Report on Hydroacoustics, Bioacoustics, and Noise Thresholds for Fish "Best Available Science"

by

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Summary

A. Effects of Pile Driving Noise on Fish

The purpose of this report is to describe what is known about the effects of human-generated sound on fish and to identify studies needed to address areas of uncertainty relative to measurement of sound and the response of fishes.

A limited number of studies over the past decade provide some data on the effects of intense sounds on fishes. Results indicate that some sounds, under some circumstances, will cause a change in the hearing capabilities of the fish species tested and/or actually damage the sensory structures of the inner ear. There is also a very small body of evidence that these sounds have the potential for affecting other aspects of the physiology of fishes, and that these effects may range from the macro (destruction of the swim bladder) to the cellular and molecular.

Data from blast studies, while not readily comparable to pile driving, lead to the suggestion that very high level concussive impacts can cause structural damage to fishes. Just as in investigations using sound, however, the number of species studied is very limited, and there has been no investigation as to whether blasts that do not kill fish have any impact on short or long-term hearing loss.

Earlier studies of the effects of sound or explosive blasts on fish can provide a very preliminary indication of the potential impact of pile driving on fishes. However, there are no peer-reviewed studies on the effects of pile driving on fish hearing or on non-sensory structures. While we are able to use available data as a very preliminary indication of the kinds of effects that might be encountered as a result of pile driving, only well-controlled studies[1] of behavioral and physiological responses to pile driving or to signals specifically designed to have the same characteristics as pile driving sounds, will provide clear scientific support of any criteria.

B. Areas of Uncertainty and Studies Needed

At this stage, it is fair to say that there is substantial uncertainty with regard to the effects of pile driving on fishes and other aquatic organisms. The few data available are not peer-reviewed and often lack suitable controls. It is also very difficult to extrapolate to pile driving from studies using other signals because such signals are not analyzed or described in a format that can be interpreted in terms of a pile-driving signal (e.g., energy flux over time). Moreover, signals used in other studies often differ markedly from that emitted by pile driving in terms of duration, and in rise and decay times. Thus, specific signal components that affect the fish may be very different in, for example, a study with continuous noise than in one that uses blasts or pile driving.

[1] Controlled studies must include a double-blind paradigm where the individual(s) doing the analysis of results is (are) not aware of the nature of the stimulus given to the fish. It is only by using this method, which is widely used in large-scale and complex studies such as those required in the analysis of effects of pile driving, that one can be fully confident of results obtained. In this document, whenever we refer to "controlled" studies, it should be assumed that the studies would, as appropriate, be done "double-blind."

It is concluded that it is imperative to initiate studies that start with very basic questions on the effects of pile driving. Even before such studies get underway, however, it is critical that there be a common description of the acoustic signal being generated by the pile driving, and that such descriptions be used in all future studies. Table 1, below, gives an overview of the types of studies that need to be accomplished in order to better understand the issues of pile driving and the biological effects caused by such signals. Note that this table is presented in much greater detail in section V of this report (Table 5, Page 32).

Table 1: Outline of studies to investigate pile driving and its effects on fishes. (Also see "draft" Figure 10, page 49)
Characteristics of pile driving
Define dose/response level for pile driving sounds - Develop ways to express exposure to pile driving sounds in terms of cumulative energy over time and to define the acoustic particle velocity within the sound field.
Structural acoustic analysis of piles – Develop structural acoustics models of piles to investigate how modifications to piles could alter the sounds and potentially incur less damage to animals The acoustic analysis could also indicate how best to describe the waveform and the function of material, pile size, and environmental factors like water temp, depth, substrate. Such studies could lead to a better ability to develop attenuation of sounds produced during pile driving by modifying structural material, attenuation technologies, etc. These studies should link to the study described below to help investigators better understand wave propagation and zone-of-effect predictions to facilitate development of attenuation technology.
Characteristics of underwater sound field - Develop an underwater sound propagation model and integrate with pile structural acoustics models to estimate received levels of sound pressure and particle velocity in the vicinity of pile driving operations and define zones of impact on fishes. Verify with field measurements of underwater sound pressure measurements.
Effects on fishes
Hearing capabilities of Pacific coast fishes - Determine hearing capabilities (using ABR) of representative species[2]
Mortality of fishes exposed to pile driving - Determine short and long term effects on mortality of representative species as a result of pile driving. Measure pathology (using accepted necropsy studies) of the effects of sounds on fishes at different levels of exposure.
Effects of pile driving on non-auditory tissues - Using precisely same paradigm as used to study effects on the ear, examine other tissues using standard fish necropsy techniques to assess gross, cellular, and molecular damage to fish. Furthermore, determine stress effects on fish using appropriate stress measures (e.g., hormone levels). Do for representative species.
Effects of pile driving on hearing capabilities - Determine permanent hearing loss (PTS) and temporary hearing loss (TTS) on representative species. [TABLE CONTINUED NEXT PAGE]

[2] All studies involve what are called in this report "representative species." Representative species are defined as those that serve as models for fishes in the region of question – in this case, the Pacific coast. Species for study need to be selected to represent differences in: (a) habitat; (b) presumed hearing capabilities; (c) ear structure and connections of the ear to peripheral structures such as an air bubble; (d) bony fish vs. non-bony fish (including elasmobranches); and (e) other comparable factors. A minimum set of fishes should be defined so as to have the fewest possible studies and yet represent as many of the parameters for the fishes of the area of question as possible.

Table 1: Outline of studies to investigate pile driving and its effects on fishes. (Also see “draft” Figure 10, page 49)
Effects of pile driving on fish eggs and larvae - Determine mortality, growth rates, and pathological changes in developing fishes of representative species with exposure at different times during the development cycle
Behavioral responses of fish to pile driving - Observe, in large scale cages, the behavioral responses of representative species to pile driving sounds. Do fish attempt to swim from the source? Do they react to the sounds? Do they “freeze” in place?
Effects of pile driving on the ear and lateral line - Determine morphological changes over time for representative species on sensory cells of the ear and lateral line, and whether such changes are reversible
Effects of multiple pile driving exposures on fish - For the appropriate experiments cited above, determine effects of multiple exposures, over time, of pile driving

It is important to note, as discussed in detail in Section V, that the body of scientific and commercial data available is inadequate for the purpose of developing more than the most preliminary scientifically supportable criteria for pile driving noise that will protect fish.. As a consequence, such criteria are not proposed in this report. The information from earlier blasting and pure tone studies may be used to develop interim criteria for addressing injury and mortality, recognizing the need for well-controlled studies to provide clear direction for development of scientifically supported criteria. It is critical to note, however, that the interim criteria developed must be used with the utmost caution, and that they should not be used for any other signal than pile driving. In essence, the interim criteria developed for pile driving are *only* applicable to that source and not for other sources such as air guns or sonars.

C. Terminology

There are a wide range of acoustic and biological terms used in this report. To facilitate understanding of terminology, the terms are defined in a Glossary that appears at the end of the report (Page 40).

I. Introduction

A. Purpose

Over the past decade it has become increasingly apparent that human-generated (anthropogenic) sound has the potential to impact the health and well-being of animals as well as humans. There has been, in this same time frame, an increasing awareness of the presence of human-generated sounds in the aquatic environment, and concern has arisen that these sounds could impact aquatic mammals, fishes, amphibians, reptiles, and perhaps even invertebrates.

Despite the concerns raised by increased human-generated sound in the aquatic environment, very little is known about the effects of such sounds on marine mammals, and far less is known about the effects on fishes (see NRC 2000 2003; Popper 2003; Popper et al. 2004). And, even in the very few cases where data are available for fishes, they are so few that it is impossible to extrapolate between species, even for identical stimuli. Moreover, it is also impossible to extrapolate results between stimuli because the characteristics of the sources (e.g., seismic air gun, SONAR, ship noise, pile driving) are very different.

The purpose of this report is to describe what is known about the effects of human-generated sound on fish and to identify needed studies to address areas of uncertainty relative to measurement of sound and the response of fishes. The focus is on questions dealing with the effects of pile driving on fishes of the Pacific Coast region, including fish in bay, estuarine, lake, river, and stream habitats. Pile driving commonly occurs in water and is related to construction and repair of bridges, docks, and other infrastructure.

To date, there are exceedingly few data for fish on the effects of sound generated by pile driving. Furthermore, based on current knowledge of the effects of noise in producing acoustic traumas, there is little that can be definitively concluded with regard to the effects of pile driving on fishes. Of the data in the literature on noise effects, none have used sounds that even approximate those of pile driving. Thus, this report does not directly use results from experiments on pile driving.

This report describes the potential for effects on fish that is supported or inferred from available information and sets the stage for future studies by outlining what is known about detection of acoustic signals by fishes, sound detection by Pacific coast fishes, effects of human-generated sounds on other species of fishes, and characteristics of the sounds produced by pile driving. Far too little is known about the effects of intense sounds on fishes for definitive conclusions to be drawn from the literature. A series of well-defined research programs, with suitable and appropriate experimental design and experimental controls, would help garner needed information (see Tables 1 [page 4] and 5 [page 32]).

The material presented here, and the basis for the conclusions are, wherever possible, based upon peer-reviewed scientific literature. At the same time, there are instances when there has been little or no peer-reviewed work on topics that are important to this analysis, and so we have, with caution, used "gray" reports that have not necessarily been subject to the same kind of rigorous scientific peer-review that is the basis for scientific journals. We have, in addition, avoided use of material that is presented only as pages on the World Wide Web (WWW) because we have no basis for knowing if that material has received any review whatsoever.

In addition to primary scientific literature, we also include citations to a number of reviews and overviews of various aspects of the material presented here. In each case, the reviews have gone through appropriate peer-review. At the same time, it must be recognized that the reviews are often the opinions of the authors and may be based upon analysis of material that is peer-reviewed and/or from the gray literature.

II. Biology of Fishes

A. Fishes of the Pacific Coast and River Systems

The fishes of the Pacific Coast region that are potentially impacted by pile driving in estuaries, bays, lakes, streams and rivers are listed in Table 2.[3] There is a wide diversity of species that include both cartilaginous fishes (sharks and rays – class Chondrichthyes), and bony fishes (class Osteichthyes). Among the bony fishes are more advanced teleosts (ray-finned fishes such as salmon, tuna, perch, and most commercially important species), as well as representatives of more primitive chondrostean fishes, including sturgeons. The vast majority of fish species on the Pacific Coast (as throughout the world's oceans and fresh water systems) are teleosts.[4]

Table 2: Target Fish Species for Bioacoustics Criteria in California Estuaries, Bays, and Rivers

Species	Estuarine Life Stages	Riverine/fresh water Life Stages
	(A-adult, E-egg, L-larvae, J-Juvenile)	
Priority 1: ESA Listed Species		
Chinook Salmon *Oncorhynchus tshawytscha*	A, J	A, E, L, J
Coho Salmon *Oncorhynchus kisutch*	A, J	A, E, L, J
Steelhead *Oncorhynchus mykiss*	A, J	A, E, L, J
Delta Smelt *Hypomesus transpacificus*	A, J	A, E, L, J
Tidewater goby *Eucyclogobius newberryi*	A, E, L, J	A
Priority 2: EFH Species		
Leopard Shark	A, J	
Soupfin Shark	A, J	
Spiny Dogfish	A, J	
California Skate	A, J, E	
Ratfish	A, J, E	
Lingcod	A, J, E, L	
Cabezon	A, J, E, L	[CONTINUED NEXT PAGE]

[3] Data provided by Warren Shaul of Jones and Stokes.
[4] Indeed, teleost fishes make up approximately 23,000 of about 27,000 extant fish species (Helfman et al. 1997). It is worth noting that the number of living species of fish far exceeds the number of living species of all other vertebrate groups *combined*.

Species	Estuarine Life Stages	Riverine/fresh water Life Stages
	(A-adult, E-egg, L-larvae, J-Juvenile)	
Kelp Greenling	A, J, E, L	
Pacific Cod	A, J, E, L	
Pacific Whiting (Hake)	A, J, E, L	
Sablefish	J	
Black Rockfish	A, J	
Bocaccio	J, L	
Brown Rockfish	A, J, E, L	
Calico Rockfish	A, J	
California Scorpionfish	J, L	
Copper Rockfish	A, J, E, L	
Kelp Rockfish	J	
Quillback Rockfish	A, J, E, L	
English Sole	A, J, E, L	
Pacific Sanddab	J, E, L	
Rex Sole	A	
Starry Flounder	A, J, E, L	
Northern Anchovy	A, J	
Pacific Mackerel	A, J	
Jack Mackerel	A, J	
Pacific Sardine	A, J	
Market Squid	A, J	
Priority 2: Other Commercial Species		
Pacific Herring	A, J, E, L	
Priority 3: Sensitive Native Species		
White sturgeon—native *Acipenser transmontanus*	A, J	A, J, E, L
Green sturgeon—native *Acipenser medirostris*	A, J	A, J, E, L
Longfin smelt—native *Spirinchus thaleichthys*	A, J	A, E, L
Tule perch—native *Hysterocarpus traskii*		A, J
Priority 4: Nonnative Sport-Fishery Species		
American shad—nonnative *Alosa sapidissima*	A, J	A, J, E, L
Channel catfish—nonnative *Ictalurus punctatus*		A, J, E, L
Striped bass—nonnative *Morone saxatilis*	A, J	A, J, E, L
Bluegill—nonnative *Lepomis macrochirus*		A, J, E, L
Redear sunfish—nonnative *Lepomis microlophus*		A, J, E, L
White crappie—nonnative *Pomoxis annularis*		A, J, E, L
Black crappie—nonnative *Pomoxis nigromaculatus*		A, J, E, L
Largemouth bass—nonnative *Micropterus salmoides*		A, J, E, L
Small mouth bass—nonnative *Micropterus dolomieui*		A, J, E, L

Among the fishes, several are listed as threatened or endangered under the federal Endangered Species Act. These include three species of the genus *Oncorhynchus* (Chinook salmon, coho salmon, and steelhead), delta smelt (*Hypomesus transpacificus*), and the tidewater goby (*Eucyclogobius newberryi*). The salmonids and the smelt are all in the taxonomic order Salmoniformes, while the goby is unrelated to salmonids.

