PATHOLOGICAL EXAMINATION OF FISH EXPOSED TO EXPLOSIVE BASED INSTANTANEOUS PRESSURE CHANGE

by

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ABSTRACT

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Oil and gas exploration in Northern Canada uses explosive-based seismic techniques to locate hydrocarbon reserves beneath waterbodies not frozen to the bottom. The use of explosives in, or near, waterbodies has the potential to harm fishes, primarily through instantaneous pressure changes (IPCs) generated from the detonations. These IPCs can damage soft tissues through the rapid compression and expansion of the swimbladder as the pressure wave passes. In Canada, a document entitled *Guidelines for the Use of Explosives In or Near Canadian Fisheries Waters* recommends that peak pressures not exceed 100 kPa for the protection of fish, however damage has been reported below this level. To simulate seismic exploration and examine potential pathological changes surrounding the current *Guideline*, fish across different developmental stages and with varying degrees of swimbladder presence were exposed to a variety of explosive based IPC levels in field experiments. Early life stages of rainbow trout (*Oncorhynchus mykiss*) including eyed eggs, sac fry, and juveniles were caged and exposed to discrete detonations from 0 to 280 kPa in the Mackenzie Delta, NWT. These fish were subsequently examined for both gross pathological and histological changes to cranial structures as well as swimbladder, kidney, liver and gill tissue. Results showed changes in both the area and circumference of the cranial region of eyed eggs, as well as swimbladder, ocular and kidney damage in juveniles. Additionally, caged adult swimbladder bearing lake trout (*Salvelinus namaycush*) and non-swimbladder bearing

slimy sculpin (*Cottus cognatus*) were exposed to explosive based IPCs ranging in peak pressure from 0 to 127 kPa at the Experimental Lakes Area, Ontario. Fish were later examined grossly and blood, liver, kidney, intestine, and spleen were examined to determine the presence of any traumatic based pathological changes. Results indicated the occurrence of swimbladder hemorrhage in lake trout exposed to IPCs near the current *Guideline* level. Finally, a risk assessment for lake trout of the Mackenzie Delta exposed to IPCs was undertaken; to examine the potential for adverse risk to individuals and populations, and the likelihood of populations being unable to recover. Based on the findings of the aforementioned studies, the recommended *Guideline* level is not protective of early life stages of rainbow trout and furthermore represents the threshold at which damage to the swimbladder in adult lake trout does not occur, as such, a re-examination of the recommended *Guideline* level is warranted.

PREFACE

The following thesis represents a collection of manuscripts (Chapters 2, 3 and 4) that have either been published or are intended for submission to scientific journals for publication. As a result, certain redundancies in material may occur. All chapters within the following document were authored by Danielle R. Godard.

Chapter 2:
Godard DR, Peters L, Evans R, Wautier K, Cott PA., Hanna B. and Palace V. 2009. Development of histopathology tools to assess instantaneous pressure change-induced effects in rainbow trout (*Oncorhynchus mykiss*) early life stages. *Environmental Studies Research Funds Report*. 164: iv + 88 p.

Chapter 3:
Godard DR, Bradley J Park, Vince P Palace. Pathological assessment of adult lake trout (*Salvelinus namaycush*) and sculpin (*Cottus cognatus*) exposed to explosive based instantaneous pressure change (IPC). *Journal of Aquatic Animal Health.* In preparation May 2010.

Chapter 4:
Godard DR, Cott PA, Hanson ML. Risk Assessment for Lake Trout (*Salvelinus namaycush*) of the Mackenzie Delta Exposed to Explosive Based Instantaneous Pressure Changes. *Human and Ecological Risk Assessment*. In preparation September 2010.

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TABLE OF CONTENTS

TABLE OF FIGURES

1 INTRODUCTION

1.1 Explosives in the Aquatic Environment - Oil and Gas Exploration

Explosives within the aquatic environment have a wide variety of applications including ice management, channel and harbour construction, the decommissioning of offshore structures, as well as removal of navigation obstructions (Houghton and Munday 1987, Keevin 1998, Lewis 1996, Wiley et al. 1981, Wright 1982, Viada et al. 2008). Explosives are also used to generate seismic waves for oil and gas exploration. In fact, the use of explosives for oil and gas exploration has recently seen resurgence in Canada's Arctic region. Since 2000, seismic exploration within the Mackenzie Delta area of the Northwest Territories has reached levels not seen since the 1970s and early 1980s (Cott et al. 2003, Cott and Hanna 2004). As explained by Cott et al. (2003), previously discovered hydrocarbon reserves in the NWT remain undeveloped but demands for oil and gas have prompted new interest in exploration and development. With depleting reserves in Western Canada as well as the limited ability of Atlantic Canada to meet increased demand, the NWT is set to become a major exporter of these resources (Cott et al. 2003).

1.2 Locating Hydrocarbon Reserves

Many of the exploratory programs within the Arctic are winter based and, as such, explosives serve as energy sources to gather information on hydrocarbon reserves underneath waterbodies not frozen to the bottom (Cott et al. 2003, Cott and Hanna 2004). The gathering of this information occurs via registering shock waves, also referred to as

sound waves, generated by the explosives (Rasmussen 1964). These shock waves reflect from the underlying sediment layers of a waterbody and return to the surface where they are processed and translated into data. These data are then used to create a profile of the geology underneath the substrate, and highlight areas where potential hydrocarbon deposits exist (Falk and Lawrence 1973, Fitch and Young 1948, Fry and Cox 1953, Hubbs and Rechnitzer 1952, Kearns and Boyd 1965, McCauley et al 2000, Rasmussen 1964, Rulifson and Schoning 1963, Wright 1985). The depth and character of the sediment layers are determined by measuring the frequency of reflected seismic waves as well as the time it takes for waves to travel to and from the reflecting layers (Falk and Lawrence 1973, Rasmussen 1964, Rulifson and Schoning 1963). Refraction surveys provide information on seismic waves travelling horizontally through substrata levels. They are used for geological mapping. Reflection surveys however, gather information on seismic waves travelling vertically through sub-strata levels and are predominantly used by the oil industry (Gausland 2003, Kearns and Boyd 1965).

1.3 Anatomy of an Explosion

Within the context of this thesis, an explosive is a chemical compound which, upon detonation, creates a compression wave having an almost instantaneous rise time to a peak pressure followed by decay to below ambient pressure (Wright 1982, Wright and Hopky 1998). Explosives therefore, generate instantaneous pressure change (IPC).

Pressure waves are produced in water when an explosive charge is within the watercolumn or when the shot is beneath or adjacent to the body of water (Keevin and Hempen 1997). Furthermore, the detonation of an explosive underwater has a characteristic waveform. The waveform signature is composed of two parts, the primary

pulse (which in the context of the current text will refer to the initial shock wave followed by the rarefaction wave) and the secondary (or bubble) pulses (Sverdrup et al. 1994, Viada et al. 2008).

1.3.1 Primary Pulse

Upon initial detonation of an explosive, the solid charge gets converted to a dense gas (MacLennan and Simmonds 1992, Keevin and Hempen 1997, Rasmussen 1964, Viada et al. 2008, War Report 1949). The gaseous product of the detonation (a gas bubble) then suddenly starts to expand, compresses the water around the charge, and results in a shock wave (War Report 1949). This shock wave consists of a shock front, characterized by an almost instantaneous rise to a peak pressure followed by an equally instantaneous exponential decay in pressure (Cronin 1948, Falk and Lawrence 1973, Hill 1978, Hubbs et al. 1960, Keevin and Hempen 1997, Lavergne 1970, MacLennan and Simmonds 1992, Viada et al. 2008, War Report 1949, Wiley et al. 1981). The initial shock wave is known as a compression (or positive) pressure wave. It travels a speed greater than that of sound wave (MacLennan and Simmonds 1992). This shock wave eventually weakens and slows down to the sonic velocity of the medium, at which point it reaches pressure levels where it acts like a normal sound wave (Keevin and Hempen 1997, Viada et al. 2008).

The exponential decay of the initial shock wave continues up until the time a surface reflection wave arrives, at which point there is a sharp drop to a pressure below ambient hydrostatic pressure (Gaspin 1975, Gaspin et al. 1976, Wiley et al. 1981). This surface reflected wave is known as a rarefaction (or negative) pressure wave. The rarefaction wave assumes the opposite sign of the initial compression wave, and is

generated from the seismic shock wave reflecting off of the water surface, back into the water column. This reflected wave has as a negative pressure pulse and causes a drop in pressure (Cronin 1948, Christian 1973, 1974, Falk and Lawrence 1973, as described in Hubbs and Rechnitzer 1952, Hubbs et al. 1960, Trasky 1976). To summarize, an explosion generates a wave of positive pressure, which instantaneously decays and is followed by a negative pressure wave (Wiley and Wilson 1975).

A typical IPC waveform, generated as a mean composite of the measured pressure of three hydrophones during Chapter 2 of this study, is presented in Figure 1.1. As demonstrated in the figure, peak pressures typically occur in a period of milliseconds (Hill 1978, Keevin and Hempen 1997)

1.3.2 Bubble Pulse

Though the shock wave created at the onset of the explosion was created by the formation of a gas bubble, it in fact travels faster than that bubble (War Report 1949). However, the gas bubble, created at the point of detonation continues, to contract and expand and oscillate in size, owing to the surrounding water pressure (MacLennan and Simmonds 1992, Rasmussen 1964, Viada et al. 2008). Figure 1.2 demonstrates the bubble pulses generated following a primary pulse. The oscillatory action of the gas bubble transmits secondary shock waves of considerably lower peak pressures than the initial shock wave (Hill 1978, Lavergne 1970, Rasmussen 1963, Sulfredge et al. 2001). The gas bubble oscillates in size until the gas sphere breaks the surface of the water, or until it gradually diminishes in intensity to the point that it is damped out (Keevin and Hempen 1997, Lavergne 1970, Sulfredge et al. 2001). This is most likely what occurs in the case of under ice detonations. In open water, where the blast is shallow and located

near the air surface, blasts will have no oscillating bubble and the gases will be vented to the air (Keevin and Hempen 1997).

While it has been said that the bubble-wave pulse lasts for a long time period, has a low maximum pressure, and is not highly destructive to fish (Hill 1978, Hubbs and Rechnitzer 1952, Rasmussen 1964), it has been argued that the recurring pressure waves of the pulsating bubble may contribute significantly to physical damage (Viada et al. 2008).

1.4 Alternative IPC Sources

The characteristics of explosives that produce gas bubbles are similar, yet they vary in the amount and rate of energy release which affects the amplitude of the shock front (MacLennan and Simmonds 1992). To illustrate, Hubbs and Rechnitzer (1952) conducted experiments using dynamite, hercomite and black powder to determine the effects of underwater explosions on fish. Contrasting results were found between dynamite and black powder. In experiments conducted with black powder, a modest number of fish fatalities occurred when compared to dynamite charges that were damaging or fatal to fish in most cases. This was attributed to the fact that black powder burns more slowly, does not produce a shock wave with an abrupt front (rather a slow development with a rounded front) , and has a lengthened pressure pulse interval, as opposed to dynamite (Hubbs and Rechnitzer 1952, Rulifson and Schoning 1963). It was also attributed to the fact that the peak intensity (both positive and negative) is not nearly as high as that of dynamite, but rather of smaller amplitude (Hubbs and Rechnitzer 1952, Rasmussen 1964). Lavergne (1970) supports this rationale, and explains that that slow explosives, such as black powder, generate shock waves having longer pressure duration

and smaller peak pressures as compared to high explosive charges, such as TNT. Similarly, Keevin and Hempen (1997) describe that some of the difference in effects on fish between black powder (a low explosive) and high explosives seems to be related to the waveform produced by each explosive source, with black powder producing a pressure waveform having slow rise time and low amplitude, as opposed to high explosives with an abrupt rise time, high amplitude and short frequency.

Rate of pressure change is the main factor determining the effect on fish (Tsvetkov et al. 1972); therefore, slow explosives, with slow rising times to maximum pressure, will not generate the same damage responses as explosives that generate instantaneous pressure change, and as such, will lessen impacts to fish (Goertner et al. 1994, Rulifson and Schoning 1963). Paterson and Turner (1968) explain that a slower increase in pressure within an organ such as the swimbladde will result in a decrease in the possibility of damage or rupture. Moreover, Rulifson and Schoning (1963) (based on the work of Hubbs and Rechnitzer 1952) demonstrate that dynamite peak pressures between 276 and 483 kPa kill fish as opposed to pressures from black powder of 855 to 1103 kPa that kill no fish.

Slow explosives are noted for generating poor seismographic records (Rulifson and Schoning 1963), as abrupt waves of great pressure intensity produce better quality seismographic records (Kearns and Boyd 1965). However, the opposite has also been reported, that shockwaves with broader peaks and lower amplitudes, such as air guns and black powder, do produce good seismic records (Trasky 1976).

1.5 Trauma to Fish Exposed to Explosive Based IPC

1.5.1 Anatomy of the Swimbladder

The swimbladder is a soft-walled sac located above the gut and just below the spinal column (Willmer et al. 2000) (Figure 1.2a). It occupies approximately 4-5 percent of the body volume in marine teleosts (Pough et al. 1999; Steen 1970; Willmer et al. 2000) and 7 percent of the volume in freshwater teleosts (Pough et al. 1999). In some species the organ may be non-existant (Bishai 1961, Christian 1973, Falk and Lawrence 1973, Wright 1982, Yelverton et al. 1975). The swimbladder may either have an open connection via a small duct to the alimentary canal (physostomous) (Figure 1.2b), or be closed to the alimentary canal (physoclistous) (Figure 1.2c). Physoclistous species are known to have specialized regions including the gas gland, the rete mirabile and the ovale, all of which work aiding in buoyancy control. The gas gland is responsible for blood acidification (Pelster 2004), while the rete mirabile is a long and highly ordered network of blood capillaries responsible for moving gas, mainly oxygen, from the blood to the gas bladder (Berenbrink et al. 2005; Pough et al. 2002; Willmer et al. 2000) and for enabling blood gases to accumulate at high concentrations so that they can diffuse into the swimbladder (Willmer et al. 2000). Finally, the ovale is responsible for the diffusion of gas back into the bloodstream through the resorbing section of the swimbladder (Pelster 1998).

The swimbladder has a major function in enabling fish to regulate their buoyancy (Campbell and McLean 1994, Falk and Lawrence 1973, Fänge 1983,Yelverton et al. 1975), but also has known functions (depending on the species of fish) as a respiratory organ (Helfman et al. 1997, Datta Munshi and Hughes 1992), as an accessory auditory

organ (Popper and Platt 1993), and as an organ used for sound production (Ladich and Fine 2006; Tavolga 1971).

1.5.2 The Swimbladder as a Primary Site of Damage

Due to the sensitivity of the air filled swimbladder to extreme pressure change, it is the primary site of damage in fish (Wright and Hopky 1998). Several studies have demonstrated that the swimbladder is a key organ that can be damaged by detonations from seismic activity (Aplin 1947, Coker and Hollis 1950, Cronin 1948, Falk and Lawrence 1973, Ferguson 1962, Fitch and Young 1948, Hubbs and Rechnitzer 1952, Kearns and Boyd 1965, Linton et al 1985, Roguski and Nagata 1970, Settle et al. 2002, Wiley et al. 1981, Wright 1982, Yelverton et al 1975). During a series of underwater explosions in Northern Canada, Paterson and Turner (1968) reported that mortality occurred following detonation of 60% carbonitronitrate explosive (peak pressure not reported) in species possessing a swimbladder including burbot (*Lota lota*), lake whitefish (*Coregonus clupeafomis*), trout-perch (*Percopsis omiscomaycus*) and cisco (*Coregonus artedii*). Similarly, Cronin (1948) examined the traumatic effects of explosive detonation in fish, notably trout (*Cynoscion regalis* [Bloch and Schneider]) and rock (*Roccus saxatilis* Walbaum), exposed to TNT/nitramon charges varying in peak pressure from approximately 620- 4544 kPa and determined that one of the main organs affected was the swimbladder. Kearns and Boyd (1965) studied the effect of a marine seismic exploration on fish populations in British Columbia coastal waters. Following a series of detonations of nitrone seismic marine (S.M.) (of unknown peak pressure) the researchers reported that the most commonly damaged organ was the swimbladder. Finally, Linton et al. (1985) examined the extent of injury in red drum (*Sciaenops*

ocellatus) and black drum (*Pogonias cromis*) following exposure to Primacord explosive (peak pressure levels not indicated). The authors reported that in addition to kidneys, swimbladders were the most frequently damaged organ in the fish.

1.5.3 Fish Lacking a Swimbladder

It is generally accepted that fish with gas filled swimbladders are more vulnerable to explosions than those without (Aplin 1947, Bishai 1961, Christian 1973, 1974, Fitch and Young 1948, Goertner et al. 1994, Gaspin 1975, Ogawa et al. 1978, Paterson and Turner 1968, Rasmussen 1964, Roguski and Nagata 1970, Rulifson and Schoning 1963, Settle et. al 2002, Wright 1982). Following exposure to pentolite charges, Hogchokers (*Trinectes maculatus*), a species that lack a swimbladder, were unaffected while spot (*Leiostomus xanthurus*) and white perch (*Morone americana*), both possessing swimbladders, were affected (Gaspin 1975). Pressures within the study varied from approximately 200 kPa-2861 kPa (Gaspin 1975). Hogchokers were also extremely tolerant to underwater explosions from cylindrical cast pentolite charges with maximum pressures between 951 and 1303 kPa (Gaspin et al. 1976). Aplin (1947) found that sculpin (*Scorpaena guttata*) and cabezone (*Scorpoenicthys marmoratus*), two species lacking a swimbladder, showed no damage following exposure to explosive detonations of 60% petrogel in 10 pound sticks (pressure level not indicated). Goertner et al. (1994) conducted a study using hogchokers (*Trinectes maculates*) and summer flounder (*Paralichthys dentatus*), both fish lacking a swimbladder, and spot (*Leiostomus xanthurus*), a species with a swimbladder. Following detonation of pentolite charges (pressure not indicated), all spot were killed while none of the hogchockers or flounders sustained injury. The researchers explained that the lack of significant gas cavities within

the hogchokers is probably the reason for their lack of vulnerability to underwater explosions. Finally, Kearns and Boyd (1965) reported that a 50 pound charge of nitrone S.M (Seismic Marine) had sufficient force to remove shellfish from rocks and split large boulders, but it had no effect on lingcod (*Ophiodon elongatus*) and sculpin (species not identified), both species lacking a swimbladder, that were found 15.2-30.5m away from the shot point.

1.5.4 Reaction of the Swimbladder to IPC

The instantaneous generation of a peak positive pressure and subsequent negative peak pressure wave, immediately following the detonation of an explosive, triggers oscillation of the organ (Wiley et al. 1981) resulting in potential damage. Muir (1959) demonstrated the physical reaction of the swimbladder in fish exposed to positive and negative pressure changes. Using an apparatus designed to subject fish to pressures above and below atmospheric level, Muir (1959) exposed the internal organs of four dead cohoe salmon (*Onchorhynchus kisutch*) and clamped the front ends of the swimbladders of the fish. Three slow cycles of pressure were applied (between 0 and 414 kPa). As the pressures increased, the volumes of the swimbladders decreased. Subsequently, partial vacuum was slowly applied. With this, the swimbladders distended and in all cases ruptured. Figure 1.3 illustrates the response of a swimbladder to an underwater blast. Though swimbladders are able to respond to a wide array of pressure changes, when exposed to pressures beyond what they are able to accommodate, or at rates beyond what they are able to accommodate, the swimbladder can experience trauma ranging from slight tissue strain, to complete rupture (Alaska Department of Fish and Game 1991).

1.5.5 Effects of Negative Pressure on the Swimbladder

Specifically, the negative pressure produced during a blast is deleterious to fish and the prime cause of damage to the swimbladder is its expansion from the sudden reduction in pressure (Gaspin 1975, Hill 1978, Hubbs and Rechnitzer 1952, MacLennan and Simmonds 1992, Rasmussen 1964, Wiley 1981, Wright 1982). Christian (1973, 1974) explains that the negative pressure associated with explosions are more damaging than the shock waves and that fish are especially sensitive to tension due to the swimbladder's vulnerability to overextension. Although swimbladders may be able to adjust to a wide variety of changes in volume and pressure gradients in nature, unnatural stresses (such as explosives) into the system can cause the bladder to be overexpanded and burst (Christian 1973, 1974). Muir (1959) and Christian (1973) relate that when fish are unable to release gas from the swimbladder under conditions of negative pressure, the walls of that organ become susceptible to rupture from the tensile stress induced by the expansion of gases (Muir 1959, Christian 1973). The aforementioned is demonstrated in a study simulating changes of hydrostatic pressure from turbines, in which death from pressure injury occurred after abrupt discharge from a state of increased pressure. A pressure discharge of 304kpa/sec, following a state of increased pressure, resulted in 100% mortality in roach fingerlings [*Rutilus rutilus* (L.)] while 40-72% mortality was observed at 10.1-50.7kpa/sec (Tsvetkov et al. 1972). The authors concluded that not only were faster rates of negative pressure exertion most injurious to these fish, but death occurred subsequent to rupture of swimbladder walls due to a sizeable and sharp increase in swimbladder volume from abrupt discharge of increased pressure.

The effect of negative pressure on swimbladders is most commonly associated with swimbladders exhibiting tears with the edges turned outwardly (Rasmussen 1964). Blood from broken blood vessels may be expelled into the abdominal cavity and holes within swimbladders turned outward, indicating that rupture occurred from an increase of pressure within the organ, rather than from external forces, in which case blood would be found in the lumen of the swimbladder (Cronin 1948, Hubbs and Rechnitzer 1952, Kearns and Boyd 1965). Teleki and Chamberlain (1978) confirm that common injuries in fish (see Table A1) exposed to blasts between 8.61 kPa and 158 kPa were a function of not only the compression but also the rarefaction wave, highlighted by swim bladders of fish burst outward, indicating a negative pressure effect. In a caged fish experiment undertaken in the Bow River during construction of the Alaska Highway Gas Pipeline in Alberta, Fernet (1982) noted that swimbladders of rainbow trout (*Salmo gairdneri*) exposed to 60% Geogel appeared to have burst outward indicating negative pressures as the causative factor. Hubbs and Rechnitzer (1952) confirm the outward explosion of air bladders with the sudden application of negative pressure. Finally, though Thompson (1958) reported that four specimens of Rockfish (*Sebatodes spp*.) had their swimbladders broken inwards following detonation of DuPont Nutramex 2-H explosive (peak pressures not indicated), the author went on to later indicate that two fish had their swimbladders blown outwards, and proposed that it is an effect of rarefaction wave and resulting expansion of the organ.

Above all, the effect of decompression on a fish is thought to be determined by: the form of the swimbladder, the tensile strength of the swimbladder, the resistance that the body wall and internal organs offer to the expansion of the swimbladder, and the

percentage volume of the swimbladder gas relative to the ambient pressure (Simenstad 1973).

1.5.2 Damage to Other Organs

The detonation of explosives has been well documented to cause injury and mortality in fish. Blasting effects reported in the literature include external trauma, broken bones, rupture, protrusion and hemorrhaging of organs, as well as death. A summary is provided in Table A1. The oscillatory response of the swimbladder as the pressure wave of an IPC passes has been linked with internal trauma in fish (Fernet 1982, Goertner 1978, as described in Govoni et al 2003; Keevin 1997, Wiley et al 1981). More specifically, the rapid expansion of the swimbladder during sharp drops in pressure can greatly compress internal organs (Fernet 1982, Tsvetkov et al. 1972) adjacent to the swimbladder causing injury. Such internal damage has been described in tissues both surrounding and in contact with the swimbladder (Houghton and Munday 1987, Tyler 1960).

1.5.3 Factors Influencing Extent of Trauma in Fish

Responses to IPC may vary as a result of a multitude of factors. Responses to IPC may vary among fish species with differences in body rigidity and swimbladder thickness. Cronin (1948) reports that rock (*Roccus saxatilis* Walbaum), being sturdy with firm flesh, are more susceptible to instant death in contrast to trout (*Cynoscion regalis*), a softer bodies species, that show a higher percentage of severe injury rather than death. The response of the trout is attributed to soft flesh that may receive shock waves with less immediate damage (Cronin 1948). In the same way, fish with more flexible bodies,

including catfish (*Ictalurus catus*) and toadfish (*Opsanus tau*) are probably more resistant to damage because their bodies are able to cushion internal organs from rapid fluctuations in swimbladder size (Gaspin et al. 1976, Wiley et al. 1981). Further to body rigidity, thickness of swimbladders may influence the incidence of damage to the organ. Falk and Lawrence (1973) suggest reduced damage to the swimbladder arising from pressure changes in fish with thicker walled swimbladders. Fitch and Young (1948) report that fish such as the barracuda (*Sphyraena argentea*), kingfish (*Genyonemus lineatus*), and queenfish (*Seriphus politus*) tend to have thick-walled swimbladders that are more resistant to pressure than fish, such as the salt-water perch (*Brachyistius frenatus*), which have thinner swimbladders. Trasky (1976) relays that fish with thin walled swimbladders are more subject to damage than those with thicker walls. Finally, during a study evaluating effects of pentolite explosives and spot (*Leiostomus xanthurus*) and white perch (*Morone americana*) in the Chesapeake Bay, Wiley and Wilson (1975) describe that white perch were less damaged than spot owing to their strong construction, including thicker walls within the swimbladder.

Differences in species susceptibility have also been linked with fish shape. The laterally compressed bodies of the fish in the study of Teleki and Chamberlain (1978) exposed more surface area to oncoming pressure fronts and were found to be the most sensitive to a blast as opposed to the fusiform bodied fish that were least affected. Roguski and Nagata (1970) highlight that fish with larger surface areas absorb more shock energy than smaller fish and, therefore, tend to be more greatly injured. Finally, Fitch and Young (1948) and Alaska Department of Fish and Game (1991) explain that

species such as barracuda, kingfish, queenfish are cylindrical bodied and, as such, are more resistant to pressure than laterally compressed fish.

Basic internal anatomy differences among fishes can affect the responses to IPCs, including both swimbladder affixation and swimbladder form. For instance, rupture could be observed at several points along the ventral surface of the swimbladder in fish with a more fixed swimbladder, such as rock (*Roccus saxatilis* Walbaum), while those with less surface attachment, such as croaker (*Micropogon undulates* Linnaeus), weakfish (*Cynoscion regalis*), and spot (*Leiosomus xanthurus* Lacépède) tended to show a single lengthwise split (Cronin 1948). In addition to swimbladder affixation, swimbladder form is also a factor in fish response to IPC. There are differences in the effects of IPCs between physoclists and physostomes. Physoclists are unable to quickly discharge excess gas from the swimbladder during excess pressure events while physostomous fish adapt more readily (Tsvetkov et al. 1972). Teleki and Chamberlain (1978) noted that physoclistic fish (including pumpkinseed [*Lepomis gibbosus*], white bass [*Morone chrysops*] and crappie [*Pomoxis sp*]) were the most sensitive to the blast as opposed to the physostomes of their study (rainbow trout [*Salmo gairdneri*], white suckers [*Catostomus commersoni*], and yellow bullheads [*Ictalurus natalis*]), which were the least affected by the blast. In terms of physostomes, Hogan (1941) showed that among fish exposed to experimental vacuum pressures between 50.8 kPa and 91.43 kPa, physostomous species were capable of quickly releasing air from their swimbladder and were better able to withstand the treatments than physoclistous fish. To note however, pressures were not instantaneous in nature but varied between exposures of 10-55 seconds. Trasky (1976) and Falk and Lawrence (1973) relay that fish possessing and open swimbladder are not as

sensitive as those with a closed swimbladder since they may compensate for the pressure change. Although physoclists and physostomes may be differently affected upon exposure to explosive IPC, Christian (1973) speculates that near the lethal zone, physostomes may be more resistant to pressure change but that under conditions of severe shock both swimbladder types would suffer equal damage. Differences in swimbladder infrastructure may not even be relevant because the pressure changes during an explosion take place within microseconds, proving too rapid for normal gas exchange mechanism to function (Christian 1973). In support of this, Yelverton et al. (1975) found little or no difference in the impulses needed for an LD_{50} between fish with ducted versus non-ducted swimbladders exposed to high explosive charges.

Differential placement of seismic sources can have an impact on fish. Lavergne (1970) suggests that surface charges can cause more damage to fish, while Kearns and Boyd (1965) report that detonations originating at depth are more damaging to fish than those at shallow depths. Of note, often charges are not placed within the water column but are buried. The *Guideline,* in fact, provides charge burial depth suggestions to avoid exceeding the 100kPa threshold, as shock waves generated by explosives within the water have a greater lethal effect than waves propagated from the ground into the water as they exhibit a sharper pressure-time signature (Alaska Department of Fish and Game 1991).

Apart from the purely physical considerations of shock waves in waterbodies and how their strength may be affected by depth, there are also reports that fish at the surface or at depth can be differentially affected. Specifically, fish in deeper water at the time of detonation may be less vulnerable than fish nearer to the surface (Christian 1973, Coker and Hollis 1950, Wiley et al 1981). Hubbs and Rechnitzer (1952) reported that fish, held

in cages at the surface (including anchovy [*Engraulis mordax mordax* Girard], jack mackerel [*Trachurus symmetricus* Ayres], kingfish [*Genyonemus lineatus* Ayres], sardine *Sardinops caerulea* Girard], grunion [*Leuresthes tenuis* Ayres], pompano [*Palometa simillima* Ayres]) and free-swimming fish near the surface suffered more damage than fish at the bottom (including anchovy [*Engraulis mordax mordax* Girard], sardine *Sardinops caerulea* Girard], jack mackerel [*Trachurus symmetricus* Ayres], kingfish [*Genyonemus lineatus* Ayres], pacific mackerel [*Pneumatophorus japonicus diego* Ayres], slim midshipman [*Porichthys myriaster* Hubbs and Schultz], pipefish [*Syngnathus californiensis* Storer], shiner seaperch [*Cymatogaster aggregate* Gibbons]). These fish were exposed to 60 percent gelatine dynamite at blast levels varying from 297 kPa to 1117 kPa. Similarly, Arctic cisco (*Coregonus autumnalis*) held in cages at the surface (3 ft.) were more susceptible to injury than those caged near the bottom (7 ft.) when exposed to high explosives Aquaflex and Geogel (Falk and Lawrence 1973). Pressure measurements were not indicated in the aforementioned study. Moreover, Ferguson (1962) documented that, following exposure to a variety of explosive combinations (see Table A1) of unknown peak pressure, swimbladder injuries were greater among yellow perch (*Perca flavescens*) caged at depths of 10 feet from the surface compared to perch exposed in bottom cages, 42 or 58 feet from the surface. In keeping with the aforementioned studies, Kearns and Boyd (1965) evaluated the effects of nitrone S.M explosives on a variety of fish (see Table A1) during a marine seismic exploration in British Columbia. Following detonations of the explosive the researchers reported that the incidence of fish mortality is greater in shallow water. Linton et al. (1985) however reported conflicting results noting survival of black drum (*Pogonias*

cromis) was greatest among cages held at the surface following exposure to underdetermined peak pressure blasts of Primacord explosive. In fact, Wiley et al. (1981) suggests that at shallower depths, the bladder does not have time to respond fully to the positive portion of the wave and thus, at shallow depth fish are in effect protected from harm (Wiley et al. 1981)

Regardless of which direction body rigidity, swimbladder thickness, shape, anatomy, placement of charges or fish depth influences the impacts of IPCs on fish, interpreting and comparing results from different studies, and regulating the use of explosives for seismic exploration needs to consider the possibility that a multitude of factors may affect the impacts explosives and resultant IPCs have on fish.

A full list of factors influencing trauma in fish can be found in Table A2.

1.5.4 Pathological Evaluation

A body of literature exists investigating the effects of blasting on fish. The evaluation of effects in most of these studies is via gross pathological observation alone. Given that most studies have focused on gross examination, information gaps exist regarding more subtle effects. Recent evidence has shown that some sub-lethal effects of IPCs are detectable using histopathology (Godard et al. 2008, Govoni et al. 2003). In fact, following examination of trauma to juvenile fish subjected to submarine detonations, Govoni et al. (2003) reported that most trauma within their study could not have been adequately detected by gross anatomical examination. Instead, the trauma was observed histopathologically. Therefore, histological methods were used to evaluate damage to fish tissues in the current studies.

Evidence has also shown that damage to specific organs can also be detected by assessing changes in blood chemistry (Casillas et al. 1993). Interpretation of blood based chemistries in fish is not as advanced as in other areas of veterinary or human medicine (Harmes 2003). Routine methods for biochemical evaluation of mammalian blood however do appear to be useful for fish blood (Campbell 2004). Piscine based clinical research to assess and establish baseline blood chemistry values in various fish species (Asadi 2006, Çelik 2004, Edsall 1999, Manera and Britti 2006, Sakamoto et al. 2001, Shahsavani et al. 2008) has been used to determine the effects of such things as capture and handling stress, nutrition, starvation, temperature, water quality, infectious disease and toxicants on blood chemistry values (as reviewed by Bowser 1993, Bucher 1990, as reviewed in Chen et al. 2004, Congleton and Wagner 2006). Blood biochemistry in fish has also been used to evaluate tissue damage from toxicant exposure (Bernet et al. 2001, Chen et al. 2004, Folmar 1993, Young et al. 1994), bacterial infection (Grizzle and Kiryu 1993, Chen et al. 2004), viral infection (Řehulka 2003), algicides and antibiotics (Nelson et al. 1999) and trauma (Grizzle et al. 1992, Wagner and Congleton 2004) (as described by Congleton and Lavoie 2001 and Wagner and Congleton 2004). Few studies have evaluated changes in blood chemistry following exposure to blasts similar to those used for seismic exploration. The exception is a study by Sverdrup et al. (1994) examining the effects of seismic shock on stress hormones in Atlantic salmon (*Salmo salar*). The study describes and relates vascular endothelium injury and the impairment of stress hormone release. Most of the aforementioned studies used clinical chemistry to support histological evidence of tissue damage (Bernet et al. 2001, Chen et al. 2004, Folmar et al. 1993, Grizzle et al. 1992, Grizzle and Kiryu 1993, Nelson et al. 1999, Řehulka 2003,

Young et al. 1994). The evaluation of blood biochemistry in the current study was also used to assess any changes indicative of specific tissue damage between control and exposure groups.

To allow more in depth investigation, histology and clinical pathology served as an extension to gross observations and enabled detection of subclinical, more subtle, tissue damage.

1.6 Guidelines for Fish Protection

Prior to the resurrection of seismic exploration in the North, concern regarding negative impacts to fish from the use of explosives in the aquatic environment arose (Wright 1985). Consequently, in 1998 the Department of Fisheries and Oceans developed the *Guidelines for the Use of Explosives in or Near Canadian Fisheries Waters* proposing that, for the protection of fish, no explosive is to be detonated in or near fish habitat that produces, or is likely to produce and instantaneous pressure change greater than 100 kPa in the swimbladder of a fish (Wright and Hopky 1998). Other jurisdictions are more restrictive on the allowable IPC levels in fish bearing waters. For example, in Alaska, IPC levels have to remain below 18.6 kPa unless the waterbody and its substrate are frozen (Alaska Department of Fish and Game 1991; Alaska Department of Fish and Game 2009).

There is concern regarding the protective nature of the suggested *Guideline* IPC level. Experience in the NWT has shown that 100 kPa is not adequately protective of fish with developed swimbladders (Pete Cott, personal communication, November 2010). Moreover, numerous studies have found tissue damage and mortality in fish below the current protective *Guideline* level (Houghton and Munday 1987, McAnuff et al.1994,

Sakaguchi 1976, Teleki and Chamberlain 1978). It has been suggested that the *Guidelines* should be reevaluated (Cott et al. 2003).

1.7 The Study

The *Guideline* document serves as a directive for the conservation and protection of fish and is intended to prevent or avoid the destruction of fish (Wright and Hopky 1998). A detailed evaluation of the pathological effects surrounding the suggested *Guideline* level is needed as a means to ensure its effectiveness or as a means of aiding managers in refining the threshold that would offer protection for all affected life stages of fish species. Therefore, the objective of the current study was to evaluate pathological effects in fish at different life stages exposed to explosive instantaneous pressure changes surrounding the current recommended Canadian *Guideline* level. In order to accomplish this objective a series of studies were conducted.

Chapter 2 of this thesis examines tissue level effects on early life stages of fish exposed to explosive based IPCs surrounding the current *Guideline* level. A field experiment was conducted to examine the potential for histolopathologies to occur in early life stages of rainbow trout (*Oncorhynchus mykiss*) exposed to IPCs from 0 to 280 kPa. Larval and young fish have long been noted for their sensitivity to increases in pressure (Bishai 1961), explosive force (Coker and Hollis 1950) and pressure waves (Fitch and Young 1948). Tissues from IPC exposed eyed eggs, sac fry and juvenile fish exposed to the IPCs were examined microscopically to determine potential damage to craniofacial features as well as other internal tissues (e.g. liver, kidney, gill and swimbladder). Quantitative histopathology measures to specific soft tissues and organs were applied to improve objectivity of comparisons among groups of fish exposed to

different IPCs. Results of the study suggested potential differences in pathology as a function of degree of structural and swimbladder development and therefore examination of larger swimbladder bearing fish and fish lacking a swimbladder was warranted. To this end, Chapter 3 evaluates the pathological effects from explosive based IPC exposure in adult fish and compares effects in a swimbladder and a non-swimbladder bearing species. Lake trout (*Salvelinus namaycush*) and sculpin *(Cottus cognatus)* were exposed to explosive blasts from 0 to 127 kPa. Gross pathology and histopathology (liver, kidney, intestine) were assessed. Blood biochemical variables were also evaluated as markers of specific tissue damage and to supplement tissue histopathological analysis. With results from both Chapter 2 and Chapter 3 suggesting damage in young and adult swimbladder bearing trout near and below the *Guideline* level, an evaluation of risk to trout populations exposed to explosives during oil and gas exploration was necessary. A risk assessment for lake trout (*Salvelinus namaycush*) exposed to explosive based IPCs surrounding the current recommended *Guideline* level in the Mackenzie Delta is presented in Chapter 4. The Mackenzie Delta serves as an important area to assess, not only for being a region of noted seismic exploration, but also for being a region in which lake trout are valued as an important ecological and social component of the ecosystem that are likely to be exposed to explosive based IPC. The objective of the risk assessment was to determine risk to lake trout and lake trout populations exposed to explosive based exploratory events surrounding 100 kPa, through problem formulation, risk analysis and the characterization of risk, to be considered in the case that the *Guideline* document is re-evaluated.

The null hypothesis of Chapter 2 is that explosive based IPCs surrounding the current *Guideline* level are not harmful to early life stages of rainbow trout. The null hypothesis of the Chapter 3 is that the IPCs near the *Guideline* level are not harmful to adult lake trout or sculpin, a species lacking a swimbladder. Finally, the null hypothesis of Chapter 4 is that no risk exists to lake trout or lake trout populations near the 100 kPa *Guideline* level.

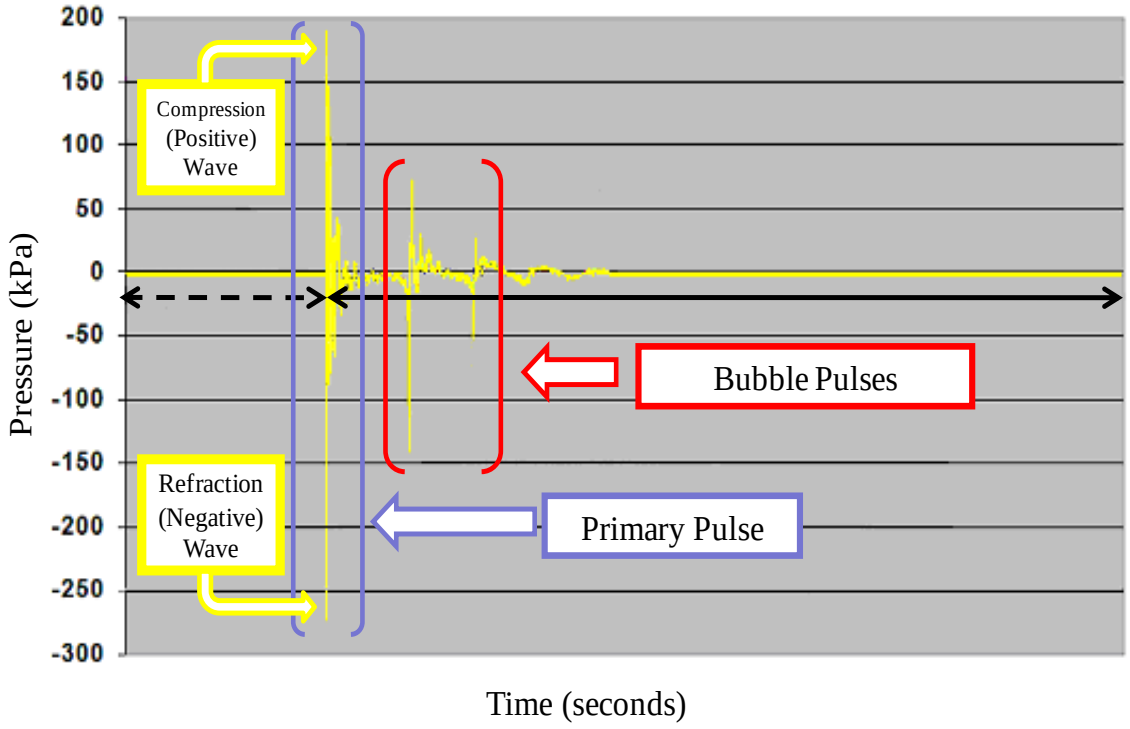

Figure 1.1: Representative waveform following explosive detonation of approximately 180 kPa in pressure. Dashed timeline (◄- -►) represents 0.25 second pre-detonation record; solid timeline (◄—►) represents 1 second detonation record. The figure illustrates positive and negative waves of the primary pulse and distinguishes between primary pulse and bubble pulses within an explosive signature characteristic of an explosive detonation.

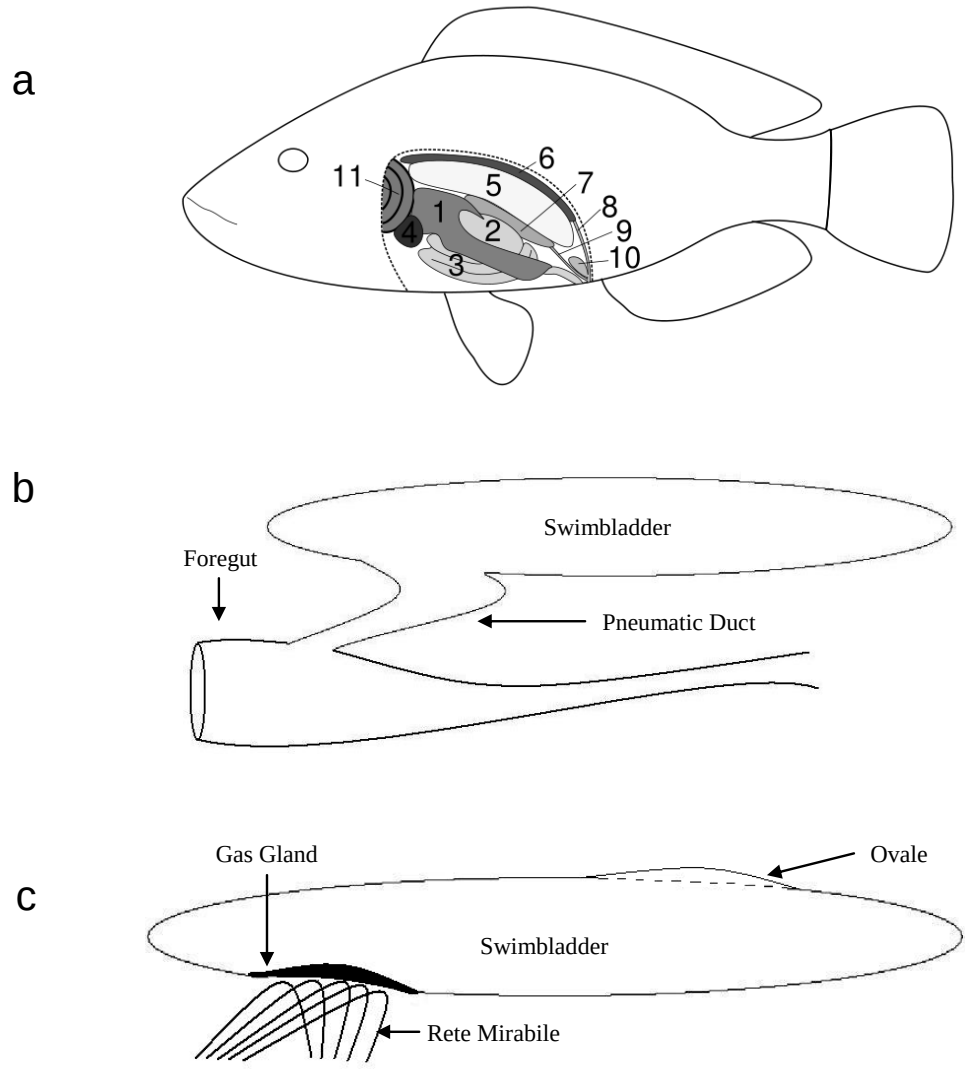

Figure 1.2: Typical teleost body plan (a) showing the swimbladder location; 1= Liver, 2= Stomach, 3= Intestine, 4= Heart, 5= Swimbladder, 6= Kidney, 7= Gonad, 8= Ureter. 9= Efferent Duct, 10=Urinary Bladder, 11= Gills (Image is licensed under Creative Commons Attribution 2.5 License and used with permission under the terms of the GNU Free Documentation License, Version 1.2, November 2002, Copyright (C) 2000,2001,2002 Free Software Foundation, Inc. 51 Franklin St, Fifth Floor, Boston, MA 02110-1301 USA). Example of a physostomous swimbladder (b) including the pneumatic duct connecting the gut and swimbladder (after Denton 1961; Campbell and McLean 1994). Example of a physoclistous swimbladder characterized by the lack of a pneumatic duct in adults. but possessing a gas gland, rete mirabile, and resorptive region (ovale) for diffusion of gas back into the bloodstream (after Denton 1961; Pough et al. 2002).

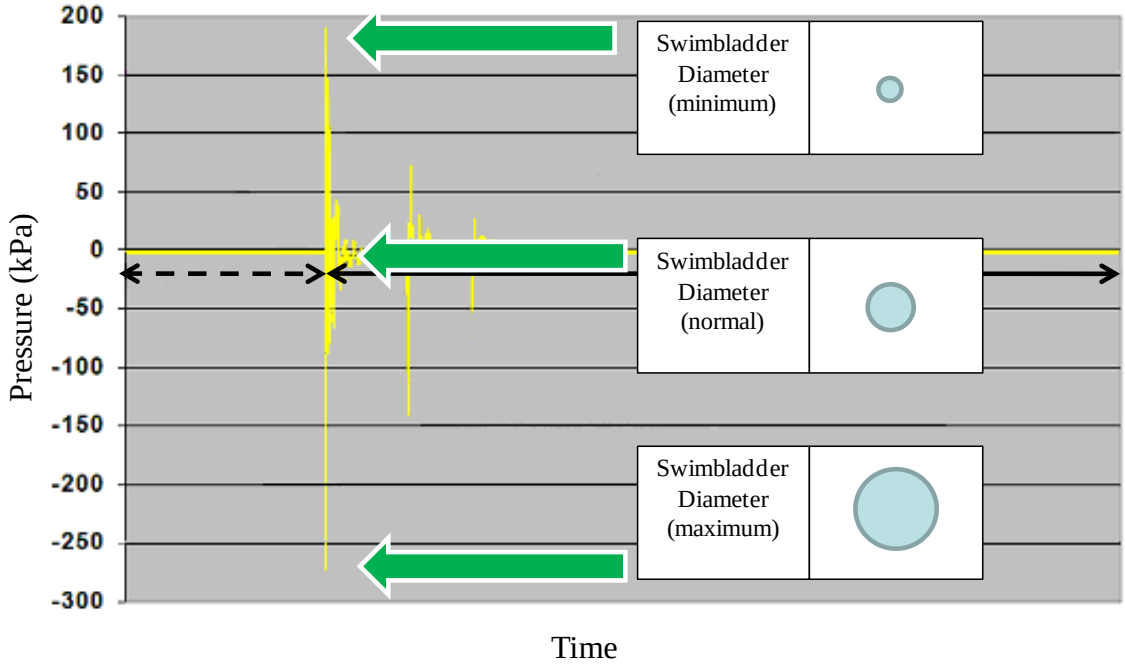

Figure 1.3: Response of swimbladder to underwater detonation. Swimbladder compression (corresponding with maximum positive pressure) occurs initially when the shock front passes, while swimbladder expansion occurs at rarefaction, after the passage of the shock front (as described by Govoni et al. 2003, Yelverton 1975).

2 HISTOPATHOLOGICAL EXAMINATION OF EMBRYOS, SAC FRY AND JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EXPOSED TO EXPLOSIVE BASED INSTANTANEOUS PRESSURE CHANGE (IPC)

2.1 Abstract

The oil and gas industry uses explosive-based seismic techniques to explore for reserves beneath waterbodies. In Northern Canada and Alaska, industry and regulatory agencies acknowledge that the use of explosives in, or near, waterbodies has the potential to harm fishes. In Canada, *Guidelines* recommend that peak pressures not exceed 100 kPa. To re-examine these *Guidelines* and simulate seismic exploration activities, a winter field experiment was undertaken in the Mackenzie Delta, NWT. Early life stages of rainbow trout (*Onchorhynchus mykiss*), including eyed eggs, sac fry and juveniles were exposed to IPCs ranging from 0 to 280kPa. All life stages were held simultaneously in cages and subjected to explosive detonations giving rise to measured IPCs below the ice. After detonations of each magnitude, all three life stages were preserved. Microscopic damage to craniofacial features were examined in eyed eggs, while swimbladders were examined in sac fry. Furthermore, tissues including eyes, swimbladder, liver, kidney, and gill were evaluated in juveniles. Results of this study showed the occurrence of negative effects in early life stages of rainbow trout exposed to IPCs below and surrounding the current *Guideline* level. Significant effects included changes in both the area and circumference of the cranial region of eyed eggs, as well as swimbladder, ocular and kidney damage in juveniles. Results suggest that the current *Guidelines* are not protective of early life stages of fish.

2.2 Introduction

Explosive-based seismic techniques are used by the oil and gas industry in Canada's North to explore for reserves beneath waterbodies that do not freeze to the bottom. In addition to applications for geophysical exploration, explosive based seismic techniques are used for ice management, general construction and for scientific applications (Wright 1982). Seismic-based exploration activities have recently increased to levels not seen since the 1970s in Canada's arctic (Cott et al. 2003). Upon detonation of an explosive within a waterbody, pressure waves are produced in the watercolumn (Keevin and Hempen 1997). These pressure waves are characterized by a sharp rise in peak pressure immediately followed by sharp drop in pressure below ambient hydrostatic pressure (Cronin 1948, Falk and Lawrence 1973, Gaspin 1975, Gaspin et al. 1976, Hill 1978, Hubbs et al. 1960, Keevin and Hempen 1997, Lavergne 1970, MacLennan and Simmonds 1992, Viada et al. 2008, War Report, Wiley et al. 1981). There is concern regarding these instantaneous pressure changes (IPCs) that arise in the water column from the detonation of explosives and their potentially harmful effects to fish (Wright 1982; Wright and Hopky 1998). Industry, environmental managers and regulatory bodies all acknowledge that the use of explosives is potentially harmful to fish (Wright and Hopky 1998).

