Histopathological Assessment of the Sub-lethal Effects of Instantaneous Pressure Changes (IPCs) on Rainbow Trout (*Onchorhynchus mykiss*) Early Life Stages Following Exposure to Detonations Under Ice Cover.

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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. Instantaneous pressure change (IPC) from detonations can damage soft tissues due to expansion beyond their elastic capacities. In Canada, guidelines stipulate that peak pressures not exceed 100 kPa. However, from a review of the literature, it is clear that this guideline is not precautionary. To re-examine these guidelines, early life stages of rainbow trout (*Onchorhynchus mykiss*), including eyed eggs (degree days or days post fertilization?), sac fry (degree days or days post fertilization?) and juveniles (degree days, days post fertilization , length mean 5.10 cm ± standard error 0.10) were exposed to IPCs ranging from 0 to 280kPa. To simulate winter seismic exploration activities, 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 examined for evidence of gross effects then subsequently preserved. Microscopic damage to craniofacial features were examined in eyed eggs, while swimbladders were examined in sac fry. Furthermore, soft tissues including eyes, swimbladder, liver, kidney, and gill were evaluated in juveniles. Impacts to eye structures, swimbladder integrity, and kidney tissue were observed in fish exposed to IPCs that were ≥69 kPa. While these results suggest that the current guidelines may not be protective of early life stages of fish, additional studies are required before the protective nature of the guidelines for larger fishes can be assessed.

Keywords: histology; arctic; fish; swim bladder; early life stages; explosives; seismic;

SOMMAIRE

L’industrie pétrolière et gazière utilise des techniques sismiques par explosion pour explorer des réserves sous les plans d’eau. Dans le Nord du Canada et de l’Alaska, l’industrie et les organismes de réglementation reconnaissent que l’usage d’explosifs dans ou proches des plans d’eau pourrait faire du mal aux poissons. Une variation instantanée de la pression (VIP) causée par des détonations peut endommager des tissus mous qui s’étendent alors au-delà de leur élasticité. Au Canada, les directives stipulent qu’une pression de crête ne doit pas dépasser 100 kPa. Toutefois, d’après une revue de la documentation, il est clair que cette limite n’est qu’à titre de précaution. Pour réexaminer ces directives, on a exposé les stades précoces de l’existence de la truite arc-en-ciel (*Onchorhynchus mykiss*), notamment les œufs embryonnés (degrés-jours ou jours après la fertilisation?), les alevins vésiculés (degrés-jours ou jours après la fertilisation?) et les juvéniles (degrés-jours, jours après la fertilisation, longueur moyenne de 5,10 cm ± une erreur type de 0,10) à des VIP allant de 0 à 280 kPa. Pour simuler des activités d’exploration sismique en hiver, tous les stades de l’existence du poisson ont été placés ensemble dans des cages et soumis à des détonations à l’explosif provoquant sous la glace des VIP mesurées. Après des détonations à chaque magnitude, tous les trois stades ont été examinés pour déterminer l’ampleur des effets; ils ont été ensuite préservés. Des dommages microscopiques ont été trouvé au niveau des éléments cranofaciaux des œufs embryonnés alors que c’était les vessies natatoires ont été endommagées chez les alevins vésiculés. Par ailleurs, on a évalué les tissus mous, y compris les yeux, la vessie natatoire, le foie, le rein et les branchies chez les juvéniles. On a observé des répercussions sur les structures oculaires, l’intégrité de la vessie natatoire et le tissu rénal des poissons exposés à des VIP supérieures ou égales à 69 kPa. Bien que ces résultats suggèrent que les directives courantes pourraient ne pas protéger les stades précoces de l’existence des poissons, d’autres études sont nécessaires avant de pouvoir évaluer la nature protectrice des directives concernant les poissons plus gros.

Mots clés : histologie; arctique; poisson; vessie natatoire; stades précoces de l’existence; explosifs; sismique;

INTRODUCTION

Explosive-based seismic techniques are used by the oil and gas industry in Canada's north to explore for reserves beneath water bodies that do not freeze to the bottom. In addition to applications for geophysical exploration, seismic techniques are used for ice management, general construction and for scientific applications (Wright 1982). Seismic-based exploration activities have recently reached levels not seen since the 1970s in Canada's arctic (Cott et al. 2003). There is concern regarding the 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 under ice could be potentially harmful to fishes (Wright and Hopky 1998), but important knowledge gaps exist. For example, when charges of a given size are buried in the sediment to a measured depth in order to generate a specific peak pressure, the actual IPC is often unpredictable (Cott and Hanna 2004). In addition, there is considerable biological variability among fish species and life stages that have not been considered in the impact assessment for IPCs.

Most of the existing information regarding damage to fishes from IPCs has been determined from in-hand gross examinations of adult fish larger than 54 mm in length (Govoni et al. 2003). While mortality, injury and secondary infection have been considered, little is known regarding potentially more subtle effects of IPC's on tissue structure or on early life stages of fish. To that end, 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 pancreas). These injuries arise as a result of rapid expansion of that organ during the negative pressure phase of the IPC (Govoni et al. 2003). Temporary and permanent damage to sound sensory capabilities have also been documented in fish exposed to intense sound waves (McCauley et al. 2003; Smith et al. 2004, 2004b; Popper et al. 2005). Additional injuries that have arisen in fish exposed to IPCs are noted in Appendix 1.

Due to concerns regarding the potential effects of IPCs to fish, the Canadian Department of Fisheries and Oceans (DFO) has established a *Guideline for the Use of Explosives In or Near Canadian Fisheries Waters* (Wright and Hopky 1998) hereafter the "*Guidelines*". These *Guidelines* stipulate that in order to protect fish, maximum peak pressures are not to exceed 100 kPa. This value, however, was derived from the literature and can represent an LD_{50}. By definition the LD_{50} means that 50% of the exposed fish would be killed. An IPC at the *Guideline* therefore, is not precautionary. In fact, recent monitoring of pile driving operations, observed fish kills of Pacific herring (*Clupea harengus*) and juvenile Pacific salmon (*Oncorhynchus* sp.) when IPCs were <65kPa (Cott et al. 2003).

Mortality in roach (*Rutilus rutilus*) fingerlings exposed to IPCs between 10.1-50.7 kPa (Tsvetkov et al. 1972) were also observed. Finally, mortality among young salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) exposed to pressure increases as low as 20 kPa (Bishai 1961) were also documented. Before revisions to the existing IPC *Guideline* can be considered, scientifically defensible data is urgently needed.

The present study addresses the question of whether IPCs surrounding the current guideline have the potential for harmful tissue level effects on early life stages of fish. Because the effects of IPCs are most likely to be detected first at the cellular 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 surrounding the current guideline (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 eyed eggs, sac fry and juvenile fish exposed to the IPCs were examined microscopically to determine potential damage to craniofacial features, spinal curvature, as well as other internal tissues (e.g. liver, kidney, gill and swimbladder). Quantitative histopathology measures specific to each soft tissue were applied to facilitate subjective comparisons among groups of fish exposed to different IPCs.

METHODS

Fish and Fish Holding

Three different early life stages of rainbow trout were exposed in situ to IPCs in this experiment. Eggs (approximately 230-270 degree days at 10°C), sac fry (approximately 224-266 degree days, between 16°C-19°C, respectively) and juveniles (approximately ≤336-399 degree days, between 16°C-19°C, respectively) 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 in the same holding containers. Once at the site, all life stages were held in their shipping containers, and aerated at room temperature in a heated shelter until they were deployed for exposure to a specific IPC.

For exposure to an IPC, twenty eyed eggs, sac fry and juveniles were selected, and each life stage was placed in separate 18 oz. Whirl-Pak sterile plastic bags (Nasco, Fort Atkinson, WI, U.S.A) that were filled with water in which they 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 and 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). Cages were suspended from rope so that they hung 2 m below the 2m thick ice platform and 2 m from the lake bottom (Figure 1).

Study Site

The study was performed in Lake 24 on Richards Island, Inuvik (53720E, 7702404N) in the Mackenzie Delta during March 2004. 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 utilized heavily by 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. The lake is isolated and the substrate is predominantly clay overlain with organic material (Clayton and Cott 2004).

IPC Exposures

Explosive charges were buried 1 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).

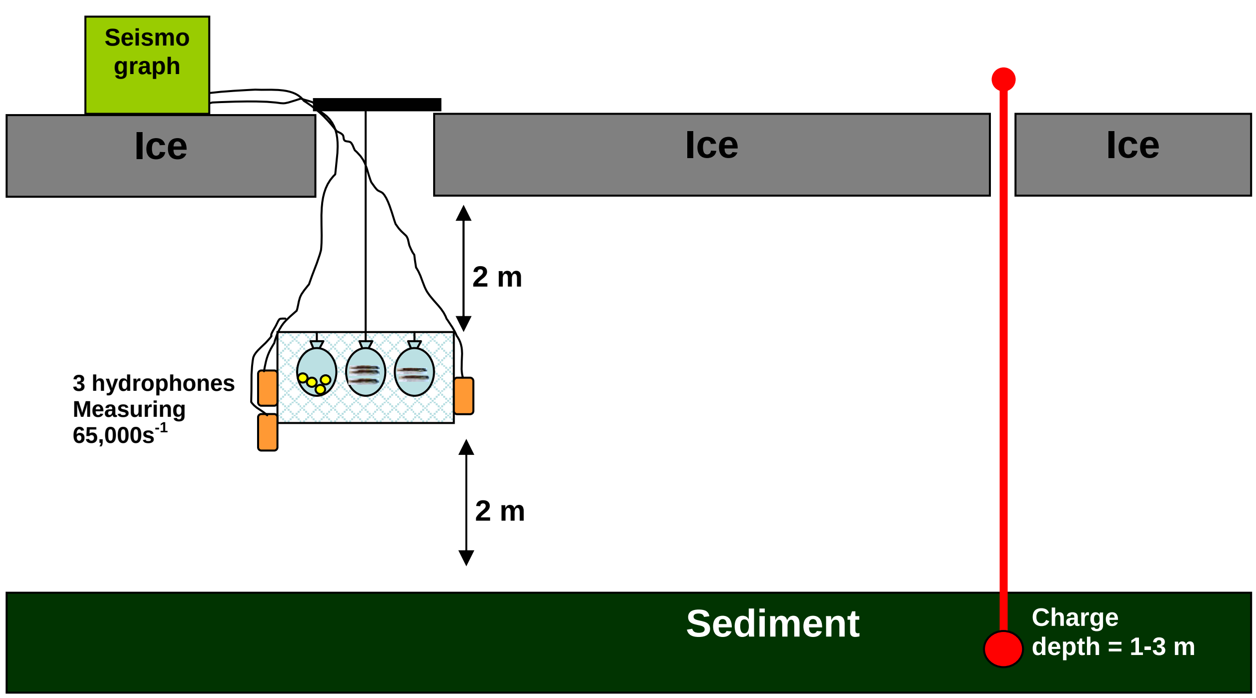


Figure 1: Schematic of IPC exposures.

IPCs were monitored at the side of the suspended cage using seismographs and hydrophones. The IPCs measured were the average of 3 hydrophones capable of measuring at a frequency of 65,000 cycling samples per second per channel. The hydrophones were deployed on the outside of the cage to avoid trauma to the fish inside the cage. Shot number 1 (Table 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. 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 0, 64, 105, 228 and 280 kPa detonations (Table 1) while juveniles were exposed to detonation charges of 0, 69, 239 and 280 kPa (Table 1).

Fish Sampling and Effects Endpoints

Immediately following each detonation, cages were removed from the water into a heated shelter (5 meters away) where eggs and fish were assessed for morbidity and obvious gross lesions. After removing the bags from the cages, eggs and sac fry were placed directly into fixative (10% ph buffered formalin) for later laboratory analysis. Juvenile fish were anesthetised using pH buffered tricaine methanesulfonate (MS-222; 0.3g/L) and examined for gross pathologies. A midline incision was made in the juveniles and once the general conditions of the internal organs had been noted for each individual, the viscera and swim bladder were carefully moved to expose the underlying kidney tissue, to 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 anesthesia 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.

a) Eyed Eggs

Eyed eggs were assessed to note any visible disruptions to gross anatomical structures. An incision was then made in the chorion and this egg layer was manually removed to allow a more thorough examination of any anomalies that may have not been visible through the chorion.

Following chorion removal, exposed embyros were oriented in several planes under a dissecting microscope with an attached PC image processor to obtain digital images. Potential disruptions to the shape of the cranium were investigated by measuring: the cranial width at eye midline, the eye midline to highest peak on head (a), width between orbits (b), and the area of upper cranium from eye midline (Figure 2). 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.

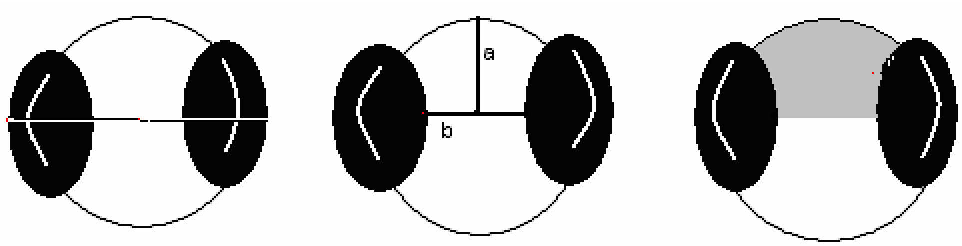


Figure 2: Cranial measurements for dechorionated eyed egg evaluations.

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,

b) Sac Fry

At the laboratory swim bladders were removed from sac fry with the aid of a dissecting microscope. The intestine was dissected free distally using scissors and manoeuvred to allow an initial investigation of swim bladder integrity. The swimbladder was subsequently removed and examined immediately.

Following swimbladder removal, the organ was 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).

c) Juveniles

With the aid of a dissecting microscope, the gastrointestinal tract was removed by severing the distal end of the intestine and the anterior end of the esophagus. Careful manoeuvring allowed the structure to be freed from the body cavity in its entirety. Removal of the gastrointestinal tract allowed the gross examination of organs surrounding the swim bladder, except for the kidney. The swimbladder was then removed. Swimbladder condition was examined immediately. Extraction of the swim bladder allowed the kidney to be inspected for hemorrhages. Dark brown to black spots were noted as possible sites of hemorrhage. Using a scalpel and scissors, the liver and kidney were dissected in their entirety 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 in individual biopsy envelopes and placed within histocassettes which where 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 2), 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).

Juvenile swim bladders were assessed and scored using the same procedure as sac fry.

Initial gross examinations indicated that eye distension in juveniles may have been affected. This was quantitatively assessed by measuring the areas of left and right eyes digitally, from the dorsal perspective (Figure 3). Right and left areas were averaged.

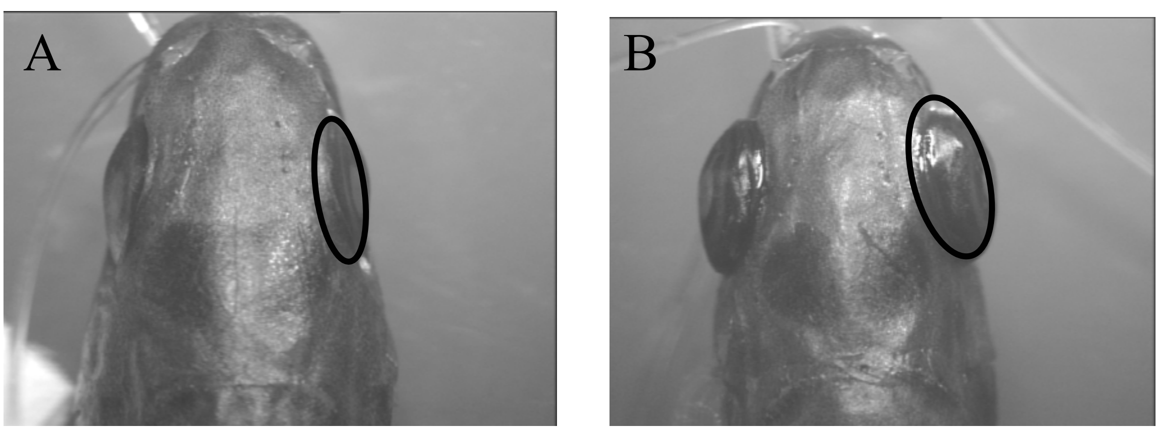


Figure 3: Digitized image analysis of dorsal eye areas in juvenile rainbow trout not exposed to an IPC (Panel A) or exposed to an IPC of 250 kPa (Panel B) and expressed relative to body length to account for differences related to body size**.** Intact heads from each fish were also sectioned in the transverse plane to histologically examine potential effects of eye displacement.

Kidney tissues were processed, embedded, sectioned and stained as previously described. For the analysis of juvenile kidney tissue, three sections from each prepared slide were randomly selected using a random number generator. Two fields of view were examined (=141 X 106 mm) for every section resulting in 12 fields of view 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). Furthermore, the presence of either single or multiple red blood cells (RBCs) in the lumen of the kidneys were noted as there are occasional red blood cells that appear in the renal tubules of apparently healthy kidneys via passage through the glomerulus or tubule wall into the lumen of the tubules (R. Evans, DFO Winnipeg, personal communication).

