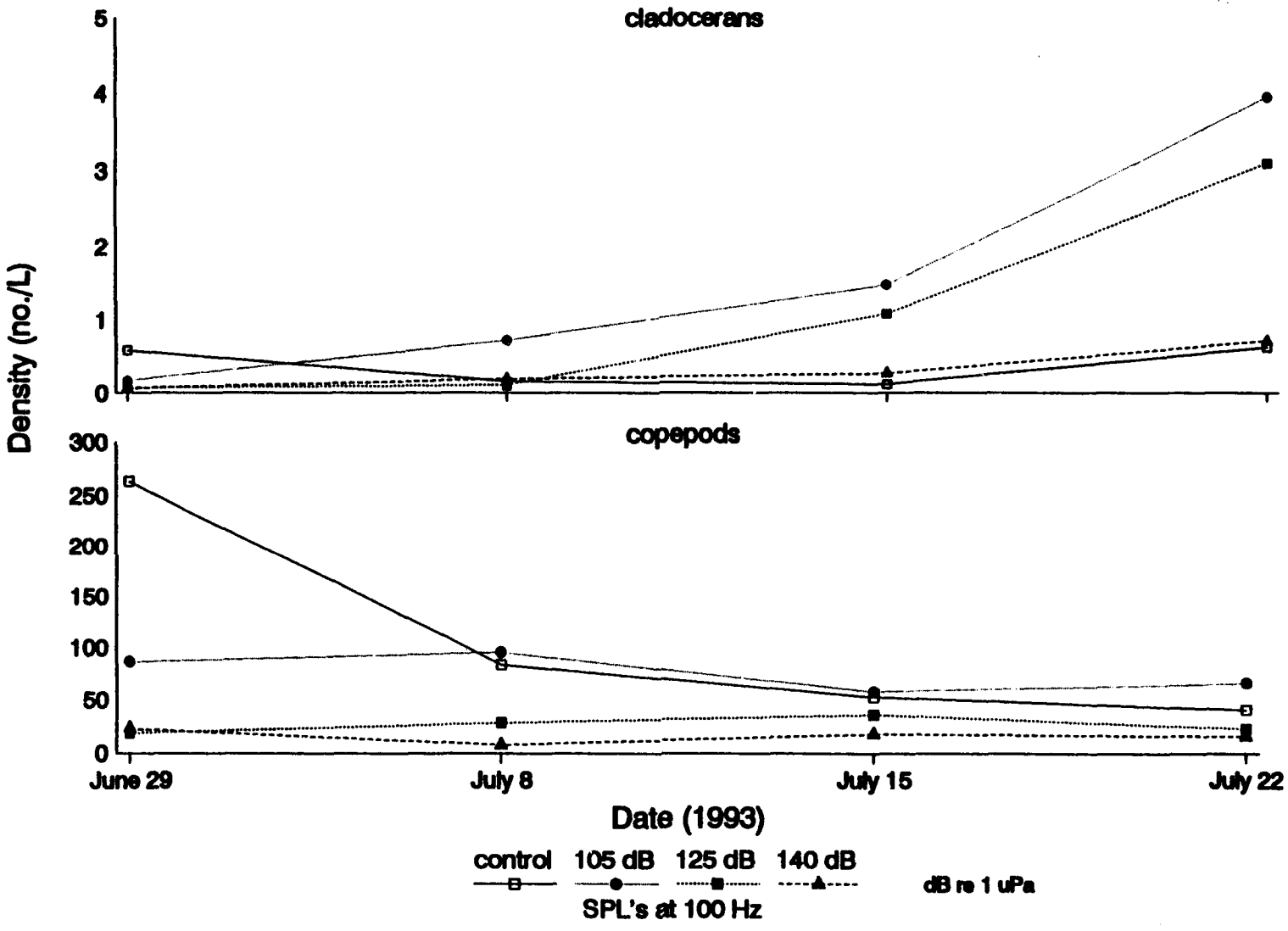


Figure 29. Zooplankton densities inside net pens for cladocerans (top) and copepods (bottom) during July 1993.

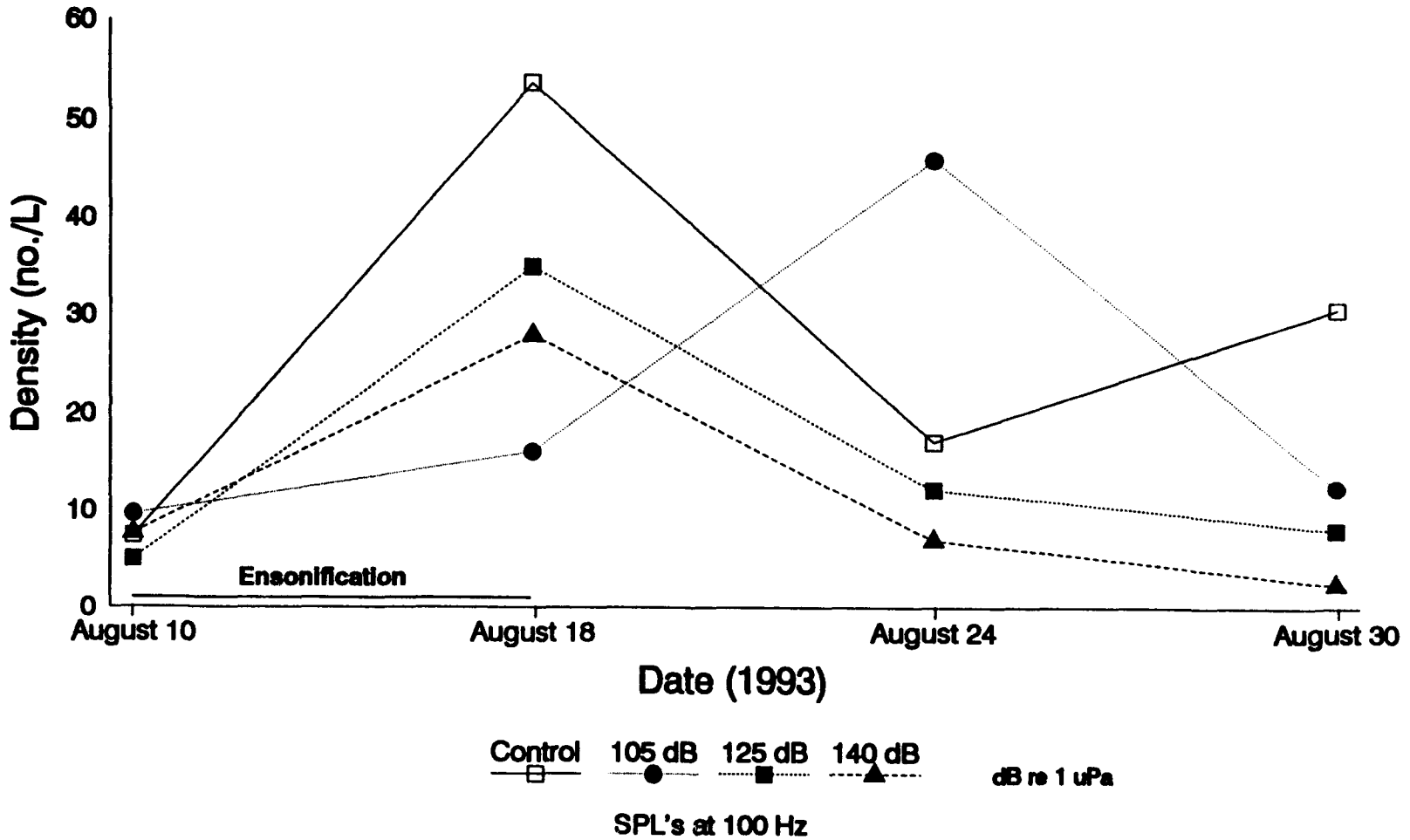


Figure 30. Total zooplankton densities inside net pens for August 1993.

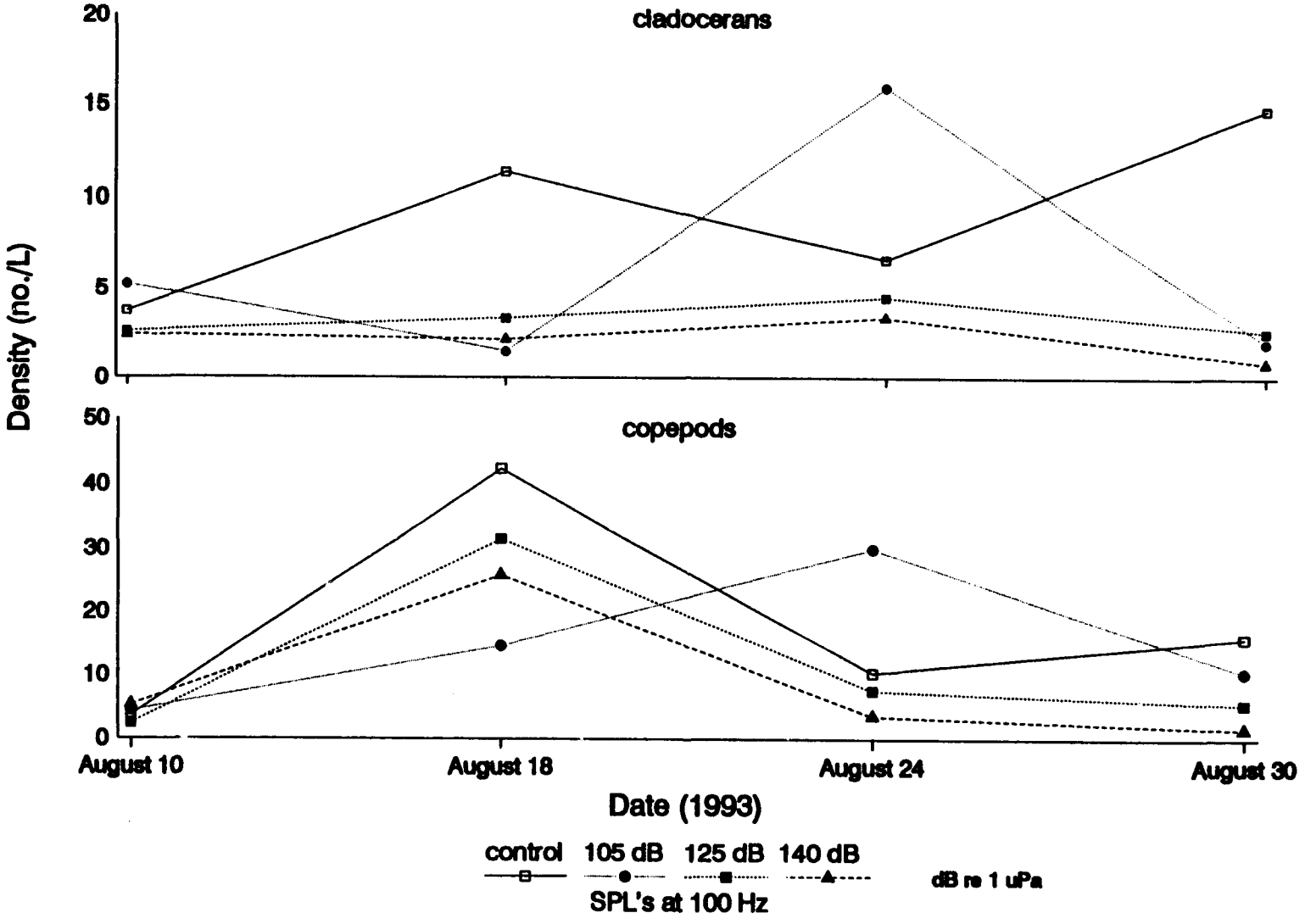


Figure 31. Zooplankton densities inside net pens for cladocerans (top) and copepods (bottom) during August 1993.

among all arcs by week 1 of the experiment (September 14) (Figure 32). Though initial variability was high among arcs, we observed similar patterns of abundance for ensonified and non-ensonified zooplankton. Additionally, lake samples obtained during September indicated a similar decline in zooplankton abundance by week 1 of the experiment, consistent with observations inside the pens (Figure 25). Cladocerans were abundant in zooplankton samples during September and exhibited abundance trends similar to copepods (Figure 33).

With the exception of the first arc, during October we observed similar trends in zooplankton abundance (Figure 34). Pens at the 165 dB sites exhibited higher zooplankton abundance by week 1 (October 8) whereas abundance at other arcs declined. Lake samples indicated that patterns in zooplankton abundance were variable during the October experiment (Figure 25). We observed a slight increase in zooplankton abundance in lake samples near the first arc (165 dB) and at the control area whereas zooplankton abundance at other arcs tended to decline by week 1 (October 8). Copepod and cladoceran densities exhibited similar patterns during the October experiment (Figure 35).

Water Chemistry

Monthly temperature and dissolved oxygen data collected from May-October 1993 were similar among test and control sites. Water temperature ranged from 4 • C in May to 16.6 • C in September, 1993 (Figure 36). Dissolved oxygen ranged from 13.8 mg/L in May to 7.9 mg/L in October, 1993 (Figure 37). Chlorophyll "a" data were also similar among test and control sites during May-October 1993 ranging from 1.0 μg/L in October to 5.6 μg/L in July 1993 (Figure 38). We observed very high chlorophyll "a" levels compared to data previously reported for Lake Pend Oreille (Rieman 1980).

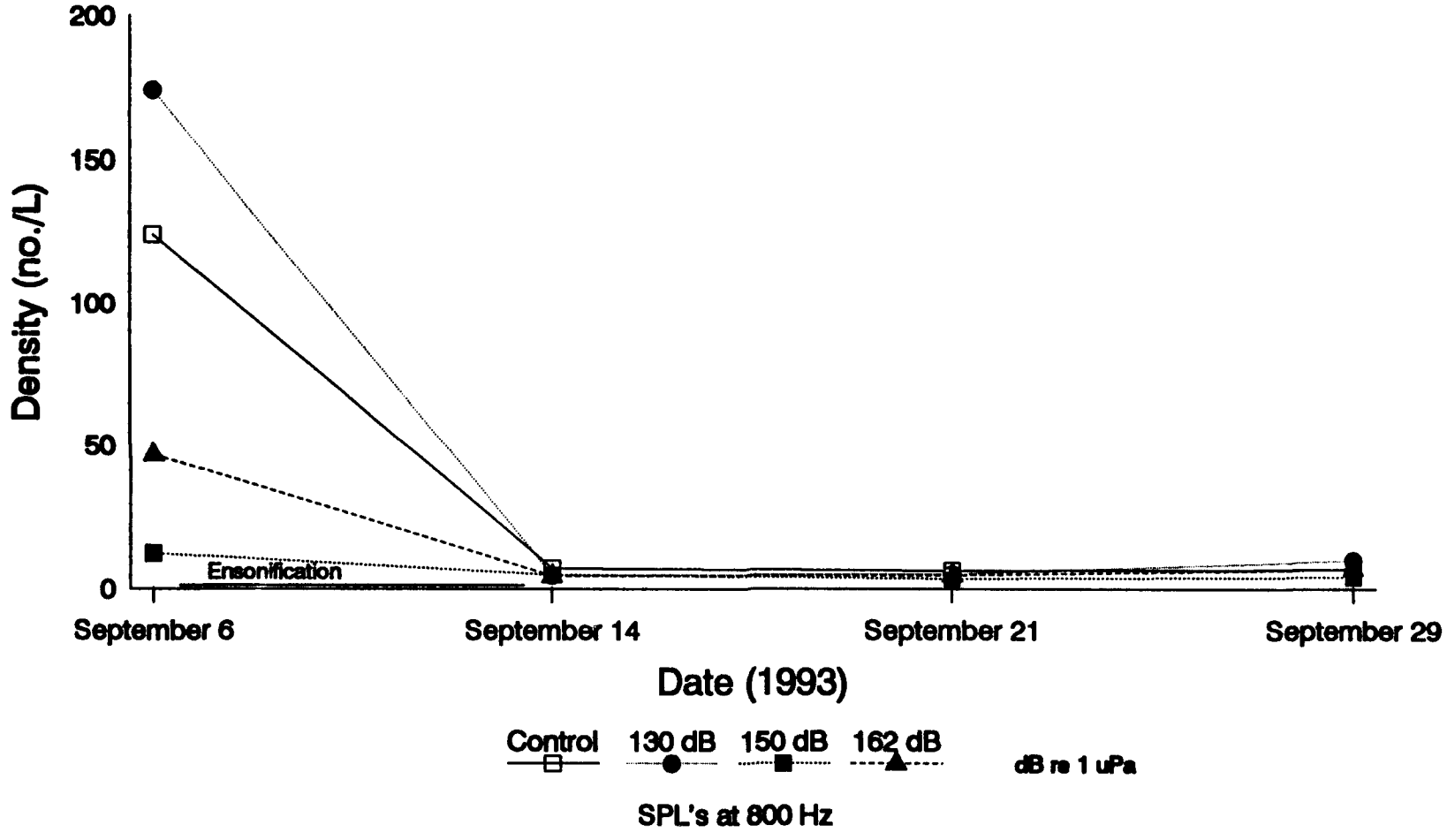


Figure 32. Total zooplankton densities inside net pens for September 1993.

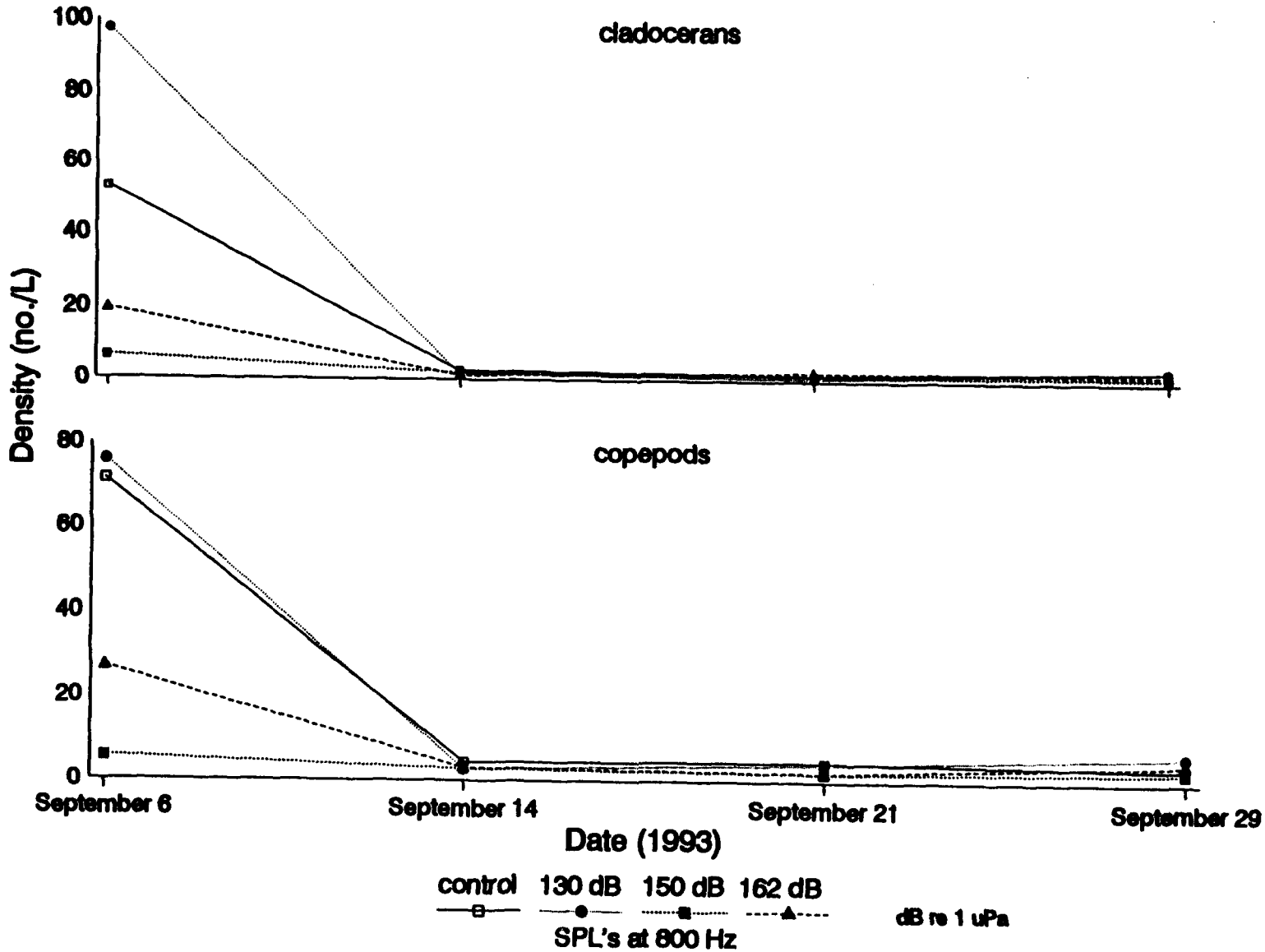


Figure 33. Zooplankton densities inside net pens for cladocerans (top) and copepods (bottom) during September 1993.

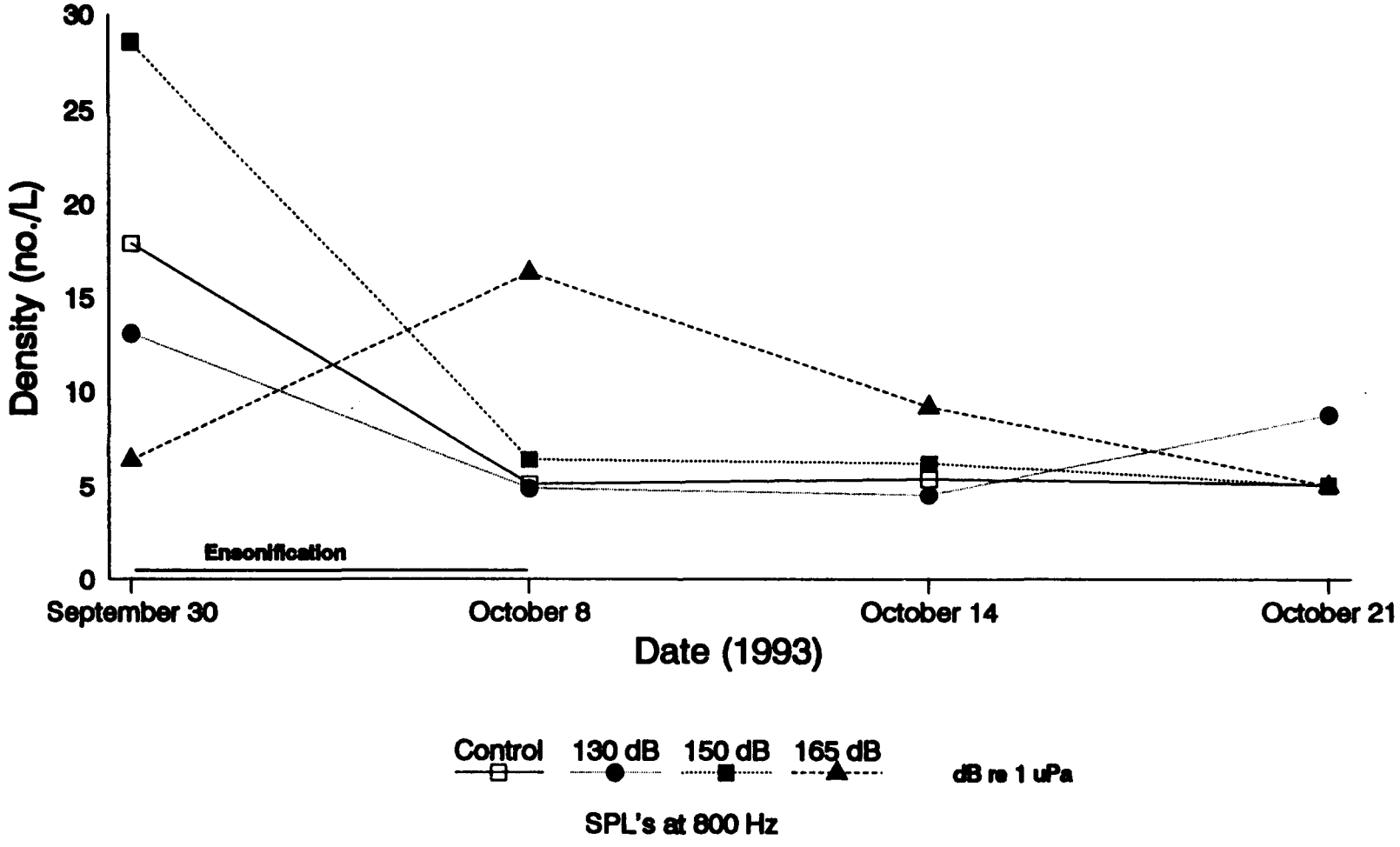


Figure 34. Total zooplankton densities inside net pens for October 1993.