Most of the existing information regarding damage to fishes from IPC has been determined from in-hand gross examinations of adult fish (Govoni et al. 2003). Little is known regarding more subtle effects of IPCs on tissue structure or on early life stages of fish. Recent evidence has shown that some sub-lethal effects of IPCs are only detectable using histopathology, microscopic examination of tissues at the cellular structure level.

The specific effects of IPCs include injuries to the swimbladder and vasculature and secondary damage to tissues near the swimbladder (ie: liver, kidney and intestine). These injuries arise as a result of rapid expansion of that organ during the negative pressure phase of the IPC (Fernet 1982, Tsvetkov et al. 1972). Additional injuries from other studies that have arisen in fish exposed to IPCs are noted in Table A1.

Due to concerns regarding the potential effects of IPCs to fish, the Canadian Department of Fisheries and Oceans (DFO) established a *Guideline for the Use of Explosives In or Near Canadian Fisheries Waters* (Wright and Hopky 1998) hereafter the "*Guideline*s". These *Guidelines* stipulated that to protect fish, maximum peak pressures are not to exceed 100 kPa. However, damage to fish and mortality has been documented near and below the currently recommended *Guideline* level (Godard et al. 2008, Houghton and Munday 1987, McAnuff et al.1994, Sakaguchi 1976, Teleki and Chamberlain 1978). Before revisions to the existing IPC *Guideline* can be considered, scientifically defensible data are needed.

To address these concerns, an investigation into the effects of explosive based IPC was undertaken on eggs, sac fry and juvenile rainbow trout with the objective of addressing whether explosive based IPCs surrounding the current suggested *Guideline* level have the potential for harmful effects on early life stages of fish detectable at either a gross or microscopic level. The null hypothesis is that explosive based IPCs surrounding the current *Guideline* level are not harmful to early life stages of rainbow trout.

2.3 Materials and Methods

2.3.1 Fish and Fish Holding

Three different early life stages of rainbow trout were exposed *in situ* to IPCs in this experiment. Eggs, sac fry and juveniles were obtained from a certified disease free hatchery (Ackenberry Trout Farms, Camrose, Alberta). Fish and fish eggs were shipped by air from the hatchery to Inuvik in insulated containers. The fish were then transported from Inuvik to the study site by truck. Once at the site, the fish, still in their insulated containers, were placed in a heated shelter for less than three hours and aerated. The fish were held until they were deployed for exposure to a specific IPC.

2.3.2 Study Site

The study was performed in Lake 24 on Richards Island, Inuvik (53720E, 7702404N) in the Mackenzie Delta during March 2004 (Figure 2.1). The study location was selected based on traditional ecological knowledge through consultation with the Tuktoyaktuk Hunters and Trappers committee. This lake was chosen because it is not a lake commonly fished by the community nor is it popular for breeding waterfowl in the spring. Lake 24 has a surface area of 317, 869m^2, a maximum depth of approximately 11m and a total estimated volume of 1,106,080m^3.

2.3.3 Experimental Design

For exposure to an IPC, twenty each of eyed eggs, sac fry and juveniles were selected, and each life stage was placed in a separate 18 oz. sterile Whirl-Pak plastic bag (Nasco, Fort Atkinson, WI, USA) filled with water in which the fish had been

transported (4 ºC). Bags were sealed to exclude any air in the head space and the three bags containing the different life stages were then suspended in a PVC framed cage (81 X 81 X 46 cm) covered with 1/8" nylon mesh with Velcro closures at one end (for a more detailed description of the cages see Palace et al. 2005). The suspended plastic bags served to minimize temperature fluctuations as well as potential collisions of fish with the cage frame as a result of flight responses during the experimental detonations (Govoni et al. 2003). A reference group was put into bags and suspended in the lake, without detonation, for the same timeframe as exposed fish (~10 min). The cage was suspended from rope so that it hung 2 m below the 2m thick ice platform and 2 m from the lake bottom (Figure 2.2).

2.3.4 IPC Exposures

Dynamite charges were buried 2 to 3 m into the sediment and were placed 1.5 to 6 m away from the cages, depending on the IPC that was being targeted (Figure 1.1). IPCs were monitored at the side of the suspended cage using a seismograph (Blastware® MiniMate Plus^{TM} Monitor, Instantel Inc., Canada) and corresponding hydrophones (Geo Space, Texas). The IPCs measured were the average of 3 hydrophones capable of measuring at a frequency of 65,000 cycling samples per second per channel (Table A3). The hydrophones were deployed on the outside of the cage to avoid trauma to the fish inside the cage via collision with the units. Shot number 1 was used as a reference to establish that IPCs measured inside plastic bags in the cage were not different than those outside the cage. For this shot, hydrophone 1 was inside a plastic bag similar to that used for fish exposures, hydrophone 2 was in the cage and hydrophone 3 was beside the cage. Eyed eggs and sac fry were exposed to peak pressures measuring 0, 64, 105, 228 and 280

kPa while juveniles were exposed to detonation charges measuring 0, 69, 239 and 280 kPa (Table A3).

2.3.5 Fish Sampling and Effects Endpoints

Immediately following each detonation, the cage was removed from the water into a heated shelter (5 meters away). Eggs and sac fry were placed directly into fixative (10% pH [7.0] sodium hydroxide buffered formalin) for later laboratory analysis. Juvenile fish were euthanised using pH buffered tricaine methanesulfonate (MS-222; 0.3g/L). A ventral midline incision was made in the juveniles and once the general condition of the internal organs had been noted for each individual, the viscera and swim bladder were carefully moved to expose the underlying kidney tissue and allow the fixative to penetrate that tissue (Palace et al. 2006). Each individual juvenile was then uniquely tagged and placed whole in the fixative. The time from beginning of euthanasia to immersion in fixative was less than 5 minutes. After 24 hours in fixative, all samples were rinsed, and stored at room temperature in 70% ethanol for transport to the laboratory. Eggs and fish were sampled in the laboratory and prepared for analysis as explained below. Quantitative histological criteria were developed in consultation with a histologist with 35 yrs of experience in the analysis of fish tissue pathologies (Mr. Robert Evans, DFO Winnipeg).

2.3.5.1 Eyed Eggs

Eyed eggs were assessed for visible disruptions to gross anatomical structures. An incision was then made in the chorion and this layer was manually removed to allow a more thorough examination.

Exposed embyros were oriented face onwards to a dissecting microscope (Motic SMZ-143 Series, China) with an attached PC image processor (Olympus America Inc., Q Color 3, Canada) to obtain digital images. All measurements were made using computer image analysis. Potential disruptions to the shape of the cranium were investigated by measuring: the cranial width at eye midline (Figure 2.3, i), the eye midline to highest peak on head (Figure 2, iia), width between orbits (Figure 2.3 iib), and the area of upper cranium from eye midline (Figure 2.3 iii). Head circumference and S Factors, a measure of how close the cranium approximates a perfect circle (0-1, with 1 = perfect circle) were also determined. Because of difficulties in fixing, staining and sectioning eggs with large amounts of yolk, no further tissue level assessments were performed on the eyed eggs.

2.3.5.2 Sac Fry

At the laboratory, swim bladders were removed from sac fry with the aid of a dissecting microscope (Motic SMZ-143 Series, China). The intestine was resected at the distal end using scissors and reflected cranially to allow an initial investigation of swim bladder integrity.

The swimbladder was removed and placed in a 70% ethanol-filled Petri dish and examined for structural damage. Further evaluation of swim bladder integrity was conducted using a dissecting microscope and a blunt probe. Swimbladders were scored for the presence or absence of tears (0= no tears; 1= presence of tears), represented by the occurrence of any foreign laceration. Care was taken during dissections to avoid iatrogenic tears (tears resulting from the dissector).

2.3.5.3 Juveniles

With the aid of a dissecting microscope (Motic SMZ-143 Series, China), the gastrointestinal tract was removed by severing the distal end of the intestine and the anterior end of the esophagus. Careful manoeuvring allowed the intact gastrointestinal tract to be freed from the body cavity which allowed the gross examination of organs surrounding the swim bladder, except for the kidney. The swimbladder was then removed. Swimbladder condition was examined as described for sac fry. Extraction of the swim bladder allowed the kidney to be inspected for hemorrhages. Using a scalpel and scissors, the liver and kidneys were dissected from surrounding tissues, ensuring that tissues remained moist during dissection. The first and second gill arches were dissected from the right side of the fish. All tissues, except swimbladders, were placed within histocassettes (Simport, M490-2, China) which were then submerged in 70% ethanol. Additionally, intact heads of fish were removed and stored in 70% ethanol for later examination of potential eye displacement.

Only tissues from juveniles were processed for histopathology. Tissues were processed through an ethanol-toluene-paraffin series, overnight, in a Tissue Tek Vacuum Infiltration Processor 5A-F1 (Sakura Finetek, Torrence, California, USA) (Table A2), and were subsequently embedded individually in paraffin using a Tissue-Tek embedding center (Miles Scientific, Model 4585, Naperville, Illinois, USA). Sections of each tissue (7 µm) were cut with a rotary microtome (ThermoShandon, Finesse 325, UK) and the tissue ribbons were affixed onto two to three microslides per tissue for each fish. The tissues on the slides were then stained with hematoxylin and eosin (H&E) for general evaluation of histopathology as described by Edwards (1967).

Gross examinations indicated that eyes of juveniles may have been displaced. This was assessed by measuring the areas of left and right eyes digitally from digital images taken from the dorsal perspective (Figure 2.4).

Kidneys were processed, embedded, sectioned and stained as previously described. For the analysis of juvenile kidney tissue, three sections, from an average of 16 sections, were randomly selected from each slide using a random number generator. Two 40x fields were examined (=141 X 106 mm) for every section resulting in 12 high powered fields (HPFs) for each fish. Kidney tissues were examined and scored for the incidence of hematuria (red blood cells in tubule lumen/urine) as well as for the presence or absence of hemorrhaging (red blood cells in the insterstitium). Hematuria was further grouped into single or multiple occurrences. Single occurrence indicated one of 12 HPFs showed hematuria, versus multiple occurrence in which more than one HPF showed hematuria.

Livers tissues were prepared with H&E stain as previously described and the presence of thromocytes, cellular components responsible for blood clotting, was assessed blindly (Roberts and Ellis 2001). Thrombocytes were chosen for evaluation as they were thought to be markers of tissue damage, indicating areas where blood clotting was necessary in the organ due to damage from the rapid expansion of the swimbladder. Four or six randomly selected digitized sections (=141 X 106 mm) on 2 or 3 slides respectively, each with an average of 16 sections, were subjectively scored for the degree of thrombocyte infiltration within the entire section. Using 40x magnification, the presence of thrombocytes within each section was assessed quantitatively and scored using three categories, 1 = nil or very few thrombocytes (1-4 congregations), 2 = a

moderate numbers of thrombocytes (5-10 congregations), 3 = extensive thromobocytes (>10 congregations). Congregations were defined as groupings of 12-15 thrombocytes. Thrombocyte scores for each fish were subsequently averaged for statistical analysis.

Gill tissues were prepared as previously described. Hyperemia (increased congestion in a tissue) and hemorrhaging (the escape of large quantities of red blood cells from tissue) were recorded in lamellae as well as the presence of thrombocytes within the tissue. Hyperemia and hemorrhage were each scored using two categories, 1=present, 0=not present. Four or six sections per fish were scored from 2 or 3 respective slides, each with an average of approximately 14 sections. Scores for each fish were subsequently averaged for statistical analysis. Thrombocytes were similarly scored.

2.3.6 Statistical Analysis

2.3.6.1 Eyed Eggs

Head measurements in eyed eggs were tested using a Kruskal-Wallis followed by Dunnett's post-hoc test. Statistical significance was set at $p<0.05$. Kruskall Wallis is a non-parametric method for testing equality of population medians among groups. It is identical to a one-way ANOVA with the data replaced by their ranks. The Dunnett's test is a multiple comparison test used for comparisons of all treatments against a control (SAS Institute Inc. 1989b, StatSoft 2007, Winer et al. 1991).

2.3.6.2 Sac Fry

Incidence of swimbladder tears in sac fry were tested using a two-tailed Cochran-Armitage test for discrete variables. Statistical significance was set at $p<0.05$. The Cochran-Armitage procedure designates the treatment effect as a class variable not a

numeric value. Therefore, for the current study, the class variables are the kPa pressures. This test answers the question of whether or not there is a linear trend in effect associated with different treament levels. Results of the Cochran-Armitage test will therefore indicate the presence or absence of a significant linear trend in the incidence of swimbladder tears as a response to treatment levels. An example of the application of the Cochran-Armitage test, applied to clinical categorical data, can be found in Schaaf et al. (2003).

2.3.6.3 Juveniles

2.3.6.3.1 Swimbladder

Incidence of swimbladder tears in sac fry were tested using a two--tailed Cochran-Armitage test for discrete variables. Statistical significance was set at $p<0.05$.

2.3.6.3.2 Eyes

Eye measurements in juveniles were tested using a Kruskal-Wallis followed by Dunnett's post-hoc test. Statistical significance was set at $p<0.05$.

2.3.6.3.3 Kidneys

Incidence of hematuria and hemorrhage were tested using a two-tailed Cochran-Armitage test for discrete variables using a Bootstrap P-value adjustment. Statistical significance was set at $p<0.05$.

2.3.6.3.4 Liver

Incidence of thrombocytes in junvenile livers were tested using a Kruskal-Wallis test followed by Dunnett's t-test. Statistical significance was set at $p<0.05$.

2.3.6.3.5 Gills

Presence of hyperemia, hemorrhage, and thrombocytes in juvenile gills were each tested using a Kruskal-Wallis test followed by Dunnett's t-test. Statistical significance was set at $p<0.05$.

2.4 Results and Discussion

2.4.1 Eyed Eggs

A gross examination of eyed eggs revealed no obvious disruptions arising from any of the IPC intensities tested. There were significant differences in area of the upper cranium in embryos, but multiple comparison tests did not resolve differences between exposure groups and the reference group making conclusions difficult. Contrastingly, inter-orbital, outside orbital, mid-orbital to cranial peak and S factor measurements were not significantly different (Table A6). Head circumference was significantly lower in the 64 kPa treatment group compared with the reference group. This may be attributed to slight size differences between groups rather than a treatment level effect. Unfortunately, no data were collected on size of the eyed embryos. Discrepancies may have also arisen through slight variation in embryo orientations during image processing.

The susceptibility of fish eggs to mechanical shock varies according to their age and stage of development (Battle 1944, Fitzsimons 1994, as described in Faulkner et al. 2007). Salmonid eggs are especially vulnerable to physical shock during the gastrulation (Jensen and

Alderice 1983,Smirnov 1954) with extreme sensitivity to shock during epiboly (as described in Jensen 2003, Jensen and Alderice 1983, 1989). Gastrulation (the developmental stage during which epiboly occurs) is five days in duration and lasts from approximately 40 degree days until 90 degree days (at 10°C) in rainbow trout (Salmo gairdneri Richardson 1836) (Vernier 1969). Lowest survival from physical shock and mechanical stress in lake trout (*Salvelinus namaycush*) has been correlated with this stage of gastrulation (Eshenroder et al. 1995; Fitzsimons 1994). Epiboly is characterized by the blastodermal germ ring overgrowing the yolk (Jensen and Alderice 1983, Faulkner et al. 2007, Velsen 1987). In fact, increase in sensitivity has been attributed to the adherence of the blastoderm to the perivitelline membrane as it surrounds the yolk. Mechanical shock pulls on the newly formed blastoderm and tears the perivitelline membrane, allowing yolk contents to release into the perivitelline space, causing the egg to die (Smirnov 1955 as cited by Post et al. 1974). At more advanced stages of development, however, the embryonic tissue has already surrounded the yolk, making it less susceptible to rupture (Battle 1944).

Mechanical sensitivity of eggs declines immediately after epiboly, and continues to decline until the early eyed stage (Battle 1944; Jensen and Alderice 1983, 1989). The eyed eggs in our study were approximately 230-270 degree days (at 10°C). Hence, in the current experiment, the eyed eggs were developed beyond epiboly and this may partly explain their resistance to shock.

In addition to the stage of egg development, the makeup of lake sediments can have a bearing on the potential effects of blasting on egg viability. To examine the effects of spawning gravel on the survival of rainbow trout eggs, samples corresponding to middle and late epiboly (75 and 95 degree days respectively; stages 11 and 14) were

placed in containers with spawning gravel consisting of smooth river rock, 3cm in diameter (Faulkner et al. 2007). Controls were placed in containers containing no gravel. The presence of spawning gravel increased both egg mortality and variability in mortality compared to those in non-gravel environments following exposure to simulated blasting. The reason for this was not known, but may have associated with movement of the gravel (Faulkner et al. 2008). Damage contributed by the lake sediments and particulate matter would have been eliminated in the current study because the eggs were held in plastic bags.

The sensitivity of eggs may also be related to chorion thickness in teleosts. A study was conducted to assess the susceptibility of various types of fish eggs to physical external influences. Following mechanical shock, a thick chorion was protective in the mummichog (*Fundulus heteroclitus*), while the thinner chorion of the four-bearded rockling (*Enchelyopus cumbrous*) resulted in a greater degree of abnormal development (Battle 1944). Salmonids typically have relatively thick chorions (55 to 70 µm for an egg diameter of 7mm) probably limiting the effects of IPC mediated damage in this study (Depêche and Billard 1994).

Further variables thought to influence shock sensitivity of eggs include temperature and mineral content of the water, egg size, egg mass and species of fish (Jensen and Alderice 1983, 1989)

Other studies have reported no effects from blasting on fish egg viability. For instance, Thompson (1958) reported that, following detonation of DuPont Nutramex 2-H explosive (peak pressures not indicated) for the removal of Ripple Rock on the coast of British Columbia, samples of herring spawn were collected before and after the blast, and

they found no difference in the number of dead eggs in each sample. Conversely, the opposite has also been reported. Kostyuchenko (1973) evaluated impacts to fish eggs exposed to elastic waves from sources that included an air gun, an electric pulse generator and 50g charges of Trinitrotoluene (TNT), of which pressure measurements are not indicated. All sources, including TNT, were attributed to causing mechanical injury in the form of deformation and compression of the egg membrane, spiral curling of the embryo, migration of the embryo, and a reduction in vitelline membrane integrity (Kostyuchenko 1973). Kostuchenko (1973) furthermore reported that the number of dead eggs was greater with the use of the TNT charge than any of the other sources of elastic waves.

Because several factors that could contribute to egg damage were not considered in the current study (eg. sediment mediated effects, pre-epiboly development stages, species differences in chorion thickness) and because significant changes in upper area cranium and head circumference measurements could not be attributed to pressure level effects, additional study is required.

Of importance to discuss, the *Guideline* document recommends that no explosive is to be detonated that produces, or is likely to produce, a peak particle velocity (PPV) greater than 13 $mm \bullet s^{-1}$ in a spawning bed during the period of egg incubation (Wright and Hopky 1998). This peak particle velocity has been found to provide ample protection to fish eggs (Faulkner et al 2006, 2007). Though the recommended *Guideline* PPV level has proven protective, it was valuable to investigate impacts to fish eggs exposed to pressure, as opposed to particle velocity, in order to determine damage to the eggs from IPC exposure approximating the current recommended *Guideline* level and to get an overall sense of IPC damage across all life stages.

2.4.2 Sac Fry

The incidence of a linear trend in swimbladder tears in the sac fry of this study (Figure 2.7, Table A7) was not significant (Table A6), which is likely a function of underdeveloped swimbladders.

While no significant treatment related swimbladder tears were detected in sac fry from this study, an early study by Bishai (1961) noted that responses in larval and young fish to pressure are dependent on the species, the age of the fish, and the presence or absence of a swimbladder. Bishai (1961) stressed this point by demonstrating the effects of pressure on the survival of larval and young fish at different stages of development. In young salmonids (*Salmo salar* L. *Salmo trutta* L., and *Salmo trutta* f. *fario*) pressures of 507 kPa were tolerated in alevins (a developmental stage in which the swimbladder is immature) until the absorption of the yolk-sac. Similar findings were shown in newly hatched herring larvae (*Clupea harengus* L.) that were viable even at pressures of 405 kPa (Bishai 1961). Both species were unaffected by either compression or decompression pressures. Older fish with well developed swimbladders withstood pressures of 203 kPa, but were affected by decompression due to the incomplete formation of the sphincter controlling the release of gas bubbles from the pneumatic duct (Bishai 1961). The incomplete sphincter did not allow gas from the swimbladder to be released, and consequently, the swimbladder became distended (Bishai 1961). The study of Bishai (1961) helps highlight that the specific age of larval and young fish, and hence degree of swimbladder development, may strongly affect the impacts of pressure on fish. This is supported by Wright (1982) who states that larval fish are less sensitive to the effects of shock waves than eggs or post larval fish in which the swimbladder has developed

(Alaska Department of Fish and Game 1991, Wright 1982). It is also supported by Tsvetkov et al. (1972) who observed no effect in 16 day old Atlantic salmon (*Salmo salar* (L.)) larvae with undeveloped swimbladders upon exposure to near instantaneous changes in hydrostatic pressure.

In contrast to the aforementioned however, smaller fish are thought to be more at risk from explosive impact than larger fish (Alaska Department of Fish and Game 1991, as described by Baxter et al. 1982, Continental Shelf Associates, Inc 2004, Govoni et al. 2008). Tsetkov et al. (1972) noted that the sensitivity of fish with swimbladders was higher in young compared to older individuals. This is attributed to the weakness of the swimbladder wall in earlier stages (Tsvetkov et al. 1972). Likewise Govoni et al. (2008), following an experimental exposure of larval spot and pinfish to explosive blasts, concluded that larval and recently transformed small juvenile spot and pinfish were more vulnerable than large juveniles and adult fish.

Accounts of both sensitivity and insensitivity of sac fry and larva to explosive blasts have been reported. For instance, Fitch and Young (1948) noted death of larval anchovies (*Engraulis mordax*) following explosive based exploratory work in the Newport-Huntington Beach area. No pressure data or description of explosive is provided. Contrastingly, Bird and Roberson (1984) examined coho salmon (*Oncorhynchus kisutch)* and chum salmon (*O. keta*) fry and fingerlings, as well as wild coho and Dolly Varden (*Salvelinus malma*) fry in three tests conducted during highway realignment in Keystone Canyon near Valdez Alaska. These fish were exposed to Tovex T-1, Tovex 930, and Anfo-P explosives. All fish were observed after each test for 24

hours and selected specimens dissected. Results showed that fish had no discernible external or internal trauma. Peak pressures were low and ranged from 5.51-18.6 kPa.

2.4.3 Juveniles

2.4.3.1 Swimbladders

The Cochran-Armitage test indicates a significant linear trend in swimbladder tears as a function of pressure level (Table A6). Ten (10) percent of the swimbladders in juvenile rainbow trout exposed to the 69kPa IPC were torn, and thirty (30) percent of fish exposed to 280 kPa blasts had torn swimbladders (Figure 2.8, Table A8). However, the highest percentage, sixty (60) percent of ruptured swimbladders was found in the juvenile rainbow trout exposed to the 239 kPa detonation. This is thought to be related to developmental differences among the fish. Those with more developed swimbladders may show more marked effects to IPCs than those with lesser developed swimbladders. Further investigation is warranted.

Swimbladder tissue was not evaluated histologically in this study. In fact, literature regarding the histological effects of instantaneous pressure on juvenile swimbladders is generally scarce. However, Govoni et al. (2002, 2003) histologically examined trauma to juvenile pinfish (*Lagodon rhomboids*) and spot (*Leiostomus xanthurus*) exposed to submarine detonations of varying intensities. They reported trauma within the swimbladder of both species exposed at 636.92 kPa including hyperemia within the swimbladder serosa, the muscosa of the gas gland, and the rete mirabile of exposed fish. This trauma was attributed to the rapid compression and expansion of the swimbladder as the pressure wave passed (Govoni et al. 2003). However, in constrast

with the current study, they found no damage to the swimbladder of fish exposed to 109.93 kPa of pressure, a level nearer to the *Guideline*. Settle et al. (2002) histologically examined larval and early juvenile spot and pinfish exposed to underwater blasts varying in intensity from 79.4 kPa to 866.0 kPa. They reported tissue damage that included swimbladder hemorrhage in both species of exposed fish.

Other studies have determined juvenile swimbladders to be sensitive to explosive exposure. During a test program at Parsons Lake in August and September 2000, juvenile lake whitefish (*Coregonus clupeaformis*) that died following exposure to charges of dynamite powder (overpressure at the detonation site measured 174 kPa) were all found to show ruptured swimbladders (Golder Associates, 2000). Roguski and Nagata (1970) investigated the physical effects of blasting on caged yearling king salmon (*Oncorhynchus tshawytscha*) exposed to under ice detonations of C-4 explosives (peak pressure not indicated). Autopsy results confirmed the most frequent injury was rupture of the swimbladder.

Similar to the findings in this study, detrimental effects to juvenile swimbladders have been reported below the current recommended *Guideline* IPC level. Houghton and Munday (1987) conducted a study investigating the effects of linear explosive detonations on juvenile fish, notably on caged salmonids including coho salmon and chum salmon (*Oncorhynchus keta*) smolts, as well as on juvenile Pacific herring (*Clupea harengus pallasi*). Following exposure to Primacord of three different strengths (50, 100 and 200 grains per foot), fish were autopsied. Peak pressures measured from 37 to 331 kPa. Level 2 and 3 injuries, associated with bruised, hemorrhaged and ruptured swimbladders, were noted in 80% of coho at pressure levels as low as 37 kPa. In general,

results indicated swimbladder injuries to caged fish ranged from no observable damage to lethal damage. Lethal damage included injuries such as hemorrhage or clots within the swimbladder, visible deformities of the swimbladder as well as rupture and hemorrhage of the swimbladder.

2.4.3.2 Eyes

An initial gross examination of juvenile fish suggested that the eyes of fish exposed to some IPCs were displaced outwardly. Digitized analysis revealed significantly greater ocular areas, when viewed dorsally, among the 69 and 280 kPa groups when compared with controls, but not the 239 kPa exposed fish (Figure 2.9, Table A6 and A9). This occurrence in juvenile eye data is thought to be related to slight developmental differences amongst the fish, specifically the development of ossified ocular support. More developed fish, as may exist the 239 kPa exposed group, might possess more developed ocular support than less developed fish and resultantly, show less damage from IPCs.

To further investigate the underlying pathology associated with eye displacement, histological sections, through the transverse plane of intact heads, were prepared for each fish as shown in Figure 2.10. Sections from the 239 kPa group appeared to contain more cartilaginous support and ossification surrounding the eye. Greater support surrounding the eye might have contributed to the more limited displacement among the fish in the 239 kPa group compared with the other treatment groups. Different responses among fish that varied only slightly may underscore the importance of developmental stages in mediating effects.

Exopthalmos (or pop-eye) in fish exposed to partial vacuums and explosive blasts has been documented in the literature albeit generally, in larger and adult fish (Kearns and Boyd 1965, Coker and Hollis 1950, Hogan 1941, Thomson 1958, Muir 1959). In fact, it is described as a characteristic sign of pressure injury (Tsvetkov et al. 1972). The cause of eye protrusion due to explosive based IPCs is not known, however it may be related to either gas bubble accumulation behind the eye, the negative forces exerted during the blasts, or direct physical trauma.

Bishai (1961) noted that larval and young fish may develop protruding eyes due to the accumulation of gas behind them, a symptom of decompression sickness caused by rapid pressure reduction. Tsvetkov et al. (1972) and Dukes (1975) further support this theory. However, Simenstad (1973) questions whether the decompression phase of a shock wave is long enough to allow nitrogen or other gas bubbles to accumulate to the point of damage, as the short period of decompression may limit the volume of gas coming out of solution.

Exopthalomos may have also been a function of the negative forces exerted during the blasts. Muir (1959) subjected four dead coho salmon (*Oncorhynchus kisutch*) to a slowly applied partial vacuum using a test apparatus. In two cases, one eye was partially extruded for its socket, however, unlike in the current study, the eye repositioned with the restoration of atmospheric pressure. Hogan (1941) constructed an apparatus to test the effects of vacuum pressure on a variety of fish including bluegill bream (*Lepomis macrochirus*), golden shiners (*Notemigonus crysoleucas*), crappies (*Pomoxis sp*.), largemouth black bass (*Micropterus salmoides*), carp (*Cyprinus carpio*), bullhead catfish (*Ameiurus sp*.) and long-nosed gar (*Lepisosteus osseus*). The tests examined various

negative pressure levels (to a maximum of 91.4 kPa) for 10-55 seconds. Many of the fish exhibited eye protrusion during the tests. Though the application of vacuum pressure was not instantaneous in nature, it confirms the ease of deforming a soft, fluid-filled structure with vacuum, which is not likely relevant to other types of trauma.

Finally, the onset of eye protrusion may have been due to direct physical trauma caused by the explosions, as Dukes (1975) explains that mechanical injury from a blow to the head could produce exopthalmos.

2.4.3.3 Kidneys

Juvenile fish exposed to IPCs exhibited hematuria (Figure 2.11, Table A10). Single RBCs were routinely detected in tubule lumens yet results of the Cochran-Armitage test indicate no significant linear trend associated with treatment levels (Table A6). Of greater physiological relevance, however, were the fish with increased multiple hematuria at 280 kPa, and to an even greater extent at 239 kPa. A significant linear trend was seen in the percentage of fish with hematuria as a function of pressure level (Table A6). Results are similar to those of Govoni et al. (2003) who reported hematuria in the proximal tubules of kidney in juvenile spot (*Leiostomus xanthurus*) and pinfish (*Lagodon rhomboides*) following exposure to submarine detonations of 230.86 kPa in peak pressure.

Hemorrhage was observed among fish exposed to 69, 239 and 280 kPa and showed a significant linear trend with pressure (Figure 2.12, Table A6 and A9). These observations, similar to those see in swimbladders and eyes, are attributed to developmental differences amongst the fish. In this case, fish with more developed swimbladders showed greater kidney impacts than those with lesser developed

swimbladders. For instance, the higher incidence of hemorrhage among fish from the 239 kPa group is likely related to increase in swimbladder rupture in this group of potentially more developed fish.

Many studies have highlighted the impact to piscine kidneys from exposure to explosive blasts. Roguski and Nagata (1970) investigated the effects of blasting on caged yearling king salmon (*Oncorhynchus tshawytscha*) exposed to under ice detonations of C-4 explosives (peak pressure not indicated) and described renal damage, either in the form of rupture or hemorrhage. Likewise, Houghton and Munday (1987), who conducted a study investigating the effects of linear explosive detonations on caged juvenile fish including coho salmon (*Oncorhynchus kisutch*), chum salmon (*Oncorhynchus keta*) and Pacific herring (*Clupea harengus pallasi*) reported kidney impact ranging from no observable damage to lethal damage (bruising, hemorrhage and liquefaction of the organ) following exposure to Primacord of three different strengths (50, 100 and 200 grains per foot). Peak positive pressures ranged from 37 kPa to 331 kPa, with level 2 and 3 injuries, associated with bruised and hemorrhaged kidneys, noted in 80% of coho at pressure levels as low as 37 kPa.

2.4.3.4 Liver

There were no significant differences in thrombocyte aggregations (Figure 2.13 and 2.14; Table A6 and A11) in livers of juvenile fish among any of the treatment groups in this study. As illustrated by the reference group in Figure 2.13, thrombocyte aggregations are routinely detected in rainbow trout livers. This indicates they are normal findings. The lack of differences found indicates no impact from swimbladder expansion on livers from IPCs at the tested levels.

Several studies have shown the liver to be a sensitive organ to tissue damage in young fish from IPC exposure. (Govoni et al. 2003, Houghton and Munday 1987, Roguski and Nagata 1970, Settle et al. 2002), however most damage reported is concerned with bruising, necrosis or hemorrhaging of liver tissue. A paucity of information regarding changes in thrombocyte presence exists concerning tissue damage and IPC exposure.

2.4.3.5 Gills

No statistical significance was found for any of the gill variables assessed in juvenile rainbow trout from any of the treatment groups in this study (Figures 2.15-2.17, Table A6 and A12-A14). Non-linearity indicates no damage to gills from IPCs.

While previous studies have confirmed damage in gill tissues following exposure to explosive pressure changes, such as Thompson (1958) who confirmed frayed gills in fish following the explosion of the British Columbia navigation hazard known as Ripple Rock, more detailed information is lacking.

2.5 Conclusion

The results of this initial study indicate that effects can occur in early life stages of rainbow trout exposed to IPCs below the current DFO *Guideline* of 100 kPa. The null hypothesis is therefore rejected.

The current study enabled preliminary conclusions to be drawn regarding the effects of IPCs on early life stages as well as the protective nature of the current DFO G*uideline*. Further research will serve as an extension to our findings and allow for more definitive conclusions to be made. Different responses among fish may have underscored

the importance of developmental stages in mediating effects. A more thorough investigation of the effects of IPCs in fish with more developed structures (i.e. swimbladders, support surrounding the eye) is therefore warranted. Also valid for future research is the comparison of a swimbladder species to a species lacking a swimbladder. As the swimbladder is thought to be the prime organ damaged during explosive blasts as well as a major contributor to secondary tissue damage, an examination of the potential effects surrounding the current *Guideline* level in a species lacking this organ would be worthwhile.

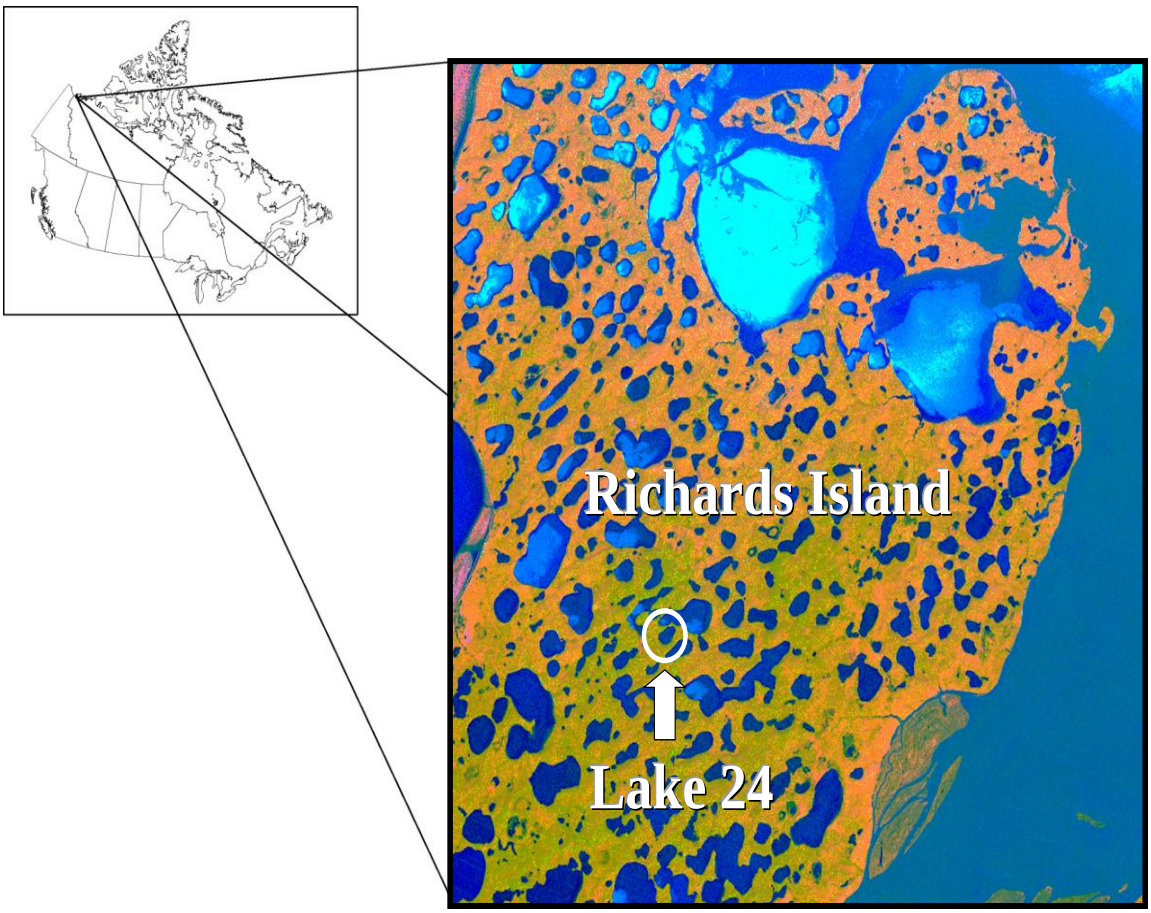

Figure 2.1: Map of study site (Lake 24) on Richard's Island, Inuvik, Northwest Territories (Clayton and Cott 2004; Cott and Hanna 2004)

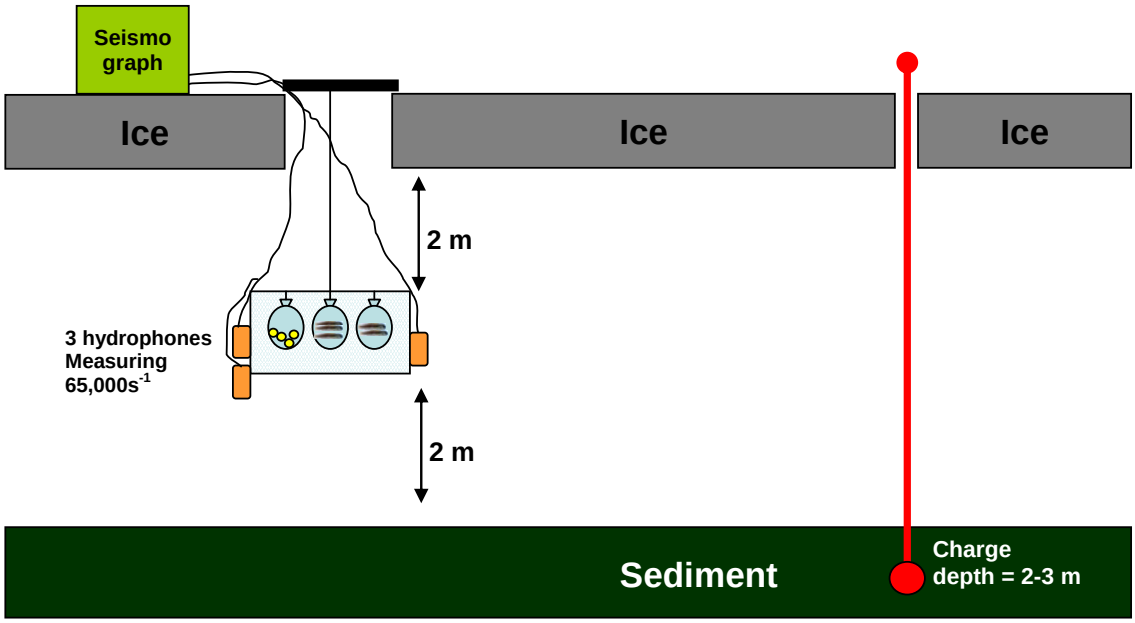

Figure 2.2: Schematic of experimental design and instantaneous pressure change exposures.

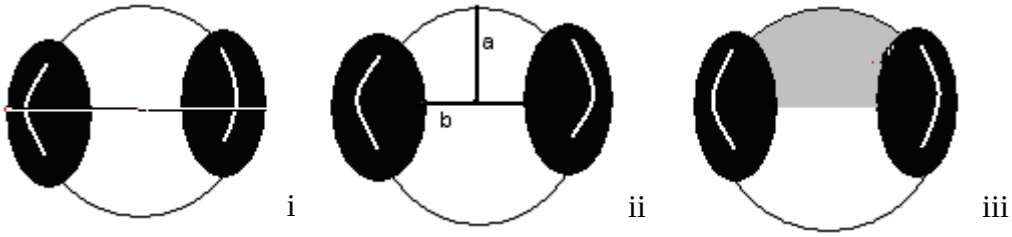

Figure 2.3: Cranial measurements for de-chorionated eyed egg evaluations.

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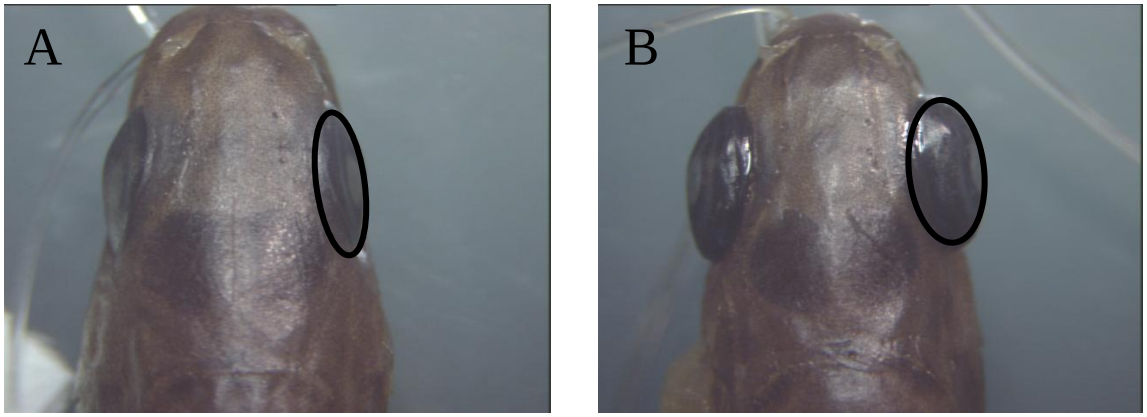


Figure 2.4: Digitized image analysis of dorsal eye areas in juvenile rainbow trout not exposed to an IPC (Panel A) or exposed to an IPC of 280 kPa (Panel B). Intact heads from each fish were also sectioned in the transverse plane to histologically examine potential effects of eye displacement.

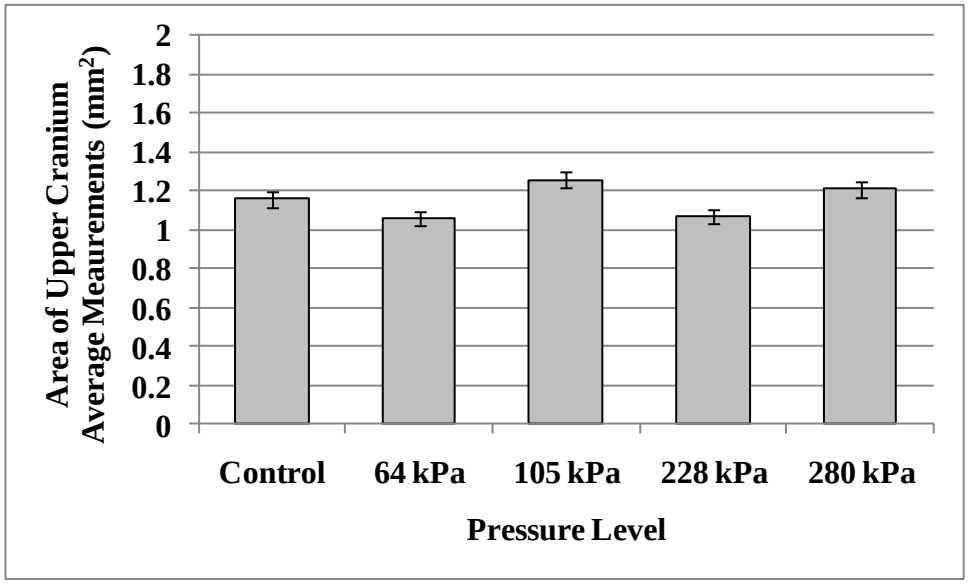


Figure 2.5: Average upper cranium area measurements of eyed egg rainbow trout exposed to varying IPCs. Data are expressed as mean (± SE) for n=10 (control, 106, 225, 280 kPa) and n=11 (62 kPa) fish exposed to blasts per pressure level.

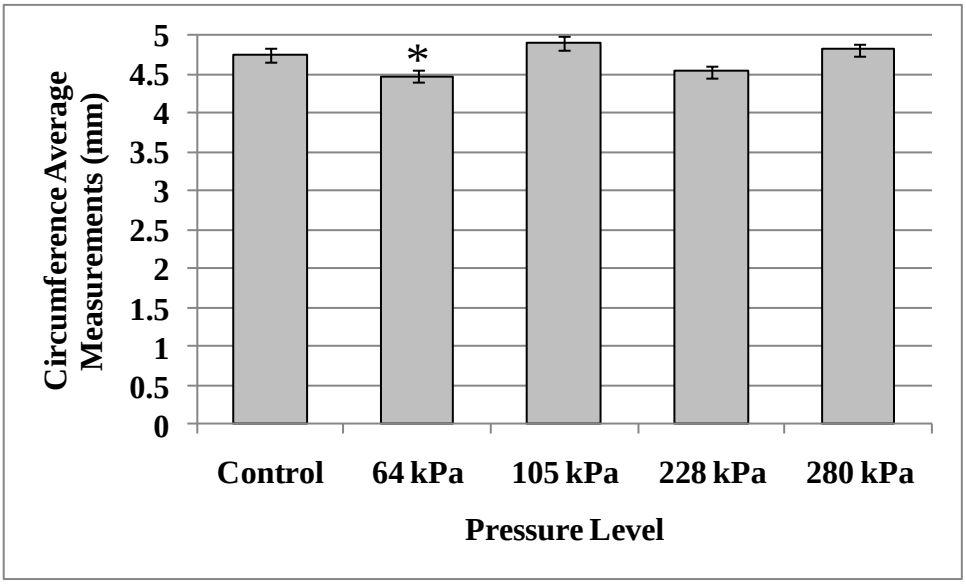


Figure 2.6: Average head circumference measurements in eyed egg rainbow trout exposed to varying IPCs. Data are expressed as mean (± SE) for for n=10 (control, 106, 225, 280 kPa) and n=11 (62 kPa) fish exposed to blasts per pressure level. Bars labeled with asterisks are significantly different from control.

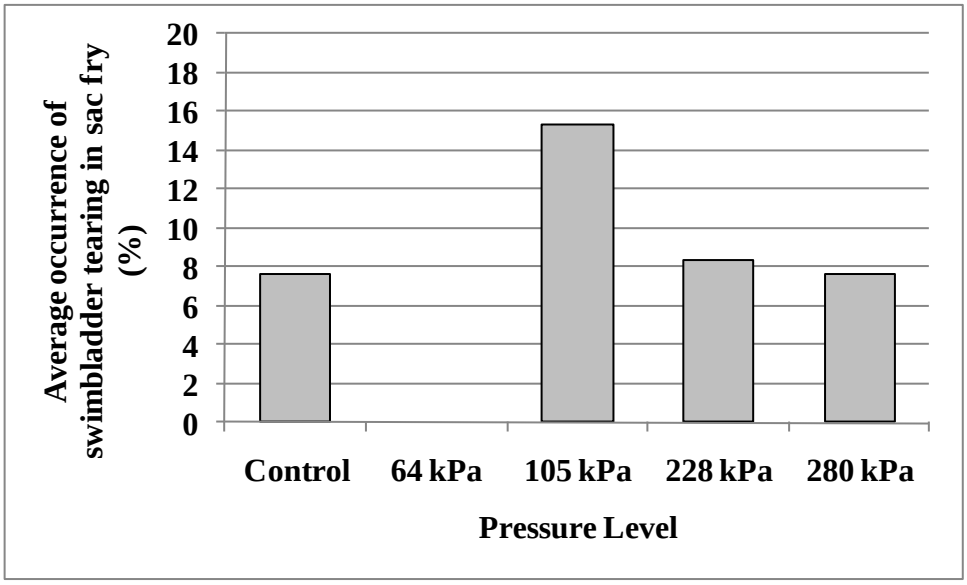


Figure 2.7: Percentage of sac fry rainbow trout exposed to detonations with torn swimbladders. Data are expressed as percentage of fish with non-intact swimbladders for n=12 (64, 228 kPa) and n=13 (control, 105, 280 kPa) fish exposed to blasts per pressure level

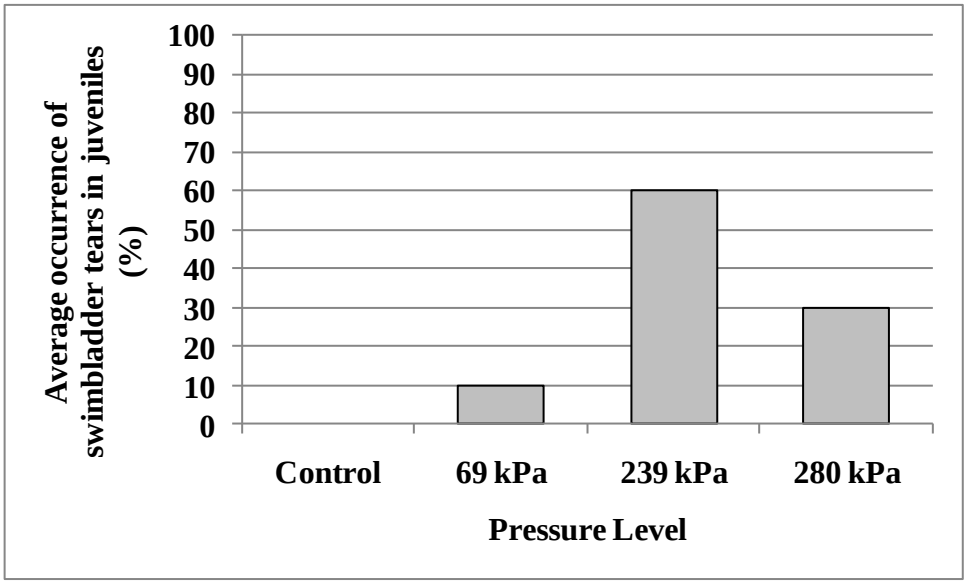


Figure 2.8: Percentage of juvenile rainbow trout exposed to detonations with torn swimbladders. Data are expressed as percentage of fish with non-intact swimbladders for n=9 [control] and n=10 [69, 239, 280 kPa) fish exposed to blasts per pressure level.