Livers tissues were prepared as previously described and the presence of thromocytes was assessed blindly. Four or six randomly selected digitized sections (=141 X 106 mm) on 2 or 3 respective separate slides, each with an average of 16 sections, were subjectively scored for the degree of thrombocyte infiltration within the entire section (total average area = 11.5mm^2). The presence of thrombocytes were assessed quantitatively and scored using three categories, 1 = 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. During microscopic observation, general notes were made regarding the presence of cellular particles other than thrombocytes and red blood cells, as well as the general locations of thrombocyte concentrations within the tissue. Thrombocyte scores for each fish were subsequently averaged for statistical analysis.

Gill tissues were prepared as previously described. Hyperemia (increased congestion of blood in a tissue), hyperplasia (proliferation of cells) 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, hyperplasia and hemorrhage were each scored using two categories, 1=present, 0=not present. Four or six determinations were scored on 2 or 3 respective separate slide sections, and then subsequently averaged for statistical analysis. Thrombocytes were similarly scored and additional notes were made indicating the locations of observed thrombocytes (primary or secondary lamellae).

Data Analysis

a) Eyed Eggs

Measurements of cranial parameters in eyed eggs were tested using an ANOVA followed by Tukey's. Statistical significance was set at $p<0.05$. ANOVA is a general statistical technique used to test the hypothesis that the means among two or more groups are equal, under the assumptions that the sampled populations are normally distributed and variance is homogeneous. Tukeys is used in conjunction with an ANOVA to find which means are significantly different from one another

b) Sac Fry

Measurements of the 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 therefore evaluates whether a linear response exists in the response variable across different levels of the treatment. In the case of sac fry, 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).

c) Juveniles

Swimbladder

Measurements of the incidence of swimbladder tears in sac fry were tested using a two-tailed Cochran-Armitage test for discrete variables. Statistical significance was set at $p<0.05$. Results of the Cochran-Armitage test indicate the presence or absence of a significant linear trend in the incidence of swimbladder tears as a response to treatment levels.

Size

Length measurements in juveniles were tested using ANOVA followed by Tukey’s. Statistical significance was set at $p<0.05$.

Eyes

Measurements of ocular areas in juveniles were tested using ANOVA followed by Tukey’s. Statistical significance was set at $p<0.05$.

Kidneys

Measurements of the 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 $p<0.05$. Results of the Cochran-Armitage test indicate the presence or absence of a significant linear trend in tested variables as a response to treatment levels.

Liver

Measurements of the incidence of thrombocytes in junvenile livers were tested using a Kruskal-Wallis test followed by Dunnett’s t-test. Statistical significance was set at $p<0.05$. 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. Dunnett's test compares group means.

Gills

Measurements of the incidence of hyperemia, hyperplasia, hemorrhage, and thrombocytes in junvenile gills were each tested using a Kruskal-Wallis test followed by Dunnett’s t-test. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Instantaneous pressure changes (IPCs)

A typical IPC waveform, generated as a mean composite of the measured pressure by the three hydrophones, is presented in Figure 4. Negative peak pressures typically occurred in less that 0.2 milliseconds for each of the detonations, regardless of intensity, and the peak intensity measured by each of the three hydrophones never differed by more than 5%.

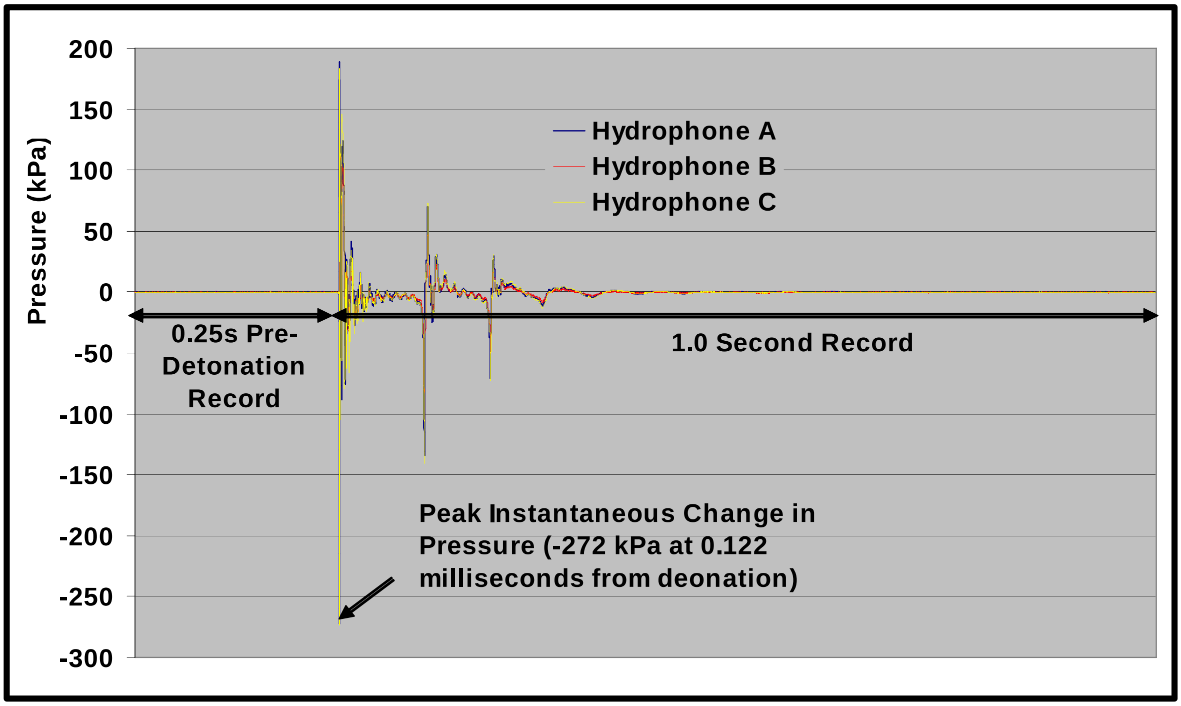


Figure 4: Pressure waveform for a detonation of 272 kPa, as measured by 3 hydrophones**.**

Chemical explosives and non-chemical explosives, including airguns, represent the most common means of generating underwater shock waves for seismic exploration purposes (Wright 1982). Detonating explosives under water generates an almost instantaneous pressure or shock wave which rises to a peak pressure, and continues by decaying exponentially (Cronin 1948). The steep fronted shockwave and subsequent decay is followed by a rarefaction wave. The rarefaction wave is a wave that assumes the opposite sign of the initial pressure (compression) wave, and arises from the seismic shock wave reflecting off of the water surface, back into the water column (Christian 1973, Hubbs and Rechnitzer 1975, Hubbs et al. 1960). Otherwise stated, the positive pressure pulse reflects at the water surface as a negative pressure pulse (Christian 1973, Cronin 1948, Falk and Lawrence 1973, Hubbs and Rechnitzer 1975). If the surface reflected negative pressure is larger than a critical breaking pressure an area of cavitation may form (Christian 1973). Following the IPC generated from the steep shock wave and subsequent rarefaction wave, the oscillatory action of a gas bubble formed at the point of explosion transmits secondary shock waves of considerably lower peaks of pressure than the initial shock wave (Rasmussen 1963, Sulfredge et al. 2001). Bubbles formed during the detonation process can be a source of significant pressure peaks (Wright 1982). However, the bubble-wave pulse lasts for a long time period, has a low maximum pressure without a steep front, and is not highly destructive to fish (Rasmussen 1964). It should be noted however that the gas bubbles from shallow explosions immediately break into the atmosphere and no subsequent bubble oscillation waves are generated (Sulfredge et al. 2001). Wave reflection can also emanate from reflection off of the bottom as well as from enclosed areas such as underwater canyons and shallows (Kearns and Boyd 1965, Yelverton et al. 1975). Therefore, a given point in the water column will be exposed to a direct wave, a smaller amplitude wave reflected off of the bottom, a surface reflected wave (Cronin 1948), and secondary shock waves due to cavitation and the formation of bubbles.

In the case of the current study, it is especially important to note that a charge detonated under thick ice will produce a positive, rather than a negative, surface-reflected wave (Hill 1978, Wright 1982). Consequently, this will increase the impulse and the lethal range of the detonation (Hill 1978, Wright 1982). The under-ice detonations of the current study may have lead to results that would have been different than had the detonations occurred within an ice-free environment.

To note, the interpretation of results for each analysis should be made with caution due to limitations imparted by small sample sizes, including the power and level of confidence of the findings.

a) Eyed Eggs

A gross examination of eyed eggs revealed no obvious disruptions arising from any of the IPC intensities that were examined. Cranial measurements of embryos however (Table 3), proved significant for area measurements of the upper cranium (Figure 5, Table 14) and circumference measurements (Figure 6, Table 14). Contrastingly, inter-orbital, outside orbital, mid-orbital to cranial peak and S factor measurements were not significant (Table 14).

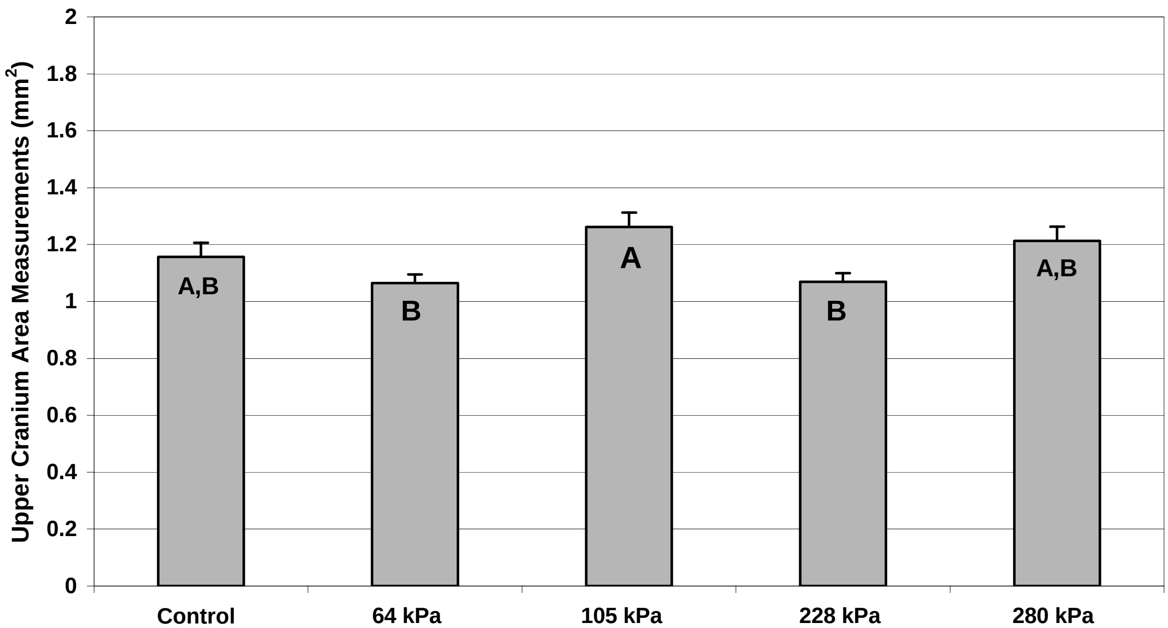


Figure 5: Upper cranium area of eyed egg rainbow trout exposed to varying IPCs. Data are expressed as mean (± SE) for n=10 (control, 106, 225, 280 kPa) and n=11 (62 kPa) fish. Bars labeled with different letters are significantly different from each other.

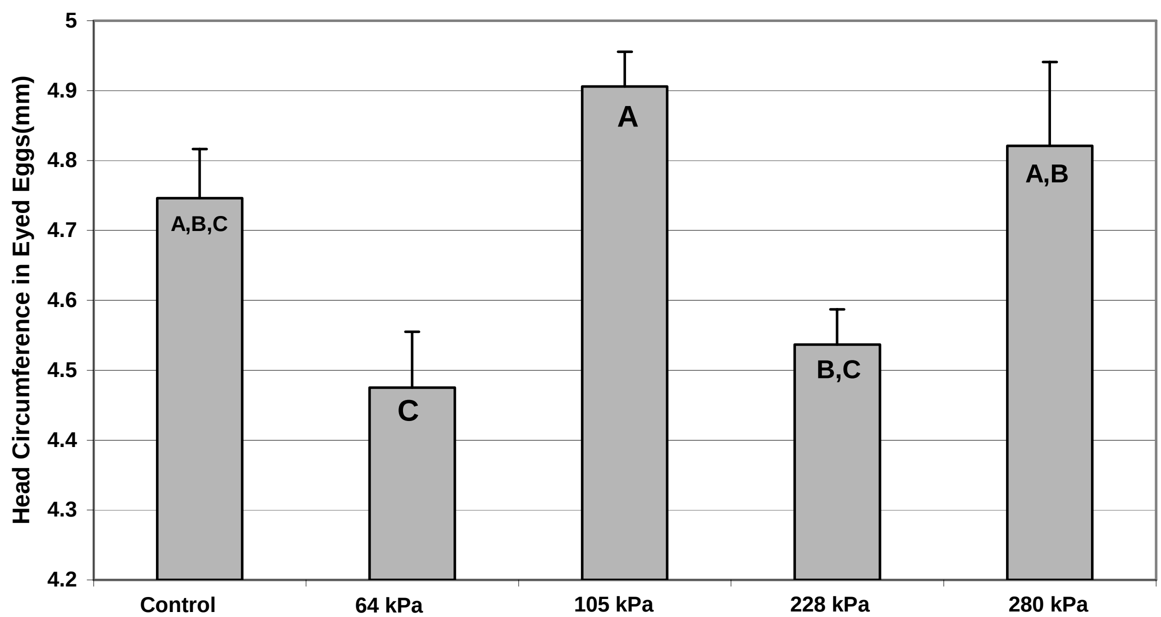


Figure 6: Head circumference in eyed egg rainbow trout exposed to varying IPCs. Data are expressed as mean (± SE) for for n=10 (control, 106, 225, 280 kPa) and n=11 (62 kPa) fish. Bars labeled with different letters are significantly different from each other.

The susceptibility of fish eggs to mechanical shock varies according to their specific stage of development (Battle 1944). Salmonid eggs are especially vulnerable to physical shock during gastrulation (Smirnov 1954,1955) with sensitivity beginning soon after fertilization and peaking at epiboly (Jensen 2003; Jensen and Alderice 1983, 1989). Lowest survival from physical shock and mechanical stress in lake trout (*Salvelinus namaycush*) were correlated with these stages of gastrulation (Eshenroder et al. 1995; Fitzsimons 1994). It is likely that during the early stages of egg development, mechanical shock or pressure ruptures the cytoplasmic layer surrounding the yolk, causing the yolk to infiltrate the perivitelline space. At more advanced stages of development 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 throughout gastrulation until the early eyed stage (Battle 1944; Jensen and Alderice 1983, Smirnov 1954). In the current experiment, the eyed eggs were developed beyond epiboly and this may partly explain why no significant impacts were evident.

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 (*Oncorhynchus mykiss*) 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. Spawning gravel increased both egg mortality as well as the variability in mortality compared to those in non-gravel environments following exposure to simulated blasting (Faulkner et al. 2007). Damage contributed by the lake sediments would have been eliminated in the current study because the eggs were held in plastic bags, removed from the lake sediments.

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 found to limit the impact 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).

Similar to our results, other studies have reported no effects of pressure changes from blasting on fish egg viability. No mortality increase was observed in rainbow trout eggs at varying stages of development (37.5, 75, 125 or 250 degree days postfertilization) exposed to peak vertical accelerations equivalent to up to 10 times the force of gravity (Faulkner et al. 2006; Post et al 1974) In a study designed to evaluate the effects of explosives on incubating lake trout eggs, a blast more than double the current guideline (13mm/s peak particle velocity), resulted in mortality that was similar to reference fish located outside of the blast zone (Faulkner et al. 2006).

At least one study has reported detrimental 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. Exposed eggs showed deformation and compression of the egg membrane, spiral curling, migration of the embryo, and a reduction in vitelline membrane integrity (Kostyuchenko 1973).

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) additional study is required before recommendations can be made regarding the general adequacy of the current IPC Guideline for protecting fish eggs.

Summary of Results in Eyed Eggs

Gross examination of eyed eggs revealed no obvious disruptions arising from any of the IPC intensities. Cranial measurements of embryos proved significant for area measurements of the upper cranium and circumference measurements in contrast to inter-orbital, outside orbital, mid-orbital to cranial peak and S factor measurements which were not significant.

b) Sac Fry

Swimbladder

The swimbladder is a hydrostatic, gas filled organ that enables fish to regulate their buoyancy (Falk and Lawrence 1973, Yelverton et al 1975). It may either have an open connection via a small duct to the alimentary canal (physostomous), be closed to the alimentary canal (physoclistous), or in some species the organ may be non-existant (Bishai 1961, Christian 1973, Falk and Lawrence 1973, Wright 1982, Yelverton et al 1975). Rainbow trout, used in the current study, have physostomous swimbladders.

