Effect of Seismic Energy on Snow Crab (*Chionoecetes opilio*)

By

and

For

Environmental Studies Research Fund
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by

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EXECUTIVE SUMMARY

Introduction

LGL Limited of St. John's, Newfoundland and Labrador was contracted by the Environmental Studies Research Fund (ESRF) to conduct a study of the effects of seismic air gun noise on snow crab (*Chionoecetes opilio*) and snow crab catches. Loud noise has the potential for detrimental effects on animals by physical damage to sensitive organs (e.g., ear structures in the case of vertebrates), by causing increased stress, or by causing changes in behaviour. At present, the snow crab fishery in Atlantic Canada is concentrated in Newfoundland and Labrador and it is the most important commercial fishery there. The snow crab is also an important commercial species for fishers in the southern Gulf of St. Lawrence and off southern Nova Scotia, particularly for those who fish the waters off Cape Breton's west coast.

The study was one of the recommendations of an ESRF workshop convened in Halifax, Nova Scotia (September 2000) where seismic issues were discussed by local, national and international experts. Until recently, most of the concerns related to seismic exploration have focused on certain species and/or life stages of marine mammals and fish that are known to have sensitive hearing abilities. Little research has been conducted on the effects of seismic noise on invertebrates in general, and crab in particular, because these species do not have hearing structures, although some can detect pressure waves. Furthermore, unlike vertebrates, the bodies of marine invertebrates are generally the same density as the surrounding water and therefore, theoretically speaking, sudden changes in pressure, such as that caused by sudden loud noise, would be unlikely to cause physical damage. Nonetheless, concern has been expressed by commercial fishers in Cape Breton, Nova Scotia that seismic surveys may affect the health and/or behaviour of snow crab. In addition, there also have been anecdotal suggestions in Newfoundland that seismic surveys may affect crab trap catch efficiency. Given the concerns of the fishers, the high value of the fishery, the general lack of hard evidence for or against effects, and the increasing amount of seismic exploration in waters inhabited by snow crab, the ESRF commissioned the present study.

The study, conducted in the fall of 2002, examined a number of health, behavioural, and reproductive variables before, during, and after, seismic shooting in a preliminary attempt to assess potential effects on physical, biochemical, or activity patterns in the subject animals. The research had four main objectives:

1. to conduct an experimental commercial fishery for snow crab before and after commencement of seismic shooting to determine whether there was a change in snow crab catch rate,

2. to observe snow crab during initial exposure to seismic sound to determine their responses to the sound,

3. to monitor snow crab activity using telemetric techniques to determine whether post-seismic activity patterns were different from pre-seismic activity patterns, and

4. to expose snow crabs and fertilized eggs to various seismic noise exposure levels to determine whether the seismic energy had any acute and/or chronic physiological and/or pathological effects on haemolymph (invertebrate 'blood'), various organs and associated tissues, adult crab and egg mortality, and embryo development.

Methodology

The study required a wide variety of expertise. LGL Limited assembled a team composed of experts in decapod crustacean biology and behaviour (LGL), histopathology (Oceans Limited), seismic noise (Jasco), and the snow crab fishery (H. Thorne and C. Hyde, commercial crab fishers).

Study Sites

A study site was selected in Conception Bay about five kilometers north-northeast of Cape St. Francis, after screening of potential sites. The site was chosen partly on logistic considerations such as proximity to the St. John's base of operations and a sheltered location with electrical power (Cape St. Francis). It was also considered to be typical 'prime' crab grounds by the commercial fishers, with mud-gravel substrate, a relatively level bottom, and a water depth of 165-175-m. Some of the video work was conducted in Freshwater Bay, just outside St. John's Harbour at a water depth of 50-m.

Logistic support was provided by two commercial fishing longliners (10-m and 15-m in length) operated by registered crab fishers.

Noise Sources

Seismic air guns were used for noise sources, including two 10 cubic inch (in^3) sleeve air guns, one 20-in^3 gun, and four 40-in^3guns. The air guns were used singly (40-in^3) and combined in an array of seven guns (200-in^3). For each firing session, the guns were set at two metres below the surface of the water and fired 200 times at 10-second intervals. The number of shots was chosen to be representative of the dosage that an individual animal might be exposed to during an operational seismic program.

Temperature Profiling

Water column temperature data were collected on each day of seismic shooting using a CTD.

Catchability

Fishing was conducted in a controlled manner using both standard (14-cm mesh) and modified (5-cm mesh) crab traps, baited with squid, and set in lines ('fleets') of 40 traps each by the commercial fishers. Seven sets were made prior to shooting and six after shooting. The sets were made close to the sound source and start and end points recorded using a GPS.

Crabs available to the experimental fishery were exposed to shots with the seven gun array (200-in^3). Numbers of shots that crabs were exposed to varied from 200 to 1,000 and times after exposure varied between two and 292 hours.

Captured crabs were counted, measured and categorized into four groups based on carapace width (legal >95-mm carapace width; sublegal <95-mm), shell condition, and sex.

Behaviour

Movements of crabs after exposure to air gun shots were to be monitored by four Lotek wireless hydrophones deployed on moorings several hundred meters apart and data retrieved via radio and stored at a land-based station. A manual tracking hydrophone on the boat was also deployed. Eight crabs (legal-size adults, not previously exposed to seismic) were tagged with acoustic transmitters manufactured by Lotek of St. John's. However, due to technical difficulties and severe weather-related problems, only one hydrophone was operational during firing and thus movement data were much coarser than originally intended.

Video (Silicon Intensified Target or SIT camera) was also used to observe potential startle behaviour of crabs deployed in cages, first in the water column, and then sitting on the bottom in 50-m of water.

Crab Health

The following experiments were conducted to assess potential indictors of stress (e.g., haemolymph chemistry) and physical damage (e.g., injury to statocysts, hair-like structures that crabs may use to detect vibration and balance) that might result from exposure to seismic surveys.

A series of experiments were conducted in the field by exposing caged crabs (legal and sublegal size males) to either the single 40-in^3 gun at 2, 10, or 15-m depths or the 200-in^3 array at 4, 50, 85, or 170-m depths. Each exposure consisted of 200 air gun shots fired at a rate of one every 10 seconds for a total exposure of 33 minutes. Excluding the fishing and telemetry experiments a total of 92 crabs were exposed to seismic and 92 were handled in a similar manner but not exposed (i.e., the controls). Crabs were processed after exposure for subsequent lab analyses but twenty animals plus controls were brought to the Fisheries and Oceans (DFO) wet lab in St. John's for long term observation and sampling.

In addition to the above, DFO provided six egg-carrying females. One batch of eggs (about 4,000) showing a similar level of development were divided into two groups that were handled in an identical manner except that one group was exposed to 200 shots with the 40-in^3 gun at a distance of two metres. Eggs were held in the DFO aquarium for 12 weeks before fixing and preserving.

Crab were sampled for haemolymph, hepatopancreas and heart, heads (statocysts, green glands, and brains), gills and gonads. Unanalyzed samples were archived.

Haemolymph was analyzed for the following variables:

- Relative concentrations of total dissolved substances in serum
- Serum proteins
- Serum enzyme activity screening
- Haemocyte types

Crustacean haemolymph represents the analogue of blood in vertebrates and can be inexpensively analysed for various chemical parameters such as dissolved substances (solutes) in haemolymph, and proteins and enzymes in serum. The blood cells or haemocytes found in haemolymph play a pivotal role in disease defence and can be examined by light microscopy for overt change in structure and/or abundance.

Haemolymph solute contains a composite of dissolved sugar, minerals, proteins, etc., and can be used to assess changes in haemolymph dilution or concentration, possibly an indication of disturbances in important physiological functions such as osmoregulation. Changes in solute concentration can be measured through changes in refractive indices by refractometry. This method is commonly used in studies of blood and other body fluids in human and veterinary medicine (George 2001).

Serum or urine protein is a classical analyte in clinical chemistry, and its determination in crustacean haemolymph can shed light on stress related to changes in haemolymph dilution and concentration (as discussed above with total dissolved substances). Based on concepts derived from mammalian studies, protein level determination has the potential to indicate either non-normal release of relatively large amounts of protein from other body compartments into the haemolymph or non-normal clearance of protein from the haemolymph.

Serum enzymes are widely used in human medicine to diagnose damage to specific organs and tissues. Their measurement is based on the principle that cells of various organs contain characteristic enzymes which leak into the blood in highly elevated concentrations due to various types of tissue damage or disease states (Ettlinger and Feldman 1995; Kaneko et al. 1997). For example, in the case of humans, enzymes associated with cardiac infarction can reach levels 10 times the normal level, and enzymes associated with liver damage can reach levels 150 times the normal level.

Haemocytes are believed to play a key role in crustacean immunology and have considerable potential for assessing animal health or specific pathological processes (Noga 2000). Three major types of haemocytes (hyalinocytes, semi-granulocytes and granulocytes) have been described on the basis of morphological and functional characteristics in most crustacean species (Bauchau 1981; Soderhall and Cerenius 1992). Changes in relative numbers of different haemocyte types (differential haemocyte counts), a possible threat to immunodefence (Baier-Anderson and Anderson 2000) are useful indicators of stress or physiological dysfunction in crustaceans (Jussila et al. 1997; Fotedar et al. 2001).

Organ and tissue pathology consisted of examination of statocysts for physical damage by light microscopy and scanning electron microscope and examination of hepatopancreas and heart for physical damage by light microscopy.

Pathology has traditionally been a pillar of human and veterinary medicine. Pathology is also proposed to be one of the most reliable indicators for assessing health impairment in aquatic organisms caused by anthropogenic activities (Myers et al. 1987; Hinton et al. 1992). Pathological parameters studied in snow crab included visible signs of external damage to the carapace and appendages, and microscopic analysis of selected organs including statocysts, hepatopancreas and heart.

Immediate lethal effects appear to be relatively uncommon in invertebrates exposed to even high levels of acoustic energy. Delayed mortality of adult animals has received virtually no attention to date and an important component of this study was to determine if lethality occurred three months after exposure.

Effect on reproduction and productivity is one of the main concerns when evaluating potential impacts on animal populations. Early developmental stages of marine organisms are commonly known to be sensitive to stressors. Embryonic development in snow crab includes a number of stages based on morphological and physiological features and it lasts approximately two years in Atlantic waters (Moriyasu and Lanteigne 1998). This long incubation period is unique in crustaceans and most likely increases the probability of egg mortality due to parasitism and physical damage as compared to the other species with shorter reproductive cycles. Additional stressors would increase this probability more.

Results and Discussion

Acoustics

The received air gun sound levels were estimated based upon a combination of direct measurements and interpolation. Depending upon water depth under the 40-in^3 gun, experimental animals were exposed to peak received broadband sound levels of 201 to 227 dB re 1 µPa and energy densities of 183 to 187 dB re 1 μPa^2Hz at frequencies between 24 and 31 Hz. Received levels from the 200-in^3 array were 197 to 237 dB re 1 µPa, depending upon distance from the source, and a maximum energy density of 175 dB re 1 $\mu Pa^2/Hz$ within the 17 to 19 Hz frequency range.

The above-received sound levels, at least on the high end of the ranges, are representative of the levels that would be encountered by an adult crab sitting on the bottom while seismic surveys were being conducted at the surface.

Temperature Profiles

During the course of the study, bottom temperatures ranged from -0.5 to -1.5ºC. The primary thermocline was located at about 20-m at the beginning of the study but moved to 40-85-m by the latter part of October. It is likely that the severe prolonged winds experienced in the fall of

2002 hastened this deepening of the thermocline. The position of the thermocline was monitored to help explain some of the acoustic dynamics observed during the telemetry program.