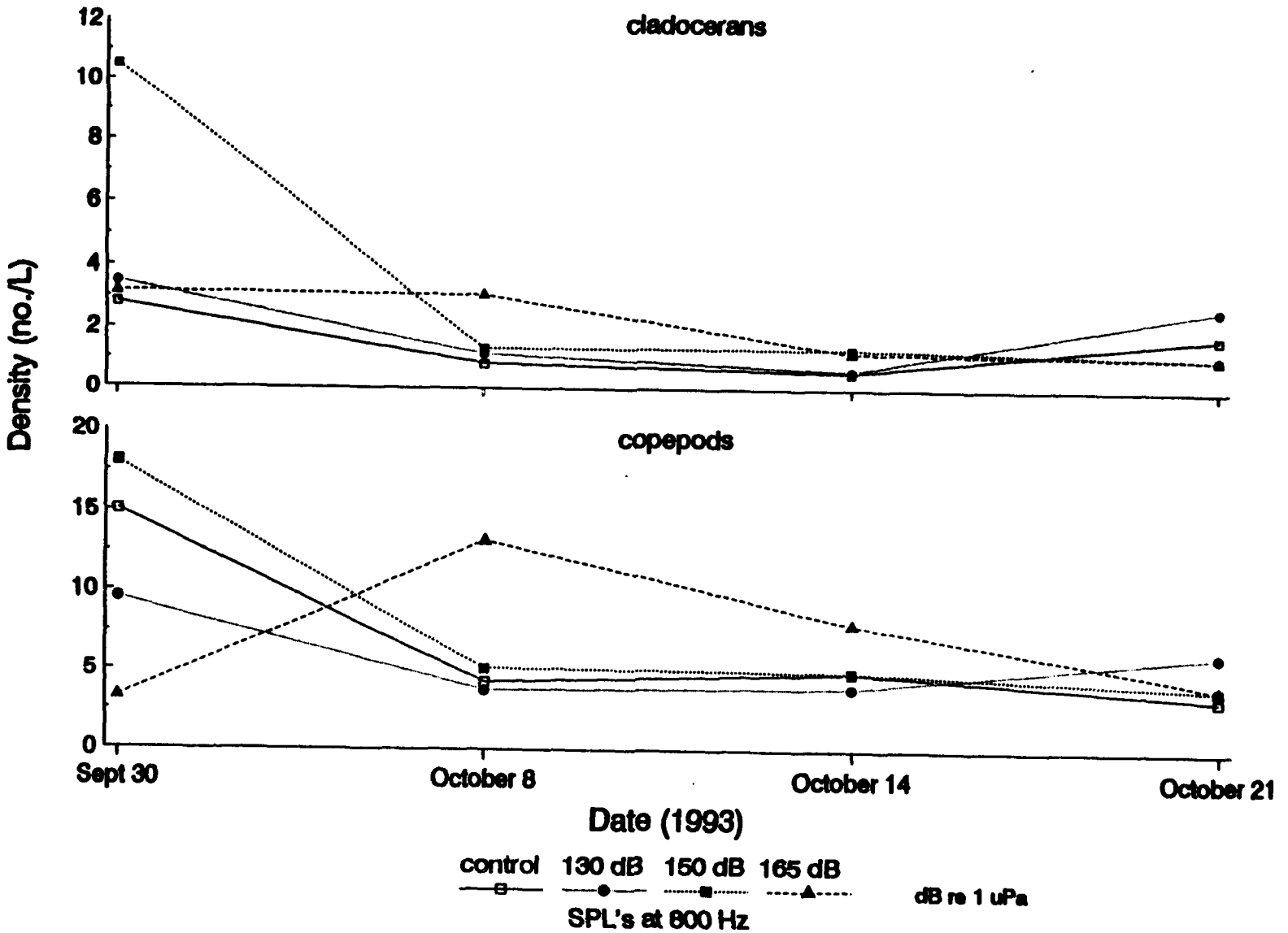


Figure 35. Zooplankton densities inside net pens for cladocerans (top) and copepods (bottom) during October 1993.

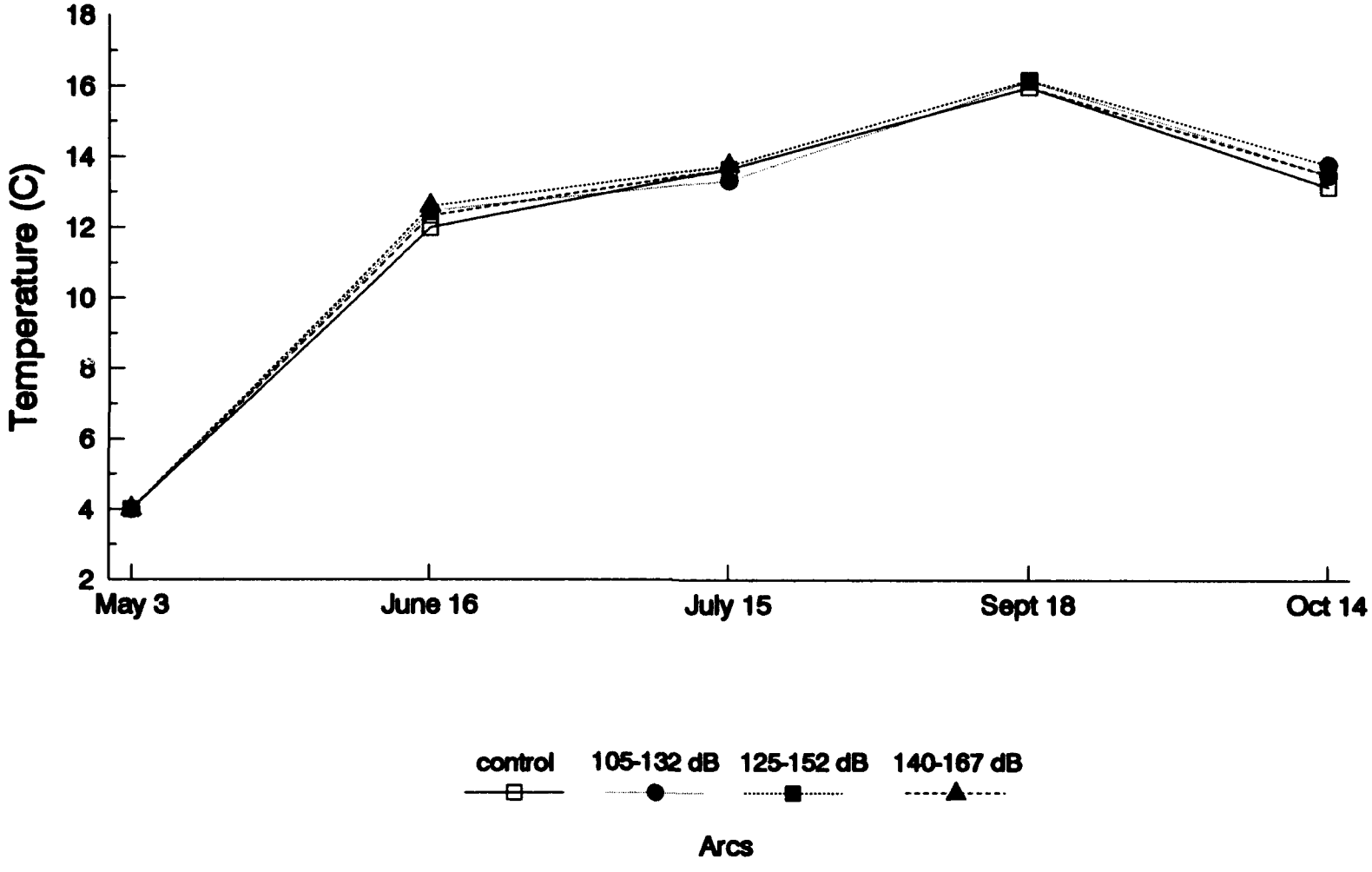


Figure 36. Mean monthly water temperatures (C) at test and control sites, May through October 1993.

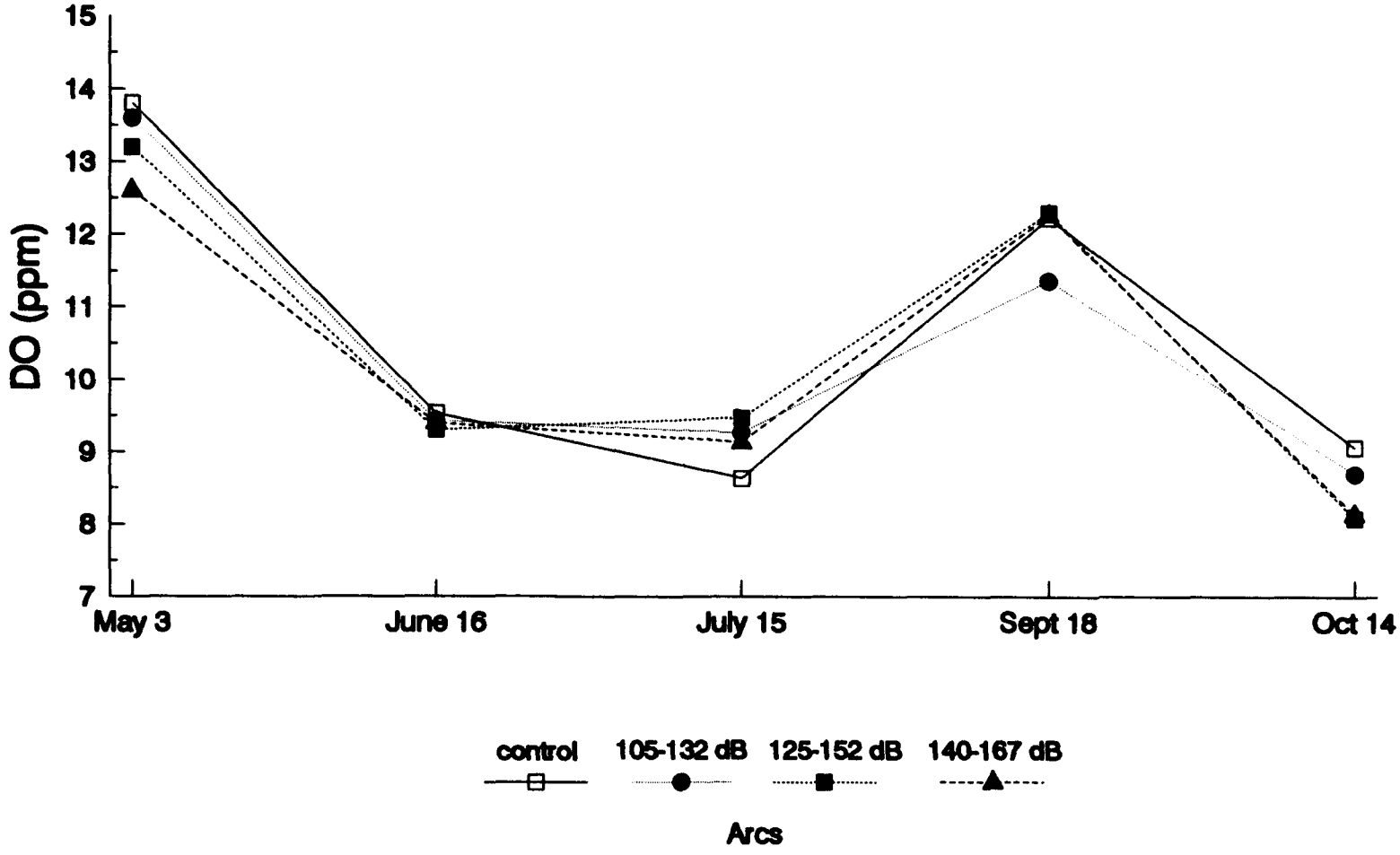


Figure 37. Mean monthly dissolved oxygen (DO) levels at test and control sites, May through October 1993.

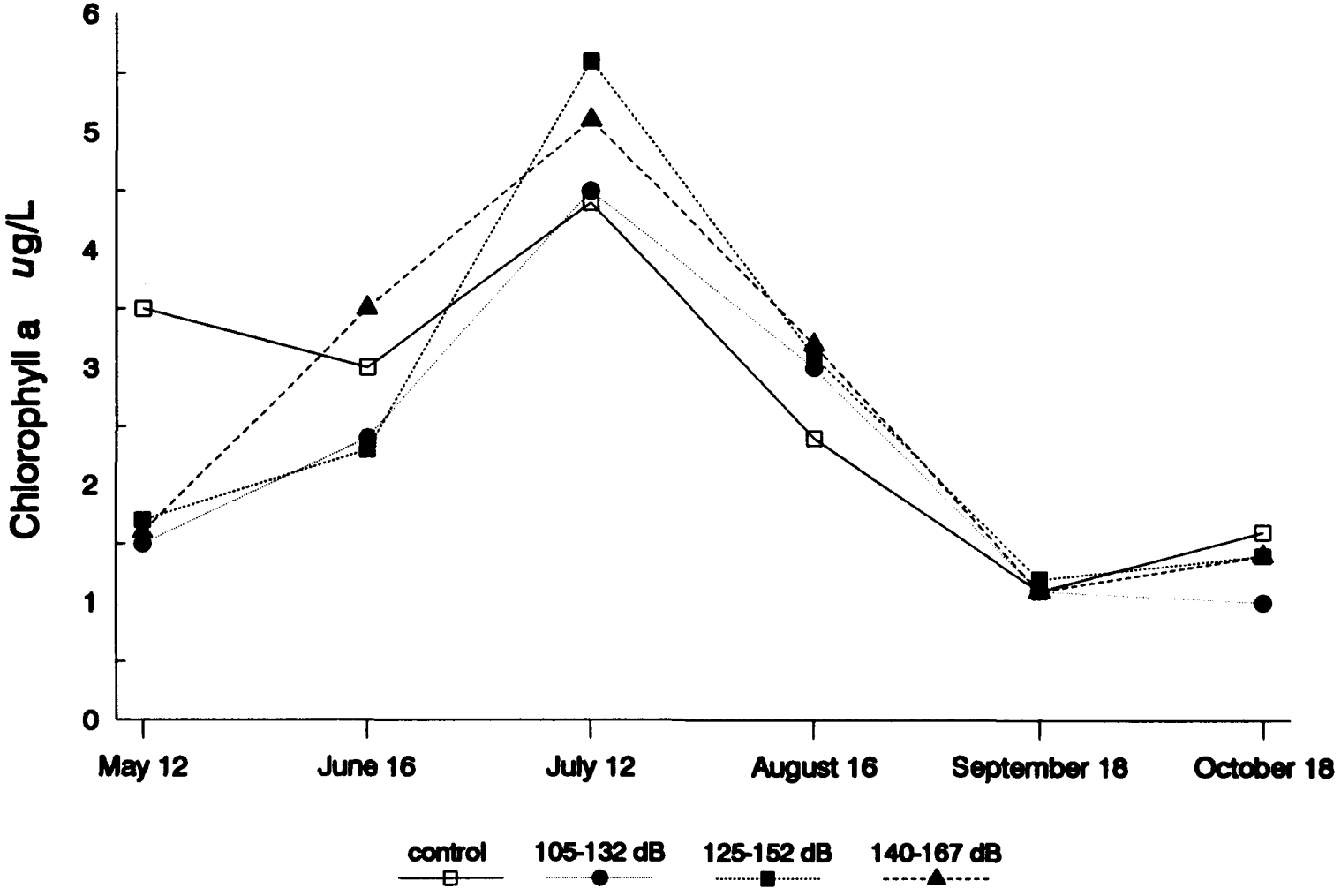


Figure 38. Mean monthly chlorophyll "a" concentrations at test and control sites, May through October 1993.

DISCUSSION

We found no evidence that zooplankton densities (total, copepods, or cladocerans) decreased or increased as a result of simulated ISMS sound at frequencies of 100, 800, or 5,600 Hz and sound pressure levels ranging from 105-167 dB. Ensonified zooplankton communities exhibited recruitment and mortality cycles similar to the control communities. Additionally, trends in abundance within the pens were similar to trends in zooplankton abundance in the lake. Based on these results, we do not anticipate that simulated ISMS sound at frequencies of 100, 800, or 5,600 Hz and sound pressure levels of 140-167 dB would significantly affect zooplankton abundance or biomass in the areas ensonified by ISMS projectors.

Trends in zooplankton densities and biomass were generally similar among arcs during the monthly experiments and were consistent with observations of concurrently sampled pelagic zooplankton. The similarity between trends in zooplankton abundance and biomass among arcs indicated that captive zooplankton were responding naturally to factors affecting recruitment and mortality.

Monthly water chemistry data were similar among arcs though we observed high chlorophyll "a" levels compared to other studies in Lake Pend Oreille. These observations were consistent between arcs during May-October 1993 and may be related to bias associated with large kokanee feeding and growth cages (see Task 1.1). For example, monthly water samples were collected by attaching the boat to mooring lines suspending the fish and zooplankton pens. Attached algae was often thickly growing on the sides of the pens. Algae may have been shaken from the large pens as a result of jostling the pens during sampling hence increasing chlorophyll "a" levels in the immediate vicinity where water samples were obtained.

We frequently observed high variability in initial zooplankton densities between arcs during the study. Environmental conditions such as light, food availability, and temperature regimens all affect zooplankton distribution and abundance in natural

environments (Hall et al. 1970). Additionally, surface disturbance from external sources and physical factors such as internal wave action and upwelling can cause variation in zooplankton abundance. The latter two are especially important in a large, deep lake such as Pend Oreille. Variation in initial zooplankton abundance is probably related to spatial or "patchy" distribution of zooplankton in Lake Pend Oreille (Lewis 1979) and we hypothesize that physical forces play a very important role in zooplankton distribution in Lake Pend Oreille.

Differing life cycles also contribute to the natural variability in zooplankton abundance and composition within Lake Pend Oreille. Life cycles of cyclopoids, for example, range from 7 days to approximately 180 days, whereas some species of diaptomids have life cycles ranging up to 1 year (Pennak 1989). Cladoceran life cycles are also extremely variable and are species- and temperature- dependent. Life cycles for *D. magna* are 26, 42, and 108 days at temperatures of 28, 18, and 8 • C, respectively (Pennak 1989). Hence, differences in species composition and temperature regimens can create variability in localized zooplankton abundance.

During the July experiment we observed relatively high zooplankton abundance in pens at the control area. Abundance declined by week 1 (July 8) and was similar to the ensonified communities during the remainder of the experiment. This decline may be related to density-dependent factors affecting captive zooplankton popu[illegible]ns inside the pens. Factors such as competition and predation are important mechanisms regulating zooplankton densities and may be responsible for the observed decline in zooplankton abundance at the control area during July. Predation by predatory zooplankton (i.e. copepods) inside the pens may have been more pronounced than in the natural environment, creating bias in zooplankton density (i.e. reducing numbers at a faster rate). This is only a hypothesis since we have no data bearing on effects of predation (or competition) within the net pens.

During August, we observed higher zooplankton abundance in net pens at the 105 dB sites by week 2 (August 24) whereas abundance among other arcs generally declined by week 2. Variation also appears in the lake samples during this period and may be related to micro-environmental differences between arcs (i.e. food availability and light). The variation observed during October indicated that zooplankton abundance along the first arc (165 dB) increased by week 1 (October 8) whereas abundance at other locations tended to decline. These patterns were not seen in ambient lake samples. During early October, relocation and handling of zooplankton pens was necessitated as a result of other acoustic testing on the ARD Kamloops barge (late September-October 1). The Kamloops barge was outfitted with fluorescent lights which remained on, for security reasons, throughout the night. Pens were moved to a different location on the barge on October 1 after being inoculated on September 30. Varying light intensities provided on the Kamloops barge may have enabled zooplankton to feed more efficiently during the night. Additionally, pens at the 165 dB sites were relocated again on October 11 due to the ARD Kamloops barge being moved and remoored in Bayview. These pens were subsequently attached to a cable located approximately 15 m from their original location. Other pens were not moved or relocated during the October experiment and handling or repositioning may be a factor related to the observed variation during October.

Task 1.2

Kokanee Growth in Response to Simulated ISMS Ensonification.

INTRODUCTION

Kokanee represent an important fishery in Lake Pend Oreille. The Idaho Department of Fish and Game annually releases hatchery reared, age-0 kokanee in attempts to enhance the kokanee fishery. Hatchery-reared kokanee are stocked in mid-summer to maximize growth and subsequently increase fry survival (M. Maiolie, Idaho Fish & Game, Coeur d' Alene, pers. comm.).

To date, we are aware of no literature describing effects of *sustained* underwater sound on feeding and growth of fishes. Underwater sound has been shown to elicit a variety of behavioral responses among fishes that include startle response, avoidance reactions, and changes in schooling behavior (Blaxter and Hoss 1981; Dunning et al. 1992; Knudsen et al. 1992). Effects on feeding behavior and subsequent growth of fishes, however, is an area requiring considerable basic research. Nestler et al. (1992) describes a hypothesis based on work of Johnson et al. (1947) who demonstrated that *Alpheus* spp. (Decapoda: Alpheidae) can produce sounds with frequencies as high as 50 kHz. From this work, Nestler et al. (1992) hypothesized that other species of zooplankton might produce low intensity, high-frequency sounds. Fish such as *Alosa* spp. may be able to detect these sounds hence increasing their ablity to locate zooplankton prey. Several studies have shown acoustic stimuli are used by predators to locate prey (see Schwarz 1985 for review) indicating that underwater sound may be an important mechanism affecting feeding behavior and thus growth of some fishes. Growth of fishes is important to their survival, particularly during early life stages (Van Den Avyle 1993) and therefore represents an important ecological element among the Lake Pend Oreille fishery.

Salmonid fishes reportedly have poor hearing compared to other fishes and may be sensitive to a narrow range of frequencies (VanDerwalker 1967; Hawkins and Johnstone 1978; Knudsen et al. 1992). Studies of Atlantic salmon report a hearing threshold below 380 Hz (Hawkins and Johnstone 1978) and avoidance responses to infrasound frequencies from 5 to 10 Hz (Knudsen et al. 1992). Additionally, Knudsen et al. (1992) found that a frequency of 150 Hz did not elicit an avoidance response from Atlantic salmon even at intensities of 100-150 dB above physiological awareness thresholds.

Recent research indicates that salmonid fishes respond consistently to low frequency infrasound below 20 Hz (Mike Curtin, SONALYSTS Inc. Waterford CT, pers. comm.). In many fishes, low-frequency sensitivity is believed to be associated with the lateral line system (i.e. particle displacement) and not the inner ear (i.e. hearing) (Sand 1984; Karlsen 1992a; 1992b). However, recent studies indicate that the inner ears of Atlantic cod and perch are extremely sensitive to low frequency underwater sounds (Sand and Karlsen 1986; Karlsen 1992a; 1992b).

To better understand the potential effects of ISMS underwater sound on feeding and growth of kokanee, the objective of this aspect of the study was to examine growth of age-0 kokanee in repsonse to simulated ISMS underwater sound. We conducted in-situ experiments to examine ISMS-related frequency and sound pressure level effects on kokanee growth.

METHODS

Kokanee holding cages

Kokanee fry (n=10,000) were obtained from the Cabinet Gorge Fish Hatchery, Cabinet Gorge, Idaho in early May 1993 and maintained in holding pens in Scenic Bay, Lake Pend Oreille. Four cylindrical holding cages 2 m diameter x 9 m long, constructed of 3 mm nylon mesh, were attached to ARD pilings in Scenic Bay. Kokanee were

placed in the cages in early May and allowed to acclimate to natural food sources (i.e. zooplankton). Cages were maintained at a depth of 9 m below the water surface (i.e. top of cages) and were checked weekly for dead fish. Cages became fouled during late July when ducks and seagulls began to perch more frequently on the pilings suspending the cages. The cages were cleaned and moved in mid-August due to fouling and warm water temperatures and deployed from the ARD Green barge at a depth of 12 m. Approximately 450 kokanee were removed from a cage(s) at the beginning of each ensonification experiment and used in growth experiments conducted in the southern basin of Lake Pend Oreille. At completion of ensonification experiments, kokanee were released into Lake Pend Oreille.

Ensonification

The test area was partitioned into three concentric arcs surrounding the sound source (Figure 22). Each arc represented a different sound pressure level. Four treatments--three sound pressure levels and the control--were tested for each of three frequencies 100, 800, and 5,600 Hz. These frequencies represented the range of ISMS frequencies. Each frequency was tested twice at monthly intervals from May through October 1993 for a total of six test cycles. Kokanee feeding and growth experiments were conducted concurrently with zooplankton experiments described in Task 1.1.

We randomly selected three of four established sites under each treatment condition. Rearing cages, measuring 2 m diameter x 9 m height and constructed of 3 mm nylon mesh, were deployed at each site. We attached cages (n = 12) to mooring lines placed at each site (Figure 21). Mooring lines were anchored in water depths ranging from 66-360 m. Cages were suspended along the mooring line at a depth of 5 m below water surface except during September when cages were lowered 10 m to provide kokanee with thermal refuge. Each mooring line was fitted with one fish rearing pen. Cages were deployed and retrieved using an outrigger mounted to the side of a 7.3 m

Monark research vessel (Figures 39 and 40). Cages located along the closest arc (i.e. highest sound pressure levels) were deployed from established sites on the Kamloops barge. To monitor daily water temperatures throughout the study, one thermograph (Ryan Tempmentor) that recorded hourly water temperatures was attached to a cage at the test area and one attached to a cage at the control area.