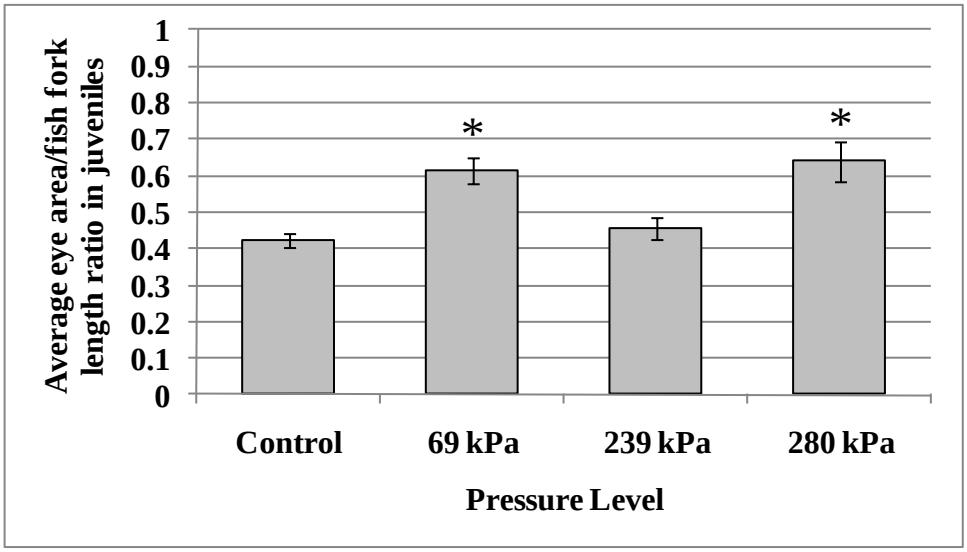


Figure 2.9: Dorsally viewed ocular areas of juvenile rainbow trout exposed to varying IPCs. Data are expressed as mean (± SE) for digitized areas divided by fork length for n=9 (control) and n=10 (69, 239, 280 kPa) fish. Bars labeled with asterisks are significantly different from controls.

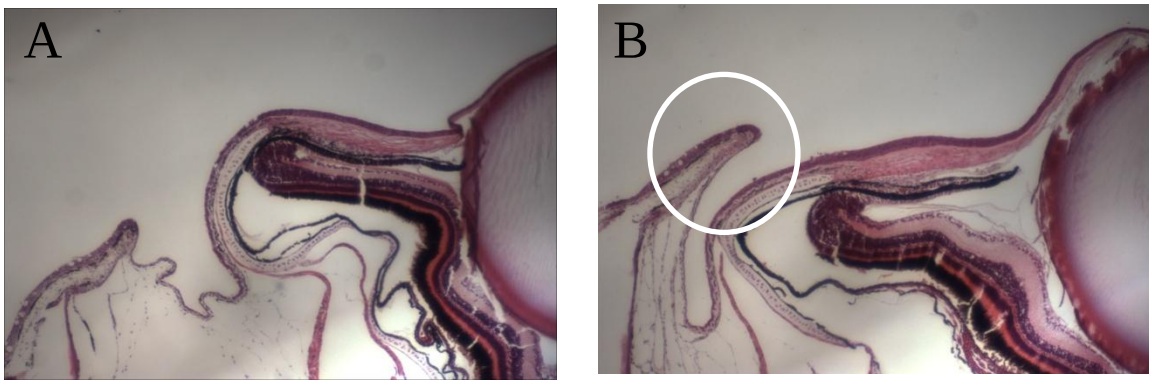


Figure 2.10: Histological sections of eyes (transverse) of juvenile rainbow trout exposed to varying IPCs. Panel A is a frontal eye section of juvenile at 280 kPa. Panel B is a frontal eye section of juvenile at 239 kPa. The circled region shows the presence of increased ocular support.

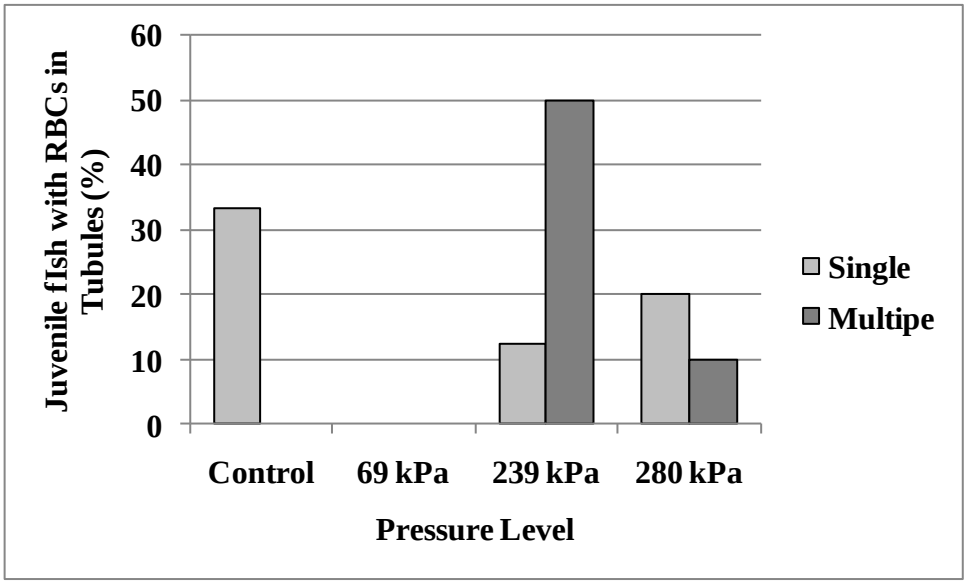


Figure 2.11: Incidence of hematuria in juvenile rainbow trout kidneys exposed to varying IPCs. Data are expressed as percentage of fish with single or multiple occurrence of red blood cells (RBCs) in kidney tubules for n=8 (239 kPa), n=9 (control, 69 kPa) and n=10 (280 kPa) fish.

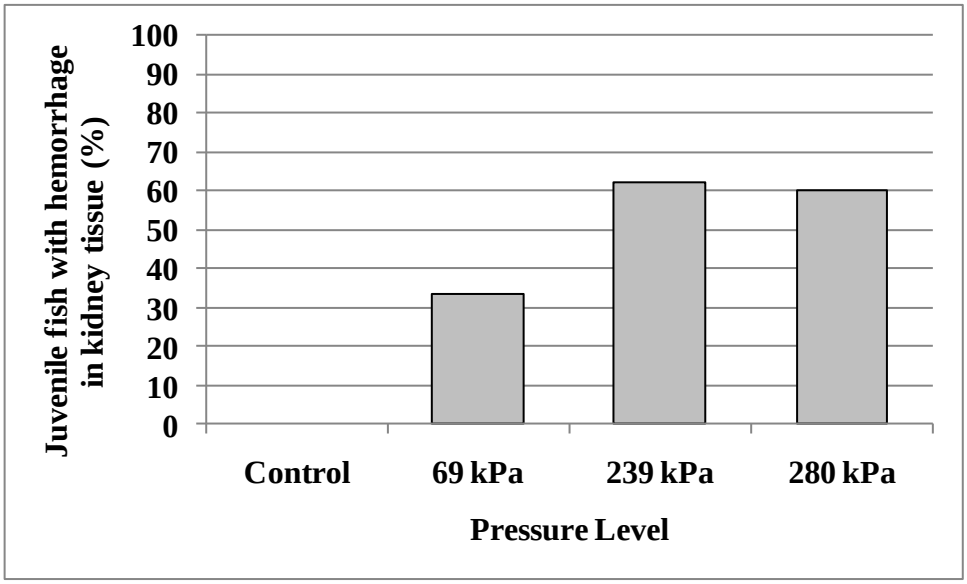


Figure 2.12: Incidence of hemorrhaging in juvenile rainbow trout kidneys exposed to varying seismic peak pressures. Data are expressed as percentage of fish with RBCs in the insterstitium of kidney tubules for n=8 (239 kPa), n=9 (control, 69 kPa) and n=10 (280 kPa) fish.

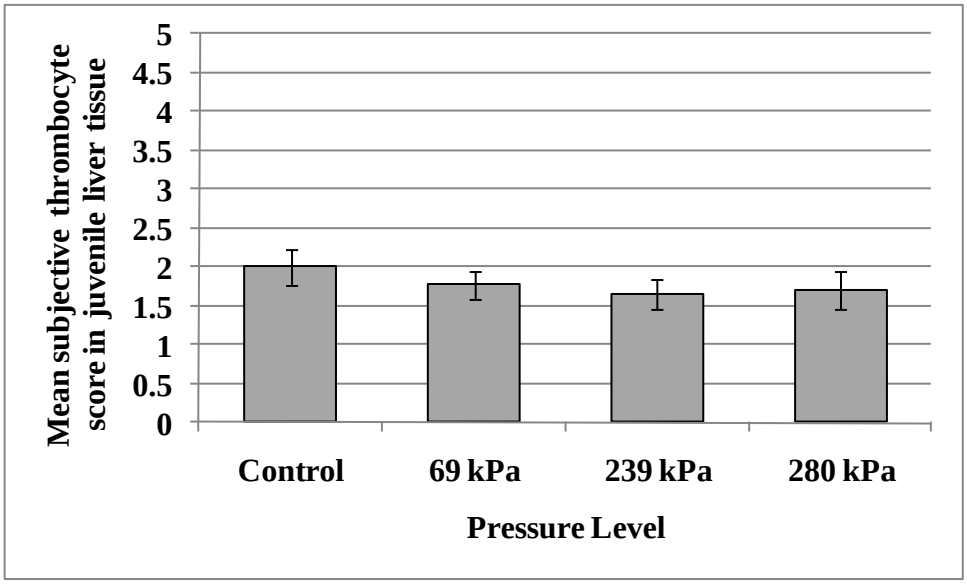
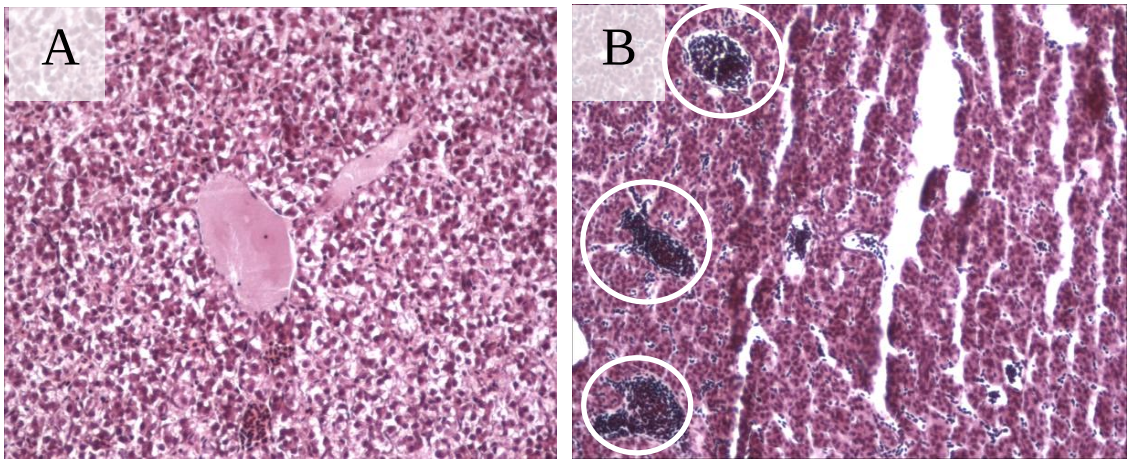


Figure 2.13: Mean subjective thrombocyte scores in liver of juvenile rainbow trout exposed to varying seismic peak pressures. Data are expressed as mean (± SE) subjective thrombocyte score for n=9 (control) and n=10 (69, 239, 280 kPa) fish. A score =1 represents nil or very few thrombocytes (1-4 congregations), 2 = an intermediate amount of thrombocytes (5-10 congregations), 3 = extensive thromobocytes (>10 congregations). Congregations were defined as groupings of 12-15 thrombocytes.



Thrombocyte Score of 1 Thrombocyte Score of 3

Figure 2.14: Liver tissue from juvenile rainbow trout exposed to IPCs, depicting thrombocyte scores (20x magnification). Panel A is a low incidence of thrombocytes and was scored as 1. Panel B is a high incidence of thrombocytes and was scored as 3.

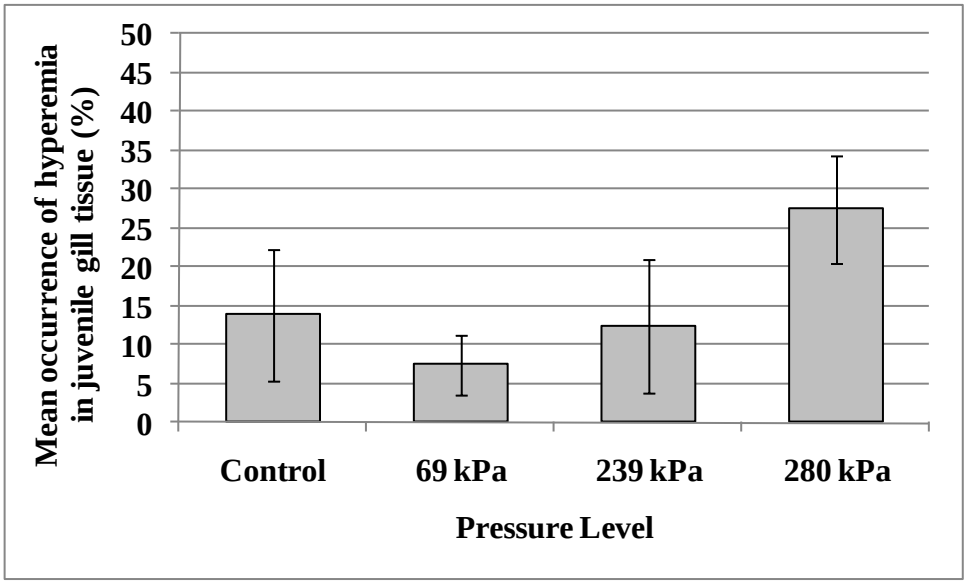


Figure 2.15: Mean occurrence of hyperemia in gills for juveniles exposed to varying seismic peak pressures. Data are expressed as mean percentage (± SE) hyperemia score for n=9 (control) and n=10 (69, 239, 280 kPa) fish.

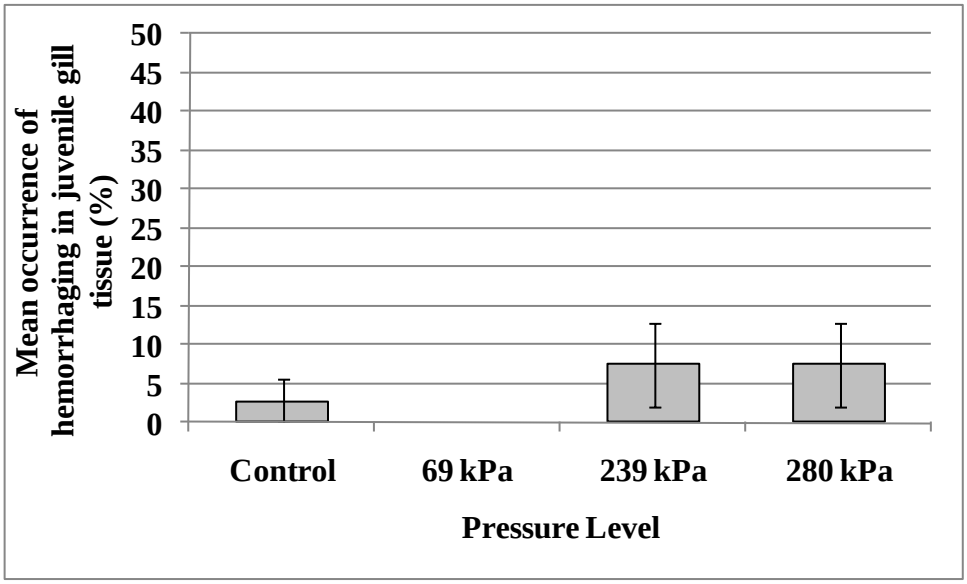


Figure 2.16: Mean occurrence of hemorrhaging in gills for juveniles exposed to varying seismic peak pressures. Data are expressed as mean percentage (± SE) hemorrhage score for n=9 (control) and n=10 (69, 239, 280 kPa) fish.

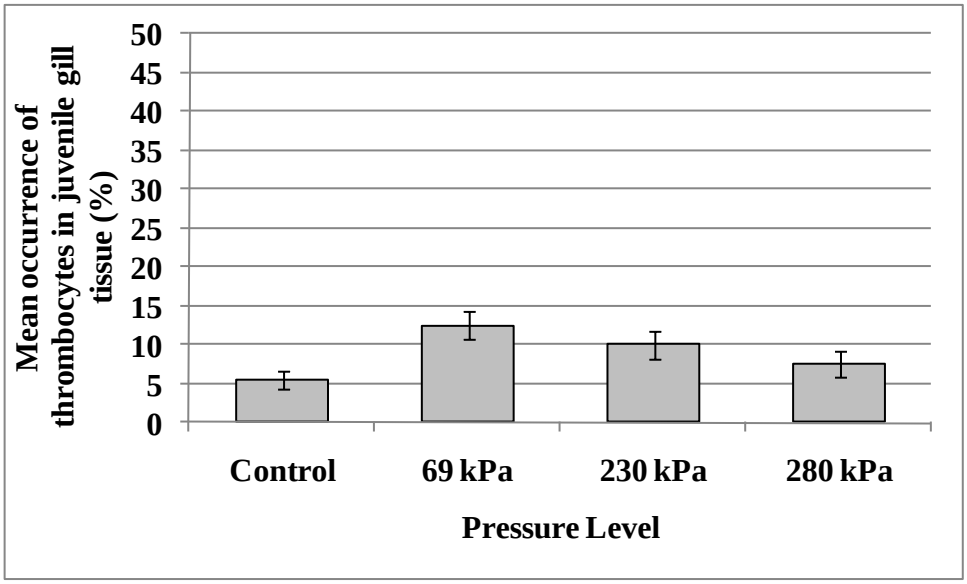


Figure 2.17: Mean occurrence of thrombocytes in gills for juveniles exposed to varying seismic peak pressures. Data are expressed as mean percentage (± SE) thrombocyte score for n=9 (control) and n=10 (69, 239, 280 kPa) fish.

3 Pathological Assessment of Adult Lake Trout (*Salvelinus namaycush*) and Sculping (*Cottus cognatus*) Exposed to Explosive Based Instantaneous Pressure Change (IPC)

3.1 Abstract

Winter based seismic activities in Northern Canada use explosives to detect oil and gas reserves. For the protection of fish the Department of Fisheries and Oceans (DFO) Canada recommends a maximum peak pressure of 100 kPa; however previous research has reported damage to early life stages of rainbow trout (*Onchorhynchus mykiss*) below this level. The damage was thought to be mediated by developmental stage and swimbladder presence. To evaluate the protective ability of this pressure limit in fish with more developed swimbladders as well as in fish lacking a swimbladder, a study was undertaken at the Experimental Lakes Area, northwestern Ontario. Swimbladder bearing adult lake trout (*Salvelinus namaycush*) and sculpin (*Cottus cognatus*), a fish species lacking a swimbladder, were exposed to IPCs ranging from 0 to 127 kPa. Fish were held in cages and subjected to discrete IPC inducing explosive based detonations. Fish were then examined grossly and sampled for blood and tissues including liver, kidney and intestine, to determine resultant pathological changes. Our results indicate hemorrhaging in the swimbladder of lake trout was caused near the 100 kPa limit.

3.2 Introduction

Explosives are employed as seismic sources for winter hydrocarbon exploration in Canada's North, specifically in waterbodies not frozen to the bottom. In response to the potential for damage to fish from explosive detonations, the Department of Fisheries and

Oceans (DFO) Canada released a *Guideline* document stipulating a maximum IPC of 100 kPa for the protection of fish.

A preliminary investigation was undertaken to determine effects to eggs, sac fry and juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to explosive based IPCs using levels surrounding the *Guideline* (Godard et al. 2009). This study investigated gross pathology as well as histopathology. Results determined cranial changes in eggs, as well as swimbladder tears, exopthalmos, and hematuria and hemorrhaging of the kidneys in juveniles. Interestingly, in swimbladders, eyes and kidneys of juvenile fish, the study noted differences between treatment groups thought to be due to differences in development. Further investigation into effects of explosive exposure in larger, more developed fish was warranted. Moreover, because the swimbladder is thought to cause much of the damage from an IPC, the effects of explosives in a swimbladder bearing species and a species lacking a swimbladder were included (Fernet 1982, Goertner 1978, as described in Govoni et al 2003; Keevin 1998, Wiley et al 1981, Wright and Hopky 1998). The current experiment was conducted on adult lake trout, a species with a swimbladder, and sculpin, a species lacking a swimbladder. As in the previous chapter, histopathology of tissues was examined in addition to gross pathology. Blood biochemistry was added to investigate subclinical or subtle damage to organs and tissues from exposure to explosive based IPC in fish.

The main intent of the study was to evaluate effects surrounding the current suggested DFO *Guideline* IPC level, however, peak IPC levels currently in use surrounding northern oil and gas exploration served as a general guide for target exposure levels to be tested during the current study (Table A15). The null hypothesis of this

study is that the IPCs near the *Guideline* level are not harmful to adult lake trout or sculpin, a species lacking a swimbladder.

3.3 Materials and Methods

3.3.1 Study Site

The experiment was performed in Lake 382 at the Experimental Lakes Area (ELA) (49° 42' 17.65" N, 93° 40' 39.73" W) (Figure 3.1). The lake has an area of approximately 232 hectares, a volume of approximately 2,126,468 m^3 and a maximum depth of approximately 13.1m (ELA Data Retriever provided by Susan Kasian, Department of Fisheries and Oceans, Winnipeg, June 2009). Prior to the experiment, an underwater survey of the lake was performed to evaluate substrate characteristics. The substrate was predominantly soft organic sediment with some areas of sand and rocky outcroppings.

ii) Experimental Design

Two areas on Lake 382 were designated for the experiment: a control area for fish holding, and a blasting area for exposing fish to selected IPCs (Figure 3.2). Preliminary blasting and monitoring of seismic pressure recordings ensured that these areas were isolated from each other.

3.3.1.1 Control Area

Holding pens were anchored within the control area. One lake trout pen (1.2 x 1.2 x 3.7 metres) and one sculpin pen (0.8 x 0.8 x 0.5 metres) were deployed for fish holding after capture (primary holding pens) (Figure 3.2). Additionally, six lake trout pens (1.2 x

1.2 x 3.7 metres) and six sculpin pens (0.8 x 0.8 x 0.5 metres) were deployed as secondary holding pens for fish following their exposure to an IPC (Figure 3.2).

Two types of pens were used within the control area. Lake trout pens were made from 5/16" atlas mesh and had a large velcro opening for easy access to fish (Figure 3.3). Sculpin pens within the control area were sinking cages framed in PVC pipe and covered with 1/8" nylon mesh with Velcro closures at one end (for a more detailed description of the cages see Palace et al. 2005) (Figure 3.4).

Secondary pens were deployed by coupling one lake trout pen with one sculpin pen (Figure 3.5). The paired pens were then segregated according to increasing blast intensity exposure. For instance, as shown in Figure 3.2, the pair of pens labelled 1 housed lake trout and sculpin not exposed to a blast (controls), the pair of pens labelled 2 housed lake trout and sculpin exposed to the lowest blast intensity, the pair of pens labelled 3 housed lake trout and sculpin exposed to the next highest blast intensity, and so on.

Prior to deployment of pens into the lake, concrete anchors were attached to the bottom of the pens with enough rope to reach the sediment, while floats were tied to the top loops. Subsequently, two wooden boards (2x4) were attached lengthwise along the top edge of the pens to enhance buoyancy and enable the pens to maintain their shape. Sculpin pens were fitted with floats to allow easy retrieval.

Within the control area in which pens were set, water depths ranged from 1-2 metres.

3.3.1.2 Blasting Area

Holding pens for lake trout (1.2 x 1.2 x 2.4 metres) and sculpin (1.2 x 1.2 x 1.2 metres) were deployed 15m from the intended detonation location which was marked with a float. The water depth at the blasting site was approximately 5 metres. See Figure 3.6

Pens within the blasting area were sunk halfway down the water column. Prior to deployment, concrete anchors were attached to the bottom loops of the pens with enough rope for the anchors to reach the sediment. Floats were attached to the top loops of the pen with enough rope to ensure that the centre of the pens would be at a depth of 2.5 metres. Subsequently, PVC pipes were attached along the top edge of the pens to allow them to sink as well as maintain their shape (Figure 3.6).

3.3.2 Fish Capture and Holding

The project was undertaken during a period which coincided with historic fall ELA lake trout catches (late September through mid-October). Lake trout were captured with trap nets set in known spawning habitats in Lake 382. Most of the lake trout were caught the day prior to blasting, with the final fish being caught the morning of the experiment. Lake trout were retrieved from trap nets, transported in tubs filled with aerated lake water and released into the initial holding pen in the control area. Care was taken to minimize their time in this pen following capture as they were not being fed.

For eight consecutive days in advance of blasting, baited minnow cages with phosphorescent stick lights were set to capture sculpin. As there was no known area of sculpin aggregation, minnow traps were set arbitrarily around the lake, checked daily, and

reset until a total of 30 sculpin were caught. Once captured, sculpin were placed in their respective holding pen in the control region.

3.3.3 IPC Exposures

Dynamite (POWERFRAC, 75% Nitroglycerine) was used as the explosive source. Preliminary test detonations served as range finding experiments to determine the quantity of dynamite needed to attain target peak pressures. Once the test period was completed, the first group of fish (five lake trout and five sculpin) were removed from their respective initial holding pens within the control area and transported in tubs containing aerated lake water to their respective blasting area cages (<5 minutes total). For each blasting event, a pre-determined quantity of dynamite was buried in the sediment and remotely detonated by a crew in a boat anchored 15m away from the cage area. IPCs from were monitored with Blastware® software (Version 7.1, Instantel Inc., Canada) and a Blastware® MiniMate Plus^{TM} Monitor (Instantel Inc., Canada) capable of measuring at a frequency of 65,000 cycling samples per second per channel. The monitor was connected to 2-3 hydrophones (Geo Space, Texas). To avoid trauma from fish colliding with the hydrophones, the hydrophones were secured on top of the cages. See Figure 3.7 and 3.8.

Following each detonation, fish were removed from the cages, placed in a tub filled with aerated lake water, and transported back to the control area where they were dispatched to the appropriate secondary holding pens. Additional detonations were performed in the same manner for all levels of treatment. Control fish were handled in the same manner as exposed fish, but were not subjected to explosive blasts.

3.3.4 Fish Sampling and Pathological Assessment

Once exposures were complete, fish were allowed to recover in their holding pens for 36 hours to allow any sub-acute pathologies (bruising, hemorrhaging, etc) to develop. Fish were then euthanized with an overdose of pH buffered tricaine methanesulfonate (MS-222; 0.4-0.5 g/L). Details on examination of gross pathology, as well as tissue dissection, blood preparation and analysis are provided below.

3.3.4.1 Lake Trout

3.3.4.1.1 Eyes

Eye measurements were made with callipers, extending between the orbit of the eye and the outside of the cornea (Figure 3.9) to determine the occurrence of eye protrusion (exopthamia).

3.3.4.1.2 Blood

Blood was obtained by either dorsal aortic puncture or caudal venous puncture (Department of Fisheries and Oceans 2004) using a pre-heparinized (2500 U/mL) 20 gauge needle (Becton, Dickenson and Company, USA) and 10 ml syringe (Becton, Dickenson and Company, USA). Whole blood was placed into 10 ml labelled sodium heparin vacutainers (Becton, Dickenson and Company, USA) and centrifuged at 3000 g for 10 minutes. Plasma was recovered with a 1000 µL plastic pipette (Fisherbrand, USA), aliquoted evenly into three 1.5mL microcentrifuge tubes (Fisherbrand, USA) and frozen on dry ice.

Clinical blood biochemistry was determined with the use of multilayered, analytical element coated VITROS slides that measure enzyme activity as well as

nitrogenous compound or mineral concentrations in plasma (Ortho-Clinical Diagnostics Inc. 2004a-f). Slides were analyzed with the VITROS 250 chemistry system (Johnson and Johnson, New York). The layers found on each slide are differentially coated according to the enzyme, breakdown product, waste product, or mineral to be determined. In each case a drop of plasma (5.5-11 µL, test dependent) is deposited on the slide and is evenly distributed by the first layer (the spreading layer), to the underlying layers (Ortho-Clinical Diagnostics Inc. 2004a-f). Figure 3.10 provides an example of the specific layering of the VITROS slide, reaction processes and how changes are measured for aspartate aminotransferase (AST).

Blood biochemistry was investigated as a means of detecting biochemical changes indicative of tissue damage and to relate the results to tissue findings. Specific blood components indicative of tissue damage were selected through consultation with veterinarians. These analytes can be used as markers of damage to a particular organ. For instance AST and alanine aminotransferase (ALT) are markers of liver damage but they also are interpreted in conjunction with other analytes to determine damage to other organs as well. For instance, increased AST and ALT in conjunction with elevated creatinine and phosphorus can denote damage to the kidney. AST and ALT were analyzed to assess liver damage. They were also used to in conjunction with creatinine and phosphorus levels to determine renal damage (See Table A16). Gill damage would be indicated by increased blood urea nitrogen (BUN) levels and skeletal muscle damage would be suggested with increased AST and creatine kinase (CK) levels. Assessment was made by comparing quantitative findings between control and exposed fish. Control fish

were used to provide the normal, or reference range for these variables against which treatment groups could be compared.

3.3.4.1.3 Tissue

Following blood collection, a ventral midline incision from the anus to the esophageal region was made to expose the internal organs and allow gross examination of the swimbladder. This was done to detect damage (notably hemorrhage) that may have occurred due to explosive based IPC. Hemorrhage was scored as present (1) or absent (0).

After examining for evidence of gross damage, tissues including liver, spleen, intestine and kidney were dissected from each carcass. Liver tissue was biopsied from the region nearest the swimbladder, while intestine and kidney tissues were sampled at their mid-point. Due to the small size of the spleen, the whole organ was sampled. Tissue sample sizes varied between 10-15mm. All tissues were placed in individual histocassettes (Simport m490-2, Canada) which were then submerged in Bouin's solution and taken to the lab for processing and analysis.

Tissues were fixed in Bouin's solution for 48 hours before transfer to 70% ethanol. The tissues were rinsed and stored in the alcohol three times at 2 day intervals. Tissues were processed through an ethanol-toluene-paraffin series, overnight, in a Tissue Tek Vacuum Infiltration Processor 5A-F1 (Sakura Finetek, Nagano, Japan) (Table A17), and were subsequently embedded individually in paraffin using a Tissue-Tek embedding center (Miles Scientific, Naperville, Illinois, USA). Sections of each tissue (5 µm) were cut with a rotary microtome (American Optical, Model 820, Buffallo, NY, USA). They were generally oriented in either a transverse or sagittal plane to allow views of both the dorsal and ventral regions of the organ during microscopic analysis. In most cases tissues

were step sectioned which involved taking two consecutive tissue slices from a representative area followed by another two consecutive tissues slices that were 12-20 cuts apart until a total of six tissues pairs (twelve tissue sections total) were collected and affixed onto two or three microslides. The exception to this were spleens which were serial sectioned from different regions of the tissue, so that five consecutive tissue sections from a representative area were mounted onto a slide followed by another five consecutive tissue sections from a different representative region. These ribbons were also affixed onto two or three microslides. The tissues on the slides were then stained with hematoxylin and eosin (H&E) for general evaluation of histopathology as described by Edwards (1967).

Six sections per tissue were scanned using Pathscan Enabler IV (Meyer Instruments, China). Each scanned image generally coincided with one section per tissue pair of the step sectioned series and every second section of the serial sectioned series. Subsequently, five high powered fields (HPFs) to be analyzed were generated using a random point generator (SigmaScan Pro 5.0) which superimposed a grid mask of random points over selected tissue images. A grid mask of 5-25 random points was applied until 5 random points laid over the image. In the case where random points fell over non-representative tissue (an area not made up of parenchemal tissue or tissue damaged by preparation) the nearest representative tissue to that point was assessed. Using a microscope (Nikon Eclipse 50i, Japan), 30 HPFs were analyzed per tissue at 40x magnification. To avoid bias in the interpretation of findings, histopathology slides were analyzed blindly. This was done by assigning each slide a random number.

Potential responses to traumatic injury to be assessed were developed through consultation with fisheries and veterinary pathologists. These injuries were also based on previous histological work on trauma inflicted by detonations (Godard et al. 2008, Govoni et al. 2003) and on preliminary comparisons of control and IPC exposed tissues. Table A18 identifies and describes traumatic based pathologies assessed, details how the pathologies were identified, and explains the assessment method. However, a summary is provided in Table A19.

3.3.4.2 Sculpin

Following euthanasia, sculpin were processed by making a mid-ventral incision from the anus to the esophageal area to allow fixative to penetrate tissues in the body cavity. Fish were submerged whole in Bouin's solution and taken to the lab for rinsing, dissection, processing and analysis. Sculpin were treated in a similar manner to lake trout, however blood samples were not taken and no swimbladder scores were made. Additionally, due to the small size of the fish, organs were removed and embedded whole. Tissue processing was the same as for lake trout.

3.3.5 Statistical Analysis

Statistical analysis was performed using The SAS system (Version 8.0.2). In cases of continuous data, a non-parametric test was chosen to compare data sets. The non-parametric test chosen to compare the data for each parameter assessed was the Kruskal-Wallis Test. Statistical significance was set at $p<0.05$.

If significance was determined in the Kruskall-Wallis, the Dunnett's post-hoc test (two-tailed) was subsequently performed. Statistical significance was set at $p<0.05$.

Swimbladder data were in the form of frequency data (presence/absence), as such, the Fisher's exact test was used for the analysis of damage. The Fisher's exact test is a test of independence used to compare frequencies (McDonald 2009). Statistical significance was set at $p<0.05$.

3.4 Results and Discussion

In practice, the actual IPCs generated from charges are often variable and unpredictable. As such, the IPC exposure levels generated during the current experiment were 33 kPa, 57 kPa, 59 kPa, 72 kPa and 127 kPa (Table A20).

Non-caged, resident lake trout were attracted to the blasting area after detonations occurred. While no specific pressure levels were correlated with this phenomenon, attraction of lake trout was thought to be a result of small uncaged fish seen stunned or killed and floating to the surface near the blast site. This in effect, created a feeding area for the fish. Neither caged lake trout nor sculpin were observed to be stunned or killed, suggesting that the most susceptible fish and those likely to be primarily impacted were small bodied, swimbladder bearing resident fish.

Fitch and Young (1948) reported similar results during blasting in the Santa Barbara, California area. While pressures and explosive type were not indicated, kelp bass (*Paralabrax clathratus*) were observed to feed on small perch (*Brachyistius frenatus*) behaviourally affected by a detonated charge. In contrast however, Houghton and Munday (1987) reported that larger predatory fish (species not specified) were not attracted to a test area of incidental fish kill following testing of linear Primacord explosives.

Attraction of predatory fish to the blasting site in the current experiment has implications for mitigative strategies that are used to deter fish from inhabiting a blasting area. Weak pre-blasts, also known as repelling charges, detonated prior to the onset of full scale blasting events, have been both proposed and employed as a method of deterring fish from the proximity of seismic explorations (as reviewed in Golder Associates 2008, Hill 1978, Keevin 1998). Theoretically, the noise and pressure produced from these repelling charges serves to scare fish from an exploratory area (Keevin 1998). The effectiveness of repelling charges is controversial as many consider them ineffective as well as potentially harmful (Keevin 1998). Aplin (1947) reported that a school of anchovies (genus and species not indicated) circling a survey ship during detonation of 60% petrogel explosives (peak pressure not indicated) remained in the area. The author further related an incident in the Philippine Islands where three hundred pound charges of explosives were being used to clear reefs. Schools of tuna (genus species not provided) remained in the blasting region. Coker and Hollis (1950) also reported that fish, in general, were not driven from a test area following detonation of HBX explosive charges (peak pressures not indicated). Though the aforementioned explosive blasts were not used as repelling charges, they help to illustrate that the detonation of explosives is not necessarily the most suitable means of detracting fish from inhabiting an area.

The potential effectiveness of pre-blasting to remove fish from a blasting site is further compromised by the potential for mortality resulting from repelling charges. This has been documented in several instances (Keevin and Hempen 1997, McAnuff et al. 1994, Nix and Champman 1985). Nix and Chapman (1985) monitored fish during a blasting project in False Creek, B.C. Efforts were made to mitigate the potential effects of

blasting on fish through the detonation of small preliminary charges prior to the detonation of larger charges (peak pressure not indicated). This method was deemed not only ineffective in deterring fish to inhabit the blasting area, but was in fact noted to kill fish. Observations such as that in the aforementioned study may have prompted revision of the original drafts of the Canadian *Guideline* document where it was suggested that the proponent consider detonation of small scareing charges, because the final version of the *Guidelines* omitted this recommendation (Keevin 1998).

The significance of our anecdotal observations may underscore the susceptibility of smaller swimbladder bearing fish to explosive blasts and further validate that explosive charges are not effective in removing fish from a blasting area. That predatory fish were attracted to the area of blasting is important because of the potential for increased numbers of fish being exposed to detonations. Indeed, Rulifson and Schoning (1963) explain that fish mortality may be linked to fish moving in to explosive areas to feed on those previously killed. However, as this portion of the study was not quantitative additional research is required to make definite statements or recommendations.

3.4.1 Gross Pathology

3.4.1.1 Swimbladder

There was significantly more swimbladder damage among fish exposed to 127 kPa, than in other groups (Figure 3.11; Table A21, A22). One of the most commonly reported injuries is rupture, which is described in several studies. For example, Tyler (1960) conducted an experiment on the Kvichak River in Igiugig, Alaska to determine the effect of underwater explosives on adult red salmon (*Oncorhynchus nerka*). Following

the detonation of 40% gelatine dynamite of unknown peak pressure, internal damage showed ruptured swimbladders. Similarly, Aplin (1947) examined damage to fish from the use of explosives to locate oil deposits in California. Though no peak pressures are indicated, following exposure to 60% petrogel in 10 pound sticks, all fish examined (including anchovies [*Engraulis mordax*], kingfish [*Genyonemus lineatus*], sardines [*Sardinops caerulea*], queenfish [*Seriphus politus*] and smelt [*Atherinopsis californiensis; Leuresthes tenuis*]) exhibited torn swimbladders. Roguski and Nagata (1970) investigated the physical effects of blasting on captive northern pike (*Esox lucius*) exposed to under ice detonations of C-4 explosive (peak pressures not indicated). Indeed, fish were reported to have ruptured air bladders. Ferguson (1962) also noted swimbladder rupture in freshwater fish including yellow perch (*Perca flavescens*) and American smelt (*Osmerus mordax*) collected at the lake surface following the detonation of various explosive combinations (see Table A1) of unknown peak pressure. Furthermore, in the same experiment, 67% of caged yellow perch exhibited swimbladder rupture. Finally, Falk and Lawrence (1973) investigated the effects of Aquaflex and 60% Geogel explosives on caged Arctic cisco (*Coregonus autumnalis*) and also examined non-caged fish killed as a result of experimental detonations (no peak pressures were recorded). Results confirmed swimbladder rupture in both caged Arctic cisco as well as in free ranging coregonids (*C.nasus, C. autumnalis*) with exposure to each type of explosive. Other studies in which similar findings are reported include Fitch and Young (1948), Teleki and Chamberlain (1978), Thompson (1958), as well as Hubbs et al. (1960). That swimbladder rupture was not identified in the current study may be attributed to peak

pressures not being sufficient to extend the organ beyond its eleastic capabilities or to the fact that the swimbladder was more developed, and hence less sensitive to rupture.

Though swimbladder rupture was not detected in the current study, hemorrhage was identified. Linton et al. (1985) examined the extent of injury in red drum (*Sciaenops ocellatus*) and black drum (*Pogonias cromis*) following exposure to Primacord explosives (peak pressure levels not indicated). Injuries to the fish included capillary rupture within the swimbladder wall. Similarly, Houghton and Munday (1987) autopsied wild fish incidentally exposed to linear explosives of different strengths (50, 100 and 200 grains per foot). Peak positive pressures during the study ranged from approximately 37-331 kPa. Juvenile walleye pollock (*Theragra chalcogramma*) and coho salmon (*Oncorhynchus kisutch*), as well as rockfish (*Sebastes spp.*), Pacific tomcod (*Microgadue proximus*), Pacific cod (*Gadus macrocephalus*), Pacific herring (*Clupea harengus pallasi*), and dolly varden (*Salvelinus malma*) showed haemorrhaging of the swimbladder. Finally, Coker and Hollis (1950) investigated the effects of blasting on fish following 21 explosions of HBX charges in Chesapeake Bay (peak pressures not reported). A list of the fish investigated can be found in Table A1. Their investigation confirmed that following the blasts, the swimbaldder injuries of examined fish included some degree of vasculature system hemorrhaging. Though expansion of the swimbladder at the levels tested in the current study were not enough to induce tears, at 127 kPa, the highest peak pressure used, the expansion of the swimbladder was enough to induce hemorrhage.

Most other studies have not focused on effects of IPCs near the *Guideline* on the swimbladder. However, a number of studies have described damage to the organ

surrounding and even below this suggested maximal IPC level. For instance, during a test program at Parsons Lake in August and September 2000, lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*) that died following exposure to dynamite at 174 kPa had ruptured swimbladders (Golder 2000). Sakaguchi et al. (1976), reported swimbladder congestion at a peak pressure of 54 kPa in rock fish (*Sebastiscus marmoratus*) exposed to explosive detonations during borehole blasting at Bisan strait. Similarly, Teleki and Chamberlain (1978), who examined acute effects of underwater construction blasting on fish exposed to blasts between 8.61 kPa and 158 kPa in Lake Erie reported that pressures between 30-85 kPa were associated with type 2 injury, characterized by stretching of the swimbladder lining and rupture of fibrous connective tissue. A high explosive (Hydromex), with a detonation velocity 1.5 times that of dynamite, thought to have lowered the minimum lethal pressure from 276 kPa (dynamite) to 30 to 150 kPa was used in this latter study. The lack of damage detected at lower pressures levels in the current study may be attributed to differences between this study and those of other researchers, including species of fish, depth of the waterbody, and swimbladder anatomy (i.e swimbladder thickness) (See Table A2). However, similar to the findings of the current study, Traxler et al. (1993) examined the effects of subsediment detonation of dynamite on caged fish, including bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*) and largemouth bass (*Micropterus salmoides*) in Lake of the Pines, Texas. Exposure of fish ranged from pressure levels of 3.92 kPa to 36.3 kPa. No gross evidence of swimbladder damage was evident at these lower pressures.

Only one other study described the effect of blasting on fish while considering the recommended maximum current *Guideline* IPC level. During the summers of 1992 and 1993, installation of a natural gas pipeline necessitated the crossing of waterbodies including the Nipigon and Winnipeg rivers (McAnuff et al.1994). Excavation of the trench was carried out following, what was at the time, draft *Guidelines* (which still included the condition that instantaneous overpressures do not exceed 100 kPa). McAnuff et al. (1994) reported that 88 fish were killed on Nipigon River, even though blasts were limited to 17 to 97 kPa. Autopsies carried out by the DFO and Ministry of Natural Resources determined that the majority of the fish died as a result of a ruptured swimbladder.

Attempts in the current study were made to examine swimbladder tissue histologically, however due to the nature of the tissue and fixation procedures, fixed swimbladders were unsuitable for analysis. Specifially, swimbladders did not retain their shape in the fixative, which hindered proper orientation of the tissues and hence proper comparison between samples. Govoni et al. (2003) however, histologically examined trauma to juvenile pinfish (*Lagodon rhomboids*) and spot (*Leiostomus xanthurus*) exposed to submarine detonations of varying intensities. The authors reported trauma in the swimbladder of both species exposed at 636.92 kPa including hyperemia of the swimbladder serosa, the muscosa of the gas gland, and the rete mirabile of exposed fish.

No damage was reported to the swimbladder at 109.93 kPa, a level near to the recommended 100 kPa *Guideline*.

Of significance, damage to the swimbladder may result in the upset of hydrostatic ability of the fish, especially should damage (such as hemorrhaging) occur to sensitive

areas such as the gas gland or associated rete system. Damage to the swimbladder may also impede, depending on the species, auditory function, sound production or respiration.

The data of our study show that the swimbladder is indeed a sensitive organ to explosive based IPC, as hemorrhaging occurred near the recommended *Guideline* IPC level (127 kPa). While the data support that pressures at and below the *Guideline* level are protective of adult swimbladders, caution is strongly warranted as this value seems the threshold at which damage does not appear. As previously mentioned the nature of blasting is unpredictable and as such, if pressure were to even slightly exceed 100 kPa, damage would be likely to occur.

3.4.1.2 Eye

Eye damage in the form of exopthalmia was not significantly different among exposure groups for lake trout (Figure 3.12; Table A21 and A23) and sculpin (Figure 3.13; Table A21 and A24).

Various studies have reported protruding eyes in fish exposed to explosive blasts. For instance, Kearns and Boyd (1965) studied the effect of a marine seismic exploration on fish populations in British Columbia coastal waters. Following a series of detonations of nitrone seismic marine (S.M.) of unknown peak pressures, the researchers reported protruding eyes in several species of exposed fish (list of fish found in Table A1). Coker and Hollis (1950) describe that following detonation of charges of HBX (unreported peak pressure), menhaden (*Brevoortia tyrannus*) were seen with protruding eyes. Finally, Thompson (1958) reported that, following detonation of DuPont Nutramex 2-H explosive

(peak pressure not indicated) for the removal of Ripple Rock on the coast of British Columbia, rockfish (*Sebatodes spp.*) showed eye protrusion.

Exopthalmos may arise following explosive events as a result of either gas bubble accumulation behind the eye, from the negative forces exerted during the blasts, or by direct physical trauma (Dukes 1975, Hogan 1941, Muir 1959).

Apart from external measurements, attempts to measure internal eye pressure with the use of a Tonometer (Bio-Rad Tono-Pen XL, California, USA) were made. This was based on a hypothesis that changes in eye pressure may occur due to damage of the rete system behind the eye of the fish upon exposure to IPC. This method however proved unsuitable due to the lack of sufficient eye pressure in the fish and the resultant inability of the tonometer to make a numerical reading.

3.4.2 Tissue Pathology

Certain points should be kept in mind when considering histological assessment of tissue. Firstly, autolytic post-mortem changes can be confused with pre-mortem pathological changes. Ideally tissues for post-mortem examination should be taken from freshly dead specimens or sampled immediately after death (Roberts and Rodger 2001). In the current study, precautions to avoid post mortem pathological mis-interpretations were taken by fixing samples immediately following euthanasia. Secondly, artifacts of fish handling, tissue fixation, tissue preparation and tissue dissection can account for certain non-pathological tissue damage. These artifacts may resemble trauma and result in overinterpretation. To avoid any such artifacts and maximize accuracy and proper identification of pathologies, it was important to ensure that representative tissue was being assessed, not tissue damaged by processing (i.e ripped from the microtome,

damaged by histocassette). This was addressed by avoiding areas with apparent artifactual changes. Furthermore, it was important to evaluate suspected pathologies in relation to surrounding tissue and other sections of the same tissue, to ensure that the pathology was consistent, showed a pattern, and was not an outlier. It was also important for all tissues and slides to be treated and analyzed in the same manner. Finally, it was important to determine what was histologically normal for a particular tissue by studying tissues from control animals prior to designating them with a random number for blind analysis.

3.4.2.1 Liver

Liver tissue in both lake trout and sculpin were not negatively affected by IPCs for any of the parameters assessed (Figure 3.14; Table A21 and A25-A30). However, there are indications that liver is a sensitive organ to the impacts of explosive detonations, especially in swimbladder bearing fish. Cronin (1948), examined the trout (*Cynoscion regalis* [Bloch and Schneider]) and rock (*Roccus saxatilis* Walbaum) exposed to TNT/nitramon charges (of approximately 620- 4544 kPa in peak pressure), and determined that in addition to the swimbladder, one of the main organs affected was the liver. Yelverton et al. (1975) examined the effects in various fish (see Appendix I) from exposure to underwater blasts of Pentolite charges ranging in peak pressure from 530 kPa-9025 kPa and noted that, besides the swimbladder and kidney, livers were most commonly damaged. Coker and Hollis (1950) investigated fish following 21 explosions of HBX charges in Chesapeake Bay (peak pressure levels not indicated) (Table A1). Their investigation confirmed evidence of ruptured blood vessels in the liver. Similarly, Linton et al. (1985) reported liver damage in red drum (*Sciaenops ocellatus*) and black

drum (*Pogonias cromis*) following exposure to Primacord explosive (peak pressure levels not indicated). Liver hemorrhage was also observed in wild fish (including Pacific cod [*Gadus macrocephalus*], tomcod [*Microgadus proximus*] and walleye pollock [*Theragra chalcogramma*]) incidentally killed by detonations of linear explosive ranging from approximately 37-331 kPa in peak positive pressure (Houghton and Munday 1987). During a test program at Parsons Lake in August and September 2000, lake whitefish (*Coregonus clupea)* and northern pike (*Esox lucius*) exposed to charges of dynamite at a pressure of 174 kPa exhibited hepatic bruising (Golder 2000). Finally, Roguski and Nagata (1970) reported ruptured livers in captive northern pike (*Esox lucius*) exposed to under ice detonations of C-4 explosives (peak pressure not indicated) while Thomson (1958) noted blood clots during post mortem examination of the livers of resident banded rockfish (*Sebastodes nigrocinctus*), red snappers (*Sebastodes ruberrimus*), orange-spotted rockfish (*Sebastodes maliger*) and copper rockfish (*Sebastodes caurinus*) after a blast from DuPont Nitramex 2-H explosive (peak pressure not indicated).

Damage to the liver has been reported at IPCs below the recommended Canadian *Guideline* level. Sakaguchi et al. (1976), examined the influence of underwater explosions on common carp (*Cyprinus carpio*) and rock fish (*Sebastiscus marmoratus*) and reported congestion within the liver of rockfish at a peak pressure of 54 kPa during borehole blasting at Bisan strait, Japan (unknown explosive type).

Lack of trauma to the liver of fish in the current study is attributed to lack of overextension of the swimbladder at tested pressure levels, and hence no collateral damage to visceral tissue. The degree of swimbladder overexpansion at 127 kPa may have been enough to cause hemorrhage to the organ, but not enough to cause damage to

other tissues, including the liver. Though damage to the liver has been reported at IPCs below the 100 kPa *Guideline* level, this damage could have resulted due to any one of the influential factors presented in Table A1 including species of fish, positioning of fish relative to the blast at the time of detonation, and orientation of the fish relative to the blast.

3.4.2.2 Spleen

There were no significant negative effects of IPC on any of the parameters assessed in spleen (Table A21 and A31, A32).

Spleen tissue was not assessed in sculpin due to the small size of the tissue and potential damage that would have been inflicted during dissection, resulting in compromised samples.