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). Fish with swimbladders are almost certainly more vulnerable to pressure changes from underwater explosions than those lacking a swimbladders (Rassmussen 1964, Wiley et al 1981, Wright 1982, Aplin 1947, Bishai 1961, Kearns and Boyd 1965, Wiley et al 1981, Wright 1982). Figure 7 illustrates the response of swimbladders from an underwater blast. Kearns and Boyd (1965) reported that a 50 pound charge of nitrone S.M (Seismic Marine) at a distance of 40 feet had sufficient force to remove shellfish from rocks and split large boulders, but it had no effect on lingcod (*Ophiodon elongatus)* and sculpins (species not identified), both species lacking a swimbladder that were found 50-100ft away.

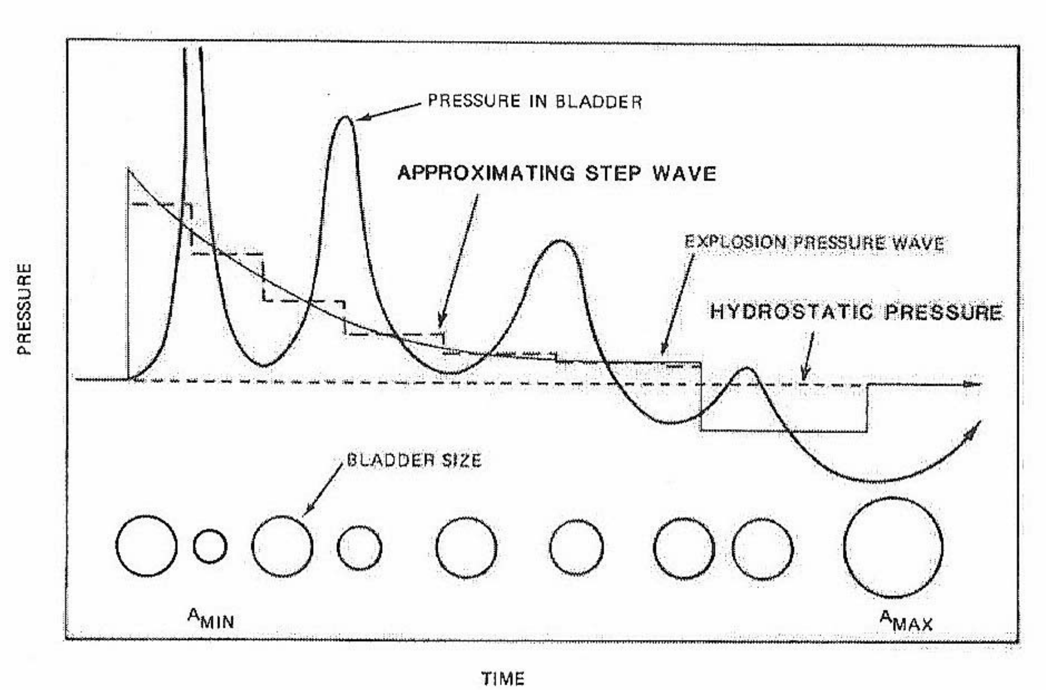


Figure 7: Pressure signature representative of an underwater explosion test coupled with the calculated pressure inside a swimbladder responding to pressure changes. Underneath pressure outline are circles representative of the changing diameter of the swimbladder.
(Wiley et al. 1981)

The incidence of swimbladder tears in the sac fry of this study (Figure 8, Table 4) was not significant (Table 14). The non-linearity of sac fry data is thought to be a function of poorly developed swimbladders which vary in their response to IPC's. Tsvetkov et al. (1972) also observed no swimbladder trauma to rapid positive pressure changes, up to 294 kPa in 16 day old Atlantic salmon (*Salmo salar* (L.)) larvae with undeveloped swimbladders upon exposure to changes in hydrostatic pressure. However, even where trauma to the swimbladder itself is not evident, the rapid expansion of the swimbladder during the negative pressure phase can mediate damage to surrounding tissues. These mechanisms are discussed further in the section of the juvenile results section. Tsetkov et al. (1972) noted that the sensitivity of fish, possessing a swimbladder, (see species list Appendix

I) to pressure changes was higher among early young of fish as compared to older individuals. Furthermore, larval fish are more sensitive to the effects of shock waves compared to eggs or post-larval fish that do not have a developed swimbladder (Wright 1982, Settle et al. 2002). While no hemorrhages or tears in the swimbladder were evident in sac fry from this study, an early study by Bishai (1961) noted that responses in larval and young fish to pressure were 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 up to 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 up to pressures of 405 kPa (Bishai 1961). Both species were unaffected by either compression or decompression pressures. Older fish (56 days) with a well developed swimbladder 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). Finally, Bishai (1961) notes that 98-178 day old salmon and brown trout died within 24 hours after being subjected to pressure increases of more than 20 kPa, while older fish (272 days) survived at pressures up to 405 kPa. The study of Bishai (1961) helps highlight that the specific age of larval and young fish may strongly affect the impacts of pressure on fish. The absence of damage to swimbladders of the rainbow trout sac fry from this study may have arisen because the specific developmental stage was insensitive to changes in pressure.

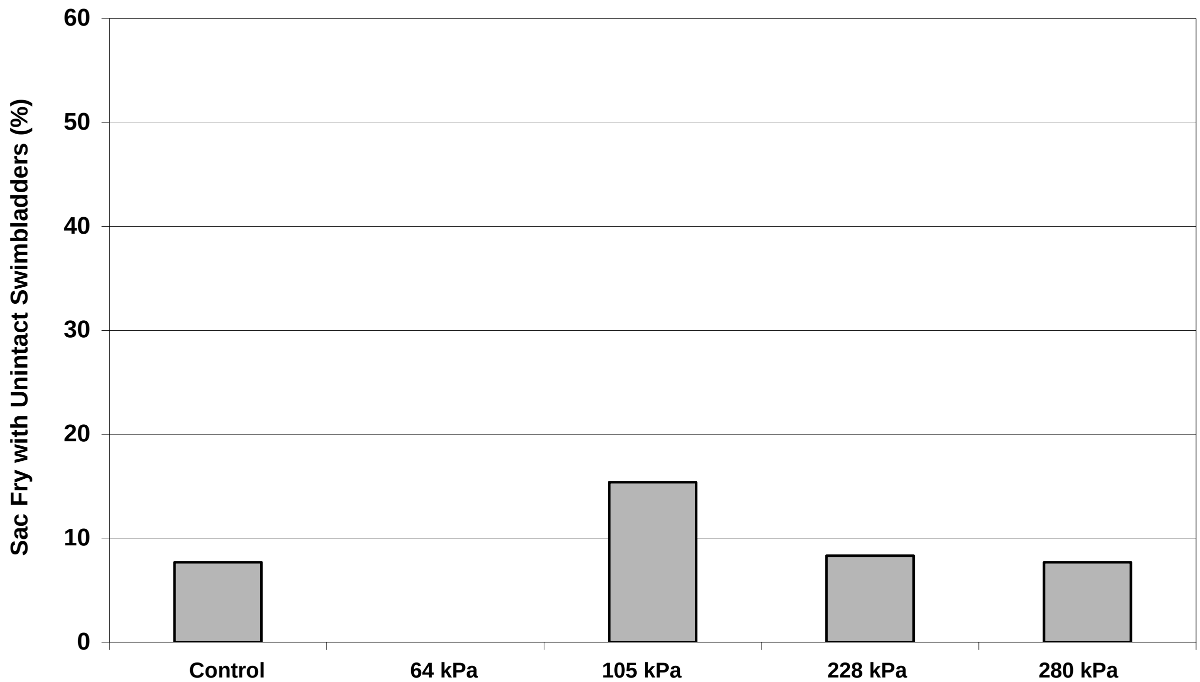


Figure 8: Percentage of sac fry rainbow trout exposed to detonations with torn swimbladders. Data are expressed as percentage of fish with unintact swimbladders for n=12 (64, 228 kPa) and n=13 (control, 105, 280 kPa) fish.

Summary of Results in Sac Fry

Swimbladder tears in the sac fry of this study were not significant.

c) Juveniles

Swimbladders

The results of the Cochran-Armitage test indicate that there is a significant linear trend in swimbladder tears as a function of pressure level (Table 14). 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 9, Table 5 & 13). However, the highest percentage, sixty (60) percent of ruptured swimbladders were found in the juvenile rainbow trout exposed to the 239 kPa detonation. Non-linearity of the juvenile swimbladder data set is thought to be related to slight size differences, and hence stage of swimbladder development, amongst the fish. Those fish possessing more developed swimbladders may show more marked effects to IPCs than those with lesser developed swimbladders. Though differences in the size of the fish proved statistically non-significant (Table 14), further investigation is warranted.

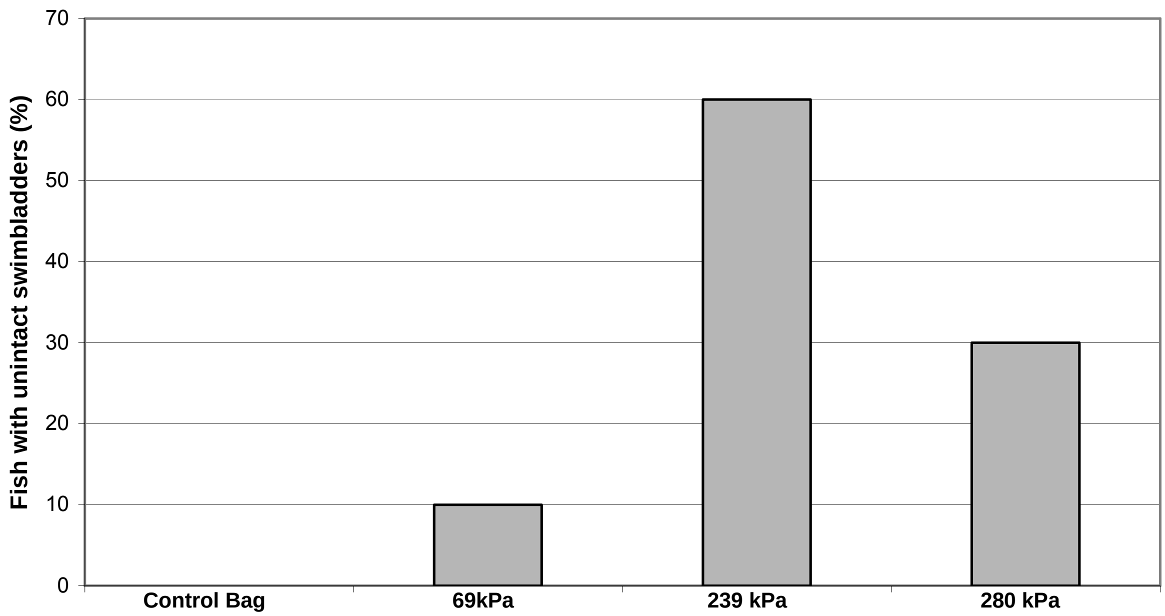


Figure 9: Percentage of juvenile rainbow trout exposed to detonations with torn swimbladders. Data are expressed as percentage of fish non-intact swimbladders for n=9 [control] and n=10 [69, 239, 280 kPa) fish.

Swimbladder tissue was not evaluated using histology in fish from this study. In fact, literature regarding the histological effects of instantaneous pressure on swimbladders is scarce, and limited to adult fish larger than 5.4cm (Govoni et al. 2003). However, some information regarding mechanisms for swimbladder disruption and the modes of action for secondary damage to other tissues arising from these effects can be gleaned from that literature.

The creation of a rapid negative pressure from the rarefaction wave of an IPC is particularly deleterious to fish (Hubbs and Rechnitzer 1952, Rasmussen 1964). Settle et al. (2002) demonstrated that fish were more resistant to compression exerted by seismic forces than to negative pressure. Using a vacuum apparatus, Muir (1959) subjected swimbladders dissected from cohoe salmon fingerlings (*Oncorhynchus kisutch*) to three cycles of pressures between 0 to 414 kPa. Rupture occurred at an absolute vacuum pressure of 41 to 52 kPa. The author also reported deflated swimbladders with tiny holes when the organ was subjected to an abrupt pressure decline equivalent to 54.2 kPa. In a study simulating changes of hydrostatic pressure from turbines, 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). Death in these fish occurred subsequent to rupture of swimbladder walls due to a sizeable and sharp increase in swimbladder volume from abrupt discharge of increase pressure (Tsvetkov et al 1972). It appears that faster rates of negative pressure exertion are most injurious to fish. When fish are unable to release gas from the swimbladder under conditions of partial vacuum, the walls of that organ become susceptible to rupture from the tensile stress induced on it by the expansion of gases (Muir 1959, Wiley et al 1981, Christian 1973). Swimbladders ruptured in this way have tears with the edges turned outwardly (Rasmussen 1964). Blood from broken blood vessels may be expelled into the abdominal cavity, 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 1975, Kearns and Boyd 1965).

Cavitation may also prove to exhibit effects in fish. Muir (1959) exposed cohoe salmon fingerlings (*Oncorhynchus kisutch*) to cavitation using a vacuum apparatus, simulating the passage of fish through turbines. Following a 0.4 second exposure to a partial vacuum, 10 of 20 fish died a short time following the exposure and 2 more within 4 hours. Microscopic examination revealed the presence of hemorrhages in one or both eyes and gills.

Recuperation and survival in fish with damaged swimbladders varies. While Wiley et al. (1981) speculated that fish in the natural environment would have difficulty recovering from a ruptured swimbladder and have very little chance at survival, they showed that spot (*Leiostomus xanthurus*) swimbladders could heal after bursting from rapid decompression. Spot were exposed to a pressure acclimation test at 138 kPa. Four days into the test, swimbladders had begun to heal. This healing was attributed to retained gas secretion within the swimbladder. Swimbladders, however, later reopened and gas escaped (Wiley et al. 1981). Cronin (1948) suggested that moderate damage to the swimbladder does not necessarily lead to mortality given that the vascular system of the organ can remains intact. Similarly, Yelverton et al. (1975) showed that carp (*Cyprinus carpio*) and catfish (*Ictalurus punctatus*) could survive with damaged swimbladders. These fish, exposed to LD_{50} level blasts from high explosive Pentolite charges at impulse strengths averaging 177.2 kPa/msec and 253.8 kPa/msec, respectively, survived for two weeks with ruptured swimbladders before they were autopsied.

Regardless of whether mortality results, injury to the swimbladder can greatly reduce the fitness of fish (Wiley et al 1981). Because swimbladders are essential for buoyancy control, damage can render fish more vulnerable to predators (Wiley et al 1981, Govoni et al. 2002, 2003) and unable to feed effectively (Falk and Lawrence 1973, Settle et al 2002). Compression of internal organs by the swimbladder may also interfere with their normal functioning, impacting overall fitness (Tsvetkov et al. 1972).

Responses to IPC may vary among fish species with differences in body rigidity, thickness and volume of the swimbladder and surrounding internal anatomy. 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 (Wiley et al. 1981). Reduced damage to the swimbladder arising from pressure changes are evident in fish with thicker walled swimbladders (Falk and Lawrence 1973). Similarly, Fitch and Young (1948) report that cylindrical fish such as the barracuda (*Sphyraena argentea*), kingfish (*Genyonemus lineatus*), and queenfish (*Seriphus politus*) tended to have thick-walled swimbladders that are more resistant to pressure than laterally compressed fish, such as the salt-water perch (*Brachyistius frenatus*), which had thinner swimbladders. However, there are exceptions to these generalized statements, as Cronin (1948) found weakfish (*Cynoscion regalis*), with soft bodies were more susceptible to injury upon exposure to underwater TNT/Nitramon explosions, at peak pressures from 0 to ≥ 5516 kPa compared to the more firm-fleshed rock (*Roccus saxatilis* Walbaum).

Basic anatomy differences among fishes can affect the responses to IPCs. For example, internal anatomy can affect the sites of swimbladder rupture (Wiley et al 1981). 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). The swimbladders of Atlantic menhaden (*Brevoortia tyrannus*), blueback herring (*Alosa aestivalis***)** and striped killifish (*Fundulus majalis*) tended to burst along the lateral edges of the ventral surface, but in white perch (*Morone americana)* the break tended to be along the midventral surface due to the swimbladders dorsal attachement to the rib cage (Wiley et al 1981). External anatomy can also have impacts on the swimbladders' response to IPCs. Fish with larger surface areas absorb more shock energy than smaller fish and, therefore, tend to be more greatly injured (Roguski and Nagata 1970).

There are differences in the effects of IPCs between physoclists, with closed swimbladder chambers, and physostomes, with open swimbladder configurations. 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). Lower pressures, and lesser rates of pressure discharge, are lethal to fingerlings and larvae of physostomous fish as they have more difficulty releasing gas than older fish (Tsvetkov et al 1972). Lethal pressure changes in physostomous larvae cause excess gas to pass from the swimbladder to the gut (Tsvetkov et al 1972). Hogan (1941) also showed that among fish exposed to experimental vacuum pressures between 50.8 kPa and 91.4 kPa, physostomous species were capable of quickly releasing air from their swimbladder and were better able to withstand the treatments than physoclistous fish, which suffered ruptured swimbladders and hemorrhaging. Christian (1973) speculated that near the lethal zone, physostomes may be more resistant to pressure change but that under severe shock conditions both swimbladder types would suffer equal damage. Furthermore, 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.