Effects on Behaviour

Snow crab were observed reacting slightly to sound in the laboratory when sharp sounds were made near them. However, in the field while being observed with a video camera, caged crab sitting on the bottom showed no readily visible reactions to the 200-in^3 array being fired 50-m above them.

Due to technical difficulties and weather-related problems, the telemetry system was unable to discriminate fine scale movements. Of the six tagged crabs that were being monitored clearly by the one remaining wireless hydrophone prior to shooting with the 200-in^3 array, all six were still being received 48 hours after shooting, all within the approximately 200-m radius of the range of the hydrophone. The tagged crabs did not undergo any large-scale movements out of the area.

Effects on Fishing

There was considerable variability in the durations of the experimental fishing periods of the commercial fishery traps (i.e., soak times), the likely received levels of sound experienced by the crab, and the experimental fishery catch data. The catch data were examined using a number of standardization, filtering and statistical techniques. Post-seismic catches were higher than pre-seismic catches but this was likely due to physical, biological, or behavioral factors unrelated to the seismic source. There was no significant relationship between catch and distance from the seismic source.

Effects on Health

A wide variety of health variables were examined using chemical and biochemical techniques. The data were subjected to statistical analyses (mostly Unpaired t-test or Mann-Whitney Rank Sum Test). It was concluded that there were no seismic effects on the health of the snow crabs, as measured by this suite of variables, prior to and after shooting.

The preliminary experiment conducted with 4,000 eggs from one female suggested that exposure to high levels of received sound (221 dB at 2-m depth) may retard the development of eggs. This result, if it is further confirmed, is similar to results with other marine animals and other types of disturbances that the young stages are often the most sensitive part of a species life history. Kostyuchenko (1973) and Booman et al. (1996) found indications of seismic effect on fish eggs when shot at close distance. While Holliday et al. (1987) and Saetre and Ona (1996) observed effects of seismic on fish larvae, Pearson et al. (1994) did not observe any statistically significant effects on Dungeness crab larvae shot as close as 1-m from a 231 dB source.

Other life stages of fish have also been investigated in seismic effect studies. Fish fry were the subjects of work by Booman et al. (1996) while effects on fish eggs and larvae were studied by a number of other researchers (Kostyuchenko 1972; Holliday et al. 1987; Booman et al. 1996; Saetre and Ona 1996).

Conclusions

The present study was a pilot study that evaluated a wide variety of techniques for relevance and viability in testing hypotheses concerning the potential effects of seismic surveys on snow crabs and on the snow crab fishery. The study showed no obvious effects on crab behaviour and no significant effects on the health of adult crabs. While the study did not indicate an overall reduction in snow crab catch rate due to seismic shooting, post-seismic catches were greater than pre-seismic catches, probably due to biological, behavioural, or physical factors unrelated to the seismic source. Statistical analyses of the catch data by distance from the seismic source indicated that there was no significant relationship between these variables. The eggs of one female showed significant effects on development when exposed at a very close range of 2-m. The exposed eggs were much slower to develop than were the unexposed controls. It should be noted that the females carry the eggs on the bottom where received sound levels are much lower than at the 2-m test distance. Future research should probably focus on the sensitive reproductive stages and determine at what distance the effect on eggs, if it occurs in all cases, tapers off. These data would be useful for both impact assessment and mitigation design purposes.

RÉSUMÉ

Introduction

LGL Limited, de St. John's (Terre-Neuve et Labrador), a été contactée par les responsables du Fonds pour l'étude de l'environnement (FEE) pour mener une étude portant sur l'effet de l'utilisation des canons sismiques à air sur le crabe des neiges (*Chionoecetes opilio*) et la pêche de ce crustacé. Les bruits intenses peuvent nuire aux animaux en entraînant des lésions dans les organes sensibles (l'oreille interne dans le cas des vertébrés), un stress croissant ou des changements de comportement. La pêche du crabe des neiges dans les eaux canadiennes de l'Atlantique est présentement centrée sur les côtes de Terre-Neuve et du Labrador où elle est la plus importante des pêches commerciales. Le crabe des neiges est également une espèce commerciale importante pour les pêcheurs qui travaillent dans le sud du golf du Saint-Laurent et au large du sud de la Nouvelle-Écosse, en particulier pour ceux qui pêchent dans les eaux de la côte ouest du cap Breton.

L'étude faisait partie des recommandations formulées à l'issu de l'atelier du FEE organisé à Halifax (Nouvelle-Écosse) en septembre 2000, au cours duquel les enjeux liés à la prospections sismique ont été débattus par des experts locaux, nationaux et internationaux. Jusqu'à récemment, la plupart des préoccupations concernant ce type de prospection avaient été émises à l'égard de certaines espèces et/ou étapes de vie de mammifères marins et de poissons réputés avoir une ouïe sensible. Très peu d'études ont été conduites sur les effets que pourrait avoir la prospection sismique sur les invertébrés en général et sur les crabes en particulier, parce que ces espèces ne possèdent pas de structures spécialisées pour l'ouïe même si certaines d'entre elles sont capables de détecter les ondes de pression. De plus, contrairement au corps des vertébrés, celui des invertébrés marins a généralement la même densité que l'eau environnante et en théorie, les changements brusques de pression dans l'eau, tels que ceux causés par des bruits intenses, ne sont donc pas susceptibles d'entraîner des dommages physiques. Les pêcheurs commerciaux du cap Breton (Nouvelle-Écosse) ont cependant fait part de leurs préoccupations concernant les relevés sismiques qui, selon eux, pourraient affecter la santé et/ou le comportement des crabes des neiges. De plus, on a suggéré plusieurs fois à Terre-Neuve que les relevés sismiques pourraient nuire à l'efficacité des pièges à crabe utilisés par les pêcheurs. Compte tenu de ses préoccupations, de la valeur de cette pêche, du manque général de preuves solides établissant l'existence ou l'absence d'effets et de l'intensification de l'exploration sismique dans les eaux fréquentées par le crabe des neiges, le FEE a demandé que soit effectuée la présente étude.

Cette étude, menée à bien au cours de l'automne 2002, a consisté à examiner un certain nombre de variables sanitaires, comportementales et reproductives avant, pendant et après le déclenchement de plusieurs déflagrations sismiques afin de tenter, dans un premier temps, d'évaluer les effets potentiels de tels bruits sur le physique, la biochimie et l'activité des animaux étudiés. Les quatre objectifs principaux suivants avaient été fixés pour l'étude :

1. effectuer une pêche commerciale expérimentale des crabes des neiges avant et après le début des déflagrations sismiques afin de mettre en évidence un éventuel effet sur le rendement des pièges à crabe,

2. observer les crabes des neiges pendant l'exposition initiale aux ondes sismiques afin de déterminer la manière dont ces animaux répondent à de telles perturbations,

3. surveiller l'activité des crabes des neiges à l'aide de techniques télémétriques afin de déterminer si cette activité était affectée par les ondes sismiques,

4. exposer des crabes des neiges et des œufs fécondés à différents niveaux d'ondes sismiques afin de déterminer dans quelle mesure l'énergie sismique avait des effets physiologiques ou pathologiques aigus ou chroniques sur l'hémolymphe (le «sang » des invertébrés), sur divers organes et les tissus associés, sur la mortalité des crabes adultes et des œufs et sur le développement des embryons.

Méthodologie

L'étude a nécessité la mise en œuvre d'une vaste gamme d'expertises. LGL Limited a mis sur pied une équipe composée d'experts en biologie et en comportement des crustacés décapodes (LGL Limited), en histopathologie (Oceans Limited), en bruits sismiques (Jasco) et en pêche du crabe des neiges (H. Thorne et C. Hyde, pêcheurs de crabes professionnels).

Site étudié

Un site d'étude a été choisi dans la baie Conception, à environ cinq kilomètres au nord-est du cap St. Francis, après évaluation d'un certain nombre de sites potentiels. Le site a été choisi en partie à cause de ses avantages, soit la proximité de la base d'opération de St. John's et le caractère abrité du site qui bénéficie en outre de l'électricité (Cap St. Francis). Les pêcheurs commerciaux considèrent de plus ce site comme un habitat idéal typique pour les crabes des neiges, doté d'un substrat de vase et de graviers, d'un fond relativement plat et d'une profondeur variant entre 165 et 175 m. Certaines séquences vidéo ont été tournées dans la baie Freshwater, juste à l'extérieur du port de St. John's, à une profondeur de 50 m.

Le soutien logistique a été assuré par deux palangriers commerciaux (de 10 m et de 15 m) pilotés par des pêcheurs de crabe inscrits.

Sources de bruit

Des canons à air sismiques ont été utilisés pour générer les ondes sonores. Il s'agissait de deux canons de 10 pouces cubes (po^3), d'un canon de 20 po^3 et de quatre canons de 40 po^3. Les canons de 40 po^3 étaient utilisés individuellement ou combinés en réseau de sept cannons (200 po^3). Lors des séances de tirs, les canons étaient plongés à deux mètres sous la surface et 200 coups étaient tirés à la cadence d'un coup toutes les 10 secondes. Le nombre de coups tirés était censé être représentatif de l'exposition subie par un animal marin durant un programme réel d'exploration sismique.

Profil de la température

Les données décrivant le gradient de température dans la colonne d'eau ont été enregistrées à l'aide d'un capteur CTD à chaque fois qu'une série de tirs étaient effectuée.

Rendement des pièges

La pêche a été effectuée d'une manière contrôlée à l'aide de pièges à crabe traditionnels (mailles de 14 cm) et de pièges modifiés (mailles de 5 cm), appâtés avec des calmars et disposés en lignes de 40 pièges chacune par les pêcheurs commerciaux. Sept lignes ont été relevées avant les déflagrations et six après. Les lignes ont été posées près de la source des déflagrations et la position exacte de leurs extrémités enregistrée à l'aide d'un GPS.

Les crabes accessibles à la pêche expérimentale ont été exposés à des ondes sismiques provenant du réseau de sept cannons (200 po^3). Les crabes ont été exposés à un nombre de tirs variant entre 200 et 1 000 et la durée après l'exposition variait entre 2 et 292 heures.

Les crabes capturés ont été comptés, mesurés et répartis en quatre groupes suivant la largeur de leur carapace (taille réglementaire : largeur supérieure à 95 mm; taille non réglementaire : largeur inférieure à 95 mm), l'état de leur carapace et leur sexe.

Comportement

Le mouvement des crabes après l'exposition à un tir de canon à air devait être surveillé par l'intermédiaire de quatre hydrophones Lotek sans fil déployés le long du mouillage, à plusieurs centaines de mètre d'intervalle, les données étant transmises par radio et enregistrées dans une station terrestre. Un hydrophone de suivi manuel avait également été installé sur le bateau. Huit crabes (adultes de taille réglementaire, n'ayant pas été exposés à des ondes sismiques) ont été munis d'émetteurs acoustiques fabriqués par Lotek (St. John's). Des difficultés techniques et des problèmes liés à une météo peu clémente ont cependant fait que seulement un hydrophone s'est avéré opérationnel durant les déflagrations. Les données décrivant les mouvements ont dont été beaucoup plus grossières que prévu à l'origine.

Des séquences vidéo (caméras «SIT » [Silicon Intensified Target]) ont également été utilisées pour observer les sursauts éventuels de crabes installés dans des cages, d'abord entre deux eaux, puis sur le fond, à une profondeur de 50 m.

Santé des crabes

Les expériences suivantes ont été conduites pour évaluer les indicateurs potentiels du stress (p. ex. chimie de l'hémolymphe) et des changements physiques (p. ex. lésions des statocystes, ces sortes de poils que les crabes peuvent utiliser pour détecter les vibrations et pour s'équilibrer) qui peuvent résulter de l'exposition aux relevés sismiques.