Studies have reported the sensitivity of the spleen to blast exposures, especially within swimbladder bearing fish species. Spleen was affected in trout (*Cynoscion regalis* [Bloch and Schneider]) and rock (*Roccus saxatilis* Walbaum) exposed to TNT/nitramon charges (peak pressures of approximately 620- 4544 kPa) (Cronin 1948). Tyler (1960) conducted an experiment on the Kvichak River in Igiugig, Alaska to determine the effect of underwater explosives on adult red salmon (*Oncorhynchus nerka*). Following detonation of 40% gelatine dynamite of unknown peak pressure, internal examination revealed torn spleens. Houghton and Munday (1987) examined wild fish incidentally exposed to linear explosives varying in peak positive pressures from approximately 37-331 kPa. Wild fish affected by the detonations included juvenile walleye Pollock (*Theragra chalcogramma*) and coho salmon (*Oncorhynchus kisutch*), as well as rockfish (*Sebastes spp.*), Pacific tomcod (*Microgadue proximus*), Pacific cod (*Gadus*

macrocephalus), Pacific herring (*Clupea harengus pallasi*), and Dolly Varden (*Salvelinus malma*). The authors reported that herring (*Clupea pallasii)* autopsied following the blasts showed injured spleens, though no detail was provided on the type of injury seen. Furthermore, in the same experiment, the researchers investigated the effects of linear explosive detonations on caged juvenile fish, including coho salmon (*Oncorhynchus kisutch*) and chum salmon (*Oncorhynchus keta*) smolts and juvenile pacific herring (*Clupea harengus pallasi*). Hemorrhages in the spleen of coho salmon were detected. Coker and Hollis (1950) also investigated the effects of blasting on fish (see Table A1) following 21 explosions of HBX charges in Chesapeake Bay (pressure levels not indicated). Their investigation confirmed evidence of ruptured blood vessels in the spleen. Finally, during a test program at Parsons Lake adult northern pike (*Esox lucius*) as well as adult and juvenile lake whitefish (*Coregonus clupea*) that died following a dynamite blast measuring 174 kPa were found to show damage to the spleen. No further detail however is reported.

Spleens within the current study were undamaged most likely because effects to the swimbladder were minimal, and resultantly the spleen was unaffected.

3.4.2.3 Intestine

There were no negative impacts of IPCs on intestine of lake trout (Table A21 and A33-A35) or sculpin (Table A21 and A36-A37) for any of the parameters assessed. There is however suggestion of an increase in the occurrence of RBCs within the intestinal lumen at upper IPC exposures (Figure 3.15), justifying the need for further research at IPCs surrounding the current *Guideline*.

Previous studies have reported the intestine to be a vulnerable organ to explosive based pressure changes, mostly in swimbladder bearing species. Intestinal damage was reported in caged Arctic ciscos (*Coregonus autumnalis*) exposed to blasts from Aquaflex and 60% Geogel explosives (unknown peak pressure) (Falk and Lawrence 1973). Kostyuchenko (1973) also reported injury to the anterior part of the intestine in anchovy (*Engraulis encrasicholus ponticus* Aleksandrov) and blue runner larvae (*Caranx crysos*) exposed to TNT (no peak pressure indicated). Linton et al. (1985) noted rupture of the intestinal wall following the evaluation of injury to red drum (*Sciaenops ocellatus*) and black drum (*Pogonias cromis*) from detonation of Primacord explosive (peak pressure levels not indicated). Roguski and Nagata (1970) investigated the physical effects of blasting on caged yearling king salmon (*Oncorhynchus tshawytscha*) exposed to under ice detonations of C-4 explosives (peak pressure not indicated). Autopsy results confirmed ruptures and hemorrhaging of the intestine in exposed fish.

A lack of damage to the intestinal tract following low level IPC exposure has also been reported. Traxler et al. (1993) studied the effects of subsediment detonations of dynamite on caged fish, including bluegill (*Lepomis macrochirus*), channel catfish (*Ictaluris punctatus*) and largemouth bass (*Micropterus salmoides*) in Lake of the Pines, Texas. Exposure of fish ranged from pressure levels of 3.92 kPa to 36.3 kPa. Following necropsy of fish exposed at all pressure levels, no gross evidence of digestive tract damage was determined. Govoni et al. (2003) investigated the histology of intestinal tissue in juvenile spot (*Leiostomus xanthurus*) and pinfish (*Lagodon rhomboids*) following average submarine detonations of 109.93 kPa, 230.86 kPa, and 636.92 kPa. Though liquefactive necrosis was identified, it was concluded that the observed trauma

was not due to shock wave exposure, but more likely due to high food intake prior to the experiment. Noted lesions resembled those reported in studies by Mobin et al. (2000, 2001) who evaluated feeding levels and associated pathological alteration in the digestive system in Japanese flounder (*Paralichthys olivaceous*) and seabream (*Pagrus major*).

That no damage was determined at any of the peak pressure levels in the current study is accredited to negligible expansion of the swimbladder.

3.4.2.4 Kidney

Kidney tissue was not negatively impacted by IPCs in lake trout (Figure 3.16-3.18; Table A21 and A38-A43) or sculpin (Figure 3.19-3.20; Table A21 and A44-A49) for any of the variables assessed. As observed in figure 3.16, 3.18, 3.19, 3.20, RBCs and erythrophages are routinely observed in reference conditions, highlighting that these alone do not necessarily indicate damage and it is therefore necessary to examine for abnormal presence of RBCs or erythrophages, beyond that which is found in reference samples. Figure 3.17, with the exception of the 72 kPa exposed group, suggests a trend in kidney hemorrhage in lake trout. Though not significant, it does however warrant further investigation.

Contrary to our findings, many studies have reported damage to kidneys following explosive blasts. Yelverton et al. (1975) examined the effects in various fish (see Table A1) from exposure to underwater blasts of Pentolite charges ranging in peak pressure from 530 kPa-9025 kPa and noted that kidneys commonly sustained damage. Ferguson (1962) noted kidney hemorrhage in freshwater fish including yellow perch (*Perca flavescens*), emerald shiner (*Notropis atherinoides*) and American smelt (*Osmerus mordax*) collected at the lake surface following detonation of various explosive

combinations (see Table A1) of unknown peak pressure. In the same study, 74.3% of yellow perch caged during the experiment exhibited hemorrhage in the kidney tissue. Linton et al. (1985) examined the extent of injury in red drum (*Sciaenops ocellatus*) and black drum (*Pogonias cromis*) following exposure to Primacord explosive (peak pressure levels not indicated). The nature of injuries noted to the fish included renal portal vein hemorrhage, as well as rupture of kidney tubules, mesovarium and mesorchium. In fact, kidney damage was one of the most frequently recorded injuries. Thompson (1958) reported that, following detonation of DuPont Nutramex 2-H explosive (peak pressures not indicated) for the removal of Ripple Rock on the coast of British Columbia, rockfish (*Sebatodes spp*.) showed blood clots associated with the kidney. Finally, Houghton and Munday (1987) examined wild fish incidentally killed during exposure to experimental detonations of linear explosives (varying in peak pressure from 37-331 kPa). Species including gadid species (including Pacific cod [*Gadus macrocephalus*], tomcod [*Microgadus proximus*] and walleye pollock [*Theragra chalcogramma*]) exhibited hemorrhaged kidneys. In the same study rockfish (*Sebastes sp*.) showed hemorrhage and rupture of the kidney. The aforementioned list of studies illustrating damage to kidneys from explosive events is not exhaustive. Other studies that exemplify the sensitivity of the kidney to explosives include Tyler (1960), Fernet (1982), Golder (2000), Falk and Lawrence (1973), Hubbs et al. (1960).

Damage to kidneys has also been reported near and below the 100 kPa *Guideline* level. Teleki and Chamberlain (1978) examined acute effects of underwater construction blasting on fish in Lake Erie. Fish exposed to blasts between 8.61 kPa and 158 kPa, in addition to swimbladder rupture, exhibited kidney hemorrhage as one of the most

common recurring fatal injuries. A test program at Parsons Lake revealed ruptured kidneys in lake whitefish (*Coregonus clupea)* and northern pike (*Esox lucius*) that died following a dynamite blast of 174 kPa (Golder 2000). Sakaguchi et al. (1976) examined the influence of underwater explosions on common carp (*Cyprinus carpio*) and rock fish (*Sebastiscus marmoratus*). The researchers reported both tissue damage and congestion within the kidney of rockfish at a peak pressure of 54 kPa during borehole blasting at Bisan strait.

Results from this study are similar to those reported by Traxler et al. (1993) who found no gross evidence of kidney damage in bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*) and largemouth bass (*Micropterus salmoides*) in Lake of the Pines, Texas following exposure to dynamite ranging in pressure from 3.92 kPa to 36.3 kPa. A lack of damage to kidneys can be attributed to minimal expansion of the swimbladder at tested pressure levels. Though damage has been reported below the *Guideline* level, similar damage may not have arisen in the current study due to any one of the influential factors presented in Table A1.

3.4.2.5 Conclusion (Tissue Pathology)

In conclusion, the liver, spleen, intestine and kidney tissue in both lake trout and sculpin were not negatively impacted for any of the factors evaluated. This was the case even though all tissues have been shown to be sensitive to the effects of IPCs in previous studies. The most likely reason is a lack of effect from explosive blasts surrounding the *Guideline* level, notably the minimal expansion of the swimbladder to a volume that would cause visceral damage. The incidence of damage reported below the *Guideline*

level may differ from the results of the current study as a result of various influential factors (Table A1). Histological findings in the livers, spleen, intestine and kidney of adult fish and fish without a swimbladders exposed to explosive based IPC is generally lacking making the quantitiative histological comparisons in our study unique.

The absence of damage in the tissues of the scuplin may simply be a function of not having a swimbladder to cause damage to adjacent tissues as a result of rapid expansion. Results from the current study suggest that visceral tissues are not especially sensitive to IPCs near the current *Guideline*. However, additional research is required to examine suggested trends surrounding the current *Guideline.*

3.4.3 Blood Pathology

There were no significant differences among exposure and control groups for AST, ALT, CK, creatinine and phosphorus (Figure 3.21-3.25; Tables Table A21 and A50-A55). BUN levels were below detection limits. These results corroborate the histological results from liver and kidney tissues which determined that these organs were not negatively affected by IPCs for any of the parameters assessed. Though muscle and gill were not assessed histologically, evaluation of additional blood analytes suggested a lack of damage to these tissues. Variation in concentration levels between exposure groups however can be attributed to the potential presence of disease, parasites or injury in fish unrelated to exposure, which could alter blood parameters. Variation can also occur as a result of handling (Campbell 2004).

Analysis focussed on blood enzymes (AST, ALT, CK), nitrogenous compounds (BUN, creatinine) and minerals (phosphorus) that are specific indicators of tissue damage in fish.

All tests were performed at a routine measurement temperature of 37 ºC. Measurement of enzyme activities at 37°C however may inactivate the enzymes of fish especially in cold water species including salmonids. Mammalian enzyme analysis protocols may therefore not be recommended for fish (Groff and Zinkl 1999). However, conditions in the current study were similar to previous studies that have investigated blood based clinical biochemistry in fish (Canfield, 1994, Christensen et al. 1994, Folmar et al. 1992, 1993, 1995)

Though it has been argued that blood markers are non-specific and limited as indicators of damage (Wagner and Congleton 2004), studies exist to the contrary, highlighting their applicability as relevant indicators of damage.

3.4.3.1 Enzymes (AST, ALT, CK)

AST and ALT are found in liver, kidney and muscle tissue of fish (Bucher 1990, Stoskopf 1993). They are key enzymes in protein to carbohydrate metabolism (Oluah 1999). These enzymes were used as indicators of tissue damage from IPC exposure in liver, kidney and muscle tissues in fish from this study. The use of AST and ALT as indicators of liver, renal and muscle damage is supported by numerous studies. Casillas et al. (1983) injected English sole (*Parophrys vetulus*) with carbon tetrachloride, a known hepato/nephrotoxin, to determine relationships between lesions in specific organs and serum indicators of tissue damage. AST and ALT both increased correlating with liver lesions. Carbon tetrachloride injected pinfish (*Lagodon rhomboids*) also had elevated serum AST and ALT (Folmar et al.1993). Young et al. (1994) related histopathology and blood chemistry in normal and moribund striped bass (*Morone saxatilis*) involved in summer die-off in the Sacramento-San Joaquin Delta of California. Levels of marker

enzymes for liver function, including AST, were significantly higher in moribund fish and reflected the histopathological damage seen in the livers. Grizzle and Kiryu (1993) found no relationship between AST activity and hepatic necrosis in channel catfish (*Ictalurus punctatus*) infected with bacteria (*Aeromonas hydrophilia* complex). However, Řehulka (2003) examined biochemical profiles of rainbow trout (*Oncorhynchus mykiss*) infected with viral hemorrhagic septicaemia (VHS) virus and determined that increased concentration of AST and ALT in the diseased fish were related to damage to the hepatocytes. Folmar et al. (1995) investigated whether serum chemistry measurements could be used as predictive indices of neoplasia in tumour bearing brown bullheads (*Ameriurus nebulosus*). Fish from a contaminated lake exhibited significantly higher levels of AST and ALT when compared to the reference site. This was considered to be consistent with liver damage. Chen et al. (2004) conducted a comparative study of blood chemistry and histopathology of Nile tilapia (*Oreochromis niloticus*) after infection with bacteria (*Vibrio vulnificus, Streptococcus iniae*) or exposure to either carbon tetrachloride, gentamicin or copper sulphate. The severity of liver and kidney histopathology was associated with elevated AST and ALT. Chen et al. (2003) looked at the blood chemistry of healthy and nephrocalcinosis affected tilapia (pure strain *Oreochromis niloticus*) and deduced that nephrocalcinosis affected tilapia differed significantly from healthy fish in AST and ALT activities. Specifically, as it relates to AST, Canfield et al. (1994) noted extreme variability for AST in a study investigating hematological and biochemical reference values for captive Australian snapper (*Pagrus auratus*). This was thought to reflect muscle damage associated with blood collection. Similarly, Messager et al. (1992) studied the effects of a modified diet on histopathology,

hematology, tissue and plasma biochemistry in sea bass (*Dicentrarchus labrax*). In fish fed the modified diet, skeletal muscle degeneration was observed and AST was increased.

CK is an enzyme that plays a role in ATP generation as it catalyzes the reaction ADP + phosphocreatine → ATP + creatine (Barrantes et al. 1983, Weng et al. 2002). In tissues of channel catfish (*Ictalurus punctatus*), Liu et al. (2001) reported highest CK activity within muscle tissue. CK was used as an indicator of muscular damage for the current study based on its high activity in that tissue and that fact that it has previously been utilized as an indicator of muscle damage in fish (Canfield et al. 1994, Messager et al. 1992, Rodger et al. 1991). Rodger et al. (1991) researched acute skeletal myopathy in farmed Atlantic salmon (*Salmo salar*) affected by 'sudden death syndrome" (SDS). Pathological findings in the fish were located predominantly in skeletal muscle and significantly elevated creatine kinase levels confirmed the severe myopathy (Rodger et al. 1991). Canfield et al. (1994), in addition to correlated AST levels, showed extreme variability in CK levels reflecting muscle damage associated with blood collection in captive Australian snapper (*Pagrus auratus*). Finally, Messager et al. (1992) reported increased CK levels along with increased AST activity upon observation of skeletal muscle degeneration.

3.4.3.2 Nitrogenous Compounds (BUN, Creatinine)

Urea nitrogen is a nitrogenous waste produced in small quantities in fish (Campbell 2004). Often used as a measure of renal function, disease and injury in mammals (Nelson et al. 1999, Ragan 1989), in fish, urea is excreted primarily by the gills (Campbell 2004, Stoksopf 1993). An elevated BUN in bony fishes therefore associated with branchial compromise (Campbell 2004, Nelson et al. 1999, Stoksopf 1993). Nelson

et al. (1999), in examining the effects of copper-induced gill proliferation and gentamicin-induced renal tubular injury in goldfish, explained that changes in BUN levels were not noted in control fish or fish with kidney damage alone, but were instead seen only in fish affect by gill epithelial proliferation. Bernet et al. (2001) found changes in serum chemistry in brown trout (*Salmo trutta* L.) exposed to effluent from a sewage treatment plant. A significant correlation between histological gill lesions and BUN concentration was demonstrated, and it was determined that BUN most strongly indicates gill lesions (Bernet et al. 2001). The aforementioned studies therefore support evidence that changes in BUN correlates to gill as opposed to kidney damage and that BUN in fish is not a relevant marker of kidney upset in fish. In the following study, BUN was used to evaluate gill damage.

Creatinine is an indicator of renal damage in fish (Casillas et al. 1983, Sakamoto et al. 2001) and was therefore used to indicate kidney damage within the current study. Creatinine is formed from creatine and is also secreted by the kidneys of fish (Campbell 2004, Stoksopf 1993). Once formed it is excreted unmetabolized (Stoksopf 1993). In mammalian models, creatinine is used as a measure of renal function and disease (Fettman and Rebar 2004,Wardrop and Van Hoosier 1989). This has also been demonstrated in fish such as the English Sole (*Parophrys vetulus*) in which increases in plasma creatinine concentrations have been associated with renal disease (Campbell 2004).

3.4.3.3 Minerals (Phosphorus)

Based on mammalian organisms, phosphorus (as phosphate) is a mineral found in bone and components such as nucleic acids and cell membrane phospholipids and is

furthermore involved in energy metabolism (Porter and Kaplam 2009). Plasma phosphorus/phosphate is an inorganic mineral associated with the piscine kidney (Casillas et al. 1983,, Renfro 1997, Vielma and Lall 1998). Phosphorus was used as supplement to AST, ALT, and creatinine as a marker of kidney damage. Further to AST, ALT and creatinine, phoshorous/phosphate has been studied in relation to renal damage. Casillas et al. (1983), who injected English sole (*Parophrys vetulus*) with carbon tetrachloride, found that increased serum phosphate in fish was related to lesions in the kidney. In fact, based on data from the English sole used in the study, phosphate may be the most sensitive indicator of kidney damage (Casillas et al. 1983). In contrast to the findings of Young et al. (1994) and Casillas et al. (1983), Amend and Smith (1975), determined that phosphorus in rainbow trout (*Salmo gairdneri*) was significantly reduced in fish injected with infectious hematopoietic necrosis virus (IHNV), a virus in which the primary pathological lesion in diseased fish is extensive necrosis of the hematopoietic tissue. Also, John (2007) noted significant decline in phosphorus level of blood in the striped dwarf catfish (*Mystus vittatus*) following chronic exposure to the pesticides Metasystox and Sevin. These decreases were associated with kidney dysfunction.

3.4.3.4 Conclusion (Blood Parameters)

Blood biochemistry proved beneficial for supplementing histological findings in fish exposed to explosive based IPC. The study not only supported and strengthened our histological findings, but provided baseline blood profile values in lake trout both unexposed and exposed to blasting based IPCs.

Lack of significant variation for blood parameters is attributed to the fact that no damage to tissues from IPC exposures existed. Further research should be conducted to ensure that, in fact, blood parameters were not altered or inactivated due to analysis at 37°C.

3.5 Conclusion

Resident lake trout appeared to be attracted to the blasting area when small fish were stunned or killed following blasting events. Hemorrhaging in the swimbladder was significantly elevated in caged lake trout at 127 kPa as compared to controls. There were no significant differences in eye position, tissue pathology or blood biochemistry among the treatment groups for lake trout or sculpin. The most notable effects from IPC exposure was hemorrhaging in the swimbladder of lake trout.

The current study shows that effects from IPCs can occur in adult swimbladder bearing lake trout at levels surrounding the current recommended *Guideline* level of 100 kPa. Sculpin, fish that lack a swimbladder, seem more tolerant of blasting events, as no damage for any of the parameters assessed was detected. Therefore, the null hypothesis is rejected for lake trout while the null hypothesis is accepted for sculpin.

Further research is required to enable more definitive conclusions regarding the impact of explosive based IPC on fish. Future research should also evaluate blood biochemistry analyzed at 37°C versus optimal temperatures for fish. Finally, pathology in fish with a closed connection between the gut and swimbladder (physoclists) and those with an open connection to the gut (physostomes) should be evaluated.

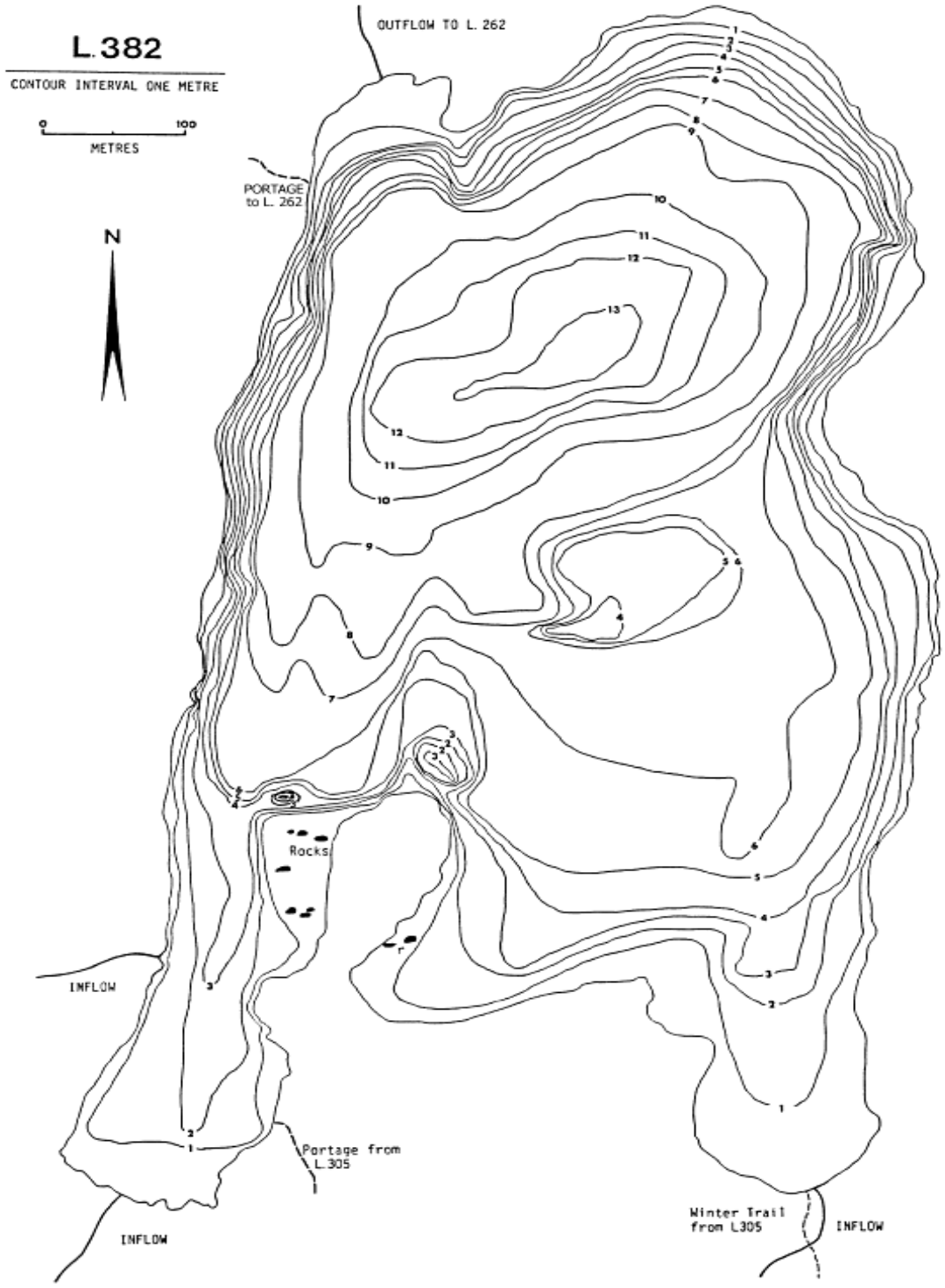


Figure 3.1: Bathymetric map of Lake 382 (ELA Data Retriever provided by Susan Kasian, Department of Fisheries and Oceans, Winnipeg, June 2009). Numbers represent depths in metres

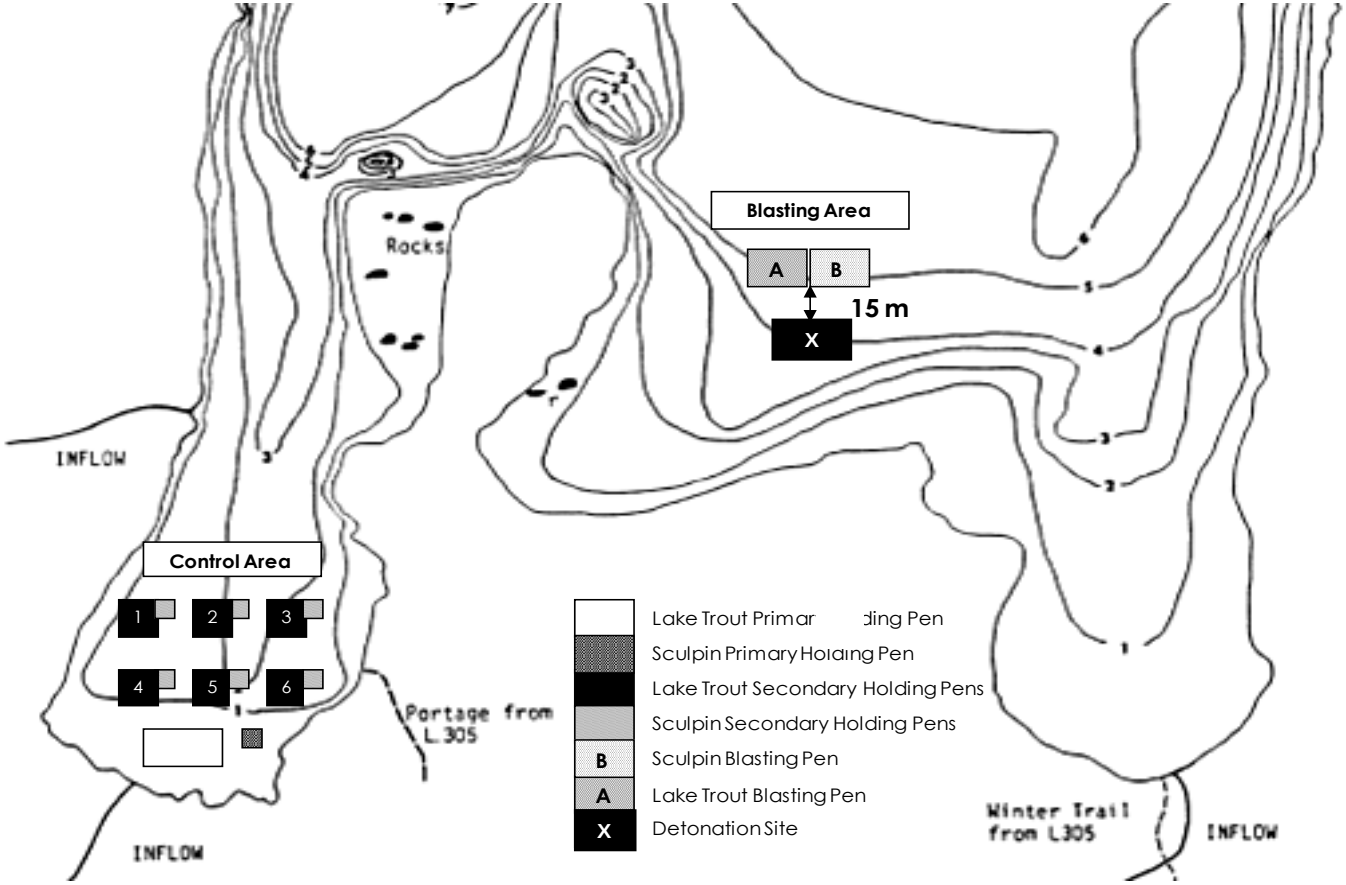


Figure 3.2: Lake 382 Pen Setup

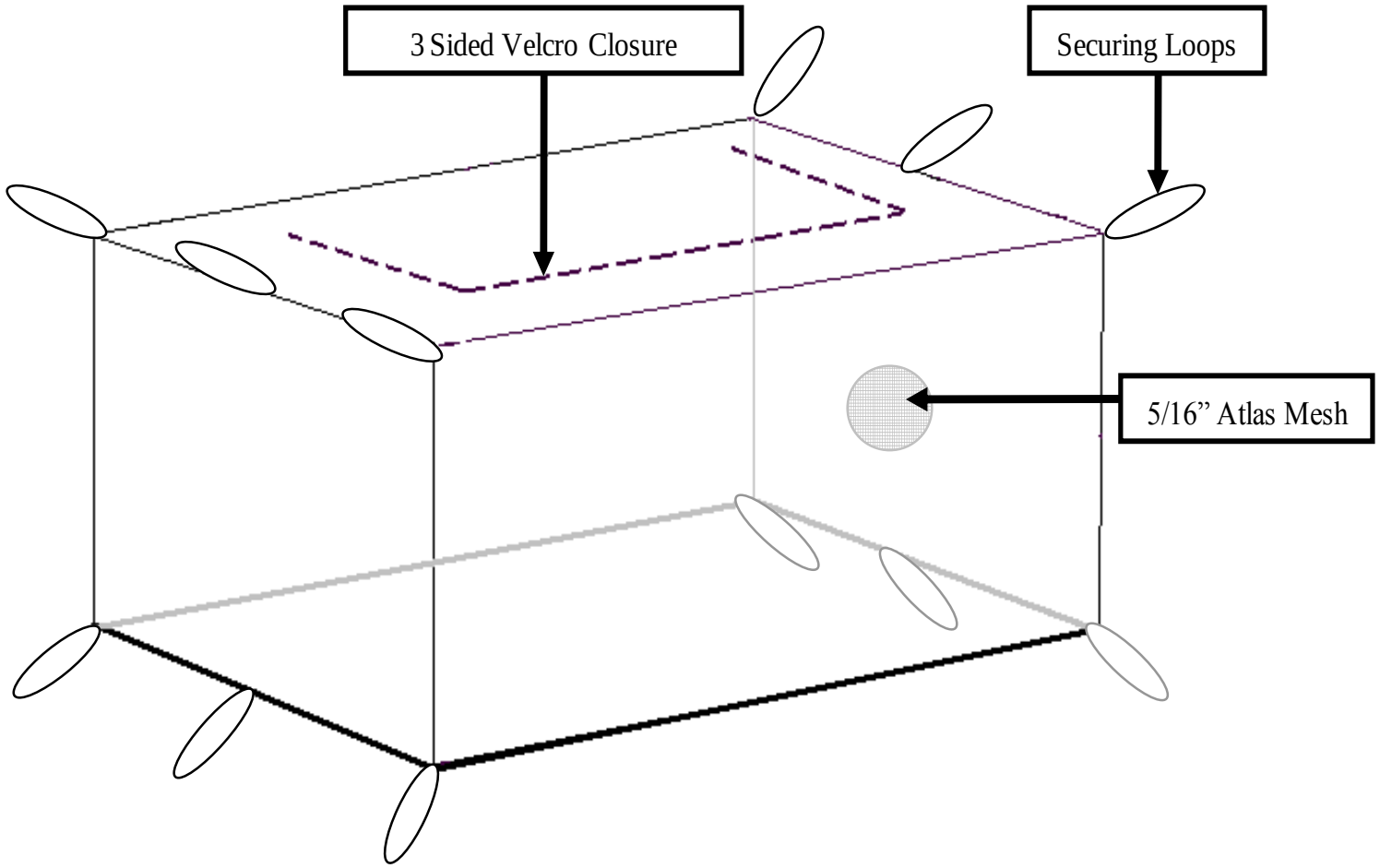


Figure 3.3: Design of fish holding pens for lake trout in control and blasting area as well as for sculpin in blasting area. Pens were of the same design, differing only in dimension.

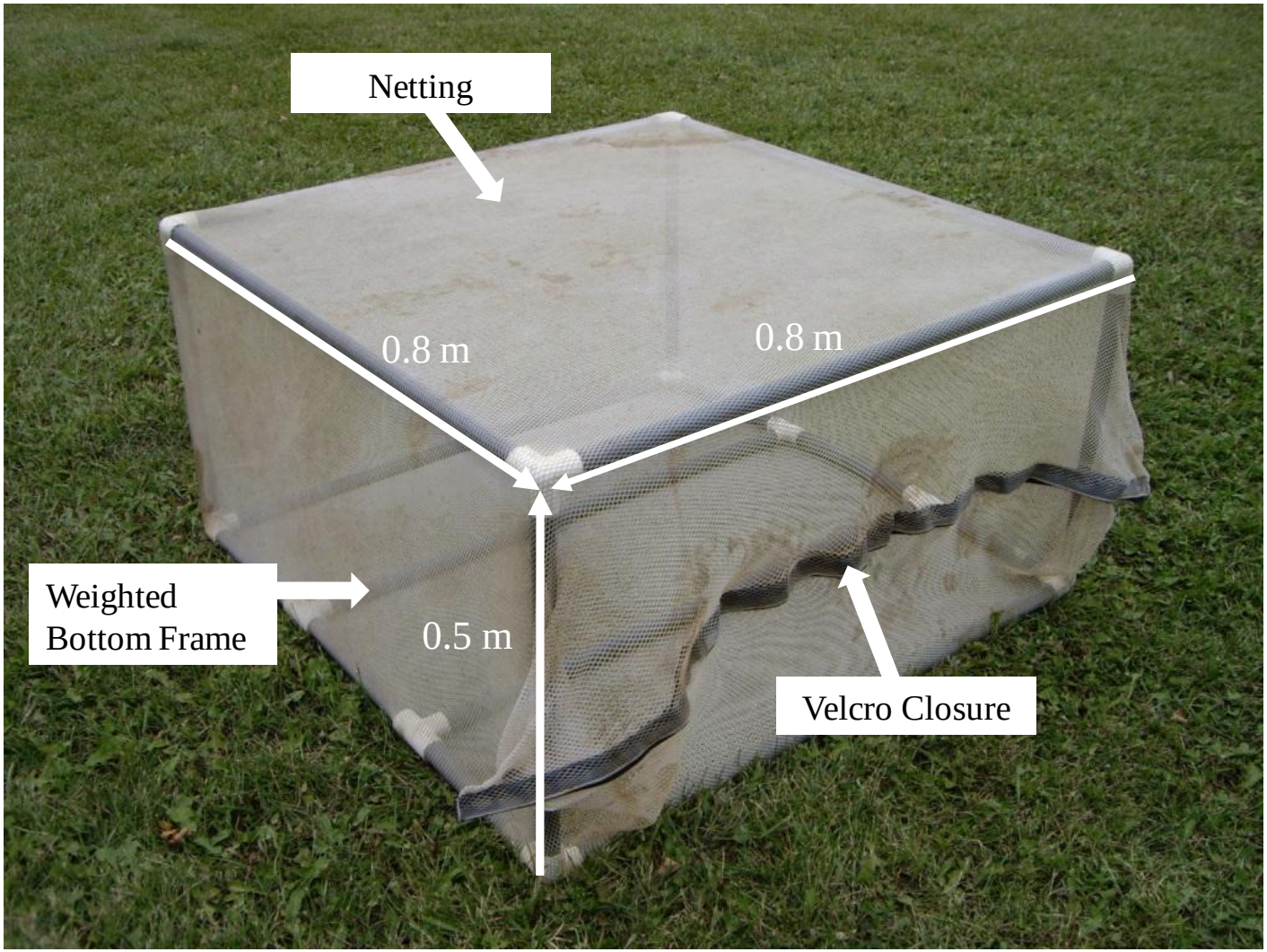


Figure 3.4: Sculpin Cage

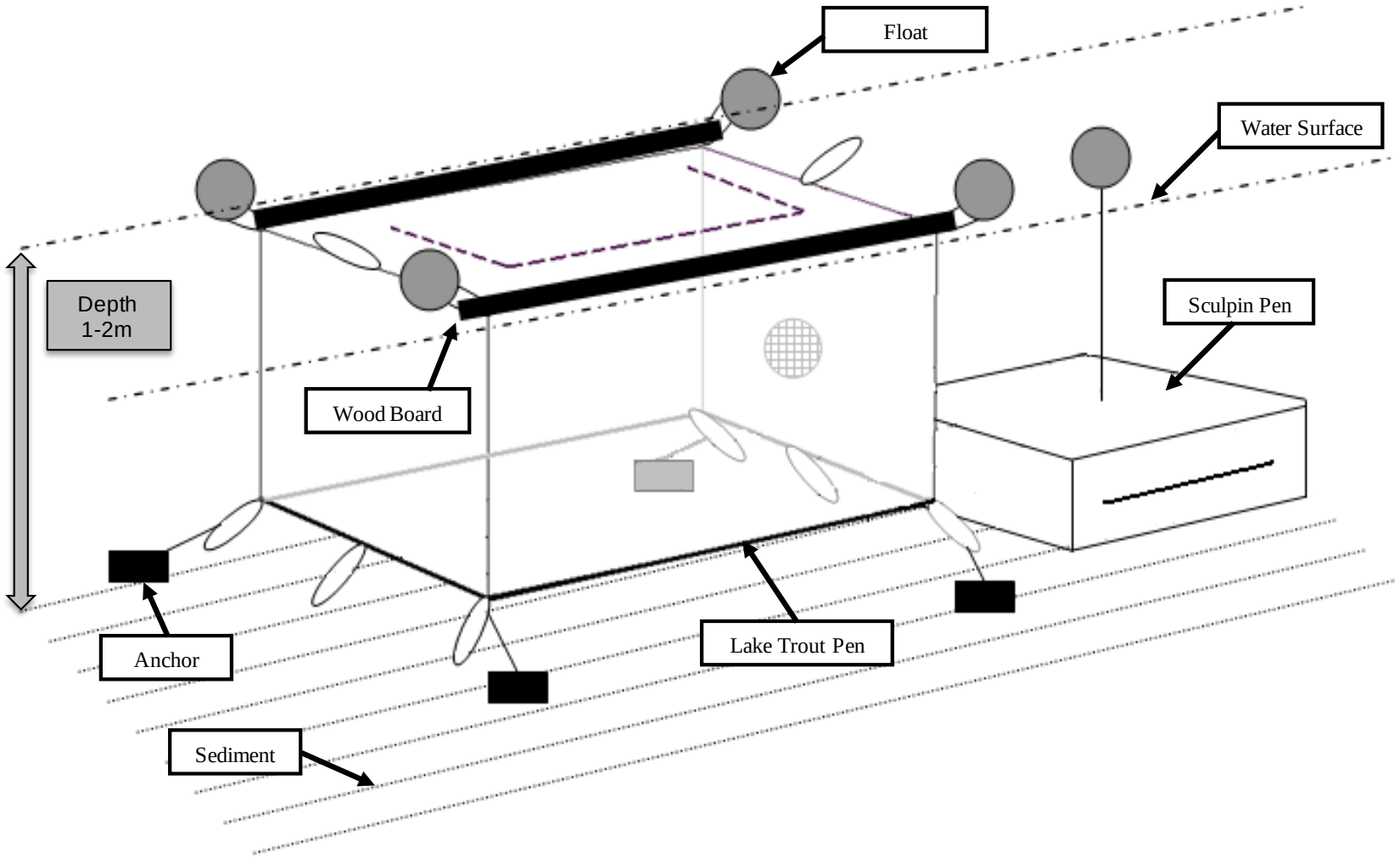


Figure 3.5: Image depicting underwater setup of a lake trout and sculpin pens within control area

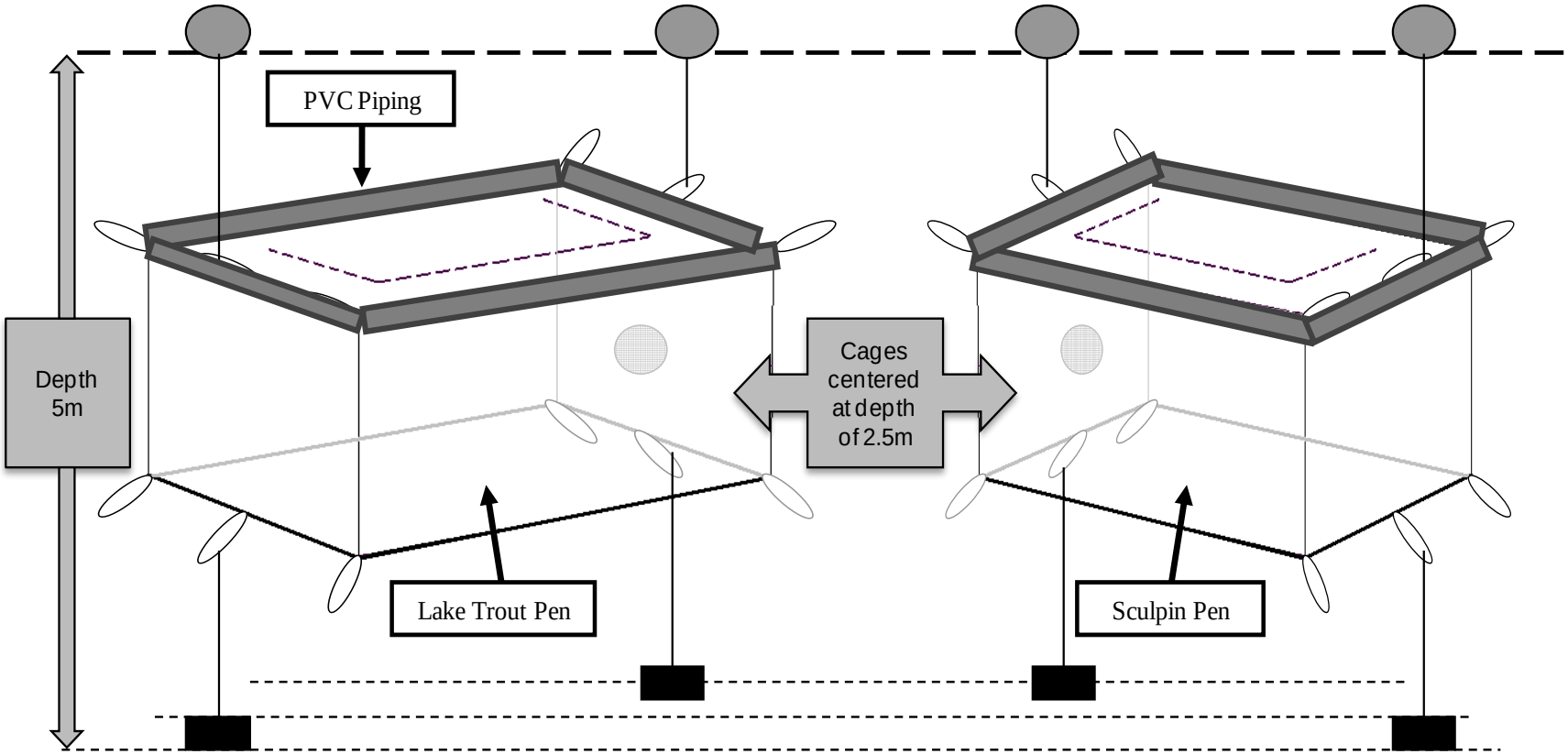


Figure 3.6: Image depicting underwater setup of a lake trout and sculpin pens within blasting area.

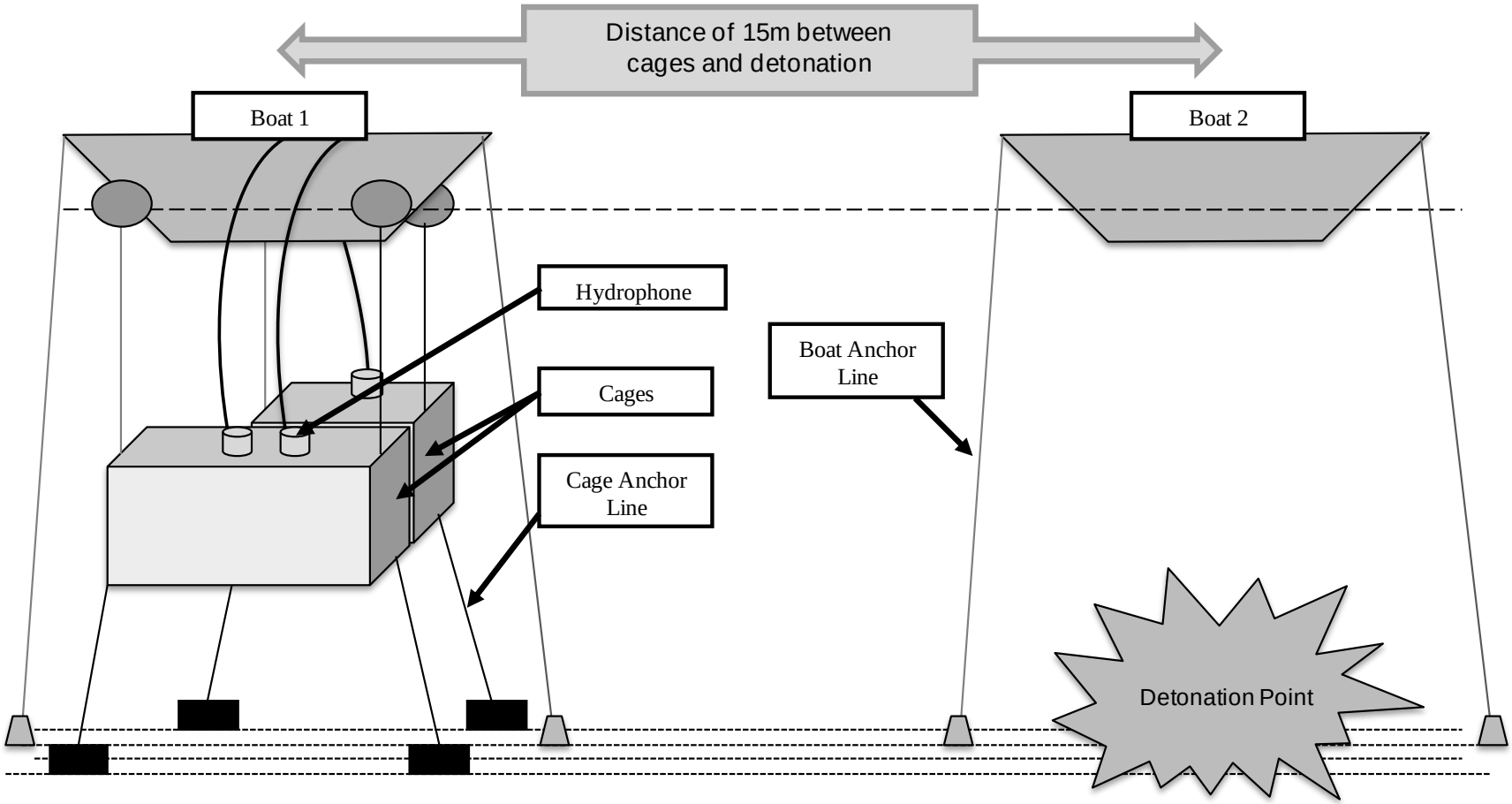


Figure 3.7: Image depicting a boat anchored over the cages for monitoring of IPC with hydrophones (Boat 1) and a second boat (Boat 2) for setting up and detonation of charges, within blasting area.

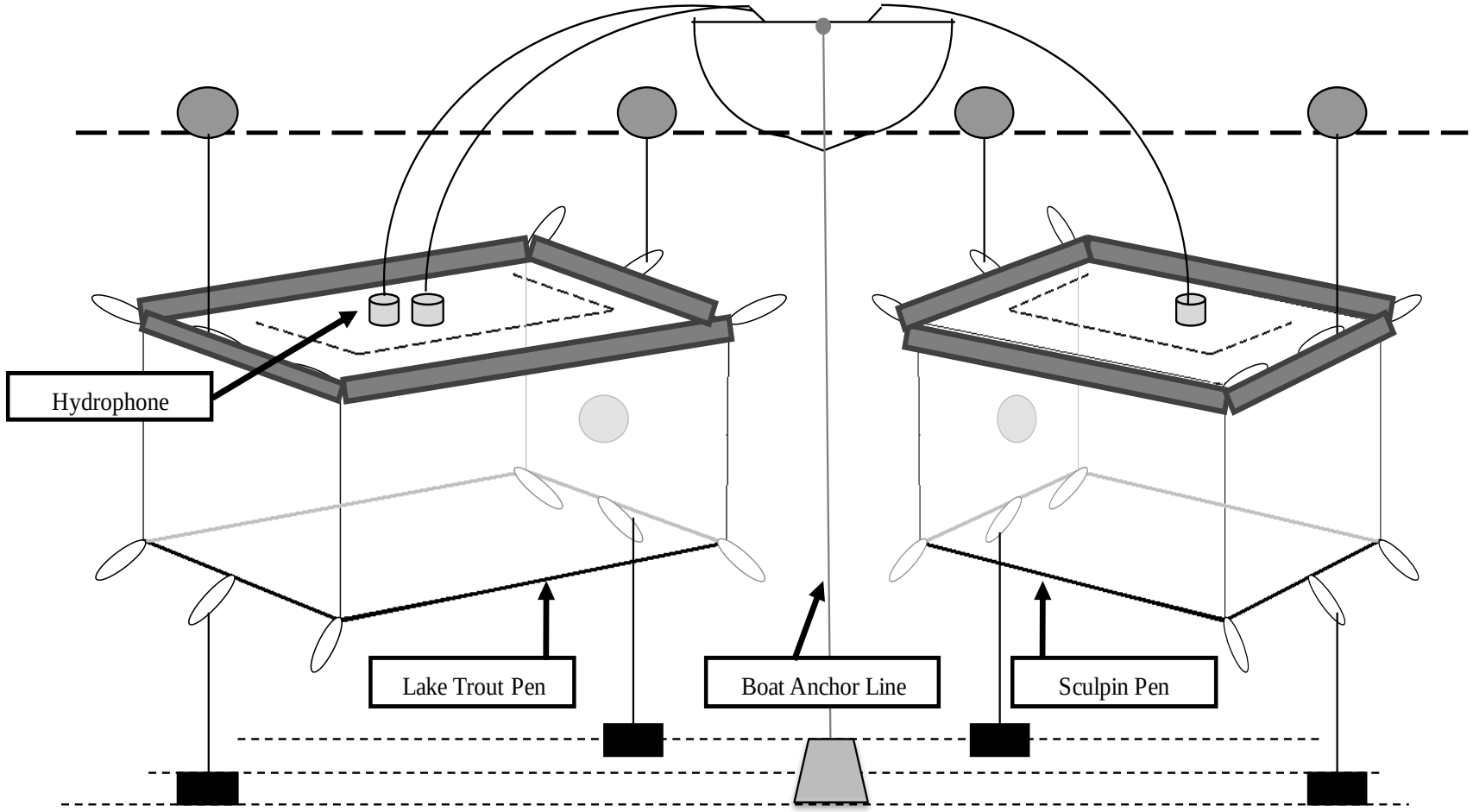


Figure 3.8: Frontal view of cage setup and pressure monitoring devices (hydrophones) within blasting area during detonation event.