Body structure and anatomy can affect susceptibility to IPCs, but differential placement of seismic sources can have an impact. For example, different effects have been postulated to be induced in fish inhabiting different depths. Hubbs and Rechnitzer (1975) contend that the formation of a negative pressure pulse is intensified at the water surface. While Lavergne (1970) suggested that surface charges can cause more damage to fish, Kearns and Boyd (1965) report that detonations originating at depth are more damaging to fish than those at shallow depths, because more of the shock wave energy is lost at the surface, resulting in less being directed below the surface.

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 (1975) 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 the linear high explosive, Aquaflex (Falk and Lawrence 1973). Pressure measurements were not indicated in the aforementioned study. Ferguson (1962) documented that, following exposure to both a nitrone explosive and black powder 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.Linton et al. (1985) reported conflicting results noting that black drum

(*Pogonias cromis*) held at the bottom (2.4m), closest to the site of detonation (one and 23 meters away), exhibited greater mortality than those in surface waters following exposure to underdetermined peak pressure blasts of Primacord explosive.

Regardless of which direction 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 depth may affect the peak pressures and their impacts. Furthermore, the effects of negative pressure on swimbladders, cavitation, and anatomy need to be considered major factors affecting impacts to fish exposed to seismic detonations.

Eyes

An initial gross examination of juvenile fish suggested that the eyes of fish exposed to some IPCs were displaced in an outward direction. Digitized analysis revealed significantly greater ocular areas, when viewed dorsally, among the 69 and 280 kPa groups, but not the 239 kPa exposed fish (Figure 10, Table 6,13 and 14). Non-linearity of juvenile eye data is thought to be related to slight size differences amongst the fish, and hence slight differences in the development of ocular support. Slightly larger fish may possess more developed ocular support than smaller fish and resultantly, show a lesser impact to IPCs. As previously mentioned, though differences in the size of the fish proved statistically non-significant, further investigation is warranted.

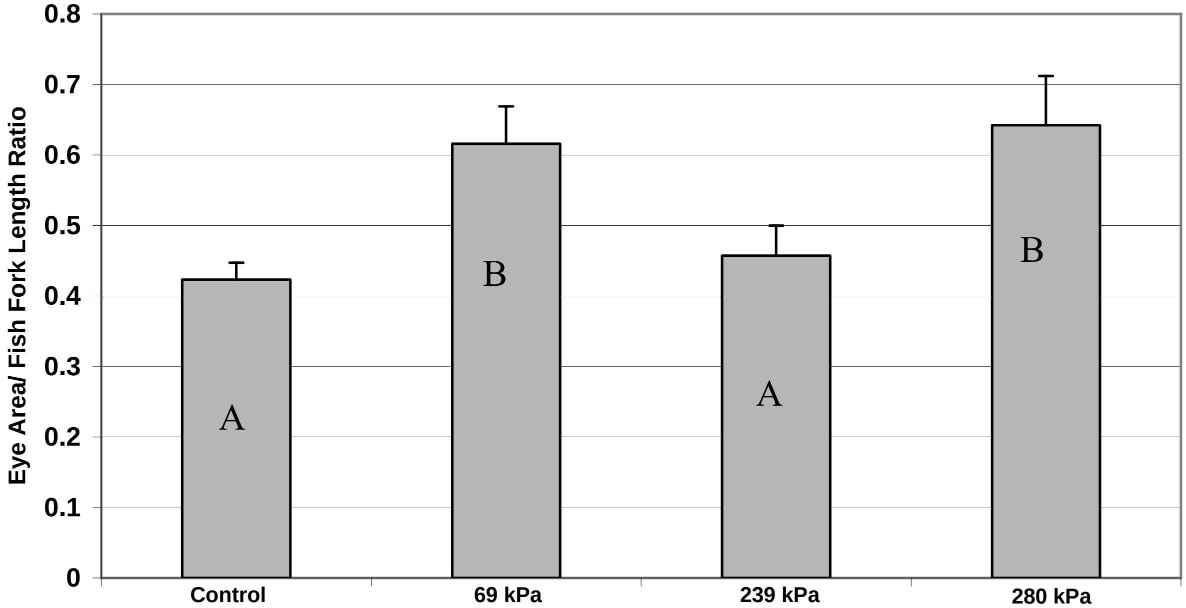


Figure 10: Dorsally viewed ocular areas of juvenile rainbow trout exposed to varying IPCs. Data are expressed as mean (± SE) for digitized areas divided by fork length for n=9 (control) and n=10 (69, 239, 280 kPa) fish. Bars labeled with different letters are significantly different from each other.

To make an initial investigation of the underlying pathology associated with eye displacement, histological sections, through the transversal plane of intact heads, were prepared for each fish as shown in Figure 11. While no quantitative analysis was performed, the 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 in size may underscore the importance of developmental stages in mediating effects.

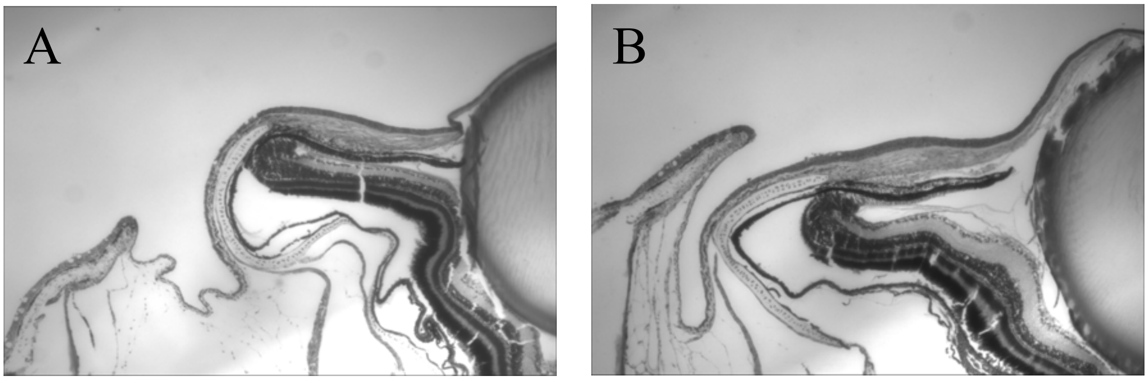


Figure 11: Histological sections of eyes (frontal plane) of juvenile rainbow trout exposed to varying IPCs. Panel A is a frontal eye section of juvenile at 280 kPa. Panel B is a frontal eye section of juvenile at 239 kPa

Eye displacement 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). 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 (Bishai 1961). Tsvetkov et al (1972) further support this theory in fish ranging from fingerling to adult.

Kidneys

Juvenile fish exposed to IPCs exhibited hematuria (Figure 12, Table 7 & 13). Single RBCs were routinely detected in tubule lumen yet results of the Cochran-Armitage test indicate that there was no significant linear trend (Table 14). Of greater physiological relevance, however, was the appearance of multiple RBCs in several tubules which was observed at 280 kPa, and to an even greater extent at 239 kPa. The presence of multiple RBC's showed a significant linear trend as a function of pressure level (Table 14). Furthermore hemorrhages were observed among fish exposed to 69, 239 and 280 kPa and the extent of the hemorrhages as well showed a significant linear trend as a function of pressure level (Figure 13, Table 7,13 & 14). Non-linear data sets for juvenile kidneys, similar to those of swimbladders and eyes, are attributed to slight size differences amongst the fish. In this case, those fish possessing more developed swimbladders will show greater kidney impacts than those with lesser developed swimbladders. For instance, the higher incidence of hematuria among fish from the 239 kPa group may have been related to the greater incidence of increased swimbladder rupture in this group of slightly larger fish.

Our results are consistent with previous studies showing hematuria in the proximal tubules of kidney in spot (*Leiostomus xanthurus*) and pinfish (*Lagodon* *rhomboides*) following exposure to submarine detonations measured at peak pressures of 230.86 and 636.92 kPa generated from 12-grain Primadet PDT 1403 detonators (Govoni et al, 2003). Blood clots were also been noted by Thomson (1958), during post mortem examination, in the kidneys of resident banded rockfish (*Sebastodes nigrocinctus*), red snappers (*Sebastodes ruberrimus*), orange-spotted rockfish (*Sebastodes maliger*) and copper rockfish (*Sebastodes caurinus*) following a blast from DuPont Nitramex 2-H explosive (peak pressure not indicated).

Damage to the kidneys, even slight bruising, has the potential to interfere with osmoregulatory (maintenance of the water-salt balance) efficiency in fish, causing heightened energy expenditure (Wiley et al. 1981). The trauma evident in the tubules of the kidney is likely caused by the passing pressure wave affecting the serosa and surrounding mesentery of the pancreas and endothelium of arterioles and venules causing erythrocyte migration into the lumen of the kidney tubules (Govoni et al. 2003). As a side note, Wiley et al (1981) indicate that bruising to the kidneys is much more prominent in rigidly structured species of fish in which the swimbladder closely adheres to the kidney, than flexible bodied species in which the swimbladder is in less direct contact with the dorsal body wall.

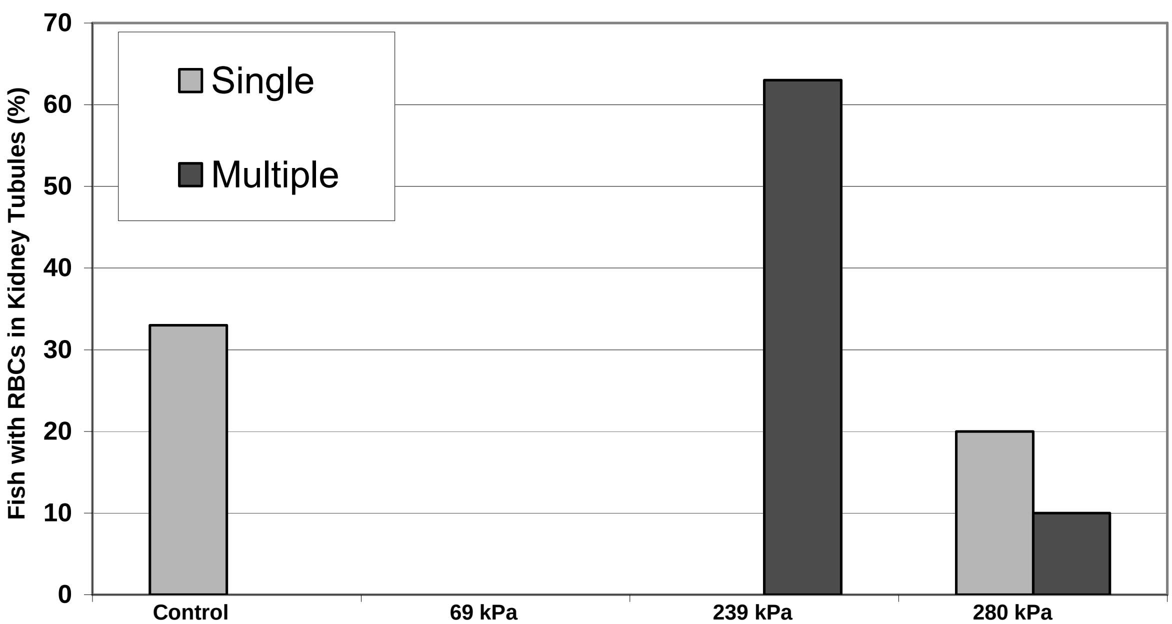


Figure 12: Incidence of hematuria in juvenile rainbow trout kidneys exposed to varying IPCs. Data are expressed as percentage of fish with Red Blood Cells (RBC) in kidney tubules for n=8 (239 kPa), n=9 (control, 69 kPa) and n=10 (280 kPa) fish.

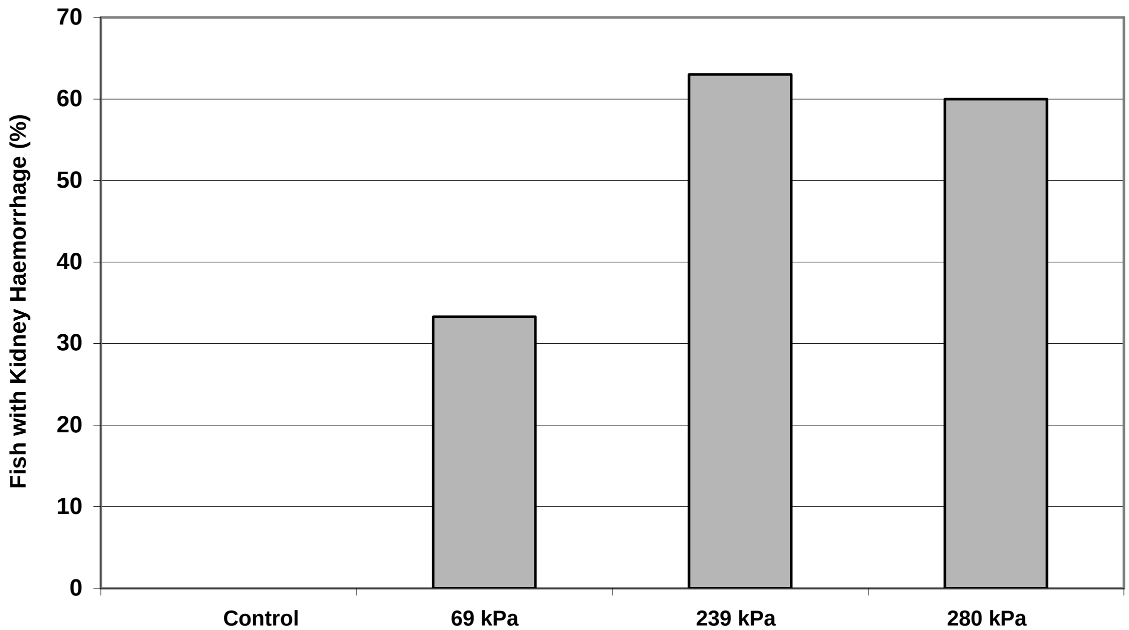
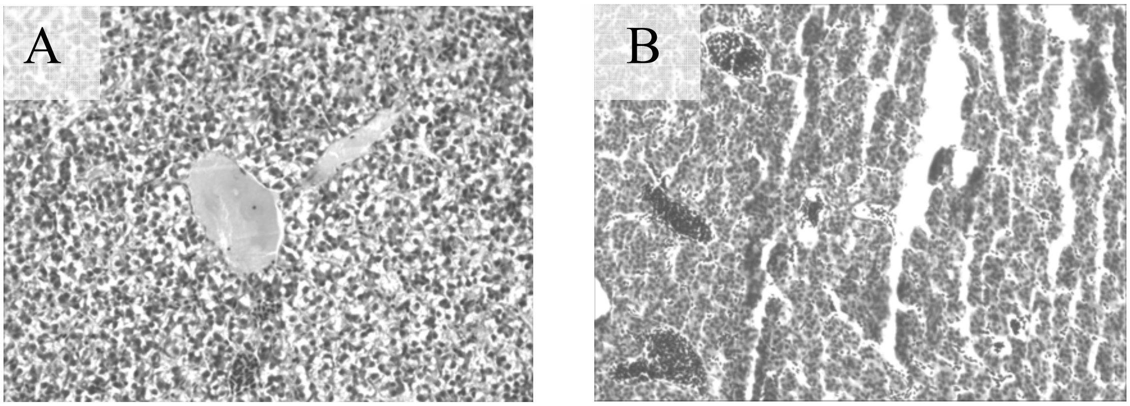


Figure 13: Incidence of hemorrhaging in juvenile rainbow trout kidneys exposed to varying seismic peak pressures. Data are expressed as percentage of fish with RBC's in the insterstitium of kidney tubules for n=8 (239 kPa), n=9 (control, 69 kPa) and n=10 (280 kPa) fish.

Liver

There were no significant differences in the degree of thrombocyte aggregations (Figure 14, Table 14) in livers of juvenile fish from any of the treatment groups in this study (Figure 15, Table 8 & 13). Non-linearity of the data is attributed to the lack of impact on livers from IPCs. Govoni et al (2002, 2003) found hyperemia and contusions within the liver in regions proximal to the swimbladder in juvenile pinfish (*Lagodon rhomboids*) and spot (*Leiostomus xanthurus*) following exposure to submarine explosions measured at peak pressures of 230.86 and 636.92 kPa generated from 12-grain Primadet PDT 1403 detonators, and further found evidence of coagulative necrosis proximal to the swimbladder. Blood clots have also been noted by Thomson (1958) during post mortem examination, in 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).



Thrombocyte Score of 1 Thrombocyte Score of 3

Figure 14: Liver tissue from juvenile rainbow trout exposed to IPCs, depicting thrombocyte scores. 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.