B. Fish Hearing and Importance

There is a long historic record of human awareness that fishes produce and use sounds in their behavior (Moulton 1963). Fish hearing and sound production (bioacoustics), and the importance of sounds to the lives of fishes, did not really get studied, however, until the early part of the 20th century (see Moulton 1963 and Tavolga 1971 for historic reviews). The level of investigation rose considerably in the second half of the 20th century (see Popper and Fay 1999; Zelick et al. 1999; Popper et al. 2003).

It was also in the latter part of the 20th century that investigators became more acutely aware of the idea that human-generated sounds may have an effect on the lives of aquatic organisms (see reviews in NRC 1994, 2000, 2003; Richardson et al. 1995), and that the organisms affected not only include marine mammals (the subjects of greatest interest) but also fishes and other aquatic organisms. The concerns about potential effects of human-generated sounds include impacts on communication with conspecifics (members of the same species), effects on stress levels and the immune system, temporary or permanent loss of hearing, damage to body tissues, effects on survival, and mortality or damage to of eggs and larvae.

1. Sound Production and Communication

Teleost fishes produce sound in several ways, none of which involves a larynx or syrinx-like structure as used by terrestrial vertebrates. Instead, fishes use a variety of different methods to produce sounds that range from moving two bones together to more complex mechanisms involving exceptionally fast muscles connected to the swim bladder. In this latter instance, the muscles contract at frequencies high enough to produce sound (see Zelick et al. 1999). The gas-filled swim bladder (or gas bladder) in the abdominal cavity may serve as a sound amplifier (although it has other functions as well -- see Steen 1970). Sounds produced in this way usually have most of their energy below 1,000 Hz.

Fish use sounds in a wide variety of behaviors including aggression, defense, and reproduction (reviewed in Tavolga 1971; Demski et al. 1973; Zelick et al. 1999). There is also evidence that at least one species of marine catfish uses a form of "echolocation" to identify objects in its environment by producing low frequency sounds and listening to their reflections from objects (Tavolga 1976). Data in the literature suggest that it is the temporal pattern of fish sounds, rather than their frequency spectrum, that is most important for acoustic communication by fishes (Winn 1964; Spanier 1979).

2. *Hearing Capabilities of Fishes*

Fishes are able to detect and respond to a wide range of sounds. The mechanism for determining hearing capabilities of fishes is not unlike that used in humans. One set of measures involves "asking" a fish what it hears and then measuring some kind of response whenever a sound is detected. Such responses may be conditioned (trained, such as hitting a paddle when a sound is detected) or unconditioned (untrained, such as change in heart rate). Alternatively, the response of the fish can be determined by measuring electric potentials in the brain that are generated when the ear detects a sound.

In either case, the first goal of measuring hearing is to determine the range of frequencies (or bandwidth) over which a fish will respond, and then the lowest pressure level of the sound detected at each frequency (the "threshold").[5] The graphic representation of the threshold as a function of frequency is called an "audiogram." Figure 1 (Page 43) shows audiograms for fishes similar to those found in the Pacific Coast region, or that have ears with similar structures to those species.

Several aspects of fish hearing are apparent from Figure 1. The figure clearly shows that these fishes have some variability in the range of frequencies, or bandwidth, that they are able to detect, and in their thresholds. The fish with the widest bandwidth is the scaled sardine (a species that is probably representative of the sardines and anchovies on the Pacific Coast). Greatest sensitivity (lowest threshold) is found in the Atlantic cod, a relative of the Pacific cod on the Pacific Coast.

It has been generally argued that fish are divisible into two non-taxonomic groups – hearing generalists (or "non-specialists") and hearing specialists (see Popper et al. 2003 for detailed discussion). The hearing specialists have special adaptations (discussed briefly below) that enhance their hearing bandwidth and sensitivity. Examples of specialists include goldfish, catfish, some squirrelfish, and many other taxonomically diverse species. Quite often, hearing specialists will detect sounds at frequencies up to 3,000 – 4,000 Hz and have sensitivity that is 20 dB better, or greater, than the generalists.[6]

Based upon taxonomic relationships, it appears that the majority of the native fishes on the Pacific Coast are hearing generalists. The known hearing specialists include the sardine and related species of the order Clupeiformes.[7] While there are no data in the literature for a number of species on the Pacific Coast, knowledge of the auditory anatomy of a number of these species indicates that they are most likely generalists. At the same time, it must be pointed out that data exist for perhaps only 100 of the 25,000 or more extant species of fish and so any extrapolation of hearing capabilities between different species, and especially those that are taxonomically distant, must be done with the greatest caution. Thus, studies of hearing capabilities of at least a number of the species on the Pacific Coast (especially rockfish) may be of considerable value in

[5] The threshold generally represents the lowest sound pressure level an animal will detect in some statistically predetermined percent of presentations of a signal. Most often, the threshold is the sound level at which a fish will respond 50% of the time.

[6] Note, however, that not all of the thresholds for hearing generalists plotted in Figure 1 may be quantitatively valid because a number of these species probably do not respond to sound pressure (except, possibly the scaled sardine and Atlantic cod). It is likely, however, that the frequency range of best sensitivity of the generalists is reasonably accurate. Furthermore, the relatively poor sensitivity in a number of these species is probably qualitatively correct. To do more accurate measures, one would need to determine not only sound pressure, as done in the studies reported here, but also particle motion because that is what these fishes most likely are detecting.

[7] Clupeiformes include herrings, shads, menhaden, anchovies, sardines, and related species.

trying to understand whether or not the sounds generated by pile driving are within the hearing range of the species in question,[8] and whether there are other hearing specialists in the region.

As indicated above, there are no data on hearing capabilities specifically for any of the fishes in Pacific Coast estuaries and bays that are potentially of concern with regard to human-generated sound (Table 2, page 7). It is likely that the hearing generalists among this group of fishes detect sounds only to 1,000 – 1,500 Hz (with the one clear exception being the clupeids – sardines and alewives). Behavioral evidence (albeit very limited and very much in need of replication) is that the sharks and rays probably do not detect sounds at frequencies above 800 to 1000 Hz (e.g., Banner 1967; Nelson 1967; Myrberg 2001; Casper et al. 2003). No data are available in the literature for any of the rockfish, nor for hearing by Pacific Coast mackerel, although the Japanese horse mackerel (*Trachurus japonicus*) is reported to be able to detect sounds from 70 to 3,000 Hz (Chung et al. 1995).[9]

The very limited data in the literature on plaice and other related species of flatfish suggest that the Pacific Coast species are likely to have poor hearing sensitivity (high thresholds) and a relatively narrow bandwidth. For example, Chapman and Sand (1974) reported that the plaice, *Pleuronectes platessa* is able to detect sounds at frequencies up to only 200 Hz. In contrast, Zang et al. (1998) suggest that the marbled sole (*Pleuronectes yokohamae*) can detect sounds up to 1,000 Hz with best sensitivity around 300 Hz. This relatively poor hearing sensitivity is likely related to these fishes not having a swim bladder, a structure that appears to widen the bandwidth and increase sensitivity in many species.

Salmonids are one of the most important groups of fishes commercially, and yet the extent of data on their hearing is limited to the Atlantic salmon (*Salmo*).[10] Earlier data (Hawkins and Johnstone 1978) showed that this species can detect sounds to frequencies somewhat above 600 Hz, while more recent data show that it is also able to detect sounds to well below 20 Hz (Knudsen et al. 1992, 1994). It has been suggested that this infrasound response could be useful as a way of keeping fish from entering small areas such as irrigation ditches (Knudsen et al. 1994). It appears, however, that these fish only respond when they are very close to the infrasound source, most likely because very low-frequency sound will not propagate in shallow water (Rogers and Cox 1988).

One must be careful about extrapolating from Atlantic salmon to Pacific Coast salmonids. Data on the anatomy of the ear of several species (Popper 1976, 1977) suggest that

[8] Species recommended for such studies would include select species of: rockfish, sole, mackerel, salmonid, goby, and perhaps an elasmobranch. To facilitate getting data, the best approach might be to use physiological recording from the brain as opposed to the far more time-consuming behavioral studies done in the past.

[9] This work, and that of Zhang et al. on flatfish were only seen in abstract form and it was therefore not possible to determine the methods used in the study, which was written in Japanese. The hearing bandwidth of the mackerel in the Chung study is substantially wider than for any other non-specialist fish. Moreover, the bandwidth for the flounder reported by Zhang et al. (1998) is far wider than that reported for another species of the same genus by Chapman and Sand (1974). Therefore, without a careful analysis of the methods and results these data must be viewed with considerable caution.

[10] Most likely because most of the work on this group has been done in Europe where this species is commercially most important.

the auditory system is similar in all of them, but without at least some additional behavioral data this extrapolation must be done with great caution. Thus, it would be of great value to have hearing data on at least a few of the species in Pacific Coast aquatic habitats. Moreover, such data would be of particular value if it were for animals of different life stages and sizes. While there are no data to suggest that hearing changes with age, there is such a dearth of data on this topic that this becomes a totally open question.

There are no data whatsoever on mackerels or scorpion fish or related species, and it is not possible to predict their hearing capabilities even based on morphology because there are no such data in the literature. Sturgeon is also an unknown with regard to hearing capabilities.

While not as extensively studied, a variety of behavioral and physiological investigations of fish hearing show that a number of species (and perhaps all) are able to perform basically the same acoustic functions as found in other vertebrates, including mammals (see Popper et al. 2003 for review of fish hearing capabilities). Thus, fishes are able to discriminate between sounds of different levels or frequencies and, most importantly, detect a sound in the presence of other signals (noise). Fishes are also able to determine the direction of a sound source (sound source localization). Indeed, these higher level capabilities are far more important to a fish than just detection of sound (as illustrated by the threshold measures) because fishes must discriminate between sounds of predator vs. those of prey, determine the direction of a sound made by a potential predator or potential prey, and determine the nature of one sound source in the presence of others. Most importantly, fishes must detect the presence of a signal that is important to them even when there are extraneous background noises.[11] Clearly, adding to the background noise (such as noise from pile driving, although not continuous) can make the environment so loud that fish are not able to detect important signals (e.g., that of a predator) because of the strong anthropogenic masking sound.

3. Auditory Structures

The basic mechanism for transducing the mechanical signals of sound into electrical signals compatible with the nervous system is the sensory hair cell (Figure 2, Page 44). This cell is ubiquitous in the ears of all vertebrates. The same cell is also found in the lateral line, a series of detectors along the body of the fish that determines water motion relative to the fish that arise from sources within a few body lengths of the animal.

The body of the sensory hair cell is typical of most other cells; however, the hair cell also has an apical group of projections called the ciliary bundle that extends above the surface of the epithelium in which the cell lies (the sensory epithelium, or macula). Bending of the cilia causes the opening of very tiny channels in the cilia and the entry of ions from the surrounding fluid into the cell (e.g., Hudspeth and Corey 1977). Bending results in a series of very rapid chemical events that culminate in the release of chemicals called neurotransmitters from the cell body.

[11] A relevant analogy here is the well-known cocktail party effect. A person at a cocktail party is able to hear sounds of a person with whom they are talking regardless of the high level of background noise. This, as well as general detection of sounds in any noisy environment, is a function of extensive processing of signals by the auditory system.

The neurotransmitters then stimulate the neurons, which contact (innervate) the sensory cells. The neurons, in turn, send electrical signals to the brain that provide information about the sound.

Fishes, like other vertebrates, have two inner ears that lie within the cranial (brain) cavity just lateral to the brain as shown in Figure 3 (Page 44). Unlike terrestrial vertebrates, however, fishes have no middle or external ear.[12] The structure of the fish inner ear is similar to that found in all other vertebrates (Ladich and Popper 2004), and the basic mechanisms of stimulation of the hair cells in the inner ear and the conversion of acoustic energy to electrical signals compatible with the nervous system are the same in all vertebrates.

The inner ear (Figure 4, Page 45) has three semicircular canal ducts, which are small looping tubes that lie in nearly orthogonal planes to one another. These canals serve to detect angular acceleration (e.g., rotational acceleration of the head). In addition, fishes have three fluid-filled otolith organs (utricule, saccule, and lagena), each of which contains a dense calcified otolith that overlies a sensory epithelium (often referred to as the "macula") that contains numerous sensory hair cells. These otolith organs subsume two roles for fish. First, they serve as vestibular organs and measure the position of the head relative to gravity.[13] Second, they are involved in sound detection. The earliest work suggested that the primary auditory end organs in fishes were the saccule and lagena, but there is a growing body of evidence that now suggests that all three of the otolithic end organs have roles in hearing (reviewed in Popper et al. 2003).

Each otolithic end organ may have many thousands of sensory hair cells. Fishes, unlike most tetrapods other than amphibians, continue to produce sensory hair cells throughout much of their lives (Lombarte and Popper 1994, 2004; Higgs et al. 2003).[14] In addition, there is evidence that fishes can replace sensory cells that have been damaged as a result of exposure to certain drugs (Lombarte et al. 1993), although there have been no studies to determine if fishes can replace sensory cells that have been killed as a result of stimulation by intense sounds.

Hearing is based on the detection of the mechanical motions in the medium imparted by sound. In fishes, the otolith organs are stimulated directly by the particle motions associated with underwater sound fields. In addition, the organs can be stimulated indirectly by particle motions created when sound pressure fluctuations from the sound source are transformed into motion by a gas-filled accessory organ such as the swim bladder (see below).

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[12] The middle ear and the external ear and canal are needed in terrestrial vertebrates to transform sound pressure in the air to motion in the fluid of the inner ear so that air-borne sounds are detectable. In contrast, because the bodies of fishes have the same density and compressibility as water, there is no need to make such a trasnformation for the sound to stimulate the inner ear.

[13] The function and role of the semicircular canals in fishes is identical to that of the canals in terrestrial vertebrates. The gravistatic role of the otolith organs in fishes is the same as in terrestrial animals as well, and there are some terrestrial animals (e.g., amphibians) that may use these end organs for hearing, as in fishes.

[14] It should be noted that one reason for hearing loss in humans is the death of sensory hair cells due to aging and/or the effects of killing by certain classes of medications or intense sounds. Humans (and other mammals) produce all of the sensory hair cells they will ever have before birth, whereas fishes increase the number of hair cells in their ears with growth in addition to regenerating hair cells damaged by exposure to certain drugs (Lombarte and Popper 1993, 2004).