Each cage was stocked with 25 kokanee (n=300) at the beginning of each month. Fish were measured for total length to the nearest millimeter and individual weights (g) were estimated from length/weight curves established at the time of stocking from a random sample of at least 100 kokanee. Kokanee were exposed to a typical ISMS sound regimen for 7 days at a preselected frequency and sound pressure level. Kokanee were sampled weekly by removing them from the cages via a zipper sewn near the bottom of the cage. Fish were anesthetized with MS-222 and total length of each fish was measured weekly for 2-3 weeks after ensonification.

We tested the hypothesis that kokanee growth was equal among treatments (three sound pressure levels and control). Differences in growth were determined using covariance analysis (test for homogeneity of slopes) and considered significant at $P<0.05$. Specific growth (G) and condition factors (Fultons K) were calculated where,

$$G=\log(W_t)-\log(W_o)/T_t-T_o \times 100$$

W_o = weight (g) at beginning of experiment
W_t = weight at end of experiment
T_o = beginning day
T_t = ending day

and

$$K=W_t/L^3 \times 10^5$$

L = total length (mm) at end of experiment.

We examined effects of long-term exposure on growth of kokanee in six rearing cages. Cages were deployed using the same protocol as the 7 day experiments. Long-

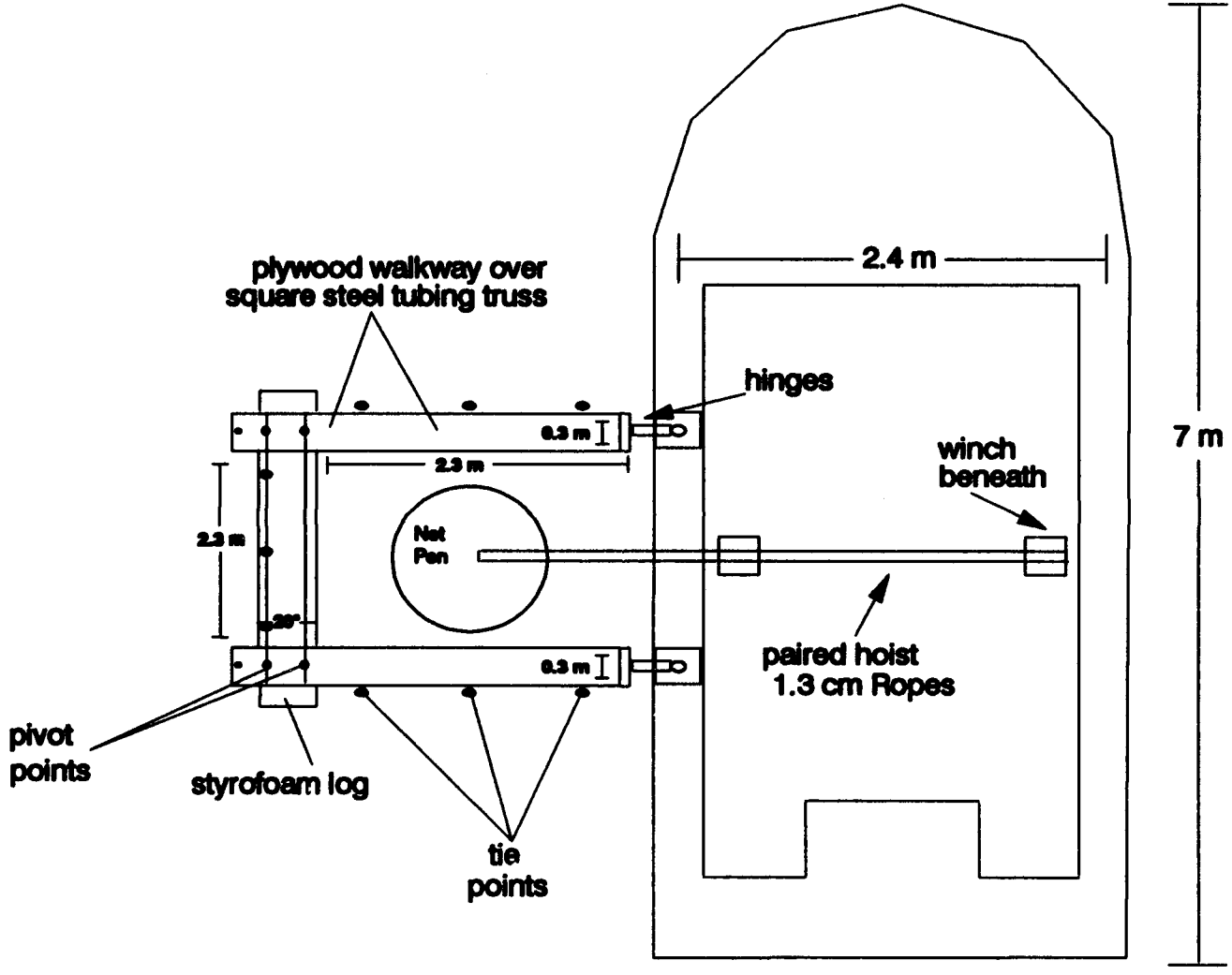


Figure 39. Top view of research vessel showing side-mounted outrigger. Outrigger was used to deploy and retrieve rearing cages.

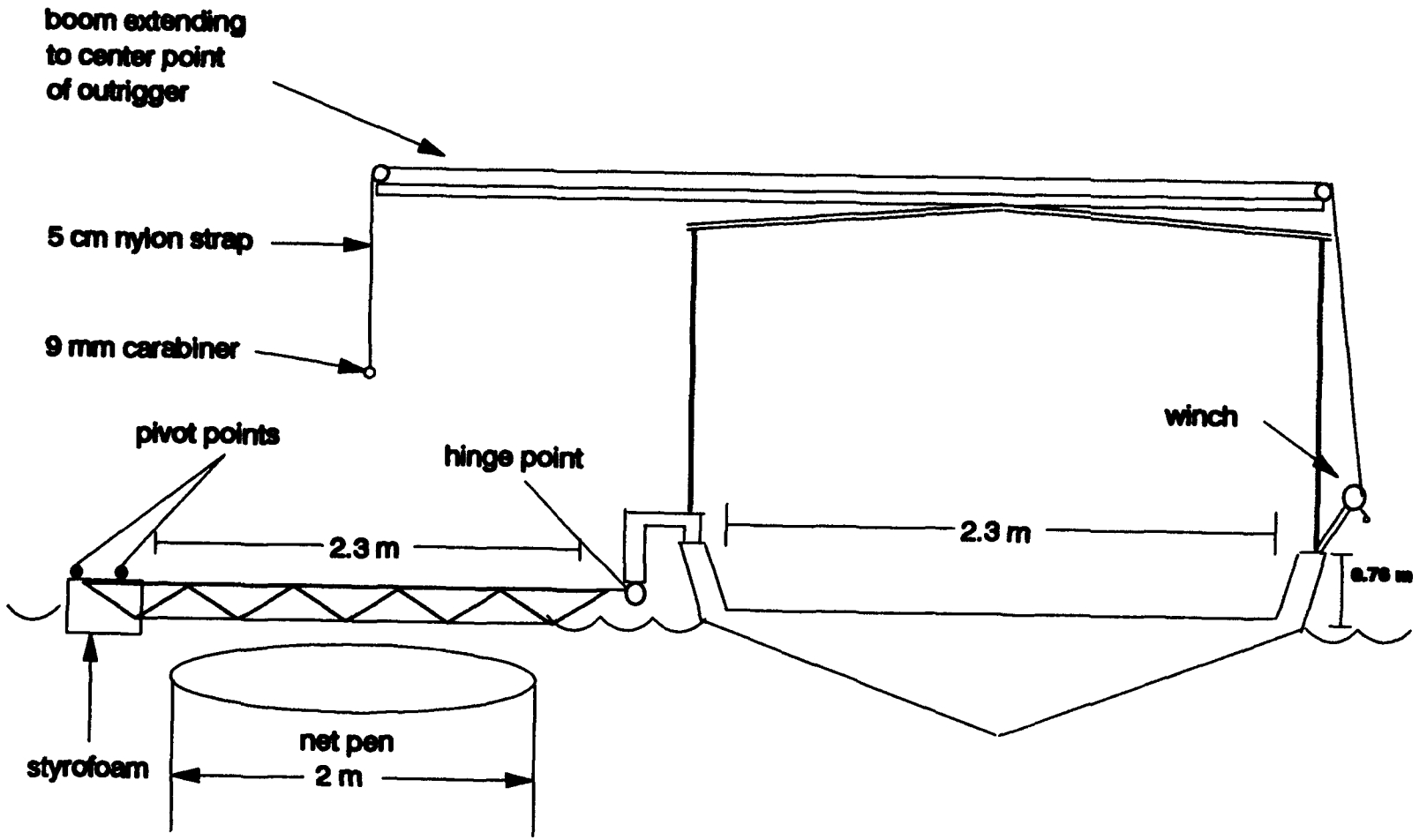


Figure 40. Bow view of research vessel showing side-mounted outrigger. Winch system was used to raise and lower outrigger as well as retrieve cages during high winds.

term rearing cages (n=3) used for ensonification were deployed from the ARD Kamloops barge and cages used as controls (n=3) were deployed at the control area. Each cage was stocked with 25 kokanee (n=150) in May 1993 and individual lengths were recorded monthly. Cages were restocked in July 1993 due to fish escape and/or damage to cages so two, 3-month exposure periods were examined. Growth of long-term control fish during August through October was not quantified because mooring systems suspending the cages disappeared and could not be located. We compared growth of ensonified kokanee to the controls (T-test) for the period of May-July 1993.

RESULTS

Kokanee growth during May 1993 did not differ among treatments (P=0.051, df=3, F=2.59) (Figure 41). Kokanee were ensonified at a frequency of 5,600 Hz and sound pressure levels of 167 dB at the first arc, 152 dB at the second arc, and 152 dB at the third arc. Specific growth (G) was highest among kokanee at the 167 dB sites (2.2%·day^{-1}) and lowest among kokanee at the controls (1.2%·day^{-1}) (Table 9). Condition factors in May ranged from 0.76-0.79.

Growth rates of kokanee ensonified at 5,600 Hz during June 1993 were not significantly different from those of the controls (P=0.72, df=3, F=0.44) (Figure 42). Specific growth (G) was similar for all treatments and ranged from 2.1-2.3%·day^{-1} (Table 9). Condition factors in June 1993 ranged from 0.80-0.81.

In July and August 1993, we ensonified kokanee at a frequency of 100 Hz. Sound pressure levels at 100 Hz were 140 dB at the first arc, 125 dB at the second arc, and 105 dB at the third arc. Kokanee growth did not differ among treatments during July 1993 (P=0.21, df=3, F=1.49) (Figure 43). Specific growth (G) was highest among the 140 dB sites (2.5%·day^{-1}) and lowest among the controls (1.9%·day^{-1}) (Table 9). Condition factors in July 1993 ranged from 0.66-0.68.

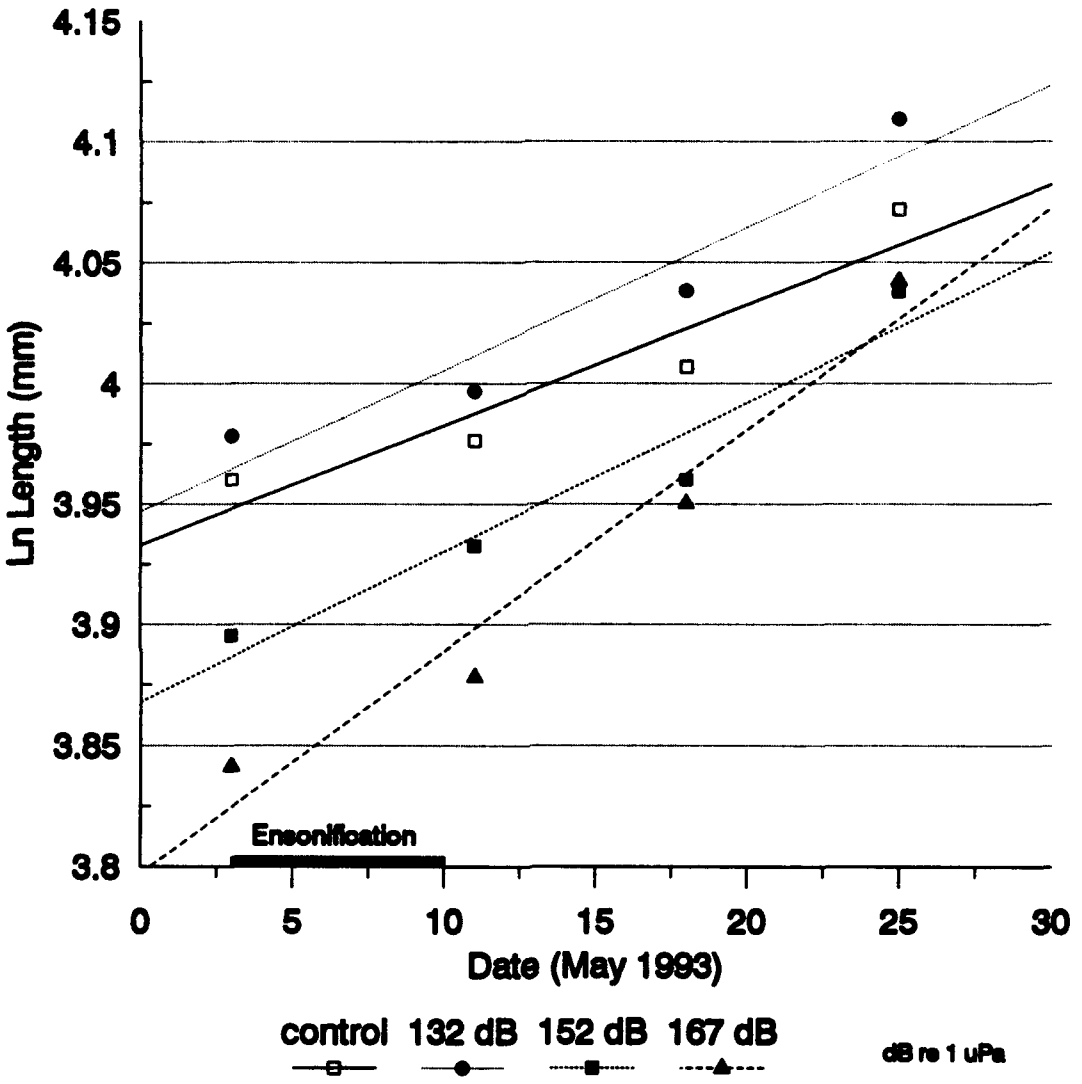


Figure 41. Growth of age-0 kokanee in Lake Pend Oreille during May 1993. Test fish were ensonified from May 3 to May 10 at a frequency of 5,600 Hz.

Table 9. Specific growth rates (G) and condition factors (K) for age-0 kokanee during monthly ensonification experiments.

Month	Frequency (Hz)	Sound pressure level (dB)	G (%·day^{-1})	K (Fulton's)
May	5,600	167	2.2	0.79
		152	1.5	0.79
		132	1.4	0.76
		control	1.2	0.78
June	5,600	167	2.3	0.80
		152	2.1	0.81
		132	2.1	0.81
		control	2.3	0.81
July	100	140	2.5	0.67
		125	2.4	0.66
		105	2.3	0.67
		control	1.9	0.68
August	100	140	-	-
		125	1.0	0.59
		105	1.7	0.57
		control	1.4	0.56
September	800	162	3.3	0.68
		150	1.8	0.68
		130	1.4	0.68
		control	1.2	0.69
October	800	165	2.6	0.71
		150	2.5	0.71
		130	2.7	0.70
		control	2.0	0.71

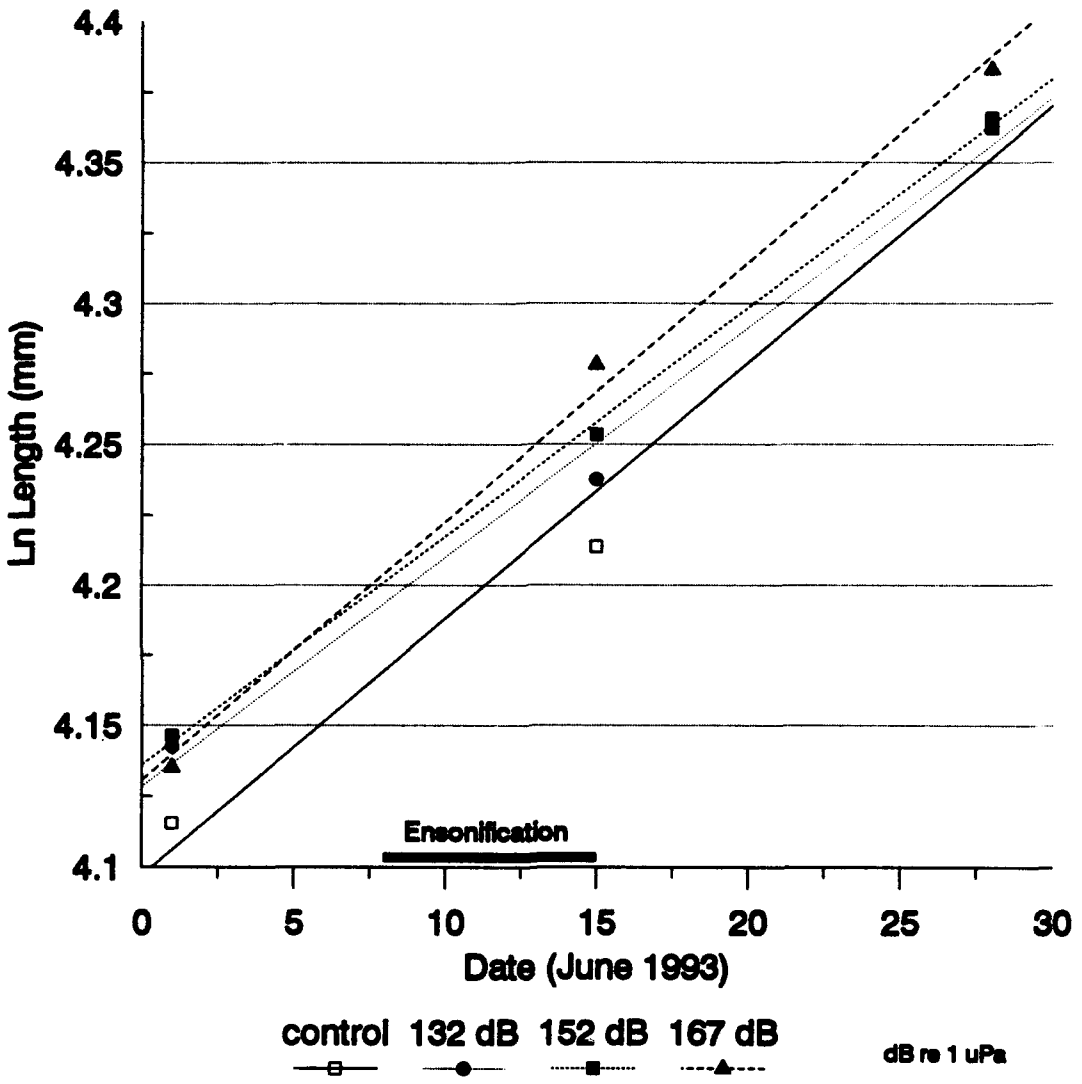


Figure 42. Growth of age-0 kokanee in Lake Pend Oreille during June 1993. Test fish were ensonified from June 8 to June 15 at a frequency of 5,600 Hz.

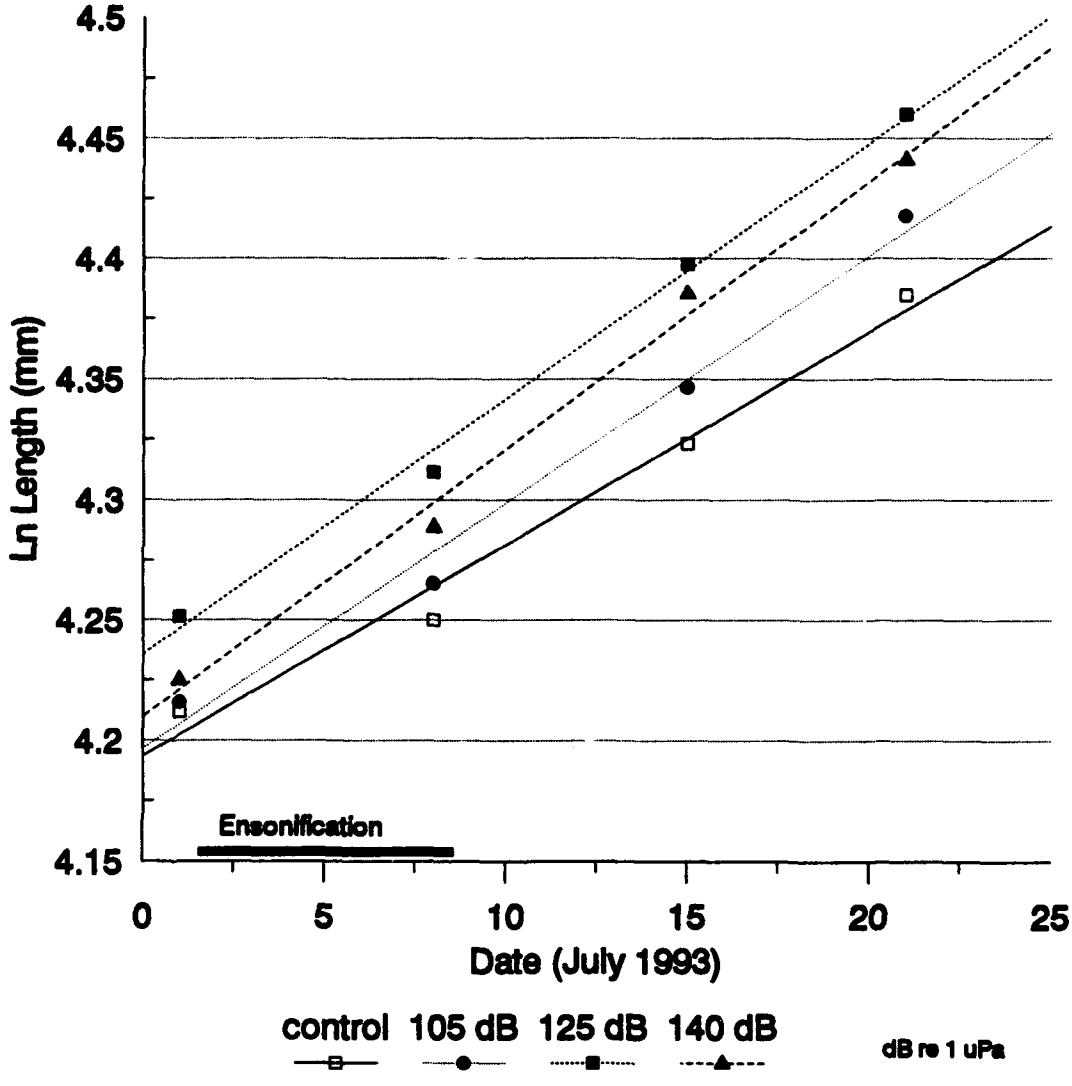


Figure 43. Growth of age-0 kokanee in Lake Pend Oreille during July 1993. Test fish were ensonified from July 1 to July 8 at a frequency of 100 Hz.

Growth during August 1993 was not significantly different among treatments at 100 Hz ($P=0.55$, $df=2$, $F=0.60$) (Figure 44). The power of the statistical analysis, however, was reduced due to low sample sizes. An epizootic outbreak of *F. columnaris* in August reduced samples sizes considerably. Kokanee in all treatments were affected by *F. columnaris* with mortality ranging from 62 to 80% by week 2 of the experiment (August 20, 1993). Cages deployed from the Kamloops barge (140 dB) were not included in the analysis due to insufficient sample size at the end of the experiment ($n=5$). Specific growth rates during August 1993 were low relative to other months and ranged from 1.0-1.7%·day^{-1} (Table 9). Condition factors for August were also low compared to other months and ranged from 0.56-0.59.

We ensonified kokanee at a frequency of 800 Hz during September and October 1993. Sound pressure levels during September were 162 dB at the first arc, 150 dB at the second arc, and 130 dB at the third arc. Sound pressure levels tested during October were 165, 150, and 130 dB. In September, we lowered cages 10 m to provide thermal refuge for kokanee and circumvent problems with *F. columnaris*. The acoustic source was lowered 10 m in conjunction with lowering the cages. Cages deployed from the ARD Kamloops barge were not lowered due to obstructions under the barge, thus the difference of 3 dB in sound pressure levels of 162 dB in September and 165 dB in October.