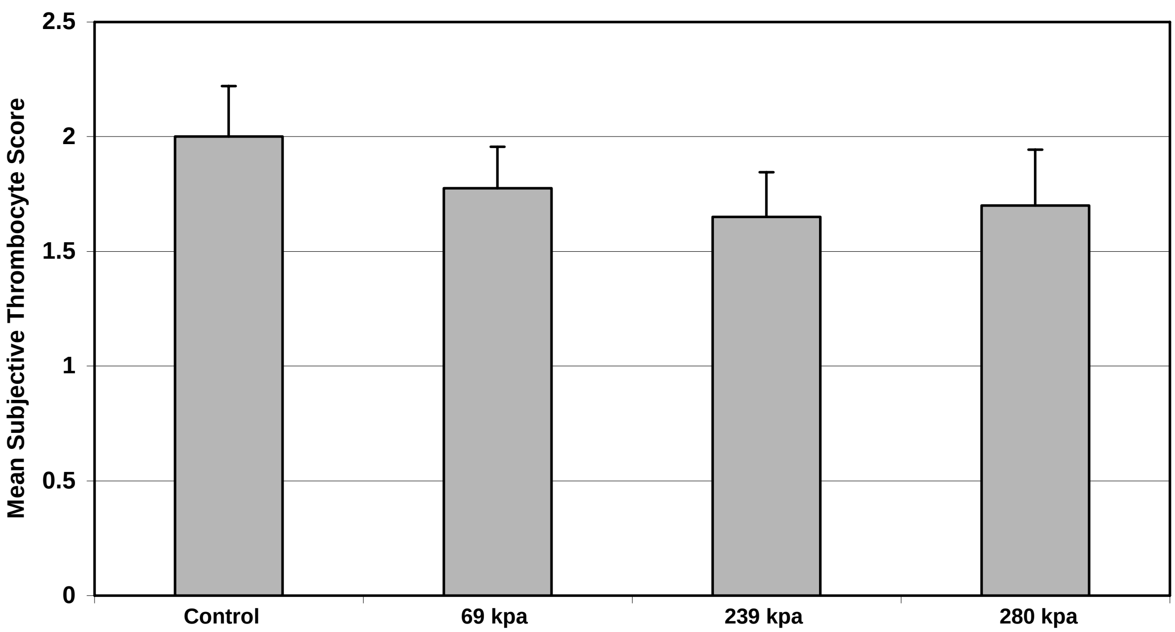


Figure 15: Subjective thrombocyte scores in liver of juvenile rainbow trout exposed to varying seismic peak pressures. Data are expressed as mean (± SE) subjective thrombocyte score for n=9 (control) and n=10 (69, 239, 280 kPa) fish. A score =1 represents nil or very few thrombocytes (1-4 congregations),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

Gills

No statistical significance was found for any of the gill parameters assessed in juvenile rainbow trout from any of the treatment groups in this study (Figures 16-19, Table 9-14). Non-linearity of the data is attributed to the lack of impact on gills from IPCs.

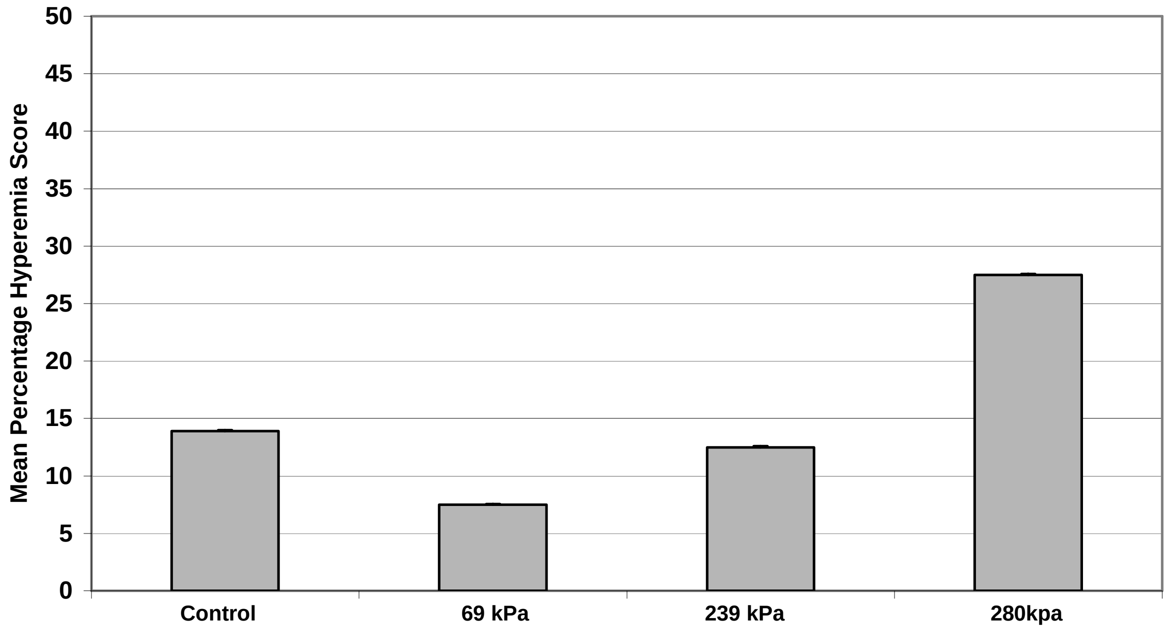


Figure 16: Mean percentage hyperemia scores in gills for juveniles exposed to varying seismic peak pressures. Data are expressed as mean percentage (± SE) hyperemia score for n=9 (control) and n=10 (69, 239, 280 kPa) fish.

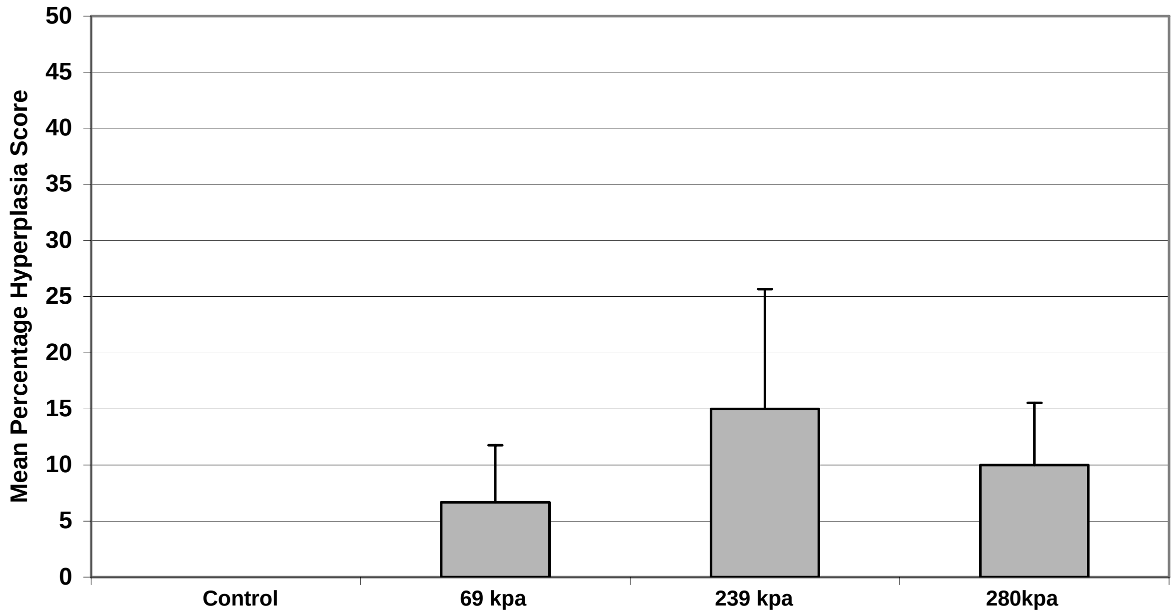


Figure 17: Mean percentage hyperplasia scores in gills for juveniles exposed to varying seismic peak pressures. Data are expressed as mean percentage (± SE) hyperplasia score for n=9 (control) and n=10 (69, 239, 280 kPa) fish.

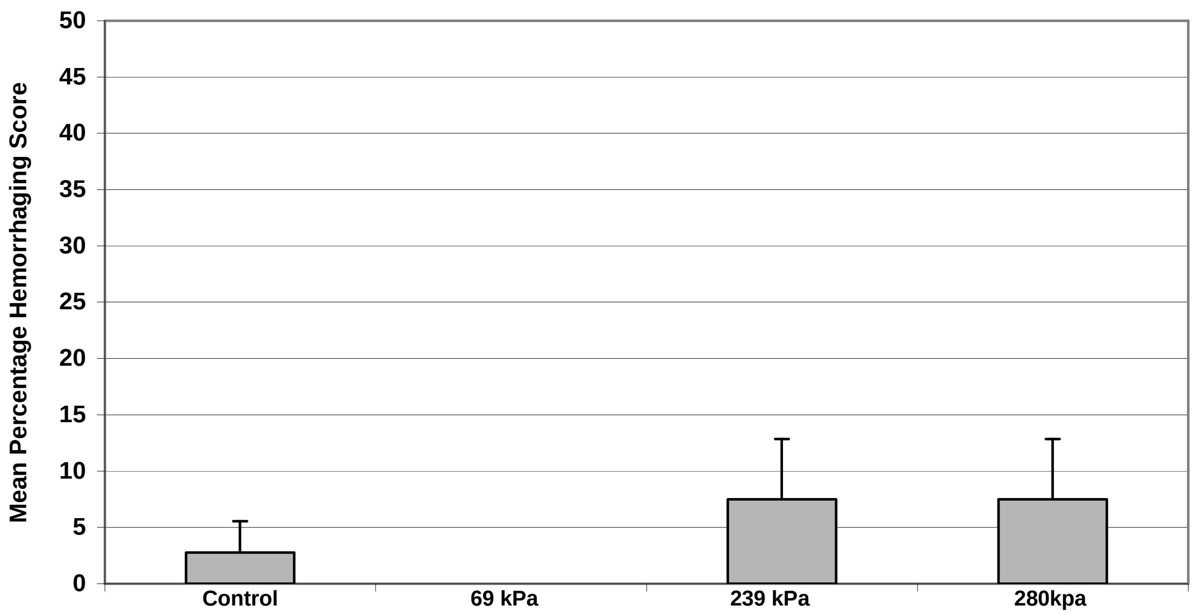


Figure 18: Mean percentage hemorrhaging score in gills for juveniles exposed to varying seismic peak pressures. Data are expressed as mean percentage (± SE) hemorrhage score for n=9 (control) and n=10 (69, 239, 280 kPa) fish.

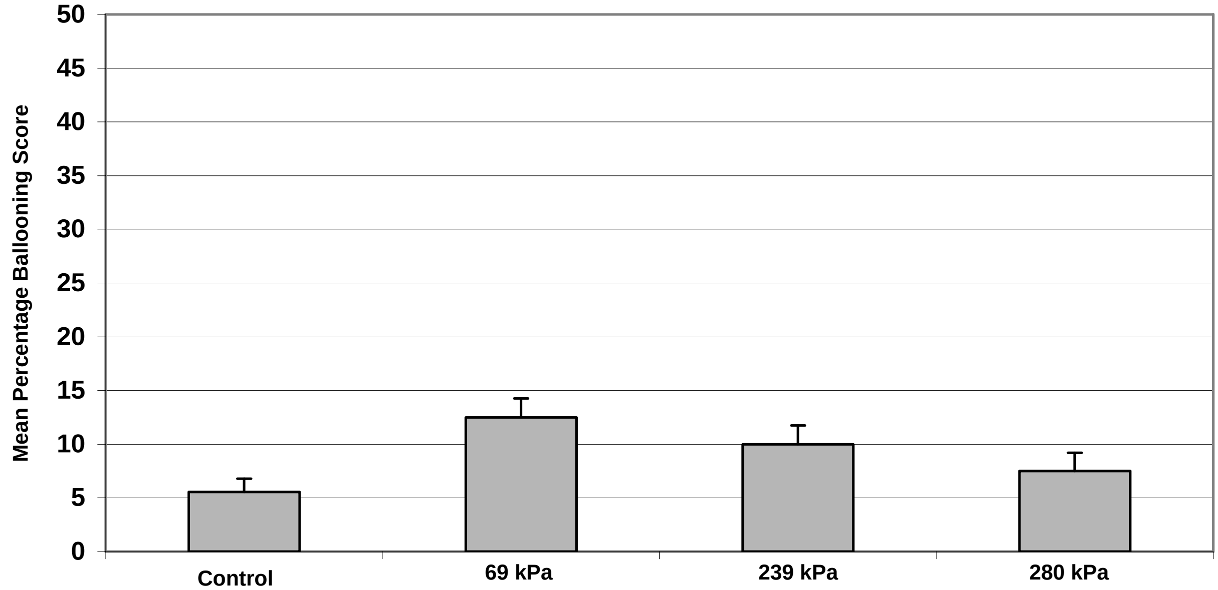


Figure 19: Mean percentage thrombocyte score in gills for juveniles exposed to varying seismic peak pressures. Data are expressed as mean percentage (± SE) thrombocyte score for n=9 (control) and n=10 (69, 239, 280 kPa) fish.

Muir (1959) reported damage to gill plates of fingerling cohoe salmon (*Oncorhynchus kisutch*) following their exposure to cavitation in a vacuum apparatus designed to simulate the passage of fish through turbines (partial vacuum pressure not indicated). Thompson (1958) confirmed the presence of frayed gills in fish following the explosion of the British Columbia navigation hazard known as Ripple Rock. This finding was noted during post mortem examinations of resident fish, including banded rockfish (*Sebastodes nigrocinctus*), red snappers (*Sebastodes ruberrimus*), orange-spotted rockfish (*Sebastodes maliger*) and copper rockfish (*Sebastodes caurinus*), found floating on the surface after a blast from DuPont Nitramex 2-H explosive (peak pressure not indicated).

Future experiments evaluating the effects of IPCs on gill tissue, should evaluate the pseudobranch, the reduced first gill arch of a fish. Evidence suggests that this structure is linked with the choroid rete, believed to create high oxygen pressures behind the retina of the fish, and the supply of oxygen to the retina of the fish eye (Bridges et al. 1998). The function of the pseudobranch could be susceptible to impacts from varying pressure changes during seismic activity.

Summary of Results in Juveniles

A significant linear trend was present in swimbladder tears as a function of pressure level. Digitized analysis of juveniles revealed significantly greater ocular areas among the 69 and 280 kPa groups, but not the 239 kPa exposed. This is thought to be due to greater support surrounding the eye contributing to a more limited displacement among the fish in the 239 kPa group. Further to this, while single RBCs detected in the tubule lumens of kidneys showed no significant linear trend, the appearance of multiple RBCs in several tubules, observed at 280 kPa and to an even greater extent at 239 kPa, showed a significant linear trend as a function of pressure level. Hemorrhages were observed among juvenile fish exposed to 69, 239 and 280 kPa and the extent of the hemorrhages as well showed a significant linear trend as a function of pressure level. Finally, no significant differences were found in the degree of thrombocyte aggregations in livers of juvenile fish nor in any of the gill parameters assessed.

While it was originally thought that juveniles exposed to the 239kpa detonation had greater swimbladder and kidney damage as well as lesser ocular damage due to being slightly larger than the other groups, difference in the size of the fish proved statistically non-significant. Further investigation however is warranted.

CONCLUSIONS AND RECOMMENDATIONS

To summarize, a gross examination of eyed eggs revealed no obvious disruptions arising from any of the IPC intensities that were examined. Cranial measurements of embryos however proved significant for area measurements of the upper cranium and circumference measurements. Contrastingly, inter-orbital, outside orbital, mid-orbital to cranial peak and S factor measurements were not significant. In sac fry, the incidence of swimbladder tears was not significant. In juveniles however, a significant linear trend was present in swimbladder tears as a function of pressure level. Additionally, digitized analysis of juveniles revealed significantly greater ocular areas, when viewed dorsally, among the 69 and 280 kPa groups, but not the 239 kPa exposed. This is thought to be due to greater support surrounding the eye contributing to a more limited displacement among the fish in the 239 kPa group compared with the other treatment groups. Further to this, juvenile fish exposed to IPCs exhibited hematuria. While single RBCs detected in tubule lumens showed no significant linear trend, the appearance of multiple RBCs in several tubules, observed at 280 kPa and to an even greater extent at 239 kPa, showed a significant linear trend as a function of pressure level. Furthermore, hemorrhages were observed among juvenile fish exposed to 69, 239 and 280 kPa and the extent of the hemorrhages as well showed a significant linear trend as a function of pressure level. Finally, no significant differences were found in the degree of thrombocyte aggregations in livers of juvenile fish nor in any of the gill parameters assessed. The most notable effects on rainbow trout from exposure to IPCs from our study are therefore changes in both the area and circumference of the cranial region of eyed eggs, as well as swimbladder, ocular and kidney damage in juveniles.

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.

Because of this new data, DFO Western Arctic Area has recommended that the maximum peak pressure never exceed 50kPa (Cott et al. 2003; Cott and Hanna 2004). 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 (Alaska DFG 1991; Alaska DNR 2003).

While sample sizes in this study were small and both the power and level of confidence of the findings were not optimal, this study enabled preliminary conclusions to be drawn regarding the effects of IPC's 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 that varied only slightly in size may have underscored the importance of developmental stages in mediating effects. A more thorough investigation of the effects of IPCs in fish with well developed structures (i.e. swimbladders, support surrounding the eye) as compared to those with lesser developed structures is therefore warranted. This is owing to the greater effects of IPCs in juvenile fish that may be linked to more advanced swimbladder development as well as to the lesser effects of eye displacement that may be linked to greater development of support surrounding the eye.