Une série d'expériences a été effectuée sur le terrain par l'exposition des crabes encagés (mâles de taille réglementaire et non réglementaire) à des tirs du canon de 40 po^3 à des profondeurs de 2, 10 et 15 m et à des tirs du réseau de 200 po^3 à des profondeurs de 4, 50, 85 et 170 m. Chaque exposition consistait à tirer 200 coups de canon à air à la fréquence d'un coup toutes les 10 secondes pendant 33 minutes. Sans compter les expériences de pêche et de télémétrie, un total de 92 crabes ont été exposés aux ondes sismiques et 92 ont été manipulés de la même manière mais n'ont pas été exposés (groupe témoin). Après exposition, les crabes ont été envoyés au laboratoire pour subir les analyses prévues; vingt d'entre eux ainsi que des crabes «témoins » ont été envoyés au laboratoire humide de Pêches et Océans Canada (MPO) de St. John's pour faire l'objet d'une observation et d'un échantillonnage à long terme.

MPO a en plus fourni six femelles porteuses d'œufs. Un ensemble d'œufs (environ 4 000) apparemment au même niveau de développement a été divisé en deux groupes ultérieurement manipulés de la même façon sauf que l'un des groupes a été exposé à 200 tirs du canon de 40 po^3 situé à 2 m des oeufs. Les œufs ont été gardés 12 semaines dans l'aquarium de MPO avant d'être fixés et préservés.

Des échantillons d'hémolymphe, d'hépatopancréas, de cœur, de tête (statocystes, glandes vertes et cerveau), de branchies et de gonade ont été prélevés sur les crabes. Les échantillons non analysés ont été archivés.

Les variables suivantes ont été mesurées dans l'hémolymphe :

- Concentration relative des substances dissoutes dans le sérum
- Protéines sériques
- Activité des enzymes sériques
- Typologie des hémocytes

L'hémolymphe des crustacés est l'analogue du sang des vertébrés. On peut y mesurer à peu de frais la concentration de divers produits chimiques tels que les substances dissoutes (en solution) ainsi que les protéines et les enzymes sériques. Les cellules sanguines (hémocytes) présentes dans l'hémolymphe jouent un rôle crucial dans la défense des organismes contre les vecteurs pathogènes. On peut les examiner à l'aide d'un microscope optique afin de déceler d'éventuels changements évidents de structure ou d'abondance.

Les matières dissoutes dans l'hémolymphe sont entre autres des sucres, des minéraux et des protéines qui peuvent être dosées pour évaluer les changements de concentration et du taux de dilution dans l'hémolymphe. De tels changements peuvent refléter des perturbations au niveau d'importantes fonctions physiologiques telles que l'osmorégulation. Les changements de concentrations peuvent être mesurés par réfractométrie, en évaluant le changement d'indice de réfraction. Cette méthode est communément utilisée pour l'étude du sang et d'autres fluides corporels en médecine humaine et en médecine vétérinaire (George 2001).

Les protéines présentes dans le sérum et dans l'urine sont couramment mesurées dans le domaine des analyses cliniques chimiques. Leur concentration dans l'hémolymphe des crustacés peut renseigner sur le degré de stress qui a pu entraîner des changements à ce niveau (comme discuté

ci-dessus au sujet des substances dissoutes). Grâce aux concepts dérivés des études sur les mammifères, la détermination de la concentration de ces protéines peut révéler la sécrétion anormale de quantité relativement importante de protéines dans l'hémolymphe ou l'élimination anormale de ces mêmes protéines de l'hémolymphe.

Les enzymes sériques sont utilisés à grande échelle en médecine humaine pour diagnostiquer la présence de lésions dans des organes ou des tissus. Leur mesure est basée sur le principe selon lequel les cellules des organes contiennent des enzymes spécifiques qui se répandent dans le sang en concentrations élevées à la suite d'un dommage ou d'un état pathologique (Ettlinger et Feldman 1995; Kaneko et al. 1997). Dans le cas des humains, par exemple, les enzymes associées à un infarcissement peuvent atteindre une concentration dix fois plus élevée que la concentration normale tandis que les enzymes associées aux liaisons hépatiques peuvent atteindre des concentrations cent cinquante fois plus élevées que la normale.

On pense que les hémocytes jouent un rôle clé dans le système immunitaire des crustacés et qu'elles nous permettraient donc d'évaluer de façon très efficace la santé de ces animaux ou de diagnostiquer des processus pathologiques spécifiques (Noga 2000). Trois types principaux d'hémocytes (hyalinocytes, semi-granulocytes et granulocytes) ont été décrits en fonction de leurs caractéristiques morphologiques et fonctionnelles chez la plupart des espèces de crustacés (Bauchau 1981; Soderhall et Cerenius 1992). Les changements observés au niveau du nombre relatif des différents types d'hémocytes (comptage différentiel des hémocytes), qui peuvent refléter une attaque sur le système immunitaire (Baier-Anderson et Anderson 2000) sont des indicateurs utiles du stress ou de disfonctionnements physiologiques chez les crustacés (Jussila et al. 1997; Fotedar et al. 2001).

La pathologie des organes et des tissus a consisté à détecter des dommages physiques éventuels dans les statocystes à l'aide d'un microscope optique et d'un microscope électronique à balayage et dans l'hépatopancréas et le cœur à l'aide d'un microscope optique.

La pathologie a toujours été un des piliers de la médecine humaine et de la médecine vétérinaire. Elle est aussi l'instrument de choix pour évaluer les effets sanitaires des activités anthropogéniques sur les organismes aquatiques (Myers et al. 1987; Hinton et al. 1992). Les paramètres pathologiques étudiés dans le cas du crabe des neiges comprennent entre autres les signes visibles de dommages sur la carapace et les pattes et l'analyse microscopique d'organes choisis tels que les statocystes, l'hépatopancréas et le cœur.

Il semble que les ondes acoustiques, même de forte intensité, n'entraînent que très rarement la mort immédiate des invertébrés exposés. Cependant, la mortalité différée des animaux adultes n'a pratiquement jamais été étudiée jusqu'à présent et une composante importante de cette étude consistait à détecter une éventuelle mortalité trois mois après l'exposition.

L'effet éventuel des ondes sismiques sur la reproduction et la productivité est l'une des principales questions qui doivent être abordées lors de l'évaluation des impacts potentiels sur les populations animales. Les premières phases de développement des organismes marins sont souvent sensibles aux facteurs de stress. Chez le crabe des neiges, le développement embryonnaire comprend un certain nombre d'étapes faisant intervenir différentes caractéristiques

morphologiques et physiologiques et il dure approximativement deux ans dans les eaux de l'Atlantique (Moriyasu et Lanteigne 1998). Cette période d'incubation est unique chez les crustacés et sa durée contribue probablement à augmenter la probabilité que les œufs meurent à cause d'agents parasites ou de dommages physiques, comparé à d'autres espèces pour lesquelles le cycle de reproduction est plus court. L'existence de facteurs de stress supplémentaires contribuerait encore à augmenter cette probabilité.

Résultats et discussion

Acoustique

Les niveaux sonores émis par les canons à air ont été estimés à partir de mesures directes et à partir d'interpolations. Suivant la profondeur de l'eau sur le site de tir du canon de 40 po^3, les animaux étudiés ont été exposés à des niveaux de son de large bande de 201 à 227 dB à 1 µPa et à des densités d'énergie de 183 à 187 dB à 1 μPa^2Hz, avec des fréquences allant de 24 à 31 Hz. Les niveaux reçus du réseau de 200 po^3 variaient entre 197 et 237 dB à 1 µPa, suivant la distance de la source, avec une densité d'énergie maximale de 175 dB à 1 $\mu Pa^2/Hz$ entre 17 et 19 Hz.

Les niveaux sonores reçus décrits ci-dessus, du moins pour ce qui est des valeurs supérieures, sont représentatifs des niveaux auxquels serait exposé un crabe adulte évoluant sur le plancher océanique tandis que des relevés sismiques seraient effectués en surface.

Profils de température

La température de l'eau, au fond, est restée entre –0,5 et –1,5° C pendant toute la durée de l'étude. La thermocline primaire était située à environ 20 m de profondeur au début de l'étude puis à 40-85 m vers la fin octobre. Il est probable que les vents forts et prolongés qui ont soufflé pendant l'automne 2002 ont précipité la descente de la thermocline. La position de la thermocline a été suivie pour mieux comprendre la dynamique acoustique observée dans le cadre des mesures télémétriques.

Effets sur le comportement

En laboratoire, les crabes des neiges ont réagi faiblement à des bruits intenses produits près d'eux. Sur le terrain, les crabes encagés au fond de la mer et surveillés par caméra vidéo n'ont cependant pas réagi de manière visible aux détonations produites par le réseau de 200 po^3 situé 50 m au-dessus d'eux.

Des problèmes techniques et des problèmes liés à la météo ont fait qu'il a été impossible d'enregistrer précisément les mouvements des crabes à l'aide du système de télémétrie. Les six crabes porteurs d'un émetteur qui étaient suivis clairement par le seul hydrophone sans fil restant avant les tirs du réseau de 200 po^3 étaient toujours détectés, 48 heures après les détonations, à moins de 200 m de l'hydrophone. Les crabes porteurs d'un émetteur ne se sont donc pas systématiquement éloignés du secteur des détonations après le début des tirs.

Effets sur la pêche

La durée d'immersion des pièges utilisés pour la pêche commerciale expérimentale a varié de manière considérable, tout comme le niveau sonore probablement reçu par les crabes et, en général, les données issues des prises capturées dans le cadre de la pêche expérimentale. Ces données ont été examinées en mettant en œuvre un certain nombre de techniques de normalisation, de filtrage et d'analyse statistique. Le nombre de crabes capturés après les tirs s'est avéré supérieur au nombre de crabes capturés avant mais cette différence est probablement attribuable à des facteurs physiques, biologiques et comportementaux qui ne sont pas liés aux tirs. Aucune corrélation n'a été relevée entre le nombre de crabes capturés et la distance de la source sismique.

Effets sur la santé

Une grande variété de variables sanitaires ont été examinées à l'aide de diverses techniques chimiques et biochimiques. Les données ont été soumises à une analyse statistique (mettant principalement en jeu le test t et le test de Mann-Whitney). Il a été conclu que l'analyse de ces variables mesurées avant et après les tirs montrait que les ondes sismiques n'avaient aucun effet sur la santé des crabes des neiges.

L'expérience préliminaire menée avec 4 000 œufs provenant d'une femelle a montré que l'exposition à de hauts niveaux d'ondes sonores (221 dB à 2 m) peut retarder le développement des œufs. Ce résultat, s'il est confirmé, serait similaire à ceux observés chez d'autres animaux marins avec d'autres types de perturbations, c'est-à-dire que les premières phases de développement des espèces sont souvent les plus sensibles aux perturbations extérieures. Kostyuchenko (1973) et Booman et al. (1996) ont relevé des signes montrant que les ondes sismiques avaient un effet sur les œufs de poisson lorsque ces ondes étaient générées près des œufs. While Holliday et al. (1987) et Saetre et Ona (1996) ont quant à eux observé des effets sur des larves de poisson mais Pearson et al. (1994) ont conclu qu'une source de 231 dB située à 1 m de larves de crabes dormeurs n'avait aucun effet statistiquement significatif sur ceux-ci.