We observed significant variation in kokanee growth during September 1993 ($P<0.001$, $df=3$, $F=11.69$) (Figure 45). Kokanee at the 162 dB sites grew faster than kokanee at 150 dB, 130 dB, and the controls, although growth at 150, 130 dB, and the controls was not significantly different ($P>0.05$). Specific growth (G) was high (3.3%·day^{-1}) among fish maintained at the 162 dB sites whereas G was similar among other treatments ranging from 1.2-1.8%·day^{-1} (Table 9). Condition factors in September ranged from 0.68-0.69.

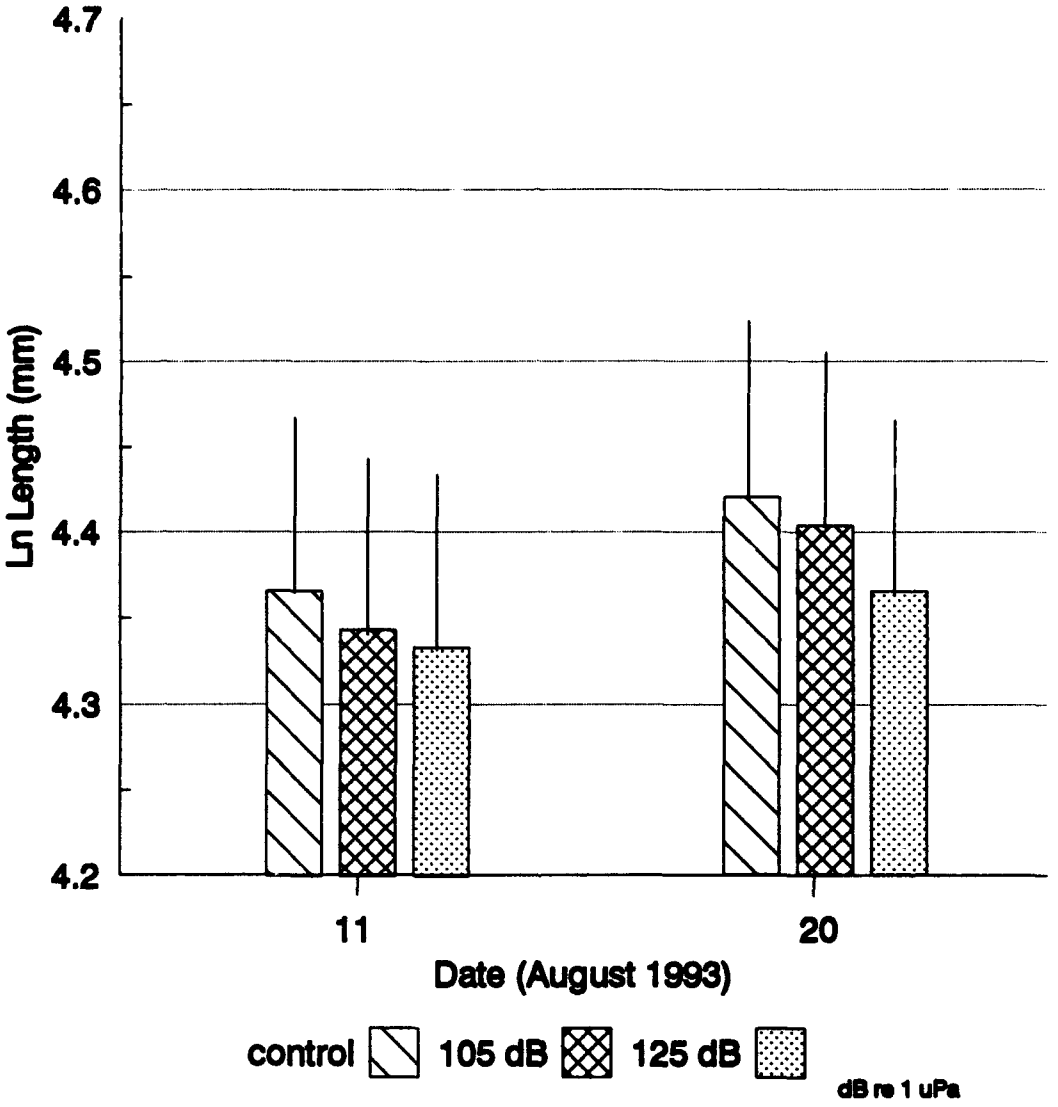


Figure 44. Growth of age-0 kokanee in Lake Pend Oreille during August 1993. Test fish were ensonified from August 11 to August 18 at a frequency of 100 Hz. Vertical lines represent 95% confidence intervals.

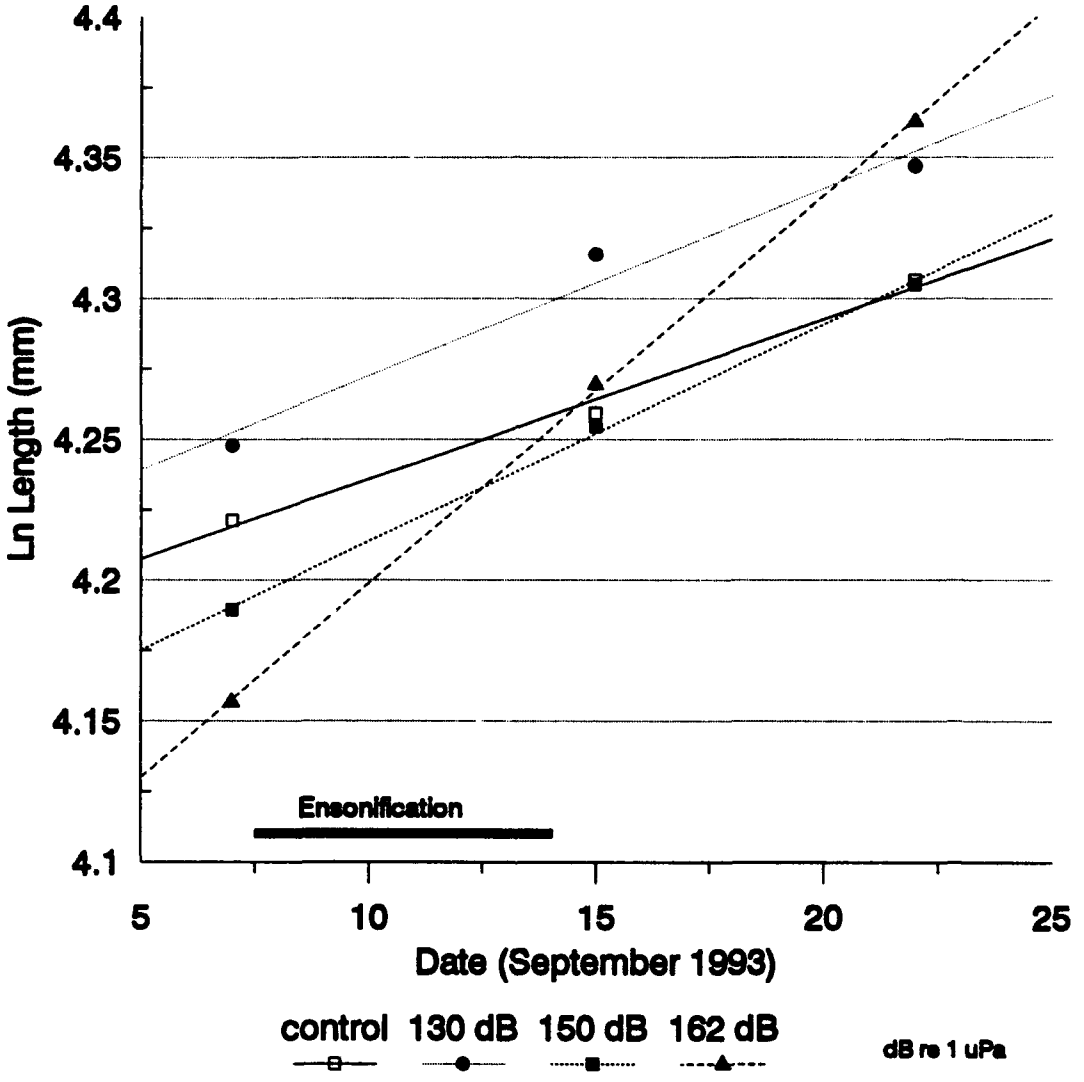


Figure 45. Growth of age-0 kokanee in Lake Pend Oreille during September 1993. Test fish were ensonified from Sept. 7 to Sept. 14 at a frequency of 800 Hz.

We observed growth differences among treatments during October 1993 ($P=0.009$, $df=3$, $F=3.84$) (Figure 46). Kokanee at the control sites grew slower than kokanee at 130, 150, and 165 dB. Specific growth (G) was higher during October relative to other months (2.0-2.7%·day^{-1}) and condition factors ranged from 0.70-0.72 (Table 9).

Growth of kokanee reared in the long-term exposure cages did not differ from controls during May-July 1993 (T-test; $P>0.05$) (Figure 47). Kokanee were ensonified during the first weeks of May, June, and July 1993 (total=21 days) at sound pressure levels of 167 dB (May and June) and 140 dB (July) and frequencies of 5,600 (May and June) and 100 Hz (July). Specific growth (G) for ensonified kokanee was 2.4%·day^{-1} compared to specific growth of 2.0%·day^{-1} for kokanee maintained at the control area.

Growth of ensonified kokanee during August-October 1993 was slightly higher than growth during May-July 1993 (Figure 47). Kokanee were ensonified a total of 21 days at sound pressure levels of 140 (August), 162 (September), and 165 dB (October) at frequencies of 100 (August) and 800 Hz (September and October). Specific growth of ensonified kokanee was 2.5%·day^{-1}.

DISCUSSION

We found no evidence that ensonified kokanee grew less than non-ensonified kokanee at frequencies of 100 and 5,600 Hz and sound pressure levels ranging from 105-167 dB. Feeding and growth studies indicated that kokanee growth was similar among all sound pressure levels and controls at 100 and 5,600 Hz.

We observed significant variation in kokanee growth during the 800 Hz experiments. In September 1993, kokanee growth was significantly higher in rearing cages deployed from the Kamloops barge and in October 1993, kokanee growth was lower in rearing cages deployed at the control sites.

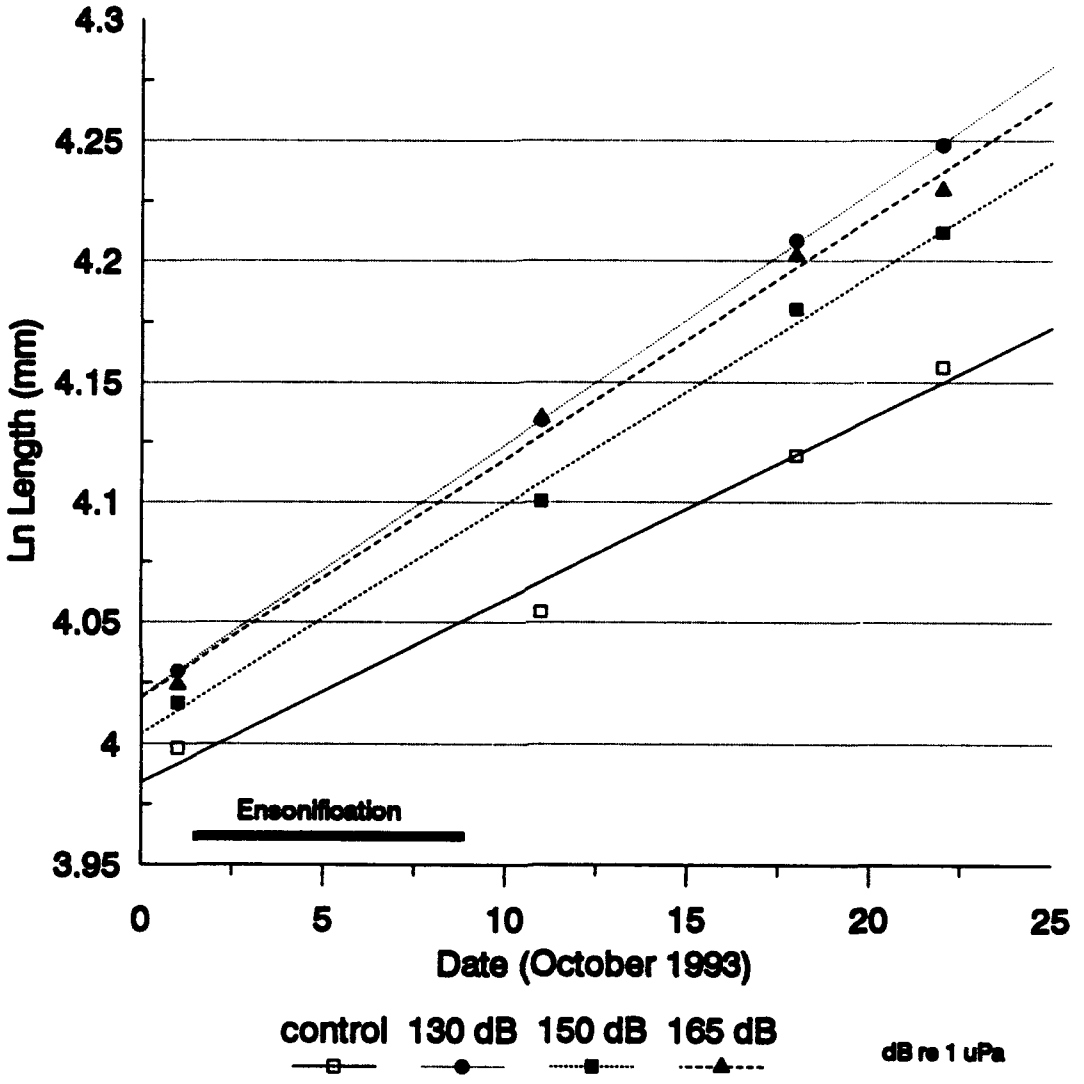


Figure 46. Growth of age-0 kokanee in Lake Pend Oreille during October 1993. Test fish were ensonified from Oct. 1 to Oct. 8 at a frequency of 800 Hz.

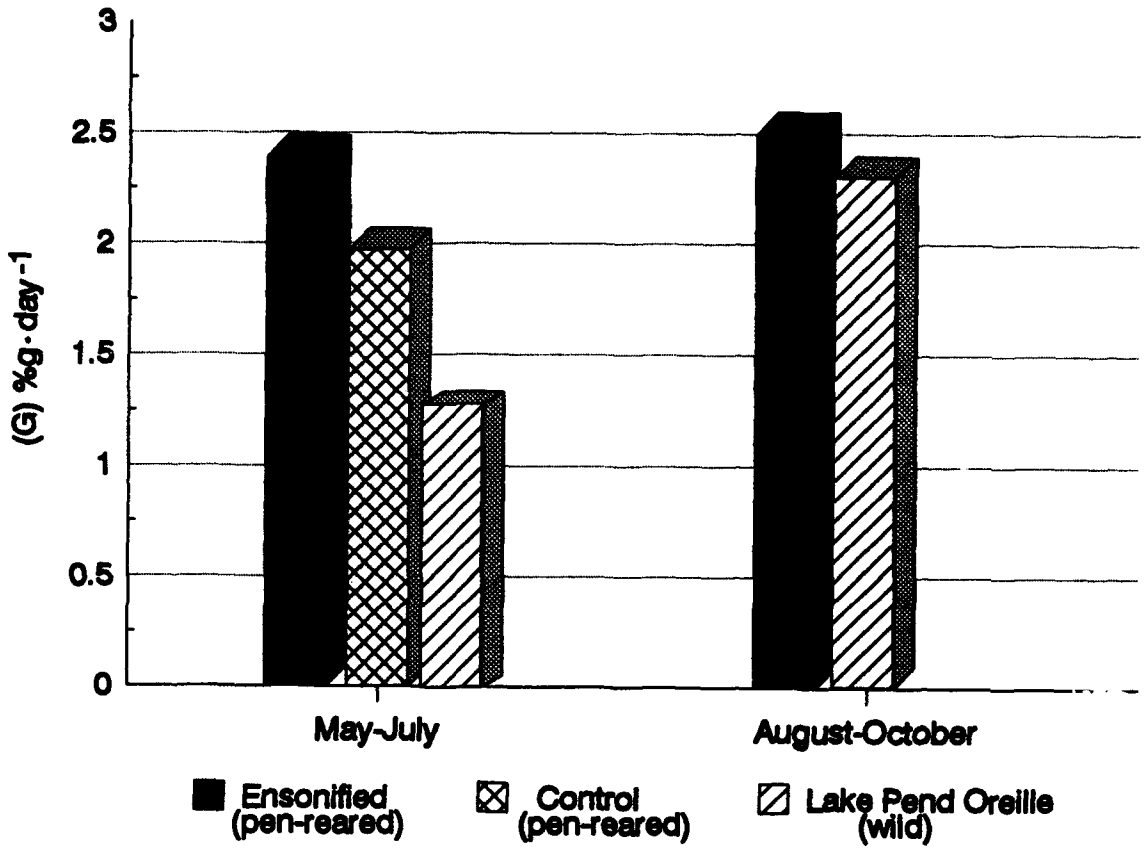


Figure 47. Specific growth rates (G) for age-0 kokanee reared in long-term exposure cages. Lake Pend Oreille data from Rieman (1980).

Kokanee growth in the long-term exposure cages was similar to the controls during May through July 1993, despite 21 days of ensonification (14 @ 5,600 and 7 @ 100 Hz). Growth of ensonified kokanee was slightly higher during August-September and consistent with other observations in Lake Pend Oreille that growth of age-0 kokanee is highest during late August-early September (Rieman 1980). Additionally, growth of pen-reared kokanee used during our experiments was slightly higher than growth rates reported for age-0 kokanee (Rieman 1980) in Lake Pend Oreille. These observations are similar to Johnston's (1990) who reported higher growth rates of pen-reared versus wild kokanee in Kootenay Lake, B.C. We found no evidence that 21-day exposure to the frequencies and sound pressure levels tested affected kokanee growth.

The epizootic outbreak of the bacterium *F. columnaris* in August 1993 was attributed to stress associated with warm water temperatures and handling. Kokanee reared in cages on the ARD Kamloops barge were particularly susceptible to warm water temperatures during August 1993. These cages exhibited considerable movement within the water column as a result of wind and wave action against the ARD Kamloops barge. We sometimes observed these cages extending horizontally along the surface of the water at times of high velocity, longshore surface currents.

Environmental differences among treatment locations appear to be responsible for variation in observed growth patterns during September and October 1993. Realizing that environmental factors are difficult (or sometimes impossible) to compensate for in field situations and that 'micro'-environmental differences among treatment locations probably existed, we monitored physical and biological characteristics at each location throughout the study. Since all other characteristics among treatments were similar (i.e. cages, fish densities, handling, etc.), we considered differences in water temperature and food availability to be the two more important factors affecting kokanee growth during the monthly experiments.

We used a bioenergetics model to evaluate effects of water temperature on kokanee growth (Hewett and Johnson 1987; Beauchamp et al. 1989). Average daily water temperatures at the test and control areas were generally similar during the study (Figure 48), although hourly temperatures at the control area were more variable. We used pooled energy density values for prey which represented proportions of copepods and cladocerans in the diet. Summer diet data for age-0 kokanee were obtained from Rieman (1980) and verified from specimens collected during July and August 1993 (Table 10).

Bioenergetics simulations indicated that water temperature variation had minimal influence on kokanee growth. Using the control area temperatures as baseline, we observed, at most, a 1.6% change in growth due to water temperatures (Table 11). Water temperatures at the control area favored higher growth for all months except July.

The second important factor that could affect kokanee growth is food density. Patterns of zooplankton abundance, obtained from lake samples during Task 1.1, were generally similar during summer 1993 (Figure 25). Zooplankton densities at sites closest to the Kamloops barge (high SPL's) were slightly (but not significantly) higher than densities at the control sites during July through October 1993. Effects of subsurface water currents and upwelling on the cage mooring systems were more noticeable at the control area during May through October 1993. Surface currents and upwelling of water masses are important factors affecting the spatial and temporal distribution of zooplankton.

We frequently observed higher growth rates in rearing cages deployed from the ARD Kamloops barge. Higher growth among kokanee maintained at these sites may be related to increased photoperiod. The ARD Kamloops barge is equipped with florescent lights that remained on (for safety reasons) throughout the night. Cages deployed from the ARD Kamloops barge were within range of incident light whereas

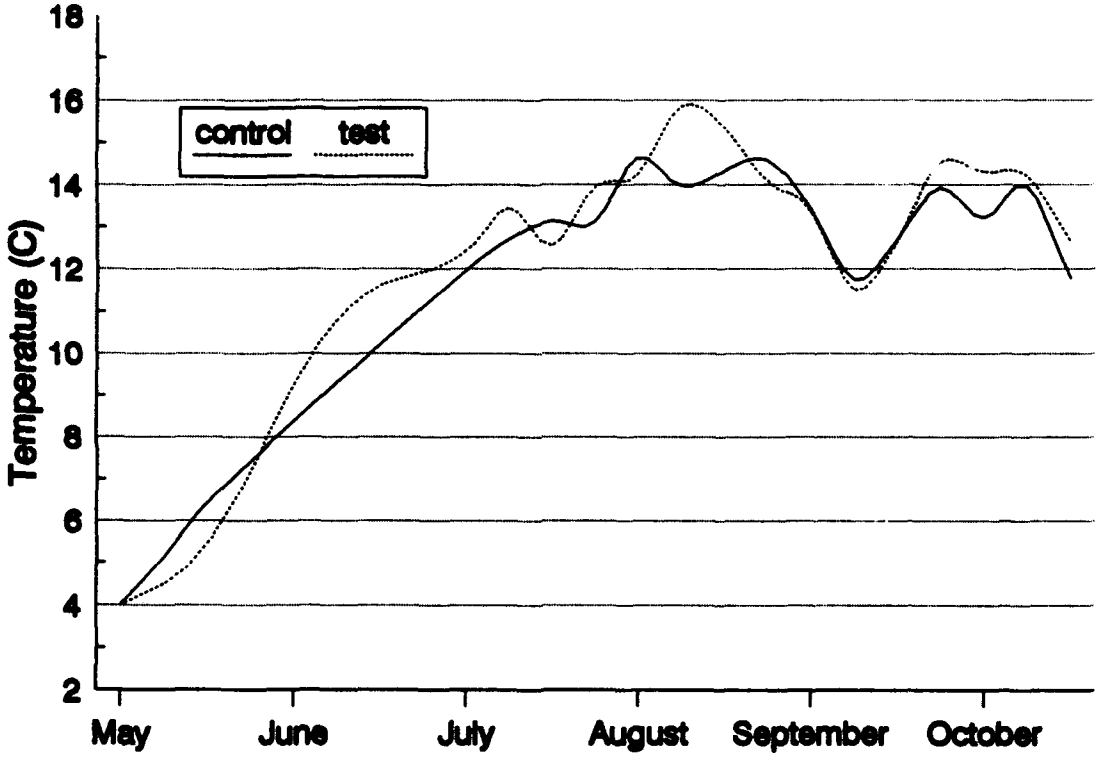


Figure 48. Mean daily water temperatures (C) at test and control areas in Lake Pend Oreille, Idaho, 1993. Data represent water temperatures at a depth of 10 m, except during Sept. 6-15 when cages were lowered 10 m.

Table 10. Proportions of copepods, cladocerans, and mysids in the summer diet of age-0 kokanee in Lake Pend Oreille. Data from Rieman (1980).

Month	Copepods	Cladocerans	Mysids
May	0.85	0.08	0.07
June	0.85	0.08	0.07
July	0.86	0.11	0.03
August	0.07	0.86	0.07
September	0.14	0.78	0.08
October	0.70	0.23	0.07

Table 11. Observed and simulated growth of age-0 kokanee. Observed weight represents growth of ensonified kokanee at test area water temperatures. Simulated weight represents growth of ensonified kokanee at control area water temperatures.

Month	Sound pressure level (dB)	Begin wt(g)	Observed end wt(g)	Simulated end wt(g)	Weight change
May	167	0.90	1.51	1.52	+0.01
	152	1.04	1.49	1.50	+0.01
	132	1.26	1.77	1.78	+0.01
June	167	2.18	3.98	4.01	+0.03
	152	2.24	4.00	4.03	+0.03
	132	2.23	4.16	4.18	+0.02
July	140	2.37	4.14	4.13	-0.01
	125	2.54	4.33	4.33	0
	105	2.35	3.92	3.91	-0.01
August	140	-	-	-	-
	125	2.65	2.94	2.97	+0.03
	105	2.69	3.20	3.23	+0.03
September	162	1.88	3.30	3.30	0
	150	2.05	2.80	2.80	0
	130	2.51	3.20	3.20	0
October	165	1.26	2.32	2.35	+0.03
	150	1.24	2.21	2.24	+0.03
	130	1.27	2.41	2.45	+0.04

other cages were not exposed to light during darkness. Because water temperature effects were minimal during the experiments, we hypothesize that longer photoperiods and localized zooplankton abundance are probably responsible for the observed variation in kokanee growth during September and October 1993. Although we have no data on effects of increased photoperiod on kokanee growth, kokanee are known to be sight feeders and studies of feeding chronology indicate that kokanee reduce feeding rates during darkness (Narver 1970; Doble and Eggers 1978). Additionally, Nemeth and Anderson (1992) demonstrated that activity of juvenile coho *O. kisutch* and chinook salmon *O. tshawytscha* increased 90% when exposed to underwater light at nighttime.