In effect, hearing is based upon relative motion between the fish's body[15] and the overlying otolith. As indicated earlier, the sensory hair cells have an apically located tuft of "cilia" (Figure 2, Page 44). Because the body of fish is primarily composed of water, it will move at the same amplitude and phase as the impinging sound. The otoliths, however, which are about three times denser than the rest of the body, will move at different amplitude and phase, and this causes the intervening ciliary bundles on the sensory hair cells to move, and the resultant detection of sound.

Similarly, the air-filled swim bladder (or other gas bubble in the body) is stimulated by the pressure component of the sound field. The swim bladder then serves as a small transducer that re-radiates energy in the form of particle motion, which is again detectable by the inner ear. In hearing generalists, the primary acoustic energy is provided by the direct stimulation of the ear, though it is possible that some additional energy is re-radiated from the swim bladder and that this could enhance hearing sensitivity and/or bandwidth. In contrast, hearing specialists have evolved a number of different mechanisms to acoustically couple the swim bladder (or other gas-filled structure) to the ear. These mechanisms directly transmit motion of the swim bladder or other gas-filled structure, induced by sound pressure, to the inner ear, thereby providing a substantial pressure input to supplement the direct detection of particle displacement. This coupling increases hearing sensitivity and bandwidth as compared to generalists (see Popper et al. 2003 for review).

Specializations that enhance hearing vary among different species. They may range from having an extension on the swim bladder that results in its rostral termination being very close to the ear, as in some croakers and drums (family Sciaenidae) (Ramcharitar et al. 2001) to a direct mechanical connection between the swim bladder and ear as found in the otophysan fishes (catfish, goldfish, and relatives). Finally, there are some species that have an extension of the swim bladder, or a separate bubble of gas, that is tightly associated with the ear, or which lies near the ear (e.g., all herrings and shads and relatives, mormyrids).

III. Effects of Human-Created Sound on Fish

Interest in the effects of human-generated sound on aquatic organisms has grown considerably in the past decade (e.g., NRC 1994, 2000, 2003; Richardson et al. 1995; NRDC 1997). While these reports, and a handful of research studies, have primarily focused on marine mammals, several have raised the issue that the very sounds that potentially affect marine mammals may also affect other aquatic organisms, including fishes and invertebrates (e.g., NRDC 1994, 2000; Popper 2003; Popper et al. 2004). The basis for concern about the effects of sound with regard to fishes are the well-documented effects of intense and/or prolonged sounds on hearing and overall physiology of humans and other terrestrial animals (Lenhardt 1986; NIH 1990).

[15] The fish's body is approximately the same density as the water.

Results of the few peer-reviewed studies on the effects of sound on fishes are discussed in this section. The specific studies are outlined, by type, in Table 3 (below) in order to give an overview of the investigations and *to show gaps* in the literature that must be filled if we are to understand overall effects of sound on fishes, and the specific effects of pile driving. The information in this table should be used with that of Table 4 (page 30) to understand specific needs with regard to pile driving.

Table 3: Citations of studies examining the effects of sound on fishes

Issue	Hearing Generalists	Hearing Specialists	Non-Teleosts (e.g. sturgeon, sharks)
Death	Yelverton 1975 (guppy, bluegill, trout, bass, carp; explosive blasts)	Yelverton 1975 (goldfish, catfish, minnow; explosive blasts) Hastings 1995 (goldfish and gouramis, pure tones)	
Non-Auditory Tissue Damage	Yelverton 1975 (guppy, bluegill, trout, bass, carp; explosive blasts)	Yelverton 1975 (goldfish, catfish, minnow; explosive blasts) Hastings 1995 (goldfish and gouramis, pure tones)	
Auditory Tissue Damage	Enger 1981 (cod, pure tones for 1 – 5 hr) Hastings et al 1996 (oscar, pure tones, 1 hr) McCauley et al. 2003 (pink snapper, air-gun)	Hastings 1995 (goldfish, pure tones)	
Permanent Threshold Shift (PTS)			
Temporary Threshold Shift (TTS)		Smith et al. 2004 (goldfish, white noise) Scholick and Yan 2002 (fathead minnow, white noise) Popper and Clarke 1976 (goldfish, pure tones)	
Behavioral Changes	Skalski et al. 1992 (*Sebastes* catch decreased after one air-gun blast of 186-191 dB re: 1 µPa) Engås et al.1996 (Haddock and cod catch reduction after seismic blasts) Wardle et al. 2001 (Exposed fish and invertebrates on reef to continuous air gun with no significant behavioral changes) Engås and Løkkeborg 2002 (Haddock and cod catch reduction area after seismic blast Slotte et al. 2004 (herring & blue whiting, fish do not enter the area of air gun during use)		

Issue	Hearing Generalists	Hearing Specialists	Non-Teleosts (e.g. sturgeon, sharks)
Eggs and Larvae	Banner and Hyatt 1973 (*Cyprinidon* and *Fundulus* showed somewhat decreased egg viability and larval growth in tanks with increased noise) Kostyuchenko 1973 (Increased egg mortality up to 20 m from seismic source) Booman et al. 1996 (eggs and larvae of various species were exposed to air guns at over 220 dB re: 1 µPa. Results variable with some stages showing decreased growth in a few species)		
Miscellaneous	Lagardère and Régarde 1981 (Shrimp show increased metabolic rate when subject to increased ambient noise levels) Lagardère 1982 (Shrimp showed decreased reproductive rates and growth with continuous increased background noise)	Smith et al. 2004 (no change in corticosteroid levels after continuous exposure to white noise)	

A. Behavioral Responses and Masking of Biologically Relevant Sounds

Several studies have demonstrated that human-generated sounds may affect the behavior of at least a few species of fish. For example field studies by Engås et al. (1996) and Engås and Løkkeborg (2002) showed that there was a significant decline in catch rate of haddock and cod that lasted for several days after termination of air gun use, after which time the catch rate returned to normal.[16] The conclusion was that the catch decline resulted from the sound of the air guns, and that the sound probably caused the fish to leave the area of insonification. More recent work from the same group (Slotte et al., 2004) showed parallel results for several additional pelagic species including blue whiting and Norwegian spring spawning herring. Slotte et al. found that fishes in the area of the air guns appeared to go to greater depths after insonification compared to their vertical position prior to the air gun usage. Moreover, the abundance of animals 30-50 km away from the insonification increased, suggesting that migrating fish would not enter the zone of seismic activity. Similarly Skalski et al. (1992) showed a 52% decrease in rockfish catch when the area of catch was exposed to a single air gun emission at 186-191 dB re: 1 µPa (mean peak level).

A study by Wardle et al. (2001) examined the behavior of fish and invertebrates on a coral reef using a TV system. These investigators continuously set off air guns that had a peak level of 210 dB re: 1 µPa at 16 m from the source. They found no permanent changes in the behavior of the fish, or invertebrates throughout the course of the study, and no animals appeared

[16] Studies were done on only two species, so these results must be taken with some caution in any attempt to extrapolate to other species.

to leave the reef. There was no indication of any observed damage to the animals; however, sound levels were not recorded.

While not totally germane to fishes, there is some evidence that an increased background noise (for up to three months) may affect at least some invertebrate species. Legardère (1982) demonstrated that sand shrimp (*Crangon crangon*) exposed in a sound proof room to noise that was about 30 dB above ambient for three months demonstrated decreases in both growth rate and reproductive rate. In addition, Legardère and Régnault (1980) showed changes in the physiology of the same species with increased noise, and that these changes continued for up to a month following the termination of the signal.

There is also considerable concern regarding the effects that increased human-generated sounds may have on detection of a broad range of environmental sounds that are of critical importance to the survival of fishes (e.g., Fay and Popper 2000; Popper et al. 2003). An increased level of background may not affect the physiology of the receiving animal, but such sounds may prevent the animal from hearing biologically relevant sounds. In such cases, animals may not hear the sound of a predator, or be able to hear a potential mate. While not necessarily having an immediate effect on an animal, the long-term implications for an animal or, more importantly, a population of animals, could be detrimental.

Indeed, we are now aware that fishes, as mammals and probably all other vertebrates, glean a great deal of information about their environment from the general sound field. In other words, whereas visual signals are very important and useful for things close and in the line of sight, the major information about the unseen part of an animal's world comes from acoustic signals. One may therefore think of fishes as using two "classes" of sound. The first is the well-known group of communication signals used to keep in touch with other members of a species and detect the presence of predator or nearby prey. The second are the sounds of the environment that, for a fish, might include the sounds produced by water moving over a coral head, waves breaking on shore, rain, and many more physical and biological sources. Bregman (1991) coined the term "Auditory Scene" to describe the acoustic environment. The acoustic environment has become of increasing importance in the overall understanding of hearing for all animals during the past 10 years. Moreover, it is becoming increasingly clear that one of the major roles of the auditory system is to discriminate between, and determine the position, of sounds in the auditory scene, using a mechanism called "stream segregation" (Bregman 1991; Fay and Popper 2000; Popper et al. 2003).

B. Stress – Physiological Responses

The impact of stress is much more difficult to define because it is hard to quantify this measure in fish (or marine mammals, for that matter) as it has not been extensively studied; however, increased background noise is known to increase stress in humans (e.g., NIH/CDC 1990; von Gierke and Eldred 1993; Pearsons et al. 1995). There is evidence that effects on non-auditory aspects of an animal's physiology can come from increased background noise or sudden intense sounds (e.g., Hattingh and Petty 1992), such as an increase in stress levels. Physiological responses to sudden intense noise in humans may include constriction of peripheral blood vessels, reduced breathing, shifts in heart rate, and shifts in the electrical resistance of the skin and muscle tension (Davis et al. 1955). In turn, increased stress does impact overall human health and well-being, and it is reasonable to suggest that the same would occur in fishes. Thus, a considerable concern with regard to aquatic organisms, as it is to humans and other terrestrial organisms, is not only the impact of very loud acoustic stress on the function of the auditory receptor, but also the impact of any sounds that are above ambient levels on overall health and well-being.

In fact, an early study by Gilham and Baker (1984) used crude vibratory noise (electric motors fixed to aquaria) to measure stress responses in rainbow trout. Although the stressors were not quantifiable, this study demonstrated that a general stress response occurred in fish between 1 and 5 days after signal onset that was shown by significant increases in serum cortisol levels. Other studies have also demonstrated that exposure to non-traumatic stressors (i.e., crowding, spawning, rapid environmental changes, suboptimal water quality or physical environment, altered conductivity, and pollution) can predispose fish to opportunistic infections (e.g., Walters and Plumb 1980; Noga et al. 1998; Wedemeyer 1999; Pickering 1981).

Work with goldfish (*Carassius auratus*) demonstrated that corticosteroid levels do not necessarily change in the presence of high sound levels in at least in this species (Smith et al. 2004). Corticosteroid level is a measure of stress, and suggests that stress levels in these animals were not influenced by continuous exposure to white noise in the 0.1 – 10 kHz frequency band with an overall pressure level of 170 dB re: 1 µPa. At the same time, while it is relatively easy to measure the steroid levels, controls are very difficult because the handling involved in taking the samples needed to asses steroid levels may affect the steroid level shown by the fish. Smith et al. (2004) suggest that additional studies are needed on the goldfish. Moreover, one must be cautious in extrapolating between species and between different experimental paradigms in trying to understand the effects of potential stressors on physiology.

C. Temporary and Permanent Hearing Loss

There are two classes of effects of sound exposure on the ear. Exposure to low levels of sound for some period of time may result in temporary hearing loss, referred to as temporary threshold shift or TTS (e.g., Lonsbury-Martin et al. 1987). The level and duration of sound exposure that causes TTS varies widely and can be affected by factors such as repetition rate of the sound, pressure level, frequency, duration, health of the hearer, and many other factors. By definition, hearing recovers after TTS. The extent of hearing loss (how many dB of hearing loss) and the duration of the TTS may extend from minutes to days, again depending on many variables. The second possible effect is referred to in the literature as permanent threshold shift or PTS. PTS is a permanent loss of hearing and is generally accompanied by death of the sensory hair cells of the ear (e.g., Saunders et al. 1991).

Laboratory studies have been used to determine whether there may be temporary or permanent changes in hearing ability in animals exposed for short or long duration to different types of sound (e.g., pure tones or white noise). TTS has been found using behavioral or physiological tests for several fish species, including goldfish (*Carassius auratus*), tilapia (*Oreochromis niloticus*), and fathead minnows (*Pimephales promelas*) (e.g., Popper and Clarke 1976; Scholik and Yan 2002; Smith et al. 2004). These experiments demonstrated the presence of TTS immediately after exposure to loud sounds. In all cases, hearing sensitivity returned to normal over time.[17]

In a recent set of studies, Smith et al. (2004) tested hearing in goldfish and tilapia to determine more detailed parameters of hearing loss, including the effects of different exposure durations and recovery times. They demonstrated that goldfish had significant threshold shifts after only 10 min of exposure to white noise (0.1 to 10 kHz bandwidth), and that fish with a three-week exposure at moderate sound levels (170 dB re: 1 µPa overall sound pressure level) took over two weeks to return to normal hearing (Smith et al. 2004). Similarly, Scholik and Yan (2001) demonstrated by behavioral experiments that fathead minnows did not recover to control levels even as long as 14 days after the termination of 24 hours of exposure to white noise from 0.3 to 2.0 kHz with an overall sound pressure level of 142 dB re: 1 µPa.

Finneran et al. (2002) found that for odontocetes (whales with teeth such as dolphins, belugas and killer whales), a total cumulative sound exposure level (or total energy flux based on the plane-wave assumption) of about 190 dB re: 1 μPa^2-s does not create a TTS in the hearing of these animals. According to Finneran et al. (2002) this holds true for exposure to explosive type sounds, pure tones of 1-s duration, and band-limited noise. For extremely fast rise times, however, they indicate peak pressure must still be considered.

In humans and other terrestrial vertebrates, exposure to intense sounds for even a short period of time may result in permanent hearing loss. This occurs because the sounds serve to destroy the sensory hair cells of the inner ear and/or fracture or dislocate the ossicular chain of the middle ear (Roberto et al. 1989; Patterson and Hamernik 1997). It is significant that exposure to lower intensity sounds for longer periods, as in a noisy work environment, can also lead to permanent hearing loss through death of sensory cells (Kryter 1985; Hamernik et al. 1994).