Des études ont porté sur l'effet des ondes sismiques sur d'autres étapes du cycle de vie des poissons. Booman et al. (1996) ont par exemple étudié les effets de telles perturbations sur des alevins tandis que d'autres chercheurs (Kostyuchenko 1972; Holliday et al. 1987; Booman et al. 1996; Saetre et Ona 1996) ont étudié comment réagissaient les œufs et les larves.

Conclusions

Ces travaux ont été réalisés dans le cadre d'une étude pilote visant à évaluer une vaste gamme de techniques permettant de tester la viabilité d'hypothèses concernant les effets possibles des relevés sismiques sur les crabes des neiges et sur la pêche de ces crustacés. L'étude n'a pu mettre en évidence aucun effet évident sur le comportement des crabes ni aucun effet important sur la santé des crabes adultes. L'étude n'indique pas que les ondes sismiques entraînent une réduction du rendement des pièges à crabe, et au contraire, les prises effectuées après les tirs étaient supérieures aux prises réalisées avant, probablement à cause de facteurs biologiques, comportementaux et physiques qui n'étaient pas liés aux tirs. L'analyse statistique du nombre de

crabes capturés en fonction de la distance à laquelle se trouvait la source sonore a montré qu'il n'existait aucune corrélation significative entre ces deux variables. Des effets significatifs ont cependant été observés sur des œufs prélevés sur une femelle et exposés à une source sonique intense placée très près (2 m). Les œufs exposés se sont développés beaucoup plus lentement que les œufs témoins qui n'avaient pas été exposés. Il faut noter que les femelles transportent leur sac sur le fond, où les niveaux sonores reçus sont bien inférieurs à ceux reçus dans le cadre de l'exposition en laboratoire, lorsque la source est située à 2 m des oeufs. Des travaux supplémentaires devraient être effectués pour étudier les effets sur les étapes sensibles de la reproduction et déterminer à quelle distance les effets sur les œufs, si présents dans tous les cas, commencent à diminuer. Ces données pourraient être utiles pour les évaluations d'impacts et l'élaboration de mesures destinées à atténuer ceux-ci.

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INTRODUCTION

LGL Limited, environmental research associates of St. John's, Newfoundland and Labrador, conducted a pilot study during the fall of 2002 to assess the effects of seismic energy on the behaviour and physiological health of the snow crab (*Chionoecetes opilio*). The study, funded by Environmental Studies Research Fund (ESRF), was conducted on a natural snow crab ground off the Newfoundland coast near St. John's. Such a study had been suggested during a September 2000 Halifax workshop conducted to develop methodologies for conducting research on the effects of seismic exploration on the Canadian East Coast Fishery (Thomson et al. 2001).

There were two main phases to the pilot study.

1. Determination of whether or not commercial-sized snow crab can 'hear' seismic sounds, and whether or not there are any discernible changes in behaviour and physiology.

2. Based on lessons learned from the first phase, the design and execution of a field study to determine the potential effects of seismic pulses on the snow crab fishery.

Phase 1 involved a combination of laboratory work (e.g., preliminary exposure of snow crabs to non-seismic underwater noises; post-treatment holding, observation and sampling of egg-bearing females and egg masses; physiological and pathological effects analyses) and fieldwork (e.g., videography of snow crab behaviour in response to onset of seismic shooting; observation of acute behavioural effects of seismic pulses). Phase 2 was accomplished primarily through fieldwork (e.g., experimental commercial snow crab fishery; telemetric monitoring of snow crab behaviour before and after exposure to seismic pulses).

The overall null hypothesis (H_0) tested during the study was as follows:

> ***H_0: Sounds emitted during seismic exploration do not cause a significant decrease in the catch per unit effort of snow crabs.***

In the event that the overall null hypothesis was rejected, the following overall alternative hypothesis (H_A) would be accepted.

> ***H_A: Sounds emitted during seismic exploration do cause a significant decrease in the catch per unit effort of snow crabs.***

The overall null hypothesis was evaluated by testing a series of null sub-hypotheses related to the two main phases of the study. The five relevant null and alternative sub-hypotheses are stated below.

Phase 1

Detection of sound and subsequent reactions to sound

1. *Snow crabs do not exhibit observable reactions to sound, including those kinds of sounds emitted by seismic sources.*

   OR

   Snow crabs do exhibit observable reactions to sound, including those kinds of sounds emitted by seismic sources.

Physiological effects of sound

2. *Seismic sounds do not damage snow crab statocysts and associated tissues.*

   OR

   Seismic sounds do damage snow crab statocysts and associated tissues.

3. *Seismic sounds do not cause other physiological damage to snow crabs.*

   OR

   Seismic sounds do cause other physiological damage to snow crabs.

Phase 2

Effects on activity patterns

4. *Snow crabs remain in an area when exposed to seismic sounds regardless of received sound levels.*

   OR

   Snow crabs leave an area when exposed to seismic sounds.

5. *Snow crabs do not return to their original area within a few days of leaving that area in response to seismic sound.*

   OR

   Snow crabs do return to their original area within a few days of leaving that area in response to seismic sound.

The specific objectives of the study were as follow:

- To conduct an experimental commercial fishery for snow crabs before and after commencement of seismic shooting to determine whether there was a change in snow crab catch rate (i.e., catch per unit effort – CPUE). The pre-seismic fishery also collected the crab specimens required for other components of the pilot study.

- To observe the initial responses of snow crabs to seismic sounds.

- To monitor snow crab activity using telemetric techniques to determine whether pre-seismic activity patterns were different from post-seismic activity patterns.

- To treat snow crabs and fertilized eggs with various seismic exposure levels to determine whether the seismic energy had any acute and/or chronic physiological and/or pathological effects on haemolymph, various organs and associated tissues, adult crab and egg mortality, and embryo development.

THE FOLLOWING SECTIONS DETAIL THE MATERIALS AND METHODS USED DURING THIS PILOT STUDY; RESULTS ARE PRESENTED AND INTERPRETED AND CONCLUSIONS DRAWN. APPENDIX 1 CONTAINS SOME OF THE RAW ACOUSTIC DATA.

MATERIALS AND METHODS

Study Site

The study was conducted on a commercially fished snow crab ground off the northeast Avalon Peninsula, Newfoundland, approximately five kilometres north-northeast of Cape St. Francis in the mouth of Conception Bay (47º 50.7' N, 52º 45.4' W). The fieldwork occurred between mid-September and mid-December, 2002. Water depth at the study site was approximately 165 to 175-m. One aspect of the study was performed in Freshwater Bay, located immediately south of the St. John's Harbour Narrows, at a site with a water depth of 50-m (Figure 1).

The principal vessel used during the study was a 15-m longliner, the *FV Rough and Wild* (Figure 2). A 10-m longliner, the *FV Melanie and Jasmine*, was used as a secondary vessel.

Seismic Array

The seismic system used in this study consisted of a Price Compressor Co. A-300 4-Stage 300 SCFM compressor (Figure 2), four 40-in^3 Texas Instrument (TI) sleeve air guns, one 20-in^3 TI sleeve air gun, two 10-in^3 TI sleeve air guns (Figure 3), a 5,000 psi air tank with pressure regulator set @ 2,000 psi, a 50-m multihose and firing line umbilical via distribution panel, and a Macha International Inc. gun firing box, Model TGS-8.

Each shooting session consisted of 200 shots at a firing rate of one every 10 seconds (i.e., approximately 33 minutes per session). The seismic source was set at a two-metre depth during all shooting sessions. The number of shots was chosen to be representative of the dosage that an individual animal might be exposed to during an operational seismic program.

Measurement of Seismic Acoustic Signals

A RESON TC4014 hydrophone was attached to the topsides electronics via a 200-m cable. The hydrophone output was fed to a Wavetek System 816 multi-channel filter that was configured as a 48dB/octave, 8-pole Butterworth low pass filter with a 500 Hz cutoff frequency. This band-limited the analog hydrophone signals prior to sampling and the analog-to-digital conversion to prevent 'aliasing' of out-of-band components into the frequency band of interest.

The filtered hydrophone signal was brought to a Datel PCI-416 data acquisition board housed in an Advantech industrial computer. The Datel C++ source code was modified for this project to provide the necessary data collection, graphics and storage routines for the hydrophone data.

The data were collected in 16-second file blocks at a sampling rate of 2,048 Hz resulting in at least one seismic shot (1 shot every 10 seconds) being included in each data file. The data files were scanned for the start of the sleeve gun burst and this offset in the file was recorded with the filename for the subsequent data processing. Many of the files contained data from two shots. The graphic plots (time-amplitude signature, and energy density spectrum) for each shooting session are representative of the mean values obtained from that specific session.

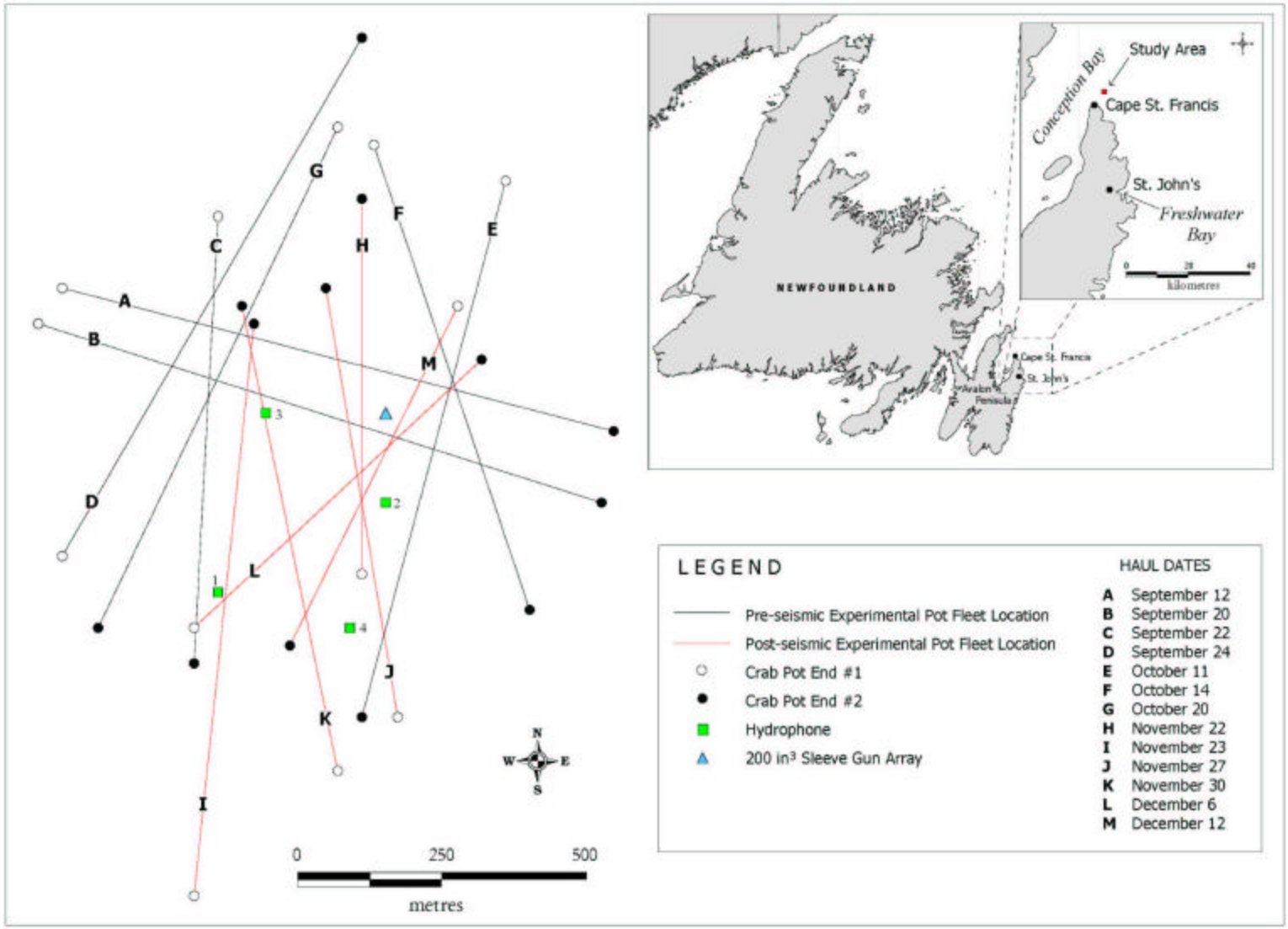

Figure 1. Study area site locations and experimental fishery set locations.