With the exception of kokanee reared at the ARD barge, we observed low growth rates in September relative to other months. This appears to be related to a concomitant decrease in zooplankton abundance in Lake Pend Oreille in mid September. We observed low zooplankton densities in lake samples during the second week of September, indicating an overall reduction in food availability, although zooplankton abundance was generally higher than during May when we observed similar growth rates.

We found no evidence from the September and October experiments, that pulsed underwater sound at 800 Hz and sound pressure levels of 105-165 dB reduced growth of age-0 kokanee. Rather, we frequently observed higher growth rates among kokanee ensonified at the highest sound pressure levels (i.e. September).

Our findings indicated that sustained exposure to simulated ISMS sounds between 100-5,600 Hz at sound pressure levels of 140-165 dB would not affect kokanee feeding behavior in the BIZ region based on the absence of reduced growth among ensonified kokanee.

Task 1.3

Predator/Prey Interactions in Response to Simulated ISMS Ensonification

INTRODUCTION

Acoustic stimuli are used by prey fishes to avoid capture and by predator fishes to locate prey (Schwarz 1985). Fish generate low frequency sound by the motion of their body through water. Prey and predator fishes use these and other acoustic stimuli to initiate respective escape and attack responses (Hawkins and Johnstone 1978; Enger et al. 1989; Montgomery et al. 1988; Schwarz 1985). Exposure to sounds generated during ISMS testing may act as "noise", effectively canceling or confusing the "natural" sounds used by fish.

During operation of the ISMS test system on Lake Pend Oreille, underwater sounds will be generated at various frequencies and sound pressure levels. To better understand the potential effects of these sounds on predator/prey interactions of fishes in Lake Pend Oreille, this aspect of the study examined effects of simulated ISMS underwater sounds on the interactions between the piscivorous predators, bull trout and northern squawfish *Ptychocheilus oregonensis* and salmonid prey, kokanee and cutthroat trout.

Predator/prey bioassays have been widely used to evaluate performance after exposures to sublethal conditions. Hatfield and Anderson (1972) investigated the effects of insecticides on the vulnerability of Atlantic salmon to predation by brook trout. Bams (1966) used predation bioassays to test the vulnerability of sockeye salmon *O. nerka* to predation by cutthroat trout. The ability of thermally shocked prey to escape capture by predators has been investigated among a variety of species (Coutant 1973; Deacutis 1978; Sylvester 1972; Yocum and Edsall 1974).

METHODS

Bioassessments to evaluate effects of simulated ISMS sounds on predator/prey interactions were examined in three series of tests. Tests examined the hypotheses that: 1.) ensonified prey were not captured and consumed more readily than nonensonified prey; 2.) ensonified predators could capture and consume equally as many prey as nonensonified predators, and 3.) predation levels between simultaneously ensonified predator and prey fishes did not differ from nonensonified controls.

Prey fish used in testing were juvenile cutthroat trout and kokanee, two species of interest to the Lake Pend Oreille sport fishery. Young cutthroat trout were obtained from the Sandpoint Fish Hatchery and young kokanee were obtained from the Cabinet Gorge Fish Hatchery. Kokanee ranged from 45 to 100 mm in total length whereas cutthroat trout ranged in length from 61 to 127 mm. All prey fish were acclimated to net pens located at the ARD Green barge prior to testing (Figure 11). All prey fishes were small enough to be consumed by predators.

Bull trout were used in predator prey testing during May, June, and July 1993. Bull trout were obtained from stock raised at the Cabinet Gorge Fish Hatchery. Bull trout ranged in total length from 205 to 332 mm. Because of their limited availability, consideration as a threatened species, and health problems arising at the hatchery in mid-June 1993, bull trout were not available after July 1993. Northern squawfish, because of their availability and reputation as a voracious predator on young salmonids, were used in predation bioassays during July, August, September, and October 1993. Northern squawfish were captured using boat electrofishing equipment near Scenic Bay and along the shoreline of Lake Pend Oreille, near Sandpoint, Idaho. Northern squawfish used as predators ranged from 190 to 543 mm. Predators were used only once and were returned to the hatchery (i.e. bull trout) or sacrificed (i.e. northern squawfish).

In-situ predation experiments were conducted within eight in-lake net pens located on the ARD Green barge (Figure 49), or during simultaneous ensonification on the ARD Kamloops barge. Net pens were cylindrical in shape with a diameter of 1.9 m and a height of 2 m and covered with 3 mm nylon mesh. Refuge areas were not available to the prey fish inside the net pens.

Predator/prey experiments were conducted at the ARD Green barge in Scenic Bay and the ARD Kamloops barge. Ensonification of fishes at 100, 800, and 5,600 Hz was conducted concurrently with ensonification cycles for Tasks 1.1 and 1.2 (Figure 23). Predator/prey experiments were conducted at the highest sound pressure level for each frequency tested. Effects of simulated ISMS sounds at 5,600 Hz were tested during May and June 1993, 100 Hz ensonification was investigated during July and August, and ensonification at 800 Hz was examined during September and October. Sound pressure levels were 140 dB at 100 Hz and 167 dB at 5,600 Hz. Sound pressure levels at 800 Hz were 162 dB during September and 165 dB during October 1993.

Prey Ensonification

All prey used in testing the ability of ensonified prey to avoid predation were anesthetized with MS-222 and measured for total length (mm) to evaluate possible size-specific effects of ensonification on their ability to avoid capture by predators. Prey fish were isolated for 0.25-2 h of recovery before adding them to the test chambers. Prey fish which did not recover from anaesthetic were replaced prior to testing.

To begin a trial, prey fish (n = 100-150) were transported to the ARD Kamloops barge where they were subjected to simulated ISMS ensonification for a period of 24 h (Figure 50). Immediately following ensonification, ensonified fish were transported to the ARD Green barge where they were marked with a randomly chosen clip of the upper or lower caudal fin. Ten control fish were handled in the same manner (i.e. transported to the ARD Kamloops barge and back to the ARD Green barge) but were

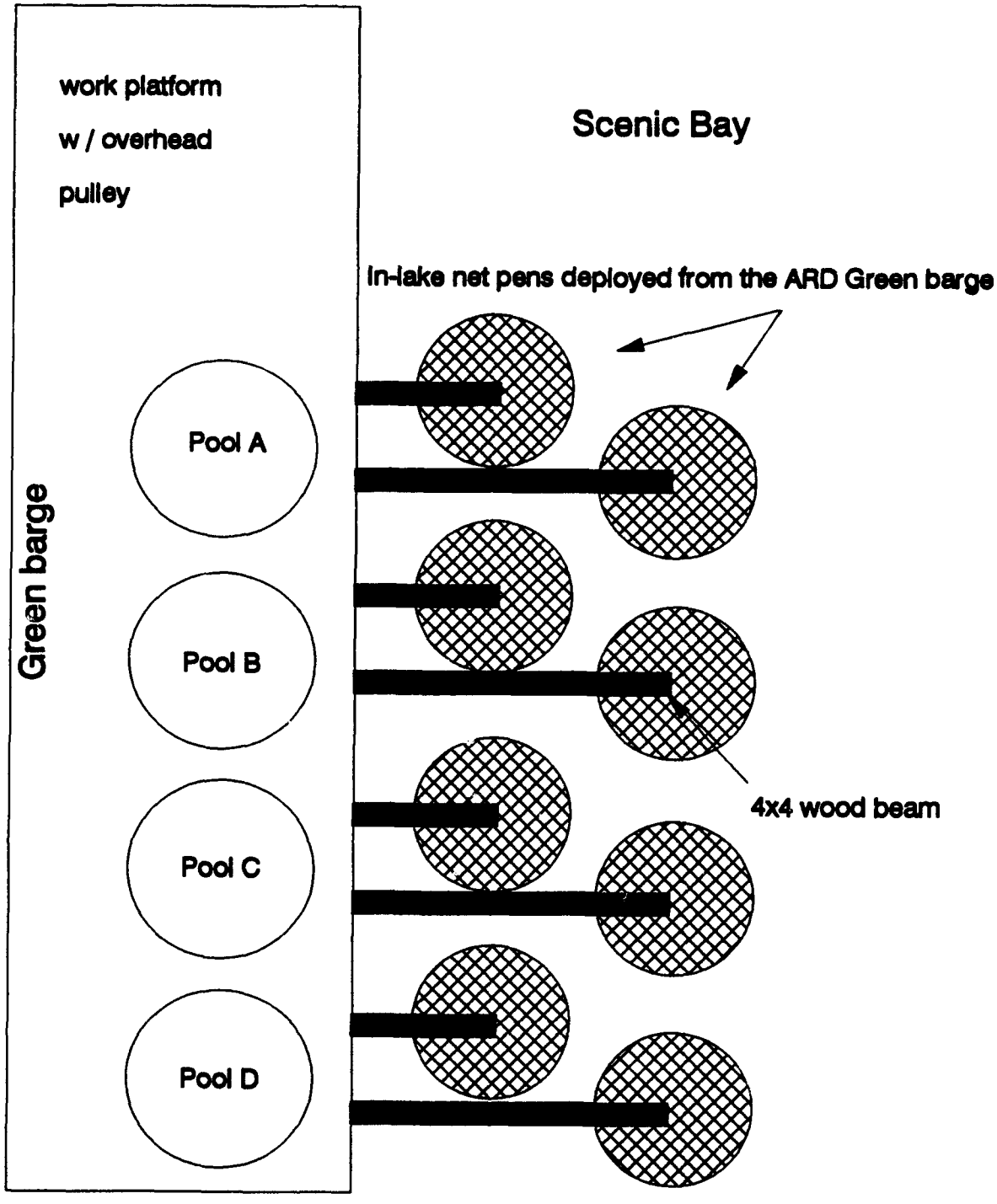


Figure 49. Diagram of predator/prey test facilities located on the ARD Green barge.

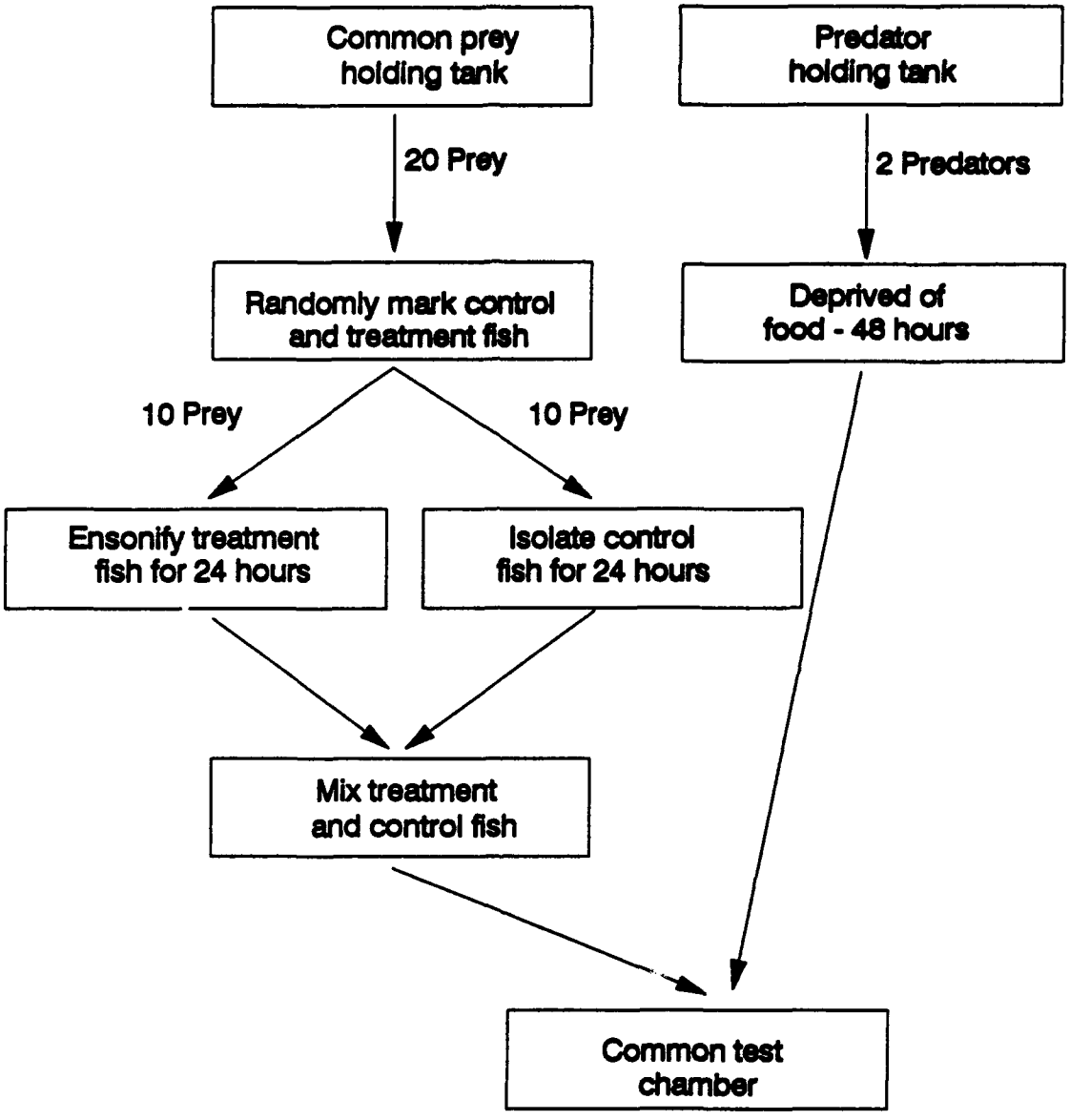


Figure 50. Flow diagram of ensonified prey test.

not subjected to ISMS underwater sound. Treated (n=10) and control (n=10) fish were added to a common test chamber with two predators, deprived of food at least 48 h. Predation tests were allowed to continue until 15-85% of the prey fish were consumed (Bams 1966) or until 48 h. Based on these criteria, tests lasted between 18 to 48 h. Testing was terminated by rapidly netting predator and prey fishes from the test chamber. The remaining ensonified and control prey fish were then enumerated and measured for total length. We compared lengths of prey before and after testing to determine if predation was size-selective (i.e. more smaller prey consumed). We analyzed 'before and after' lengths using a Student's T-test and considered differences significant at $P<0.05$.

Predator Ensonification

The ability of ensonified predators to capture and consume prey was evaluated in a second series of trials. When bull trout were used as predators (i.e. tests conducted at 5,600 Hz), a test was initiated by transferring food-deprived bull trout (n=8) to the ARD Kamloops barge for 24 h of ensonification (Figure 51). Following ensonification, bull trout were transferred to the ARD Green barge where they were marked with a randomly assigned upper or lower caudal fin clip. Bull trout used as controls (n=8) were handled similarly except they were not subjected to simulated ISMS ensonification. At the ARD Green barge, treated and control predators were transferred to one of eight test chambers each containing 20 prey fish. Predation continued until either 15-85% of the prey fish were consumed or until the end of 48 h. Testing was terminated by removing predator and prey fishes from the test chamber. Surviving prey fish were enumerated. The number of prey eaten by both treated and control fish were counted by examining stomach contents of bull trout using the lavage technique (Seaberg 1957).

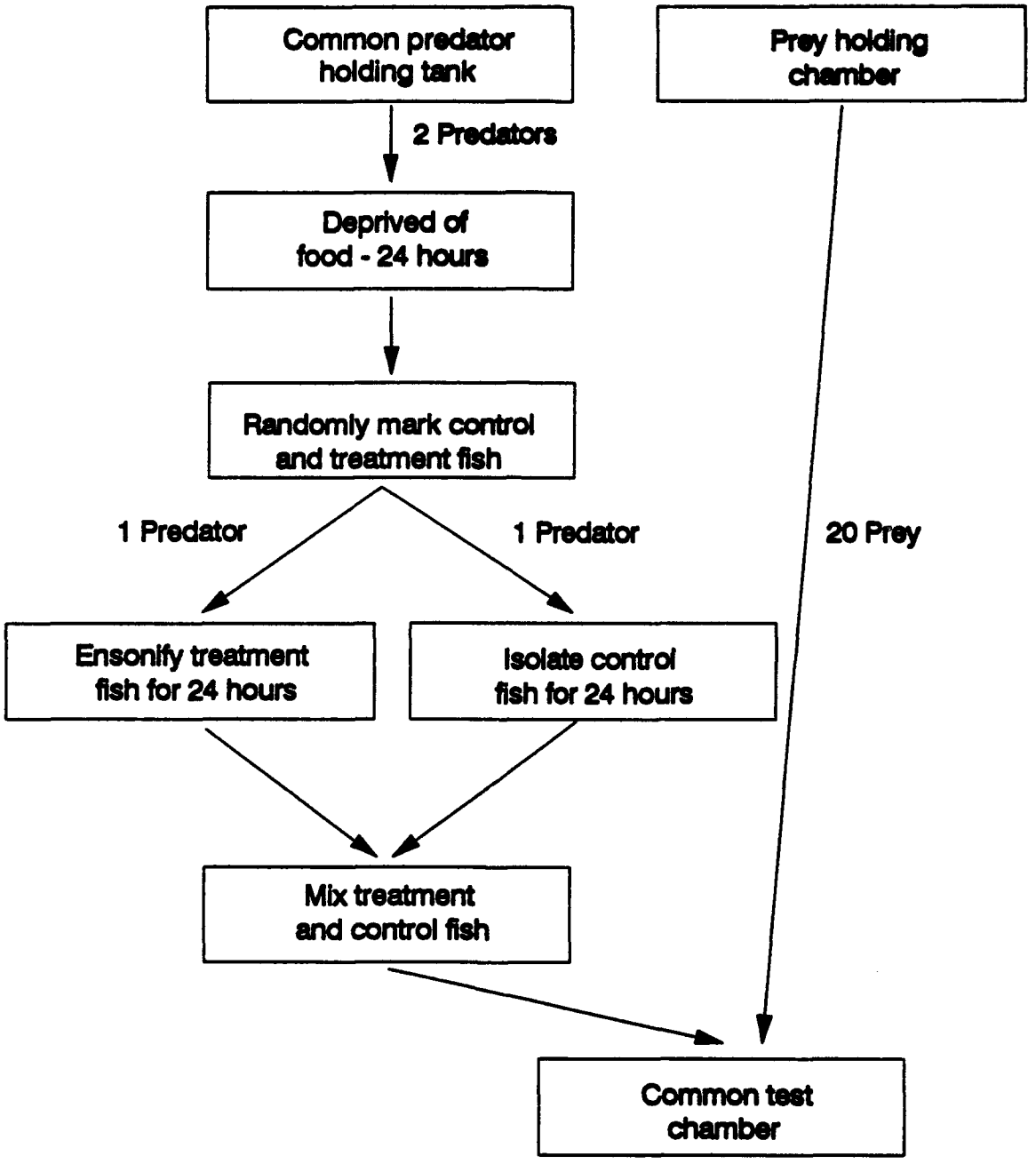


Figure 51. Flow diagram of ensonified predator test using bull trout as predators.

Tests utilizing northern squawfish as predators (i.e. 100 and 800 Hz experiments) were initiated by transferring food-deprived squawfish (n=8) to the ARD Kamloops barge for 24 hours of ensonification (Figure 52). Immediately following ensonification, ensonified squawfish were transferred to the ARD Green barge where they were added to a test chamber with 20 prey fish. Northern squawfish used as controls for these tests were handled similarly and tested simultaneously with ensonified squawfish but in separate cages. For example, two ensonified squawfish were placed with 20 prey fish in one cage and two squawfish used as controls were placed in a separate cage with 20 prey fish. This allowed us to count the number of prey fish remaining without having to pump stomachs of squawfish. Squawfish proved to be a voracious predator and exhibited high evacuation rates during July-September 1993 (i.e. 10 prey fish could be digested within 18-24 hours). Predation tests using squawfish were terminated at the end of 24 h. Testing was terminated by removing predator and prey fish from the test chambers and surviving prey fish were enumerated.

Simultaneous Ensonification

Effects of simultaneous ensonification of predators and prey on the level of predation were also evaluated (Figure 53). To begin a test, two predators were deprived of food for a period of 48 h. Following deprivation, the predators were transferred to the ARD Kamloops barge and gently added to a net pen containing 20 prey fish. Predation continued concurrently with ISMS sound generation for a period of 24 h. At the conclusion of testing, predators were removed from the test chamber and the remaining prey were enumerated. Controls for the simultaneous treatment tests were conducted immediately prior to or following ensonification in the same location and in a similar manner except that predation took place in net pens subjected only to background noise.

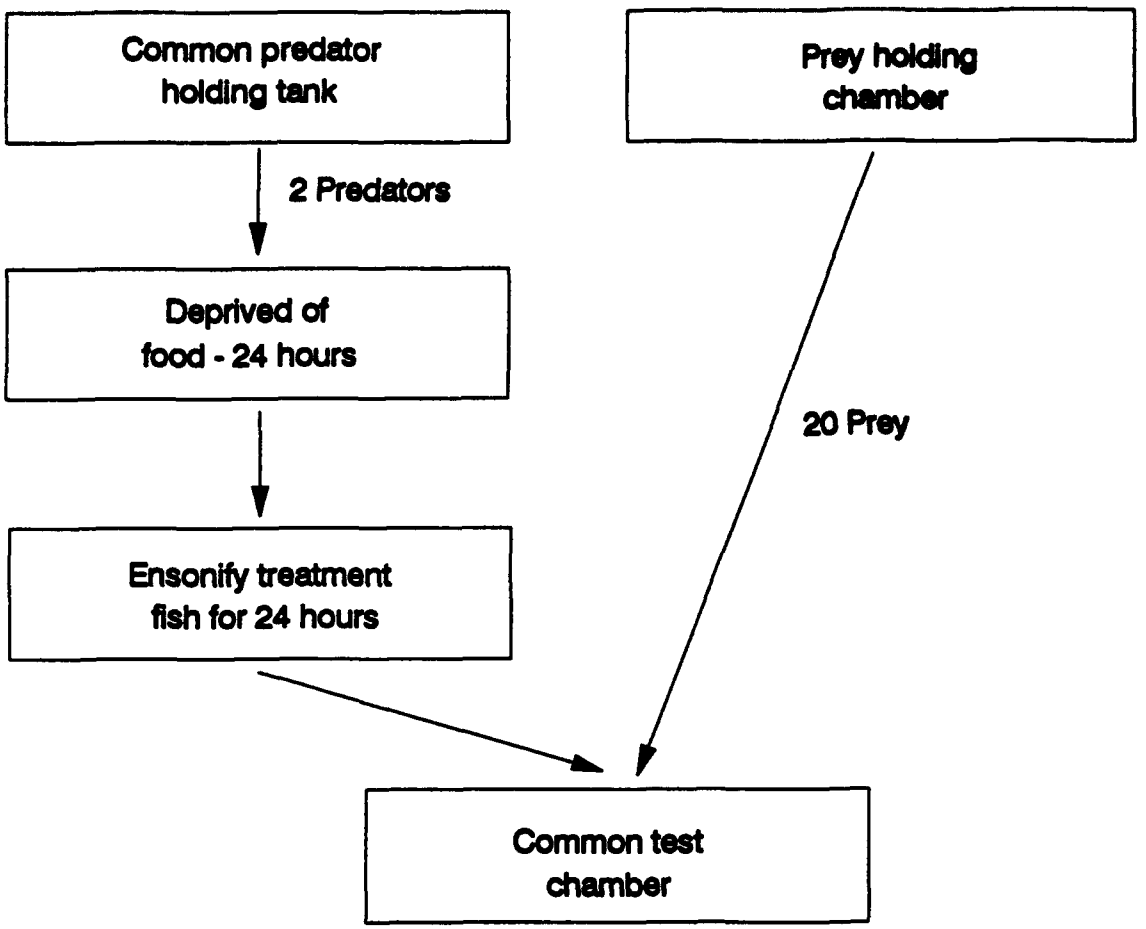


Figure 52. Flow diagram of ensonified predator test using northern squawfish as predators.