As a consequence, the issues that lead to the concerns of the effects of human-generated sounds on marine and terrestrial mammal hearing can be extended to fishes (Popper 2003; Popper et al. 2004). At the same time, the data on the effects of these sounds on fishes are very limited as compared to data for terrestrial vertebrates and even marine mammals. However, there is a small but growing body of peer-reviewed literature showing that such sounds can destroy the sensory cells in fish ears and that long-term exposure to even moderate level sounds will cause temporary loss of hearing (Popper 2003; Smith et al. 2004).

While looking for evidence of frequency discrimination in the peripheral auditory organs, Enger (1981) found that some sensory cells of the ears of codfish (*Gadus morhua*) were damaged after 1 – 5 hours exposure to pure tones at frequencies from 50 to 400 Hz with a sound pressure level of 180 dB re: 1 µPa (rms). Enger used a waveguide instrumented with a sound projector at each end to produce an exposure that had negligible particle velocity. In a similar

[17] It is important to note that the sound levels expressed in TTS studies were done based on sound pressure level, but should more correctly be determined in terms of cumulative energy exposure. Future experiments need to be done in such context to allow comparison between studies, animal groups, and, most importantly, different signal parameters (e.g., bandwidth, duration, duty cycle). The importance of the studies cited here lie with the observations that TTS does take place in fish, and that the effects of TTS may last for a considerable time after the termination of the noise source.

study, Hastings (1995) reported damage to auditory hair cells in goldfish (*Carassius auratus*) exposed to continuous tones of 189, 192, and 204 dB re: 1 μPa (peak) at 250 Hz and 197 dB re: 1 μPa (peak) at 500 Hz for approximately two hours. Four fish were exposed at each set of conditions and damage was found to correlate with sound pressure level at a 95% confidence level. This study also included several controls (fish placed in the waveguide and held for 2, but not exposed to sound).

Hastings et al. (1996) demonstrated similar effects on the ears of the oscar (*Astronotus oscellatus*). Sensory cells of the ears were damaged after one hour of continuous exposure to a 300-Hz pure tone at 180 dB re: 1 μPa (peak); however, the particle velocity in their waveguide was about five times that which would be associated with the same acoustic pressure in open water. This would be equivalent to the same particle velocity associated with an unbounded plane wave or spherical wave with a peak pressure of 194 dB re: 1 μPa.

It is important to note that in the Hastings et al. study, damage did not show up in animals after one day, but only in the animals that were kept alive for four days following exposure. These results suggest that damage from noise exposure takes some time to become visually apparent. At the same time, if the investigators were measuring hearing, hearing loss would have been apparent well before damage was physically visible, and perhaps immediately after noise exposure.

McCauley et al. (2003) investigated the effects of exposure to the sounds of a seismic air gun on the Australian fish, the pink snapper (*Pagrus auratus*). Fish were in a cage and exposed to several air gun emissions at different distances. After survival for different time intervals post-exposure, the ears were examined for signs of damage, using electron microscopic techniques identical to those used by Hastings et al. (1996). The results clearly showed extensive damage to the sensory hair cells of the ear. The extent of damage increased with the post-exposure period up to at least 58 days (the maximum survival interval described).

While the McCauley et al. (2003) study further substantiated the potential for the effects of intense noise on fish, both the McCauley and the Hastings et al. (1996) studies were careful to provide a number of caveats to their work. These included (a) use of only a few species which may not be representative of other species, (b) the inability of the fish to escape the intense sounds – they were caged, and (c) the relatively long duration of exposure as compared to exposures to more "realistic" human-generated sounds at the high levels used in the studies.

One difference between these studies that needs to be controlled for in future investigations is the relationship between acoustic pressure and particle velocity in the sound stimulus. While it was possible for Hastings et al. (1996) to calibrate both pressure and particle velocity in their stimulus, this was not done by McCauley et al. (2003). The importance of having full characterization of the stimulus in these and future studies is to enable correlation of results with the specific component(s) of the sound stimulus and thus comparison of results between studies.

It again needs to be pointed out that damage observed in these three species was only a visual manifestation of what may have been a much greater effect, and that observable physical evidence took days to show up. It may be more important to ask about the more immediate effects of the sounds on hearing capabilities of the fish. Even if there is only TTS as a result of a loud sound, temporary deafness could result in a fish unable to respond to environmental sounds that indicate the presence of predators and facilitate the location of prey and mates. Effects, however, depend on the use of sound by that species in those situations.

While it is clear from the data discussed above that intense sounds of some types can affect the ear and hearing, it is important to note that at this stage of our knowledge, and the very limited data, that it is not possible or reasonable to extrapolate results between species or sound sources. Thus, results for one species may not be indicative of the results one would obtain for another species using the same type of signal, and the results from one type of signal (e.g., air gun) may not be germane to another signal (e.g., pile driving).

The reasons for not being able to extrapolate results are many but include: (a) differences in the hearing systems of different fish species and too little knowledge of the effects of intense signals on such different systems; (b) limited data on the precise nature of a stimulus (e.g., pressure and/or particle motion) which might affect the hearing apparatus; and (c) the time course of different signals (e.g., continuous noise vs. impulsive signals). To be able to extrapolate between species and signals, much more will need to be known about the effects of sounds on different auditory systems and it will be imperative to have a common way of expressing noise exposure (e.g., energy flux) so that it is possible to compare stimulus parameters between signals of different types.

Finally, it should be noted that the same concerns regarding stimulus parameters and extrapolation between species applies to all other aspects of the effects of sound on fishes (or any animal, for that matter). Some of these other effects are discussed below.

D. Structural and Cellular Damage of Non-Auditory Tissues

Compared to data for the effects of human-generated sounds on fish hearing capabilities and the ear, there are even fewer peer-reviewed data on the effects of such sounds on other aspects of fish biology, and little work has been done to determine the non-auditory effects of sound on fish. It is widely known that intense sounds can alter the physiology and structure of terrestrial vertebrates (e.g., Fletcher and Busnel 1978; Saunders et al. 1991). Indeed, there are strong standards set by the Occupational Safety and Health Administration (OSHA) recognizing that high levels of background sound has an impact on human well-being (e.g., NIH 1990; von Gierke and Eldred 1993; Pearsons et al. 1995). These changes may include cellular changes, organ system changes, or stress level effects caused by exposure to sound. Intense sounds at ultrasonic frequencies (~ 750 kHz and higher) have even induced cardiac arrhythmias in humans and premature ventricular contractions in frogs (Dalecki et al. 1991); however, these effects have not been observed at lower frequencies that characterize the sound produced by pile driving.

While there are far fewer data on the impact of intense sounds on the health and well being of laboratory animals, and far less known about the impact of such sounds on wild animals (including aquatic animals), it is reasonable to suggest that the long-term exposure to high levels of sound impact all sound-detecting vertebrates (e.g., Richardson et al. 1995). The major concern with regard to human-generated sound and aquatic organisms lies with marine mammals. One of the organ systems of most concern with marine mammals is the lungs, and the resultant damage that may occur in this organ due to the presence of air. Most fishes do have at least one large air chamber, the swim bladder, which provides the same discontinuity between water and air as does the lung in marine mammals.

Studies on terrestrial mammals have indicated that gas-filled structures (i.e., lung) or gas pockets (such as could occur in the gastrointestinal tract) within a body make it susceptible to damage by sound (Richmond et al. 1973; Fletcher et al. 1976; Yang et al. 1996; Bauman et al. 1997; Dodd et al. 1997; Elsayed 1997). Tissue damage can occur when sound passes through the interface from a fluid tissue structure (e.g., adipose tissue and muscle) to a gas void because the gas is more compressible then the fluid, and this results in a relatively large increase in the motion of the connective tissue between the two. In addition, sound will cause gas organs such as the swim bladder and lung to oscillate and push on the surrounding tissues. The amplitude of these oscillations can be quite large at high sound pressure levels or even at lower sound pressure levels if the gas organ is excited at its resonance frequency. In fishes, gas oscillations induced by intense sound can even cause the swim bladder to tear or rupture.

Other structures within the body can be affected by sound because of their small size or dynamic characteristics. There is some evidence to suggest that sound at sufficiently high-pressure levels can generate bubbles from micronuclei in the blood and other tissues such as fat (ter Haar et al. 1982). In fish, blood vessels are particularly small in diameter so bubble growth by rectified diffusion (Crum and Mao 1996) at low frequencies can create arterial air embolism or burst small capillaries to cause superficial bleeding. This type of bubble growth may also occur in the eyes of fish where the tissue can have high levels of gas saturation (see non peer-reviewed reports by Turnpenny et al. 1994; Gisiner 1998).

Another type of tissue damage caused by intense sound pressure waves is traumatic brain injury (TBI) or neurotrauma. In humans, TBI can occur with no marks of external injury, but manifests itself with instantaneous loss of consciousness or sustained feelings of anxiety and confusion, or amnesia, and may result in death (Elsayed 1997; Knudsen and Oen 2003). The underlying physical mechanisms for these manifestations are cerebral edema, contusions and lacerations, as well as hemorrhages in the meninges (protective tissues around the brain), brain substance, nerve roots, and ventricles (fluid-filled spaces within the brain and spinal cord) that may result from extreme relative motion between the skull and brain during exposure to high overpressures. Hastings (1990, 1995) reported “acoustic stunning” in four gouramis (*Trichogaster trichopterus*) exposed for approximately eight minutes to a 150-Hz pure tone with a peak pressure of 198 dB re: 1 μPa. Three out of four of these fish recovered. The loss of consciousness exhibited by these fish could have been caused by neurotrauma, especially since this species has a bubble of air in the mouth cavity near each inner ear and located near the brain. This bubble of air enhances hearing capability of this species (Yan 1998). Thus fish with swim bladder projections or other air bubbles near the ear (e.g., butterfly fish, squirrel fish, and many other species) could be susceptible to neurotrauma when exposed to high sound pressure levels.

Elsayed (1997) conducted a series of investigations using terrestrial animal models to examine biochemical responses in tissues to blast overpressures. He and his colleagues have found two responses that correlate with blast overpressure: (1) depletion of antioxidants and (2) lipid pre-oxidation. Cernak et al. (1996) also examined biochemistry related to neurotrauma in blast injury. They also found lipid pre-oxidation products as well as increased levels of lactate and calcium ions and decreased levels of glucose and magnesium and zinc ions. Changes in lactate and glucose levels indicate changes in metabolism and energy in the damaged tissue, while changes in ion concentrations indicate cellular disruption and damage. Cernak et al. postulate that afferent neural impulses from injured organs (such as lungs) could impair CNS function and contribute to further damage over time. The biochemical mechanisms of acoustic traumas and barotraumas, as well as their acoustic thresholds, remain undefined. Understanding these mechanisms, however, could provide new means for treatment and intervention for these injuries.

Studies reported by Hastings (1990, 1995), Turnpenny et al. (1994), and Abbott (2002)[18] also describe non-auditory damage to fish caused by sound including evidence of capillary rupture in the skin, neurotrauma, eye hemorrhage, swim bladder rupture, and death. Hastings' work was with pure tones on goldfish, gouramis, and oscars. Her work showed that pond-size goldfish could not survive 2-hour continuous wave exposures at 250 Hz and a sound pressure level of 204 dB re: 1 μPa (peak), and gouramis could not survive 0.5-hour continuous wave exposures at 150 Hz and 198 dB re: 1 μPa (peak).

1. Juvenile and Adult Fish

Key variables that appear to control the physical interaction of sound with fishes is the size of the fish relative to the wavelength of sound, mass of the fish, anatomical variation, and location of the fish in the water column relative to the sound source. Yelverton et al. (1975) provide the most definitive study of the gross effects of sound generated by underwater blasts on fishes.[19] These sound waves consist of an extremely high peak pressure (called overpressure) with very rapid rise times (< 1 ms). Yelverton et al. exposed eight different species of fish, five with ducted swim bladders (physostomes) and three with non-ducted swim bladders (physoclists)[20] to blasts. The former were top minnow (*Gambusia affinis*), goldfish (*Carrasius auratus*), carp (*Cyprinus carpio*), rainbow trout (*Salmo gairdneri*), and channel catfish (*Ictalurus punctatus*), and the latter guppy (*Lebistes reticulates*), bluegill (*Lopomis macrochirus*), and large mouth bass (*Micropterus salmoides*). The test specimens ranged from 0.02 g (guppy fry) to 744 g body mass (large carp) and included small and large animals from each species. The fish were exposed to blasts having extremely high peak overpressures with varying impulse lengths.

[18] Neither Turnpenny et al. (1994) or Abbott (2002) were peer-reviewed.

[19] While an extremely important paper, it should be noted that the work does not appear in the peer-reviewed literature. And, the experiments were performed without having controls in which animals were handled in precisely the same way as the experimental animals, but without blast. While it is clear that blast effects are real in the Yelverton experiments, any replication of this (or similar) work requires extensive controls. In particular, without controls it is impossible to quantify results since some portion of the effects and mortality may result from fish handling and not the blast exposure.

[20] Physostomes are species in which the swim bladder is connected to the esophagus by a thin tube. Air to fill the swim bladder is swallowed by the fish and is directed to the swim bladder. Air removal from the swim bladder is by expulsion through this tube to the esophagus. Physoclistus fishes have no such connection. Instead, they add gas to the swim bladder using a highly specialized gas secreting system called the rete mirabile which lies in the wall of the swim bladder and extracts gas from the blood using a counter-current system, much like that found in the kidney to remove wastes from the blood. Removal of gas from the swim bladder occurs by reabsorbtion into the blood.

Yelverton et al. found a direct correlation between body mass and "impulse" as characterized by the product of peak overpressure and the time it took the overpressure to rise and fall back to zero (units in psi-ms) as shown in Figure 5 (Page 45).

Their results indicate that a sound energy metric, such as the sound exposure level or cumulative energy flux, rather than just peak pressure correlates with tissue damage in fish. In fact Yelverton et al. (1975) concluded that peak pressure alone did not correlate with damage because they kept peak pressure constant and varied the pulse width or vice versa in their study. The injuries they observed included swim bladder rupture, kidney damage, and liver damage.

2. Eggs and Larvae

In considering fishes, it is important to not only think in terms of adults, but also in terms of fish eggs and larvae. Whereas it is possible that some (though not all) species of fish would swim away from a sound source, thereby decreasing sound exposure, larvae and eggs are often at the mercy of currents and move very slowly, if at all. Eggs are often stationary and thus could be exposed to extensive human-generated sound if it is presented in the area, including sound transmitted through water (i.e., eggs within the water column) or substrate (e.g., eggs deposited within substrate, such as salmonid redds).