Figure 2. Price compressor situated at the stern of *FV Rough and Wild*.

Figure 3. 200-in^3 seven-air gun array in frame.

Water Column Profiling

On each day of seismic shooting, water column temperature profiling was conducted with a CTD. These data provided real time measurements of bottom water temperature and indicated the position of the primary thermocline. Knowledge of the position of the thermocline was needed to help to explain certain aspects of the telemetry program.

Experimental Commercial Fishing

Controlled experimental commercial snow crab fishing was conducted at the study site before and after experimental seismic shooting. Crab traps were set and retrieved by Skipper Henry Thorne and crew of the longliner *FV Rough and Wild*, and Skipper Clyde Hyde and crew of the longliner *FV Melanie and Jasmine*. Both skippers have over 20 years experience as commercial fishers, many of those as snow crab fishers. Both crews baited, set and retrieved the traps in the same manner they would during the regular commercial snow crab fishery. Mr. Thorne and his crew conducted most of the experimental fishing. The study was conducted after closure of the regular snow crab commercial fishing season to eliminate any interference from normal crab fishing activities. Each conical crab trap was 1.25-m in diameter at the base, 1-m in height and baited with squid (Figure 4). For each experimental set, 40 crab traps were deployed approximately 36-m apart on a line approximately 1.4-km in length, although the line was never perfectly straight on the bottom. In crab fishing terminology, each of these lines is called a fleet. All but one of the traps of the experimental fleet were made up with 14-cm mesh. The eighth trap was made up with five-centimetre mesh. The small mesh trap was designed to fish for female crabs that are substantially smaller than the males. Seven sets were made before seismic

Figure 4. Example of crab trap used during the study.

shooting activity and six after. All sets were made such that the centre of each fleet was closest to the seismic source. The 200-in^3 seven-gun array was the seismic source used in the commercial fishing experiment. Prior seismic shooting with the single gun was performed at least five kilometers away from the fishing area. Snow crabs in the experimental fishery after the commencement of seismic shooting were exposed to 200 shots on 21 November (shooting day 1), 400 shots on 27 November (shooting day 2), and 400 shots on 30 November (shooting day 3). Three sets were conducted between shooting days 1 and 2: (1) from two to 18 hours after exposure, (2) from 20 to 45 hours after exposure, and (3) from 45 to 143 hours after exposure. One set was conducted between shooting days 2 and 3: (4) from 40 to 60 hours after exposure. Two sets were conducted after shooting day 3: (5) from two to 146 hours after exposure, and (6) from 148 to 292 hours after exposure. Obviously, there was cumulative exposure for the animals not caught during the first post-seismic fleet set. The timing of sets after shooting days was determined by the weather in the area.

Crabs captured in each retrieved pot were counted, measured and categorized into one of four groups based on carapace width, shell condition and sex: (1) hard shelled legal-sized males: = 95-mm; (2) hard shelled sublegal-sized males: < 95-mm; (3) soft shelled males, all sizes; and, (4) females. The number of animals in each category was recorded for each trap. Carapace widths were measured with calipers provided by the company that provides personnel for the Crab Observer's Program. Shell condition was determined by observation of the animal and by application of pressure with the thumb to the underside of the right cheliped. As they grow, crabs discard their old shell and the new larger shell is soft for several weeks after this molt. These assessment methods are the standard ones employed by Department of Fisheries and Oceans (DFO) observers when sampling crab catches. All snow crabs caught during the post-seismic fishing were released at a location at least five kilometers from the experimental fishing site.

In order to standardize catch per unit effort (CPUE), catch rates were calculated as the number of crabs caught per trap per 24-hour period. Fleets spent a variable amount of time in the water (i.e., soak time) so this temporal standardization was necessary (Table 1). Crab fishermen indicated to us that squid bait generally does not last longer than 48 to 96 hours. Thus, most of the CPUE statistics were based on data from sets that were in the water no longer than 96 hours. Because of the mesh size used, catch rates of only legal-sized crab (= 95-mm) were considered reliable if soak times exceeded 48 hours. Smaller animals could escape through the mesh and bias the number of sublegal-sized crab (< 95-mm) in the trap.

Hard shelled legal-sized and sublegal-sized crab were retained from the pre-seismic exposure fishing and kept in small meshed baited holding traps on the ocean bottom to be used as experimental animals in subsequent experiments. Some of these unexposed hard-shelled legal-sized males were also transported to the Department of Fisheries and Oceans, St. John's, for holding in saltwater tanks.

Table 1. Dates, soak times and positions of experimental commercial snow crab fishery fleet sets.

Designation On Map	Set Date (d/m/y)	Haul Date (d/m/y)	Soak Time (hr)	Position of End #1	Position of End #2	Distance Between Ends (m)
A	18/09/02	19/09/02	19	47º 50' 58'' 52º 45' 39''	47º 50' 50'' 52º 44' 53''	990
B	19/09/02	20/09/02	27	47º 50' 56'' 52º 45' 41''	47º 50' 46'' 52º 44' 54''	1,025
C	20/09/02	22/09/02	44	47º 51' 02'' 52º 45' 26''	47º 50' 37'' 52º 45' 28''	775
D	22/09/02	24/09/02	45	47º 50' 43'' 52º 45' 39''	47º 51' 12'' 52º 45' 14''	1,040
E	09/10/02	11/10/02	43	47º 51' 04'' 52º 45' 02''	47º 50' 34'' 52º 45' 14''	965
F	11/10/02	14/10/02	68	47º 51' 06'' 52º 45' 13''	47º 50' 40'' 52º 45' 00''	855
G	14/10/02	20/10/02	141	47º 51' 07'' 52º 45' 16''	47º 50' 39'' 52º 45' 36''	960
H*	21/11/02	22/11/02	16	47º 50' 42'' 52º 45' 14''	47º 51' 03'' 52º 45' 14''	650
I*	22/11/02	23/11/02	25	47º 50' 24'' 52º 45' 28''	47º 50' 56'' 52º 45' 23''	995
J*	23/11/02	27/11/02	96	47º 50' 34'' 52º 45' 11''	47º 50' 58'' 52º 45' 17''	750
K*	29/11/02	30/11/02	20	47º 50' 31'' 52º 45' 16''	47º 50' 57'' 52º 45' 24''	825
L*	30/11/02	06/12/02	144	47º 50' 39'' 52º 45' 28''	47º 50' 54'' 52º 45' 04''	680
M*	06/12/02	12/12/02	144	47º 50' 57'' 52º 45' 06''	47º 50' 38'' 52º 45' 20''	660

Sets marked with an '*' caught snow crabs exposed to seismic energy

Field Observations of Behaviour

Behavioural reactions to seismic were observed using acoustic tags and video cameras.

Telemetry

Crabs were tagged with acoustic transmitters. The signals emitted by the transmitters were to be picked up by hydrophones on 4 moorings. Data received by each mooring was to be transmitted to a base station. Crab movements were estimated by examination of signals received at each mooring.

Crabs used in the telemetry study were caught during the pre-seismic experimental commercial fishery and held in baited crab traps at a depth of 170-m. Eight legal-sized male crabs were tagged with Lotek Model CAFT16-1 acoustic transmitters. Transmitters were attached to the crab carapaces using a 'transmitter saddle' (Figure 5).

Figure 5. Snow crabs outfitted with 'transmitter saddles'.

It was necessary to develop a technique for attachment of the acoustic transmitters to the crabs. Cold resistant vinyl tubing with an inner diameter slightly less than the transmitter outer diameter was used to make the saddle. Pieces of tubing approximately 10-cm long were longitudinally split open at one end (~ 4-cm length) to form four separate strips hanging from the unaltered piece of tubing. A transmitter was then inserted into the unaltered end of the tubing leaving only the transmitting end of it exposed. After drying, lightly sanding and cleaning the crab carapace, the four 'tendrils' on the altered end of the tubing were spread out in roseate fashion and attached to the top of the carapace with Loctite 454 epoxy. All inner surfaces of the tubing were lightly washed with acetone before application of the epoxy to them. This 'transmitter saddle' resulted in the transmitter being oriented upwards and slightly angled to the back of the carapace. There remained enough flexibility in the section of tubing between the bottom of the transmitter and the top of the carapace to allow for some front to back flex in the saddle. This method was successfully tested in the Fisheries and Oceans laboratory before the field study to ensure that the saddle remained attached to the carapace and that crab behaviour was not drastically altered.

Four moorings were deployed at the study site in a diamond-like configuration. Distances between adjacent moorings were approximately 250 to 300-m (see Figure 1). Each mooring consisted of a Lotek WHS_1000 Series wireless hydrophone that was electronically cabled to an inflatable surface buoy-radio antenna unit. The hydrophone was about seven to eight metres below the surface. Wire rope was used as the main mooring line between the surface buoy-radio antenna unit and a point approximately four to five metres below the hydrophone. Half-inch polypropylene rope made up the remainder of the mooring line between lower end of the wire rope and the 250 lb anchor at bottom. An additional surface buoy with high-flyer was added to

each hydrophone mooring. Mooring positions were determined using differential GPS. The Canadian Coast Guard was notified and marine advisories were subsequently issued to warn marine traffic.

Assuming each hydrophone could pick up a transmitter within a radius of at least 200-m, the spacing of the hydrophones in the diamond-shaped configuration would theoretically provide a coarse bottom grid based on the overlap regions of the four areas of coverage. While not able to determine fine-scale positions of the tagged animals with the equipment used in this study, we hoped to be able to locate the animals within the grid (see Figure 1). There are radio acoustic positioning systems available that do provide more finely resolved positional data but they were not believed to be appropriate for the present study given the considerable water depth and high energy at the study site.

In addition, a manual tracking system was deployed from a boat. It consisted of a Lotek LHP_1 hydrophone, SRX_400 receiver with antenna, and an Upconverter.

A base station was established in the remaining structure of a lighthouse that had once been located at Cape St. Francis. A six-element YAGI antenna was secured outside the structure in order to pick up the radio signals transmitted from the hydrophone buoys at the study site. Signals detected by the YAGI antenna were sent to a Lotek SRX_400 radio receiver established within the structure. Absence/presence data were stored in the receiver unit.

Video Camera

A SIT (Silicone Intensified Target) camera was used to monitor the behaviour of the experimental animals caged within a crab trap. Specifics of the camera include 400-line resolution, zero lux @f/2.6 light sensitivity, 85º wide angle field of view, and a six inch to optical infinity focal range.

A rigid bracket was fabricated and mounted onto the crab trap used to hold the animals during experimentation. The bracket was fully adjustable, allowing the video technician to angle the camera in order to optimize the field of view. The experimental snow crabs were recorded in real time VHS format on topsides electronic equipment.

Monitoring of the crab with the SIT camera was initially attempted with traps suspended in the water column beneath the seismic source but there was far too much motion due to water movement to detect any observable startle response by the crab.