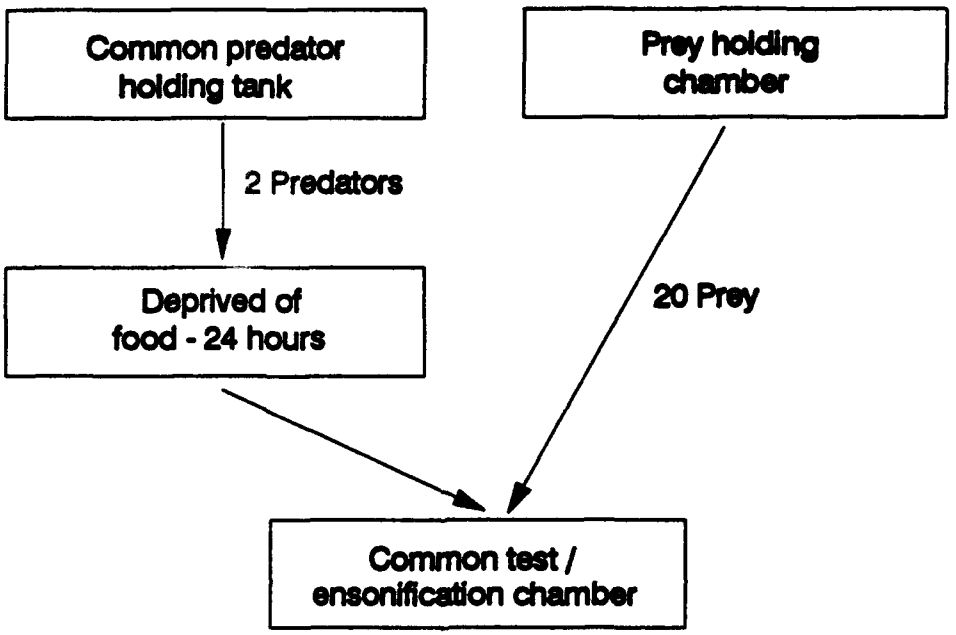


Figure 53. Flow diagram of simultaneous ensonification test.

Comparisons between ensonified and control fish were tested for significance using a Wilcoxon signed-rank test and Mann-Whitney nonparametric statistics. Successful predator/prey bioassays where treated and control fish were tested together in a common test chamber were defined by consumption of 15-85% of the available prey. Bams (1966) and Coutant (1973) discussed the reasons for omitting trials in which levels of predation are extremely low (<15%) or high (>85%). When treatment and control trials were conducted in separate test chambers, all replications were used for analysis.

RESULTS

Prey Ensonification

Mean number of prey fish consumed in successful tests for the control and treated fishes at the three frequencies tested ranged from 1.57 to 9.66 (Table 12). Highest variability in number of treatment and control fish was at 100 Hz.

Ensonified kokanee and cutthroat trout prey showed no reduction in their ability to avoid capture by predators compared to control fish. In 11 replicates conducted at 100 Hz, an average of 2.58 ensonified kokanee were consumed by bull trout and squawfish predators compared to an average of 2.91 untreated kokanee ($P=0.350$). At 800 Hz, squawfish consumed an average of 4.50 ensonified cutthroat trout in 12 replicates compared to an average of 4.58 cutthroat trout used as controls ($P=0.285$). In 12 replicate tests conducted at 5,600 Hz, bull trout predators consumed an average of 3.60 ensonified kokanee compared to 3.33 naive kokanee ($P=0.455$).

Bull trout and squawfish predators did not preferentially select any sizes of kokanee or cutthroat trout for either ensonified or control fishes ($P>0.05$) (Figure 54).

Table 12. Mean number of prey fish consumed at each frequency. Sound pressure levels (SPL) measured in dB re 1 μPa. SD represents standard deviations for the number of fish consumed. Asterisk indicates significant difference at P=0.058.

Test	Frequency (Hz)	SPL (dB)	Mean no. of test fish consumed	SD	Mean no. of control fish consumed	SD	n
Ensonified prey	100	140	2.58	1.62	2.91	1.72	12
Ensonified predator	100	140	9.66	5.50	14.66	5.88	6
Simultane	100	140	5.87	6.22	5.62	7.09	14
Ensonified prey	800	162/165	4.50	2.78	4.58	2.07	12
Ensonified predator	800	162/165	4.16*	3.10	6.00	3.03	22
Simultane	800	162/165	4.62	3.07	2.60	2.74	16
Ensonified prey	5600	167	3.60	1.98	3.33	1.61	11
Ensonified predator	5600	167	1.57	1.81	3.42	2.37	7
Simultane.	5600	167	5.66	2.33	5.66	4.63	6

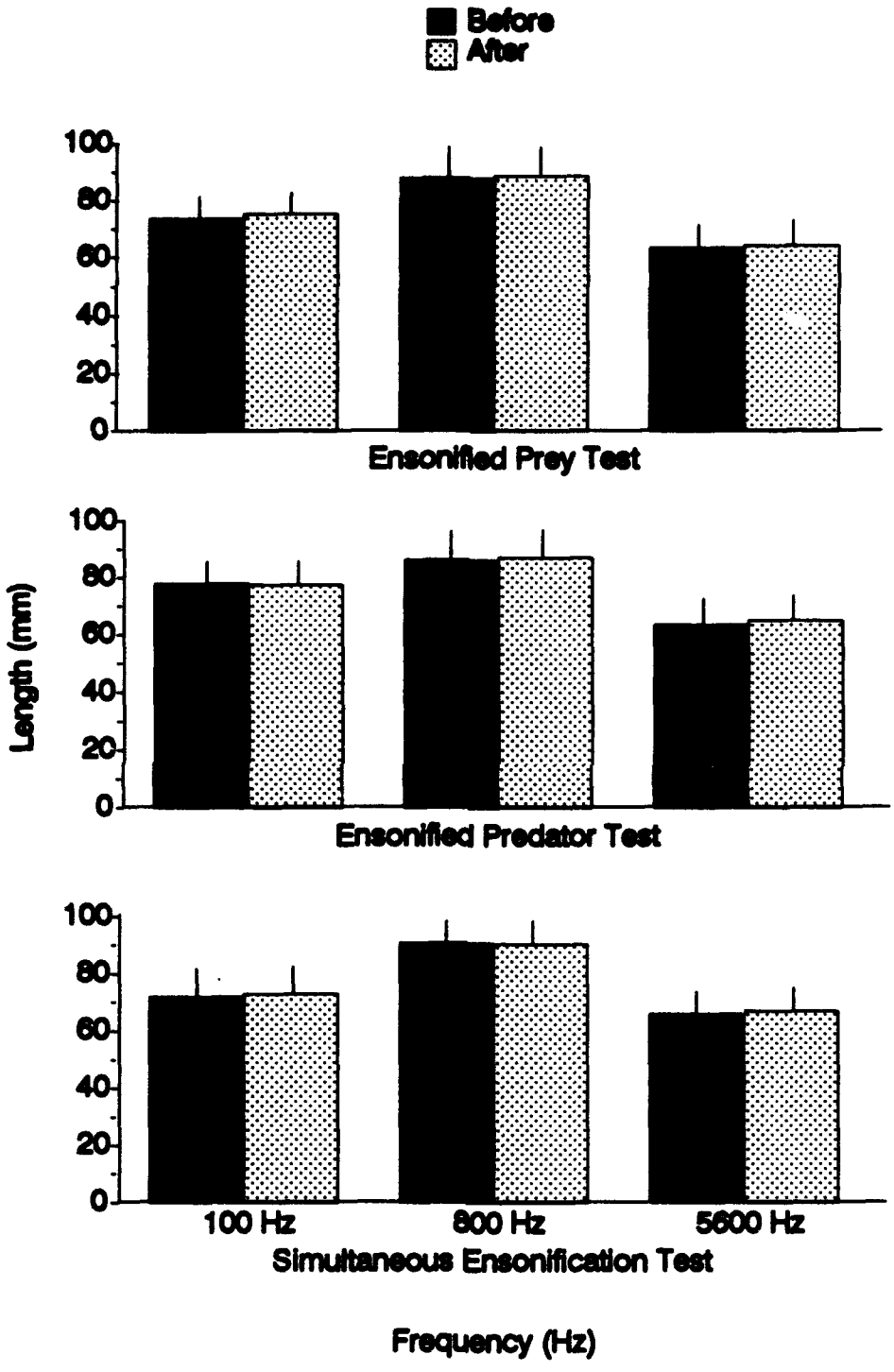


Figure 54. Mean lengths and standard deviations of prey fish before and after ensonified prey test, ensonified predator test, and simultaneous ensonification test.

Predator Ensonification

Bull trout ensonified at 5,600 Hz and northern squawfish ensonified at 100 Hz did not show a significant alteration in their ability to capture prey compared to control predators (Table 12). Northern squawfish ensonified at 100 Hz consumed an average of 9.66 kokanee compared to 14.66 consumed by untreated northern squawfish ($n=6$, $P=0.256$). Following ensonification at 5,600 Hz, bull trout consumed an average of 1.57 kokanee compared to a mean consumption of 3.42 for untreated bull trout ($n=7$, $P=0.125$).

Predation by northern squawfish ensonified at 800 Hz was lower than untreated northern squawfish at $P=0.058$. In 22 trials, ensonified squawfish consumed an average of 4.16 prey compared to an average of 6.00 prey consumed by untreated squawfish. Variability in consumption was similar between ensonified and control groups.

Simultaneous Ensonification

We found no significant difference ($P<0.05$) in numbers of fish consumed between simultaneously ensonified predator and prey fishes as compared to control groups (Table 12). At 100 Hz, an average of 5.87 kokanee were consumed during treatment trials compared to an average of 5.62 for control tests ($n=14$, $P=0.275$). In 16 simultaneous ensonification tests conducted at 800 Hz, squawfish consumed an average of 4.62 cutthroat trout fry compared to an average of 2.60 cutthroat trout used as controls ($n=16$, $P=0.276$). In simultaneous tests conducted at 5,600 Hz, bull trout predators consumed an average of 5.66 kokanee in both the treatment and control tests ($n=6$, $P=0.999$).

DISCUSSION

We found no significant alteration of predator/prey interactions resulting from either ensonification of prey fishes or from simultaneous ensonification of both

predator and prey fishes. Consumption of kokanee or cutthroat trout ensonified at 100, 800, and 5,600 Hz by northern squawfish and bull trout predators was similar to levels of consumption of untreated prey. Predation was also similar between simultaneously ensonified predator and prey fishes and their corresponding control groups.

Bull trout and squawfish predators ensonified at frequencies of 100 and 5,600 Hz did not exhibit a reduced ability to capture kokanee or cutthroat trout fry. Results of predator/prey testing at 800 Hz, however, indicated that consumption by ensonified squawfish was lower than controls at $P=0.058$. Consumption of prey fishes was variable among predator bioassays, although the variability in number consumed was similar between tests and controls. If ensonification at 800 Hz somehow "debilitates" northern squawfish, we would expect to observe similar results during experiments where predators and prey were ensonified simultaneously. We did not observe reduced predation among simultaneously ensonified northern squawfish and cutthroat trout at 800 Hz. However, in a study describing sounds produced by cutthroat trout, Stober (1969) indicated that cutthroat trout produced low-intensity "thumps" with a principal frequency 150 Hz and "squawks" with principal frequencies in the band of 600-850 Hz (Figure 55). Northern squawfish may detect underwater sounds produced by prey, enhancing their predatory abilities. In light of the limited amount of available data, we cannot dismiss the possibility that northern squawfish use underwater sounds to cue in on prey fishes and that sounds of similar frequency may interfere with this ability. Northern squawfish, as well as other fishes in the order Cypriniformes, have an elaborate connection of ligaments and bones connecting the gas bladder to the inner ear (Lagler et al. 1977). This structure, the Weberian apparatus, presumably enhances their sensitivity to sound frequencies and intensities (Platt and Popper 1981). Sounds within their hearing range and similar to those produced by prey fish may interfere with their ability to detect prey, though this hypothesis is not definitive and requires research describing hearing thresholds and sensitivities of northern squawfish as well as

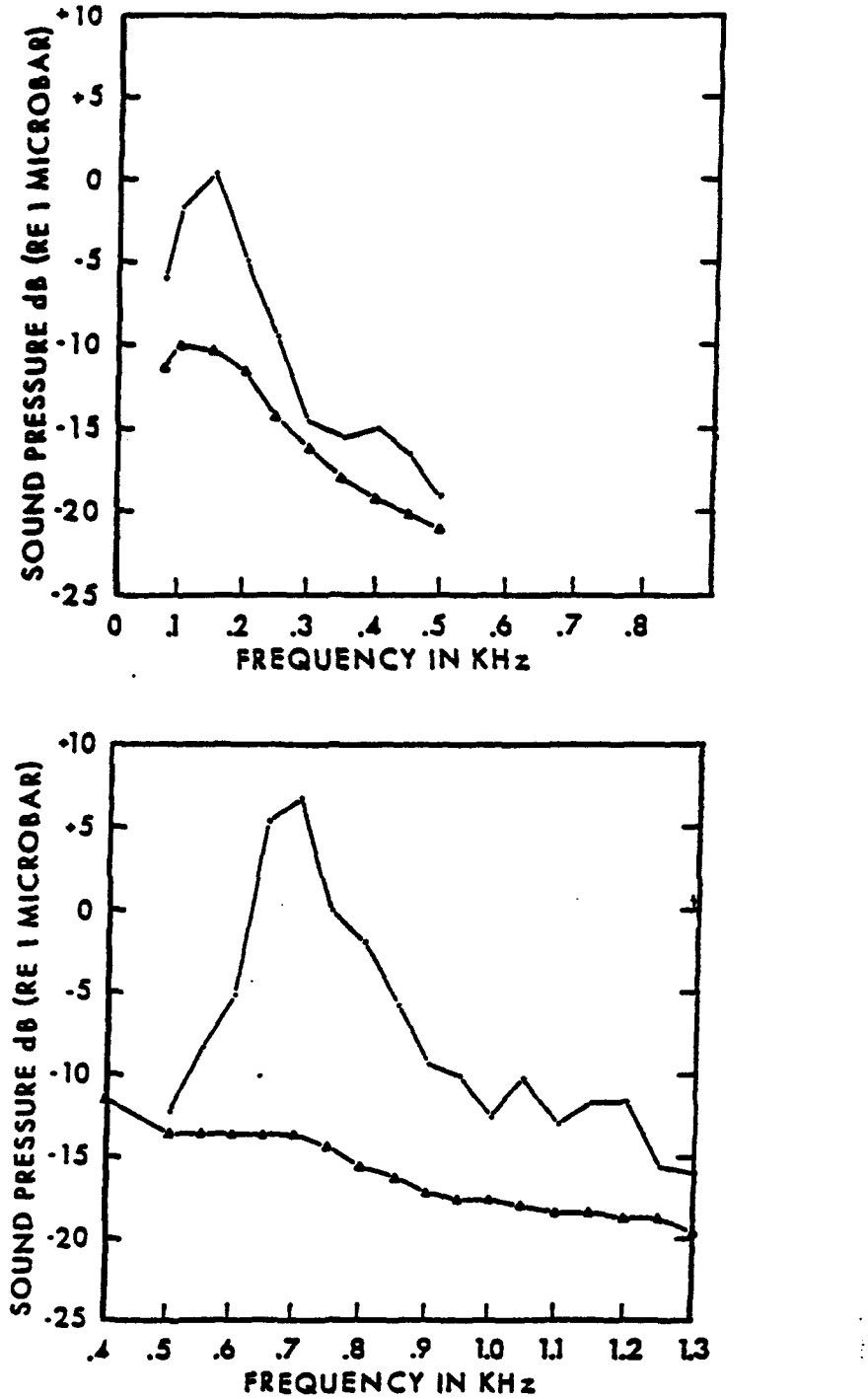


Figure 55. Analysis of sounds produced by cutthroat trout. Upper graph indicates "thump" sounds with a principal frequency range of 100-150 Hz. Lower graph shows "squawk" sounds with a principal frequency range of 600-850 Hz (from Stober 1969). Dotted lines connecting triangles represent average system noise. Sound pressure level in microbars can be converted to sound pressure level in uPa by adding 100 to the graph levels.

verification of earlier studies describing sounds produced by cutthroat trout. We do not anticipate that ensonification of northern squawfish will have impacts at the population level. Northern squawfish are littoral predators and likely would not be exposed to sound intensities produced in the open water during testing. Our experiments were conservative in so much as we ensonified northern squawfish at intensities that were at least 15 dB higher than they would experience during ISMS testing anywhere in the BIZ region.

In contrast to cyprinids, salmonids have comparatively poor hearing and are sensitive to frequencies below about 380 Hz (Hawkins and Johnstone 1978). Assuming that 800 Hz may affect northern squawfish predation on cutthroat trout, we anticipate that this effect might be attenuated, to some degree, in salmonids. Available literature suggests that salmonids are sensitive to frequencies up to 600 Hz. Recent evidence indicates hearing sensitivities in the infrasound range for salmonid fishes (Knudsen et al. 1992; Mike Curtain, SONALYSTS, Waterford, CT, pers. comm.). We have no data bearing on effects of 800 Hz on salmonid predators but caution against interspecific generalizations. We are aware of no studies describing hearing thresholds and sensitivities of northern squawfish. Their presumed sensitivity is based on studies describing hearing in similar species. Platt and Popper (1981) described inconsistencies in hearing sensitivities within the same taxa (i.e. Family) and caution interspecific generalizations.

We found no indication that the ability of ensonified prey to avoid predation was impaired by simulated ISMS testing at 100, 800 and 5,600 Hz. Also, bull trout and northern squawfish exposed to similar sound pressure levels at 100 and 5,600 Hz did not exhibit reduced feeding. Northern squawfish ensonified at 800 Hz may exhibit reduced predation on cutthroat trout though these impacts are not expected to be significant since northern squawfish in Lake Pend Oreille are littoral predators and would

probably not be exposed to localized ensonification at a frequency of 800 Hz and sound pressure level of 165 dB.

Task 2.1

Kokanee Behavior in Response to Simulated ISMS Ensonification

INTRODUCTION

Effects of underwater sound on fish behavior have been well documented for several species. Recent studies of blueback herring, Pacific herring, alewife, and Atlantic salmon have shown that underwater sound can elicit behavioral changes (Schwarz and Greer 1984; Dunning et al. 1992; Knudsen et al. 1992). The relevance of behavioral changes can be positive or negative. The use of underwater sound to repel alewife from hydropower intakes can have beneficial effects at the population level (Dunning et al. 1992). In contrast, the disruption of natural behavior patterns may make fishes more vulnerable to predation or put them at other survival disadvantages (Schwarz 1985). Documented behavioral responses to underwater sound include startle response (Blaxter et al. 1981; Blaxter and Hoss 1981), avoidance/attraction reactions (Haymes and Patrick 1986; Dunning et al. 1992; Nestler et al. 1992), schooling behavior (Hawkins and Johnstone 1978; Blaxter and Hoss 1981), and increased/decreased activity (see review by Schwarz 1985).

The objective of this aspect of the study was to examine behavioral responses of small (age-0) and large (adult) kokanee to simulated ISMS underwater sound.

METHODS

We conducted in-situ behavior experiments from the ARD Kamloops barge during July 26 through 30, 1993. We examined fish behavior using videocinematography. Two FC-1000 underwater cameras (DeepSea Power and Light Co., Seattle, WA) were mounted to each end of a specially designed camera cage. Each camera's field of view covered approximately 90% of the opposite end of the cage. The cylindrical cage was constructed with 3 mm nylon mesh and measured 1.8 m diameter x

2 m long (Figure 56). The top of the cage was covered with black canvas to provide a background for the bottom-mounted camera. The white ventral surface of the fish contrasted with the black background. The bottom of the cage was covered with white canvas to provide background for the top-mounted camera. The dark, dorsal surface of the fish contrasted with the white background. We recorded observations from both cameras during the experiments.

We examined the behavior of small (50-80 mm) and large (200-250 mm) kokanee in response to underwater sound at 100, 800, and 5,600 Hz (Table 13). The sound pressure level at 100 Hz was 140 dB whereas sound pressure levels at 800 and 5,600 Hz were 165 dB. Acoustic Research Detachment personnel generated the acoustic signals using the same equipment and protocols as the monthly test cycles (see Acoustic Methods). The ISMS sound source was located 30.8 m below the camera cage.

Fish were maintained in holding tanks on the ARD Kamloops barge during testing and returned to holding cages in the lake at the end of each day. To begin an experiment, we added either 50 small or 10 large kokanee to the camera cage and monitored their behavior. Based on observed swimming activity, small and large kokanee acclimated quickly to the camera cage (within 10 minutes). After a 15-minute acclimation period, we began recording kokanee behavior on video tape. We recorded behavior for 5 minutes with the sound "off". At the end of the 5-minute control (sound "off") we ensonified kokanee using a 20 ms pulse and a repetition rate of 6 s for a period of 5 minutes. We repeated this sound "off", sound "on" cycle three times allowing 10 minutes between cycles. We removed kokanee from the cage at the end of the third cycle and restocked the cage with a new group of the same size kokanee (i.e. either 50 small or 10 large kokanee). We repeated the same sound "off", sound "on" cycles again using the new group of kokanee (Figure 57).

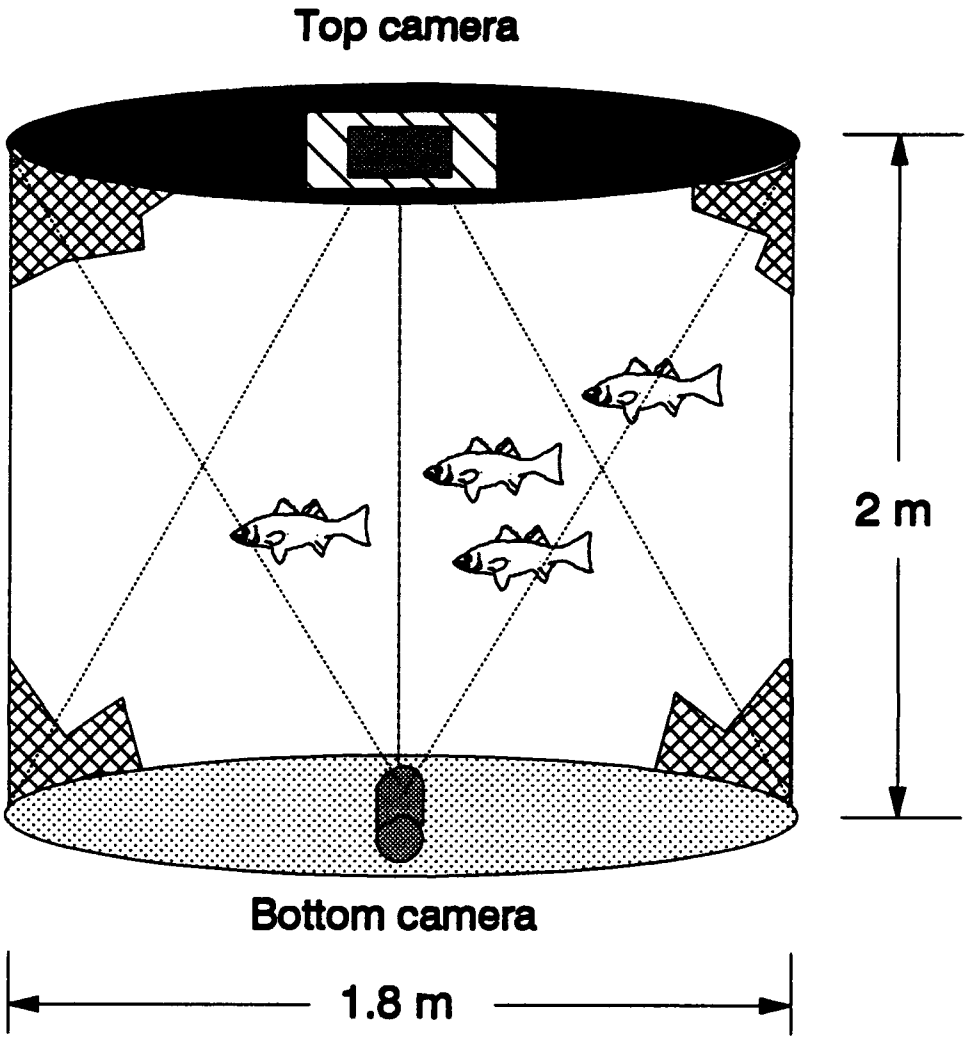


Figure 56. Camera cage used in behavior experiments. Cameras were mounted perpendicular to each other in order to view the entire cage.

Table 13. Frequencies and sound pressure levels used to ensonify kokanee during behavior experiments. Large kokanee ranged in total length from 200-250 mm and small kokanee ranged in total length from 50-80 mm.