Data on effects of sound on developing eggs and larvae are very limited. There is some suggestion in the literature that developing larvae have different levels of sensitivity to mechanical stimulation at different stages of development (e.g., Piper et al., 1982; Dweyer et al. 1993). The only peer-review study on the effect of sound on eggs and development[21] was done by Banner and Hyatt (1973) and it was never followed up with additional investigations. Banner and Hyatt found an increased mortality of eggs of and embryos of *Cyprinodon variegates* exposed in 20-litre glass aquaria to broadband noise (100-1,000 Hz) that was about 15 dB above ambient sound level. The sound did not affect hatched fry of *C. variegates*, and neither eggs nor fry of *Fundulus similes* were affected. Banner and Hyatt also found that the larval growth was significantly less in the noise-exposed larvae of both species than in the larvae raised in ambient noise.[22] While these results are of considerable interest, they were from only two species subject to relatively low noise levels and for a limited time period.

Indeed, there are several issues that must be considered with regard to the effects of sound on eggs and larvae. These include: (a) immediate effects as measured by mortality; (b) long term effects, even after the termination of the insonification, as measured by mortality; (c) long term effects from which recovery is possible if the fish is not subject to predation or other factors that kill it during the recovery time; (d) effects on egg development and viability, (e)

[21] Jensen and Alderdice (1983) investigated the effects of mechanical shock on fish egg development. However, the study involved direct "banging" of the eggs on a surface and so the nature of the stimulus was totally unrelated to any sound or blast signal. Therefore, results from that study have no bearing on our understanding of how pile driving or other stimuli that move the water mass might affect fish, or fish eggs and larvae.

[22] Interestingly, these findings parallel the afore cited studies showing that shrimp exposed to noise have slower growth than controls not exposed to noise (Lagardère 1982).

effects on short and long-term growth of the developing larvae and young fish in the presence of sound and/or after termination of sound; and (f) effects of the sounds on the development and function of various organ systems.

Several other sets of data are worth noting. A more recent non peer-reviewed study on the effects of sounds from 115-140 dB (re: 1 µPa) on eggs and embryos in Lake Pend Oreille (Idaho) reported no effects of the sounds on survival or hatching (Bennett et al. 1994). However, few data were provided that could be used to evaluate the results. In contrast, Kostyuchenko (1973) worked with marine fishes, none of which are related to the species on the Pacific coast, to determine the effects of seismic air gun sounds on eggs. Kostyuchenko reported damage to eggs at up to 20 m from the source. Similarly, a Norwegian group (Booman et al. 1996) investigated the effects of seismic air guns on eggs, larvae, and fry and found significant mortality in several different marine species (cod, saithe, herring) at a variety of ages to source levels as high as 242 dB (peak) re: 1 :Pa, but only when the specimens were within about 5 m of the source, and the most substantial effects were within 1.4 m of the source. These authors also reported damage to neuromasts (sensory structures with sensory hair cells) of the lateral line and to other organ systems; however, data are limited to just a few species and need replication, and the received sound pressure and particle velocity were not measured.

There are a number of other gray literature studies of the effects of sound on developing eggs and larvae; none provide conclusive evidence on this topic that is germane to most Pacific Coast species. Indeed, one can conclude that there is a total dearth of material on this topic and it is an area of research that needs rigorous experimental evaluation.

In summary, the few studies on the effects on eggs, larvae, and fry are insufficient to reach any conclusions with respect to the way sound would affect survival. Moreover, most of the studies were done with seismic air guns and these are sounds that are very different than those from pile driving. The results suggesting some damage and death need to be followed up in a way that would be relevant to pile driving and the characteristic sound transmitted through water and substrate.

IV. Sound Generated by Pile Driving

A. Characterization of Pile Driving Sound

Impact noise results from a rapid release of energy when two objects hit one another. The physical characteristics of impact sounds primarily depend upon the mechanical properties of the impacting objects. When a pile driving hammer strikes a pile, the impact creates a pulse that propagates through the pile. If the pile is a hollow steel cylinder with a wall thickness that is very small relative to its diameter, then the impact will also create flexural waves in the wall of the pile which couple with the surrounding fluids (air and water) to radiate sound into the water as well as the air. In addition to the flexural waves, the hammer impact also creates a longitudinal pulse that propagates down the length of the pile and couples to the substrate at the water bottom. The resulting pulse on the substrate causes waves to propagate outward through the bottom sediments. These sound waves in the substrate can be transmitted from the bottom into the water some distance away from the pile to create localized areas of high sound pressure and particle motion, especially if they constructively interfere with the sound pulse that is traveling outward through the water directly from the pile.

Sound pressure pulses as a function of time are referred to as waveforms. The passage of a waveform at some point away from the pile can be measured at a selected location in the water column using a hydrophone (an underwater microphone) or sound level meter with an underwater probe. Pile driving sounds underwater are characterized by a multiple sharp increases and decreases in sound pressure over time as shown in the measured waveform displayed in Figure 6(a) (Page 46). The peak pressure is the highest absolute value of the measured waveform, and can be a negative or positive pressure peak.

The root-mean-square or "rms" level is determined by analyzing the waveform and computing the square root of the average of the squared pressures over the time period that comprises that portion of the waveform containing 90 percent of the sound (pressure squared) energy.[23] This calculated rms sound pressure level (SPL) is described as $RMS_{90\%}$ and is used to report an overall average SPL for a single pile driving pulse.[24] The frequency content of the sound pressure level shown in Figure 6(b) provides some indication of the bandwidth of the pile driving pulse. The frequency band for pile driving sounds is typically below 1,000 Hz, the same bandwidth as hearing in many species of fish (see Figure 1, Page 43).

Another measure of the pressure waveform that can be used to describe the pile driving pulse is the sound energy. Typically, the effects of impulsive type sounds are characterized by not only their rise time, duration (impulse width), and peak pressure, but also total energy dose over time. While the effects are described most often in terms of humans, all indications are that the same effects occur with all animals. The energy contained in a sound wave is a measure of the amount of work it does pushing on the fluid (or substrate material) as it travels. The sound wave "pushes" with pressure, or force acting over a unit area, and this force causes the fluid to move. This fluid motion is called acoustic "particle velocity." If the sound impinges on an aquatic animal, the energy will create forces and motions inside its body just as it does in the fluid.

For a sound wave traveling in open space without any interaction with objects or boundaries, such as a plane wave or spherical wave, the relationship between sound pressure (p) and particle velocity (v) is $p = (\rho c)v$, where ρ (kg/m^3) is the density of the fluid and c (m/s) is the speed of sound in the fluid (or substrate). Then the energy dose (e) contained in the sound wave is just the pressure multiplied by the particle velocity, or $e = p^2/(\rho c)$, which has the units of Joule per square meter per second (J/m^2-s). Thus energy dose, e, is the amount of energy in Joules passing through a unit area per unit time as the sound wave travels unbounded in the fluid. It is called the "acoustic energy flux" (see for example, Johnson and Robinson 1969; Hamernik and Hsueh 1991). How rapidly the energy accumulates may be significant in assessing the potential effects of impulses on fish and other aquatic animals.

Because pressure is usually the only quantity measured to determine the effects of sound and the pressure squared is proportional to the acoustic energy flux for a plane-traveling wave,

[23] As suggested by Richardson et al. 1995 and C. Greene, personal communication to MCH.
[24] Personal communication, J. Reyff, Illingworth & Rodkin, Inc.

pressure squared (p^2) is often used as an indication of the energy dose. The time-integrated (or cumulative) squared sound pressure is called "sound exposure." The total cumulative sound exposure spectrum level is called the sound exposure level (SEL), a common unit indicative of sound energy used in airborne acoustics to describe short-duration signals. The unit for SEL is dB re: 1μPa^2-s. The cumulative sound exposure (also commonly referred to as accumulated sound energy) plotted in Figure 6(c) currently provides the clearest comparison of the differences between impulses because it depicts the effects of both peak pressure and rise time. If a sound pulse contains higher pressure peaks and faster rise and fall times, then the cumulative sound exposure will increase at a greater rate than for a pulse with lower peak pressure and longer rise and fall times.

The "total energy flux," however, is *not* equivalent to the sound exposure levels based only on pressure squared, unless the sound wave is a plane or spherical wave traveling in a fluid (or substrate) without boundaries. In the case of pile driving, there is rarely a plane or spherical traveling wave because the sounds are produced in shallow water near shore with numerous boundaries that interact with sound traveling in the substrate. These pile driving conditions produce a very complex sound field that does not have a simple relationship between sound pressure and particle velocity, as do plane- and spherical-traveling waves. Moreover, we need to also know the sound particle velocity because particle velocity is detected by the ears of fishes, especially in hearing generalists (e.g., Popper et al. 2003). Because of the complexity of the sound field produced in pile driving environments, relatively simple models based on spherical spreading, such as the one developed by Dzwilewski and Fenton (2003), are not very useful in predicting the impact zones for aquatic animals.

B. Comparison of Pile Driving Sound Waveforms with an Ideal Impulse Wave

Impulse noise is a transient sound that also arises from a rapid release of energy, usually electrical or chemical such as circuit breakers or explosives. Although impact and impulse noise result from different processes, they share many characteristics: initial high peak overpressure, rapid rise and fall times, and relatively short duration. Thus "impulsive" and "impact" are often used interchangeably to describe many intense, short duration sounds.

The ideal impulse is described by the Friedlander wave (Hamernik and Hsueh 1991), which provides a mathematical description of impulsive sounds so they can be modeled and studied. If pile driving sounds could be characterized using a waveform similar to this type, then effects of pile driving noise on aquatic animals could potentially be extrapolated using data from effects studies based on other impulsive sources (e.g., explosives and sonic booms).

Figure 7 (Page 47) shows an approximation of a pile driving sound using a Friedlander wave. Figures 7(a), (b), and (c) compare the temporal characteristics, sound exposure spectral density and cumulative sound exposure over time, respectively, for the idealized and actual pile driving sound characterized in Figure 6. These waves are very close in exposure characteristics, which indicate that the key metrics for pile driving may be the peak pressure and its impulse width, which are combined in a single measure, the cumulative sound exposure level. Thus a systematic approach to approximate pile driving signals using mathematically modeled

Friedlander type waves could provide a way to determine how data, which have been obtained in effects studies using blasts or other impulsive sources, relate to different pile driving scenarios.

A mathematical model that captures the essential characteristics of pile driving sounds could also be used to investigate the effects of changes in the pulse that could be created by modifications in the structural acoustics design of the pile. Such an approach was used to investigate the reshaping of sonic booms to achieve both reduced loudness and sound exposure level (Leatherwood and Sullivan 1994).

V. Areas of Uncertainty and Studies Needed

A number of questions need to be asked relevant to the effects of sound generated by pile driving. Three areas of study and evaluation include definition of interim thresholds for fish protection from sound generated by pile driving using the best available science, studies to provide a clear characterization of pile driving sound, and studies to provide a more succinct description of fish injuries resulting from pile driving sound. To make these studies useful, they need to be done in a very highly specified sound paradigm and with species that are appropriate for study on the Pacific Coast (Table 2, page 7).

A. Fish Protective Criteria for Pile Driving Noise

Minimal data are available about the effects of noise on fish species in the Pacific Coast region and the information available is of questionable relevance to effects of pile driving noise. To use the existing scientific literature to address potential effects of sound caused by impact pile driving on Pacific Coast species, it is not sufficient to simply extrapolate information by comparing species that are taxonomically related. However, it is probably more appropriate to extrapolate between species that have somewhat similar auditory structures or pressure detecting mechanisms (most notably the swim bladder) and species of similar size, mass, anatomical variation, and behavior relative to location of the fish in the water column. This would enable at least a first-order approximation of extrapolation to fishes such as Salmoniformes and other teleost fishes that do not have hearing specialization (e.g., rockfish, bass). The results are less easily extrapolated to teleosts without a swim bladder (e.g., the flatfishes such as plaice, sole, and flounder, and gobies) and to fishes with very different ear structures than teleosts such as the sharks and rays, and the chondrosteans such as sturgeon. There are several hearing specialists found on the Pacific Coast, including sardines and cod, and it may be possible to get some indication on the effects of noise on these species from the few noise studies on hearing specialists. But again, extrapolation must be done with considerable caution.

The body of scientific and commercial data available is inadequate for the purpose of developing final scientifically supportable criteria for pile driving noise that will protect fish. Protective criteria developed from available data will be highly unreliable given that such data were obtained in experiments in which the sounds have only the most tenuous (at best) relationship to those produced during pile driving. The information from blasting and pure tone studies may be of some use to enable development of interim, and preliminary, criteria addressing injury and mortality. At the same time, it is imperative to recognize the need for well-controlled studies to provide clear direction for development of scientifically supported criteria. This conclusion is based upon several factors.

(1) Most importantly, the signals used in all of the earlier potentially relevant studies are completely different from the signals emitted by pile driving (Table 3). As a consequence, the effects of such sounds, whether they be from air guns, blasts, or pure tones, are likely to be very different on both hearing and physiology from sounds produced by pile driving.

(2) There are insufficient data on the effects of any sound exposure on fish. The data in the literature (and even data in the “gray literature”) are incomplete, only relevant to specific species, and not easily extrapolated to other species. Moreover, each of the studies, including those of the authors of this report, was not focused on issues that relate to pile driving. As a consequence, the results are not directly applicable to deriving fish protective criteria for pile driving.

(3) None of the earlier studies used species that are necessarily similar to those found on the Pacific coast. Because there is wide diversity in ear structure among fishes, and potentially in other aspects of their physiology, it is not reasonable to use the very small body of literature currently available to attempt to extrapolate to Pacific coast fishes. In effect, the data in the literature pertain to the species studied, and none others.

(4) It is likely that thresholds for hearing effects and effects on other aspects of fish physiology will differ. Whereas there are significant differences in how fishes hear, the responsiveness of other tissues (e.g., blood vessels, kidneys) are not likely to be very different between species (at least based upon current knowledge). Therefore, fishes with different auditory sensitivity may show very different auditory system damage attributable to the same pile driving signal, whereas all fishes may show the same kinds and level of damage to other organs and systems attributable to a similar pile driving signal.[25]

(5) Analysis of effects may not only be species specific, but also size specific. The very limited but important explosive blast data demonstrate that there are differences in the effects of blasts on fishes of different sizes. Whether the same findings would hold up for pile driving sounds is totally unknown, but the possibility of such an effect precludes trying to define final fish protective criteria for pile driving.