Instead, a shooting session was conducted in Freshwater Bay located just outside the St. John's Narrows. We conducted the session in a 50-m water depth and rested the experimental crab trap on the ocean bottom in relatively calm water. Twelve hard shelled legal-sized male snow crabs were placed in the trap and observed during the shooting session.

Field Experiments on Crab Health

The field experiments on crab health using a single 40-in^3 air gun were conducted a minimum of five kilometers from the experimental fishing site prior to the post-seismic component of the

experimental fishery. It was assumed that energy from the single gun at such a distance would have negligible effects on the snow crabs occurring at the experimental fishing site.

Male snow crabs used in the field experiments on effects of seismic on crab health belonged to two size classes; (1) legal-sized animals ranging between 95 and 115-mm carapace width (CW), and (2) sublegal-sized animals ranging between 75 and 95-mm CW (Figure 6). The mean carapace widths of all experimental groups are presented in Table 2.

Figure 6. Experimental male snow crab.

Two types of seismic shooting were conducted during this phase of the study:

1. Hard shelled legal-sized crabs, hard shelled sublegal-sized crabs, egg-bearing female crabs and detached fertilized eggs were exposed to the seismic energy from a single 40-in^3 sleeve air gun (~ 226 dB re 1 µPa-m 0 to peak). A trap holding these animals/eggs was placed at distances from the air gun ranging between 2 and 15-m.

2. Only hard shelled legal-sized crab were exposed to seismic energy from the 200-in^3 seven-air gun array. The array was suspended within a rectangular steel frame (1.25-m width x 1.25-m height x 2.5-m length). Four large inflatable buoys were attached to the top corners of the frame to provide floatation. The experimental trap was suspended directly below this array at depths appropriate for the specific treatments (i.e., 4 to 170-m).

Table 2. Specific details of seismic shooting, including date, exposure volume, exposure distance, number of specimens and the mean crab sizes for all experimental groups.

Date (d/m/y)	Treatment [T] Or Control [C]	Seismic Source (cu. in.)	Distance Between Seismic Source and Crabs (m)	Crab Number (n)	Crab Category	Mean Carapace Width ± s.d. (mm)
03/10/02	T1	40	10	4	L	102.8 ± 5.7
03/10/02	C1	-	10	4	L	109.3 ± 3.9
03/10/02	T2	40	10	4	L	103.3 ± 2.8
03/10/02	C2	-	10	4	L	107.8 ± 4.3
26/10/02	T3	40	10	4	SL	86.5 ± 3.4
26/10/02	C3	-	10	4	SL	89.5 ± 1.3
26/10/02	T4	40	10	4	SL	86.3 ± 5.9
26/10/02	C4	-	10	4	SL	88.3 ± 3.1
26/10/02	T5	40	2	4	SL	84.8 ± 3.6
26/10/02	C5	-	2	4	SL	85.8 ± 2.2
26/10/02	T6	40	2	4	SL	86.8 ± 3.6
26/10/02	C6	-	2	4	SL	88.5 ± 2.6
10/11/02	T7	40	2	4	L	104.8 ± 4.6
10/11/02	C7	-	2	4	L	106.5 ± 4.8
10/11/02	T8	40	2	4	L	103.8 ± 1.7
10/11/02	C8	-	2	4	L	103.5 ± 2.6
10/11/02	T9	40	15	4	L	104.8 ± 5.1
10/11/02	C9	-	15	4	L	111.5 ± 1.7
10/11/02	T10	40	15	4	L	113.5 ± 1.3
10/11/02	C10	-	15	4	L	106.8 ± 6.2
21/11/02	T11	40	15	4	SL	89.0 ± 4.2
21/11/02	C11	-	15	4	SL	92.0 ± 2.7
21/11/02	T12	40	15	4	SL	91.0 ± 1.6
21/11/02	C12	-	15	4	SL	92.8 ± 1.0
21/11/02	T13	200	170	4	L	97.3 ± 1.0
21/11/02	C13	-	170	4	L	96.8 ± 1.5
27/11/02	T14	200	170	4	L	106.5 ± 5.7
27/11/02	C14	-	170	4	L	108.3 ± 8.2
27/11/02	T15	200	85	4	L	100.5 ± 3.1
27/11/02	C15	-	85	4	L	105.8 ± 5.2
30/11/02	T16	200	4	4	L	105.3 ± 7.9
30/11/02	C16	-	4	4	L	108.0 ± 8.5
30/11/02	T17	200	50	4	L	113.5 ± 6.4
30/11/02	C17	-	50	4	L	107.3 ± 7.8
01/12/02	T18	200	50	4	L	102.5 ± 5.8
01/12/02	C18	-	50	4	L	100.5 ± 5.8

'L' = legal-sized male, 'SL' = sublegal-sized male

During each experiment, crabs were exposed to 200 air gun shots at a firing rate of one shot every 10 seconds for a period of 33 minutes.

Other than the snow crabs exposed to seismic energy during the post-seismic experimental commercial fishing and the telemetry component, a total of ninety-two snow crabs were treated with seismic energy and then either processed onboard or returned to DFO to be held alive in

tanks. An equal number of control animals were also retained. Treatments using the 40-in^3 gun included fifty-nine snow crabs (thirty-two legal-sized males, twenty-four sublegal-sized males, and three females bearing eggs). All were processed onboard the study vessel except for eight legal-sized males and the three females. These 11 animals were taken to DFO and placed into holding tanks. One sample mass of fertilized eggs was also treated with seismic energy and returned to DFO. There were controls for all 59 crabs and the egg mass. Treatments using the 200-in^3 seven-gun array included only legal-sized male crabs. Thirty-three legal-sized male crabs were treated during the second phase. Twenty-four of them, along with 24 control animals, were processed onboard the vessel. The remaining nine treated animals and their controls were taken to DFO and placed into holding tanks. The breakdown of each shooting session is provided below in Table 3.

Table 3. Specifics of the experimental seismic treatments, including dates, exposure volumes, exposure distances, number of specimens and subsequent sampling/handling of the specimens.

Date (d/m/y)	Seismic Source (in^3)	Distance between gun and crabs (m)	Number of treated crabs	Number of control crabs	Processing specifics
03/10/03	40	10	8 (L)	8 (L)	OB(16L)
26/10/03	40	10	8 (SL)	8 (SL)	OB(16SL)
26/10/03	40	2	8 (SL)	8 (SL)	OB(16SL)
02/11/03	40	2	3 (F)	3 (F)	DFO(6)
10/11/03	40	2	16 (L) 1 (EM)	16 (L) 1 (EM)	OB (16L) DFO (16L) DFO (2EM)
10/11/03	40	15	8 (L)	8 (L)	OB (16L)
21/11/03	40	15	8 (SL)	8 (SL)	OB (16L)
27/11/03	200	170	8 (L)	8 (L)	OB (16L)
27/11/03	200	85	4 (L)	4 (L)	OB (8L)
30/11/03	200	4	13 (L)	13 (L)	OB (8L) DFO (18L)
30/11/03	200	50	4 (L)	4 (L)	OB (8L)
01/12/03	200	30-50	4 (L)	4 (L)	OB (8L)

'L' denotes legal-sized males; 'SL' denotes sublegal-sized males; 'F' denotes females
'EM' denotes fertilized egg mass; 'OB' denotes full onboard processing;
'DFO' denotes placement in holding tank at DFO.

Exposure of Male Crabs to a 40-in^3 Air Gun

Two replicate samples of four animals each of both legal-sized males and sublegal-sized males were caged and suspended at depths of 2, 10 and 15-m below a 40-in^3 air gun (Table 4). After each 33 minute exposure to seismic sounds, four control animals were lowered to the same depth as the four treated animals for thirty minutes. After thirty minutes, both the treatment and control animals were brought to surface. Samples of haemolymph (crustacean blood) and tissue were taken immediately from the eight animals.

Table 4. Specifics of exposure of male crabs to the seismic energy of a 40-in^3 air gun from various distances.

Distance between seismic source and crabs (m)	**Gun volume (in^3)**	**Number of crab samples**			
		Legal-sized males		**Sublegal-sized males**	
		Control (N x number of replicates)	Treatment (N x number of replicates)	Control (N x number of replicates)	Treatment (N x number of replicates)
2	40	4 x 2	4 x 2	4 x 2	4 x 2
10	40	4 x 2	4 x 2	4 x 2	4 x 2
15	40	4 x 2	4 x 2	4 x 2	4 x 2

Exposure of Male Crabs to a 200-in^3 Air Gun Array

Samples of four legal-sized male crabs were caged and suspended at distances of 4, 50, 85 and 170-m below the 200-in^3 seven-gun array. Exposures at 50-m and 170-m were replicated twice (Figure 7; Table 5). After each treatment, four control crabs were lowered to the same depth as the treatment animals for thirty minutes. After thirty minutes, both the treatment and control animals were brought to surface. All eight crabs were immediately sampled for haemolymph and tissue required for the subsequent analyses.

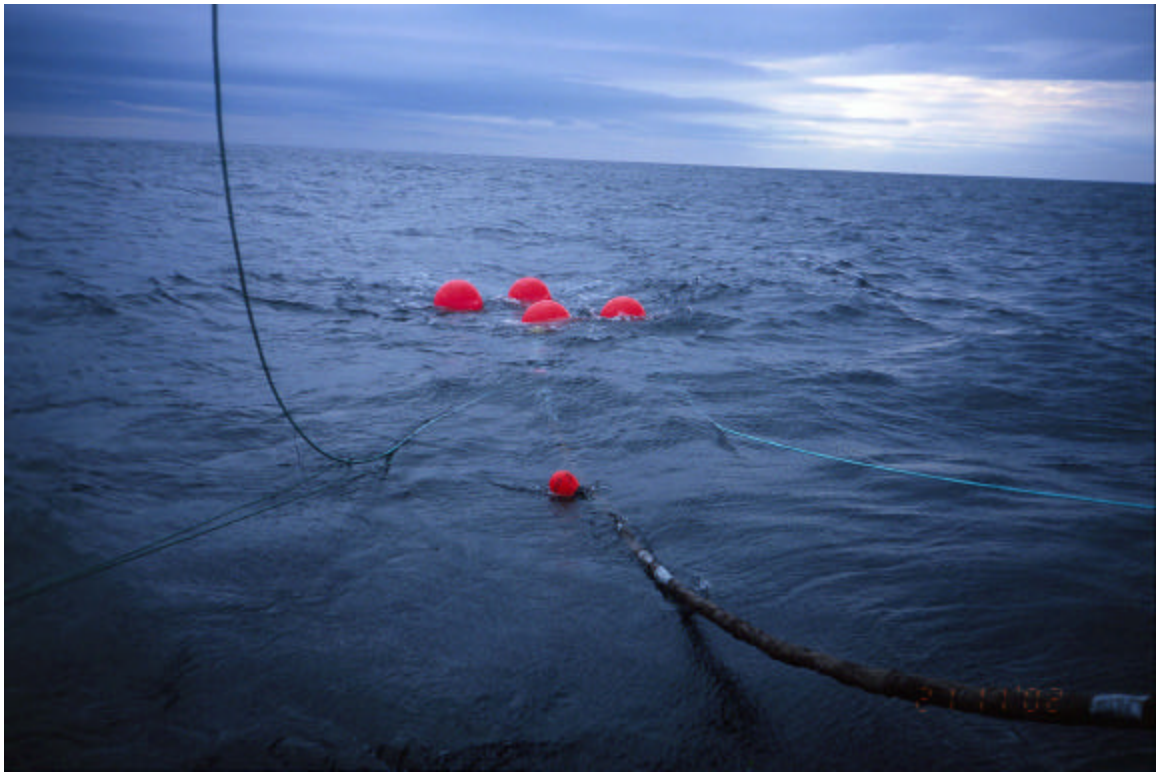

Figure 7. 200-in^3 array firing off the stern of *FV Rough and Wild*.