Frequency (Hz)	SPL (dB)	n	Kokanee size
100	140	20	large
100	140	100	small
800	165	20	large
800	165	100	small
5,600	165	20	large
5,600	165	100	small

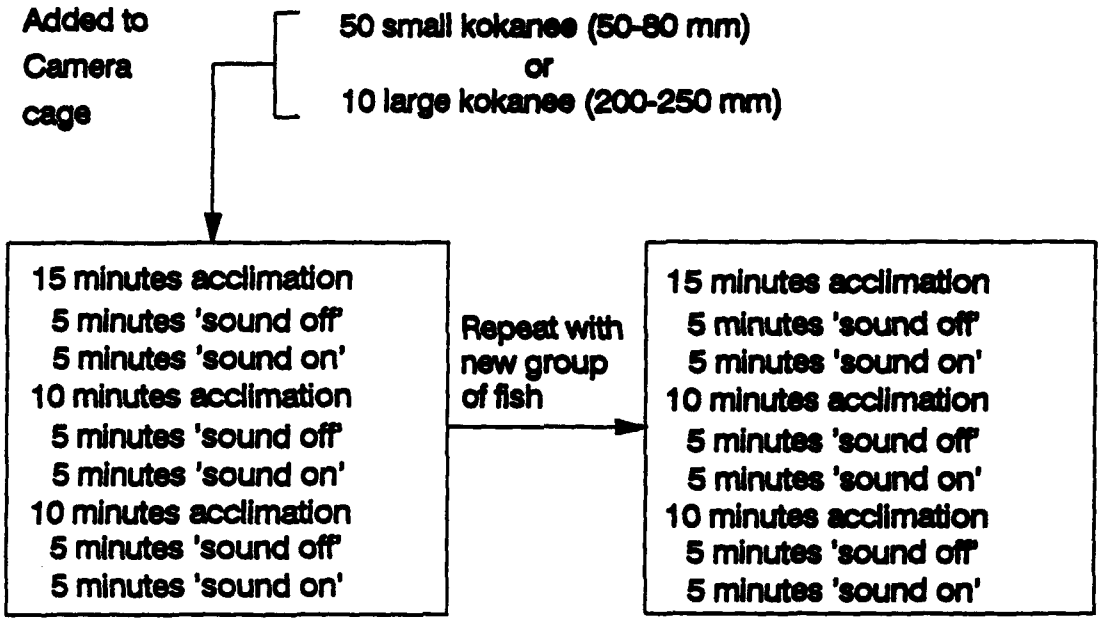


Figure 57. Flow diagram outlining control and test periods for behavior experiments. 'Sound off' represents controls and 'sound on' represents test periods.

Response variables analyzed from the video tapes included startle response, attraction/avoidance reactions, schooling behavior, and increased/decreased activity. Startle response was quantified as an "all or nothing" response initiated during the first few seconds of ensonification. We categorized startle response as a binomial variable and indicated our observations as YES or NO. Attraction/avoidance reactions were quantified by counting the number of fish in a camera's field of view. We assumed attraction if fish swam toward the bottom camera (i.e. toward sound source) and thereby decreased the number in the field of view from the bottom camera. Conversely, we assumed fish exhibited avoidance reactions (away from the sound source) by swimming toward the top camera thereby decreasing the number in the field of view from the top camera. We paused the video tape at 10 s intervals during the first minute and 30 s intervals thereafter for the remaining 4 minutes and counted fish in the field of view. Schooling behavior was quantified by pausing the video (same intervals as above) and counting the number of fish polarized. We divided the number of fish polarized by the number of fish in the field of view and multiplied by 100 to obtain a value of percent fish polarized. Activity was quantified at the same time intervals by randomly selecting a fish and counting the number of tail-flips until the fish disappeared from the screen. Tail-flip counts (tail flips/s) were restricted to large kokanee. Tail flips of small kokanee were extremely quick and could not be counted accurately. We measured activity of small and large kokanee by counting the number of times fish crossed a visible bisect. The bisect was a piece of 2.54 cm diameter PVC used to mount the camera equipment and was clearly visible in the opposite camera's field of view.

We pooled fish group data and compared response variables between controls (sound "off") and tests (sound "on"). Camera data for attraction/avoidance reactions (no. in field of view) and swimming activity (no. crossed bisect) were analyzed separately. Raw data were transformed before analysis using a square-root

transformation (Krebs 1989) and analyzed using repeated-measures analysis of variance ($P=0.05$).

RESULTS

Small Kokanee

We did not observe a startle response from small kokanee (50-80 mm) at any of the frequencies tested. Small kokanee showed no visible awareness to the first few "pings" of sound and were never startled as a result of ensonification. The number of fish in the field of view (test vs. control) was not significantly different at any of the frequencies or sound pressure levels examined (Table 14). We analyzed data by camera since the number of fish in the field of view for one camera was inversely related to the number of fish in the field of view for the other camera. Fish were often observed close to either end of the net and rarely occupied the center of the cage for longer than 45 s. Small kokanee showed no tendency to be attracted to or avoid the sound when ensonified at frequencies of 100, 800, and 5,600 Hz and sound pressure levels of 140 (100 Hz) and 165 dB (800 and 5,600 Hz).

Schooling behavior was well defined in observations of large kokanee. In contrast, small kokanee did not exhibit well-defined schooling behavior although they did tend to move together as a group within the cage. We did not observe any polarization of small kokanee throughout the experiments and consequently could not quantify a schooling response for age-0 kokanee.

Small kokanee did not exhibit significant differences ($P>0.05$) in the number of times crossed bisect indicating that swimming activity neither increased nor decreased during periods of ensonification (Table 14).

Table 14. Statistical summary of behavioral responses for small kokanee. Treatment x time interactions (control vs. test) considered significant at $P < 0.05$.

Frequency (Hz)	SPL (dB)	Camera[a]	(No. in field of view) P>F	(No. crossed bisect) P>F
100	140	top	0.10	0.89
		bottom	0.83	0.95
800	165	top	0.84	0.87
		bottom	0.69	0.32
5,600	165	top	---	---
		bottom	0.34	0.88

[a]Camera data for top at 5,600 Hz were not included in the analysis. The camera was panned toward the side of the cage during much of the experiment documenting behavior of individual fish.

Large Kokanee

We did not observe a startle response from large kokanee (200-250 mm) at any of the frequencies examined. Large kokanee, like small kokanee, showed no characteristic startle response during any of the experiments. The number of fish in the field of view was not significantly different at any of the frequencies or sound pressure levels examined (Table 15). Large kokanee swam freely throughout the cage during periods of both ensonification and controls. Large kokanee showed no tendency to be attracted to or avoid the sound during ensonification periods at frequencies of 100, 800, and 5,600 Hz and sound pressure levels of 140 (100 Hz) and 165 dB (800 and 5,600 Hz).

Schooling behavior during ensonification and controls was not significantly different ($P>0.05$) at 800 or 5,600 Hz (Table 16). Differences in schooling behavior were observed at 100 Hz for large kokanee ($P=0.01$). More fish schooled during ensonification than during periods when the sound was off, particularly during the first 60 s (Figure 58). Kokanee responded to the sound stimulus by grouping more tightly and remaining schooled for a longer period of time.

We did not observe significant differences ($P>0.05$) in swimming speed (tail-flips) for large kokanee ensonified at 100, 800, and 5,600 Hz (Table 16). Additionally, we found no significant differences ($P>0.05$) between the number of times kokanee crossed a bisect at any of the frequencies or sound pressure levels tested (Table 15). Ensonification did not increase or decrease swimming activity of large kokanee compared to the controls.

DISCUSSION

We found no evidence that behavior of small kokanee was affected by underwater sound at frequencies of 100, 800 or 5,600 Hz and sound pressure levels of 140 and 165 dB. We frequently observed small kokanee feeding during periods of

Table 15. Statistical summary of attraction/avoidance reactions (no. in field of view) and activity responses (no. crossed bisect) for large kokanee. Treatment x time interactions (control vs. test) considered significant at $P < 0.05$.

Frequency (Hz)	SPL (dB)	Camera	(No. in field of view) P>F	(No. crossed bisect) P>F
100	140	top	0.37	0.77
		bottom	0.74	0.14
800	165	top	0.13	0.18
		bottom	0.50	0.10
5,600	165	top	0.64	0.35
		bottom	0.20	0.21

Table 16. Statistical summary of schooling behavior (% polarized) and swimming speed (no. tail flips/s) for large kokanee. Treatment x time interactions considered significant at $P < 0.05$. Asterisk indicates significant difference.

Frequency (Hz)	SPL (dB)	(% polarized) P>F	(No. tail flips/s) P>F
100	140	0.01*	0.53
800	165	0.12	0.55
5,600	165	0.61	0.10

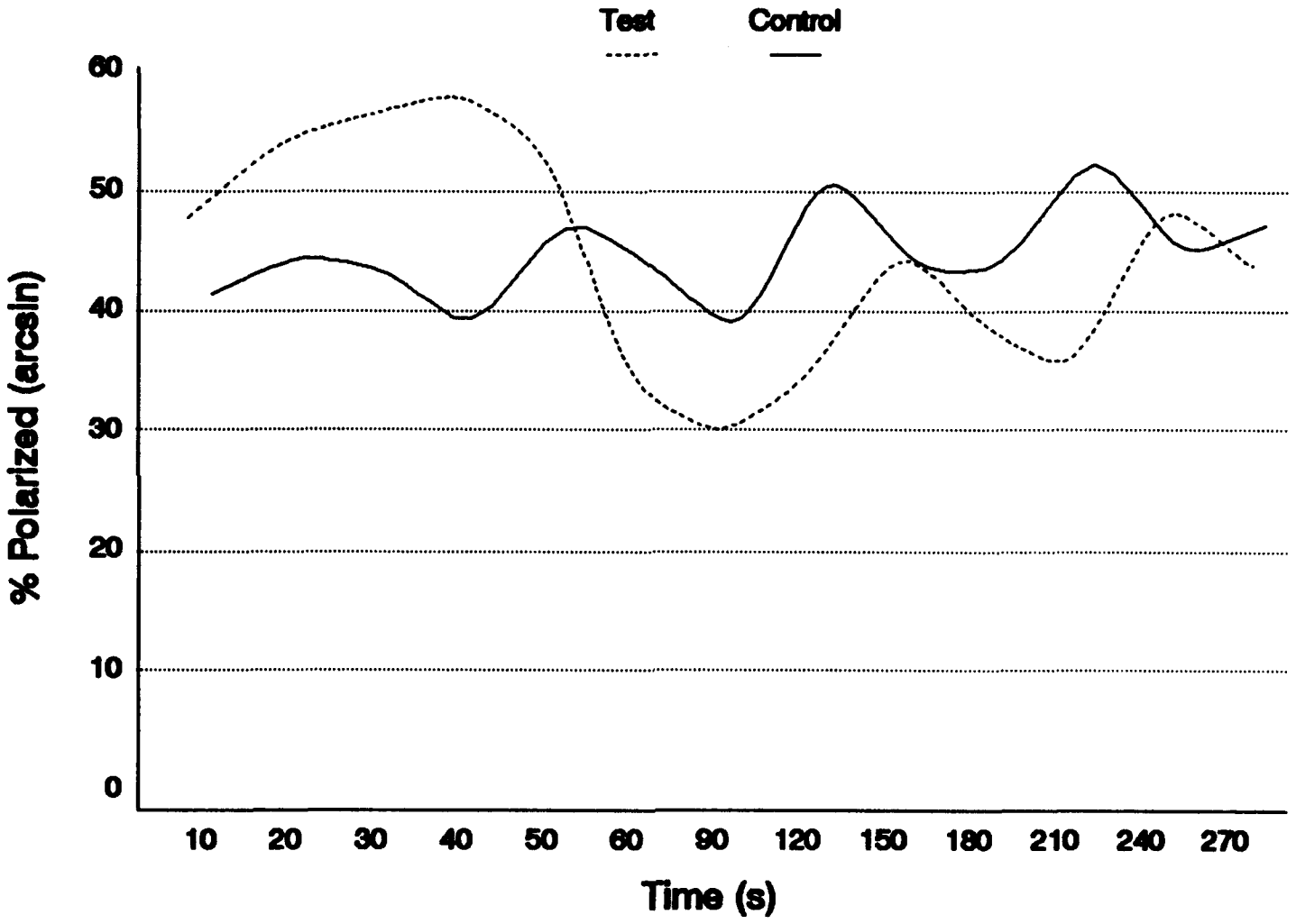


Figure 58. Schooling response of adult kokanee at 100 Hz. Treatment x time interaction significant at P=0.01.

ensonification and controls. Small kokanee did not exhibit schooling behavior prior to and during tests and were not startled by the underwater sound.

Large kokanee did not exhibit startle responses, attraction/avoidance reactions or changes in swimming activity as a result of ensonification. However, large kokanee did exhibit changes in schooling behavior at 100 Hz. Kokanee responded to the sound stimulus by remaining schooled for a longer period of time. A sound stimulus at 100 Hz may elicit a "safety" response from large kokanee, causing fish to school more tightly; a response similar to when kokanee and other schooling species encounter predators. In a study of cutthroat trout, Stober (1969) observed low intensity "thump" sounds produced by caudal fin movements, in the frequency range of 100-150 Hz. Additionally, Stober (1969) noticed that initiation of this sound by one fish caused other fish to dart off and suggested that this sound may serve as a warning signal. Our observations indicated that schooling behavior differed during the first minute and became similar to the controls for the remainder of the test period. Assuming that kokanee respond to a new sound stimulus, we might expect acclimation. Chapman (1975) suggested that fishes acclimate quickly to underwater sounds. This appears to be a plausible hypothesis since we did not observe increased schooling throughout the 5 minutes of ensonification. This hypothesis is not definitive since we do not have data on acclimation thresholds at 100 Hz.

A transient change in schooling behavior will likely not have a significant impact at the population level. In contrast to studies of Hawaiian spinner dolphins *Stenella* spp. and bottlenose dolphins *Tursiop truncatus* where it was demonstrated that they depolarize or disorient prey using high frequency echolocation (Norris and Mohl 1983; Hult 1982), we did not observe fish becoming disoriented or depolarized. Rather, our observations indicated more fish schooled and remained schooled for a longer period of time. The schooling behavior we observed at 100 Hz was not indicative of disorientation or depolarization as the case with prey ensonified by dolphins and

therefore will likely pose no threat to kokanee in the area ensonified by ISMS projectors. Additionally, we did not observe any effects on kokanee growth and predation at 100 Hz though we caution linking these results since growth and predation studies were conducted using small age-0 kokanee. Nestler et al. (1992) suggested that blueback herring may exhibit size-dependent responses to high frequency underwater sound due to anatomical development (i.e. bullae) and that large fish with large bullae may be more sensitive to underwater sound. Additionally, Loffelman et al. (1991) demonstrated that signals of 120/200/400 Hz were effective in deterring adult chinook salmon but were not as effective as higher frequencies (i.e. 500/700/900 Hz) in deterring chinook smolts. We have no data bearing on sensitives of large kokanee versus small kokanee to underwater sound at a frequency 100 Hz but do not dismiss the potential for size-dependent sensitivity.

Our data indicated that large kokanee responded to simulated ISMS underwater sound at a frequency of 100 Hz by exhibiting transient changes in schooling behavior. Additionally, kokanee may exhibit size-related behavior responses to ISMS sound. Acclimation thresholds and physiological responses at 100 Hz would provide additional information concerning effects of underwater sound on kokanee behavior.

Task 3.1

Kokanee Embryo Survival in Response to Simulated ISMS Ensonification

INTRODUCTION

Effects of underwater sound on survival and hatching success of fish embryos is not well documented. Kostyuchenko (1973) exposed four species of fish eggs to sound wave oscillations from an air gun, an electric pulse generator, or a TNT charge. All three sound sources resulted in significant effects to fish eggs but only more intense TNT charges affected fish larvae. Kostyuchenko (1973) concluded that fish eggs are more sensitive to sound impulses than fish larvae. Death and sublethal structural abnormalities resulted from the sound wave impacts.

We are aware of no studies examining effects of pulsed, high frequency underwater sound on fish embryos. Embryonic development represents a critical period in the life history of kokanee in Lake Pend Oreille. Most kokanees spawn among lake shore gravels in the southern basin of the lake during November-December and incubate during the winter and hatch in early May. Survival and hatching success of kokanee embryos can have important ramifications on kokanee recruitment and thus represents an important aspect of Lake Pend Oreille fisheries.

The objectives of this aspect of the study were to evaluate effects of simulated ISMS ensonification on kokanee embryo survival. Specific objectives of this study were to determine hatching success (% survival) for embryos exposed to a variety of sound pressure levels at frequencies of 100 Hz, 800 Hz, and 5,600 Hz. A conservative approach was taken in examining potential effects of simulated ISMS sound on kokanee embryos. In addition to placing embryos along the shoreline where they naturally occur, we also placed embryos close to the sound source where they experienced higher sound pressure levels than they would otherwise experience under natural conditions. We compared hatching success between ensonified and nonensonified embryos incubated both within the lake and in the laboratory.

METHODS

Embryo ensonification was based from the ARD Yellow barge located in Scenic Bay of Lake Pend Oreille (Figure 7). Four field sites (3 test and 1 control) and one laboratory site were used during the egg ensonification test (Table 17).

Approximately 30,000 recently fertilized kokanee embryos were obtained from Cabinet Gorge Fish Hatchery, Idaho, on December 2, 4, and 7, 1992. These embryos were obtained in three groups of about 10,000 each prior to experiments conducted on December 4, 7, and 10, respectively. Each group was maintained in the lake for 2-3 days before testing. Eggs had been obtained from kokanee collected from tributaries of Lake Pend Oreille and fertilized within a few hours of stripping. Embryos were carefully packed at the hatchery in specially designed coolers (insulated and filled with an Argentine™ solution to reduce stress) to preclude water movement. After packing, the fertilized embryos were transported to the ARD where they were placed in Whitlock-Vibert incubation boxes at densities of approximately 100 embryos/box. The embryos were acclimated and maintained in Lake Pend Oreille until they were loaded into test crates (n=20). Each test crate contained four incubation boxes.

The test crates were steel-framed plywood which was drilled to allow water circulation. They were filled with washed gravel of similar size used by spawning kokanee in Lake Pend Oreille. Five test crates were deployed at each test (sites 1, 2, and 3) and control site (site 4). At sites 1 and 2, test crates were suspended at a distance of 4.5 m from the surface of the water. Test crates at sites 3 and 4 were positioned on the shoreline in approximately 2 m of water.

Three sound pressure levels and one control (ambient background) were used as treatments. Sound pressure levels were tested at frequencies of 100, 800, and 5,600 Hz. Site 1 was closest to the sound source and at each of the frequencies tested had the highest sound pressure levels (140 dB). Site 2 had the second highest sound pressure levels (132 dB). Sound pressure levels on the northern shoreline were slightly above

Table 17. Locations of test and control sites used during embryo ensonification studies. Measured sound pressure levels are given for sites 1 and 2.

Site	Freq. (Hz)	Sound pressure level (dB) Measured	Calculated	Location
1	100	139	140	Eastern end of ARD Yellow barge
	800	139	140	
	5,600	140	140	
2	100	128	132	Western edge of ARD Yellow barge
	800	133	132	
	5,600	139	132	
3	100	--	115	Northern shore of Scenic Bay about 548 m from ARD Yellow barge
	800	--	115	
	5,600	--	115	
4	CONTROL	--	--	Northern shore of Idlewild Bay about 274 m west of Eagle boatdock; and
5	NONE	--	--	University of Idaho Wet Lab, Moscow, Idaho.

background (115 dB), while those in Idlewilde Bay were considered similar to background noise levels. Embyros were ensonified using a 20 ms pulse width and a 6 s repetition rate with a cycle of 1 h "on" and 1 h "off" for 48 hours. Ensonification at 800 Hz began at approximately 0900 h on December 4 and continued until 0900 h on December 6, 1992. Ensonification at 100 Hz began at approximately 0900 h on December 7 and continued until 1030 h on December 9, 1992. During the evening of December 7, ensonification was delayed 2.5 h due to other ARD testing. This delay extended the ensonification period by 1.5 hours. Ensonification at 5,600 Hz began at approximately 0900 h on December 10 and continued until 0900 h on December 12, 1992.

Acoustic Research Detachment personnel deployed Wilcoxon model 507B hydrophones with the test crates at sites 1 and 2 to measure the sound pressure levels experienced by the embryos (Figure 59). At site 1, one hydrophone was buried in the gravel with the Whitlock boxes and another was suspended between two of the crates. At site 2, a hydrophone was suspended between two crates of gravel.

Several pulses were measured from each model 507B hydrophone with a Hewlett Packard 3563A dynamic signal analyzer, for each ensonification period. The measured sound pressure levels (dB re 1 μPa) are shown in Table 18. Deviation from calculated sound pressure levels is a result of the Lloyd Mirror Effect[2] since the hydrophones were located at a shallow depth (Urick 1967).

After each ensonification, test crates were removed from the water and the incubation boxes retrieved and placed in coolers filled with lake water. Incubation boxes were out of the water no longer than 20 s during removal of incubation boxes from test crates. New incubation boxes containing nonensonified embryos were placed into the ensonification crates for the next test.

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[2] Lloyd mirror effect refers to constructive and destructive interference based on path length differences between incident and reflected signals.

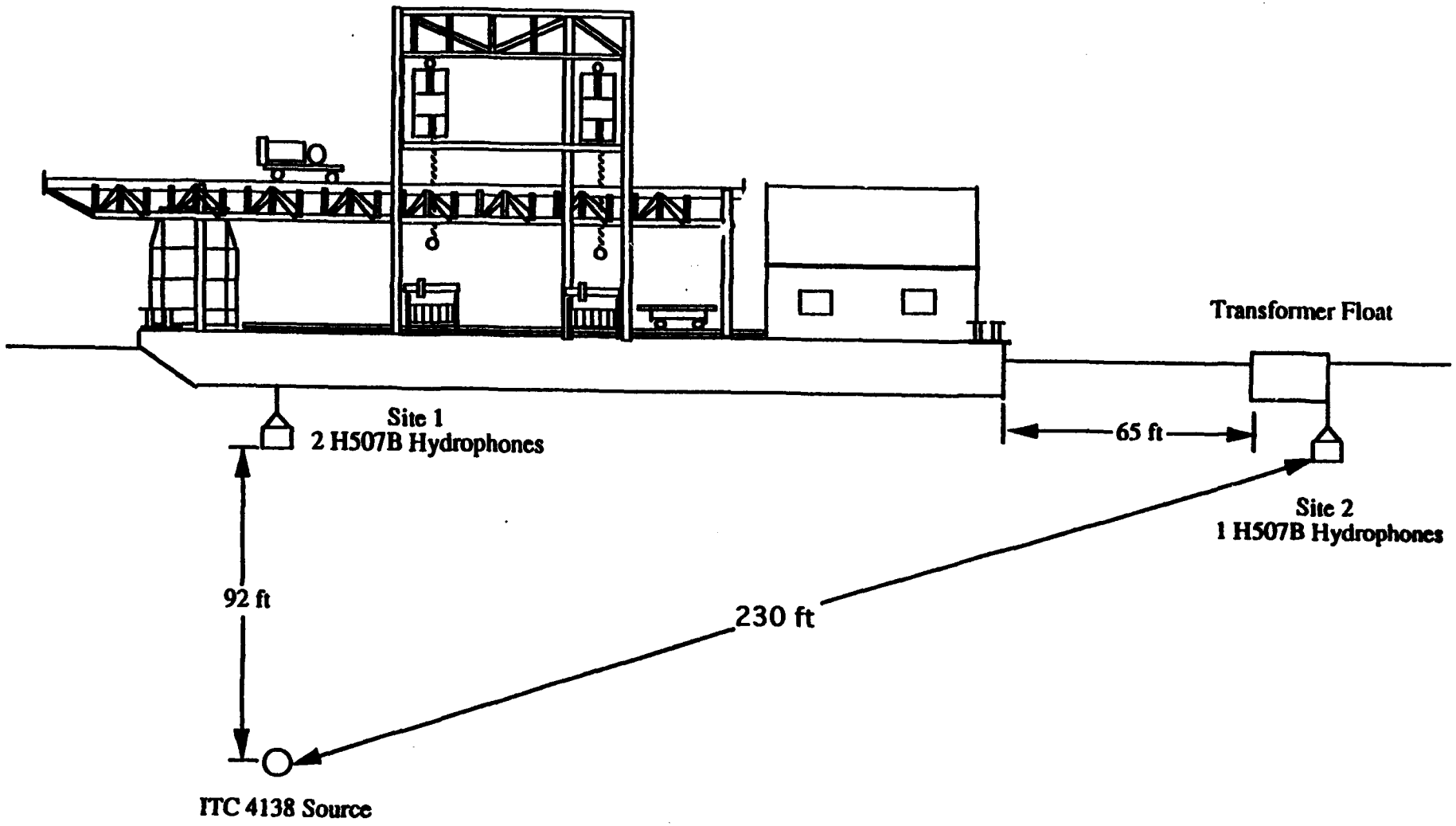

Figure 59. Acoustic source and hydrophone deployment on the Yellow barge.

Table 18. Measured and calculated sound pressure levels (±1 dB) during kokanee embryo ensonification tests. Deviation from calculated sound pressure levels is a result of the Lloyd Mirror Effect since the hydrophones were located at shallow depths.