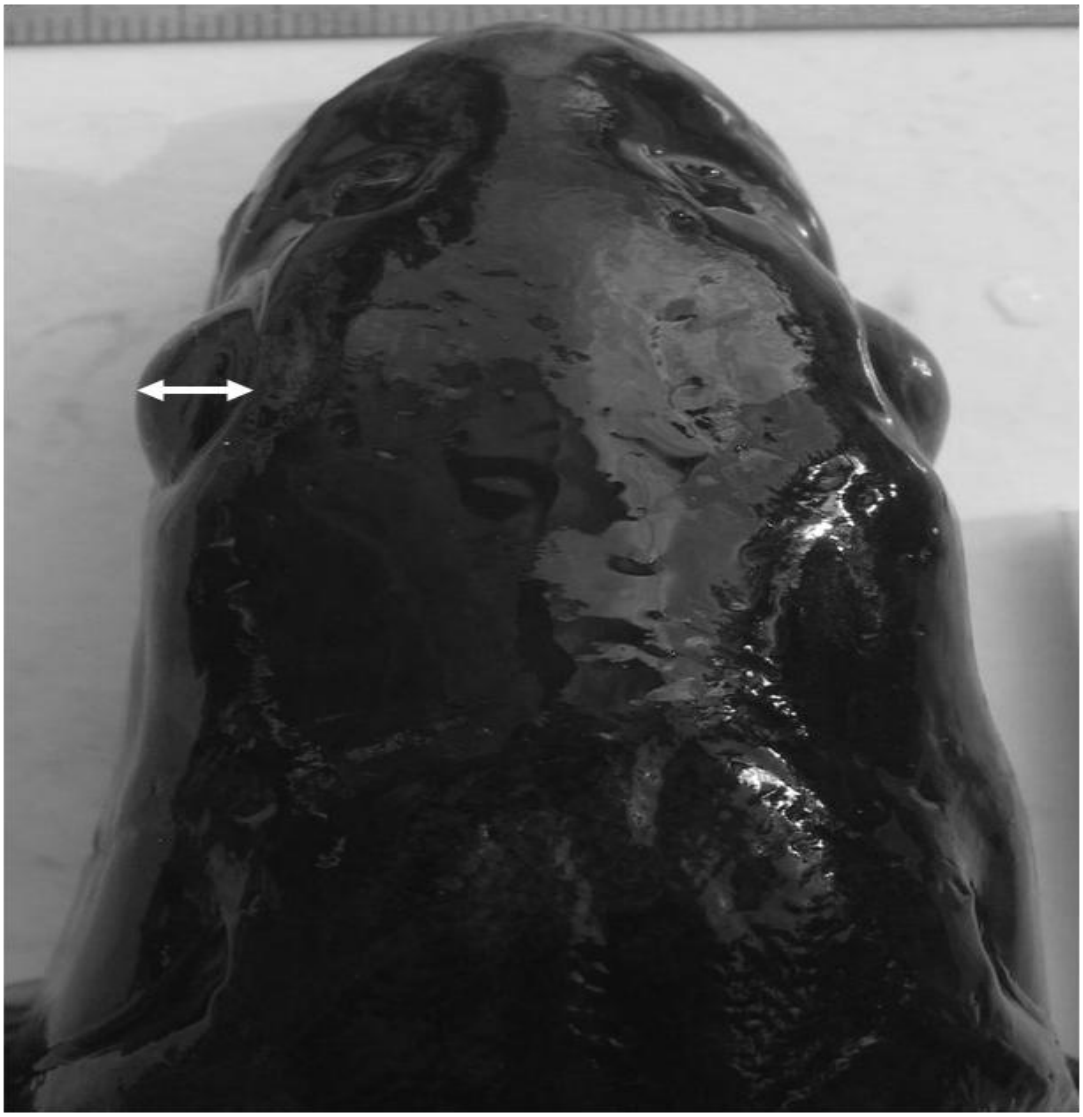


Figure 3.9: Image of test lake trout, showing location of eye measurements-extending between the orbit of the eye to the outside of the cornea.

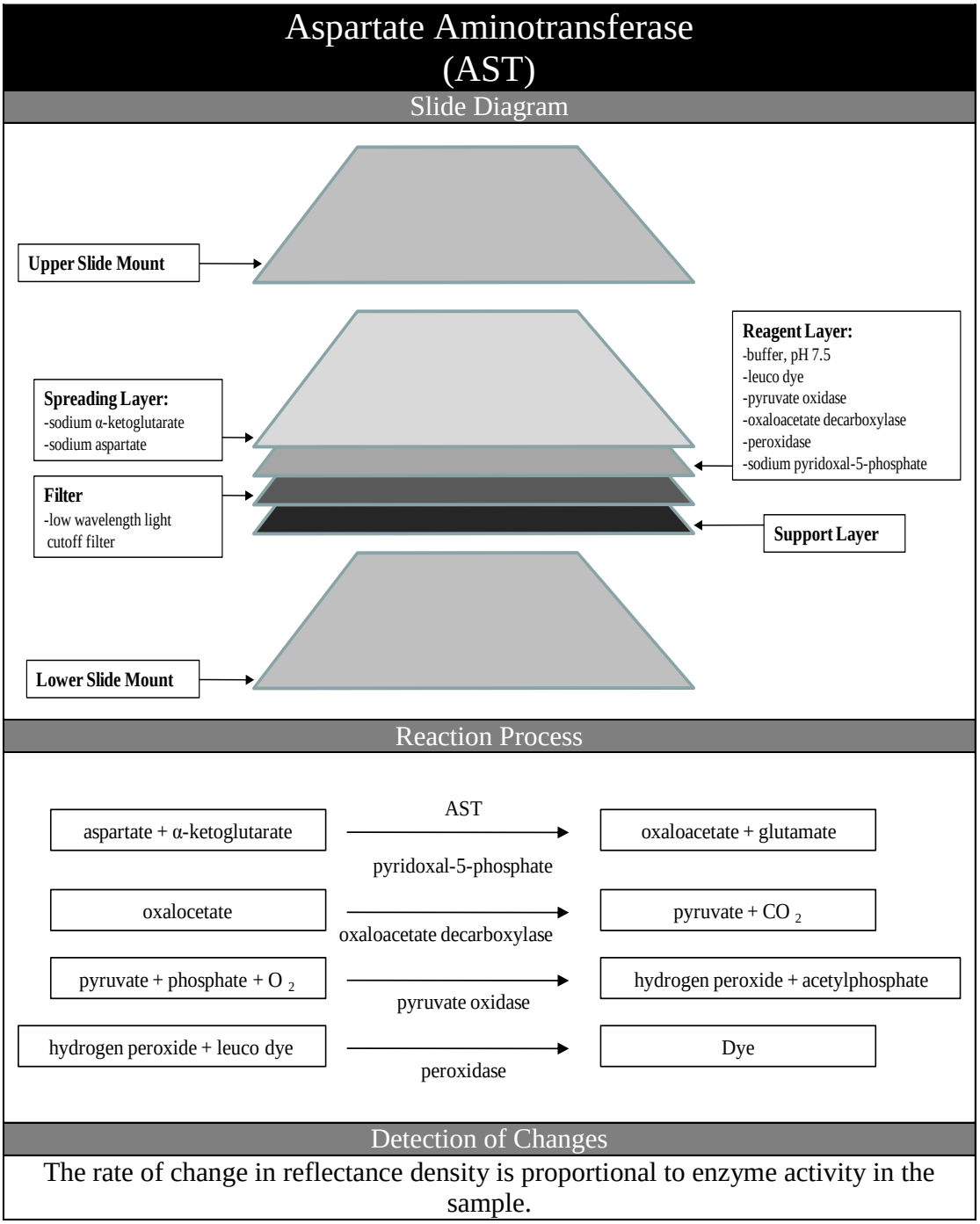


Figure 3.10: Layering of the VITROS slide, reaction processes and how changes are measured for AST analysis (Ortho-Clinical Diagnostics, Inc. 2004b)

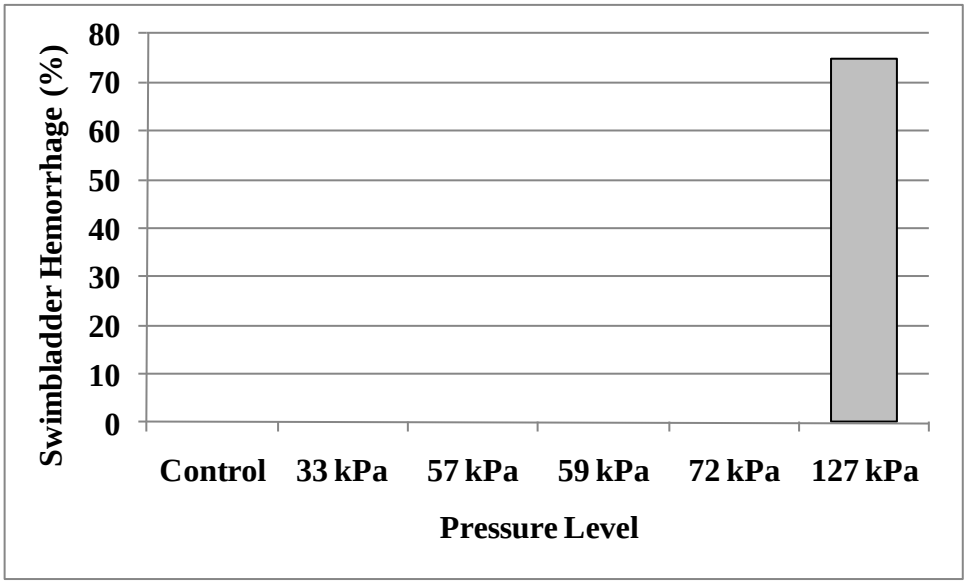


Figure 3.11: Swimbladder hemorrhage in lake trout exposed to varying blast intensities (n=5 fish per exposure level except for 127 kPa, where n=4).

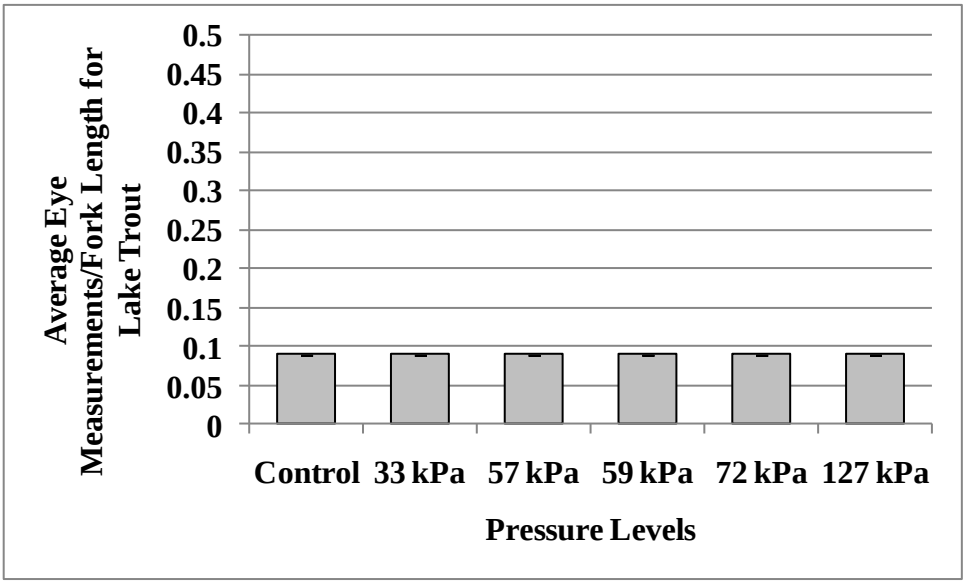


Figure 3.12: Prevalence of exopthalmia in lake trout. Data are expressed as mean (±SE=0) eye measurements/fork length for lake trout for n=5 fish per exposure level.

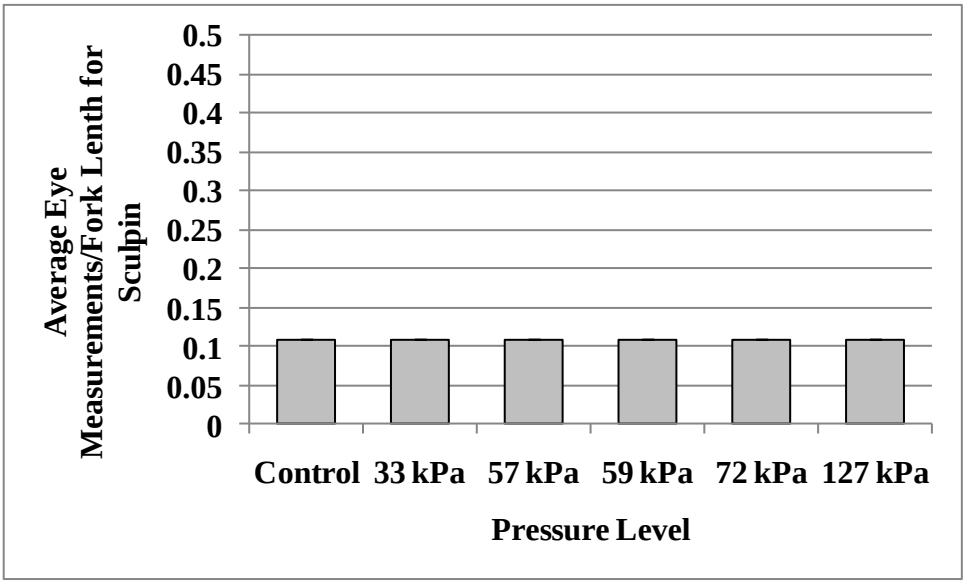


Figure 3.13: Prevalence of exopthalmia in sculpin. Data are expressed as mean (±SE=0) eye measurements/fork length for sculpin for n=5 fish per exposure level.

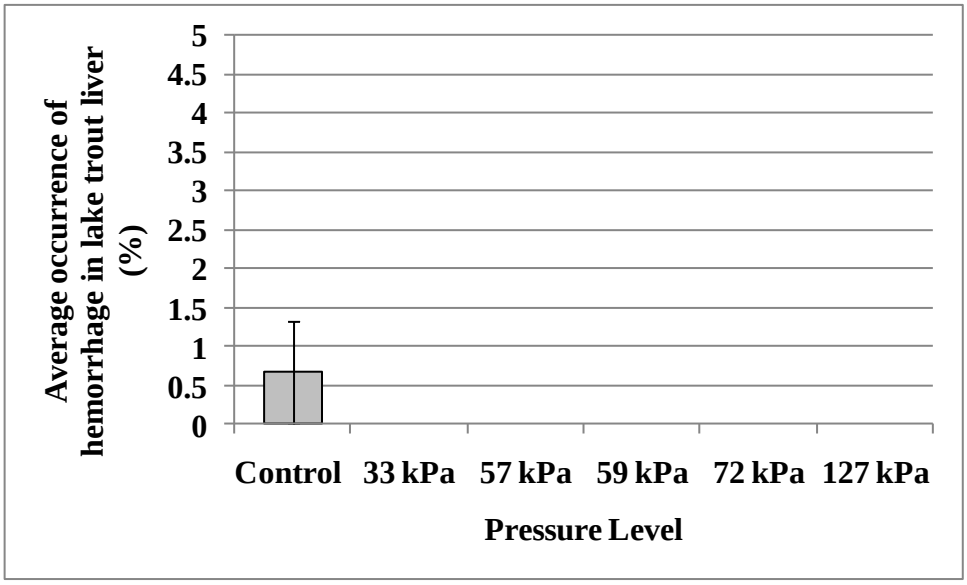


Figure 3.14: Hemorrhage in lake trout liver. Data are expressed as mean (±SE) percentage for n=5 fish per exposure level.

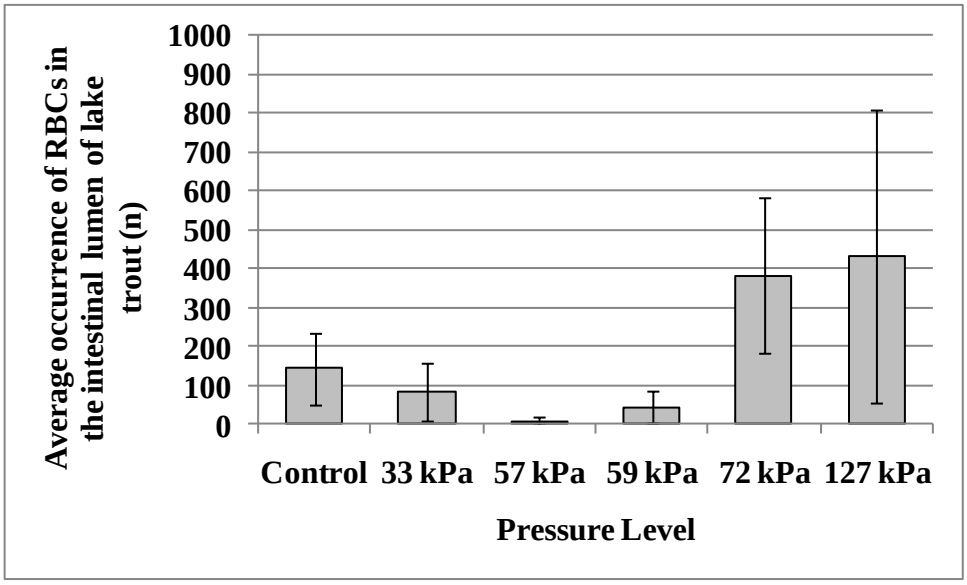


Figure 3.15: RBCs in the intestinal lumens of lake trout. Data are expressed as mean (±SE) number of RBCs for n=5 fish per exposure level.

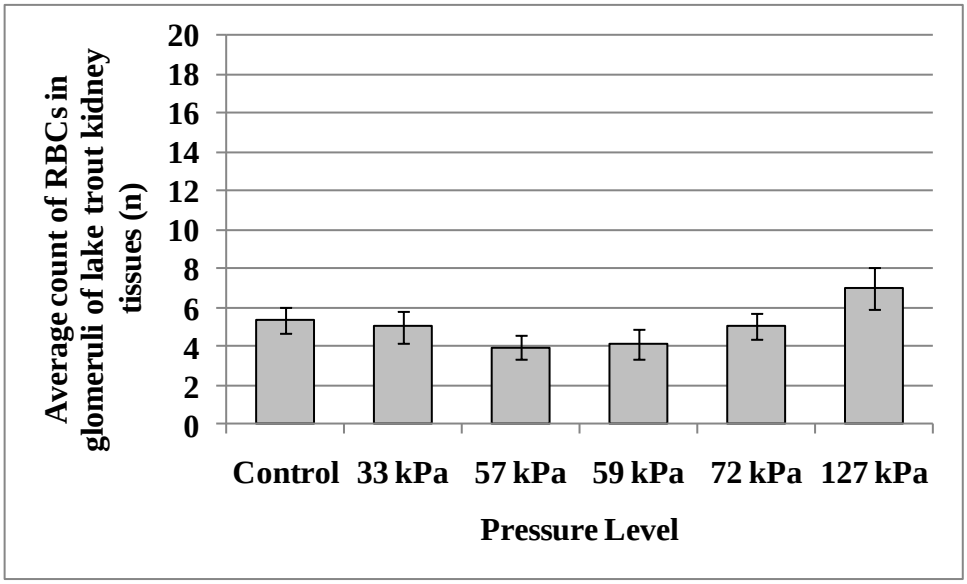


Figure 3.16: Glomerular congestion in lake trout kidneys. Data are expressed as the mean (±SE) of the average number of RBCs within glomeruli for n=5 fish per exposure level.

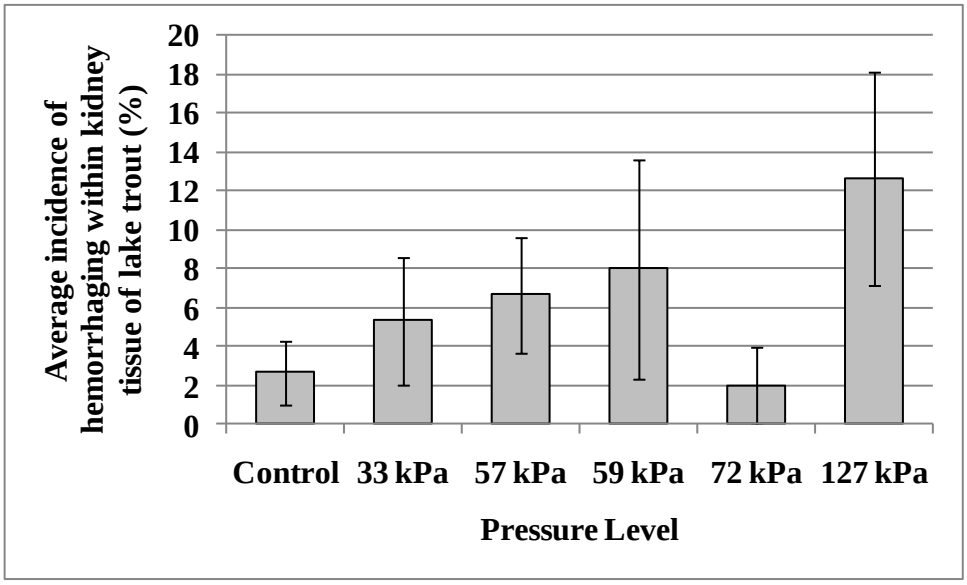


Figure 3.17: Hemorrhage within the kidney tissue of lake trout. Data are expressed as mean (±SE) percentage for n=5 fish per exposure level.

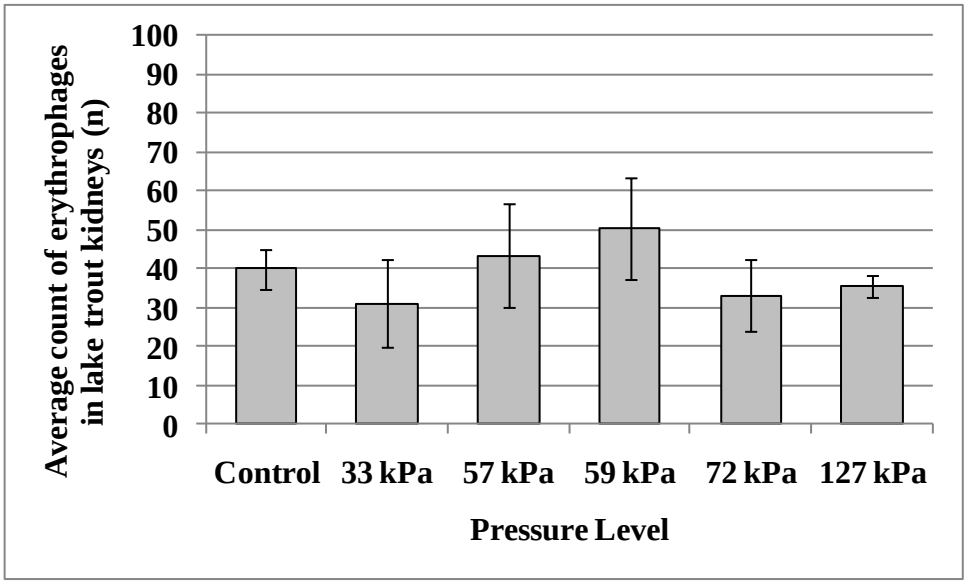


Figure 3.18: Erythrophagia in renal tissue of lake trout. Data are expressed as mean (±SE) number of erythrophages in interstices of renal tissue for n=5 fish per exposure level.

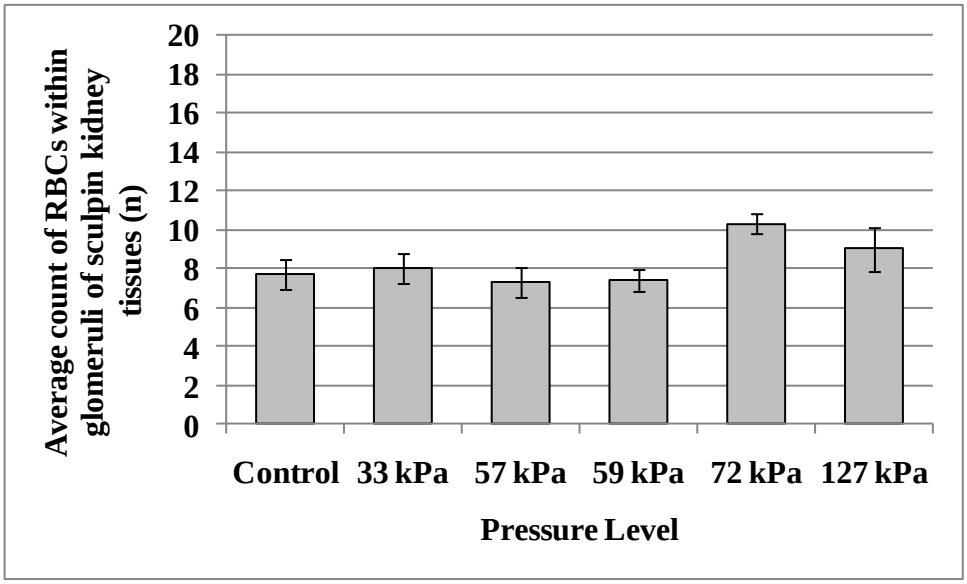


Figure 3.19: Glomerular congestion in sculpin kidneys. Data are expressed as the mean (±SE) of the average number of RBCs within glomeruli for n=5 fish per exposure level.

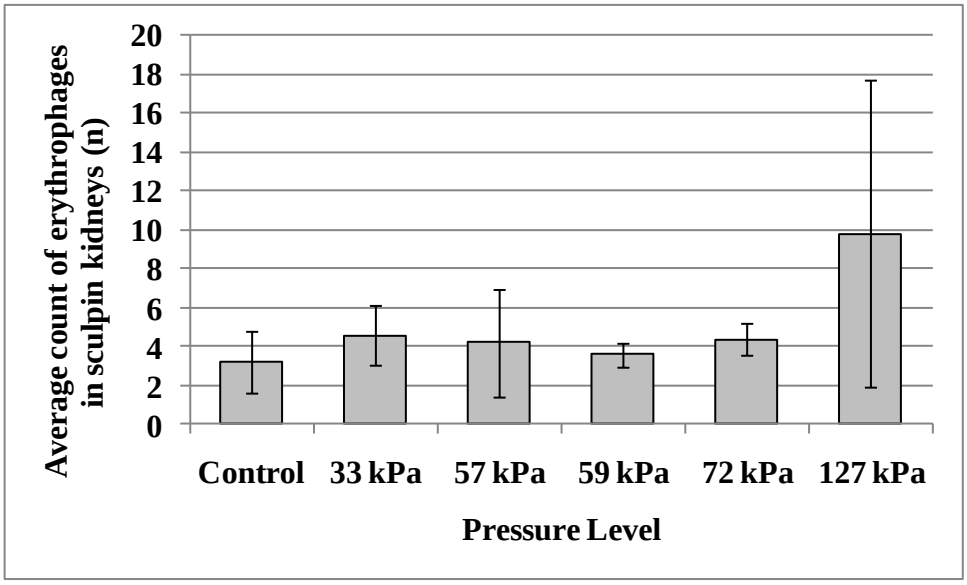


Figure 3.20: Erythrophagia in renal tissue of sculpin. Data are expressed as mean (±SE) number of erythrophages in interstices of renal tissue for n=5 fish per exposure level.

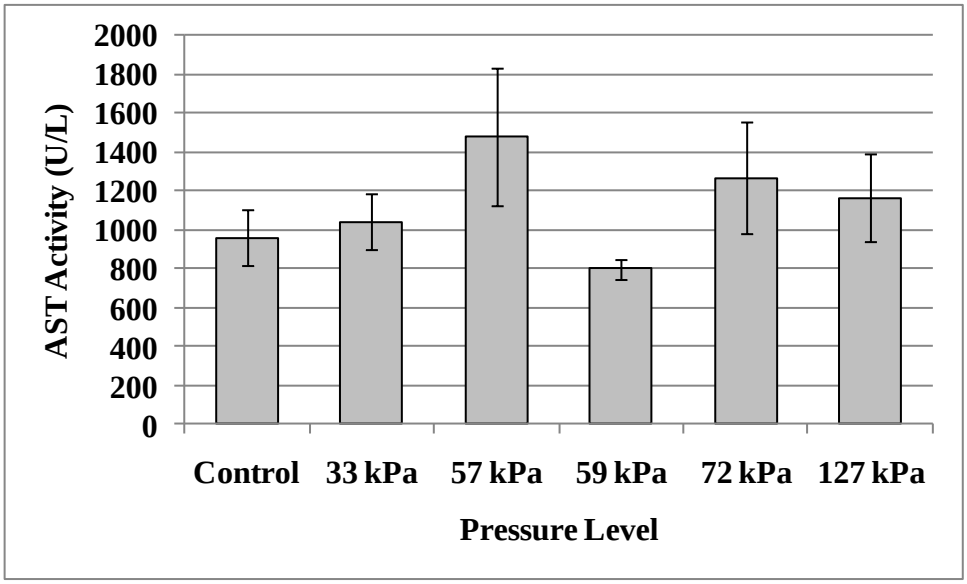


Figure 3.21: Aspartate aminotransferase activity in control lake trout and those exposed to varying IPCs. Data are expressed as mean (±SE) concentration for n=5 (except in 127 kPa group where n=4) fish per exposure level.

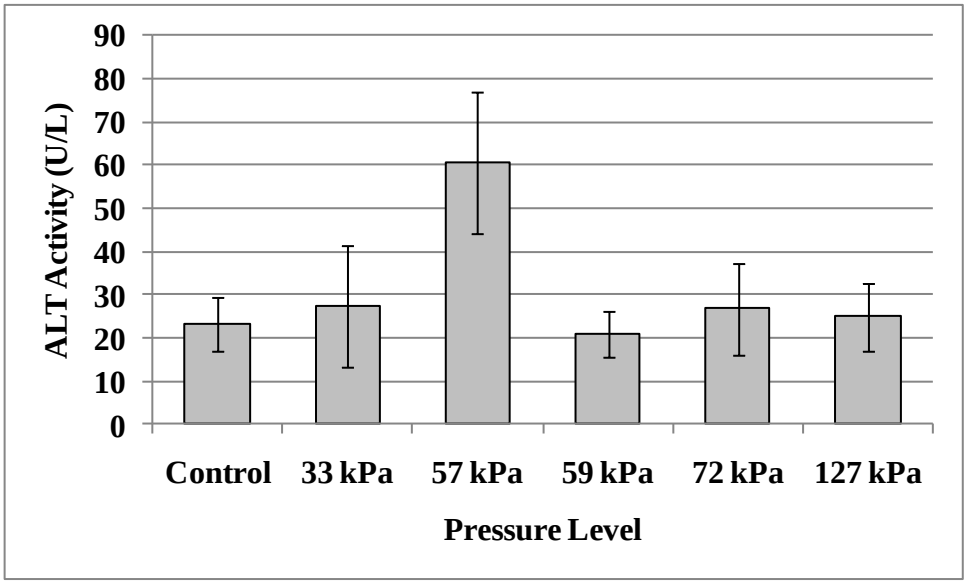


Figure 3.22: Alanine aminotransferase activity in control lake trout and those exposed to varying IPCs. Data are expressed as mean (±SE) concentration for n=5 (except in 127 kPa group where n=4) fish per exposure level.

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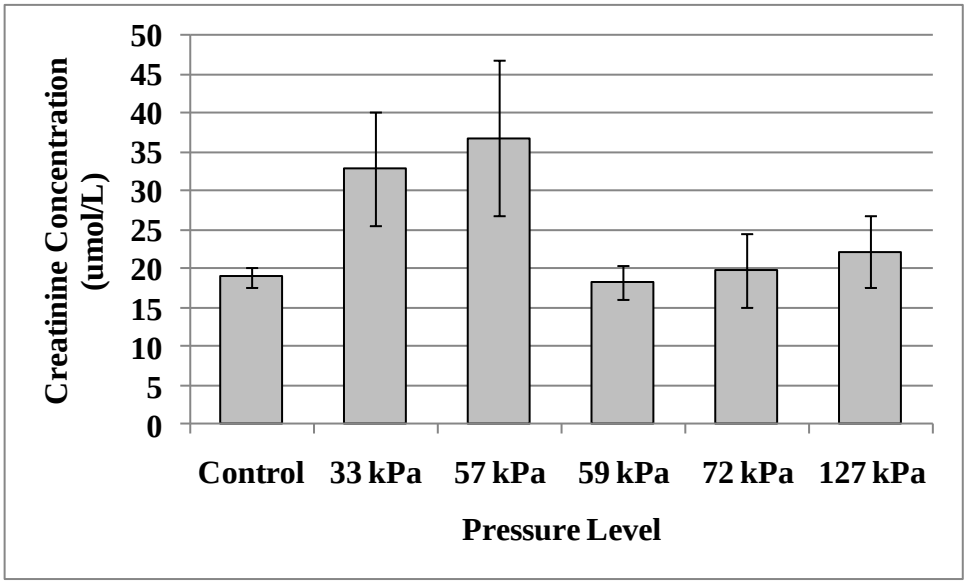

Figure 3.23: Creatinine concentration in control lake trout and those exposed to varying IPCs. Data are expressed as mean (±SE) concentration for n=5 (except in 127 kPa group where n=4) fish per exposure level.

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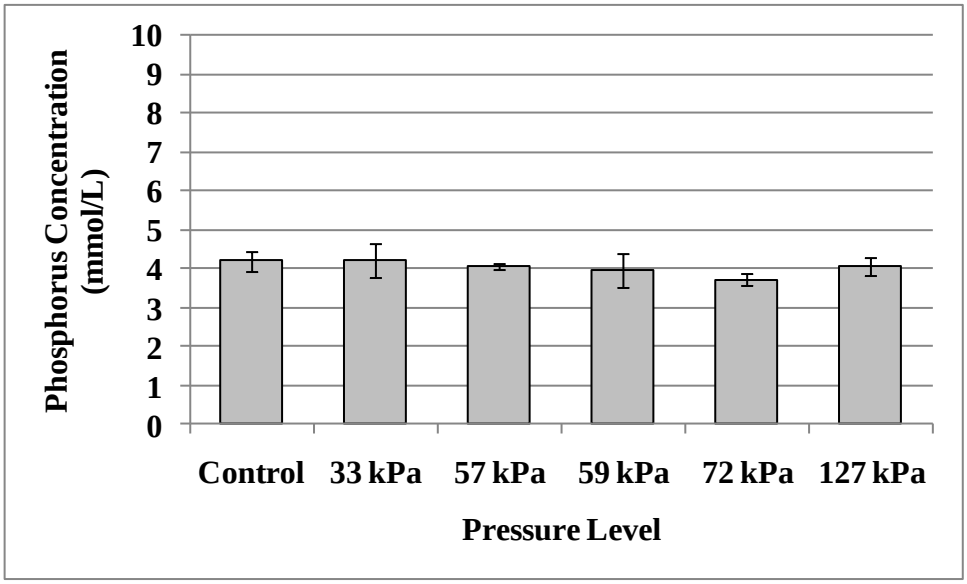

Figure 3.24: Phosphorus concentration in control lake trout and those exposed to varying IPCs. Data are expressed as mean (±SE) concentration for n=5 (except in 127 kPa group where n=4) fish per exposure level.

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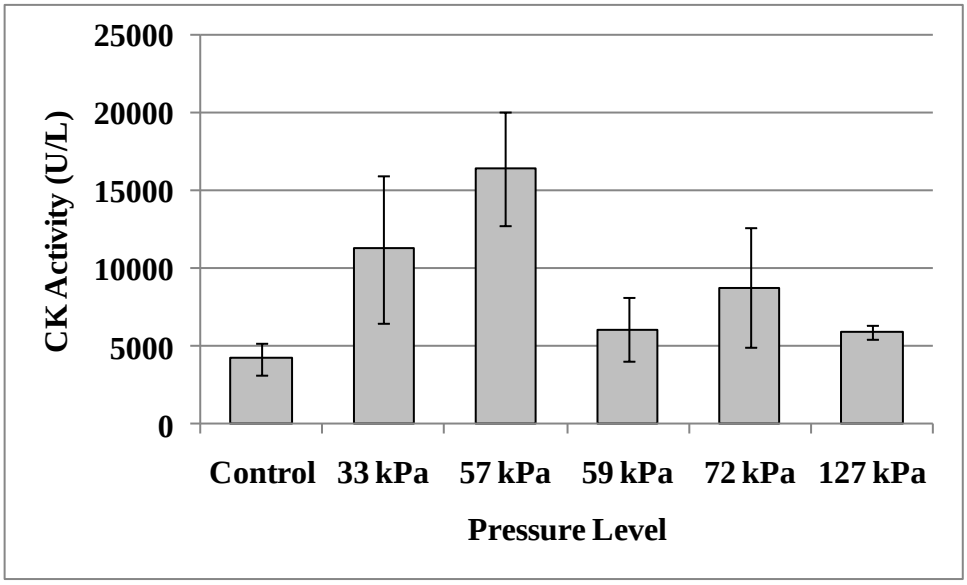

Figure 3.25: Creatine kinase activity in lake trout. Data are expressed as mean (±SE) concentration for n=5 (except in 127 kPa group where n=4) fish per exposure level.

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4 Risk Assessment for Lake Trout (*Salvelinus namaycush*) of the Mackenzie Delta Exposed to Explosive Based Instantaneous Pressure Changes

4.1 Abstract

Explosives are employed as seismic sources for oil and gas exploration in Canada's North. A *Guideline* document released by the Canadian Department of Fisheries and Oceans suggests peak pressure from these explosives not exceed 100 kPa; however the risk to fish associated with this level of pressure has not been fully explored. An ecological risk assessment was undertaken to evaluate risk of impacts to lake trout and lake trout populations of the Mackenzie Delta exposed to explosive pressure changes as a result of oil and gas exploration. The assessment suggests that lake trout are at risk of sub-lethal injury below and surrounding the *Guideline* value, and that there is low, yet plausible, risk that populations may fall below sustainable levels with impaired recovery in this highly sensitive region. We recommend that the *Guideline* peak pressure level be re-evaluated.

4.2 Introduction

From approximately December until April, lake-based explosive seismic techniques are used in Canada's North to explore for oil and gas reserves. The techniques are typically employed in waterbodies that do not freeze to the bottom. In addition to geophysical exploration, seismic techniques are used for ice management, general construction and for scientific applications (Wright 1982). Since 2000, hydrocarbon exploratory programs have expanded in the Mackenzie Delta region of the

Northwest Territories (NWT) to levels not seen since the 1970's when major seismic and drilling exploration programs were taking place (Cott et al. 2003). Negative impacts on fish from the use of explosives in aquatic environments are well documented, primarily from the instantaneous pressure changes (IPCs) that arise in the water column as a result of blasting. These IPCs can lead to negative pathologies and mortality in fish (see Table A1). In 1998, the Canadian Department of Fisheries and Oceans (DFO) established a *Guideline for the Use of Explosives In or Near Canadian Fisheries Waters*. For the protection of fish, maximum peak pressures in fish bearing waters are not exceed 100 kPa (Wright and Hopky 1998). Other jurisdictions, however, are more restrictive on the use of explosive based seismic activity in fish bearing waters. For example, in Alaska, IPC has to remain below 18.6 kPa (Alaska Department of Fish and Game 1991, 2009). The *Guideline*'s ability to be protective of fish populations is in question and a re-evaluation is warranted. To address risk from IPCs surrounding 100 kPa on lake trout, one of the most common subsistence and commercial fish species in the Mackenzie Delta, a risk assessment following the United States Environmental Protection Agency's (USEPA) framework for ecological risk assessment was undertaken. The null hypothesis is that no risk exists to lake trout or lake trout populations near the 100 kPa *Guideline* level.

4.3 Problem Formulation

4.3.1 Stressor Characterization

When explosives are detonated they create shock waves (also referred to as sound waves) that reflect from the underlying sediment layers of a waterbody, based on velocity or density discontinuities in the underlying strata. When they return to the surface they

are processed and translated into data. These data are then used to create a profile of the geology underneath the substrate, and highlight areas where potential hydrocarbon deposits exist (Falk and Lawrence 1973, Fitch and Young 1948, Fry and Cox 1953, Hubbs and Rechnitzer 1952, Kearns and Boyd 1965, McCauley et al 2000, Rasmussen 1964, Rulifson and Schoning 1963, Wright 1985).

During seismic explorations, hydrophone lines are set with source (charge) lines in different arrays depending on the type of survey being undertaken; 2 dimensional (2D) or 3 dimensional (3D) (Gausland 2003). The linear 2D surveys provide limited information on subsurface geography, but are less expensive than a 3D survey (Gausland 2003). Typically, the 2D survey is done in the early stages of exploration to determine if further exploration is warranted (Cott et al. 2003, Gausland 2003). If possible hydrocarbons are detected, a 3D survey is then undertaken (Gausland 2003). The grid pattern setup of a 3D survey enables three dimensional imagining and, therefore, better resolution of hydrocarbon reserves. It has an important application in the oil and gas industry as it provides more comprehensive coverage of the complicated geological structures of an area (Cott et al. 2003, Gausland 2003). An example of a 3D configuration is provided in Figure 4.1.

Hundreds of charges may be detonated in an area of interest (Cott et al. 2003, Cott and Hanna 2004). Blasts are typically detonated in series until sufficiently resolved images of subsurface geological structures are produced (P. Cott, personal communication, March 2009). The effects on aquatic animals from exposure to successive IPCs surrounding the *Guideline* represent a significant gap in the literature.

The instantaneous pressure wave following a blast begins by rising to a peak positive pressure that decays exponentially (Cronin 1948). This positive pressure wave almost immediately transitions to a rarefaction (negative) wave due to the seismic shock wave reflecting off of the water surface, back into the water column (Christian 1973, Hubbs and Rechnitzer 1952) (Figure 4.2). A gas bubble, created at the point of detonation, contracts and expands and oscillates, owing to the surrounding water pressure (MacLennan and Simmonds 1992, Rasmussen 1964, Viada et al. 2008). The oscillatory action of the gas bubble transmits secondary shock waves of considerably lower pressure than the initial shock wave (Hill 1978, Lavergne 1970, Rasmussen 1963, Sulfredge et al. 2001).

Based on data from field observations, negative peak pressures typically occurr less than 0.2 milliseconds following detonation. The instantaneous nature of detonations under water represents an acute stressor to fish though effects may not be manifested immediately.

The instantaneous generation of a peak positive pressure and subsequent negative peak pressure wave triggers oscillation of the swimbladder (Wiley et al. 1981), resulting in potential damage. The negative portion of the wave is particularly deleterious to fish and the prime cause of damage to the swimbladder as it expands from the sudden reduction in pressure (Gaspin 1975, Hill 1978, Hubbs and Rechnitzer 1952, MacLennan and Simmonds 1992, Rasmussen 1964, Wiley 1981, Wright 1982). Furthermore, the rapid expansion of the swimbladder during sharp drops in ambient pressure can greatly compress internal organs adjacent to the swimbladder causing injury (Fernet 1982, Tsvetkov et al. 1972).

4.3.2 Ecosystems Characterization

The Mackenzie Delta is the largest delta in Canada, covering 12,995 km^2 (Marsh 1998), and containing approximately 45,000 lakes (Squires et al. 2009) (Figure 4.3). The Delta is unusually productive for its Arctic latitude (Squires et al. 2009). Lakes in the Delta are typically shallow lakes (mean depths between 1-1.5 meters) (Squires and Lesack 2002) and are ice covered for up to 8 months of the year (Marsh 1998).

Lake trout are the focus of this risk assessment however details this species are provided in a subsequent section pertaining to the organism at risk. The number of lakes within the Mackenzie Delta region that support lake trout populations is currently unknown (P.Cott, *personal communication*, January 2009), however the species has been identified by the Inuvialuit community (the Mackenzie Delta Inuit population) as important for subsistence (Mackenzie Gas Project 2004a). Two Delta Mackenzie lakes represent systems at risk for seismic activity: Yaya Lake and Parsons Lake. Both are freshwater lakes within the Delta Mackenzie containing lake trout that, based on their size and depth, have a low likelihood of freezing to the bottom. In fact, Yaya lake is used for subsistence fishing and has already been the site of seismic exploratory work (B. Hanna, personal communication, January 2009).

4.3.2.1 Yaya Lake

Yaya Lake is one of the largest lakes in the Mackenzie Delta and is an important recreational and subsistence fishery for local communities (Mackenzie Gas Project 2004a). The lake has a surface area of 2,038 ha, a volume of 177 248 873 m^3, a recorded maximum depth of 48.8m and a mean depth of 8.7m (McCart 1976, Slaney F. F. and Company Ltd. 1974). A habitat assessment was done in the north basin of the lake in July

2002. Silt substrate with organic matter was the only substrate observed within the lake (Mackenzie Gas Project 2004a). Using fish sampling methods that included a gillnet and baited minnow traps, lake trout caught during the survey confirmed their presence in the lake (Mackenzie Gas Project 2004a), which is supported by historical data (Slaney F.F. and Company Ltd. 1974). Based on a habitat assessment for Yaya Lake, the potential habitat usage by lake trout has been predicted to include overwintering, rearing, as well as adult feeding and holding (Mackenzie Gas Project 2004a).

4.3.2.2 Parsons Lake

Parsons Lake is another large lake in the Delta region. It is located at 68º57' 13.54"N, 133º38' 29.06"W, has a surface area of 5,825 ha, a volume of 185 029 000m^3 (F.F Slaney and Company Ltd. 1974), and is relatively shallow with a maximum depth of 8.2m and a mean depth of 3.2m (Golder 2000, Mackenzie Gas Project 2004a). Summer field surveys were done at two sites on Parsons Lake in August 2002. Several regions of the shoreline were found to have cobble, boulder and gravel substrates; however, most of the substrate along the shoreline was silt, sand and organic matter. Historical records have confirmed the presence of lake trout in the system (Slaney F. F and Company 1974). They report 50 lake trout caught in a portion of Parsons Lake with a catch per unit effort (CPUE) of 0.22 fish per hour using a standard gang net of 250 feet. From a habitat assessment of this lake, lake trout could overwinter, rear, spawn and incubate eggs as well as use the lake for adult feeding and residence (Mackenzie Gas Project 2004a).

4.3.3 Organisms at Risk (Lake Trout)

Based on stressor characteristics, fish are the organism most at risk from exposure to IPCs. Explosive based seismic events may impact fish by causing both external and internal physical injury, which may in turn impact their survival. Furthermore, fish with swimbladders are thought to be more vulnerable to injury due to the compression and expansion of that organ during an explosive event (Wiley et al. 1981). Lake trout have a swimbladder and are at risk of injury. They also represent an ecologically and culturally important species.

Lake trout were chosen as the primary ecological entity of concern for this assessment as they are highly prized as a game fish and as a subsistence and commercially harvested species (Racca et al. 2004). Lake trout are widely distributed throughout North America, and are ubiquitous in the NWT (Ford et al. 1995; Martin and Olver 1980; McPhail and Lindsey 1970, Scott and Crossman 1973). They can usually be found in the cold depths of lakes in the southern part of their range, but in the northern part of their range can be found in shallow lakes and rivers (McPhail and Lindsey 1970, Scott and Crossman 1973). Though it is reported that the Mackenzie Delta represents a productive aquatic ecosystem, the growth rate of the lake trout in northern areas is observed to be slow relative to southern regions (McPhail and Lindsey 1970, Rawson 1961). For example, a lake trout of eight years in Lac la Ronge, Saskatchewan would be 20% longer and more than double the weight of a lake trout of the same age found in MacKay Lake, NWT (Rawson 1961). This phenomenon is observed in the NWT where the growth rate of lake trout in Great Bear Lake is much slower than in the more southerly Great Slave Lake (Kennedy 1954, Miller and Kennedy 1948, as described in

McPhail and Lindsey 1970). In general for all lake trout, age at sexual maturity varies from 4 to 13 years (Martin and Olver 1980). In Great Slave Lake, some lake trout mature sexually as early as 5 years but most reach maturity around 11 years (McPhail and Lindsey 1970). Northern, slow-growing lake trout live the longest (Martin and Olver 1980) with recorded ages up to 42 years in the Mackenzie Delta (F.F. Slaney and Company 1974). Lake trout generally spawn in late summer and autumn, beginning in approximately October throughout most of Canada, but sometimes as early as September in the north (Ford et al. 1995, McPhail and Lindsey 1970, Scott and Crossman 1973). At northern latitudes, lake trout spawn only every 2 to 3 years, as observed in Great Slave Lake and Great Bear Lake (Martin and Olver 1980, McPhail and Lindsey 1970). Northern populations spawn earlier than their southern counterparts (Martin and Olver 1980). In general, including in the southern range, egg incubation times take about 4 to 5 months over the winter and early spring (Ford et al. 1995; McPhail and Lindsey 1970), but this time extends in more northern populations (Ford et al. 1995, Scott and Crossman 1973). For instance, hatching generally occurs in March or April, but is delayed in the northerly Great Bear Lake until June (Scott and Crossman 1973).Young lake trout which remain in shallow water for weeks or months in the south, have been noted to remain in the shallows for months or several years in far northern lakes, before moving to deeper water (Ford et al. 1995; McPhail and Lindsey 1970, Peck 1982, Scott and Crossman 1973).

Godard et al. (2008) confirm lake trout as a sensitive species to explosive detonations, reporting hemorrhaging in the swimbladder of fish exposed to blasts of 127 kPa.

4.3.4 Assessment Endpoint and Effect Measures

Social importance, ecological relevance and sensitivity to the stressor need to be considered when developing an assessment endpoint. The assessment endpoint chosen for the current risk assessment is the maintenance of fish populations in the Mackenzie Delta region, achieved by ensuring impacts to fish populations do not exceed a sustainable level. This has societal relevance as lake trout are harvested for game, subsistence and commercial purposes. It has ecological relevance as it would maintain trout population levels. Finally, it is sensitive to the stressor as blasting may have the potential to reduce population levels below that which is sustainable.

A level of mortality from explosive based IPC similar to that experienced through harvesting, could allow populations levels to sustain themselves. Sustainable annual harvests for lake trout populations are in the order of 0.25-0.75 kg/ha (P. Cott, *personal communication*, January 2009) and few waters are capable of sustained lake trout harvest in excess of 1 kg/ha/year (Martin and Olver 1980). In fact, Healey (1978) suggests that exploitation should not exceed 0.5 kg/ha for rapid growth and large standing stocks, and 0.2 kg/ha for slow growth and low standing stocks of mature fish. All NWT waters have a sportfishing daily catch limit (the number of fish you can catch and retain over a 24 hour period) of 3 lake trout with the exception of Great Bear Lake and Great Slave Lake with daily catch limits of 1 and 2, respectively (Department of Justice 2009). Because fish in the north are slower growing and have potential for being stocks of mature fish, 0.2 kg/ha was chosen as the an allowable limit for population decline from IPC exposure in the current study.

Figure 4.4 summarizes the ecological goal, assessment endpoint and effect measures for the current risk assessment.

4.3.5 Conceptual Model

Figure 4.5 presents a conceptual model of the relationships between the stressor, the receptor and effects measures.

4.4 Risk Analysis

4.4.1 Exposure Characterization

The Mackenzie Delta region is where the majority of explosive based seismic exploration projects occur within the NWT (Cott et al. 2003).

Prior to 2003, the Canadian Department of Fisheries and Oceans (DFO) issued Section 32 (S.32) *Authorizations* allowing companies to perform seismic exploration using explosives in waterbodies not frozen to the bottom (P.Cott, *personal communication*, January 2009). An S.32 *Authorization* permits the killing of fish by means other than fishing, including the use of explosives (The Fisheries Act 2007). The issuance of an *Authorization* meant that if fish were killed as part of *Authorized* activities, the company would not be in contravention of the *Fisheries Act*. Because this was a voluntary process, if a company chose not to acquire an *Authorization* and killed fish during exploration, they would be in contravention of the *Fisheries Act* (P.Cott, *personal communication*, January 2009). In 2003, DFO began to no longer authorize the use of explosives in the NWT to conduct seismic exploration (Cott et al. 2003). Without an *Authorization*, the onus fell on the company to not violate the *Fisheries Act* by making sure that they did not kill fish. Industry was then forced to ensure that pressures generated

during exploration were below levels that could kill fish. To do this, industry had to extensively pre-test burial depth/charge size combinations and monitor pressures throughout the program, demonstrating that they would not harm fish. It was reasoned that had DFO issued *Authorizations* to kill fish there would have been no incentives, other than good corporate and environmental practices as well as public and aboriginal concern, for industry to develop mitigation (P. Cott, *personal communication*, June 2009).