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Tables

Table 1: IPC Intensities Measured By 3 Hydrophones Capable of Measuring at a Frequency of 65,000/s.

	Distance from cage (m)	Ice thickness (m)	Total Depth (m)	Burial Depth (m)	Charge size (g)	Hydrophone 1 IPC reading (kPa)	Hydrophone 2 IPC reading (kPa)	Hydrophone 3 IPC reading (kPa)	Hydrophone Averages
1	*1.5*	*1.90*	*6.80*	*2.0*	*240*	*272*	*251*	*270*	*264*
2	3.0	1.90	6.80	2.0	240	282	284	275	280
3	6.0	1.88	6.80	2.0	120	71	69	70	69
4	6.0	1.88	6.80	2.0	60	105	104	107	105
5	3.0	1.90	6.80	3.0	60	62	65	65	64
6	1.5	1.90	6.80	2.0	180	233	242	242	239
7	1.5	1.90	6.80	2.0	120	223	227	234	228

Table 2: 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 3: Summary of Cranial Measurements of Eyed Rainbow Trout Eggs Exposed to IPCs of Varying Intensity.

Peak Pressure kPa	Inter-orbital (mm)	Outside Orbital (mm)	Mid-orbital to Cranial Peak (mm)	Area Upper Cranium (mm^2)	Circumference (mm)	S Factor
0	0.61 ± 0.03	1.89 ± 0.03	1.16 ± 0.03	1.16 ± 0.05	4.75 ± 0.07	0.64 ± 0.02
64	0.61 ± 0.01	1.88 ± 0.03	1.18 ± 0.03	1.06 ± 0.03	4.48 ± 0.08	0.67 ± 0.02
105	0.62 ± 0.02	1.89 ± 0.04	1.23 ± 0.03	1.26 ± 0.05	4.91 ± 0.05	0.66 ± 0.01
228	0.63 ± 0.03	1.87 ± 0.06	1.18 ± 0.03	1.07 ± 0.03	4.54 ± 0.05	0.65 ± 0.01
280	0.67 ± 0.02	1.92 ± 0.03	1.18 ± 0.03	1.21 ± 0.06	4.82 ± 0.12	0.65 ± 0.02

Table 4: Data Summary of Swimbladder (Sb) Evaluation in Rainbow Trout Sac Fry Exposed to IPC's of Varying Intensity

Fish #	Treatment	Swimbladder	Treatment	Swimbladder	Treatment	Swimbladder	Treatment	Swimbladder	Treatment	Swimbladder
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 5: Data Summary of Swimbladder (Sb) Evaluation in Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity

Fish #	Treatment	**Swimbladder**	Treatment	**Swimbladder**	Treatment	**Swimbladder**	Treatment	**Swimbladder**
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 6: Data Summary of Ocular Data of Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity

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

Table 7: Data Summary of Histological Examination of Juvenile Rainbow Trout Kidneys Exposed to IPC's of Varying Intensity

Treatment	Fish #	Slide #	Hematuria *	Hemmorhage**
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			**33.3% (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%**	**33.3%**

Table 7: Data Summary of Histological Examination of Juvenile Rainbow Trout Kidneys Exposed to IPC's of Varying Intensity (Continued)

Treatment	Fish #	Slide #	Hematuria *	Hemmorhage**
239 kPa	1	1	3	1
239 kPa	1	2	2	1
239 kPa	3	1	3	1
239 kPa	3	2	0	1
239 kPa	4	1	0	0
239 kPa	4	2	0	1
239 kPa	5	1	3	0
239 kPa	5	2	2	0
239 kPa	6	1	0	0
239 kPa	6	2	0	0
239 kPa	7	1	0	1
239 kPa	7	2	2	1
239 kPa	9	1	0	0
239 kPa	9	2	0	0
239 kPa	10	1	2	1
239 kPa	10	2	2	1
N=8			**62.5% (multiple)**	**62.5%**
280 kPa	1	1	1	1
280 kPa	1	2	3	1
280 kPa	2	1	0	1
280 kPa	2	2	0	1
280 kPa	3	1	0	1
280 kPa	3	2	0	0
280 kPa	4	1	0	0
280 kPa	4	2	0	0
280 kPa	5	1	1	1
280 kPa	5	2	0	1
280 kPa	6	1	0	1
280 kPa	6	2	0	1
280 kPa	7	1	0	0
280 kPa	7	2	0	0
280 kPa	8	1	0	1
280 kPa	8	2	0	0
280 kPa	9	1	1	0
280 kPa	9	2	0	0
280 kPa	10	1	0	0
280 kPa	10	2	0	0
N=10			**20% (single) 10% (multiple)**	**60%**

*Value represents the total incidence of hematuria from 3 observed sections

**Value represents the total incidence of hemmorhage from 3 observed sections

Table 8: Data Summary of Histological Examination of Juvenile Rainbow Trout Livers Exposed to Varying IPC 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

Table 8: Data Summary of Histological Examination of Juvenile Rainbow Trout Livers Exposed to Varying IPC Intensities (Continued)

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-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

Table 9: Data Summary of Hyperemia in Gills of Juvenile Rainbow Trout Exposed to IPC's 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)**	v	**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)**	v	**280-1(2/2)**	v (slight)
CB-1(2/2)	X	**69-1(2/2)**	X	**239-1(2/2)**	v	**280-1(2/2)**	X
%	0		0		75%		25%
CB-2(1/2)	X	69-2(1/2)	v	239-2(1/2)	v	280-2(1/2)	X
CB-2(1/2)	X	69-2(1/2)	X	239-2(1/2)	v	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)	v	69-2(2/2)	X	239-2(2/2)	X	280-2(2/2)	X
%	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)**	v
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
%	0		0		0		25%
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	X	280-4(1/2)	v
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	X	280-4(1/2)	v
CB-4(2/2)	X	69-4(2/2)	X	239-4(2/2)	X	280-4(2/2)	v
CB-4(2/2)	X	69-4(2/2)	X	239-4(2/2)	X	280-4(2/2)	X
%	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)	v	**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)**	v
CB-5(2/2)	X	**69-5(2/2)**	v	**239-5(2/2)**	X	**280-5(2/2)**	v
%	25%		25%		0		50%
CB-6(1/2)	X	69-6(1/2)	v	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)	v
CB-6(2/2)	X	69-6(2/2)	X	239-6(2/2)	X	280-6(2/2)	X
%	0		25%		0		25%
CB-7(1/2)	v	**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)	v	**69-7(2/2)**	X	**239-7(2/2)**	X	**280-7(2/2)**	X
CB-7(2/2)	v	**69-7(2/2)**	X	**239-7(2/2)**	X	**280-7(2/2)**	v
%	75%		0		0		25%

Table 9: Data Summary of Hyperemia in Gills of Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity (Continued)

Slide #	Presence of Hyperemia	Slide #	Presence of Hyperemia	Slide #	Presence of Hyperemia	Slide #	Presence of Hyperemia
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
%	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)**	v
%	0		0		0		25%
N/A	**N/A**	69-10(1/3)	X	239-10(1/2)	X	280-10(1/2)	v
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
%	**N/A**		0		0		25%
Mean	13.9%		7.5%		12.5%		27.5%

X: Not Present v: Present

Table 10: Data Summary of Hyperplasia in Gills of Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity

Slide #	Presence of Hyperplasia	Slide #	Presence of Hyperplasia	Slide #	Presence of Hyperplasia	Slide #	Presence of Hyperplasia
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
%	0		0		0		0
CB-2(1/2)	X	69-2(1/2)	v	239-2(1/2)	X	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)	X	280-2(2/2)	X
CB-2(2/2)	X	69-2(2/2)	v(2)	239-2(2/2)	X	280-2(2/2)	X
%	0		50%		0		0
CB-3(1/2)	X	**69-3(1/2)**	X	**239-3(1/2)**	X	**280-3(1/2)**	v
CB-3(1/2)	X	**69-3(1/2)**	X	**239-3(1/2)**	X	**280-3(1/2)**	v
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
%	0		0		0		50%
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	v	280-4(1/2)	X
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	X	280-4(1/2)	v
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)	v	280-4(2/2)	X
%	0		0		50%		25%
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
%	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)	X
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)	v
%	0		0		0		25%
CB-7(1/2)	X	**69-7(1/2)**	X	**239-7(1/2)**	v	**280-7(1/2)**	X
CB-7(1/2)	X	**69-7(1/2)**	X	**239-7(1/2)**	v	**280-7(1/2)**	X
CB-7(2/2)	X	**69-7(2/2)**	X	**239-7(2/2)**	v	**280-7(2/2)**	X
CB-7(2/2)	X	**69-7(2/2)**	X	**239-7(2/2)**	v	**280-7(2/2)**	X
%	0		0		100%		0

Table 10: Data Summary of Hyperplasia in Gills of Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity (Continued)

Slide #	Presence of Hyperplasia	Slide #	Presence of Hyperplasia	Slide #	Presence of Hyperplasia	Slide #	Presence of Hyperplasia
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
%	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)**	X
%	0		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)	v(1)	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
%	**N/A**		16.67%		0		0
Mean	0%		6.67%		15%		10%

X: Not Present v: Present

Table 11: Data Summary of Hemorrhage (Hm) in Gills of Juvenile Rainbow Trout Exposed to IPC's 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
%	0		0		0		0
CB-2(1/2)	X	69-2(1/2)	X	239-2(1/2)	v	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)	v	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
%	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
%	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
%	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
%	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)	v
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
%	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
%	0		0		0		0

Table 11: Data Summary of Hemorrhage (Hm) in Gills of Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity (Continued)

Slide #	Presence of Hemorrhage	Slide #	Presence of Hemorrhage	Slide #	Presence of Hemorrhage	Slide #	Presence of Hemorrhage
CB-8(1/2)	X	69-8(1/2)	X	239-8(1/2)	v	280-8(1/2)	v (slight)
CB-8(1/2)	X	69-8(1/2)	X	239-8(1/2)	X	280-8(1/2)	v (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
%	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)	v	**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
%	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
%	**N/A**		0		0		0
Mean	2.8%		0.0%		7.5%		7.5%

X: Not Present v: Present

Table 12: Data Summary of Thrombocytes (Thr) in Gills of JuvenileRainbow Trout Exposed to IPC's of Varying Intensity

Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])	Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])	Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])	Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])
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)**	v (1° lamellae, 1)
CB-1(2/2)	X	**69-1(2/2)**	X	**239-1(2/2)**	X	**280-1(2/2)**	v (1° lamellae, 1)
%	0		0		0		50%
CB-2(1/2)	X	69-2(1/2)	X	239-2(1/2)	v(1°,1)	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)	v	239-2(2/2)	X	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
%	0		25%		25%		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)**	v(artery,1)	**239-3(1/2)**	X	**280-3(1/2)**	X
CB-3(2/2)	v	**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
%	25%		25%		0		0
CB-4(1/2)	X	69-4(1/2)	X	239-4(1/2)	v(artery,1)	280-4(1/2)	X
CB-4(1/2)	X	69-4(1/2)	v (artery, 1)	239-4(1/2)	X	280-4(1/2)	X
CB-4(2/2)	X	69-4(2/2)	X	239-4(2/2)	v(1°,1)	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
%	0		25%		50%		0
CB5(1/2)	X	**69-5(1/2)**	X	**239-5(1/2)**	v(artery,1)	**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
%	0		0		25%		0
CB-6(1/2)	v	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)	X
CB-6(2/2)	X	69-6(2/2)	X	239-6(2/2)	X	280-6(2/2)	v(1°,1)
CB-6(2/2)	X	69-6(2/2)	X	239-6(2/2)	X	280-6(2/2)	X
%	25%		0		0		25%

Table 12: Data Summary of Thrombocytes (Thr) in Gills of Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity (Continued)

Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])	Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])	Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])	Slide #	Presence of Thrombocytes (location, degree) [1-nul or little, 2-medium, 3-extensive])
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
%	0		0		0		0
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)	v(1)	239-8(2/2)	X	280-8(2/2)	X
CB-8(2/2)	X	69-8(2/2)	v	239-8(2/2)	X	280-8(2/2)	X
%	0		50%		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)**	X
%	0		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
%	N/A		0		0		0
Mean	5.6%		12.5%		10.0%		7.5%

X: Not Present v: Present

Table 13: Data Summary for Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity

			Liver	Gill				Swim-bladder*	Eyes***	Kidney^/^^		
Fish #	Treatment	Lgth (cm)	Thr Averages **/ $	Hyperplasia Percentages */**	Hm Percentages */**	Hyperemia Percentages */**	Thr Percentages */**			Hematuria (single)	Hematuria (multiple)	Hm
1	Control	5.5	1	0	0	0	0	0	0.406	0	0	0
2	Control	5.4	1.25	0	0	25	0	0	0.413	0	0	0
3	Control	4.8	2.25	0	0	0	25	0	0.441	1	0	0
4	Control	4.8	1.5	0	0	0	0	0	0.315	1	0	0
5	Control	5.4	2.75	0	0	25	0	0	0.374	0	0	0
6	Control	4.9	2	0	0	0	25	0	0.479	0	0	0
7	Control	5.5	2	0	0	75	0	0	0.345	1	0	0
8	Control	4.1	2.25	0	0	0	0	0	0.507	0	0	0
9	Control	4.3	3	0	25	0	0	0	0.531	0	0	0
1	69 kPa	5.6	1.75	0	0	0	0	0	0.546	0	0	0
2	69 kPa	6.0	2	50	0	25	25	0	0.402	0	0	1
3	69 kPa	5.4	1	0	0	0	25	0	0.715	0	0	0
4	69 kPa	5.1	2.5	0	0	0	25	1	0.716	0	0	0
5	69 kPa	5.2	1	0	0	25	0	0	0.666	0	0	0
6	69 kPa	5.0	2	0	0	25	0	0	0.908	0	0	1
7	69 kPa	5.2	2.25	0	0	0	0	0	0.359	0	0	1
8	69 kPa	5.0	2.5	0	0	0	50	0	0.626	0	0	0
9	69 kPa	4.5	1.5	0	0	0	0	0	0.500	0	0	0
10	69 kPa	4.1	1.25	17	0	0	0	0	0.724	N/A	N/A	N/A
1	239 kPa	6.9	1	0	0	75	0	0	0.400	0	1	1
2	239 kPa	6.4	1.75	0	50	50	25	1	0.266	N/A	N/A	N/A
3	239 kPa	5.3	2	0	0	0	0	1	0.404	0	1	1
4	239 kPa	6.1	1.5	50	0	0	50	0	0.387	0	0	1
5	239 kPa	5.5	1	0	0	0	25	1	0.413	0	1	0
6	239 kPa	4.3	1	0	0	0	0	1	0.573	0	0	0
7	239 kPa	5.0	3	100	0	0	0	0	0.419	0	1	1
8	239 kPa	4.7	1.75	0	25	0	0	1	0.573	N/A	N/A	N/A
9	239 kPa	4.0	2	0	0	0	0	0	0.742	0	0	0
10	239 kPa	5.5	1.5	0	0	0	0	1	0.395	0	1	1

Table 13: Data Summary for Juvenile Rainbow Trout Exposed to IPC's of Varying Intensity (Continued)

			Liver		Gill			Swim-bladder*	Eyes***	Kidney^/^^		
Fish #	Treatment	Lgth (cm)	Thr Averages **/ $	Hyperplasia Percentages */**	Hm Percentages */**	Hyperemia Percentages */**	Thr Percentages */**			Hematuria (single)	Hematuria (multiple)	Hm
1	280 kPa	5.7	1.75 (!)	0	0	25	50	1	0.679	0	1	1
2	280 kPa	5.7	1	0	0	0	0	0	0.370	0	0	1
3	280 kPa	4.9	1.5	50	0	25	0	0	0.524	0	0	1
4	280 kPa	4.6	2.5	25	0	75	0	0	0.647	0	0	0
5	280 kPa	4.7	1	0	0	25	0	0	0.680	1	0	1
6	280 kPa	4.9	1	25	25	25	25	1	0.984	0	0	1
7	280 kPa	4.7	1.25	0	0	25	0	0	0.423	0	0	0
8	280 kPa	4.6	1.25	0	50	0	0	0	1.040	0	0	1
9	280 kPa	4.4	3	0	0	25	0	0	0.527	1	0	0
10	280 kPa	5.0	2.75	0	0	25	0	1	0.545	0	0	0

* scored 1= present, 0 = not present. In the case of swimbladders, 1=non-intact swimbladder, 0= intact swimbladder
** averaged value for 4 determinations on 2 to 3 separate slide sections…
$ were scored either 1=nil or very few thrombocytes (1-4 congregations), 2 = an intermediate amount of thrombocytes (5-10 congregations), 3 = extensive thromobocytes (>10 congregations)
*** Eye area/fish fork length ratio
^ twelve random digitized fields (=141 X 106 mm) from the kidney of each fish were examined. Chart displays score of 1=present 0= not present
^^ graphed as number (present/N)*100=percentage