Despite these caveats, it is recognized that a set of interim criteria are needed to protect fish subjected to pile driving even as controlled experiments are conducted that will allow development of scientifically based criteria for pile driving. Development of interim criteria is particularly important since it is difficult to stop all pile driving until scientifically based criteria are established. Furthermore it is likely that development of such criteria for pile driving will

[25] There is some question as to whether the organ system effects would be the same in physostomus and physoclistus fishes. While data from Yelverton et al. (1995) suggest that fishes with both types of swim bladders are affected in the same way by explosive blasts, it is important to still question whether the same results would be found for both types of fishes for other types of sound exposure.

take several years of laboratory and field experiments with a number of different fish species. The following table summarizes our recommendations for interim criteria.

Table 4: Recommendations for Interim Protective Criteria

Issue	Hearing Generalists	Hearing Specialists
Death	Figure 8 (page 48) shows interim criteria based on cumulative sound exposure estimated by approximating Yelverton (1975) blast impulses for 50% mortality with an idealized Friedlander wave as described in Section IV.	See Figure 8.
Non-Auditory Tissue Damage	Figure 9 (page 48) shows interim criteria based on cumulative sound exposure estimated by approximating Yelverton (1975) blast impulses for no injury with an idealized Friedlander wave as described in Section IV.	See Figure 9.
Auditory Tissue Damage	Equivalent to 1-hour continuous exposure to a pure tone, 100 dB above auditory threshold for sound pressure, in most sensitive bandwidth (assuming relationship between sound pressure and particle velocity is equivalent to that of a plane wave propagating in open water); primarily based on Enger (1981).	Equivalent to 1-hour continuous exposure to a pure tone, 100 dB above auditory threshold for sound pressure, in most sensitive bandwidth; primarily based on Popper and Clarke (1975) and Hastings (1995).

It must be recognized that these recommended interim criteria: (a) are *only relevant to pile driving* and cannot be extrapolated to other sources of underwater sound such as air guns, ships, and sonars; (b) may not be relevant to all pile driving activities; and (c) may not be relevant to all aquatic organisms.

B. Required Studies

To better understand the effects of pile driving on fishes there are two basic sets of needs. First, a series of experiments need to be conducted that characterize the sounds emitted by pile driving in different underwater environments. These data would be used to understand the signals that could affect fish and also to define a set of signal parameters that could be used by diverse agencies to reflect the general nature of pile driving sounds. Such an analysis would enable investigators to share a common set of signals that represent the acoustics of pile driving. Equally important, various agencies interested in the effects of pile driving on fishes would not have to develop their own set of signals, and they would be assured that the signals being used would encompass those at any particular pile driving site.

Second, a series of experiments need to be conducted that use pile driving sounds to answer specific questions on the effects of pile driving on fishes. These studies would encompass behavioral to pathological effects. In all cases, the studies must be conducted under highly controlled conditions that provide data that is most useful to agencies and regulators.

More specifically, the following criteria must be followed in all experiments:

1. All studies should involve what are called “representative species.” Representative species are defined as those that serve as models for fishes in the region of question – in this case, the Pacific coast. Species are selected to represent differences in: (a) habitat; (b) presumed hearing capabilities; (c) differences in ear structure and connections of the ear to peripheral structures such as an air bubble; (d) bony fish and non-bony fish (including elasmobranches); and (e) other comparable factors. A minimum set of fishes should be defined so as to have the fewest possible studies and yet represent as many of the parameters for fishes in the area of question as possible.

2. All studies must be done double-blind so that the person(s) doing the analysis is (are) not aware of the experimental conditions of the test animals.

3. Suitable controls must be provided, subjecting animals to precisely the same experimental conditions other than exposure to the sound treatment. In addition, a second set of baseline controls is generally made up of animals that have not been subject to any manipulation whatsoever.

4. Samples must be of sufficient quantity to allow statistical analysis of results.

5. All work must be done by individuals who are expert in the appropriate techniques. In particular, pathology studies must be done by individuals who are fully familiar with fish pathology and necropsy, and they must follow accepted practice for doing necropsy.
6. All exposure experiments must be done in a chamber or facility with a defined acoustic field that has a known relationship between sound pressure and particle velocity. In a laboratory, special wave guides or larger facilities are required to achieve this underwater (see for example, Finneran and Hastings 1999; Wang et al. 1998).

The most important questions that must be asked regarding pile driving and its effect on fishes are presented in Table 5 (below).

Table 5: Research Questions on the Impact of Pile Driving on Fishes

Project title	Project Objectives	Significance	Relationship to other studies	Relationship to pile driving needs
Characterize Pile Driving Sounds				
Define dose/response level for pile driving sounds	Develop ways to express exposure to pile driving sounds in terms of cumulative energy over time, and to define the acoustic particle velocity within the sound field	This will provide a series of "standard" pile driving sounds in water and substrate that is acceptable to departments of transportation and industry for use as the stimuli with which to do studies on representative species	This study is fundamental to the investigations on fishes and could provide for laboratory signals that could be representative of the range of pile driving stimuli in different locations	Without this standardization it will be impossible to generalize between studies done in different locales and with different piles
Structural acoustic analysis of piles	Develop structural acoustics models of piles to investigate how modifications to piles could alter the sounds and potentially incur less damage to animals	This could result in potential modifications to the structure that could reshape the temporal characteristics of the pile driving stimulus without changing structural integrity	Would need to test modified sounds on animal models	Could provide ways to mitigate some effects of pile driving on aquatic organisms
Define characteristics of the underwater sound field	Develop underwater sound propagation model and integrate with pile structural acoustics models to estimate received levels in the vicinity of pile driving operations and verify with field measurements of underwater sound pressure measurements	This is the only way to define zones of impact on fishes because the sound energy received by a fish depends on not only the pile driving source, but also the size, shape, and properties of the underwater environment.	Would be able to map the impact of pile driving sounds on the underwater environment based on results of tests of pile driving sounds on animal models	Could provide environmental impact analysis and effective mitigation measures
***Characterize injury of fish exposed to pile driving sounds* (see draft Figure 10, page 49)**				
Hearing capabilities of Pacific coast fishes	Determine hearing capabilities (using ABR) of representative species	Useful for prediction of detection range of pile driving sounds and potential effects on hearing capabilities	Previous behavioral studies did not use any Pacific coast fishes or elasmobranchs	Studies would be on species that are particularly germane to those affected by pile driving [TABLE CONTINUED NEXT PAGE]

Project title	Project Objectives	Significance	Relationship to other studies	Relationship to pile driving needs
Mortality of fishes exposed to pile driving	Determination of short and long term effects on mortality on representative species as a result of pile driving. Measure pathology (using necropsy studies) of the effects of sounds on fishes at different distances from the source	Provide baseline data on effects of pile driving and the effects of such signals of different levels and spectral components	Studies of this type have, heretofore, not be done under controlled situations	Provide mortality data as well as pathology as to the effects of pile driving and determination of the cause of immediate and long-term mortality
Effects of pile driving on non-auditory tissues	Using the precise same paradigm as for effects on the ear, examine other tissues using standard fish necropsy techniques to asses gross, cellular, and molecular damage to fish. Furthermore, determine stress effects on fish using appropriate stress measures (e.g., hormone levels). Do for representative species.	Provide insight into how the sounds affect fish, even when there is no immediate mortality	The only comparable data are from blasting, which suggests significantly different effects depending upon fish size and species.	Direct measure of potential long-term damage to fishes.
Effects of pile driving on hearing capabilities	Determine TTS and PTS on representative species	Provide insight into hearing loss and possible recovery as a result of different sound levels and sound types	No studies of this type have been done using pile-driving sounds	Data that will help understand the sound levels and other parameters that could result in the loss of the ability of different species types to detect sounds, and thus detect biologically critical signals
Effects of pile driving on fish eggs and larvae	Determine mortality, growth rates, and pathological changes in developing fishes of representative species with exposure at different times the development cycle	Since eggs and larvae do not move from the sites of spawning, determine if long-term pile driving could affect fish populations	No studies done on any fish system are relevant to this investigation	If fish spawn in the vicinity of pile driving sites, or cannot be kept from spawning during pile driving operations, effects on eggs and larvae could be considerable

Project title	Project Objectives	Significance	Relationship to other studies	Relationship to pile driving needs
Behavioral responses of fish to pile driving	Observe, in large scale cages, the behavioral responses of representative species to pile driving sounds. Do fish attempt to swim from the source? Do they react to the sounds? Do they "freeze" in place?	In knowing behavioral responses, it may be possible to predict which species would remain in an area of pile driving vs. species that could be expected to leave the area after the initial pile driving activity.	None have been done to date.	This may help limit the number of species that would need to be "protected."
Effects of pile driving on the ear and lateral line	Determine morphological changes over time for representative species on sensory cells of the ear and lateral line, and whether such changes are reversible	If there is loss of sensory cells there is a loss in hearing ability or the ability of the lateral line to be used in hydrodynamic reception. If there is recovery of these cells, fishes may be able to survive (assuming they did not die prior to recovery).	A few studies suggest that intense signals will affect the sensory cells of the ear, but almost nothing is known about the lateral line. However, no studies were done with sounds comparable to those from pile driving	Loss of hearing capabilities, even for a short period of time, could dramatically affect survival of fishes.
Effects of multiple pile driving exposures on fish	For the appropriate experiments cited above, determine effects of multiple exposures, over time, of pile driving	Some fishes may stay in the pile driving area, or go between areas that have different time tables for pile driving. Thus, there may be multiple exposures over time	No data in the literature.	If fish remain in an area over time, there may be cumulative effects that need to be understood

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Glossary

Acoustic energy flux – The work done per unit area and per unit time on the fluid (or solid) by a sound wave as it travels through the medium. The units of acoustic energy flux are Joules per square meter per second (J/m^2-s).

Acoustic Pressure – The force per unit area exerted by a sound wave above and below the ambient or static equilibrium pressure is called the acoustic pressure or sound pressure. The units of pressure are pounds per square inch (psi) or, in the SI system of units, Pascals (Pa). In underwater acoustics the standard reference is one-millionth of a Pascal, called a micro-Pascal (1 µPa).

Amplitude – The maximum deviation between the sound pressure and the ambient pressure.

Arterial air embolism – The entrance of air into the arterial circulation as a result of trauma. Death can occur if an embolus of air obstructs the brain or heart circulation.

Bandwidth – The range of frequencies over which a sound is produced or received.

Continuous wave exposure – The energy received from a sound wave that is continuous in time.

Cumulative sound exposure – The integrated amount of energy received from a sound wave over certain time period.

Decibel (dB) – A customary logarithmic unit most commonly used (in various ways) for reporting measurements of sound. A difference of 10 dB corresponds to a factor of 10 in sound power. The beginning of the scale, 0 decibels, can be set in different ways, depending on exactly which aspect of sound is being measured. The actual sound measurement is compared to a fixed reference level and the "decibel" value is defined to be 10 $\log_{10}$(actual/reference), where (actual/reference) is a power ratio. Because sound power is proportional to sound pressure squared, the decibel value for sound pressure is 20$\log_{10}$(actual pressure/reference pressure). As noted above, the standard reference for underwater sound pressure is 1 micro-Pascal. The dB symbol is followed by a second symbol identifying the specific reference value (i.e., re: 1 µPa).

Fall time – The amount of time it takes to go from the peak pressure to either zero pressure or the minimum pressure in an impulsive sound wave.

Impact noise – Noise produced when two objects strike each other and release a large amount of mechanical energy. Impact noise has short duration but relatively high sound pressure level.

Impulse noise – Noise produced by a rapid release of energy, usually electrical or chemical such as circuit breakers or explosives.

Impulse length – This is the total time it takes for the impulse to occur.

Impulse width – The time required to go from a minimum or zero pressure to the peak pressure and then back to the minimum or zero again.

Insonification – Irradiation with sound.

Lagena – An otolithic end organ of the inner ear. The precise role of the lagena is not defined, but it is likely that it is involved in sound detection in many species.

Lateral line – A series of sensors along the body and head of fishes that detects water motion. The lateral line uses sensory hair cells (as in the ear) for detection. The cells are located in neuromasts which lie either in canals (e.g., along the side and head of the fish) or freely on the surface in a widely distributed pattern.

Peak pressure – The highest pressure above ambient that is associated with a sound wave.

Peak overpressures – Overpressure is the pressure above the ambient level that occurs in an impulsive sound such as an explosion. The peak overpressure is the highest pressure above ambient.

Permanent threshold shift (PTS) – A permanent loss of hearing due to some kind of acoustic or drug trauma. PTS results in irreversible damage to the sensory hair cells of the ear, and thus a permanent loss of hearing.

Plane-traveling wave – A plane wave is an idealized sound wave that propagates in a single direction along its longitudinal axis. Theoretically the sound pressure is the same over an infinite plane that is perpendicular to the direction of propagation.

Rectified diffusion – Bubble growth by rectified diffusion occurs when more gas diffuses into a bubble while it is expanded (and at lower internal pressure) than the amount of gas that diffuses out when it is compressed (and at higher internal pressure). The amount of gas inside the bubble gradually increases and the bubble grows over time as it oscillates.

Resonance frequency – The frequency at which a system or structure will have maximum response (e.g., displacement) when excited by an oscillatory sound or force.

Rise time – Is the interval of time required for a signal to go from zero, or its lowest value, to its maximum value.

Saccule – One of the otolithic end organs of the inner ear. It is generally thought that the saccule is involved in sound detection.

Sound attenuation – The reduction of the pressure level of a sound. Sound attenuation occurs naturally as a wave travels in a fluid or solid through dissipative processes (e.g., friction) that convert mechanical energy into thermal energy and chemical energy.

Sound energy metric – A value that characterizes a sound by some measure of its energy content.

Sound exposure level (SEL) – A measure of the mechanical energy associated with a noise event, based on the square of the sound pressure, which accounts for both sound intensity and duration. SEL is typically used to compare noise events having different durations and intensities.

Sound exposure spectral density – The square of the Fast Fourier Transform (FFT) of a digitized sound pressure waveform. The spectral density gives the relative energy in each narrow band of frequency that results from the FFT (a mathematical operation that is used to express data recorded in the time domain as a function of frequency).