Currently, projects in the Inuvialuit Settlement Region (ISR) go through a screening process under the Inuvialuit Final Agreement, created in 1984 (Cott et al. 2003, Hegmann et al. 2002). The ISR is a Canadian Western Arctic region that is comprised of four different geographic regions: the Beaufort Sea, the Mackenzie River Delta, the Yukon North Slope and the Arctic islands (Fast et al. 2005) (Figure 4.6). Since 2000, when seismic exploration re-commenced in the NWT (Cott and Hanna 2004), 32 seismic approvals have been issued by Indian and Northern Affairs Canada in the NWT, 26 of which were in the Mackenzie Delta, and all of which used explosives as an energy source in waterbodies that did not have groundfast ice (C. Baetz, *personal communication*, February 2009).

According to the DFO *Guidelines* document, peak pressures from the detonation of explosives no greater than100 kPa are recommended for the protection of fish. In practice, the actual IPCs generated from charges are variable and unpredictable (Cott et al. 2003, Cott and Hanna 2004). For instance, of the charges detonated in open water during an equipment test at Parsons Lake in August and September 2000, 43% had IPCs greater than 100 kPa and the range was from 30 kPa to 220 kPa (Cott et al. 2003). Eight

fish, including lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*), were killed during this test program (Golder Associates 2000) at a pressure level of 174 kPa. Later several accounts of dead fish washing up on the shore of Parsons Lakes were reported to DFO. However the reports came to the attention of DFO too late to prove a causal link to the IPCs (Cott et al. 2003) and therefore, the total number of fish killed was not documented. Monitoring of IPCs was also conducted in 2000/2001 and 2001/2002 during winter seismic exploration in the NWT. In 2000/2001, of 436 charges monitored, 50% exceeded 100 kPa, with some pressures measuring near 350kPa (Cott and Hanna 2004, Cott et al. 2003). Similarly, in 2001/2002, following charge size/burial depth adjustments implemented to avoid exceeding the 100kPa threshold, 9% of 507 detonations monitored exceeded the threshold (Cott and Hanna 2004).

4.4.2 Effect Characterization

Older literature (pre-1986) exists concerning the effects on fishes of exposure to explosives. Table A1 summarizes this literature and the effects observed in fish exposed to explosive blasts. Most effects observed concern observations of gross pathology including external trauma, broken bones, rupture, protrusion and hemorrhaging of organs, as well as death. Any fish with a swimbladder serves as a surrogate for lake trout and hence, any damage reported in swimbladder bearing species is relevant. However, a handful of these studies report similar damage to those previously listed, in trout species (Coker and Hollis 1950, Cronin 1948, Fernet 1982, Godard et al. 2008, McAnuff et al. 1994, Paterson and Turner 1968, Teleki and Chamberlain 1978). The cause of external trauma to fish has been related to impact from the passing pressure wave (particle displacement) while internal trauma has been linked to the rapid compression and

expansion of the swimbladder as the pressure wave passes (Govoni et al. 2003; Wiley et al. 1981). Pressures tested in the older literature are often at levels far higher than the current recommended *Guideline* level of 100 kPa. Therefore, due to the amplified pressure levels tested, findings from these studies lack relevance to the current recommended *Guideline* level, though they do help characterize gross effects that could occur. Of relevance however, are a few studies that have documented effects near and below the *Guideline* level. For instance, in Chapter 3, hemorrhage in the swimbladder of lake trout (*Salvelinus namaycush*) was reported at 127 kPa. Sakaguchi et al. (1976), reported swimbladder, liver and kidney congestion at a peak pressure of 54 kPa in rock fish (*Sebastiscus marmoratus*) exposed to borehole blasting at Bisan Strait, Japan. Teleki and Chamberlain (1978) examined acute effects of underwater construction hydromex explosive blasting on fish in Lake Erie. They reported that pressures between 30-85 kPa were associated with type 2 injury, characterized by stretching of the swimbladder lining and rupture of fibrous connective tissue. Finally, McAnuff et al. (1994) reported that 88 fish were killed in the Nipigon River following blasts at levels of 17 to 97 kPa during installation of a natural gas pipeline. Autopsies carried out by the DFO and Ministry of Natural Resources determined that the majority of the fish died as a result of a ruptured swimbladder (McAnuff et al. 1994).

Recently, more detailed pathological studies regarding the effects of IPCs near the *Guideline* have arisen. Govoni et al. (2002, 2003) evaluated histology in swimbladder bearing pinfish (*Lagodon rhomboids*) and spot (*Leiostomus xanthurus*) following shock waves averaging 109.93 kPa, but determined no effects. However, Godard et al. (2008) investigated embryos, sac fry and juvenile rainbow trout (*Oncorhynchus mykiss*) exposed

to explosive detonations, and noted swimbladder tears and kidney hemorrhaging beginning at 69 kPa in juveniles as well as exopthalmia significantly differening from controls in juveniles at 69 kPa.

Behavioral effects have also been recently anecdotally documented in response to IPCs. Chapter 3 described that lake trout became attracted to the area of the blasts, presumably to feed on small-bodied fish stunned by the detonations. This means that fish will likely be attracted to a blasting zone and be vulnerable to exposure from subsequent detonations.

While biological effects have been noted at pressure levels below and near the current *Guideline*, the extent of injury to the organism may depend on a variety of factors (See Table A2). For instance, Kearns and Boyd (1965) reported that detonations originating at depth are more damaging to fish than those at shallow depth where more of the shock wave energy is lost at the surface resulting in less being directed below the surface. Fish at varying depths in the water column may be differentially affected. Those in deeper water at the time of detonation may be less vulnerable than fish nearer to the surface (Christian 1973, Coker and Hollis 1950, Wiley et al. 1981). Hubbs and Rechnitzer (1975) reported that fish held in cages at the surface and free-swimming fish near the surface suffered more damage than fish at the lake bottom. Similarly, Arctic cisco (*Coregonus autumnalis*) held in cages at the surface (0.914 metres) were more susceptible to injury than those caged near the bottom (2.134 metres) when exposed to an explosive (Falk and Lawrence 1973). Ferguson (1962) documented that, following exposure to both an explosive and black powder, swimbladder injuries were greater among yellow perch (*Perca flavescens*) caged at depths of 10 feet from the surface

compared to perch exposed in bottom cages, 42 or 58 feet from the surface. Linton et al. (1985) reported conflicting results noting that black drum (*Pogonias cromis*), a swimbladder bearing species held at the bottom (2.4m), exhibited greater mortality than those in surface waters following exposure to explosives. The list of potential factors influencing the degree of impact to a fish is extensive and they should be taken into account when considering the possibility of injury to the organism.

4.5 Risk Characterization

4.5.1 Risk Estimation

4.5.1.1 Integration

A lack of definitive effects and exposure data make it difficult to measure risks from IPCs in the Canadian Arctic or to determine the protective nature of the 100 kPa *Guideline* value to lake trout populations in the Mackenzie Delta. However, predictions can be made as to the potential for risk of injury to individuals, population decline and the possibility for recovery after impact from exposure to IPCs.

Based on available information and on the information described in earlier chapters of this thesis, the 100 kPa pressure limit is not protective for juvenile and adult lake trout and other swimbladder bearing fish within the Mackenzie Delta. The risk of injury as a result of subsequent blasting may be heightened because of fish being attracted to the blasting area. Finally, the risk of injury to fish can certainly be heightened due to the unpredictable nature of blasting. In cases where 100 kPa is exceeded, risk of damage exists. The risk of adverse impacts on lake trout at different life stages is dependent on a variety of factors. For embryos factors may include specific stage of

development, chorion thickness (Battle 1944), and the makeup of lake sediments (Faulkner et al. 2007). In juveniles and adults, factors may include body size, physical condition of the fish and orientation of the fish relative to the blast (See Table A2).

Whether reported sub-lethal injuries after exposure to IPCs of approximately 100 kPa result in eventual mortality in fish is unknown. Recovery, extent and duration of damage have yet to be determined. Reported injuries near the *Guideline* level, however, are serious and may potentially lead to mortality. Injury including mild bruising of kidneys could affect osmoregulatory efficiency, causing higher expenditure of energy. Burst swimbladders could also cause fish to lose their ability to regulate buoyancy (Gaspin et al. 1976; Wiley et al. 1981). Damaged swimbladders and behavioral abnormalities from sub-lethal traumas could render fish more vulnerable to predation (Govoni et al. 2003, Wiley et al. 1981). If northern lake trout succumb to sub-lethal injuries following IPCs near 100 kPa and sufficient numbers of fish are impacted, there is a potential risk for populations to fall below a sustainable level. This concern is supported in a report generated by Continental Shelf Associates, Inc (2004) who proclaim that any effects from blasting leading to reduced survival, growth or reproductive output could lead to population impacts. In addition to the risk of direct population decline from IPC related mortality, naturally occurring intermittent spawning coupled with slower growth rates puts northern lake trout at risk of suffering from delayed recovery or of being unable to return to sustainable levels from IPC insults, should they be impacted beyond a sustainable value. As previously mentioned, northern lake trout are long lived and spawn only every 2 to 3 years (F.F. Slaney and Company 1974, Martin and Olver 1980, McPhail and Lindsey 1970). They are therefore more vulnerable to impacts than southern

populations or more fecund fish species that would be able to recover over a shorter time frame. It is important to note that the lack of information on chronic effects for fish exposed to IPCs near the *Guideline* hinders the formation of definitive conclusions regarding large scale mortality following sub-lethal injury and the likelihood of impacting lake trout populations to below sustainable levels. These assessments would need to account for fishing pressure as well.

Due to the risk of injury and potentially lethal effects to northern lake trout as well as reported behavioural effects (attraction of fish to a blasting area), risk exists to lake trout of the Mackenzie Delta, and potentially to lake trout populations of the area, from the use of the current suggested *Guideline* IPC level. Therefore, the *Guideline* value of 100 kPa of pressure should be reduced until more work can determine a truly protective value.

4.5.2 Risk Description

4.5.2.1 Ecological Risk Summary

Risk of injury exists for fish exposed to explosive based IPCs surrounding 100 kPa. Based on literature however, the degree of risk may depend on a variety of factors (Table A2). The likelihood of adverse risk to the population could exist should sub-lethal injuries be found to result in mortality and, due to the specific life history of northern lake trout, populations may be unable to recover should mortality lead to populations dropping below sustainable levels. Significant uncertainty, discussed below, exists, and further research is needed before any definitive statements regarding risks to fish or fish populations can be made.

Confidence in statements made in the current risk assessment are heightened by the fact that there has historically been a strong and consistent association between explosive based seismic activity and tissue damage in animals, and certain damage is specific to fish tissues. Additionally, findings are coherent with knowledge of effects of explosive blasts on fish and, moreover, findings of effects result from a plausible mechanism of action. Finally, confidence in the assessment is heightened by the fact that there is some experimental evidence that has observed effects in fish below the 100 kPa threshold outlined in the *Guidelines*.

4.5.3 Discussion of Uncertainty

Many uncertainties exist within the current risk assessment for effects of IPCs on lake trout of the Mackenzie Delta region exposed to blasts from seismic activity surrounding the current *Guideline*. These uncertainties prevent our ability to state definitely the IPCs that might be considered safe, i.e., impacts lake trout populations less than 0.2 kg/ha.

The scale of IPC exposure, in terms of the number of lakes impacted, the volume of the lakes impacted, and the time between possible exposures to IPCs, is not known. The average number of blasts used in a given lake and the number of lakes in which seismic exploration has taken place is unknown. A lack of basic ecological information regarding the number of lakes in the Mackenzie Delta that support lake trout hinders the risk assessment. Of those lakes with lake trout populations, how many are affected by explosive based seismic activity is unknown. Given this data, the probability of seismic activity taking place on a lake trout bearing waterbody could be calculated. For the purpose of this assessment, it was assumed that every lake with IPC exposure contained

lake trout. To begin to address these uncertainties, it would be valuable for future environmental risk assessments to conduct a detailed site-specific study of an explosive based seismic explored NWT lake. The study would document the time between exposures, lake volume, number of blasts and an estimate of the number of lake trout (or other species vulnerable to IPC) in the lake. We also recommend that DFO compile a database that reports available information on waterbodies and areas surveyed (i.e. depth of waterbody, habitat usage) as well as on sites and details of blasting operations (i.e charge type, burial depths) for all exploration projects. This would improve an assessment by providing information such as the number of lakes affected by blasting, volumes of lakes impacted, and how many of the lakes support a particular species of interest.

Uncertainty also exists in regards to both the organisms affected and explosive based effects on those organisms. The average density of lake trout in Mackenzie Delta lakes is unknown as is the sustainable yield and the average ages of the fish within the lakes. Number of lakes used by populations for overwintering is also in question. There is a paucity of research based on the current *Guideline* for chronic and long-term effects as well as differences in responses between various fish species. Information regarding effects from successive exposure to blasts surrounding the *Guideline* is also lacking. Some of these uncertainties can be addressed with continued research, notably on: 1) stock assessments in the Mackenzie Delta region, 2) on effects of explosive based activity on different life stages and species of fish surrounding and below the current suggested *Guideline* IPC level, 3) on immediate and latent mortality associated with blasts, and finally 4) on effects from successive exposure.

There is uncertainty inherent in risk estimation. It is unclear whether mortality would result following sub-lethal injury to fish from exposure to blasts around 100 kPa or if that mortality would be extensive enough to drop fish populations below sustainable levels. At this stage, only preliminary estimates and plausible scenarios of risk can be determined, therefore the presence and degree of risk can not be stated with any certainty.

These areas of uncertainty highlight the need for continued research to expand the understanding of effects of IPCs in lake trout and fish species in general, using the current *Guideline* level. A more detailed and finely tuned assessment of associated risk is needed.

4.6 Conclusion

In conclusion, risk to lake trout populations and their ability to recover, surrounding 100 kPa, cannot be stated with any certainty. However, based on the available research at this time, there is risk of injury to individuals at IPCs near and below the current suggested *Guideline* level. Attempts to ensure fish protection are currently in place. In 2004, DFO Western Arctic recommended that a more conservative threshold of <50 kPa be used as the upper limit (Cott and Hanna 2004). Currently, companies conducting seismic programs in the Delta use the 50 kPa value instead of 100 kPa. If the companies are unable to consistently keep the IPC under 50 kPa then charges are no longer detonated (B. Hanna, personal communication, March 2009).

The current assessment highlights that if population decrease is found to result from mortality following sub-lethal damage when the current *Guideline* is being followed, slow growth and intermittent spawning of northern lake trout should be taken into account when determining allowable levels of impact to these fish. Finally, the

assessment highlights that, while no formal amendments have been made to the *Guideline* IPC peak pressure value of 100 kPa, a re-evaluation of what might constitute an acceptable IPC may be warranted, especially in light of the resurgence of hydrocarbon exploration in the North.

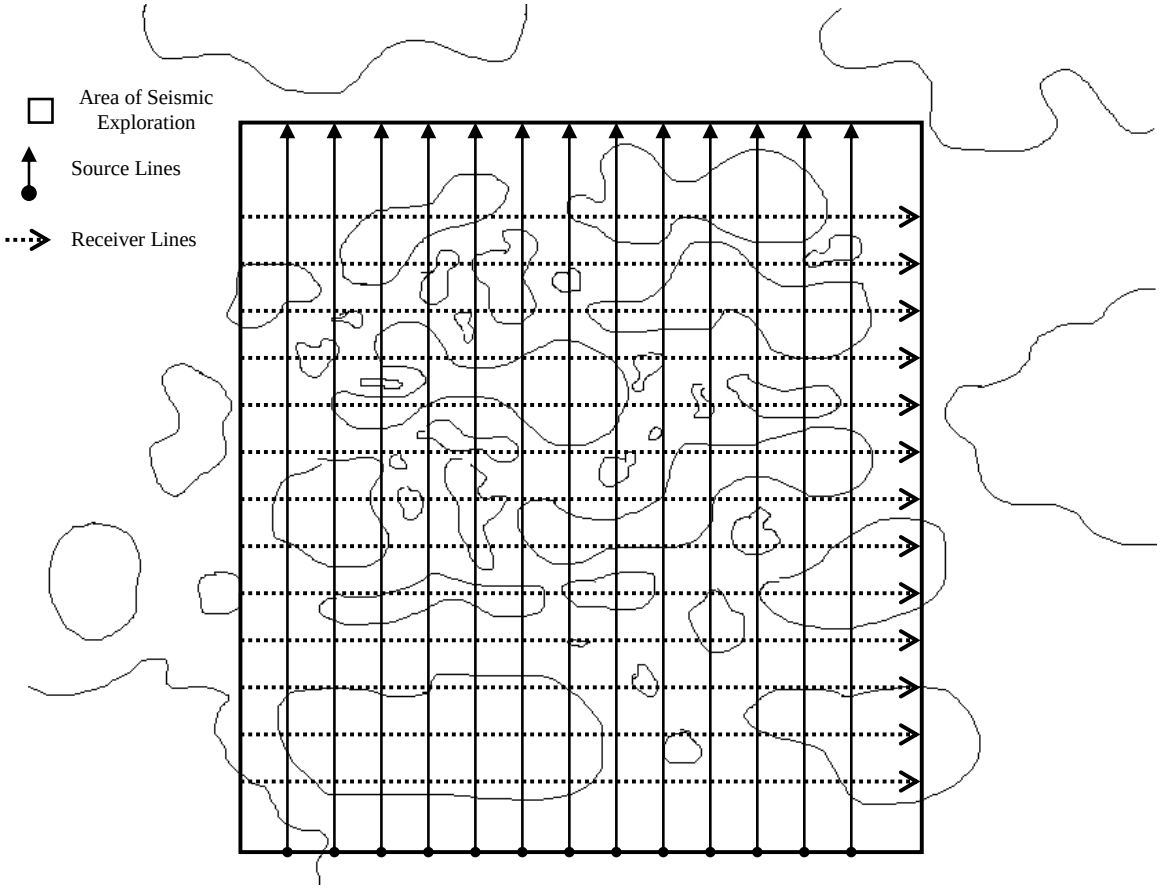

Figure 4.1: General setup of receiver and source lines during a 3D seismic based exploratory project within a lake bearing area. Note receiver lines are placed perpendicular to source lines.

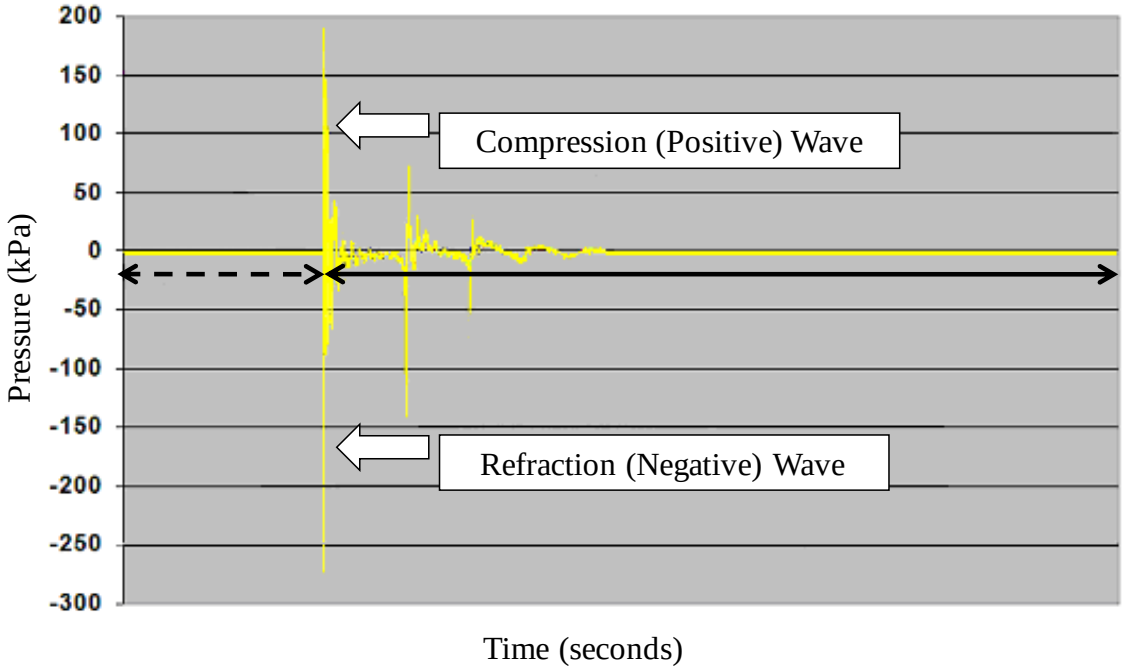

Figure 4.2: Representative waveform following explosive detonation of approximately 180 kPa in pressure. Dashed timeline (◄- -►) represents 0.25 second pre-detonation record; solid timeline (◄—►) represents 1 second detonation record; therefore waveform signature represents a total of a 1.25 second time interval. All detonations in the study were similar in duration but varied peak instantaneous pressure changes. Note the steep fronted compression (positive) peak pressure wave followed instantaneously by a rarefaction (negative) peak pressure wave.

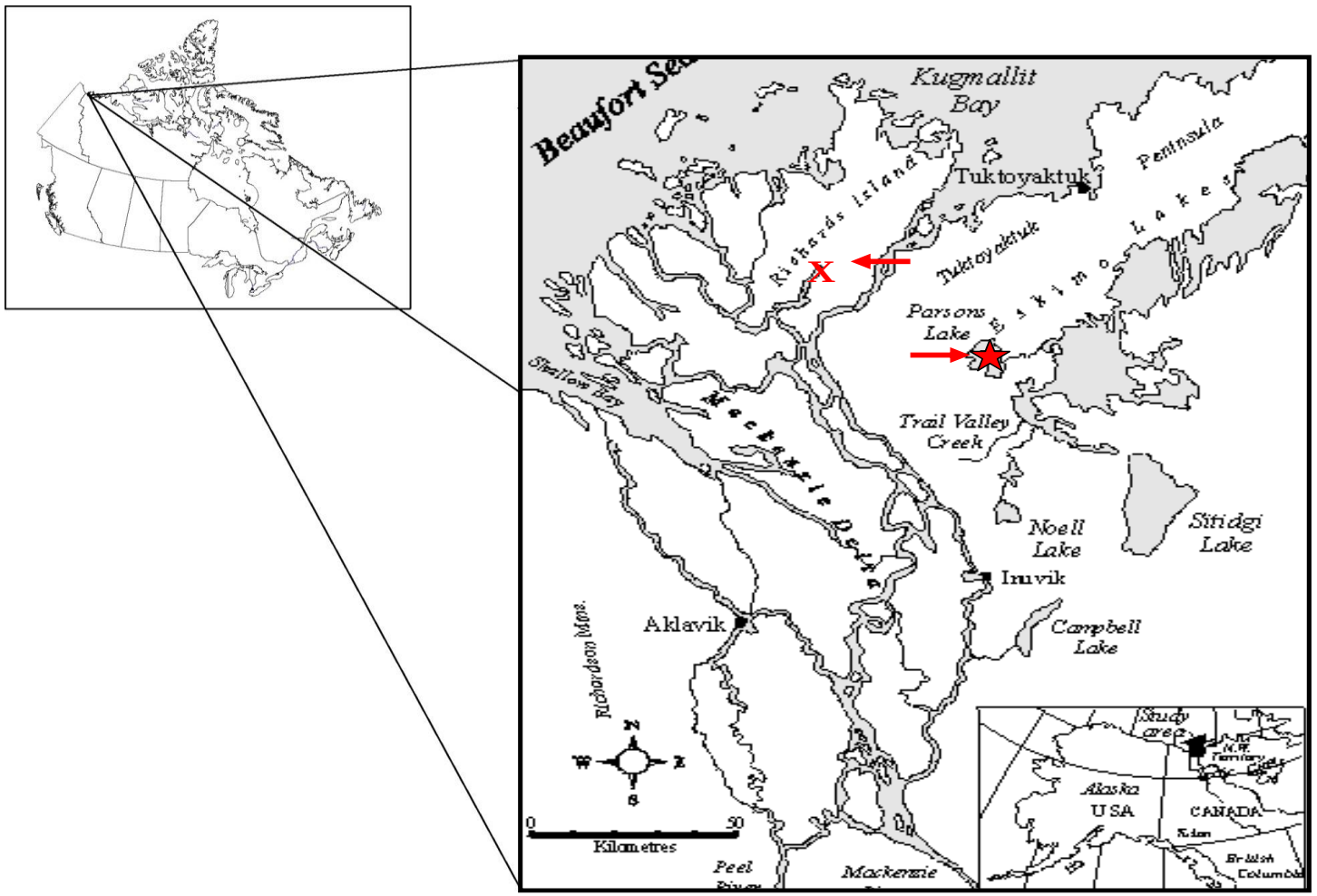

Figure 4.3: Map of Mackenzie Delta region. Parsons Lake (☆) and approximate location of Yaya Lake (X) are highlighted. (Modified from Environment Canada 1998).

Ecological Goal	Assessment Endpoint	Effect Measures
Protect fish populations in the Mackenize Delta, NWT, Canada	Maintenance of fish populations in the Mackenzie Delta region. Impacts to the population should not exceed 0.2 kg/ha.	Derivation of the level at which immediate lethality due to an IPC could be anticipated based on current literature. Derivation of the level at which latent mortality due to sub-lethal impacts could be anticipated.

Figure 4.4: Representation of ecological goals, assessment endpoint and effect measures

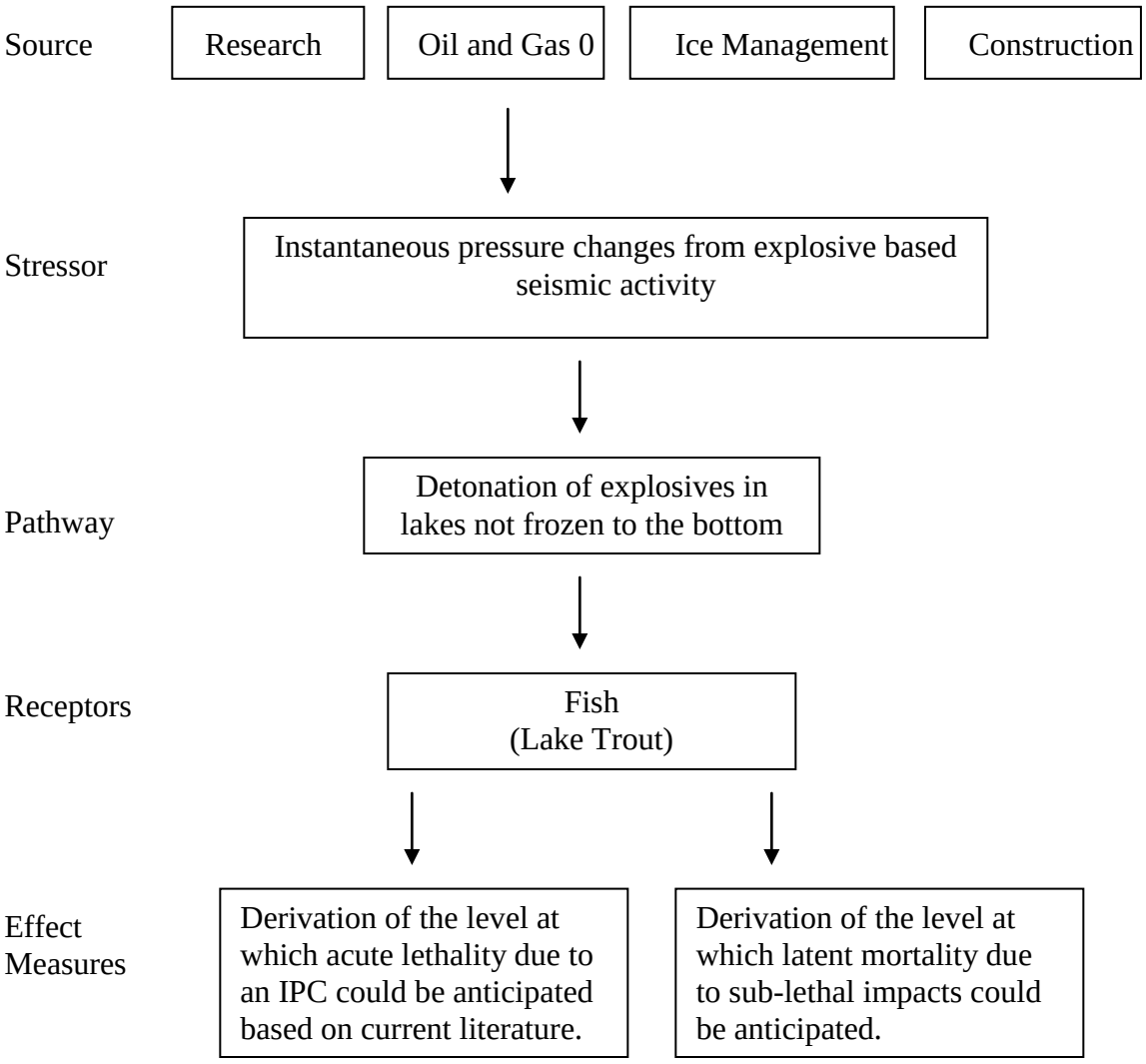

Figure 4.5: Conceptual model for risk assessment of explosive based seismic activity in the Mackenzie Delta, NWT, Canada.

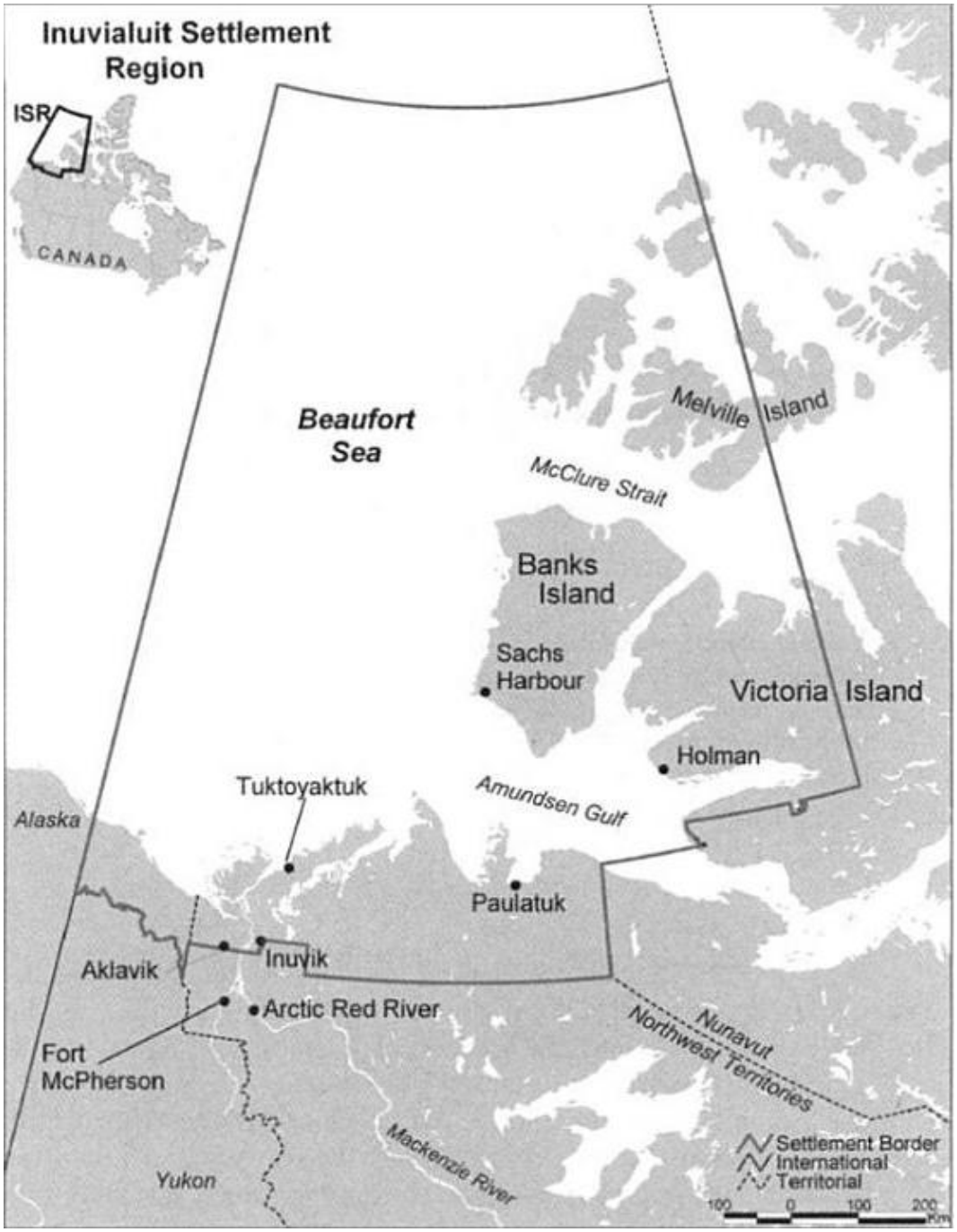

Figure 4.6: Map of Inuvialuit Settlement Region, including the Beaufort Sea, the Mackenzie River Delta, the Yukon North Slope and the Arctic islands (Fast et al. 2005)

5 General Conclusion

5.1 Summary

The overall objective of the thesis was to evaluate pathological effects in fish at different life stages exposed to explosive IPCs surrounding the current recommended Canadian *Guideline* level.

Chapter 1 evaluated effects to embryos, sac fry and juveniles exposed to explosive based IPCs from 0 to 280 kPa. Significant differences between treatments groups in cranium area and circumference was likely a product of different body sizes rather than a treatment effect. However, in juveniles, a significant linear trend in swimbladder tears as a function of pressure level was determined. Also, greater ocular areas were detected. The ocular areas, representing displacement of the eyes, were greater among juvenile fish exposed to 69 and 280 kPa but not to fish exposed at 239 kPa. Greater structural support surrounding the eye attentuated eye displacement among the fish in the 239 kPa group compared with the other treatment groups. There was a significant linear trend in renal tubules exhibiting hematuria and in the incidence of hemorrhage as a function of pressure level in juvenile kidneys. The most notable effects identified by this initial study were changes in the area and circumference of the cranium of eyed eggs, and damage to swimbladder, ocular and kidney tissue in juveniles. Data gaps identified in Chapter 1, including the effects of IPCs in fish with more developed structures as well as the effects of IPCs in fish lacking a swimbladder led to the development of the study in Chapter 2.

In Chapter 2 adult swimbladder bearing lake trout and non-swimbladder bearing sculpin were exposed to IPCs from 0 to 127 kPa. Wild, non-experimental lake trout were attracted to the blasting area to feed on stunned forage fish. In the caged lake trout,

significantly elevated hemorrhaging within the swimbladder was observed at 127 kPa. No damage was detected in sculpin. The most notable effect in this study was hemorrhaging in the swimbladder which has possible longer term impacts on morbidity and mortality. Attraction of resident fish to the blasting area may also be an important ecological finding. Chapter 1 and Chapter 2 identified that trout species are susceptible to damage from IPCs near the current *Guideline*; however little is known about the impact to wild populations of trout species in regions affected by explosive seismic exploration. This knowledge gap led to the development of Chapter 3.

A risk assessment for lake trout of the Mackenzie Delta exposed to explosive based IPCs was discussed in Chapter 3. The assessment emphasized that risk of injury exists for fish exposed to explosive based IPCs near and below 100 kPa. The degree of risk depends on a variety of factors including water depth, orientation of fish relative to the blast and substrate composition. The risk assessment highlighted that adverse risk to the population could exist if sub-lethal injuries are found to result in mortality or decreased survival due to decreased fitness. The life history of northern lake trout makes populations less able to recover if IPC related activities cause populations to drop below critical threshold numbers.

Some general statements can be made on the basis of our experimental findings. First, the *Guideline* is not generally protective to all life stages or species of fish. For example, the occurrence of swimbladder tears and kidney hemorrhaging in juvenile rainbow trout were below the *Guideline* level, and eye displacement significantly differed from controls at 69 kPa. Adult lake trout showed hemorrhaged swimbladders just above the suggested 100 kPa Guideline, at 127 kPa. Secondly, swimbladder bearing fish are

more affected by IPCs than non swimbladder bearing fish. For instance, no adverse effects from IPC exposure was documented in non-swimbladder bearing scuplin even up to 127 kPa. Third, early life stages of fish, notable juveniles, are more susceptible to damage from IPC than adult fish. While adult trout exhibited only damage to one organ (hemorrhaged swimbladder), juveniles exhibited multiple forms of damage (ocular damage, swimbladder damage, kidney damage).

5.2 Recommendations

5.2.1 Recommendations for the *Guideline*

Because of the potential for damage to early life stage and adult fish in species with swimbladders near and below 100 kPa, the *Guideline* value should be reduced. Furthermore, as a *Guideline* of 100 kPa allows variability in the strength of the actual IPCs generated, to levels that endanger fish, changes to the *Guideline* value should consider the unpredictability of blasting.

5.2.2 Recommendations for Future Research

While the current studies have determined that the *Guideline* is not generally protective, additional research is required to address remaining data gaps. Future studies need to consider fish anatomy, pathological parameters, potential for fish to recover, and in situ effects in order to effectively update the *Guidelines* and ensure their relevance to current information.

5.2.2.1 Fish Anatomy

Because differenct anatomic features can affect the susceptibility to IPCs, a range of fish species need to be evaluated. Fish to be studied should include physoclists, fish with varying degrees of swimbladder thickness, and fish with varying swimbladder function.

5.2.2.2 Pathological Variables

Future studies need to maintain an awareness that histological preparation and tissue handling methodologies can contribute to artifacts. To reduce this effect, kidneys should be removed with the surrounding tissue (i.e muscle, vertebral column) to ensure that pathology, such as hemorrhaging, is not a result of the tissue being forcibly removed from the surrounding tissue. Where small fish are being preserved, it is best to section the fish whole in either a sagittal or transverse plane to see effects in relation to other tissues.

Behavioural change may be an important but overlooked impact of IPCs. Besides observing non-experimental fish in the vicinity of blasting activity, attempts to evaluate behaviour of test fish pre and post blast would be beneficial. Methods of evaluating behaviour could include visual inspection of fish in cages by a diver, or the use of either an underwater or acoustic camera (used for high definition sonar imaging). Furthermore, monitoring of fish behaviour should not be limited to immediately pre and post blast, but should be conducted following a rest period as changes in behaviour may be latent. For instance, Goertner et al. (1994) noted changes in swimming behaviour in hogchokers 24 hours following exposure to detonation of pentolite charges.

To add to gross pathological assessment, it may be valuable to radiograph fish immediately following exposure to a detonation to evaluate swimbladder and skeletal structure integrity. Damaged and broken rib cages, for instance, have been described in northern pike (*Esox lucius*) and yearling king salmon (*Oncorhynchus tshawytscha*) following under ice exposure to C-4 explosives (peak pressure not indicated) (Roguski and Nagata 1970). Likewise, broken ribs in fish (species not indicated) exposed to plastic C-2 explosive (peak pressures not indicated) have been documented (Indrambarya 1949).

Additional tissues to those in the current study should also be examined including ovaries, brain, heart, auditory tissue, and blood vessels. As reported by Roguski and Nagata (1970), ovaries were ruptured in captive northern pike (*Esox lucius*) exposed to under ice detonations of C-4 explosive (peak pressure not indicated). It may also prove significant to assess brain tissue following an explosive event as a variety of studies have highlighted the organ's vulnerability to IPC. Ferguson (1962) noted brain hemorrhaging in American smelt (*Osmerus mordax*) collected at the lake surface following detonation of various explosive sources (see Table A1) of unknown peak pressure. Likewise, Goertner et al. (1994) following exposure to pentolite charges (peak pressure not indicated) reported brain injury to hogchokers, including blood clots and hemorrhaging in the cranium. Moreover, a caged-fish experiment was undertaken in the Bow River during construction of the Alaska Highway Gas Pipeline in Alberta (Fernet 1982). Fernet (1982) reported hemorrhage in the cerebral cavity of a rainbow trout exposed 10m from a blast of 60% Geogel (pressure level unknown). In fact, a study by Ward et al. (1948), though dated, reported that concussion in fish can occur with an explosion, and further went on

to theorize that the passage of an intense, rapid pressure wave in water can result in nervous tissue damage affecting nerve cells, fibers, synapses, and small blood vessels. Also of value to consider examining in fish following an explosive detonation is heart tissue. Ferguson (1962) noted heart rupture in caged yellow perch (*Osmerus mordax*) following detonation of various explosive sources (see Table A1) of unknown peak pressure. Thompson (1958) reported that, following detonation of DuPont Nutramex 2-H explosive (peak pressures not indicated), rockfish (*Sebatodes spp*.) showed blood clots associated with the heart. Finally, Sakaguchi et al. (1976) reported damage to heart tissue, specifically the rupture of the sinus venosus in fish exposed to explosive detonations. Consideration should also be given to the effects of blasting on auditory tissue. Seismic generating methods, such as air gun use, are known to impact hearing in fish, and studies have examined hearing tissue in fish exposed to sound sources. For instance, Song et al. (2008) examined tissues of the inner ears of lake chub (*Couesius plumbeus*), northern pike (*Esox lucius*), and broad whitefish (*Corefonus nasus*) following exposure to seismic air gun sounds, yet reported no damage in exposed species. McCauley et al. (2000) however showed pathological damage to the hearing system of pink snapper (*Chrysophrys auratus*) exposed to an operating air gun, a high energy noise source. These fish displayed damaged hair cells following exposure. One of the most powerful sources of underwater sound is explosive charges of TNT (Richardson et al. 1995 as cited in Gisiner 1998); hence, as both air guns and explosives represent sound sources, they may show similar pathological impacts. Finally, a closer examination of vascular integrity is also warranted. For instance, Sverdrup et al. (1994) examined the effects of experimental seismic shock on vasoactivity of arteries and on the integrity of the vascular endothelium

in fish exposed to experimental seismic shock in a laboratory tank. Results showed injury to the vascular endothelium of the ventral aorta and the coeliaco-mesenteric artery.

Stress hormones should also be evaluated prior to and following explosive detonation. Sverdrup (1994) evaluated primary stress hormones, adrenaline and cortisol, in fish exposed to experimental seismic shock in a laboratory tank. The hormones were not immediately elevated in plasma, but revealed patterns of delayed increases that may impact long term energy reserves and fitness in IPC exposed fish populations.

5.2.2.3 Potential for Fish to Recover

It is essential that future studies examine the implications of damage and the potential for fish to recover. Based on literature, it is difficult to determine whether recovery is possible. Most information regarding recovery in fish relates to swimbladder damage. Survival and recuperation following damage induced by explosive force has been reported. Yelverton et al. (1975) examined the effects in various fish (see Table A1) from exposure to underwater blasts of Pentolite charges ranging in peak pressure from 530 kPa-9025 kPa. Following exposure tests, 14 carp (*Cyprinus carpio*) and catfish (*Ictalurus punctatus*) were observed for a 2 week period. The carp and catfish were then autopsied and showed signs of having ruptured swimbladders, suggesting that short term survival with a damaged swimbladder is possible. However, Wiley et al. (1981) and Gaspin et al. (1976) speculated that fish in the natural environment would have difficulty recovering from a ruptured swimbladder and have very little chance of surviving. Swimbladders in spot (*Leiostomus xanthurus*) exposed to a pressure of 138 kPa appeared to show signs of healing after 4 days and this was attributed to retained gas secretion within the swimbladder. However, the tears later reopened and gas escaped from the

organ (Gaspin et al. 1976, Wiley et al. 1981). In contrast, following surgical rupture of swimbladders, Bellgraph et al. (2006) found that rainbow trout (*Oncorhynchus mykiss*) swimbladders were healed within 7-14 days and that no subsequent mortalities occurred. In general, fish may recover from minor injury, such as limited internal hemorrhaging (Hubbs et al. 1960), and it may be that with minor organ damage and limited blood loss, injuries may heal (Cronin et al. 1948).

Predation is thought to be the most likely contributor to fish mortality regardless of whether the fish is able to recover from internal damage. Bellgraph et al. (2008) suggested that the indirect effects of swimbladder injury would result in weakened and stressed fish with elevated risk of direct mortality from predation. Simenstad (1973), Wiley et al. (1981), Hubbs and Rechnitzer (1952) and Wiley and Wilson (1975) have all made similar suggestions. Moreover, Govoni et al. (2008) and Settle et al. (2002) explain that any injuries that persist for an extended period of time are probably placing fish at serious disadvantage for capturing prey, further reducing their fitness. With this in mind future studies should incorporate predator prey interactive influences.

Finally, additional studies required to address the recuperative abilities of fish following IPCs should examine effects over extended periods at similar IPCs near the *Guideline,* which would help to integrate immediate and latent mortality. Additional studies should also examine potential alterations to resident fish population numbers following IPC exposure.

5.2.2.4 In Situ Effects

The effects of sequential detonations on fish have also not been adequately studied. This is important as it most accurately represents exposure scenarios in seismic

based exploratory programs. Further studies in his area would also allow comparisons of the effects from point source explosive detonations versus continuous explosive detonations. Aplin (1947) notes that successive shots in the same area continue to damage fish. The author furthermore relates that the number of shots fired is proportionate to the number of fish that will be harmed. Moreover, Rulifson and Schoning (1963) explain that fish are more apt to be killed if several explosions occur in the same area. Related to the current study, this phenomenon was linked with fish moving in to feed on those killed from previous blasts. Caged yearling king salmon (*Oncorhynchus tshawytscha*) appeared to be more affected when exposed to repeated blasts than fish not previously exposed to a detonation (Roguski and Nagata 1970). When trout (*Cynoscion regalis Bloch and Schneider*) and rock (*Roccus saxatilis Walbaum*) were subjected to repeated shocks of TNT/nitramon fired in succession, the immediate lethal range for trout and rock was extended, by 150 feet and 50 feet respectfully, when compared to the same charges shot independently (Cronin 1948).

5.3 Conclusion

The *Guidelines for the Use of Explosives in or Near Canadian Fisheries Waters* contain provisions by which they will be reviewed and updated as necessary (Wright and Hopky 1998). Data from the current studies underscore the need for refinement of the *Guidelines.*

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7 Appendix

Table A1: Summary of literature on effects observed in fish exposed to explosive blasts.