Table 5. Specifics of exposure of male crabs to the seismic energy of a 200-in^3 sleeve gun array from various distances.

Distance between seismic source and crabs (m)	Array volume (in^3)	Number of legal-sized male crab samples	
		Control (N x number of replicates)	Control (N x number of replicates)
4	200	4 x 1	4 x 1
50	200	4 x 2	4 x 2
85	200	4 x 1	4 x 1
170	200	4 x 2	4 x 2

Testing for Delayed Effects of Exposure to Seismic Energy

Immediately following each seismic treatment, the exposed animals were observed closely for signs of morbidity and/or mortality. Male and female crabs that had been exposed to seismic energy were observed for a period of twelve weeks at the DFO in case of any delayed lethal and/or sub-lethal effects. All treated animals had associated controls. Eight legal-sized male crabs exposed to energy from the 40-in^3 sleeve gun at 2-m and 9 legal-sized male crabs exposed to energy from the 200-in^3 7-sleeve gun array at 4-m (Table 6) were returned to the Department of Fisheries and Oceans (DFO) in St. John's and held in aquaria. An equal number of control animals were also moved to the DFO facility. These animals were taken to the DFO for observation and examined to determine whether there were delayed or sub-lethal effects.

Four weeks after exposure, samples of haemolymph were taken from both the treatment and control crabs. Samples from all of the crabs were fixed for haemocyte counts while only the samples from treatment and control animals associated with exposure to the 200-in^3 7-sleeve gun array were subjected to protein and enzyme analysis. Samples of sera and fixed organ tissue have been archived at – 80°C.

Table 6. Specifics of exposure of 'delayed effects' male crabs to seismic energy from both the single 40-in^3 gun and the 200-in^3 seven-gun array.

Distance between seismic source and crabs (m)	Gun/array volume (in^3)	Post-exposure time of sampling (wks)	Number of legal-sized male crab samples	
			Control	Treated
2	40	4	8	8
4	200	2	9	9

Exposure of Fertilized Eggs and Egg Carrying (Berried) Female Crabs

The DFO, St. John's, provided the berried female crabs used in this study. The crabs were collected by the DFO in White Bay during a stock assessment survey.

Fertilized eggs containing developing embryos were stripped from the females and successfully incubated in 1-litre chambers at 4°C for a period of approximately two months. A 4°C temperature was chosen in order to enhance development (Mallet et al. 1993) to meet project timelines and to reduce the potential for onset of disease, a problem in rearing crustacean eggs

and larvae under laboratory conditions (Fisher et al. 1978; Aiken and Waddy 1985). Eggs were not disinfected in order to avoid increased potential for sub-lethal effects. Approximately 4,000 eggs showing essentially the same level of eye development were divided into two masses and placed into two small nylon mesh bags. Both the treatment and control egg samples were taken to the experimental field site. One sample was exposed to the energy from a 40-in^3 sleeve gun at two metres (Table 7). As was the case during the other shooting sessions, a total of 200 shots were fired, one every 10 seconds. The exposed and control eggs were returned to the laboratory and held in aquaria at 4°C. The eggs were monitored closely for the onset of disease. Twelve weeks after exposure to the seismic energy, the treated and control eggs were fixed in formalin for subsequent analysis.

Six female crabs that were carrying eggs were also transported to the field for experimentation. Three of the female crabs were exposed to the 40-in^3 sleeve gun (Table 7). After exposure, the control and treatment animals were returned to the DFO laboratory and held at ambient temperature in flow-through aquaria to be monitored for delayed mortality of both eggs and crabs. Pending the absence of crab mortality and egg disease, the eggs will eventually be removed from the females, fixed and archived. This will occur outside the time frame of this study because the eggs will be allowed to develop until just before larval hatching, sometime between April and June.

Table 7. Specifics of exposure of fertilized eggs and egg carrying female crabs to seismic energy from the single 40-in^3 air gun.

Distance between seismic source and crabs (m)	Gun volume (in^3)	Post-exposure time of sampling (wks)	Number and type of samples	
			Control	Treated
2	40	12	1 egg mass	1 egg mass
2	40	Ongoing*	3 berried females	3 berried females

* Outside the time frame of this study

Onboard Sampling for Physiological Analysis

Some analyses were conducted onboard the vessel immediately after each treatment, but most samples were preserved for later analysis at the laboratory. Specifics associated with each type of sampling are presented below.

Haemolymph

Haemolymph was withdrawn at the body-leg joint of the 2nd or 3rd thoracic legs of live snow crab using three millilitre sterile syringes with 23-gauge needles. The refractive index of haemolymph was recorded by placing a drop on a Westover Model RHS-10ATC hand held refractometer (s.g.1.000 to 1.070). The refractometer had automatic temperature compensation. Half a millilitre of haemolymph was gently expelled from the syringe into a tube containing 10% buffered formalin for subsequent haemocyte counts. Another 1.5-ml of haemolymph withdrawn with a syringe was centrifuged for two minutes at 2000 rpm and the supernatant or serum was frozen immediately on dry ice. Upon return to the laboratory, it was placed in –60°C freezer for subsequent biochemical analysis.

Hepatopancreas and Heart

A portion of hepatopancreas and the heart were removed from each dissected animal and placed in 10% buffered formalin for later histological processing.

Head

The head sample, which included the statocyst, green gland and brain, was fixed in 10% buffered formalin. In the laboratory, the statocysts were carefully dissected out of the sample and the other tissues were archived.

Gills and Gonads

Two gills from the right side of each crab and a portion of the gonads were placed in 10% buffered formalin and archived.

Archiving of Samples

All unanalyzed samples were fixed in 10% buffered formalin and archived. These included all samples from snow crabs exposed to the 200-in^3 array at a distance of 85-m. These samples were archived because analyses were conducted on replicate samples from animals exposed to the array at distances less than and greater than 85-m.

At some time after completion of this study, tissue samples will also be removed from the remaining live crabs still held in the laboratory for observation on delayed mortality and chronic sublethal effects. Tissues that will be sampled include hepatopancreas, heart, fertilized eggs, and head region (statocyst, green gland, brain). All of these archived tissues are fixed in 10% buffered formalin.

Laboratory Sample Analysis

Haemolymph

Haemolymph solutes

Relative concentrations of total dissolved substances in serum were determined by refractometry. Comparisons between control and treatment groups were conducted using the unpaired t-test or the Mann-Whitney Rank Sum test if the groups were not normally distributed. Probabilities = 0.05 were considered significant.

Serum proteins

The protein concentration in each serum sample was determined using the Lowry protein method (Lowry et al. 1951). Comparisons between control and treatment groups were conducted using the unpaired t-test or the Mann-Whitney Rank Sum test if the groups were not normally distributed. Probabilities =0.05 were considered significant.

Serum enzymes

Frozen serum samples were thawed, diluted to 20% with distilled water, and examined with the API ZYM^{R} system, a commercial product that provides a coarse quantitative method for rapidly screening 19 enzymatic reactions (Monget 1978; Mathieu et al. 2001). The colorimetric technique is applicable to all specimens including biological fluids and tissues homogenates. Pilot studies were initially conducted to establish that this system could also be applied to snow crab serum and tissues when it was diluted to a 20% solution of distilled water. The system consists of a strip with 20 microwells containing enzyme substrates and buffer for assaying various hydrolases including phosphatases, esterases, aminopeptidases and glycosidases. Seventy-five µl of diluted serum were placed in each microwell. The metabolic byproducts produced during the 45-minute incubation period at room temperature change the colour of the fluid. The reactions were then graded visually, depending on the intensity of colour, from 0 to 5 (0 – no enzymatic activity detected, 5 – maximum intensity of the reaction), with reference to the API ZYM colour reaction chart. These tests can detect only gross differences in enzyme activity between treatment and control groups, not subtle ones.

Relative Numbers of Haemocyte Types

Pilot studies were conducted to determine how best to preserve and fix haemocyte smears. Addition of ethylenediaminetetracetic acid, citrate or N-ethylmaleimide to haemolymph to prevent agglutination and coagulation (Bang 1970; Durliat and Vranck 1981; Martin et al. 1991) and the fixation of haemolymph in formalin produced generally unsatisfactory results. After various trials, it was determined that fixation of haemolymph in formalin followed by washing and re-suspension of pelleted haemocytes in a solution of methanol and acetic acid gave satisfactory results. It was also observed that, in order to avoid haemocyte "clumping", it was best not to prepare smears from haemolymph that had been left in formalin for extended periods. Therefore smears were prepared within a few hours of the haemolymph sampling.

Haemolymph fixed in 10% buffered formalin onboard the vessel was subsequently centrifuged in the laboratory. Resulting pellets were washed three times with distilled water. The pelleted haemocytes were then re-suspended in a 4:1 methanol/acetic acid solution. A drop of cell suspension was placed on a microscope slide and smeared, air dried, stained with GIEMSA and examined with a Wild Leitz Aristoplan bright field microscope in order to identify the three types of haemocytes (hyalinocytes, semi-granulocytes and granulocytes) described in previous studies on crustaceans (Bauchau 1981; Soderhall and Cerenius 1992). Estimations of size of the three types of haemocytes were made on 10 cells of each type using a Leitz calibrated linear scale. A differential count was performed on the three types of cells and expressed as a percentage of each type of 200 haemocytes counted per slide. Analysis scatter plots of raw and transformed data and results of regression analysis, including residuals, showed that percentages needed to be transformed using arcsin square root before comparisons between control and treatment groups could be done using the unpaired t-test or Mann-Whitney Rank Sum test (Sokal and Rohlf 1981). Probabilities = 0.05 were considered significant.

Organ and Tissue Pathology

Statocyst Examination

The head regions of crabs, including antennules, were excised in the field and immediately fixed in 10% buffered formalin for at least 96 hours. Basal segments of antennules were removed from the front of the head and, using a Wild Heerbrugg stereomicroscope, the statocysts were carefully dissected out in the following manner. The lateral outside wall of the shell of the antennular segment was removed to expose the statocyst capsule in the lumen of the segment. The interior of the capsule, including the strap of setae (sensory hairs) projecting inside the capsule, was then examined for microstructural differences between the control and treatment groups.

Preliminary studies indicated that the sensory hairs were of sufficient diameter and length to allow for inspection of sensory hair fields by light microscopy. Moreover, statocysts of individual crabs were noted to contain only one distinct sensory hair field. This permitted observation of the statocysts of a large number of crabs for visible signs of hair ablation in their sensory hair fields. Representative pictures were taken with a Nikon digital camera.

Some representative samples were also prepared for scanning electron microscopy. These samples were dehydrated in a series of ethanol solutions before being placed in 100% ethanol for 12 h. The samples were then placed in a desiccator for twenty-four hours, after which conducting graphite paint was used to glue the preparations to a scanning electron microscope stub. They were sputter coated with gold and examined with a scanning electron microscope.