Swim bladder – A gas (generally air) filled chamber found in the abdominal cavity of many species of bony fish, but not in cartilaginous fishes. The swim bladder serves in buoyancy control. In many species the swim bladder may also serve as an impedance matching device for sound production, and as a pressure receiving structure to enhance hearing bandwidth and sensitivity.

Temporary threshold shift (TTS) – Temporary loss of hearing as a result of exposure to loud sounds. The mechanisms underlying TTS are not well understood, but there may be some temporary damage to the sensory hair cells. The duration of TTS varies depending upon the nature of the stimulus, but there is generally recovery of full hearing over time.

Threshold - The threshold generally represents the lowest sound pressure level an animal will detect in some statistically predetermined percent of presentations of a signal. Most often, the threshold is the sound level at which an animal will indicate detection 50% of the time.

Total energy dose – The total cumulative energy received from a sound wave over its duration.

Utricle – An otolithic end organ of the inner ear. The utricle is probably involved in determining head position relative to gravity as well as in sound detection. It is the primary sound detection region in the Clupeiform fishes (herrings, shads, sardines, anchovies, and relatives).

Weberian ossicles – A series of bones found in the otophysan fishes (goldfish, catfish, and relatives) that connect the swim bladder to the inner ear. It is generally thought that the Weberian ossicles act to couple the motions of the swim bladder walls in response to pressure signals to the inner ear. Thus, the ossicles are functionally analogous to the mammalian middle ear bones as acoustic coupling devices.

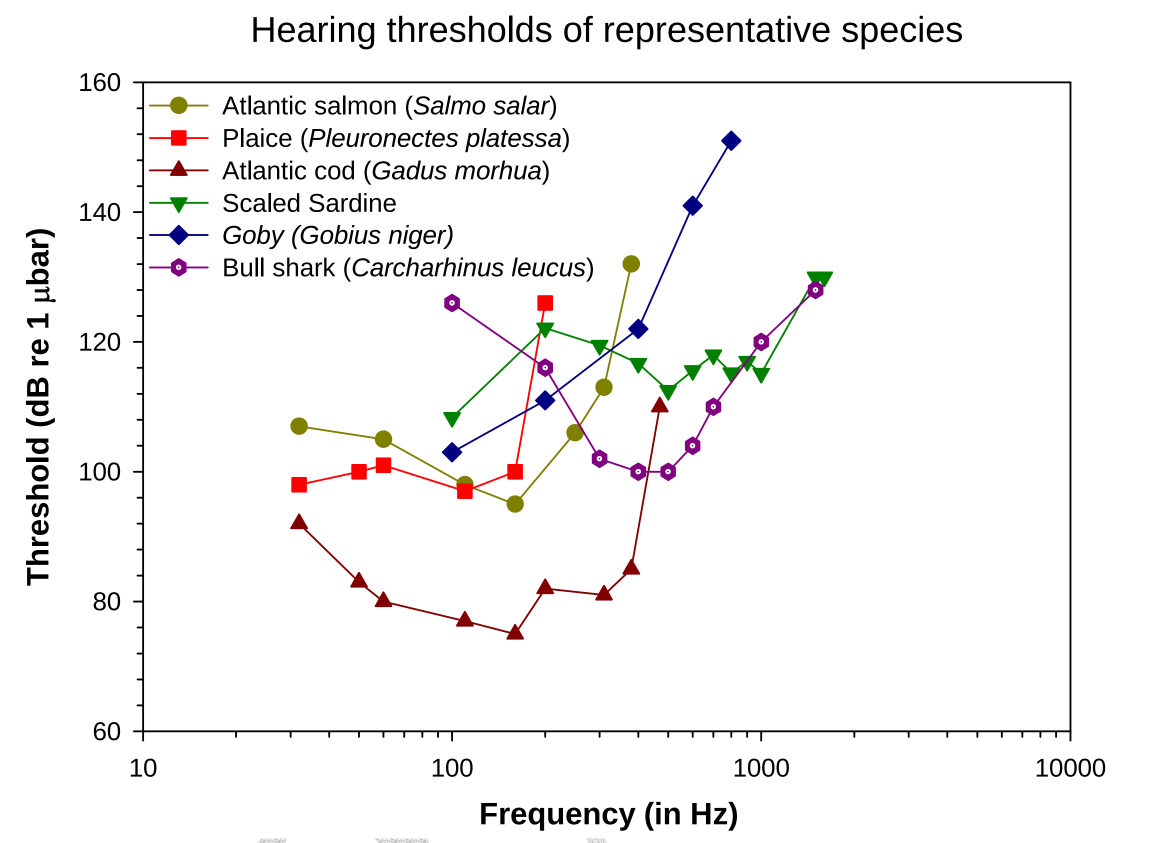

Figure 1: Hearing thresholds for species of fish that are similar to those found in San Francisco Bay. While data are not available for any of the species found in the Bay, these data suggest that none of the species, with the exception of the sardine (and related species) detects sounds much above 1000 Hz. It should be noted that the data for the bull shark are highly "suspect" and only represents determination with a few specimens. There are also recent data suggesting that salmonids (Atlantic salmon and related species) and flatfish (plaice and relatives) are able to detect infrasonic frequencies – sounds below about 35 Hz. Data in the figure were compiled from Fay 1988.

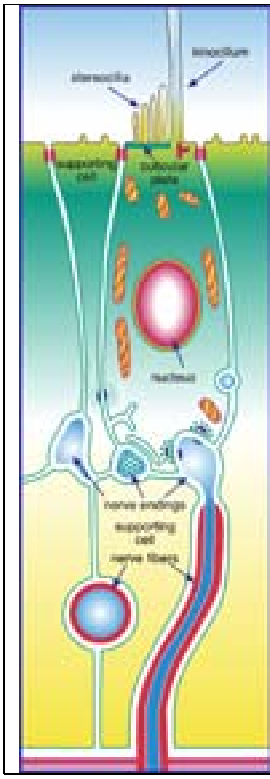

Figure 2. Schematic drawing of a sensory hair cell from a fish. The transducing element is the ciliary bundle, made up of the kinocilium and stereocilia, at the apical (top) end of the cell. This bundle is in contact with the otolith that lies in the chambers of the otolithic end organs (saccule, lagena, utricle). Relative motion between the sensory cell body sitting in the sensory epithelium and the overlying otolith results in a shearing or bending of the ciliary bundle. This causes channels (sub-microscopic holes) to open in the cilia and allowing the entry of calcium ions into the cell. This results in a cascade of events that leads to the release of chemical neurotransmitters from the base of the cell. The neurotransmitter crosses a small gap between cells and excite the endings of the nerve that innervates the cell. This, in turn, results in an electrical potential (the action potential) in the nerve which is carried to the brain. (From Popper and Coombs 1980)

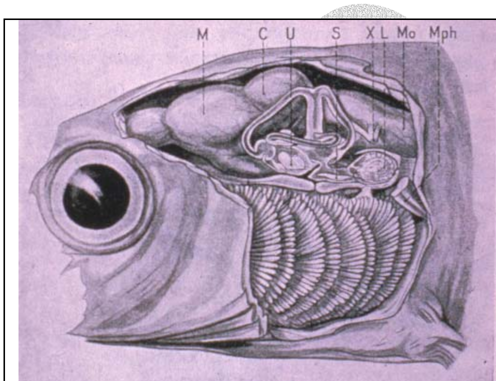

Figure 3. Lateral view of the head of a minnow *Phoxinus laevis* (from von Frisch and Stetter 1932). This picture shows the location of the ear in the brain cavity. It is located towards the rear of the brain and above the gills. This fish is a hearing specialist and so the ear is a bit different than that of a non-specialist as shown in Figure 5. M – medulla of brain; C – Cerebellum of brain; U – utricular otolithic end organ; S – saccule; L – Lagena; X – 10^{th} cranial nerve (not associated with hearing)

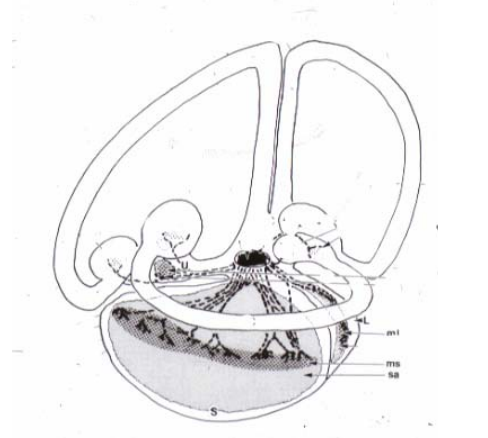

Figure 4. Drawing of the right ear of a salmon (*Salmo salar*). Anterior to the left and dorsal to the top. The drawing shows the three semicircular canals and the three otolithic end organs, the utricle (u), saccule (s), and lagena (L). The sensory epithelia of the saccule (ms) and lagena (mL) are shown, along with the saccular otolith (so). The utricle also has an epithelium and all three end organs have otoliths of different sizes. The ear is innervated by the eighth cranial nerve (the same one that innervates the mammalian ear). Drawing by Dr. Jiakun Song.

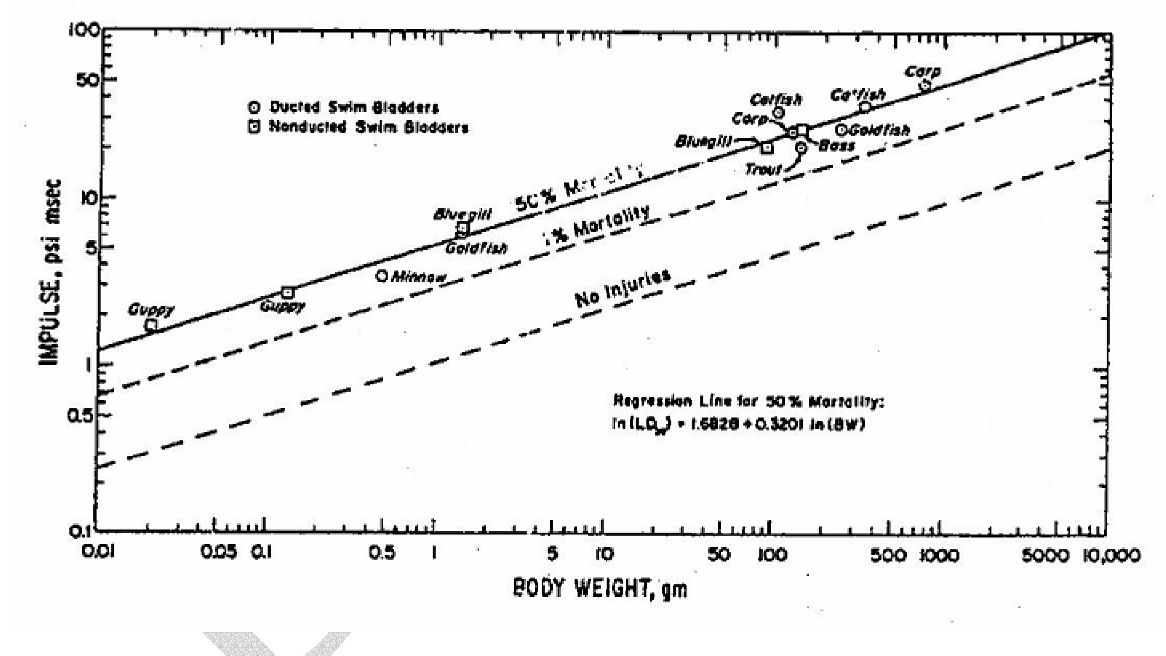

Figure 5: The results of study by Yelverton et al. (1975) to determine the effects of underwater blasts on fishes. A direct correlation was found between body mass and the impulse, characterized by psi-msec, which caused 50% mortality. The correlation was independent of peak overpressure, thus indicating that sound energy may be more indicative than peak pressure in determining damage caused by intense sound. Fish with ducted swim bladders were found to be just as vulnerable to blast injury and death as those without ducts. (Note: Yelverton et al. reported no control test specimens in this study.)

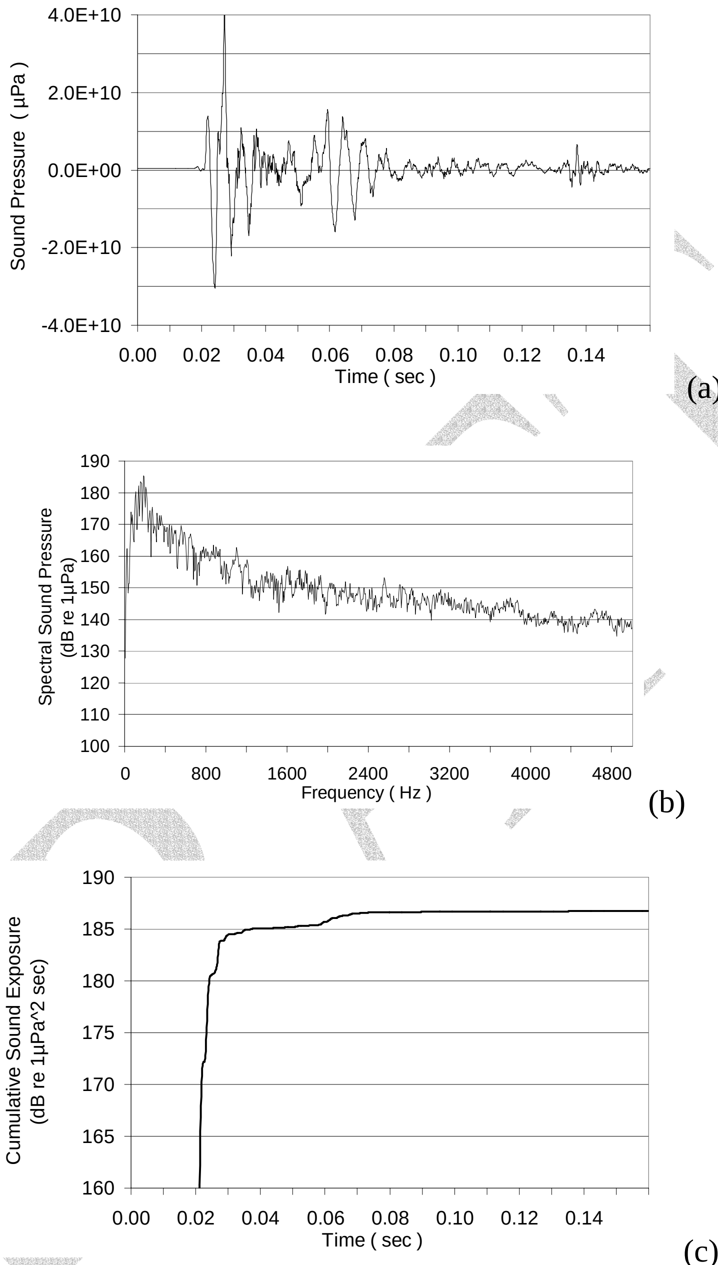

Figure 6: Measures of unattenuated pile driving sound at the San Francisco-Oakland Bay Bridge East Span Replacement project (SFOBB), Pier 3E at 50 m in relatively deep water. (a) Measured sound pressure waveform; (b) narrow-band frequency content of the waveform; (c) cumulative sound exposure over time. The sound exposure level (SEL) for this single hammer strike is 187 dB re: 1 µPa^2-s and RMS$_{90\%}$ is 200 dB re: 1 µPa (based on 0.048 s pulse width). Data provided by J. Reyff, Illingworth & Rodkin, Inc.