Table 14: Data Table of Statistical Results

Life Stage	Parameter	Test	P Value	Figure #
Eyed Eggs	Cranial width at eye midline	ANOVA	0.9500	-
	Eye midline to highest peak on head	ANOVA	0.3940	-
	Width between orbits	ANOVA	0.4469	-
	Area of upper cranium from eye midline	ANOVA	**0.0127**	5
	Head circumference	ANOVA	**0.0008**	6
	S Factors	ANOVA	0.7636	-
Sac Fry	Swimbladder Tears	Cochran-Armitage	0.7167	8
Juvenile	Length	ANOVA	0.4035	
	Swimbladder Tears	Cochran-Armitage	**0.0016**	9
	Eye Displacement	ANOVA	**0.000**	10
	Kidneys Hematuria (Single)	Cochran-Armitage with Bootstrap P-value adjustment	0.3683	12
	Kidneys Hematuria (Multiple)	Cochran-Armitage with Bootstrap P-value adjustment	**0.0010**	12
	Kidneys Hemorrhaging	Cochran-Armitage with Bootstrap P-value adjustment	**0.0053**	13
	Liver Thrombocyte	Kruskal-Wallis	0.6075	15
	Gills Hyperemia	Kruskal-Wallis	0.0646	16
	Gills Hyperplasia	Kruskal-Wallis	0.4361	17
	Gills Hemorrhaging	Kruskal-Wallis	0.5045	18
	Gills Thrombocyte	Kruskal-Wallis	0.7844	19

Text in bold represents significant P-values

Appendices

Appendix I: Seismic Effects on Fish

Authors	Effects	Affected fish including size	Pressure Source and Intensity
Alpin 1947	➢ Burst Swimbladders ➢ Ruptured blood vessels ➢ Crushed body contents ➢ Broken Ribs ➢ Death	Variety of fish of unknown weights + some ≥½ pound 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*)	<u>Explosive</u> *10 pounds (lbs) petrogel in 10 pound sticks* 10 shots of 10 lbs 18 shots of 20 lbs 18 shots of 40 lbs <u>Intensity</u> N/A
Bishai 1962 (Larval and young fish)	➢ Formation of gas bubbles in the heart and blood vessels, under the skin and over the fins ➢ Bulging of the eyes ➢ Death	*Size*: Herring *(Clupea harengus* L.*)* 6.5-8mm (average) Salmon *(Salmo salar* L.*)* 20-25mm (average) Sea Trout *(Salmo trutta* L.*)* 18-24mm (average) Brown Trout *(Salmo trutta f. fario* L.) 16-25mm Plaice *(Pleuronectes platessa*	<u>No Detonation</u> Pressures were applied using a pressure and a decompression apparatus, capable of exerting pressures of 227 kPa and 13.33 kPa, respectively

		L.) 37-50mm *Age*: -Newly hatched larvae (herring 6.5-8mm), (Salmon, sea trout, brown trout 16.20mm) -17 day old larvae (salmon, sea trout and brown trout) -30 day old larvae (salmon, sea trout and brown trout) -56 day old larvae (salmon, sea trout and brown trout) -98 day old brown trout -102 day old brown trout -103 day old brown trout -105 day old brown trout -133 day old brown trout -178 day old salmon -195 day old salmon -272 day old brown trout -220 day old salmon and brown trout	
Coker and Hollis 1950	➢ Severe body trauma (Menhaden) ➢ Dislocated mouthparts and lacerated sides (Menhaden) ➢ Protruding eyes ➢ Gut protruding 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 Spleen ➢ Death	Fry, Larvae and Adults 3-106cm (Unknown weights) Menhaden (*Brevoortia tyrannus*) Croaker (*Micropogon undulates*) Trout, gray and spotted (*Cynoscion regalis and C.nebulosus*) Rock, Striped Bass (*Roccus saxatilis*) Alewife Branch Herring (*Pomolobus pseudoharengus*) Alewife Glut Herring (*Pomolobus aestivalis*) Hickory shad Hickory Jack (*Pomolobus mediocris*)	Explosive 26 charges of Hexahydro - 1, 3, 5 Trinitro-8-Triazine (HBX), ranging from 250 to 1,200 lbs.

		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*)	
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 320mm* (*Cynoscion regalis* Bloch and Schneider) Rock 330mm* (*Roccus saxatilis* Walbaum) *N.B: These two species provided the statistical data	<u>Explosive</u> TNT/Nitram on explosions; 28-303 lbs charges <u>Intensity</u> 0-5516 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 FL *60% Geogel experiment*: Arctic Cisco (*Coregonus autumnalis*) 135mm-290mm FL *Air Gun:* Young Coregonids 70mm-100mm FL Various fish resulting from experimental shots (*C.nasus, C. autumnalis* including immature coregonids)	<u>Explosives +Air Gun</u> *Aquaflex* 200 grains/ft and was used in 165 ft lengths (4.9 lbs) *60% Geogel* supplied in 5 lb paper cartridges and charge sizes for experiments were 2.5 and 10 lbs *Air gun* Par air gun, 300in^3 <u>Intensity</u> *Aquaflex* N/A *60% Geogel*

			N/A *Air Gun* 13,789 kPa and 15,168 kPa
Ferguson 1962	Injury to fish collected at the surface: Yellow Perch ➢ 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 ➢ 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 ES 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	Fish collected at surface: -Yellow Perch *(Perca flavescens)* -Lake Emerald Shiner (*Notropis atherinoides*) -American Smelt (*Osmerus mordax)* -White Bass (*Morone chrysops*)	<u>Explosive</u> Nitrone, Nitrone primer, or 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 <u>Intensity</u> N/A
Fitch and Young 1948 (Including larval anchovies)	➢ Rupture of air bladder ➢ Damaged/crushed viscera ➢ Ribs broken from backbone ➢ Death	Variety of fish (Unknown weight/lengths) 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*)	<u>Explosive</u> Type: N/A The weight of explosive in a single shot has varied from 10 lbs to 160 lbs. Usually 40 or 80 lbs for open shots and 20 lbs for jet shots <u>Intensity</u> N/A

		Barracuda (*Sphyraena argentea*) Pacific Mackerel (*Scomber japonicus*) White Sea Bass (*Lates 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*)	
Govoni et al 2002, 2003 (Juveniles)	➢ Internal hemorrhaging ➢ Coelomic hemorrhage (in both exposed and control, could not be related to rupture of specific arteries or veins) 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	Explosive 12-grain Primadet PDT 1403 detonators Intensity Average P_{max} Values: 636.92 kPa (3.6m from blast) 230.86 kPa (7.5m from blast) 109.93 kPa (17m from blast)
Hogan 1941	➢ Loss of balance ➢ Bulging eyes	Bluegill bream (*Lepomis macrochirus*)	No Detonation Pressures

	➢ Blood drawn to towards the surface of the body ➢ Possible bursting of swimbladders ➢ Death	4 inches long Golden shiner (*Notemigonus crysoleucas*) Crappies (*Pomoxis* sp.) 4 inches long as well as others 12 oz each Largemouth black bass (*Micropterus salmoides*) 7 inches long as well as other 12 oz each and other 3 lbs each Carp (*Cyprinus carpio*) 8 and 10 inches long Bullhead catfish (*Ameiurus* sp.) 4-16 oz Long nosed gar (*Lepisosteus osseus*) 11.5 inches	were applied using a vacuum apparatus <u>Intensity</u> Siphon was operating at a vacuum pressure between 81.3 kPa and 84.7 kPa
Hubbs and Rechnitzer 1952	➢ Negative pressure may cause injury or death in part through the formation of bubbles in the body fluids, as it may liberate dissolved gases suddenly enough and in sufficient quantity to rupture the walls of unprotected blood vessels. ➢ Gas filled visceral cavity from a ruptured air bladder ➢ Hemorrhaging ➢ Death	Fish used in experimental cages: (unknown weights/lengths) 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)	<u>Explosives and Black Powder</u> *Explosive:* a)Ten (10) lb charges of 60% Dynamite b)Five (5) lb jetted charge of 60% Dynamite *Black Powder:* c)Hercules FFG or FFFG powder Up to 45 pounds <u>Intensity</u> a) 165.4-9259.6 kPa b) 951.4-1331.0 kPa c) 10.3-1103.2 kPa
Hubbs et al. 1960	➢ Light Hemorrhaging, principally in tissues covering the kidney	Northern Anchovy *(Engraulis mordax*)	<u>Explosive</u> Nitramon

	➢ Light hemorrhaging throughout the body cavity, with some damage to the kidney ➢ Burst swimbladder ➢ Gross damage to kidney ➢ Bleeding around the anus ➢ Gross damage to internal organs (lost or homogenized abdominal contents) ➢ Breaking through of body wall (partial)	half grown-small maturing to mature adults Pacific Sardine (*Sardinops caerulea*) medium to large, maturing to mature adults Jack Mackerel (*Trachurus symmtricus*) medium sized	Seismic Nitramon Water-Work Vibronite B Vibronite S <u>Intensity</u> 275.8-3792.1 kPa (includes all detonations)
Kearns and Boyd 1965	➢ Internal Ruptures ➢ Displaced Visceral Organs ➢ Torn Musculature ➢ Hemorrhaging Blood Vessels ➢ Protruding Eyes ➢ Everted Stomachs ➢ Gas Bladder Damage ➢ Rupture of Hemorrhage of: Gas bladder Abdominal vein Spleen Liver Ripe gonads ➢ Death	Unknown weights and lengths Rockfish (*Sebastodes species*) Herring (*Clupea pallasii*) Salmon (Adult and Juvenile) *(Oncorhynchus tshawytscha* and *O.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*)	<u>Explosive</u> Nitrone S.M (Seismic Marine) with nitro-carbo-nitrate (NCN) high explosive primer <u>Intensity</u> N/A Produces a detonation velocity of 15,100 feet/sec

		Sculpin (Genus/species unidentified)	
Kostyuchencko 1948 (Fish Eggs)	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 and Larvae (unknown length/weight) Anchovy (*Engraulis encrasicholus ponticus* Aleksandrov) Blue runner (*Caranx crysos*) Crucian Carp (*Carassius carassius*) Red Mullet (*Mullus barbatus ponticus* Essipov)	<u>Explosive (TNT), Air Gun, and Electric pulse Generator</u> <u>Intensity</u> N/A Air gun had a working pressure of 14,185 kPa
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*) 32cm Black Drum (*Pogonias cromis*) 23cm Anchovy*** (*Anchoa mitchilli*) Atlantic Croaker*** (*Micropogonias undulates*) ***no weights or lengths available	<u>Explosive</u> Primacord detonation cord Intensity: N/A
Muir 1959	Pressure and Vacuum Tests ➢ Ruptured swimbladders ➢ Gas bubbles issued from either/and mouth, operculum, anus ➢ Eye extrusion ➢ The bends ➢ Death Cavitation Tests (Cavitation) ➢ Hemorrhages in one or both eyes ➢ Hemorrhages in some of the gill plates ➢ Death	1957 Cohoe salmon (*Oncorhynchus kisutch*) (>1 yr) Sockeye salmon (*Oncorhynchus nerka*) (nearly 2 years) 1958 Cohoe salmon (fingerlings) (*Oncorhynchus kisutch*) 3.8-10.2cm	<u>No Detonation</u> Pressures were applied using a pressure and a vacuum apparatus <u>Intensity</u> Pressures between 152 and 745 kPa Partial Vacuum pressures between 16.9 and 98.2 kPa
Rasmussen 1964 (A review paper)	➢ Burst Swimbladder ➢ Death	N/A	N/A
Roguski and Nagata 1970	Yearling king salmon: ➢ Hemorrhaging from vent	Yearling king salmon (*Oncorhynchus*	<u>Explosive</u> 130.5-940 lbs C-

	➢ 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	*tshawytscha*) 17.8-25.4cm Northern pike (*Esox lucius*) Mean: 50.8cm	4 explosive <u>Intensity</u> N/A
Settle et al 2002	*Within 24 hours of blast* ➢ Evisceration ➢ Haemorrhaging dorsal to swimbladder and ventral to 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 (N.B: fish were examined only for a 24 hour period)	Late stage larval and recently metamorphosed fishes Spot (*Leiostomus xanthurus*) 18.0-20.1mm Pinfish (*Lagodon rhomboids*) 15.9-17.2mm	Explosive 12-grain Primadet PDT detonators Intensity Average P_{max} Values for spot and pinfish exposures resulting from each blast induced underwater shock: 639.4 kPa (3.6m from blast) 231.3 kPa (7.5m from blast) 110.6 kPa (17m from blast)
Thomson 1958	➢ Protrusion of ocular area ➢ Distended abdomen ➢ Frayed gills ➢ Ruptured air bladders ➢ Blood clots about the heart, liver and kidney ➢ Death	Variety of fish (unknown size/weight) Including herring eggs *Caged Fish:* Lingcod (*Ophiodon elongates*) Lemon Sole (*Parophrys vetulus*) Grey Cod (*Gadus macroephalus*) Blackcod (*Anoplopoma fimbria*)	<u>Explosive</u> DuPont Nitramex 2-H <u>Intensity</u> N/A

<table>
<tr><td></td><td></td><td>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)</td><td></td></tr>
<tr><td>Tsvetkov et al 1972</td><td>Under Compression:
➢ Fright response
➢ Sharp increase in motor activity and random darting movements
Under Negative Pressure:
➢ Rupture of swimbladder walls
➢ Rupture of body walls (most often along the keel), with internal organs sometimes seen through this rupture.
➢ Interference of internal organ function from compression during swimbladder expansion
➢ Death

Attributed to "Gas Disease" from pressure changes
➢ Hemorrhaging of eyes
➢ Hemorrhages and accumulation of gas bubble in fins
➢ Hemorrhages in the peritoneum and kidneys
➢ Gas bubbles ruptured the walls of vessels, expanded body tissues and emerged into the body cavity of externally beneath the skin
➢ Bulging of the eyes</td><td><u>Young Fish</u>
Larvae and Fingerling
"verkhovka"
[leucaspius delineatus (Heck.)]
15-25, 33-60mm

Roach
[Rutilus rutilus(L.)]
$B\text{-}C_2$*, 20-25, 60mm

Bleak
[(Alburnus alburnus (L.)]
C_1-F*

Perch
[(Perca fluviatilis L.)
F-G*, 25-30mm

Atlantic salmon
[Salmo salar (L.)]
20-25mm (no swimbladder)

Siberian sturgeon
(Acipenser guldenstädti Brandt)
30mm

Cicodace
(Coregonus albula (L.))
18-30mm

*= developmental stanza

<u>Older Fish</u>
Juvenile and Adult

Crucian Carp
[Carassius carassius (L.)]</td><td><u>No Detonation</u>
Pressure changes were applied using a test apparatus

<u>Intensity</u>
Pump created air pressure above the surface of the water of 101-608 kPa and thereafter, the excess pressure was reduced at rates of 10-608 kPa/sec</td></tr>
</table>

		83mm Ciscodace (*Coregonus albula* (L.)) 130mm Grayling [*Thymallus thymallus* (L.)] 150mm "Verkhovka" [*leucaspius delineatus* (Heck.)] 33-60mm Roach [*Rutilus rutilus(L.)*] 60mm Minnow [*Phoxinus phonxiuns* *(L.)*] 60-70mm Atlantic salmon [*Salmo salar (L.)*] 60-80mm, Parr, 90mm Russian Sturgeon [*Acipenser* *guldenstädti Brandt*] 90mm	
Wiley et al. 1981	- Internal Hemorrhaging\ - Bruising of the Kidneys - Burst Swimbladder - Death	8.3-24.6cm (83mm-246mm) 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	<u>Explosive</u> Cylindrical cast pentolite charges (50/50 mixture, PETN and TNT) Intensity 210 kPa-3120 kPa (Levels represent explosive pressures applied to Spot and White perch)

		(*Fundulus majalis*) 88mm Atlantic menhaden (*Brevoortia tyrannus*) N/A Blueback Herring (*Alosa aestivalis*) N/A NB : Weights and lengths are expressed as means ฆ: Primary test fish NB : While thirteen species of fish were used in explosion tests, only those presented above were discussed in the paper	
Yelverton et al 1975	➢ Disorientation ➢ Decreased movement ➢ Erratic gill movement ➢ Internal organs most commonly damaged: Swimbladders Kidneys Livers ➢ Hemorrhaging of aforementioned organs ➢ Rupture of abdominal walls ➢ Death	*Physostomes:* Top Minnow (*Gambusia affinis*) 0.47g Goldfish (*Carrasius auratus*) 1.4g and 245g Carp (*Cyprinus carpio*) 149g and 117g and 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	<u>High Explosive Charges</u> Bare spheres of Pentolite, fired by electric blasting caps (Dupont E99) <u>Intensity:</u> Peak Pressure: 530 kPa-9025 kPa Impulse: 4.1-428 kPa/msec

Authors	Effects	Affected fish including size	Pressure Source and Intensity
Alpin 1947	➢ Burst Swimbladders ➢ Ruptured blood vessels ➢ Crushed body contents ➢ Broken Ribs ➢ Death	Variety of fish of unknown weights + some ≥½ pound 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*)	<u>Explosive</u> *10 pounds (lbs) petrogel in 10 pound sticks* 10 shots of 10 lbs 18 shots of 20 lbs 18 shots of 40 lbs <u>Intensity</u> N/A
Bishai 1962 (Larval and young fish)	➢ Formation of gas bubbles in the heart and blood vessels, under the skin and over the fins ➢ Bulging of the eyes ➢ Death	*Size*: Herring *(Clupea harengus* L.*)* 6.5-8mm (average) Salmon *(Salmo salar* L.*)* 20-25mm (average) Sea Trout *(Salmo trutta* L.*)* 18-24mm (average) Brown Trout *(Salmo trutta f. fario* L.) 16-25mm Plaice *(Pleuronectes platessa* L.)	<u>No Detonation</u> Pressures were applied using a pressure and a decompression apparatus, capable of exerting pressures of 227 kPa and 13.33 kPa, respectively

		37-50mm *Age*: -Newly hatched larvae (herring 6.5-8mm), (Salmon, sea trout, brown trout 16.20mm) -17 day old larvae (salmon, sea trout and brown trout) -30 day old larvae (salmon, sea trout and brown trout) -56 day old larvae (salmon, sea trout and brown trout) -98 day old brown trout -102 day old brown trout -103 day old brown trout -105 day old brown trout -133 day old brown trout -178 day old salmon -195 day old salmon -272 day old brown trout -220 day old salmon and brown trout	
Coker and Hollis 1950	➢ Severe body trauma (Menhaden) ➢ Dislocated mouthparts and lacerated sides (Menhaden) ➢ Protruding eyes ➢ Gut protruding 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 Spleen ➢ Death	Fry, Larvae and Adults 3-106cm (Unknown weights) Menhaden *(Brevoortia tyrannus*) Croaker (*Micropogon undulates*) Trout, gray and spotted (*Cynoscion regalis and C.nebulosus*) Rock, Striped Bass (*Roccus saxatilis*) Alewife Branch Herring (*Pomolobus pseudoharengus*) Alewife Glut Herring (*Pomolobus aestivalis*) Hickory shad	Explosive 26 charges of Hexahydro - 1, 3, 5 Trinitro-8-Triazine (HBX), ranging from 250 to 1,200 lbs.