Aplin 1947	Burst Swimbladders Ruptured blood vessels Crushed body contents Broken Ribs Death	Variety of fish (unknown weights), some 0.5 lb: Anchovies(genus/species unidentified) Sardine (genus/species unidentified) Kingfish (*Genyonemus lineatus*) Queenfish (*Seriphus politus*) Perch (*Girella nigricans*) Barracuda (*Sphyraena species*) Croaker (*Roncador stearnsi*) Shad (*Alosa species*) White Sea-bass (*Cynoscion nobilis*) Smelt (*Atherinopsis californiensis*)	Explosives: 60% petrogel in 10 pound sticks: 10 shots of 10 lbs 18 shots of 20 lbs 18 shots of 40 lbs	N/A
Bird and Roberson *(Fry and Fingerlings)*	No Effects	Coho Salmon *(Oncorhynchus kisutch)* 38-80mm Chum Salmon *(Oncorhynchus keta)* 34-39mm Wild Coho Salmon *(Oncorhynchus kisutch)* 38-80mm Dolly Varden *(Salvelinus malma malma [Walbaum, 1792])* 53-80mm	Explosives: Tovex T-1 Tovex 930 Anfo-P *Maxumum loads 1065-1673 lbs*	5.51kPa, 8.96 kPa, 18.6 kPa
Coker and Hollis 1950	Severe body trauma (Menhaden) Dislocated mouthparts and lacerated sides (Menhaden) Protruding eyes Gut protrusion from mouth and anus (Croakers) Posterior gut protrusion (Trout) Distended abdomens (Croakers, Trout, Hake, small Rock) Ruptured air-bladder and haemorrhage of its vascular system Rupture of the vascular system of the abdominal cavity and organs Ruptured blood vessels in: Liver and Spleen Death	Fry, Larvae and Adults 3-106cm including: Menhaden (*Brevoortia tyrannus*) Croaker (*Roncador stearnsi*) Trout, gray and spotted Rock, Striped Bass (*Roccus saxatilis*) Alewife, Branch Herring (*Pomolobus pseudoharengus*) Alewife, Glut Herring (*Pomolobus aestivalis*) Hickory shad, Hickory Jack (*Pomolobus mediocris*) Shad (*Alosa sapidissima*) White Perch, Sand Perch (*Bairdiella chrysura*) Spot (*Leiostomus xanthurus*) Silver Hake (*Merluccius bilinearis*) Silverside (*Menidia menidia*) Half Beak (*Hyporhamphus unifasciatus*) Harvestfish (*Peprilus alepidotus*) Gizzard Shad (*Dorosoma cepedianum*)	Explosives: HBX charges Charge range: from 250 to 1,200 lbs	N/A
Cronin 1948	Main organs affected included: Swimbladder, Liver, Spleen, Unprotected blood vessels, Ripe gonads In some cases, abdominal body wall would split open Death	Trout (Cynoscion regalis Bloch and Schneider)-320mm* Rock (Roccus saxatilis Walbaum)-330mm* **N.B: These two species provided the statistical data*	Explosives: du Pont Nitramon TNT/Nitramon explosives; 28-303 lbs charges	Based on calculated peak pressures using experimental points for the test conditions ~620 kPa-4544 kPa
Falk and Lawrence 1973	Swimbladder rupture Kidney damage Internal hemorrhaging Rib cage damage Intestinal damage External damage Ovary damage Authors indicate that internal organs usually affected are: Kidney, Liver, Heart, Spleen, Gonads, Swimbladder	*Aquaflex experiment:* Arctic Cisco (*Coregonus autumnalis*) 135mm-290mm *60% Geogel experiment*: Arctic Cisco (*Coregonus autumnalis*) 135mm-290mm *Additionally:* Local Fish (*C.nasus, C. autumnalis* including immature coregonids)	Explosives: Aquaflex: 200 grains/ft; 165 ft lengths (4.9 lbs) 60% Geogel: charge sizes of 2.5 and 10 lbs	N/A

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Ferguson 1962	Yellow Perch Major ruptures of the swimbladder Severe haemorrhaging of the kidney and segmental blood vessels Accumulation of blood in the coelom resulting from hemorrhage of the kidney or rupture of the left posterior cardinal vein Free blood within the pericardium Hemorrhaging around the vent or eyes Heart Injury Death Emerald Shiner Severe and general hemorrhage of the coelomic organs and lateral body wall Hemorrhage of the kidney Rupture of the posterior cardinal vein Large amounts of blood in the coelom Death American Smelt Same injuries as Emerald Shiner but brain and eye hemorrhage more prevalent Death Injury to caged Yellow Perch: Hemorrhage of the segmental vessels Hemorrhage in the kidney tissue or left posterior cardinal vein passing through the kidney Swim bladder rupture Heart Rupture External haemorrhage	Yellow Perch (*Osmerus mordax)* Lake Emerald Shiner (*Notropis atherinoides*) American Smelt (*Osmerus mordax*) White Bass (*Morone chrysops*)	Explosives: Nitrone Nitrone primer Starter (S1) one pound sealed in can Black power (BTC-23-F-65) Squib cap (No. 89, electic) *The above were used alone or in combination*	N/A
Fernet 1982	Damage assessed using Hubbs et al. 1960 numerical criteria (see Hubbs et al. (1960). Damage levels 2,3,4,5 seen	Rainbow Trout (*Salmo gairdneri*)-15-25cm fork length Mountain whitefish (Prosopium williamsoni) *Young of year* Longnose sucker (*Catostomus catostomus*) *Juvenile*	Explosives: 60% Geogel	227.71-1996.39 kPa *N.B: Pressure level at cage where mortalities found is unknown*

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Fitch and Young 1948	Rupture of air bladder Damaged/crushed viscera Ribs broken from backbone Death	Variety of fish that include: California Halibut (*Paralichthys californicus*) Midshipmen (*Porichthys notatus* and *P. myriaster*) Jack Mackerel (*Trachurus symmtricus*) Rockfish (*Sebastodes paucispinis*) Sardine (*Sardinops species*) Kingfish (*Genyonemus lineatus*) Perch (*Brachyistius frenatus*) Barracuda (*Sphyraena argentea*) Pacific Mackerel (*Scomber japonicus*) White Sea Bass (*Laes calcarifer*) Black Sea Bass (*Stereoleopis gigas*) Queenfish (*Seriphus politus*) Anchovy (*Engraulis mordax*) Jack Smelt (*Atherinopsis californiensis*) Salt-water Perch (*Brachyistius frenatus*) Kelp Bass (*Paralabrax clathratus*) Includes larval anchovies	Explosives: Type: N/A Total weights of shot pounds from 20 lbs to 6000 lbs for open and jet shots	N/A
Gaspin 1975 (see also Wiley and Wilson 1975)	Damage assessed using Hubbs et al. 1960 numerical criteria (see Hubbs et al. (1960). All damage levels seen Includes: Light Hemorrhaging, principally in tissues covering the kidney Light hemorrhaging through body cavity, some damage to kidney Burst swimbladder Gross damage to kidney Bleeding around the anus Gross damage to internal organs (lost or homogenized abdominal contents)	Spot (*Leiostomus xanthurus*) White perch (*Morone americana*) Hogchokers (*Trinectes maculatus*)	Explosives: Pentolie spheres Charge weights: 1,8, 31, 68 lbs	~200 kPa-2861 kPa
Godard et al. (In Press) (Under Ice Study)	*Eggs* Changes in area and circumference of cranial region *Juveniles* Swimbladder damage (tears) Ocular damage (exopthalmia) Kidney damage (hemorrhage and hematuria)	Rainbow Trout (*Onchorhynchus mykiss*) eyed eggs (approx. 230-270 degree days [dd] at 10°C) sac fry (approx. 224-266 dd, between 16°C-19°C) juveniles (approx. ≤336-399 dd, between 16°C-19°C)	Explosives: N/A	Eyed eggs and sac fry 0, 64, 105, 228 and 280 kPa Juveniles 0, 69, 239 and 280 kPa
Goertner et al. 1994	Varying degrees of blood clots on gills Varying degrees of visceral hemorrhaging Varying degrees of blood within the heart chamber Varying degrees of blood clots and blood in the cranium	Hogchokers *(Trinectes maculatus)* * Summer Flounder *(Paralichthys dentatus)* Spot *(Leiostomus xanthurus)* *Predominant test species	Explosives: Cylinders of recast pentolite 10.16 +/- 0.26 lbs	N/A
Golder 2000	Swimbladder rupture Kidney damage Spleen damage Bruising on the liver Gonad rupture Bruising to eggs Death	Lake Whitefish (*Coregonus clupeaformis*) *Juveniles-170-230mm* *Adult-378mm* Northern pike (*Esox lucius*)-adult Adults-475-510mm	Explosives: Dynamite powder enclosed in a plastic tube	During test program At ~0m: 46.8-217.6 kPa At ~5m: 12.1-84.2 kPa

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Govoni et al 2002, 2003	Internal hemorrhaging Coelomic hemorrhage *Swimbladder + Liver* Hyperemia within: Swim bladder serosa ** Mucosa of the gas gland** Rete mirabile** Liver** *Kidney* Hematuria in kidney tubules** *Alimentary Canal* Liquifactive necrosis in the mucosa of the dorsal region of the anterior *intestine* (in exposed and control) *Liver* Contusions to the *liver* in region proximal to swimbladder in an exposed spot. Liquifactive necrosis of *liver* in control fish (spot only) Coagulative necrosis of liver in exposed fish, in region proximal to the swimbladder** *Pancreas* Rupture of pancreas in exposed fish, in region proximal to the swim bladder** **most recurrent and only ones attributed to exposure to sub-marine detonations	Pinfish (*Lagodon rhomboids*) 13.8-21.3mm Spot (*Leiostomus xanthurus*) 15.1-25.3mm	Explosives: 12-grain Primadet PDT 1403 detonators	Average P_{max} Values 636.92 kPa 230.86 kPa 109.93 kPa *No effects resulting from trauma at 109.93 kPa*
Houghton and Munday 1987	Test Fish Hemorrhages in the swimbladder Blood clots in the swimbladder Dilated abdominal blood vessels Swimbladder deformities Rupture of the swimbladder with hemorrhage into coelom Hemorrhaged kidneys Blood clots or hemorrhaged in the pericardium Liver damage Bleeding of anal or pectoral fins Bruising/Hemorrhaging of the kidney Hemorrhaging of the body wall near the anus Blood filled swimbladder Hemorrhage in the heart Hemorrhage in the spleen Blood clots within coelomic cavity Blood fluid within coelomic cavity Blood or bloody fluid in the gut Bleeding about the anus Ruptured peritoneum Liver discoloration Liquefied kidney Swimbladder destruction Kidney destruction Rupture of body wall Death Incidental Fish Killed Hemorrhaged swimbladders Ruptured swimbladders Hemorrhaged kidneys Liquefied kidneys Light hemorrhage of stomach Light hemorrhage of liver Light hemorrhage of heart Epidermal hemorrhages Everted stomachs Erratic swimming behavior Rupture of the body wall with ribs torn free Liver hemorrhage Death	Test Fish Coho salmon (*Oncorhynchus kisutch*) 103-146mm *Smolt* Chum salmon (*Oncorhynchus keta*) 38-59mm *Smolt* Pacific herring (*Clupea harengus pallasi*) 69-116mm *Juvenile* Incidental Fish Killed Juvenile walleye pollock (*Theragra chalcogramma*) Pacific tomcod (*Microgadus proximus*) Pacific cod (*Gadus macrocephalus*)) Pacific herring (*Clupea harengus pallasi*) Rockfish (*Sebastes spp.*) Juvenile coho salmon Dolly Varden (*Salvelinus malma*) Rock greenlings (*Hexagrammos sp.*) Species of sole (Genus and Species unknown) Copper rockfish (*Sebastes caurinus*)	Explosives: Linear primacord of three different strengths: 50, 100 and 200 grains per foot	37 kPa-331kPa Based on oberserved values for caged fish exposures only

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Hubbs and Rechnitzer 1952	Gas filled visceral cavity from a ruptured air bladder Death	Includes (but not limited to): Pacific sardine (*Sardinops caerulea* Girard) Ocean northern anchovy (*Engraulis mordax mordax* Girard) Grunion (*Leuresthes tenuis* Ayres) Jack mackerel (*Trachurus symmetricus* Ayres) California pompano (*Palometa simillima* Ayres) Kingfish (*Genyonemus lineatus* Ayres) Queenfish (*Seriphus politus* Ayres) Pacific saury (*Cololabis saira* Brevoort) Jacksmelt (*Atherinopsis californiensis* Girard) Pacific mackerel (*Pneumatophorus japonicus diego* Ayres) Shiner seaperch (*Cymatogaster aggregata* Gibbons) White seaperch (*Phanerodon furcatus* Girard)	Explosives: 60% gelatine dynamite Hercomite Hercules FFG or FFFG powder (black powder)	165.4-9259.6 kPa 951.4-1331.0 kPa 10.3-1103.2 kPa* *Deaths begin at 400 kPa
Hubbs et al. 1960	Established Criteria Levels: 0-No Damage 1-Only light hemorrhaging, principally in the tissues covering the kidney 2-Swimbladder intact, but with light hemorrhaging throughout the body cavity, with some damage to the kidney 3-No external indication of damage, but with the swimbladder usually burst. Hemorrhaging and organ disruption less extreme than in 4 and 5, but with gross damage to the kidney 4-Incomplete break-through of the body wall, but iwth bleeding about the anus. The swimbladder is almost invariably broken and the other organs damaged as noted under 5 5-Rupture of the body cavity. The break is usually a slit just to the side of the midventral line. Associated with this severe damage is a burst swimbladder and gross damage to other internal organs. The abdominal contents are often completely lost or homogenized.	Northern Anchovy (*Engraulis mordax*) half grown-small maturing to mature adults Pacific Sardine (*Sardinops caerulea*) medium to large, maturing to mature adults Jack Mackerel (*Trachurus symmetricus*) medium sized Pacific Mackerel (Scomber japonicus)	Explosives: Nitramon Seismic Nitramon Water- Work Vibronite B Vibronite S EP 198B	276-3792 kPa (includes all detonations) *Damage thought to begin at 310 kPa
Indrambarya 1949	Torn fins Detached scales Eye damage Bruising of stomach and body Bleeding Viscera protruding from the vent Broken ribs Burst swimbladders Blood from vertebral region Crushed reproductive organs Ruptured blood vessels Death	*Cacsio* sp. *Myripristis* sp. *Carapx* sp. *Calliodon* sp. *Pempheris* sp. *Dussuruieria* sp. *Scolopsis* sp. *Latianus* sp. *Abudeldus* sp. *Epinephelus* sp. *Ilenioclius* sp. *Tylosurns* sp. *Taeniura* sp. *Sphyraena* sp. *Platax* sp. *Rastrelliger* sp. *Leiognathus* sp. *Ephinephelus* sp. *Tenthis* sp.	Explosives: Plastic C-2	N/A
Kearns and Boyd 1965	Internal Ruptures Displaced Visceral Organs Torn Musculature Hemorrhaging Blood Vessels Protruding Eyes Everted Stomachs Gas Bladder Damage Death	Rockfish (*Sebastodes species*) Herring (*Clupea pallasii)* Salmon (*Oncorhynchus tshawytscha* and *Nerka*)* Miscellaneous including: Needle Fish (Genus/species unknown) Jack Mackerel (*Trachurus symmetricus*) Whiting (*Theragra chalcogrammus*) Grey Cod (*Gadus macrocephalus*) Pacific Hake (*Merluccius produtuctus*) Sea Perch (*Cymatogaster aggregata*) Pacific sandlance (*Ammodytes hexapterus*) Tube snout (*Aulorhynchus flavidus*) Sculpin (Genus/species unidentified) **Includes adult and juvenile*	Explosives: Nitrone S.M (Seismic Marine) with nitro-carbo-nitrate (NCN) high explosive primer	N/A

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Kostyuchencko 1948	*Eggs* Deformation and compression of the membrane Spiral Curling of the embryo Displacement of the embryo to one of the poles of the egg Impairment of integrity of the vitelline membrane Depression on the membrane into the egg Impairment of the plasmatic integrity of the yolk Lateral yolk shift to one of the poles in the membrane Death *Larvae* Mechanical injury to the head Mechanical injury to the anterior part of the intestine	Eggs/Larvae: Includes but not limited to: Anchovy (*Engraulis encrasicholus ponticus* Aleksandrov) Blue runner (*Caranx crysos*) Crucian Carp (*Carassius carassius*)	Explosives (TNT) Air Gun Electric Pulse Generator	N/A Working pressure 14,184 kPa N/A
Linton et al 1985	Combination of injuries in Black and Red Drum: Loss of opercular scales in black drum Stunning and distorientation in red drum Parietal peritoneum torn away from coelomic cavity Visceral peritoneum ruptured Rupture of stretching of swimbladder wall Capillary hemorrhage within swimbladder wall Renal portal vein hemorrhage Rupture of kidney tubules, mesovarium and mesorchium Rupture of liver Rupture of gall bladder wall Rupture of cystic duct Rupture of stomach wall Rupture of intestine wall Death	Red Drum (*Sciaenops ocellatus*)-average 32cm Black Drum (*Pogonias cromis*)- average 23cm Anchovy (*Anchoa mitchilli*) Atlantic Croaker (*Micropogonias undulates*)	Explosives: Primacord detonation cord	N/A
McAnuff and Booren 1989	Death	Various species common to the Lake Erie area (not specified) Yellow Perch (Perca flavescens fluviatalis)	Explosives: C.I.L Hydromex M210 and T3 Primed with C.I.L. Pentomax	8.61 -158.56 kPa *Peak overpressures associated with injury ranged from 30 kPa to 150 kPa*
McAnuff et al. 1994	Swimbladder rupture Death	*Nipigon River* Trout (genus and species unidentified) Perch (genus and species unidentified) White sucker *(Catostomus commersonii)* Whitefish (genus and species unidentified)-0.1 to 10kg Lake Herring (genus and species unidentified) Sturgeon Sucker (*Catostomus catostomus*) Rainbow trout (*Oncorhynchus mykiss*) *Winnipeg River* White Sucker (*Catostomus commersonii*) Pickerel (genus and species unidentified) Perch (genus and species unidentified) Sauger (*Sander canadensis*) Bass (genus and species unidentified) Ling (genus and species unidentified) Pike (genus and species unidentified)	Explosives: *Nipigon River* Hydromex M210 cartridges *Winnipeg River* Nitropel pellets, cardtridged Powerditch 1000 Holes were double primed with Exel Constadet detonators and Pentex primers	*Nipigon River* 17-97 kPa *Winnipeg River* 12.5-76.9 kPa

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Nix and Chapman 1985	Death	Fish collected during monitoring include: Pacific herring (Clupea harengus pallasii) Threespine stickleback (Gasterosteus aculeatus) Northern anchovy larvae (Engraulis mordax mordax) Chinook Salmon (Oncorhynchus tshawytscha) Shiner perch (Cymatogaster aggregata)	Explosives: Tovex 5000 15 pounds per hole	N/A
Paterson and Turner 1968	Death Rupture of the heart and associated vessels Damaged or ruptured swimbladder Swimbladder protrusion from the mouth	Burbot (Lota lota) Lake whitefish (Coregonus clupeaformis) Trout-perch (Percopsis omiscomaycus) Cisco (Coregonus artedii) Lake trout (Salvelinus namaycush)-uninjured	Explosives: 60% Carbonitronitrate 4000 lbs	N/A
Roguski and Nagata 1970 (Under Ice Study)	Yearling king salmon: Hemorrhaging from vent Broken ribs and rib cage Ruptured kidney Ruptured air bladder Ruptured stomach Ruptured liver Ruptured intestine Hemorrhaged body wall Kidney badly damaged Undetermined injuries Damage to body wall and ribs Hemorrhaging around heart Hemorrhaged kidney Hemorrhaged intestine Northern pike: Missing scales Badly damaged ribcage and body wall Ruptured liver Ruptured air bladder Ruptured kidney Ruptured ovaries	Yearling king salmon (*Oncorhynchus tshawytscha*) 17.8-25.4cm Northern pike (*Esox lucius*)-Mean: 50.8cm	Explosive: 130.5-940 lbs C-4 explosive	N/A
Ross et al. 1985	Death	Includes but not limited to: American sand lance (Ammodytes americanus) Cod (Gadus morhua) Silver hake (Merluccius bilinearus) Haddock (Melanogrammus aeflefinus) Pollock (Pollachius virens)	Explosives: Aquaflex Linear Explosive a series of 2 x 25 m lengths	N/A
Sakaguchi et al. 1976	Liver: Destruction of Tissue Congestion Heart: Rupture of sinus venosus Swimbladder: Destruction of Tissue Internal hemorrhage Congestion Kidney: Destruction of Tissue Congestion	Common carp (Cyprinus carpio) Rock fish (Sebastiscus marmoratus) Japanese seabass (Lateolabrax japonicus)	Explosives: Instantaneous cap CCR Unknown Dynamite	54-3590 kPa *For all pressures evaluated*

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Settle et al 2002	*Within 24 hours of blast* Evisceration Haemorrhaging dorsal to swimbladder and in region of the kidney Death *Following 24 hour period* Hematuria (damage to kidney tubules) Hemorrhage within the coelom Swimbladder hemorrhage Liver haemorrhage Coagulative liver necrosis (considered lethal) Ruptured pancreas (considered lethal) Death	*Late stage larval and recently metamorphosed fishes:* Spot (*Leiostomus xanthurus*)-18.0 to20.1mm Pinfish (*Lagodon rhomboids*)-15.9 to17.2mm	Explosives: 12-grain Primadet PDT detonators	Mean max. pressure values: 277-691.5 kPa (3.6m from blast) 146.9-290.3 kPa (7.5m from blast) 79.3-123.4 kPa (17.0m from blast)
Sverdrup et al. 1994	Injury to vascular endothelium of the ventral aorta Injury to the coeliac mesenteric artery Increase in stress hormones adrenaline and cortisol (delayed) Decline of atrial uptake of catecholamines	Atlantic Salmon (*Salmo salar*)	Explosive: Electrical percussion caps	2Mpa= 2000 kPa
Teleki and Chamberlain 1978	Swimbladder Rupture Hemorrhaging in the coelomic cavity Hemorrhaging in the paricardial cavity Death	Pumpkinseed (*Lepomis gibbosus*) Crappie Sp. (*Pomoxis sp.*) White Bass (*Morone chrysops*) Gizzard Shad (*Dorosoma cepedianum*) Yellow Perch (*Perca flavescens*) Smallmouth Bass (*Micropterus dolomieui*) Rock Bass (*Ambloplites rupestris*) Freshwater Drum (*Aplodinotus grunniens*) Quillback (*Carpiodes cyprinus*) White Sucker (*Catostomus commersoni*) Yellow Bullhead (*Ictalurus natalis*) Rainbow Trout (*Salmo gairdneri*) Common Carp (*Cyprinus carpio*)	Explosive: 22.7-272.4 kg high explosive Hydromex	8.61 kPa-158 kPa *Based on 55 readings*
Thomson 1958	Eye Protrusion Distended abdomen Frayed gills Ruptured air bladders Blood clots about the heart, liver and kidney Death	*Caged Fish:* Lingcod (*Ophiodon elongates*) Lemon Sole (*Parophrys vetulus*) Grey Cod (*Gadus macroephalus)* Blackcod (*Anoplopoma fimbria*) Ratfish (*Hydrolagus colliei*) Rock Sole (*Lepidopsetta bilineata*) *Resident Fish:* Banded Rockfish (*Sebastodes nigrocinctus*) Red Snappers (*S. ruberrimus*) Orange-spotted (*S. maliger*) Copper Rockfish (*S. caurinus*)	Explosives: DuPont Nitramex 2-H	N/A

Authors	Effects in Fish	Fish Type Affected	Pressure Source	Intensity
Tyler 1960	Disappearance of scales in the vicinity of the swimbladder Ruptured swimbladder Ribs torn from abdominal wall Kidney rupture Torn ovaries or gonads Ruptured blood vessels Damaged adipose tissue Damaged spleen	Red Salmon (Oncorhynchus nerka)	Explosives: 40% gelatin dynamite	N/A
Wiley et al. 1981 (see also Gaspin et al. 1976) N.B: Continuation of 1973 tests by Gaspin 1975 and Wiley and Wilson 1975	Internal Hemorrhaging Bruising of the Kidneys Burst Swimbladder Death	*Fish 8.3-24.6cm (83mm-246mm) in length* *Spot (*Leiostomus xanthurus*)-88mm, 9.6g *White Perch (*Morone americana*)-162mm, 79g Atlantic croaker (*Micropogonias undulatus*)-117mm, 13g Oyster Toadfish (*Opsanus tau*)-215mm White Catfish (*Ictalurus catus*)-N/A Hogchoker (*Trinectes maculatus*)-N/A Striped Killifish (*Fundulus majalis*)-88mm Atlantic menhaden (*Brevoortia tyrannus*)-N/A Blueback Herring (*Alosa aestivalis*)-N/A *NB :* *Weights and lengths are expressed as means* *While thirteen species of fish were used in explosion tests, only those presented above were discussed in the paper* **Primary test fish*	Explosives: Cylindrical cast pentolite charges (50/50 mixture, PETN and TNT)	210 kPa-3120 kPa* *Levels represent explosive pressures applied to spot and white perch in the 1973 and 1975 tests* 951 kPa-3054 kPa *(according to Gaspin etl al. 1976)*
Yelverton et al 1975	Disorientation Decreased movement Erratic gill movement Hemorrhaging of aforementioned organs Rupture of abdominal walls Death Internal organs most commonly damaged: Swimbladders Kidneys Livers	*Physostomes:* Top Minnow (*Gambusia affinis*)-0.47g Goldfish (*Carrasius auratus*)-1.4g and 245g Carp (*Cyprinus carpio*)-149g, 117g,113g,and 744g Rainbow Trout (*Salmo gairdneri*)-143g Channel Catfish (*Ictalurus punctatus*)-105g and 338g *Physoclists:* Guppy (*Lebistes reticulates*)-0.02g and 0.13g Bluegill (*Lepomis macrochirus*)-1.4g and 88g Large Mouth Black Bass (*Micropterus salmoides*)-146g *NB: Weights are expressed as means*	Explosives: Pentolite fired by electric blasting caps (Dupont E99)	Peak Pressure: 530 kPa-9025 kPa Impulse: 4.1-428 kPa/msec
Young and Willey 1977	Damage assessed using Hubbs et al. 1960 numerical criteria (see Hubbs et al. (1960). Damage levels seen include level: (2) Swimbladder intact, but with light hemorrhaging throughout body cavity with some damage to the kidney (3) No external indication of damage but with the swimbladder usually burst. Hemorrhaging and organ disruption less extreme than in (4) and (5) but with gross damage to the kidney (4) Incomplete break through the body wall but with bleeding about the anus. The swimbladder is almost invariably broken and the other organs damaged. Death	Menhaden (Brevoortia spp.) Bludeback herring (Alosa aestivalis) White Perch (Morone americana) Spot (Leiostomus xanthurus)	Explosives: Experimental explosive Pentolite (20 lbs.) TNT (91 lbs.) Baratol (2.5 lbs)	N/A

NB: Within the literature, common names were occasionally given with no mention of genus and species names. Effort however was made to provide the genus and species of the fish where possible. In certain cases, inaccuracies may exist

Table A2: Variables determining effects in and survival of fish exposed to seismic blasts.

Explosive Parameters
1) Depth of charge (Cott and Hanna 2004, Cronin 1948, Golder 2000, Lavergne 1970, O'Keefe 1984, Wright 1982)
2) Type of explosive (Cronin 1948, Fry and Cox 1953, as descrbed in Houghton and Munday 1987, Kearns and Boyd 1965, Keevin 1997, Munday et al. 1986, Nix and Chapman 1985, as discussed in Settle et al. 2002, as discussed in Wright 1982, Wright 1985)
3) Amount/Size/Weight of explosive (Christian 1974, Cronin 1948, Golder 2000, Kearns and Boyd 1965, MacLennan and Simmonds 1992, Teleki and Chamberlain 1978, as discussed in Wright 1982)
4) Detonation depth (Christian 1973, Houghton and Munday 1987, as discussed in Wright 1982)
5) Intensity of the blast (Cott et al. 2003, as discussed in Wright and Hopky 1998, Tyler 1960)
6) Detonation velocity (Teleki and Chamberlain 1978)
7) Duration of the blast (Keevin 1997)
8) Location of blast relative to fish (Bird and Roberson 1984, Houghton and Munday 1987)
9) Density of material being blasted (Teleki and Chamberlain 1978)
10) Placement of the charge (i.e buried vs. open water) (MacLennan and Simmonds 1992, McAnuff and Booren 1989, Munday et al. 1986)
11) Type and amplitude of the pressure pulse (Hubbs and Rechnitzer 1952)

Piscine Parameters
1) Location of fish in the water column (discussed in ADF&G, as described in Baxter 1985, as described in Baxter 1982, Bird and Roberson 1984, as discussed in Christian 1973, Cronin 1948, Goertner et al. 1976, Houghton and Munday 1987, Hubbs and Rechnitzer 1952, Kearns and Boyd 1965, Linton et al. 1985, Munday et al. 1986, Roguski and Nagata
2) Weight of fish (as discussed in Wright 1982, Cronin 1948, Yelverton 1975)
3) Shading effect of fish behind other fish or objects (Cronin 1948, Gaspin et al. 1976)
4) Quantity of fish (Cronin 1948)
5) Physical damage from the enclosures in which the fish are placed (Roguski and Nagata 1970)
6) Species of fish (Bishai 1961, as discussed by Continental Shelf Associates, Inc. 2004, reviewed in Hill 1978, Houghton and Munday 1987, Linton et al. 1985, as discussed in Settle et al. 2002, , Linton et al 1985, Nix and Chapman 1985, Wright 1982)
7) Distance from the site of detonation (Cronin 1948, Fitch and Young 1948, Houghton and Munday 1987, Linton et al 1985, MacLennan and Simmonds 1992, Roguski and Nagata 1970, Tyler 1960)
8) Size of fish (as described in Baxter 1982, as discussed in Christian 1973, as discussed by Continental Shelf Associates, Inc. 2004, reviewed in Hill 1978, Houghton and Munday 1987, Hubbs and Rechnitzer 1952, O'Keefe 1984, Roguski and Nagata 1970, Wright 1982)
9) Orientation of the fish (discussed in ADF&G, as discussed by Continental Shelf Associates, Inc. 2004, Cronin 1948, Falk and Lawrence 1973, Fitch and Young 1948, Goertner et al. 1994, reviewed in Hill 1978, Houghton and Munday 1987, Nix and Chapman 1985, Roguski and Nagata 1970, Sakaguchi et al. 1976, as discussed in Settle et al. 2002, as discussed in Wright 1982, Tyler 1960)
10) Shape of fish (Fitch and Young 1948, Teleki and Chamberlain 1978)
11) Age of fish/Stage of development (Birds and Roberson 1984, Bishai 1961, Faulkner et al. 2006, Faulkner et al. 2007, Govoni et al. 2003, reviewed in Hill 1978, Houghton and Munday 1987, Jensen and Alderice 1983, 1989, as reviewed in Keevin 1997, Nix and Chapman 1985, as discussed in Settle et al. 2002, Tsvetkov et al. 1972, as discussed in Wright 1982)
12) General health and physical condition of fish (Christian 1973, Cronin 1948, Falk and Lawrence 1973)
13) Swimbladder anatomy **a) Physostomous/physoclistous** (Falk and Lawrence 1973, Hogan 1941, Kearns and Boyd 1965, as reviewed in Keevin 1997, as discussed in Settle et al. 2002, as reviewed by Trasky 1976, Teleki and Chamberlain 1978, as discussed in Wright 1982) **b) Thinness/thickness of the swimbladder** (as discussed in Settle et al. 2002, as reviewed by Trasky 1976, Wiley et al. 1981) **c) Loose vs tightly adhered swimbladder** (Cronin 1948, as discussed in Settle et al. 2002, Wright et al 1981)
14) Presence/Absence of a swimbladder (Aplin 1947, Bishai 1961, as discussed by Continental Shelf Associates, Inc. 2004, Gaspin 1975, Gaspin et al. 1976, Goertner et al. 1994, as discussed in Falk and Lawrence 1973, Kearns and Boyd 1965,as reviewed in Keevin 1997,as discussed in Settle et al. 2002, Wiley and Wilson 1975, Wiley et al. 1981, as discussed in Wright 1982)
15) Anatomy of fish (Cronin 1948, Gaspin et al 1976, Wiley et al 1981)

Environmental Parameters
1) Bottom/Substrate composition (Bird and Roberson 1984, Cronin 1948, Goertner et al. 1994, Houghton and Munday 1987, as discussed in Settle et al. 2002, as discussed in Wright 1982, Hubbs and Rechnitzer 1952, Nix and Chapman 1985, Tyler 1960, Wright 1982, Yelverton et al. 1975)
2) Depth to lake substrate (Cronin 1948)
3) Effect of water temperature stratification (Cronin 1948)
4) The presence of ice cover (as discussed in Wright 1982)
5) Water depth (as discussed in Settle et al. 2002, Nix and Chapman 1985, as discussed in Wright 1982, Tyler 1960)
6) Weather (Aplin 1947, Coker and Hollis 1950)

Table A3: Instantaneous pressure change intensities recorded by 3 hydrophones

Shot #	Distance of Charge from Cage (m)	Ice thickness (m)	Total Water Depth (m)	Charge Burial Depth (m)	Charge size (g)	Hydrophone 1 IPC reading (kPa)	Hydrophone 2 IPC reading (kPa)	Hydrophone 3 IPC reading (kPa)	Average of the 3 Hydrophone Readings
1	***1.5***	***1.90***	***6.8***	***2***	***240***	***272***	***251***	***270***	***264***
2	3.0	1.90	6.8	2	240	282	284	275	280
3	6.0	1.88	6.8	2	120	71	69	70	69
4	6.0	1.88	6.8	2	60	105	104	107	105
5	3.0	1.90	6.8	3	60	62	65	65	64
6	1.5	1.90	6.8	2	180	233	242	242	239
7	1.5	1.90	6.8	2	120	223	227	234	228

Table A4: Toluene-dehydration schedule for tissue samples examined histologically.

Schedule	Solutions
1 hr	Ethanol 70%
1 hr	Ethanol 85%
1 hr	95% Ethanol
1 hr	95% Ethanol
1 hr	95% Ethanol
1 hr	100% Ethanol
1 hr	100% Ethanol
1 hr	Toluene
1 hr	Toluene
30 min	Tissue Prep II Paraffin
30 min	Tissue Prep II Paraffin
30 min	Tissue Prep II Paraffin

Table A5: Cranial measurements of eyed rainbow trout eggs exposed to instantaneous pressure changes of varying intensity.

Exposure Group	Fish #	Inter-Orbital (mm)	Outside Orbital (mm)	Mid-Orbital to Cranial Peak (mm)	Area of Upper Cranium (mm)	Circumference (mm)	S Factor
Control	1	0.59	1.94	1.20	1.20	4.86	0.64
	2	0.66	1.98	0.95	1.03	4.80	0.56
	3	0.58	1.99	1.03	0.92	4.19	0.66
	4	0.67	1.99	1.24	1.29	4.88	0.68
	5	0.75	1.88	1.22	1.36	4.92	0.71
	6	0.48	1.73	1.16	0.91	4.65	0.53
	7	0.63	1.87	1.27	1.28	4.95	0.66
	8	0.55	1.98	1.16	1.22	4.68	0.64
	9	0.52	1.71	1.19	1.10	4.75	0.61
	10	0.66	1.87	1.16	1.24	4.79	0.68
	n	**10**	**10**	**10**	**10**	**10**	**10**
	Averages	**1.46**	**2.63**	**1.16**	**1.16**	**4.75**	**0.64**
	Standard Error	**0.03**	**0.03**	**0.03**	**0.05**	**0.07**	**0.02**
64 kPa	1	0.58	1.78	1.24	1.13	4.37	0.74
	2	0.62	1.96	1.05	0.91	4.32	0.61
	3	0.58	1.82	1.08	0.95	4.25	0.66
	4	0.66	1.92	1.10	0.92	3.98	0.73
	5	0.56	1.80	1.23	1.10	4.42	0.71
	6	0.58	1.79	1.24	1.15	4.50	0.71
	7	0.67	1.99	1.29	1.07	4.77	0.59
	8	0.61	1.85	1.26	1.19	4.64	0.69
	9	0.64	1.89	1.14	1.12	4.78	0.62
	10	0.62	2.03	1.12	1.11	4.73	0.62
	n	**10**	**10**	**10**	**10**	**10**	**10**
	Averages	**0.61**	**1.88**	**1.18**	**1.06**	**4.48**	**0.67**
	Standard Error	**0.01**	**0.03**	**0.03**	**0.03**	**0.08**	**0.02**
105 kPa	1	0.63	1.67	1.36	1.60	5.26	0.73
	2	0.49	1.87	1.31	1.35	4.94	0.70
	3	0.58	1.87	1.31	1.36	4.92	0.71
	4	0.66	1.95	1.19	1.23	4.88	0.65
	5	0.65	1.92	1.18	1.21	4.80	0.66
	6	0.65	1.72	1.23	1.10	4.84	0.59
	7	0.64	1.94	1.11	1.01	4.64	0.59
	8	0.72	1.92	1.13	1.28	4.84	0.69
	9	0.50	2.11	1.31	1.27	5.05	0.62
	10	0.69	1.96	1.22	1.22	4.91	0.63
	n	**10**	**10**	**10**	**10**	**10**	**10**
	Averages	**0.62**	**1.89**	**1.23**	**1.26**	**4.91**	**0.66**
	Standard Error	**0.02**	**0.04**	**0.03**	**0.05**	**0.05**	**0.02**
228 kPa	1	0.71	1.93	1.13	1.08	4.45	0.69
	2	0.70	1.95	1.13	1.05	4.54	0.64
	3	0.62	1.93	1.08	0.96	4.40	0.62
	4	0.53	1.94	1.23	1.08	4.49	0.68
	5	0.74	2.05	1.05	1.03	4.51	0.64
	6	0.52	1.75	1.32	1.12	4.75	0.63
	7	0.52	1.48	1.22	0.94	4.49	0.59
	8	0.63	1.79	1.24	1.09	4.35	0.72
	9	0.76	2.15	1.09	1.30	4.86	0.69
	10	0.56	1.75	1.28	1.04	4.53	0.64
	n	**10**	**10**	**10**	**10**	**10**	**10**
	Averages	**0.63**	**1.87**	**1.18**	**1.07**	**4.54**	**0.65**
	Standard Error	**0.03**	**0.06**	**0.03**	**0.03**	**0.05**	**0.01**
280 kPa	1	0.58	1.80	1.30	1.32	4.71	0.75
	2	0.56	1.97	1.20	1.23	4.91	0.64
	3	0.76	1.89	1.23	1.46	4.93	0.76
	4	0.60	1.93	1.02	0.96	4.72	0.54
	5	0.72	2.00	1.17	1.20	4.94	0.62
	6	0.73	1.99	1.20	1.34	5.32	0.59
	7	0.62	1.69	1.09	0.87	4.07	0.66
	8	0.64	1.90	1.19	1.06	4.41	0.69
	9	0.76	2.03	1.16	1.33	5.05	0.66
	10	0.68	1.96	1.24	1.34	5.15	0.64
	n	**10**	**10**	**10**	**10**	**10**	**10**
	Averages	**0.67**	**1.92**	**1.18**	**1.21**	**4.82**	**0.65**
	Standard Error	**0.02**	**0.03**	**0.03**	**0.06**	**0.12**	**0.02**

Table A6: Summary of statistical parameters, tests and calculated values

Life Stage	Variable	Test	P Value
Eyed Eggs	Cranial width at eye midline	Kruskal-Wallis/Dunnett's	0.5704
	Eye midline to highest peak on head	Kruskal-Wallis/Dunnett's	0.5545
	Width between orbits	Kruskal-Wallis/Dunnett's	0.5704
	Area of upper cranium from eye midline	Kruskal-Wallis/Dunnett's	**0.0153***
	Head circumference	Kruskal-Wallis/Dunnett's	**0.0001***
	S Factors	Kruskal-Wallis/Dunnett's	0.9109
Sac Fry	Swimbladder Tears	Cochran-Armitage	0.7167
Juvenile	Swimbladder	Cochran-Armitage	**0.0016***
	Eye Displacement	Kruskal-Wallis/Dunnett's	**0.0179***
	Kidneys Hematuria (Single)	Cochran-Armitage with Bootstrap P-value adjustment	0.3683
	Kidneys Hematuria (Multiple)	Cochran-Armitage with Bootstrap P-value adjustment	**0.001***
	Kidneys Hemorrhaging	Cochran-Armitage with Bootstrap P-value	**0.0053***
	Liver Thrombocyte	Kruskal-Wallis/Dunnett's	0.6075
	Gills Hyperemia	Kruskal-Wallis/Dunnett's	0.0646
	Gills Hyperplasia	Kruskal-Wallis/Dunnett's	0.4361
	Gills Hemorrhaging	Kruskal-Wallis/Dunnett's	0.5045

Values with asterisks represent significant P values

Table A7: Swimbladder evaluation in rainbow trout sac fry exposed to instantaneous pressure changes of varying intensity.

Fish #	Treatment	Swimbladder Tears (0=absent; 1=present)	Treatment	Swimbladder Tears (0=absent; 1=present)	Treatment	Swimbladder Tears (0=absent; 1=present)	Treatment	Swimbladder Tears (0=absent; 1=present)	Treatment	Swimbladder Tears (0=absent; 1=present)
1	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
2	Control	0	64 kPa	0	105 kPa	1	228 kPa	0	280 kPa	0
3	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
4	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
5	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
6	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
7	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
8	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
9	Control	1	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
10	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	1
11	Control	0	64 kPa	0	105 kPa	0	228 kPa	0	280 kPa	0
12	Control	0	64 kPa	0	105 kPa	0	228 kPa	1	280 kPa	0
13	Control	0	64 kPa	N/A	105 kPa	1	228 kPa	N/A	280 kPa	0
Percentage (%) Summary		7.69%		0%		15.38%		8.33%		7.69%

Table A8: Swimbladder evaluation in juvenile rainbow trout exposed to instantaneous pressure changes of varying intensity.

Fish #	Treatment	Swimbladder Tears (0=absent; 1=present)	Treatment	Swimbladder Tears (0=absent; 1=present)	Treatment	Swimbladder Tears (0=absent; 1=present)	Treatment	Swimbladder Tears (0=absent; 1=present)
1	Control	0	69 kPa	0	239 kPa	0	280 kPa	1
2	Control	0	69 kPa	0	239 kPa	1	280 kPa	0
3	Control	0	69 kPa	0	239 kPa	1	280 kPa	0
4	Control	0	69 kPa	1	239 kPa	0	280 kPa	0
5	Control	0	69 kPa	0	239 kPa	1	280 kPa	0
6	Control	0	69 kPa	0	239 kPa	1	280 kPa	1
7	Control	0	69 kPa	0	239 kPa	0	280 kPa	0
8	Control	0	69 kPa	0	239 kPa	1	280 kPa	0
9	Control	0	69 kPa	0	239 kPa	0	280 kPa	0
10	Control	N/A	69 kPa	0	239 kPa	1	280 kPa	1
Percentage (%) Summary		0		10%		60%		30%

Table A9: Summary of ocular measurements in juvenile rainbow trout exposed to instantaneous pressure changes of varying intensity.

Treatment	Fish #	Area-Length Ratio		Average RT&LFT eye
		RT eye	LFT eye	
CB	1	0.389	0.422	0.406
CB	2	0.365	0.462	0.413
CB	3	0.461	0.421	0.441
CB	4	0.327	0.302	0.315
CB	5	0.353	0.395	0.374
CB	6	0.493	0.465	0.479
CB	7	0.345	0.345	0.345
CB	8	0.519	0.495	0.507
CB	9	0.549	0.512	0.531
Average				0.423
StErr				0.017
69	1	0.504	0.589	0.546
69	2	0.449	0.354	0.402
69	3	0.708	0.721	0.715
69	4	0.750	0.682	0.716
69	5	0.655	0.678	0.666
69	6	0.910	0.906	0.908
69	7	0.340	0.379	0.359
69	8	0.655	0.597	0.626
69	9	0.541	0.460	0.500
69	10	0.793	0.655	0.724
Average				0.616
StErr				0.037
239	1	0.386	0.414	0.400
239	2	0.253	0.278	0.266
239	3	0.437	0.372	0.404
239	4	0.428	0.346	0.387
239	5	0.412	0.414	0.413
239	6	0.629	0.517	0.573
239	7	0.428	0.409	0.419
239	8	0.575	0.572	0.573
239	9	0.782	0.703	0.742
239	10	0.387	0.403	0.395
Average				0.457
StErr				0.030
280	1	0.641	0.717	0.679
280	2	0.388	0.351	0.370
280	3	0.557	0.491	0.524
280	5	0.639	0.721	0.680
280	6	0.919	1.049	0.984
280	7	0.517	0.328	0.423
280	8	1.087	0.994	1.040
280	9	0.488	0.565	0.527
280	10	0.539	0.552	0.545
Average				0.641
StErr				0.055

Table A10: Histological data for kidney tissue of juvenile rainbow trout exposed to instantaneous pressures changes of varying intensity.

Treatment	Fish #	Slide #	Hematuria*	Hemorrhage**
CB	1	1	0	0
CB	1	2	0	0
CB	2	1	0	0
CB	2	2	0	0
CB	3	1	1	0
CB	3	2	0	0
CB	4	1	1	0
CB	4	2	0	0
CB	5	1	0	0
CB	5	2	0	0
CB	6	1	0	0
CB	6	2	0	0
CB	7	1	0	0
CB	7	2	1	0
CB	8	1	0	0
CB	8	2	0	0
CB	9	1	0	0
CB	9	2	0	0
N=9			**33.30% (Single)**	**0%**
69 kPa	1	1	0	0
69 kPa	1	2	0	0
69 kPa	2	1	0	1
69 kPa	2	2	0	1
69 kPa	3	1	0	0
69 kPa	3	2	0	0
69 kPa	4	1	0	0
69 kPa	4	2	0	0
69 kPa	5	1	0	0
69 kPa	5	2	0	0
69 kPa	6	1	0	1
69 kPa	6	2	0	1
69 kPa	7	1	0	1
69 kPa	7	2	0	1
69 kPa	8	1	0	0
69 kPa	8	2	0	0
69 kPa	9	1	0	0
69 kPa	9	2	0	0
N=9			**0%**	**33.30%**

Treatment	Fish #	Slide #	Hematuria*	Hemorrhage**
239	1	1	3	1
239	1	2	2	1
239	3	1	3	1
239	3	2	0	1
239	4	1	0	0
239	4	2	0	1
239	5	1	3	0
239	5	2	2	0
239	6	1	0	0
239	6	2	0	0
239	7	1	0	1
239	7	2	1	1
239	9	1	0	0
239	9	2	0	0
239	10	1	2	1
239	10	2	2	1
N=8			**12.50% (Single) 50.00% (Multiple)**	**62.50%**
280	1	1	1	1
280	1	2	3	1
280	2	1	0	1
280	2	2	0	1
280	3	1	0	1
280	3	2	0	0
280	4	1	0	0
280	4	2	0	0
280	5	1	1	1
280	5	2	0	1
280	6	1	0	1
280	6	2	0	1
280	7	1	0	0
280	7	2	0	0
280	8	1	0	1
280	8	2	0	0
280	9	1	1	0
280	9	2	0	0
280	10	1	0	0
280	10	2	0	0
N=10			**20% (Single) 10% (Multiple)**	**60%**

*Values represent total incidence of hematuria from 3 sections (2 fields of view each)
** Values represent total incidence of hemorrhage from 3 sections (2 fields of view each)

Table A11: Histological data for liver tissue of juvenile rainbow trout exposed to varying instantaneous pressure changes of varying intensities.

Slide # (# of total # of slides)	Thrombocyte Score (1:nul or very little; 2:intermediate; 3:extensive)	Slide # (# of total # of slides)	Thrombocyte Score (1:nul or very little; 2:intermediate; 3:extensive)	Slide # (# of total # of slides)	Thrombocyte Score (1:nul or very little; 2:intermediate; 3:extensive)	Slide # (# of total # of slides)	Thrombocyte Score (1:nul or very little; 2:intermediate; 3:extensive)
CB-1(1/2)	1	69-1(1/2)	1	239-1(1/2)	1	280-1(1/2)	2
CB-1(1/2)	1	69-1(1/2)	1	239-1(1/2)	1	280-1(1/2)	1
CB-1(2/2)	1	69-1(2/2)	2	239-1(2/2)	1	280-1(2/3)	1
CB-1(2/2)	1	69-1(2/2)	3	239-1(2/2)	1	280-1(2/3)	1
N/A	N/A	N/A	N/A	N/A	N/A	280-1(3/3)	1
N/A	N/A	N/A	N/A	N/A	N/A	280-1(3/3)	1
CB-2(1/2)	1	**69-2(1/2)**	2	**239-2(1/2)**	2	**280-2(1/2)**	1
CB-2(1/2)	2	**69-2(1/2)**	2	**239-2(1/2)**	2	**280-2(1/2)**	1
CB-2(2/2)	1	**69-2(2/2)**	2	**239-2(2/2)**	2	**280-2(2/2)**	1
CB-2(2/2)	1	**69-2(2/2)**	2	**239-2(2/2)**	1	**280-2(2/2)**	1
CB-3(1/2)	1	69-3(1/2)	1	239-3(1/2)	1	280-3(1/2)	2
CB-3(1/2)	2	69-3(1/2)	1	239-3(1/2)	2	280-3(1/2)	1
CB-3(2/2)	3	69-3(2/2)	1	239-3(2/2)	3	280-3(2/2)	1
CB-3(2/2)	3	69-3(2/2)	1	239-3(2/2)	2	280-3(2/2)	2
CB-4(1/2)	2	**69-4(1/2)**	2	**239-4(1/2)**	1	**280-4(1/2)**	3
CB-4(1/2)	2	**69-4(1/2)**	2	**239-4(1/2)**	2	**280-4(1/2)**	3
CB-4(2/2)	1	**69-4(2/2)**	3	**239-4(2/2)**	1	**280-4(2/2)**	2
CB-4(2/2)	1	**69-4(2/2)**	3	**239-4(2/2)**	2	**280-4(2/2)**	2
CB-5(1/2)	3	69-5(1/2)	1	239-5(1/2)	1	280-5(1/2)	1
CB-5(1/2)	3	69-5(1/2)	1	239-5(1/2)	1	280-5(1/2)	1
CB-5(2/2)	3	69-5(2/2)	1	239-5(2/2)	1	280-5(2/2)	1
CB-5(2/2)	2	69-5(2/2)	1	239-5(2/2)	1	280-5(2/2)	1
CB-6(1/2)	2	**69-6(1/2)**	2	**239-6(1/2)**	1	**280-6(1/2)**	1
CB-6(1/2)	2	**69-6(1/2)**	2	**239-6(1/2)**	1	**280-6(1/2)**	1
CB-6(2/2)	2	**69-6(2/2)**	2	**239-6(2/2)**	1	**280-6(2/2)**	1
CB-6(2/2)	2	**69-6(2/2)**	2	**239-6(2/2)**	1	**280-6(2/2)**	1
CB-7(1/2)	2	69-7(1/2)	2	239-7(1/2)	3	280-7(1/2)	1
CB-7(1/2)	2	69-7(1/2)	1	239-7(1/2)	3	280-7(1/2)	1
CB-7(2/2)	2	69-7(2/2)	3	239-7(2/2)	3	280-7(2/2)	2
CB-7(2/2)	2	69-7(2/2)	3	239-7(2/2)	3	280-7(2/2)	1
CB-8(1/2)	2	**69-8(1/2)**	2	**239-8(1/2)**	1	**280-8(1/1)**	2
CB-8(1/2)	2	**69-8(1/2)**	2	**239-8(1/2)**	2	**280-8(1/1)**	1
CB-8(2/2)	3	**69-8(2/2)**	3	**239-8(2/2)**	2	**280-8(2/2)**	1
CB-8(2/2)	2	**69-8(2/2)**	3	**239-8(2/2)**	2	**280-8(2/2)**	1
CB-9(1/2)	3	69-9(1/2)	2	239-9(1/2)	2	280-9(1/2)	3
CB-9(1/2)	3	69-9(1/2)	2	239-9(1/2)	2	280-9(1/2)	3
CB-9(2/2)	3	69-9(2/2)	1	239-9(2/2)	2	280-9(2/2)	3
CB-9(2/2)	3	69-9(2/2)	1	239-9(2/2)	2	280-9(2/2)	3
N/A	N/A	**69-10(1/2)**	1	**239-10(1/2)**	2	**280-10(1/2)**	3
N/A	N/A	**69-10(1/2)**	1	**239-10(1/2)**	2	**280-10(1/2)**	2
N/A	N/A	**69-10(2/2)**	1	**239-10(2/2)**	1	**280-10(2/2)**	3
N/A	N/A	**69-10(2/2)**	2	**239-10(2/2)**	1	**280-10(2/2)**	3

N.B: 1 = nil or very few thrombocytes (1-4 congregations), 2 = an moderate numbers of thrombocytes (5-10 congregations), 3 = extensive thromobocytes (>10 congregations). Congregations were defined as groupings of 12-15 thrombocytes.

Table A12: Hyperemia in gill tissue of juvenile rainbow trout exposed to instantaneous pressure changes of varying intensity.