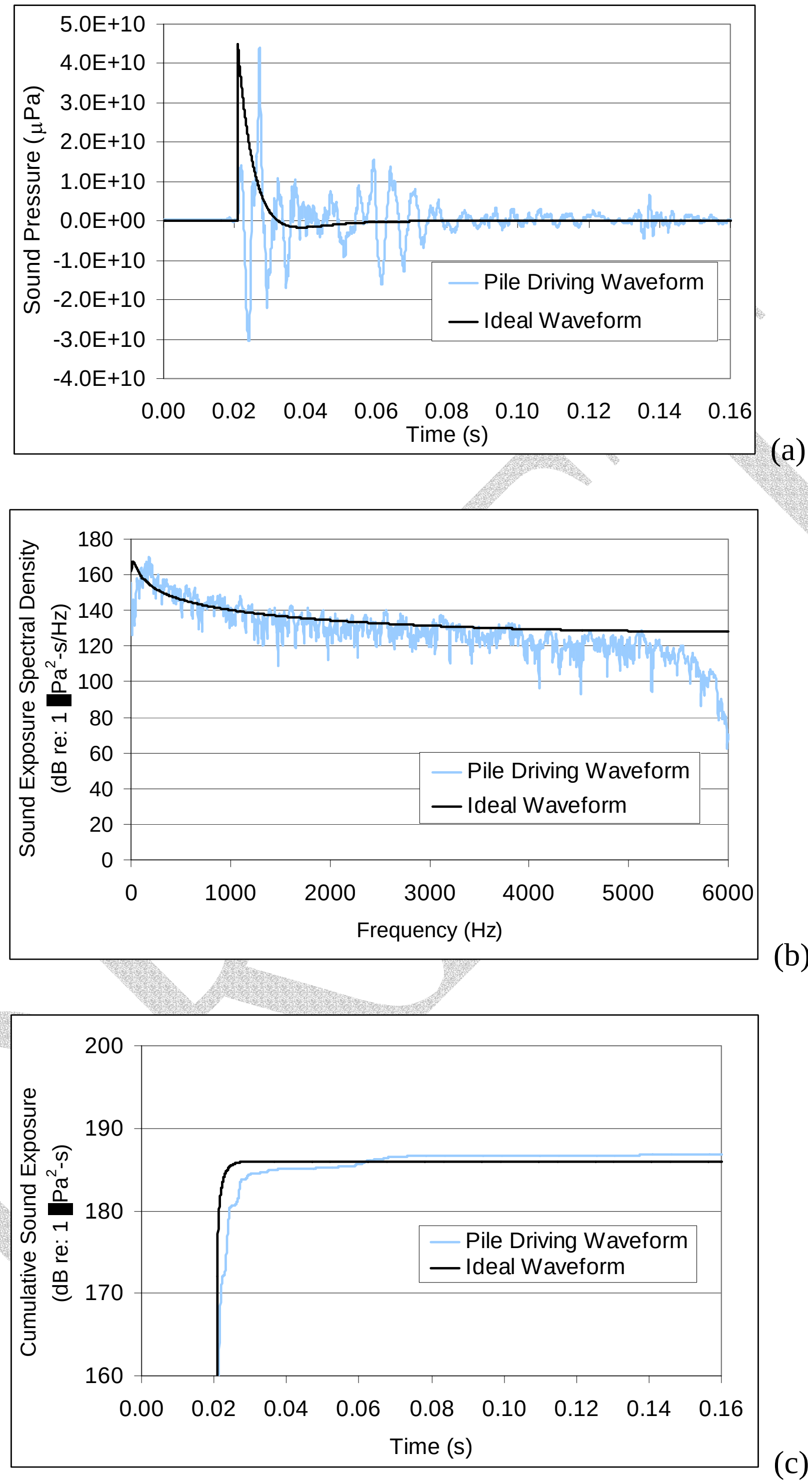

Figure 7: An ideal impulse wave, based on the Friedlander model, captures the major (a) temporal, (b) spectral, and (c) cumulative sound exposure characteristics of a real pile driving impulse. These types of analyses could be used to relate existing blast and sonic boom animal effects data to assess the impact of pile driving sounds on fishes, and to investigate the effects of shaping the pile driving sound pulse.

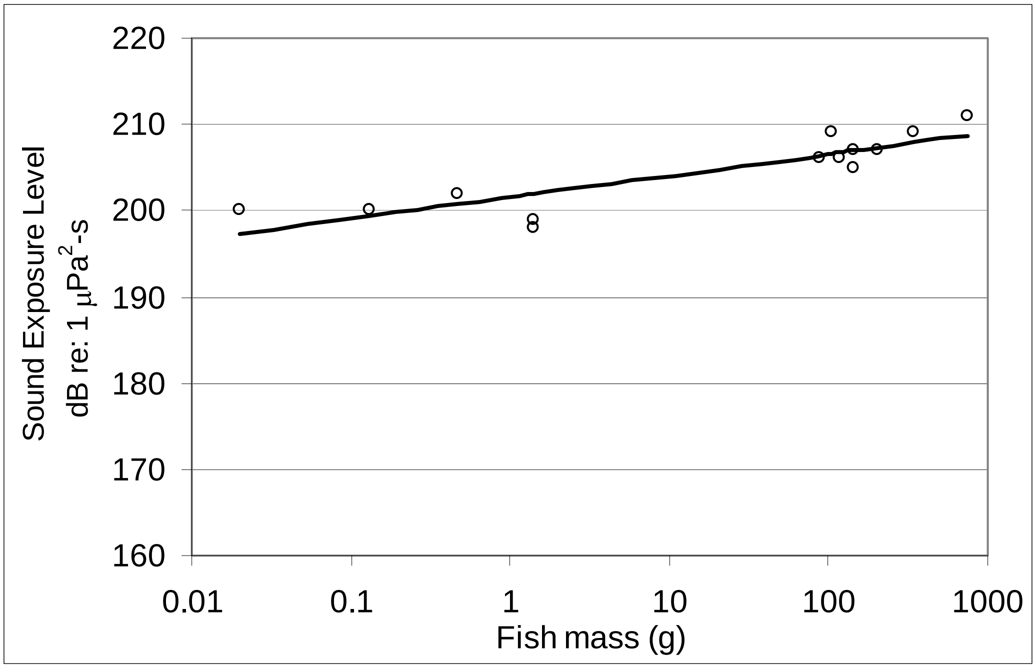

Figure 8: Estimated sound exposure level (SEL) that results in 50% mortality based on data from Yelverton et al. (1975) modeled as an ideal impulse wave (Friedlander waveform as described by Hamernikk and Hsueh 1991).

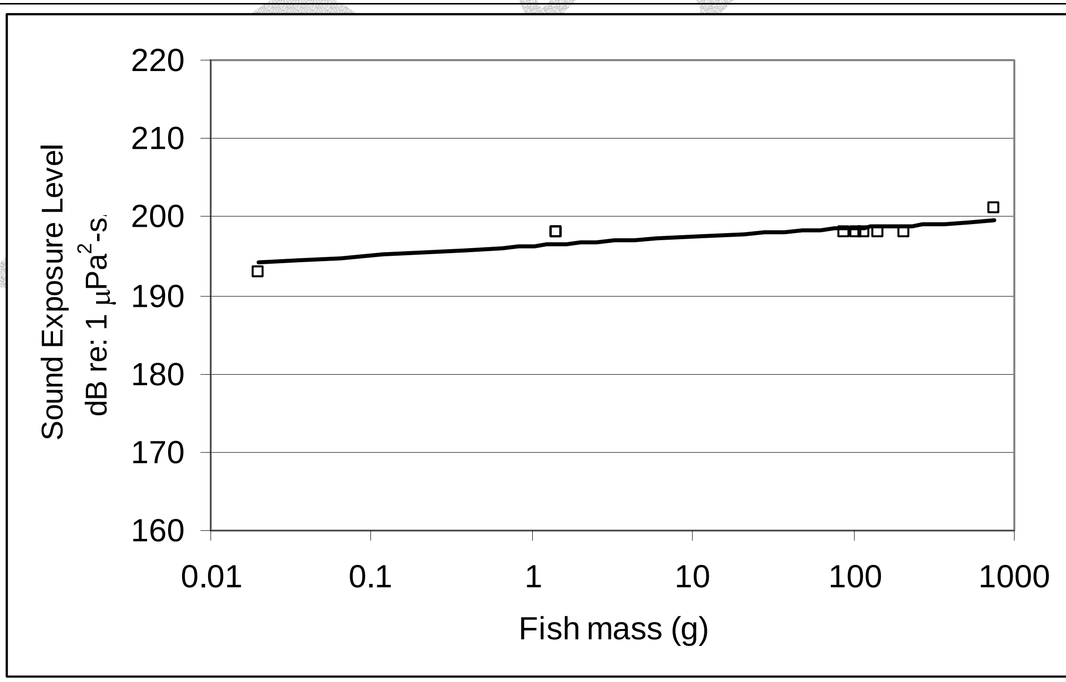

Figure 9: Estimated sound exposure level (SEL) that results in no injury to fishes based on data from Yelverton et al. (1975) modeled as an ideal impulse wave (Friedlander waveform as described by Hamernikk and Hsueh 1991).

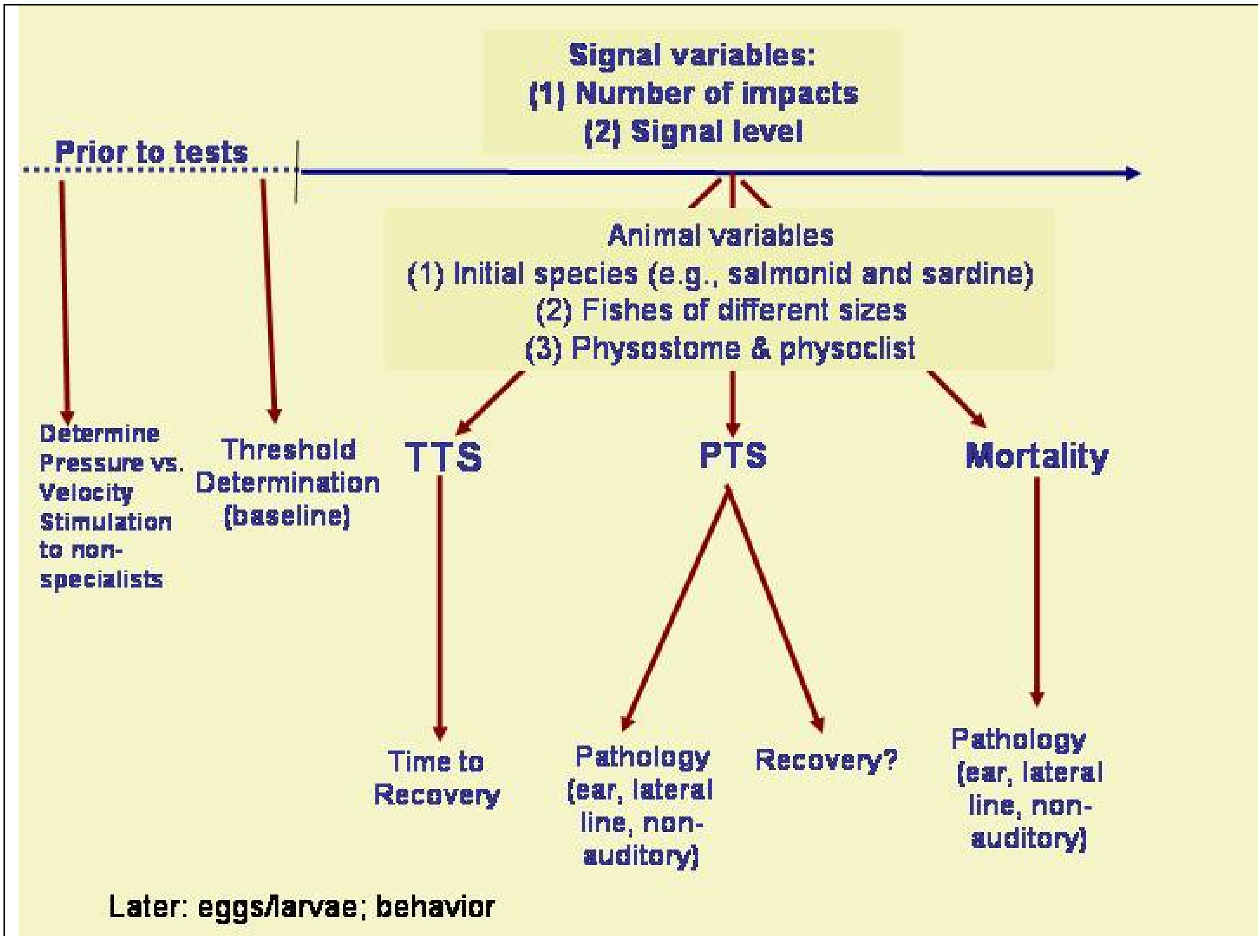

Figure 10: Diagrammatic representation of the interaction between the different proposed biological (fish) experiments. While eggs/larvae are considered for later study, they could potentially be included in some of the students proposed. Behavioral studies listed for later include areas that range from changes in response to predators to reproductive behavior and general survival. [nb: this will be upgraded in next draft and lateral line included as a point of study, in addition to further material discussed at the meeting.]