		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*)	
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 320mm* (*Cynoscion regalis* Bloch and Schneider) Rock 330mm* (*Roccus saxatilis* Walbaum) *N.B: These two species provided the statistical data	<u>Explosive</u> TNT/Nitram on explosions; 28-303 lbs charges <u>Intensity</u> 0-5516 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 FL *60% Geogel experiment*: Arctic Cisco (*Coregonus autumnalis*) 135mm-290mm FL *Air Gun:* Young Coregonids 70mm-100mm FL Various fish resulting from experimental shots (*C.nasus, C.*	<u>Explosives +Air Gun</u> *Aquaflex* 200 grains/ft and was used in 165 ft lengths (4.9 lbs) *60% Geogel* supplied in 5 lb paper cartridges and charge sizes for experiments were 2.5 and 10 lbs *Air gun* Par air gun, $300in^3$

		autumnalis including immature coregonids)	Intensity *Aquaflex* N/A *60% Geogel* N/A *Air Gun* 13,789 kPa and 15,168 kPa
Ferguson 1962	Injury to fish collected at the surface: Yellow Perch ➢ 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 ➢ 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 ES 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	Fish collected at surface: -Yellow Perch *(Perca flavescens)* -Lake Emerald Shiner (*Notropis atherinoides*) -American Smelt (*Osmerus mordax)* -White Bass (*Morone chrysops*)	Explosive Nitrone, Nitrone primer, or 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 Intensity N/A
Fitch and Young 1948 (Including larval anchovies)	➢ Rupture of air bladder ➢ Damaged/crushed viscera ➢ Ribs broken from backbone ➢ Death	Variety of fish (Unknown weight/lengths) that include: California Halibut (*Paralichthys californicus*) Midshipmen (*Porichthys notatus* and *P. myriaster*) Jack Mackerel (*Trachurus symmtricus*) Rockfish (*Sebastodes paucispinis*) Sardine	Explosive Type: N/A The weight of explosive in a single shot has varied from 10 lbs to 160 lbs. Usually 40 or 80 lbs for open shots and 20 lbs for jet shots Intensity N/A

		(*Sardinops* species) Kingfish (*Genyonemus lineatus*) Perch (*Brachyistius frenatus*) Barracuda (*Sphyraena argentea*) Pacific Mackerel (*Scomber japonicus*) White Sea Bass (*Lates 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*)	
Govoni et al 2002, 2003 (Juveniles)	➢ Internal hemorrhaging ➢ Coelomic hemorrhage (in both exposed and control, could not be related to rupture of specific arteries or veins) 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	Pinfish (*Lagodon rhomboids*) 13.8-21.3mm Spot (*Leiostomus xanthurus*) 15.1-25.3mm	Explosive 12-grain Primadet PDT 1403 detonators Intensity Average P_{max} Values: 636.92 kPa (3.6m from blast) 230.86 kPa (7.5m from blast) 109.93 kPa (17m from blast)

	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		
Hogan 1941	➢ Loss of balance ➢ Bulging eyes ➢ Blood drawn to towards the surface of the body ➢ Possible bursting of swimbladders ➢ Death	Bluegill bream (*Lepomis macrochirus*) 4 inches long Golden shiner (*Notemigonus crysoleucas*) Crappies (*Pomoxis* sp.) 4 inches long as well as others 12 oz each Largemouth black bass (*Micropterus salmoides*) 7 inches long as well as other 12 oz each and other 3 lbs each Carp (*Cyprinus carpio*) 8 and 10 inches long Bullhead catfish (*Ameiurus* sp.) 4-16 oz Long nosed gar (*Lepisosteus osseus*) 11.5 inches	<u>No Detonation</u> Pressures were applied using a vacuum apparatus <u>Intensity</u> Siphon was operating at a vacuum pressure between 81.3 kPa and 84.7 kPa
Hubbs and Rechnitzer 1952	➢ Negative pressure may cause injury or death in part through the formation of bubbles in the body fluids, as it may liberate dissolved gases suddenly enough and in sufficient quantity to rupture the walls of unprotected blood vessels. ➢ Gas filled visceral cavity from a ruptured air bladder ➢ Hemorrhaging ➢ Death	Fish used in experimental cages: (unknown weights/lengths) Pacific sardine (*Sardinops caerulea* Girard) Ocean northern anchovy (*Engraulis mordax mordax* Girard) Grunion (*Leuresthes tenuis* Ayres) Jack mackerel (*Trachurus symmetricus* Ayres)	<u>Explosives and Black Powder</u> *Explosive:* a)Ten (10) lb charges of 60% Dynamite b)Five (5) lb jetted charge of 60% Dynamite *Black Powder:* c)Hercules FFG or FFFG powder Up to 45 pounds <u>Intensity</u> a) 165.4-9259.6 kPa b) 951.4-1331.0

		California pompano (*Palometa simillima* Ayres) Kingfish (*Genyonemus lineatus* Ayres) Queenfish (*Seriphus politus* Ayres)	kPa c) 10.3-1103.2 kPa
Hubbs et al. 1960	➢ Light Hemorrhaging, principally in tissues covering the kidney ➢ Light hemorrhaging throughout the body cavity, with some damage to the kidney ➢ Burst swimbladder ➢ Gross damage to kidney ➢ Bleeding around the anus ➢ Gross damage to internal organs (lost or homogenized abdominal contents) ➢ Breaking through of body wall (partial)	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 symmtricus*) medium sized	Explosive Nitramon Seismic Nitramon Water-Work Vibronite B Vibronite S Intensity 275.8-3792.1 kPa (includes all detonations)
Kearns and Boyd 1965	➢ Internal Ruptures ➢ Displaced Visceral Organs ➢ Torn Musculature ➢ Hemorrhaging Blood Vessels ➢ Protruding Eyes ➢ Everted Stomachs ➢ Gas Bladder Damage ➢ Rupture of Hemorrhage of: Gas bladder Abdominal vein Spleen Liver Ripe gonads ➢ Death	Unknown weights and lengths Rockfish (*Sebastodes species*) Herring (*Clupea pallasii*) Salmon (Adult and Juvenile) *(Oncorhynchus tshawytscha* and *O.nerka)* Miscellaneous including: Needle Fish (Genus/species unknown) Jack Mackerel (*Trachurus symmetricus*) Whiting *(Theragra chalcogrammus)* Grey Cod *(Gadus macrocephalus)*	Explosive Nitrone S.M (Seismic Marine) with nitro-carbo-nitrate (NCN) high explosive primer Intensity N/A Produces a detonation velocity of 15,100 feet/sec

		Pacific Hake *(Merluccius produtuctus)* Sea Perch *(Cymatogaster aggregata)* Pacific sandlance (*Ammodytes hexapterus*) Tube snout (*Aulorhynchus flavidus*) Sculpin (Genus/species unidentified)	
Kostyuchencko 1948 (Fish Eggs)	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 and Larvae (unknown length/weight) Anchovy (*Engraulis encrasicholus ponticus* Aleksandrov) Blue runner (*Caranx crysos*) Crucian Carp (*Carassius carassius*) Red Mullet (*Mullus barbatus ponticus* Essipov)	Explosive (TNT), Air Gun, and Electric pulse Generator Intensity N/A Air gun had a working pressure of 14,185 kPa
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	Red Drum (*Sciaenops ocellatus*) 32cm Black Drum (*Pogonias cromis*) 23cm Anchovy*** (*Anchoa mitchilli*) Atlantic Croaker*** (*Micropogonias undulates*) ***no weights or lengths available	Explosive Primacord detonation cord Intensity: N/A

	➢ Death		
Muir 1959	Pressure and Vacuum Tests ➢ Ruptured swimbladders ➢ Gas bubbles issued from either/and mouth, operculum, anus ➢ Eye extrusion ➢ The bends ➢ Death Cavitation Tests (Cavitation) ➢ Hemorrhages in one or both eyes ➢ Hemorrhages in some of the gill plates ➢ Death	1957 Cohoe salmon (*Oncorhynchus kisutch*) (>1 yr) Sockeye salmon (*Oncorhynchus nerka*) (nearly 2 years) 1958 Cohoe salmon (fingerlings) (*Oncorhynchus kisutch*) 3.8-10.2cm	<u>No Detonation</u> Pressures were applied using a pressure and a vacuum apparatus <u>Intensity</u> Pressures between 152 and 745 kPa Partial Vacuum pressures between 16.9 and 98.2 kPa
Rasmussen 1964 (A review paper)	➢ Burst Swimbladder ➢ Death	N/A	N/A
Roguski and Nagata 1970	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	<u>Explosive</u> 130.5-940 lbs C-4 explosive <u>Intensity</u> N/A
Settle et al 2002	*Within 24 hours of blast* ➢ Evisceration ➢ Haemorrhaging dorsal to swimbladder and ventral to 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 (N.B: fish were examined only for a 24 hour period)	Late stage larval and recently metamorphosed fishes Spot (*Leiostomus xanthurus*) 18.0-20.1mm Pinfish (*Lagodon rhomboids*) 15.9-17.2mm	Explosive 12-grain Primadet PDT detonators Intensity Average P_{max} Values for spot and pinfish exposures resulting from each blast induced underwater shock: 639.4 kPa (3.6m from blast)

			231.3 kPa (7.5m from blast) 110.6 kPa (17m from blast)
Thomson 1958	➢ Protrusion of ocular area ➢ Distended abdomen ➢ Frayed gills ➢ Ruptured air bladders ➢ Blood clots about the heart, liver and kidney ➢ Death	Variety of fish (unknown size/weight) Including herring eggs *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*)	<u>Explosive</u> DuPont Nitramex 2-H <u>Intensity</u> N/A
Tsvetkov et al 1972	Under Compression: ➢ Fright response ➢ Sharp increase in motor activity and random darting movements Under Negative Pressure: ➢ Rupture of swimbladder walls ➢ Rupture of body walls (most often along the keel), with internal organs sometimes seen through this rupture. ➢ Interference of internal organ function from compression during swimbladder expansion ➢ Death *Attributed to "Gas Disease" from pressure changes* ➢ Hemorrhaging of eyes	<u>Young Fish</u> *Larvae and Fingerling* "verkhovka" [leucaspius delineatus (Heck.)] 15-25, 33-60mm Roach [*Rutilus rutilus(L.)*] B-C_2*, 20-25, 60mm Bleak [(*Alburnus alburnus* (L.)] C_1-F* Perch	<u>No Detonation</u> Pressure changes were applied using a test apparatus <u>Intensity</u> Pump created air pressure above the surface of the water of 101-608 kPa and thereafter, the excess pressure was reduced at rates of 10-608 kPa/sec

	➢ Hemorrhages and accumulation of gas bubble in fins ➢ Hemorrhages in the peritoneum and kidneys ➢ Gas bubbles ruptured the walls of vessels, expanded body tissues and emerged into the body cavity of externally beneath the skin ➢ Bulging of the eyes	[(*Perca fluviatilis* L.) F-G*, 25-30mm Atlantic salmon [*Salmo salar* (L.)] 20-25mm (no swimbladder) Siberian sturgeon (*Acipenser guldenstädti* Brandt) 30mm Cicodace (*Coregonus albula* (L.)) 18-30mm *= developmental stanza <u>Older Fish</u> *Juvenile and Adult* Crucian Carp [*Carassius carassius (L.)*] 83mm Ciscodace (*Coregonus albula* (L.)) 130mm Grayling [*Thymallus thymallus* (L.)] 150mm “Verkhovka” [*leucaspius delineatus* (Heck.)] 33-60mm Roach [*Rutilus rutilus(L.)*] 60mm Minnow [*Phoxinus phonxiuns (L.)*] 60-70mm Atlantic salmon [*Salmo salar (L.)*] 60-80mm, Parr, 90mm Russian Sturgeon [*Acipenser guldenstädti Brandt*] 90mm	

Wiley et al. 1981	➢ Internal Hemorrhaging\ ➢ Bruising of the Kidneys ➢ Burst Swimbladder ➢ Death	8.3-24.6cm (83mm-246mm) ₪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 ₪: Primary test fish NB : While thirteen species of fish were used in explosion tests, only those presented above were discussed in the paper	<u>Explosive</u> Cylindrical cast pentolite charges (50/50 mixture, PETN and TNT) Intensity 210 kPa-3120 kPa (Levels represent explosive pressures applied to Spot and White perch)
Yelverton et al 1975	➢ Disorientation ➢ Decreased movement ➢ Erratic gill movement ➢ Internal organs most commonly damaged: Swimbladders Kidneys Livers ➢ Hemorrhaging of aforementioned organs	*Physostomes:* Top Minnow (*Gambusia affinis*) 0.47g Goldfish (*Carrasius auratus*) 1.4g and 245g Carp	<u>High Explosive Charges</u> Bare spheres of Pentolite, fired by electric blasting caps (Dupont E99)

	➢ Rupture of abdominal walls ➢ Death	(*Cyprinus carpio*) 149g and 117g and 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	<u>Intensity:</u> Peak Pressure: 530 kPa-9025 kPa Impulse: 4.1-428 kPa/msec

Appendix II: Variable Determining Effects and Survival of Fish Exposed to Seismic Blasts

Environmental Parameters	1) Bottom composition (Cronin 1948, Hubbs and Rechnitzer 1952, Settle et al 2002, Wright 1982) 2) Depth of bottom (Cronin 1948) 3) Effect of water temperature stratification (Cronin 1948) 4) Water depth (Settle et al 2002, Wright 1982)
Piscine Parameters	1) Depth of fish in the water (Cronin 1948, Settle et al 2002) 2) Weight of fish * (Cronin 1948, Yelverton 1975) 3) Shading effect of fish behind other fish or objects (Cronin 1948) 4) Fish orientation (Cronin 1948) 5) Quantity of fish and physical condition (Cronin 1948) 6) Differences in shock absorption due to positioning of fish (Roguski and Nagata 1970) 7) Physical damage from the enclosures in which the fish are placed (Roguski and Nagata 1970) 8) Species of fish (Linton et al 1985, Settle et al 2002, Wright 1982)) 9) Depth and distance from the site of detonation (Linton et al 1985) 10) Size of fish (Christian 1973, Hubbs and Rechnitzer 1952, Settle et al 2002, Wright 1982) 11) Orientation of the fish relative to the shock wave ** (Fitch and Young 1948, Settle et al 2002, Wright 1982) 12) Age of fish (Bishai 1961) 13) General health of fish (Christian 1973)

	14) Difference in internal anatomy (Wright et al 1981) 15) Structure of fish (Hubbs and Rechnitzer 1952) 16) Anatomy of Fish (Settle et al 2002)
Explosive Parameters	1) Type and depth of charge (Cronin 1948, Settle et al 2002, Wright 1982) 2) Amount of explosive (Wright 1982) 3) Target depth (Wright 1982) 4) Type and amplitude of the pressure pulse (Hubbs and Rechnitzer 1952)

*Yelverton (1975) demonstrates that that the impulse for 50% lethality in fish exposed to underwater blasts increases with body weight and therefore, illustrates that mortality is weight dependant.

** Fish directly facing or turned away from a blast can be more assured of recovery compared to fish which are broadside to the pressure wave (Fitch and Young 1948).