Slide #	Presence of Hyperemia	Slide #	Presence of Hyperemia	Slide #	Presence of Hyperemia	Slide #	Presence of Hyperemia
CB-1(1/2)	X	**69-1(1/2)**	X	**239-1(1/2)**	√	**280-1(1/2)**	X
CB-1(1/2)	X	**69-1(1/2)**	X	**239-1(1/2)**	X	**280-1(1/2)**	X
CB-1(2/2)	X	**69-1(2/2)**	X	**239-1(2/2)**	√	**280-1(2/2)**	√ (slight)
CB-1(2/2)	X	**69-1(2/2)**	X	**239-1(2/2)**	√	**280-1(2/2)**	X
% Present	0		0		75%		25%
CB-2(1/2)	X	69-2(1/2)	√	239-2(1/2)	√	280-2(1/2)	X
CB-2(1/2)	X	69-2(1/2)	X	239-2(1/2)	√	280-2(1/2)	X
CB-2(2/2)	X	69-2(2/2)	X	239-2(2/2)	X	280-2(2/2)	X
CB-2(2/2)	√	69-2(2/2)	X	239-2(2/2)	X	280-2(2/2)	X
% Present	25%		25%		50%		0
CB-3(1/2)	X	**69-3(1/2)**	X	**239-3(1/2)**	X	**280-3(1/2)**	X
CB-3(1/2)	X	**69-3(1/2)**	X	**239-3(1/2)**	X	**280-3(1/2)**	√
CB-3(2/2)	X	**69-3(2/2)**	X	**239-3(2/2)**	X	**280-3(2/2)**	X
CB-3(2/2)	X	**69-3(2/2)**	X	**239-3(2/2)**	X	**280-3(2/2)**	X
% Present	0		0		0		25%
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	X	280-4(1/2)	√
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	X	280-4(1/2)	√
CB-4(2/2)	X	69-4(2/2)	X	239-4(2/2)	X	280-4(2/2)	√
CB-4(2/2)	X	69-4(2/2)	X	239-4(2/2)	X	280-4(2/2)	X
% Present	0		0		0		75%
CB5(1/2)	X	**69-5(1/2)**	X	**239-5(1/2)**	X	**280-5(1/2)**	X
CB-5(1/2)	√	**69-5(1/2)**	X	**239-5(1/2)**	X	**280-5(1/2)**	X
CB-5(2/2)	X	**69-5(2/2)**	X	**239-5(2/2)**	X	**280-5(2/2)**	√
CB-5(2/2)	X	**69-5(2/2)**	√	**239-5(2/2)**	X	**280-5(2/2)**	√
% Present	25%		25%		0		50%
CB-6(1/2)	X	69-6(1/2)	√	239-6(1/2)	X	280-6(1/2)	X
CB-6(1/2)	X	69-6(1/2)	X	239-6(1/2)	X	280-6(1/2)	X
CB-6(2/2)	X	69-6(2/2)	X	239-6(2/2)	X	280-6(2/2)	√
CB-6(2/2)	X	69-6(2/2)	X	239-6(2/2)	X	280-6(2/2)	X
% Present	0		25%		0		25%
CB-7(1/2)	√	**69-7(1/2)**	X	**239-7(1/2)**	X	**280-7(1/2)**	X
CB-7(1/2)	X	**69-7(1/2)**	X	**239-7(1/2)**	X	**280-7(1/2)**	X
CB-7(2/2)	√	**69-7(2/2)**	X	**239-7(2/2)**	X	**280-7(2/2)**	X
CB-7(2/2)	√	**69-7(2/2)**	X	**239-7(2/2)**	X	**280-7(2/2)**	√
% Present	75%		0		0		25%
CB-8(1/2)	X	69-8(1/2)	X	239-8(1/2)	X	280-8(1/2)	X
CB-8(1/2)	X	69-8(1/2)	X	239-8(1/2)	X	280-8(1/2)	X
CB-8(2/2)	X	69-8(2/2)	X	239-8(2/2)	X	280-8(2/2)	X
CB-8(2/2)	X	69-8(2/2)	X	239-8(2/2)	X	280-8(2/2)	X
% Present	0		0		0		0
CB-9(1/2)	X	**69-9(1/2)**	X	**239-9(1/2)**	X	**280-9 (1/2)**	X
CB-9(1/2)	X	**69-9(1/2)**	X	**239-9(1/2)**	X	**280-9 (1/2)**	X
CB-9(2/2)	X	**69-9(2/2)**	X	**239-9(2/2)**	X	**280-9(2/2)**	X
CB-9(2/2)	X	**69-9(2/2)**	X	**239-9(2/2)**	X	**280-9(2/2)**	√
% Present	0		0		0		25%
N/A	**N/A**	69-10(1/3)	X	239-10(1/2)	X	280-10(1/2)	√
N/A	**N/A**	69-10(1/3)	X	239-10(1/2)	X	280-10(1/2)	X
N/A	**N/A**	69-10(2/3)	X	239-10(2/2)	X	280-10(2/2)	X
N/A	**N/A**	69-10(2/3)	X	239-10(2/2)	X	280-10(2/2)	X
N/A	**N/A**	69-10(3/3)	X	N/A	N/A	N/A	N/A
N/A	**N/A**	69-10(3/3)	X	N/A	N/A	N/A	N/A
% Present	**N/A**		0		0		25%
Mean Percentage (%) Summary	**13.9%**		**7.5%**		**12.5%**		**27.5%**

Table A13: Hemorrhage in gill tissue of juvenile rainbow trout exposed to instantaneous pressure changes of varying intensity.

Slide #	Presence of Hemorrhage	Slide #	Presence of Hemorrhage	Slide #	Presence of Hemorrhage	Slide #	Presence of Hemorrhage
CB-1(1/2)	X	**69-1(1/2)**	X	**239-1(1/2)**	X	**280-1(1/2)**	X
CB-1(1/2)	X	**69-1(1/2)**	X	**239-1(1/2)**	X	**280-1(1/2)**	X
CB-1(2/2)	X	**69-1(2/2)**	X	**239-1(2/2)**	X	**280-1(2/2)**	X
CB-1(2/2)	X	**69-1(2/2)**	X	**239-1(2/2)**	X	**280-1(2/2)**	X
% Present	0		0		0		0
CB-2(1/2)	X	69-2(1/2)	X	239-2(1/2)	√	280-2(1/2)	X
CB-2(1/2)	X	69-2(1/2)	X	239-2(1/2)	X	280-2(1/2)	X
CB-2(2/2)	X	69-2(2/2)	X	239-2(2/2)	√	280-2(2/2)	X
CB-2(2/2)	X	69-2(2/2)	X	239-2(2/2)	X	280-2(2/2)	X
% Present	0		0		50%		0
CB-3(1/2)	X	**69-3(1/2)**	X	**239-3(1/2)**	X	**280-3(1/2)**	X
CB-3(1/2)	X	**69-3(1/2)**	X	**239-3(1/2)**	X	**280-3(1/2)**	X
CB-3(2/2)	X	**69-3(2/2)**	X	**239-3(2/2)**	X	**280-3(2/2)**	X
CB-3(2/2)	X	**69-3(2/2)**	X	**239-3(2/2)**	X	**280-3(2/2)**	X
% Present	0		0		0		0
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	X	280-4(1/2)	X
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	X	280-4(1/2)	X
CB-4(2/2)	X	69-4(2/2)	X	239-4(2/2)	X	280-4(2/2)	X
CB-4(2/2)	X	69-4(2/2)	X	239-4(2/2)	X	280-4(2/2)	X
% Present	0		0		0		0
CB5(1/2)	X	**69-5(1/2)**	X	**239-5(1/2)**	X	**280-5(1/2)**	X
CB-5(1/2)	X	**69-5(1/2)**	X	**239-5(1/2)**	X	**280-5(1/2)**	X
CB-5(2/2)	X	**69-5(2/2)**	X	**239-5(2/2)**	X	**280-5(2/2)**	X
CB-5(2/2)	X	**69-5(2/2)**	X	**239-5(2/2)**	X	**280-5(2/2)**	X
% Present	0		0		0		0
CB-6(1/2)	X	69-6(1/2)	X	239-6(1/2)	X	280-6(1/2)	X
CB-6(1/2)	X	69-6(1/2)	X	239-6(1/2)	X	280-6(1/2)	√
CB-6(2/2)	X	69-6(2/2)	X	239-6(2/2)	X	280-6(2/2)	X
CB-6(2/2)	X	69-6(2/2)	X	239-6(2/2)	X	280-6(2/2)	X
% Present	0		0		0		25%
CB-7(1/2)	X	**69-7(1/2)**	X	**239-7(1/2)**	X	**280-7(1/2)**	X
CB-7(1/2)	X	**69-7(1/2)**	X	**239-7(1/2)**	X	**280-7(1/2)**	X
CB-7(2/2)	X	**69-7(2/2)**	X	**239-7(2/2)**	X	**280-7(2/2)**	X
CB-7(2/2)	X	**69-7(2/2)**	X	**239-7(2/2)**	X	**280-7(2/2)**	X
% Present	0		0		0		0
CB-8(1/2)	X	69-8(1/2)	X	239-8(1/2)	√	280-8(1/2)	√ (slight)
CB-8(1/2)	X	69-8(1/2)	X	239-8(1/2)	X	280-8(1/2)	√ (slight)
CB-8(2/2)	X	69-8(2/2)	X	239-8(2/2)	X	280-8(2/2)	X
CB-8(2/2)	X	69-8(2/2)	X	239-8(2/2)	X	280-8(2/2)	X
% Present	0		0		25%		50%
CB-9(1/2)	X	**69-9(1/2)**	X	**239-9(1/2)**	X	**280-9 (1/2)**	X
CB-9(1/2)	√	**69-9(1/2)**	X	**239-9(1/2)**	X	**280-9 (1/2)**	X
CB-9(2/2)	X	**69-9(2/2)**	X	**239-9(2/2)**	X	**280-9(2/2)**	X
CB-9(2/2)	X	**69-9(2/2)**	X	**239-9(2/2)**	X	**280-9(2/2)**	X
% Present	25%		0		0		0
N/A	**N/A**	69-10(1/3)	X	239-10(1/2)	X	280-10(1/2)	X
N/A	**N/A**	69-10(1/3)	X	239-10(1/2)	X	280-10(1/2)	X
N/A	**N/A**	69-10(2/3)	X	239-10(2/2)	X	280-10(2/2)	X
N/A	**N/A**	69-10(2/3)	X	239-10(2/2)	X	280-10(2/2)	X
N/A	**N/A**	69-10(3/3)	X	N/A	N/A	N/A	N/A
N/A	**N/A**	69-10(3/3)	X	N/A	N/A	N/A	N/A
% Present	**N/A**		0		0		0
Mean Percentage (%) Summary	**2.8%**		**0.0%**		**7.5%**		**7.5%**

Table A14: Thrombocyte presence in gill tissue of juvenile rainbow trout exposed to instantaneous pressure changes of varying intensity.

Slide #	Presence of Thrombocytes	Slide #	Presence of Thrombocytes	Slide #	Presence of Thrombocytes	Slide #	Presence of Thrombocytes
CB-1(1/2)	×	**69-1(1/2)**	×	**239-1(1/2)**	×	**280-1(1/2)**	×
CB-1(1/2)	×	**69-1(1/2)**	×	**239-1(1/2)**	×	**280-1(1/2)**	×
CB-1(2/2)	×	**69-1(2/2)**	×	**239-1(2/2)**	×	**280-1(2/2)**	√
CB-1(2/2)	×	**69-1(2/2)**	×	**239-1(2/2)**	×	**280-1(2/2)**	√
% Present	0		0		0		50%
CB-2(1/2)	×	69-2(1/2)	×	239-2(1/2)	√	280-2(1/2)	×
CB-2(1/2)	×	69-2(1/2)	×	239-2(1/2)	×	280-2(1/2)	×
CB-2(2/2)	×	69-2(2/2)	√	239-2(2/2)	×	280-2(2/2)	×
CB-2(2/2)	×	69-2(2/2)	×	239-2(2/2)	×	280-2(2/2)	×
% Present	0		25%		25%		0
CB-3(1/2)	×	**69-3(1/2)**	×	**239-3(1/2)**	×	**280-3(1/2)**	×
CB-3(1/2)	×	**69-3(1/2)**	√	**239-3(1/2)**	×	**280-3(1/2)**	×
CB-3(2/2)	√	**69-3(2/2)**	×	**239-3(2/2)**	×	**280-3(2/2)**	×
CB-3(2/2)	×	**69-3(2/2)**	×	**239-3(2/2)**	×	**280-3(2/2)**	×
% Present	25%		25%		0		0
CB-4(1/2)	×	69-4(1/2)	×	239-4(1/2)	√	280-4(1/2)	×
CB-4(1/2)	×	69-4(1/2)	√	239-4(1/2)	×	280-4(1/2)	×
CB-4(2/2)	×	69-4(2/2)	×	239-4(2/2)	√	280-4(2/2)	×
CB-4(2/2)	×	69-4(2/2)	×	239-4(2/2)	×	280-4(2/2)	×
% Present	0		25%		50%		0
CB5(1/2)	×	**69-5(1/2)**	×	**239-5(1/2)**	√	**280-5(1/2)**	×
CB-5(1/2)	×	**69-5(1/2)**	×	**239-5(1/2)**	×	**280-5(1/2)**	×
CB-5(2/2)	×	**69-5(2/2)**	×	**239-5(2/2)**	×	**280-5(2/2)**	×
CB-5(2/2)	×	**69-5(2/2)**	×	**239-5(2/2)**	×	**280-5(2/2)**	×
% Present	0		0		25%		0
CB-6(1/2)	√	69-6(1/2)	×	239-6(1/2)	×	280-6(1/2)	×
CB-6(1/2)	×	69-6(1/2)	×	239-6(1/2)	×	280-6(1/2)	×
CB-6(2/2)	×	69-6(2/2)	×	239-6(2/2)	×	280-6(2/2)	√
CB-6(2/2)	×	69-6(2/2)	×	239-6(2/2)	×	280-6(2/2)	×
% Present	25%		0		0		25%
CB-7(1/2)	×	**69-7(1/2)**	×	**239-7(1/2)**	×	**280-7(1/2)**	×
CB-7(1/2)	×	**69-7(1/2)**	×	**239-7(1/2)**	×	**280-7(1/2)**	×
CB-7(2/2)	×	**69-7(2/2)**	×	**239-7(2/2)**	×	**280-7(2/2)**	×
CB-7(2/2)	×	**69-7(2/2)**	×	**239-7(2/2)**	×	**280-7(2/2)**	×
% Present	0		0		0		0
CB-8(1/2)	×	69-8(1/2)	×	239-8(1/2)	×	280-8(1/2)	×
CB-8(1/2)	×	69-8(1/2)	×	239-8(1/2)	×	280-8(1/2)	×
CB-8(2/2)	×	69-8(2/2)	√	239-8(2/2)	×	280-8(2/2)	×
CB-8(2/2)	×	69-8(2/2)	√	239-8(2/2)	×	280-8(2/2)	×
% Present	0		50%		0		0
CB-9(1/2)	×	**69-9(1/2)**	×	**239-9(1/2)**	×	**280-9 (1/2)**	×
CB-9(1/2)	×	**69-9(1/2)**	×	**239-9(1/2)**	×	**280-9 (1/2)**	×
CB-9(2/2)	×	**69-9(2/2)**	×	**239-9(2/2)**	×	**280-9(2/2)**	×
CB-9(2/2)	×	**69-9(2/2)**	×	**239-9(2/2)**	×	**280-9(2/2)**	×
% Present	0		0		0		0
N/A	N/A	69-10(1/3)	×	239-10(1/2)	×	280-10(1/2)	×
N/A	N/A	69-10(1/3)	×	239-10(1/2)	×	280-10(1/2)	×
N/A	N/A	69-10(2/3)	×	239-10(2/2)	×	280-10(2/2)	×
N/A	N/A	69-10(2/3)	×	239-10(2/2)	×	280-10(2/2)	×
N/A	N/A	69-10(3/3)	×	N/A	N/A	N/A	N/A
N/A	N/A	69-10(3/3)	×	N/A	N/A	N/A	N/A
% Present	N/A		0		0		0
Mean Percentage (%) Summary	5.6%		12.5%		10.0%		7.5%

Table A15: General target pressure levels and rationale

Target Pressure	Rationale
0	Control
17.5 kPa	Other jurisdictions are more restrictive on the use of explosive based seismic activity in fish bearing waters. For example, in Alaska seismic based exploration is generally not allowed. Where it is permitted the IPC has to remain below 17.5kPa. This level was chosen as it is the most restrictive level currently allowed.
50kPa	Based on a current DFO Western Arctic Area recommendation that the maximum peak pressure never exceed 50kPa
100kPa	Tests the current DFO guideline for the use of explosives in or near Canadian fisheries waters
280kPa	Serves as a contrast level to the control

Table A16: Summary of clinical biochemistry in plasma

Liver
Aspartate Aminotransferase (AST)
Alanine Amintotransferate (ALT)
Muscle
Creatine Kinase (CK)
AST
Kidney
AST
ALT
Creatinine
Phosphorus

Table A17: Toluene-dehydration schedule for tissue samples examined histologically.

Schedule	Solutions	Temperature
1hr	70% Ethanol	-
1hr	85% Ethanol	-
1hr	95% Ethanol	-
1hr	95% Ethanol	-
1hr	95% Ethanol	-
1hr	100% Ethanol	-
1hr	100% Ethanol	-
1hr	100% Ethanol	37
1hr	Toluene	37
1hr	Toluene	37
30 min	Tissue Prep II Paraffin	59
30 min	Tissue Prep II Paraffin	59
30 min	Tissue Prep II Paraffin	59
30 min	Tissue Prep II Paraffin	59

Table A18: Assessment Parameters for Traumatic Based Pathologies

Traumatic Based Injury	Description	Pathological Identification	Assessment Method
Liver			
a) Evaluation of Interstices and Vessels			
Hemorrhage	Hemorrhaging is the escape of blood from blood vessels (Neufeldt and Guralnik 1991) and is often associated with the escape of blood due to vessel rupture. Such rupture can occur from vascular injury as a result of trauma (Mitchell 2005).	Identified as present in any area of the tissue proper (interstices in the case of the kidney) exhibiting rupture or dissuption of vessel walls. Hemorrhage was denoted as present when an accumulation of blood was found not bordered by vascular structure.	Thirty HPFs were examined within the liver tissue of each fish. Within each HPF the incidence of hemorrhage was recorded as either present or absent. Subsequently, for each fish, the percentage of how often hemorrhage was observed within the liver was calculated. The percentages were then averaged for each respective exposure group.
Congestion	Congestion is a passive process resulting from impaired outflow from a tissue (Mitchell 2005). It is associated with systematic events such as cardiac failure, or local events due to venous obstruction (Mitchell 2005). To note, the terms hyperaemia (an active process that results from augmented tissue inflow from arteriolar dilation (Mitchell 2005)) and congestion both refer to a localized increase in blood volume within a tissue (Mitchell 2005). While it is not possible to distinguish between hyperemia and congestion within histological tissue preparations, areas showing an increased number of RBC's within an enclosed structure (i.e vasculature, glomerulus) as compared to controls, will be noted as present for congestion.	Identified as present in areas showing an increased number of RBC's within an enclosed structure (i.e vasculature, renal corpuscle) as compared to controls.	Thirty HPFs were examined within the liver tissue of each fish. Within each HPF the incidence of congestion was recorded as either present or absent. Subsequently, for each fish, the percentage of how often congestion was observed within the liver was calculated. The percentages were then averaged for each respective exposure group.
b) Evaluation of Sinusoids			
Sinusoid Damage	Sinusoids are minute endothelium-lined spaces or passages for blood in the tissues of an organ, such as the liver (Sinusoids, 2003). In the current study sinusoid damage represents disruption to sinusoidal structure.	Identified as present in areas exhbiting disruption of sinusoidal walls.	Thirty HPFs were examined within the liver tissue of each fish. Within each HPF the incidence of sinusoid damage was recorded as either present or absent. Subsequently, for each fish, the percentage of how often sinusoid damage was observed within the liver was calculated. The percentages were then averaged for each respective exposure group.

Traumatic Based Injury	Description	Pathological Identification	Assessment Method
Spleen			
Evaluation of Interstices and Vessels			
Hemorrhage			
	As Described Above	As Described Above	As described above, with the exception that assessment was within splenic tissue
Congestion			
	As Described Above	As Described Above	As described above, with the exception that assessment was within splenic tissue
Intestine			
a) Evaluation of Interstices and Vessels			
Hemorrhage			
	As Described Above	As Described Above	As described above, with the exception that assessment was within intestinal tissue
Congestion			
	As Described Above	As Described Above	As described above, with the exception that assessment was within intestinal tissue
b) Evaluation of Intestinal Lumen			
Hemorrhage into Intestinal Lumen	With trauma there is the potential for hemorrhaging into the intestinal lumen from damaged blood vessels.	Identified as present when erythrocytes were found within intestinal lumen.	Six transverse sections were examined for each fish. Erythrocytes were identified and counted within theluminal cross section and the total number of erythrocytes was calculated. Totals were averaged for each exposure group. Hemorrhage into the intestinal lumen was not reported in sculpin due to logistical and processing difficulties.

Traumatic Based Injury	Description	Pathological Identification	Assessment Method
Kidney			
a) Evaluation of Glomeruli			
Glomerular Congestion	The glomerulus is a tuft of capillaries that is covered by epithelium. It is situated at the point of origin of vertebrate nephrons (Glomerulus 2003). In the current study, glomerular congestion refers to localized increase in blood volume within the glomerular capillaries.	Identified by noting the number of erythrocytes within each glomerulus	Thirty HPFs were examined within the kidney tissue of each fish. Within each field, erythrocytes were identified and counted within each glomerulus. The average number of erythrocytes within the glomeruli were calculated for each fish. Subsequently, the mean value of these averages were calculated.
RBCs within the Bowman's Space	The renal corpuscle is made up of the glomerulus and its surrounding Bowmans's capsule (Renal Corpuscle 2003). In between these two structures lies the Bowman's space. In the current study it is thought that erythrocytes within the Bowman's space would be due to the breakage of glomerular capillaries.	Identified as present by noting if any erythrocytes were found within the Bowman's space.	Thirty HPFs were examined within the kidney tissue of each fish. Within each field, erythrocytes within the Bowman's space were noted as either present or absent. Subsequently, for each fish, the percentage of how often RBC within the Bowman's space was observed within the kidney was calculated. The percentages were then averaged for each respective exposure group.
b) Evaluation of Interstices and Vessels			
Hemorrhage	As Described Above	As Described Above	As described above, with the exception that assessment was within kidney tissue
Erythrophagia	The consumption of red blood cells by macrophages and sometimes other phagocytes (Erythrophagocytosis 2003).	Identified as pink (eosinophilic) entities that are somewhat gelatinous in appearance. The pink within these entities represents RBC debris inside of a macrophage that it has engulfed and encased.	Thirty HPFs were examined within the kidney tissue of each fish. Within each field erythrophages were counted. Subsequently, for each fish, the total number of erythrophages was calculated. The totals were then averaged for each respective exposure group.
Congestion	As Described Above	As Described Above	As described above, with the exception that assessment was within kidney tissue
b) Evaluation of Renal Tubules			
Frequency of RBCs in Tubules (Hematuria)	Hematuria is the presence of RBCs in urine (Neufeldt and Guralnik 1991).	Identified by noting the number of RBCs within the lumen of kidney tubules. RBC found within tubule lumens were counted for comparison to controls.	Thirty HPFs were examined within the kidney tissue of each fish. Within each field, the presence or absence of erythrocytes within kidney lumens was noted. Subsequently, for each fish, the percentage of how often RBC within the luminal space was observed within the kidney was calculated. The percentages were then averaged for each respective exposure group.

Table A19: Summary of variables examined histopathologically

Liver
Hemmorhage
Abnormal Congestion
Sinusoid Damage
Spleen (Lake Trout only)
Hemmorhage
Abnormal Congestion
Intestine
Hemmorhage
Abnormal Congestion
Erythrocytes within Intestinal Lumen
Kidney
Hemmorhage
Abnormal Congestion
Erythrocytes within Bowman's Space
Erythrocytes within Renal Tubules
Glomerular Congestion
Erythrophagia

Table A20: Instantaneous pressure change intensities recorded by 2-3 hydrophones in Lake 382 at the Experimental Lakes Area, Ontario

	Hydrophone 1 (kPa)	Hydrophone 2 (kPa)	Hydrophone 3 (kPa)	Average of hydrophone data (kPa)
Control	0.49	0.66	0.99	0.71
Blast 1	74.60	75.50	64.60	71.57
Blast 2	55.70	N/A	62.80	59.25
Blast 3	57.90	N/A	55.90	56.90
Blast 4	29.40	N/A	37.30	33.35
Blast 5	115.00	N/A	138.00	126.50

Table A21: Summary of statistical parameters, tests and calculated values

Parameter	Test	P-Value Lake Trout	P-Value Sculpin
Length			
Fork Length	ANOVA	0.0394*	0.4197
Behavioral Pathology			
Anecdotal Observation	N/A	N/A	N/A
Gross Pathology			
Swimbladder	Fisher's Exact	0.0008*	N/A
Eye	Kruskall-Wallis/Dunnett's	0.047*	0.6237
Blood Pathology			
AST	Kruskall-Wallis/Dunnett's	0.584	N/A
ALT	Kruskall-Wallis/Dunnett's	0.4288	N/A
CK	Kruskall-Wallis/Dunnett's	0.1195	N/A
BUN	Kruskall-Wallis/Dunnett's	N/A**	N/A
Creatinine	Kruskall-Wallis/Dunnett's	0.1365	N/A
Phosphorus	Kruskall-Wallis/Dunnett's	0.8529	N/A
Tissue Pathology			
Liver			
Hemorrhage	Kruskall-Wallis/Dunnett's	0.4389	N/A
Abnormal congestion	Kruskall-Wallis/Dunnett's	N/A	N/A
Sinusoid damage	Kruskall-Wallis/Dunnett's	0.7003	N/A
Spleen			
Hemorrhage	Kruskall-Wallis/Dunnett's	0.3164	N/A
Abnormal congestion	Kruskall-Wallis/Dunnett's	N/A	N/A
Intestine			
Hemorrhage	Kruskall-Wallis/Dunnett's	N/A	N/A
RBCs in lumen	Kruskall-Wallis/Dunnett's	0.2678	N/A
Abnormal congestion	Kruskall-Wallis/Dunnett's	N/A	N/A
Kidney			
RBCs-Bowman's capsule	Kruskall-Wallis/Dunnett's	0.4389	N/A
Congestion in Glomerulus	Kruskall-Wallis/Dunnett's	0.2651	0.1139
Hemorrhage	Kruskall-Wallis/Dunnett's	0.4317	0.1778
RBCs within tubules	Kruskall-Wallis/Dunnett's	0.0904	0.8439
Abnormal congestion	Kruskall-Wallis/Dunnett's	N/A	N/A
Erythrophagia	Kruskall-Wallis/Dunnett's	0.7798	0.8678

*Represents significant values

**Below Detection Limit

N/A: either variable was not assessed or no data were available for analysis (i.e values were 0)

Table A22: Summary of gross pathological findings of swimbladder in lake trout

Swimbladder Gross Pathology Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
ID #	Damage Level (0=no damage) (1=burst)	ID #	Damage Level (0=no damage) (1=burst)	ID #	Damage Level (0=no damage) (1=burst)	ID #	Damage Level (0=no damage) (1=burst)	ID #	Damage Level (0=no damage) (1=burst)	ID #	Damage Level (0=no damage) (1=burst)
LT1-Control	0	LT1-33	0	LT1-57	0	LT1-59	0	LT1-72	0	LT1-127	1
LT2-Control	0	LT2-33	0	LT2-57	0	LT2-59	0	LT2-72	0	LT2-127	1
LT3-Control	0	LT3-33	0	LT3-57	0	LT3-59	0	LT3-72	0	LT3-127	0
LT4-Control	0	LT4-33	0	LT4-57	0	LT4-59	0	LT4-72	0	LT4-127	1
LT5-Control	0	LT5-33	0	LT5-57	0	LT5-59	0	LT5-72	0	LT5-127	N/A
Average %	**0**	**Average %**	**0**	**Average %**	**0**	**Average %**	**0**	**Average %**	**0**	**Average %**	**75%**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**4**

Table A23: Summary of eye measurements in lake trout

Exopthalmia Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length
LTCon_1	0.09	LT33_1	0.09	LT57_1	0.09	LT59_1	0.09	LT72_1	0.09	LT127_1	0.09
LTCon_2	0.09	LT33_2	0.09	LT57_2	0.09	LT59_2	0.09	LT72_2	0.09	LT127_2	0.08
LTCon_3	0.09	LT33_3	0.09	LT57_3	0.09	LT59_3	0.09	LT72_3	0.09	LT127_3	0.09
LTCon_4	0.09	LT33_4	0.09	LT57_4	0.09	LT59_4	0.09	LT72_4	0.09	LT127_4	0.09
LTCon_5	0.09	LT33_5	0.09	LT57_5	0.09	LT59_5	0.10	LT72_5	0.09	LT127_5	0.10
Average	**0.09**	**Average**	**0.09**	**Average**	**0.09**	**Average**	**0.09**	**Average**	**0.09**	**Average**	**0.09**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A24: Summary of eye measurements in sculpin

Exopthalmia Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length		Eye Measure/ Fork length
ScCon_1	0.11	Sc33_1	0.10	Sc57_1	0.11	Sc59_1	0.11	Sc72_1	0.11	Sc127_1	0.11
ScCon_2	0.11	Sc33_2	0.11	Sc57_2	0.11	Sc59_2	0.11	Sc72_2	0.11	Sc127_2	0.11
ScCon_3	0.11	Sc33_3	0.11	Sc57_3	0.11	Sc59_3	0.11	Sc72_3	0.11	Sc127_3	0.11
ScCon_4	0.10	Sc33_4	0.11	Sc57_4	0.12	Sc59_4	0.11	Sc72_4	0.11	Sc127_4	0.12
ScCon_5	0.11	Sc33_5	0.11	Sc57_5	0.11	Sc59_5	0.11	Sc72_5	0.11	Sc127_5	0.11
Average	**0.11**	**Average**	**0.11**	**Average**	**0.11**	**Average**	**0.11**	**Average**	**0.11**	**Average**	**0.11**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	5	**n**	**5**	**n**	5	**n**	**5**	**n**	5	**n**	5

Table A25: Summary of hemorrhage in the liver of lake trout

Liver Hemorrhage Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Interstices		**Interstices**		**Interstices**		**Interstices**		**Interstices**		**Interstices**
	Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	3.33	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0.67**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.67**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A26: Summary of congestion in the liver of the lake trout

Liver Congestion Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Vessels		**Vessels**		**Vessels**		**Vessels**		**Vessels**		**Vessels**
	Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A27: Summary of sinusoid damage in the liver of the lake trout

Liver Sinusoid Damage Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Sinusoids		**Sinusoids**		**Sinusoids**		**Sinusoids**		**Sinusoids**		**Sinusoids**
	Damage (%)		Damage (%)		Damage (%)		Damage (%)		Damage (%)		Damage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	3.33	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	3.33	**57-5**	0	**59-5**	3.33	**72-5**	0	**127-5**	0
Average (%)	**0.67**	**Average (%)**	**0.67**	**Average (%)**	**0**	**Average (%)**	**0.67**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.67**	**StErr**	**0.67**	**StErr**	**0.00**	**StErr**	**0.67**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A28: Summary of hemorrhage in the liver of sculpin

Liver Hemorrhage Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Interstices		**Interstices**		**Interstices**		**Interstices**		**Interstices**		**Interstices**
	Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A29: Summary of congestion in the liver of sculpin

Liver Congestion Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Vessels		**Vessels**		**Vessels**		**Vessels**		**Vessels**		**Vessels**
	Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A30: Summary of sinusoid damage in the liver of sculpin

Liver Sinusoid Damage Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Sinusoids		**Sinusoids**		**Sinusoids**		**Sinusoids**		**Sinusoids**		**Sinusoids**
	Damage (%)		Damage (%)		Damage (%)		Damage (%)		Damage (%)		Damage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A31: Summary of hemorrhage in spleens of lake trout

Spleen Hemorrhage Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Interstices		**Interstices**		**Interstices**		**Interstices**		**Interstices**		**Interstices**
	Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	10	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	6.67	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	N/A	**59-4**	0	**72-4**	0	**127-4**	3.33
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	6.67	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**1.67**	**Average (%)**	**3.33**	**Average (%)**	**0**	**Average (%)**	**0.67**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**1.67**	**StErr**	**2.11**	**StErr**	**0.00**	**StErr**	**0.67**
n	**5**	**n**	**5**	**n**	**4**	**n**	**5**	**n**	**5**	**n**	**5**

Table A32: Summary of congestion in spleens of lake trout

Spleen Congestion Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Vessels		**Vessels**		**Vessels**		**Vessels**		**Vessels**		**Vessels**
	Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	N/A	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**4**	**n**	**5**	**n**	**5**	**n**	**5**

Table A33: Summary of hemorrhage in intestinal tissue of lake trout

Intestine Hemorrhage Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Interstices		**Interstices**		**Interstices**		**Interstices**		**Interstices**		**Interstices**
	Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**

Table A34: Summary of RBC counts in lumen of lake trout intestines

Intestine RBC in Lumen Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Lumen		**Lumen**		**Lumen**		**Lumen**		**Lumen**		**Lumen**
	RBCs within the Lumen (# of RBCs)		RBCs within the Lumen (# of RBCs)		RBCs within the Lumen (# of RBCs)		RBCs within the Lumen (# of RBCs)		RBCs within the Lumen (# of RBCs)		RBCs within the Lumen (# of RBCs)
Con-1	227	**33-1**	376	**57-1**	0	**59-1**	4	**72-1**	71	**127-1**	1932
Con-2	18	**33-2**	32	**57-2**	42	**59-2**	3	**72-2**	107	**127-2**	13
Con-3	0	**33-3**	5	**57-3**	2	**59-3**	1	**72-3**	2	**127-3**	1
Con-4	0	**33-4**	8	**57-4**	2	**59-4**	3	**72-4**	820	**127-4**	187
Con-5	477	**33-5**	2	**57-5**	1	**59-5**	212	**72-5**	916	**127-5**	22
Average (#)	**144.40**	**Average (#)**	**84.60**	**Average (#)**	**9.40**	**Average (#)**	**44.60**	**Average (#)**	**383.20**	**Average (#)**	**431.00**
StErr	**93.57**	**StErr**	**73.04**	**StErr**	**8.16**	**StErr**	**41.85**	**StErr**	**199.22**	**StErr**	**376.79**
n	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**

Table A35: Summary of congestion in intestinal vessels of lake trout

Intestine Congestion Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Vessels		**Vessels**		**Vessels**		**Vessels**		**Vessels**		**Vessels**
	Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**	**n**	**5.00**

Table A36: Summary of hemorrhage in intestinal tissues of sculpin

Intestine Hemorrhage Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Interstices		**Interstices**		**Interstices**		**Interstices**		**Interstices**		**Interstices**
	Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A37: Summary of congestion in intestinal vessels of sculpin

Intestine Congestion Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Vessels		**Vessels**		**Vessels**		**Vessels**		**Vessels**		**Vessels**
	Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A38: Summary of RBCs within the Bowman's capsule (specifically,the space between capsule and glomerulus) of lake trout kidneys.

Kidney Glomeruli I (RBCs within Bowman's Capsule Space) Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Glomeruli		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**
	RBCs within Bowman's Capsule Space (%)		RBCs within Bowman's Capsule Space (%)		RBCs within Bowman's Capsule Space (%)		RBCs within Bowman's Capsule Space (%)		RBCs within Bowman's Capsule Space (%)		RBCs within Bowman's Capsule Space (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0.00	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0.00	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0.00	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0.00	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	3.33	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0.67**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.67**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A39: Summary of RBCs in glomeruli of lake trout kidneys.

Kidney Glomeruli II (Congestion) Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Glomeruli		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**
	Approx. RBC count in glomeruli (average n)		Approx. RBC count in glomeruli (average n)		Approx. RBC count in glomeruli (average n)		Approx. RBC count in glomeruli (average n)		Approx. RBC count in glomeruli (average n)		Approx. RBC count in glomeruli (average n)
Con-1	5.67	**33-1**	7.56	**57-1**	5.55	**59-1**	5.11	**72-1**	7.07	**127-1**	10.77
Con-2	5.80	**33-2**	4.5	**57-2**	2.61	**59-2**	5.81	**72-2**	4.73	**127-2**	5.8
Con-3	5.79	**33-3**	4.57	**57-3**	5.28	**59-3**	1.3	**72-3**	4.42	**127-3**	5.02
Con-4	6.71	**33-4**	5.91	**57-4**	3.71	**59-4**	4.51	**72-4**	5.87	**127-4**	5.28
Con-5	2.74	**33-5**	2.71	**57-5**	2.75	**59-5**	4.06	**72-5**	3.36	**127-5**	8.28
Average (ave.n)	**5.34**	**Average (ave.n)**	**5.05**	**Average (ave.n)**	**3.98**	**Average (ave.n)**	**4.158**	**Average (ave.n)**	**5.09**	**Average (ave.n)**	**7.03**
StErr	**0.68**	**StErr**	**0.81**	**StErr**	**0.62**	**StErr**	**0.77**	**StErr**	**0.64**	**StErr**	**1.10**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A40: Summary of hemorrhage within the kidney tissue of lake trout.

Kidney Hemorrhage Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Interstices		**Interstices**		**Interstices**		**Interstices**		**Interstices**		**Interstices**
	Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)
Con-1	0	**33-1**	0	**57-1**	16.67	**59-1**	0	**72-1**	0	**127-1**	3.33
Con-2	0	**33-2**	13.33	**57-2**	0	**59-2**	0	**72-2**	10	**127-2**	20
Con-3	0	**33-3**	0	**57-3**	3.33	**59-3**	30	**72-3**	0	**127-3**	10
Con-4	6.67	**33-4**	0	**57-4**	3.33	**59-4**	3.33	**72-4**	0	**127-4**	30
Con-5	6.67	**33-5**	13.33	**57-5**	10	**59-5**	6.67	**72-5**	0	**127-5**	0
Average (%)	**2.67**	**Average (%)**	**5.332**	**Average (%)**	**6.67**	**Average (%)**	**8**	**Average (%)**	**2**	**Average (%)**	**12.666**
StErr	**1.63**	**StErr**	**3.27**	**StErr**	**2.98**	**StErr**	**5.64**	**StErr**	**2.00**	**StErr**	**5.52**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A41: Summary of RBCs within the tubules of lake trout kidneys.

Kidney RBCs within Tubules Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Tubules		**Tubules**		**Tubules**		**Tubules**		**Tubules**		**Tubules**
	Frequency of RBC within Tubules (n)		Frequency of RBC within Tubules (n)		Frequency of RBC within Tubules (n)		Frequency of RBC within Tubules (n)		Frequency of RBC within Tubules (n)		Frequency of RBC within Tubules (n)
Con-1	0	**33-1**	3.33	**57-1**	0	**59-1**	3.33	**72-1**	3.33	**127-1**	0
Con-2	0	**33-2**	3.33	**57-2**	0	**59-2**	0	**72-2**	3.33	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	6.67	**59-3**	6.67	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	3.33	**57-5**	3.33	**59-5**	3.33	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**1.998**	**Average (%)**	**2**	**Average (%)**	**2.666**	**Average (%)**	**1.332**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.82**	**StErr**	**1.33**	**StErr**	**1.25**	**StErr**	**0.82**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A42: Summary of congestion within the vessels of kidney tissues in lake trout.

Kidney Congestion Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Vessels		**Vessels**		**Vessels**		**Vessels**		**Vessels**		**Vessels**
	Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A43: Summary of the occurrence of erythrophagia by phagocytes in kidney tissues of lake trout.

Kidney Erythrophagia Lake Trout											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Erythrophagia (n)		Erythrophagia (n)		Erythrophagia (n)		Erythrophagia (n)		Erythrophagia (n)		Erythrophagia (n)
Con-1	60	**33-1**	74	**57-1**	9	**59-1**	87	**72-1**	65	**127-1**	39
Con-2	31	**33-2**	36	**57-2**	48	**59-2**	69	**72-2**	42	**127-2**	41
Con-3	41	**33-3**	20	**57-3**	89	**59-3**	20	**72-3**	23	**127-3**	35
Con-4	34	**33-4**	12	**57-4**	28	**59-4**	53	**72-4**	14	**127-4**	24
Con-5	34	**33-5**	14	**57-5**	43	**59-5**	23	**72-5**	22	**127-5**	39
Average (n)	**40**	**Average (n)**	**31.2**	**Average (n)**	**43.4**	**Average (n)**	**50.4**	**Average (n)**	**33.2**	**Average (n)**	**35.6**
StErr	**5.26**	**StErr**	**11.50**	**StErr**	**13.27**	**StErr**	**12.98**	**StErr**	**9.18**	**StErr**	**3.06**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A44: Summary of RBCs within the Bowman's capsule (in the space between capsule and glomerulus) of sculpin kidneys.

Kidney Glomeruli I (RBCs within Bowman's Capsule Space) Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Glomeruli		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**
	RBCs within Bowman's Capsule (approx.RBC count) (%)		RBCs within Bowman's Capsule (approx.RBC count) (%)		RBCs within Bowman's Capsule (approx.RBC count) (%)		RBCs within Bowman's Capsule (approx.RBC count) (%)		RBCs within Bowman's Capsule (approx.RBC count) (%)		RBCs within Bowman's Capsule (approx.RBC count) (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A45: Summary of RBCs in glomeruli of sculpin kidneys.

Kidney Glomeruli II (Congestion) Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Glomeruli		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**		**Glomeruli**
	Congestion (Approx. RBC count within Glomeruli) (Average n)		Congestion (Approx. RBC count within Glomeruli) (Average n)		Congestion (Approx. RBC count within Glomeruli) (Average n)		Congestion (Approx. RBC count within Glomeruli) (Average n)		Congestion (Approx. RBC count within Glomeruli) (Average n)		Congestion (Approx. RBC count within Glomeruli) (Average n)
Con-1	8.73	**33-1**	6.26	**57-1**	7.07	**59-1**	5.84	**72-1**	11.42	**127-1**	6.82
Con-2	9.6	**33-2**	7.67	**57-2**	10.43	**59-2**	8.24	**72-2**	10.79	**127-2**	11.03
Con-3	7.03	**33-3**	8.83	**57-3**	6.78	**59-3**	9.09	**72-3**	9.86	**127-3**	5.82
Con-4	5.13	**33-4**	10.58	**57-4**	6.60	**59-4**	6.84	**72-4**	8.44	**127-4**	11.33
Con-5	8.27	**33-5**	7.00	**57-5**	5.71	**59-5**	7.04	**72-5**	11.18	**127-5**	10.22
Average (ave. n)	**7.75**	**Average (ave. n)**	**8.068**	**Average (ave. n)**	**7.318**	**Average (ave. n)**	**7.41**	**Average (ave. n)**	**10.338**	**Average (ave. n)**	**9.044**
StErr	**0.78**	**StErr**	**0.76**	**StErr**	**0.81**	**StErr**	**0.57**	**StErr**	**0.54**	**StErr**	**1.14**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A46: Summary of hemorrhage within the kidney tissue of sculpin.

Kidney Hemorrhage Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Interstices		**Interstices**		**Interstices**		**Interstices**		**Interstices**		**Interstices**
	Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)		Hemorrhage (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	3.33	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	3.33
Con-4	0	**33-4**	3.33	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**1.332**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0.666**
StErr	**0.00**	**StErr**	**0.82**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.67**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A47: Summary of RBCs within the tubules of sculpin kidneys.

Kidney RBC within Tubules Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Tubules		**Tubules**		**Tubules**		**Tubules**		**Tubules**		**Tubules**
	RBC within Tubules (%)		RBC within Tubules (%)		RBC within Tubules (%)		RBC within Tubules (%)		RBC within Tubules (%)		RBC within Tubules (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	3.33	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	3.33	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	3.33	**72-5**	3.33	**127-5**	0
Average (%)	**0.67**	**Average (%)**	**0**	**Average (%)**	**0.67**	**Average (%)**	**0.67**	**Average (%)**	**0.67**	**Average (%)**	**0**
StErr	**0.67**	**StErr**	**0.00**	**StErr**	**0.67**	**StErr**	**0.67**	**StErr**	**0.67**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A48: Summary of congestion within the vessels of kidney tissues in sculpin.

Kidney Congestion Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Vessels		**Vessels**		**Vessels**		**Vessels**		**Vessels**		**Vessels**
	Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)		Congestion (%)
Con-1	0	**33-1**	0	**57-1**	0	**59-1**	0	**72-1**	0	**127-1**	0
Con-2	0	**33-2**	0	**57-2**	0	**59-2**	0	**72-2**	0	**127-2**	0
Con-3	0	**33-3**	0	**57-3**	0	**59-3**	0	**72-3**	0	**127-3**	0
Con-4	0	**33-4**	0	**57-4**	0	**59-4**	0	**72-4**	0	**127-4**	0
Con-5	0	**33-5**	0	**57-5**	0	**59-5**	0	**72-5**	0	**127-5**	0
Average (%)	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**	**Average (%)**	**0**
StErr	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**	**StErr**	**0.00**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A49: Summary of the occurrence of erythrophagia in kidney tissues of sculpin.

Kidney Erythrophagia Sculpin											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
	Erythrophagia (erythro. Count) (n)		Erythrophagia (erythro. Count) (n)		Erythrophagia (erythro. Count) (n)		Erythrophagia (erythro. Count) (n)		Erythrophagia (erythro. Count) (n)		Erythrophagia (erythro. Count) (n)
Con-1	9	**33-1**	10	**57-1**	15	**59-1**	3	**72-1**	3	**127-1**	2
Con-2	3	**33-2**	1	**57-2**	0	**59-2**	5	**72-2**	7	**127-2**	6
Con-3	1	**33-3**	5	**57-3**	4	**59-3**	5	**72-3**	5	**127-3**	0
Con-4	3	**33-4**	2	**57-4**	2	**59-4**	2	**72-4**	2	**127-4**	0
Con-5	0	**33-5**	5	**57-5**	0	**59-5**	3	**72-5**	5	**127-5**	41
Average (n)	**3.2**	**Average (n)**	**4.6**	**Average (n)**	**4.2**	**Average (n)**	**3.6**	**Average (n)**	**4.4**	**Average (n)**	**9.8**
StErr	**1.56**	**StErr**	**1.57**	**StErr**	**2.80**	**StErr**	**0.60**	**StErr**	**0.87**	**StErr**	**7.88**
n	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**	**n**	**5**

Table A50: AST activity in lake trout across exposure groups

AST (U/L)											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
LT1-Con	974.00	LT1-33	1353.00	LT1-57	1194.00	LT1-59	782.00	LT1-72	1094.00	LT1-127	1147.00
LT2-Con	1471.00	LT2-33	736.00	LT2-57	2519.00	LT2-59	833.00	LT2-72	1289.00	LT2-127	1799.00
LT3-Con	845.00	LT3-33	932.00	LT3-57	2126.00	LT3-59	625.00	LT3-72	2254.00	LT3-127	946.00
LT4-Con	908.00	LT4-33	1408.00	LT4-57	743.00	LT4-59	923.00	LT4-72	1273.00	LT4-127	769.00
LT5-Con	598.00	LT5-33	786.00	LT5-57	821.00	LT5-59	836.00	LT5-72	437.00	LT5-127	N/A
Average	**959.20**	**Average**	**1043.00**	**Average**	**1480.60**	**Average**	**799.80**	**Average**	**1269.40**	**Average**	**1165.25**
StErr	**142.88**	**StErr**	**141.76**	**StErr**	**357.50**	**StErr**	**49.23**	**StErr**	**290.99**	**StErr**	**224.92**

Table A51: ALT activity in lake trout across exposure groups

ALT (U/L)											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
LT1-Con	24.00	LT1-33	81.00	LT1-57	40.00	LT1-59	11.00	LT1-72	17.00	LT1-127	41.00
LT2-Con	40.00	LT2-33	5.00	LT2-57	112.00	LT2-59	27.00	LT2-72	24.00	LT2-127	36.00
LT3-Con	19.00	LT3-33	12.00	LT3-57	76.00	LT3-59	8.00	LT3-72	68.00	LT3-127	14.00
LT4-Con	30.00	LT4-33	32.00	LT4-57	61.00	LT4-59	21.00	LT4-72	19.00	LT4-127	9.00
LT5-Con	3.00	LT5-33	7.00	LT5-57	14.00	LT5-59	37.00	LT5-72	6.00	LT5-127	N/A
Average	**23.20**	**Average**	**27.40**	**Average**	**60.60**	**Average**	**20.80**	**Average**	**26.80**	**Average**	**25.00**
StErr	**6.14**	**StErr**	**14.23**	**StErr**	**16.55**	**StErr**	**5.30**	**StErr**	**10.71**	**StErr**	**7.93**

Table A52: CK activityin lake trout across exposure groups

CK (U/L)											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
LT1-Con	3222.00	LT1-33	22013.00	LT1-57	22818.00	LT1-59	5564.00	LT1-72	2053.00	LT1-127	5707.00
LT2-Con	7988.00	LT2-33	4432.00	LT2-57	12567.00	LT2-59	3992.00	LT2-72	11294.00	LT2-127	7116.00
LT3-Con	2216.00	LT3-33	5238.00	LT3-57	10646.00	LT3-59	3744.00	LT3-72	22858.00	LT3-127	5118.00
LT4-Con	4653.00	LT4-33	23496.00	LT4-57	27342.00	LT4-59	3206.00	LT4-72	5352.00	LT4-127	5803.00
LT5-Con	3147.00	LT5-33	1200.00	LT5-57	9077.00	LT5-59	14100.00	LT5-72	2534.00	LT5-127	N/A
Average	**4245.20**	**Average**	**11275.80**	**Average**	**16490.00**	**Average**	**6121.20**	**Average**	**8818.20**	**Average**	**5936.00**
StErr	**1013.55**	**StErr**	**4740.44**	**StErr**	**3621.49**	**StErr**	**2032.91**	**StErr**	**3876.33**	**StErr**	**421.47**

Table A53: BUN concentration in lake trout across exposure groups

BUN (mmol/L)											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
LT1-Con	<0.7	LT1-33	<0.7	LT1-57	<0.6	LT1-59	<0.7	LT1-72	<0.6	LT1-127	<0.6
LT2-Con	<0.7	LT2-33	<0.6	LT2-57	<0.6	LT2-59	<0.6	LT2-72	<0.6	LT2-127	<0.6
LT3-Con	<0.6	LT3-33	<0.6	LT3-57	<0.6	LT3-59	<0.6	LT3-72	<0.6	LT3-127	<0.6
LT4-Con	<0.6	LT4-33	<0.6	LT4-57	<0.6	LT4-59	<0.6	LT4-72	<0.6	LT4-127	<0.6
LT5-Con	<0.6	LT5-33	<0.6	LT5-57	<0.6	LT5-59	<0.6	LT5-72	<0.6	LT5-127	<N/A

N.B: Values are below detection limit

Table A54: Creatinine concentration in lake trout across exposure groups

Creatinine (umol/L)											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
LT1-Con	17.00	LT1-33	55.00	LT1-57	43.00	LT1-59	21.00	LT1-72	11.00	LT1-127	17.00
LT2-Con	21.00	LT2-33	17.00	LT2-57	19.00	LT2-59	16.00	LT2-72	14.00	LT2-127	36.00
LT3-Con	15.00	LT3-33	23.00	LT3-57	28.00	LT3-59	18.00	LT3-72	37.00	LT3-127	16.00
LT4-Con	20.00	LT4-33	45.00	LT4-57	73.00	LT4-59	12.00	LT4-72	22.00	LT4-127	20.00
LT5-Con	22.00	LT5-33	24.00	LT5-57	21.00	LT5-59	25.00	LT5-72	15.00	LT5-127	N/A
Average	**19.00**	**Average**	**32.80**	**Average**	**36.80**	**Average**	**18.40**	**Average**	**19.80**	**Average**	**22.25**
StErr	**1.30**	**StErr**	**7.30**	**StErr**	**9.98**	**StErr**	**2.20**	**StErr**	**4.66**	**StErr**	**4.66**

Table A55: Phosphorus concentration in lake trout across exposure groups

Phosphorus (mmol/L)											
Control		**33 kPa**		**57 kPa**		**59 kPa**		**72 kPa**		**127 kPa**	
LT1-Con	4.84	LT1-33	5.87	LT1-57	3.96	LT1-59	5.37	LT1-72	3.45	LT1-127	4.62
LT2-Con	4.73	LT2-33	3.43	LT2-57	3.94	LT2-59	3.97	LT2-72	3.97	LT2-127	4.22
LT3-Con	4.08	LT3-33	4.16	LT3-57	4.35	LT3-59	4.38	LT3-72	4.25	LT3-127	3.97
LT4-Con	4.04	LT4-33	3.82	LT4-57	4.21	LT4-59	3.09	LT4-72	3.55	LT4-127	3.53
LT5-Con	3.31	LT5-33	3.80	LT5-57	3.91	LT5-59	3.06	LT5-72	3.42	LT5-127	N/A
Average	**4.20**	**Average**	**4.22**	**Average**	**4.07**	**Average**	**3.97**	**Average**	**3.73**	**Average**	**4.09**
StErr	**0.28**	**StErr**	**0.43**	**StErr**	**0.09**	**StErr**	**0.43**	**StErr**	**0.16**	**StErr**	**0.23**