Frequency (Hz)	Site	Measured (dB)	Calculated (dB)
100	1 (inside crate)	138	140
	1 (outside crate)	139	140
	2 (outside crate)	128	132
800	1 (inside crate)	144	140
	1 (outside crate)	139	140
	2 (outside crate)	133	132
5,600	1 (inside crate)	139	140
	1 (outside crate)	140	140
	2 (outside crate)	139	132

Ensonified incubation boxes were randomly divided into two groups; one group was carefully placed into in-lake incubation crates and positioned on the Idlewilde Bay shoreline in about 3-4 m of water. Incubation boxes were covered by approximately 1-2 cm of gravel (6-25 mm diameter) in the incubation crates. The remaining group of ensonified embryos was taken to the University of Idaho wet lab in coolers filled with lake water.

Laboratory Incubation

At the laboratory, incubation boxes were placed in a 2.5 x 0.5 m fiberglass trough and incubated at 10 ° C. Compressed air was metered into the water to assure approximately 90% oxygen saturation. Dissolved oxygen was measured daily and temperature was recorded hourly using a Ryan TempMentor™ thermograph. During laboratory incubation, the fungus *Saprolegnia* developed in the egg boxes. As a result, we treated embryos with three Formalin drip treatments of 125 ppm for 1 h over a 2 week period. Kokanee embryo survival was first quantified on February 12, 1992 and was continuously monitored through March 24, 1993. When embryo survival was first enumerated, dead embryos were removed from the incubation boxes and live embryos were placed in Heath Techna™ trays labeled according to ensonification frequency and sound pressure level (site). The embryos were incubated until hatching was complete. During Heath tray incubation, the embryos were picked regularly for mortalities to reduce fungal infection.

In-Lake Incubation

Incubation crates were removed from Idlewilde Bay in Lake Pend Oreille on May 7, 1993. Test boxes positioned at the ARD dock indicated that hatching was complete at that time. The incubation boxes were immediately removed from the incubation crates and placed in a holding trough filled with water from the lake.

Numbers of dead and live embryos, and dead and live fry were enumerated on May 7 and 8, 1993.

We summarize results of embryo survival approximately 1 month prior to hatching and at hatching for laboratory incubated embryos and at hatching for embryos incubated in the lake. Means and 95% confidence intervals for hatching success of embryos were computed. We tested the hypothesis that survival of control embryos was higher than survival of ensonified embryos (Dunnett's one-tailed T-test).

RESULTS

Laboratory Incubation

Survival to February 12 (Prehatching).- Mean survival of kokanee embryos ensonified at 100 Hz ranged from 29 to 48% (Table 19). Survival was lowest in the control (29%; ambient background) and highest (48%) at 132 dB, although variability in hatching success was similar among sound pressure levels at 100 Hz. We found no evidence that ensonified embryos exhibit reduced survival compared to the controls (Figure 60).

Mean survival of kokanee embryos ensonified at 800 Hz ranged from 34 to 42% (Table 19). Mean survival was highest (42%) at 132 dB and lowest (34%) at 115 dB. Variability was higher in the controls than for other treatments. Hatching success of embryos ensonified at 800 Hz and incubated in the laboratory averaged about 30%. Survival of control embryos was not higher than survival of ensonified embryos at 800 Hz ($P > 0.05$) (Figure 60).

Mean survival of kokanee embryos ensonified at 5,600 Hz ranged from 41 to 49% (Table 19). Mean survival was highest (49%) at 132 dB and lowest (41%) at 115 dB. Mean survival estimates exceeded 40% in all treatments. Survival of control embryos was not greater than survival of ensonified embryos at 5,600 Hz ($P > 0.05$) (Figure 60).

Table 19. Kokanee embryo survival of laboratory incubated eggs following ISMS ensonification in Lake Pend Oreille, December 1992-March 1993.

Frequency (Hz)	Sound pressure level (dB)	% survival to Feb. 12	% survival to March 24
100	140	30	13
100	132	48	43
100	115	34	26
100	control	29	19
800	140	41	33
800	132	43	36
800	115	35	30
800	control	38	29
5,600	140	44	36
5,600	132	49	45
5,600	115	41	35
5,600	control	46	40

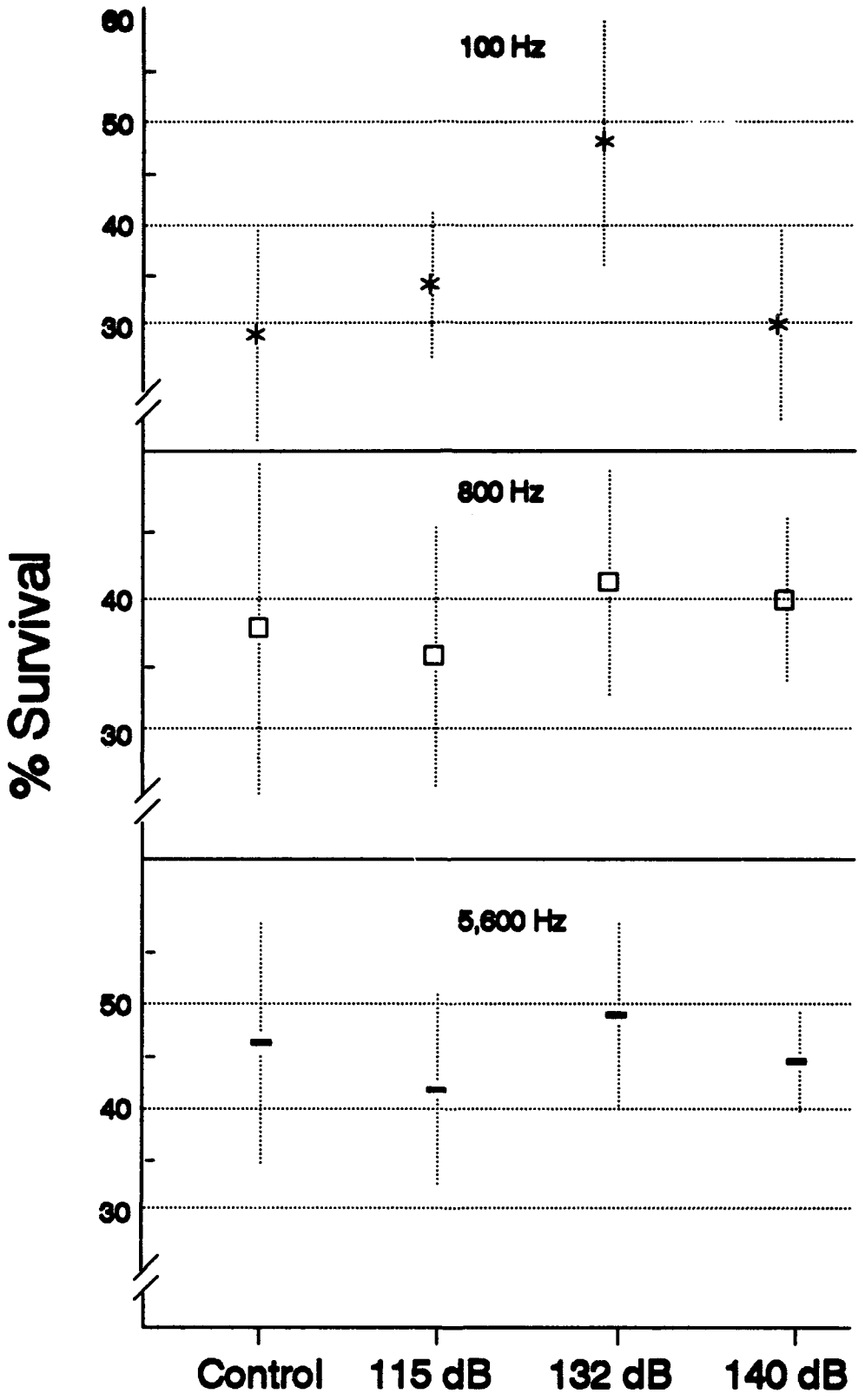

Figure 60. Survival of kokanee embryos ensonified at 100, 800 or 5,600 Hz and incubated in the laboratory December 1992-March 1993.

Survival to March 24 (Hatching).- Mean survival to hatching was highest in the 132 and 115 dB treatments at 100 Hz and lowest in the 140 dB (Table 19). Survival of eggs decreased the most from February 12 to March 24 in the 140 dB treatment (17%) followed by the control (10%).

Mean survival to hatching was highest in the 132 and 140 dB treatments at 800 Hz and lowest in the control (Table 19). Survival was similar among treatments and decreased an average of about 7% from February 12 to March 24.

Mean survival to hatching at 5,600 Hz was the highest of all frequencies tested (Table 19). Highest survival to hatching was in the 132 dB and control treatments and lowest in the 115 dB. Decreased survival from February 12 to March 24 ranged from 4 to 8%.

In-Lake Incubation

Mean survival of kokanee ensonified at 100 Hz and incubated in Lake Pend Oreille ranged from 34 to 37% (Table 20). Highest survival (37%) was at 140 and 132 dB, whereas lowest survival (34%) was observed at 115 dB. Survival of embryos ensonified at 100 Hz and a sound pressure level of 115 dB was not significantly lower than controls ($P > 0.05$) (Figure 61).

Mean survival of embryos ensonified at 800 Hz and incubated in Lake Pend Oreille ranged from 56 to 75% (Table 20). Hatching success was highest (74%) at 132 and 115 dB and lowest (56%) among the controls. We found no evidence that survival of ensonified embryos was less than the controls (Figure 61).

Mean survival of embryos ensonified at 5,600 Hz and incubated in the lake ranged from 24 to 28% (Table 20). Highest hatching success (28%) was at 132 dB followed by the control (27%). The lowest hatching success for embryos ensonified at 5,600 Hz was observed at 115 dB (24%). Survival of control embryos was not higher than survival of ensonified embryos at 5,600 Hz ($P > 0.05$) (Figure 61).

Table 20. Kokanee embryo survival of eggs incubated in Lake Pend Oreille following ISMS ensonification, December 1992-May 1993.

Frequency (Hz)	Sound pressure level (dB)	% survival
100	140	37
100	132	37
100	115	34
100	control	35
800	140	71
800	132	74
800	115	75
800	control	56
5,600	140	25
5,600	132	28
5,600	115	24
5,600	control	27

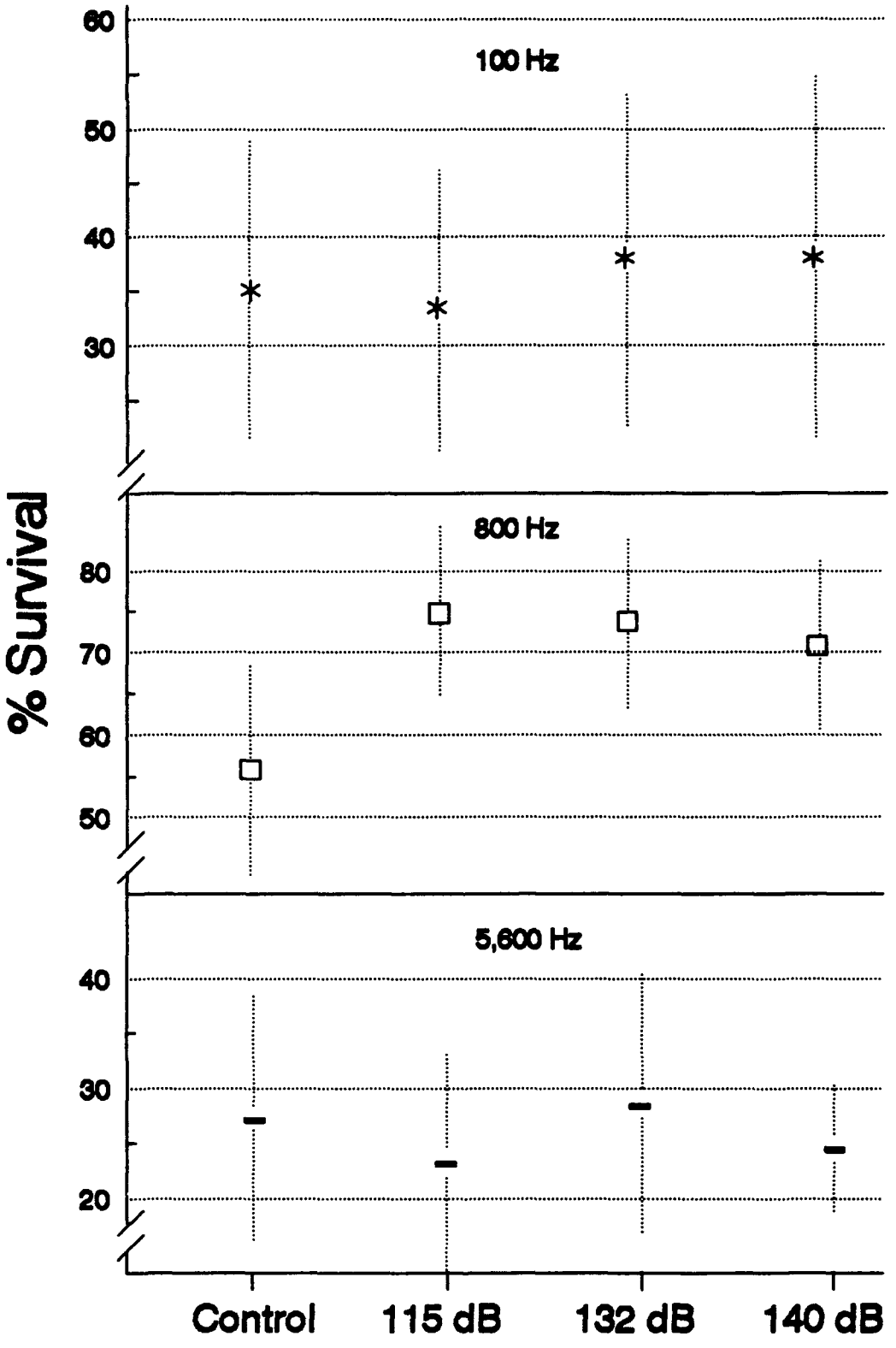

Figure 61. Survival of kokanee embryos ensonified at 100, 800 or 5,600 Hz and incubated in Lake Pend Oreille December 1992-May 1993.

DISCUSSION

Kokanee spawning and embryo incubation in Lake Pend Oreille occurs during a relatively short period in winter. For many fishes, embryo hatching success is often influenced by environmental factors whereas survival of juveniles and adults is often regulated by density-dependent factors (i.e. competition, disease, and predation). Juvenile and adult fishes are more capable of surviving or avoiding environmental influences than are eggs and larvae (Van Den Avyle 1993). Hence, density-independent environmental factors could affect fish embryos and limit fish populations, particularly in Lake Pend Oreille where the kokanee population is believed to be recruitment-limited (M. Maiolie, ID Fish and Game, Coeur d' Alene, pers. comm.).

Our observations indicated that survival of ensonified embryos was not significantly lower than survival of control embryos at frequencies of 100, 800, and 5,600 Hz and sound pressure levels ranging from 115 to 140 dB. Overall hatching success ranged from 25 to 73% and was generally lower than that reported for wild kokanee in Lake Pend Oreille (Hassemer 1984). Hassemer (1984) reported survival to the eyed-egg stage ranged from 24 to 98% and survival to pre-emergence ranged from 49 to 77%. We attribute overall low hatching success to several factors; 1.) the extreme sensitivity of recently fertilized eggs to handling 2.) harsh environmental conditions during the field experiments and 3.) *Saprolegnia* infection. Most of the handling was conducted within the first few hours following fertilization. We transported embryos immediately after fertilization to the ARD site, individually transferred them to the incubation boxes, and later transferred the incubation boxes to the ensonification crates. Following ensonification, the incubation boxes were placed into incubation crates, often under extreme environmental conditions (i.e. high wind and rough water). Movement of embryos inside the incubation boxes probably occurred as a result of crate movements and bouncing, and may have contributed to decreased overall survival.

Differences in survival of embryos incubated in the laboratory from February 12 to March 24, 1993 were relatively slight with the exception of embryos ensonified at 100 Hz. Survival decreased from 4 to 9% at 800 and 5,600 Hz, while survival decreased from 5 to 17% at 100 Hz.

Saprolegnia spp. infection was difficult to control in the laboratory and may have reduced overall survival of kokanee embryos. Additionally, we observed ensonification crates at sites 1 and 2 'bumping' on several occasions during testing due to high winds and rough water. Vibration, due to 'bumping' of ensonification crates, may have shifted gravel in the incubation boxes destroying some embryos. Positioning of the egg incubation crates was also difficult and may have contributed to relatively low survival. Incubation crates were positioned from a crane mounted to an ARD barge. Incubation crates were often positioned during high winds and rough water, making embryos susceptible to effects of gravel movement inside the incubation boxes.

Variation in incubation success between frequencies (tests) may be related to egg sensitivities between groups. For example, immediately following fertilization, embryos experience several stages of sensitivity. Depending on time after initial fertilization, some embryos may be more sensitive to handling than others. Moreover, egg quality between females can vary greatly (Craik and Harvey 1984) although rapid and reliable tests for predicting egg quality are currently lacking (Crim and Glebe 1990). This may explain differences in incubation success, for example, between embryos tested at 800 Hz and embryos tested at 5,600 Hz and incubated in the lake. We do not believe that sensitivities within lots created significant variation since embryos tested at a given frequency were all obtained at the same time and exposed to underwater sound simultaneously.

Aside from relatively low hatching success observed during the study, we found no evidence that survival of ensonified embryos was lower than control groups at frequencies of 100, 800, and 5,600 Hz and sound pressure levels ranging from 115 to 140

dB. Reduced survival from February 12 to March 24 among embryos tested during the 100 Hz experiments and incubated in the laboratory is probably related to several factors including *Saprolegnia* spp. infection, handling, or environmental conditions during ensonification. Additionally, the laboratory provided a less stable incubation environment for embryos, as the average water temperature was 8.3° C (CV = 15.7%) and ranged from 4.4 to 12.8 °C during incubation. We found no evidence that sound frequencies as low as 100 Hz and as high as 5,600 Hz at sound pressure levels up to 140 dB adversely affected incubation success of kokanee embryos.

OVERALL DISCUSSION

Simulated ISMS ensonification did not affect zooplankton densities and biomass or growth rates of age-0 kokanee. Trends in zooplankton abundance and biomass were similar among arcs during ensonification and showed no evidence that zooplankton abundance and biomass decreased as a result of ensonification. We did not observe lower growth rates among age-0 kokanee as a result of simulated ISMS ensonification. We frequently observed high growth rates among kokanee reared close to the sound source (i.e. highest sound pressure levels). Variation in kokanee growth rates during September and October (800 Hz) appears to be related to increased photoperiod and localized zooplankton abundance. Average growth rates of age-0 kokanee ensonified at 100, 800, and 5,600 Hz were slightly higher than growth rates reported for age-0 kokanee rearing in Lake Pend Oreille (see Rieman 1980). Higher growth of pen-reared versus wild kokanee was also observed in a study conducted in Kootenay Lake, British Columbia (Johnston 1990). Additionally, kokanee growth was consistent with observations of zooplankton abundance in the lake throughout the study period.

Predator/prey studies indicated that ensonified prey (kokanee and cutthroat) were not affected by simulated ISMS ensonification. We did not observe significant increases in predation among prey ensonified at frequencies of 100, 800 and 5,600 Hz and sound pressure levels of 140 dB-167 dB.

Predators ensonified at 100 and 5,600 Hz did not exhibit reduced consumption compared to the controls. We observed lower consumption among northern squawfish ensonified at 800 Hz. Differences were statistically different at $P=0.058$ (a conservative probability level for this type of data) indicating a potential for reduced consumption among northern squawfish ensonified at 800 Hz and a sound pressure level of 165 dB. This series of tests was replicated 22 times and consumption was ostensibly reduced indicating that predation on cutthroat trout by northern squawfish may be affected at this frequency and sound pressure level. Assuming these differences are real, we

speculate that impacts to squawfish populations in Lake Pend Oreille will be negligible. Our observations were made using conservative experiments (i.e. highest ISMS sound pressure levels in the upper 45 m of the lake) and any effects at these levels would likely not have significant impacts since northern squawfish are littoral predators and would probably not be exposed to a sound pressure level of 165 dB at a frequency of 800 Hz.

Unlike salmonids, cyprinids (i.e. northern squawfish) are presumably more sensitive to underwater sound and any effect of underwater sound on these species could be expected to be attenuated, to some degree, in salmonids (R.R. Fay, Loyola University, Chicago, IL, pers. comm.). However, a frequency of 800 Hz is within the hearing range of many fishes (Hawkins 1981). Stober (1969) observed that adult cutthroat trout produced "thump" sounds in the range of 100-150 Hz and "squawk" sounds in the range of 600-850 Hz. In light of the limited amount of available data, we cannot dismiss the possibility that northern squawfish may use underwater sounds as cues to increase their abilities to capture prey. Broadband noise with a principal frequency of 800 Hz and an intensity of 165-167 dB may effectively interfere or disrupt a northern squawfish's ability to detect or locate fish such as cutthroat trout that are capable of producing sounds in this frequency range. Additional research related to hearing thresholds and sensitivities of northern squawfish would permit testing this hypothesis.

Kokanee behavior studies indicated that adult kokanee exhibited increased schooling behavior at a frequency of 100 Hz. This frequency is within the hearing range reported for salmonid fishes (VanDerwalker 1967; Hawkins and Johnstone 1978). We observed increased schooling activity during the first minute of ensonification at 100 Hz. Kokanee acclimated to the stimulus after 60 s and exhibited schooling behavior similar to the controls for the remainder of the ensonification test. We did not observe kokanee becoming depolarized or disoriented. The biological impact of this transient

schooling response at 100 Hz would likely have no significant effects at the population level in the area ensonified by ISMS projectors at 140 dB.

We found no evidence that embryo survival was reduced as a result of ISMS ensonification. Despite exposure to sound pressure levels higher than they would experience under natural conditions, survival of ensonified kokanee embryos was not reduced compared to the controls.

The general lack of adverse effects associated with the simulated ISMS leads us to conclude that the frequencies and sound pressure levels tested will not adversely affect zooplankton abundance, kokanee feeding and growth, and embryo survival in Lake Pend Oreille. Predation by northern squawfish on cutthroat trout may be affected at 800 Hz and transient changes in schooling behavior by large kokanee may occur in the localized area ensonified by ISMS projectors at a frequency of 100 Hz and sound pressure level of 140 dB. Overall effects at the population level will probably not be measurable.

SUMMARY

Task 1.1 Zooplankton Abundance

We found no evidence that zooplankton densities or biomass were reduced or enhanced as a result of simulated ISMS ensonification. Natural variability in zooplankton abundance was observed during the experiments, but was similar among arcs during the study. Peaks in zooplankton abundance were generally similar for captive and pelagic zooplankton during the test cycles and consistent with results from other investigations in Lake Pend Oreille.

Task 1.2 Kokanee Feeding and Growth

We found no evidence that growth of age-0 kokanee was significantly reduced or enhanced as a result of simulated ISMS ensonification. We frequently observed higher growth rates among kokanee reared close to the sound source. Environmental factors such as food availability and increased photoperiod appear to be related to observed variation in kokanee growth during 800 Hz experiments conducted in September and October 1993.

Task 1.3 Predator/prey Interactions

We found no evidence that ensonified prey were more or less susceptible to predation as a result of simulated ISMS ensonification. Predators ensonified at 100 and 5,600 Hz consumed similar number of prey as controls, however, we observed a pattern of reduced consumption of cutthroat trout prey by northern squawfish ensonified at 800 Hz. In light of a limited amount of available data, we do not dismiss the possibility that northern squawfish use underwater sound as a cue to detect prey fishes and that ensonification at 800 Hz may interfere with predation by northern squawfish on cutthroat trout prey. Our observations were made using conservative experiments (i.e. highest ISMS sound pressure levels in the upper 45 m of the lake) and any real effects

of ensonification on northern squawfish are not anticipated to have significant impacts since northern squawfish are littoral predators and would probably not be exposed to sound pressure levels of 165 dB at a frequency of 800 Hz.

Task 2.1 Kokanee Behavior

We found no evidence that behavior of small kokanee was affected by simulated ISMS ensonification. We did observe a significant change in schooling behavior among large kokanee ensonified at a frequency of 100 Hz and sound pressure level of 140 dB. We do not expect these changes, which were transient and occurred during the first 60 s of ensonification, to result in a significant impact to the kokanee population in Lake Pend Oreille. Additional data on physiological responses and acclimation thresholds at 100 Hz (140 dB) would provide more insight into effects of ensonification on large kokanee.

Task 3.1 Embryo Survival

We did not observe reduced survival of kokanee embryos as a result of simulated ISMS ensonification. Despite exposure to sound pressure levels higher than they would experience under natural conditions, survival of ensonified kokanee embryos was not reduced compared to the controls. Low embryo survival observed during the study is attributed to extreme sensitivity of green eggs to handling and environmental conditions experienced during simulated ISMS testing. Variability between groups may be related to egg sensitivity. Additionally, transportation to the University Laboratory may have reduced survival of laboratory incubated embryos.

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