Hepatopancreas and Heart Histopathology

Both the hepatopancreas and heart samples were processed using standard histological methods (Lynch et al. 1969) with an Autotechnicon Tissue Processor. A graded ethyl alcohol series (70%, 80%, 95% and two changes of 100%) was used to dehydrate the samples. The organs were then cleared in three changes of citrisolv™. Finally, the tissues were impregnated with three changes of molten embedding media, Tissue Prep 2™. The processed tissues were embedded in steel molds using molten embedding media and topped with labelled embedding rings. After cooling, the hardened blocks of embedded tissues were removed from their base molds. The blocks were then trimmed of excess wax. Sections were cut at 6µ on a Leitz microtome, floated on a 47^0C water bath containing gelatin, and then picked up on labelled microscope slides. After air drying, the slides were fixed at 60 ^{0}C for approximately 2 hours to remove most of the embedding media and allow the sections to adhere properly to the slide. Sections were stained using Mayers Haematoxylin and Eosin method (Luna 1968). Coverslips were then applied using Entellan ® and the slides were left overnight to air dry and harden.

Slides were then examined under different magnifications by transmission light microscopy using a Wild Leitz Aristoplan bright field microscope for any microstructural differences between the control and treatment groups.

Egg Development

Fertilized eggs that had been exposed to seismic energy were held at DFO in one-litre mason jars filled with seawater at 4ºC. They were observed for delayed lethal and/or sub-lethal effects for a period of twelve weeks. After the twelve-week observation period, eggs were fixed in formalin, washed in deionised water and examined under a Wild Heerbrugg stereomicroscope. Preliminary examination indicated that the eggs could be divided into 2 categories based on colour criteria; "beige" and "orange-brown". The orange-brown eggs were further divided into 3 categories based on eye size criteria; "no eyes", "small eyes", and "big eyes". Measurements were made of the greatest width and length of one eye per embryo for 12 representative fertilized eggs for each 'eyed' category. A Leitz calibrated linear scale was used to measure the eyes.

The percentages of eggs in all categories were calculated and statistically analysed using the z-test. Resultant probabilities = 0.05 were considered to indicate statistically significant differences. Representative pictures of the different categories were taken with a Nikon digital camera.

RESULTS AND DISCUSSION

Acoustic Measurements

The sound levels received by the experimental crab and fertilized eggs from different source volumes and distances were measured in the field and are reported in two ways: (1) broadband peak pressure (0-P) in Pa and the equivalent dB level, and (2) energy density (dB re 1 µPa^2/Hz) over a frequency range up to 500 Hz. It appears that saturation of the acoustic measuring system occurred in treatments using the 40-in^3 gun at two and five metres, and the 200-in^3 array at four metres. Therefore, the broadband peak levels presented for these treatments (Table 8) are lower than the actual levels (see Appendix 1 for graphic representations of broadband signal signatures and energy density spectra).

Table 8. Results of measurements of received sound levels. Received levels at distances of 2 and 5-m from the 40-in^3 air gun were not measured accurately. Received sound levels at 2 and 5-m shown in brackets were interpolated by estimating source levels using the 10-m data and assuming spherical spreading from the source.

Date (d/m/y)	Seismic Source Volume (in^3)	Seismic Source to Hydrophone Distance (m)	Number of Shots	Mean 0-Peak (Pa)	Mean Equivalent dB Level (re 1 µPa)
03/10/02	40	10	156	11,350	201.1
03/10/02	40	10	138	11,482	201.2
26/10/02	40	10	91	15,488	203.8
26/10/02	40	10	145	14,622	203.3
26/10/02[a]	40	5	13	15,668	203.9 (213)
26/10/02[a]	40	2	6	16,032	204.1 (221)
26/10/02[a]	40	2	97	15,311	203.7 (221)
02/11/02[a]	40	2	109	15,488	203.8 (221)
10/11/02[a]	40	2	83	15,488	203.8 (221)
10/11/02[a]	40	2	46	15,668	203.9 (221)
10/11/02[a]	40	2	59	15,311	203.7 (221)
10/11/02	40	15	136	14,289	203.1
10/11/02	40	15	36	14,454	203.2
27/11/02	200	85	131	7,413	197.4
30/11/02[a]	200	4	176	15,311	203.7
01/12/02[b]	200	50	26	12,303	201.8
01/12/02[c]	200	50	72	13,804	202.8

[a] possible saturation
[b] maximum energy density in low frequency (< 100 Hz)
[c] maximum energy density in high frequency (> 100 Hz)

Unsaturated received peak broadband levels from the 40-in^3 gun at 10-m ranged from 201.1 to 203.8 dB re 1 µPa (11,350 to 15,488 Pa). Unsaturated received peak broadband levels from the 40-in^3 gun at 15-m ranged from 203.1 to 203.2 dB re 1 µPa (14,289 to 14,454 Pa). The peak source level estimated using the 10-m data and assuming spherical spreading propagation from the source was 224 to 227 dB re 1µPa at 1-m. This source level and assumed spherical spreading was used to interpolate received levels at 5-m as 213 dB and at 2-m as 221 dB. The received peak levels from the 200-in^3 array at 50 to 85-m ranged from 197.4 to 202.8 dB re 1 µPa (7,413 to 13,804 Pa). The

signals from the 200-in^3 array at 50-m was split into two frequency ranges (< 100 Hz and 100 to 500 Hz) because two energy density peaks were observed (Table 9; Appendix 1). The estimated peak source level estimated using the 50-m data and assuming spherical spreading from the source was 237 dB re 1µPa at 1-m. This is the equivalent source level for a point. A receiver 4-m from the array is with in the near field of the array and receives sound from individual air guns rather than from the integrated array as a whole; thus, the actual received levels at 4-m could not be estimated.

Table 9. Specifics of energy density spectra of measured seismic shots.

Date (d/m/y)	Seismic Source Volume (in^3)	Seismic Source to Hydrophone Distance (m)	Maximum Ambient Energy Density (dB re 1 µPa^2/Hz)	Maximum Seismic Energy Density (dB re 1 µPa^2/Hz)
03/10/02	40	10	110.7	153.5[a]
26/10/02	40	10	99.5	154.3[a]
26/10/02	40	5	99.5	170.0[a]
26/10/02	40	2	99.5	183.2[a]
02/11/02	40	2	114.9	182.6[a]
10/11/02	40	2	86.6	186.7[a]
10/11/02	40	15	86.6	146.9[a]
27/11/02	200	85	98.5	130.0[b]
30/11/02	200	4	102.0	175.2[c]
01/12/02	200	50	95.2	149.5[d]

[a] maximum energy density in frequency range 24-31 Hz
[b] maximum energy density in frequency range 106-260 Hz (mean = 170 Hz)
[c] maximum energy density in frequency range 17-19 Hz
[d] some maximum energy densities in frequency range 16-20 Hz; others in frequency range 107-244 Hz (mean = 164 Hz)

The energy density measurement results were not affected by the system saturations. The highest maximum energy density levels occurred as a result of shoots with the 40-in^3 gun at 2-m. These energy densities ranged from 182.6 to 186.7 dB re 1 µPa^2/Hz at frequencies between 24 and 31 Hz. The maximum energy density observed with the 200-in^3 array was 175.2 dB re 1 µPa^2/Hz within the 17 to 19 Hz frequency range. The maximum ambient energy densities during the days with seismic shooting ranged between 86.6 to 114.9 dB re 1 µPa^2/Hz (Table 9). Energy is a more meaningful measure of exposure because it includes an element to account for the duration of the pulse. Two pulses of equal peak pressure can, depending on their durations, have radically different energy levels.

Water Column Temperature Profiling

Bottom temperatures at the study site during the fieldwork component ranged from –0.5 to –1.5 º C. The primary thermocline was located about 20-m from surface at the onset of the fieldwork but by the later part of October to completion, it had shifted to between 40 and 85-m (Appendix 2).

Existing Knowledge of Invertebrate Sound Detection

Acoustic detection by invertebrates has been the subject of various papers over the last few decades (Wiese 1976; Janse 1980; Tautz and Sandeman 1980; Hawkins and Myrberg, Jr. 1983;

Budelmann 1988; Breithaupt and Tautz 1990; Goodall et al. 1990; Budelmann 1992; Cate and Roye 1997; Popper et al. 2001). Decapod crustaceans have a variety of external and internal sensory receptors that are potentially responsive to sound and vibration. Many of these receptors resemble vertebrate receptors that respond to hydrodynamic stimulation, particle motion and, possibly, pressure. However, by virtue of their body plan and the constraints thus imposed upon sensory structure, aquatic decapods appear specialized to respond to particle displacement components of an impinging sound field and not to the pressures. The belief that decapods have a limited ability to detect acoustic stimuli is plausible considering that aside from the exoskeleton, most decapod crustaceans are basically the same density as water and do not have any air-filled spaces such as those associated with pressure detection in fish (Hawkins and Myrberg Jr. 1983; Breithaupt and Tautz 1990; Popper and Fay 1999).

Many decapods have an extensive array of hair-like receptors both within and upon the body surface that most probably respond to water- or substrate-borne displacements (Breithaupt and Tautz 1990). Decapods also have an abundance of proprioceptive organs that could serve secondarily to perceive vibrations (Burke 1954).

Effects on Experimental Commercial Fishing

Specifics of Fishery Sets

The experimental commercial fishing was conducted by licensed commercial fishermen using their normal gear and procedures (Refer to 'Materials and Methods' for more detail). The normal complement of traps in each fleet was 40. The pre-seismic commercial fishing was conducted from 18 September to 20 October 2002 (Table 10). Seismic shooting for the crab health experiments was initiated on 3 October 2002. It was limited to two sessions with the single 40-in^3 gun and was conducted at a location at least 5-km from the experimental fishing site. No more seismic shooting occurred until 26 October. It has been assumed that those two sessions (400 shots) on 3 October had negligible impact on the behaviour of the crabs at the fishing location and did not affect the subsequent three pre-seismic fishery sets between 9 and 20 October. Therefore, the period of seismic shooting indicated on Figures 8 and 9 is from 26 October to 30 November (Table 11). Again, the adverse weather conditions during the fall months of 2002 prohibited the completion of fieldwork within a shorter time period. Unfortunately, there was a wider than intended temporal separation between the pre-seismic fishing and the post-seismic fishing.

Two of the six post-seismic fishing sets were deployed immediately after completion of shooting sessions with the 200-in^3 array, one after a 200 shot session and the other after a total of 400 shots. Another set was deployed within one day (20 hours) of a 200 shot session with the seven-gun array, and two other sets were deployed within two days (40 and 47 hours) of the completion of seismic shooting with the array. The sixth set was deployed approximately six days (148 hours) after 400 shots of the array (Table 11).

Table 10. Specifics of experimental commercial snow crab fishery before and after exposure to seismic.

Haul Date (d/m/y)	Soak Time (h)	Traps Fishing (n)	Legal-sized Males (n)	Sublegal-sized Males (n)	Number of Soft-shelled Males	CPUE for Legal-sized Males (n/trap/24h)	CPUE for Legal-sized Males (n/trap/soak time)	CPUE Sublegal-sized Males(n/trap/24 h)
Pre-Seismic								
19/09/02	19	40	195	636	10	6.2	4.9	20.1
20/09/02	27	40	82	415	6	1.8	2.0	9.2
22/09/02	44	40	103	337	6	1.4	2.6	4.6
24/09/02	45	40	284	509	8	3.8	7.1	6.8
11/10/02	43	40	128	250	83	1.8	3.2	3.5
14/10/02	68	39	100	223	41	0.9	2.6	2.0
20/10/02	141	39	104	189	22	0.5	2.7	0.8
Post-Seismic								
22/11/02	16	37	90	355	0	3.6	2.4	16.0
23/11/02	25	38	255	540	7	6.4	6.7	13.6
27/11/02	96	36	138	92	29	1.0	3.8	0.6
30/11/02	20	36	139	290	16	4.6	3.9	9.7
06/12/02	144	36	114	88	0	0.5	3.2	0.4
12/12/02	144	35	120	78	0	0.6	3.4	0.4

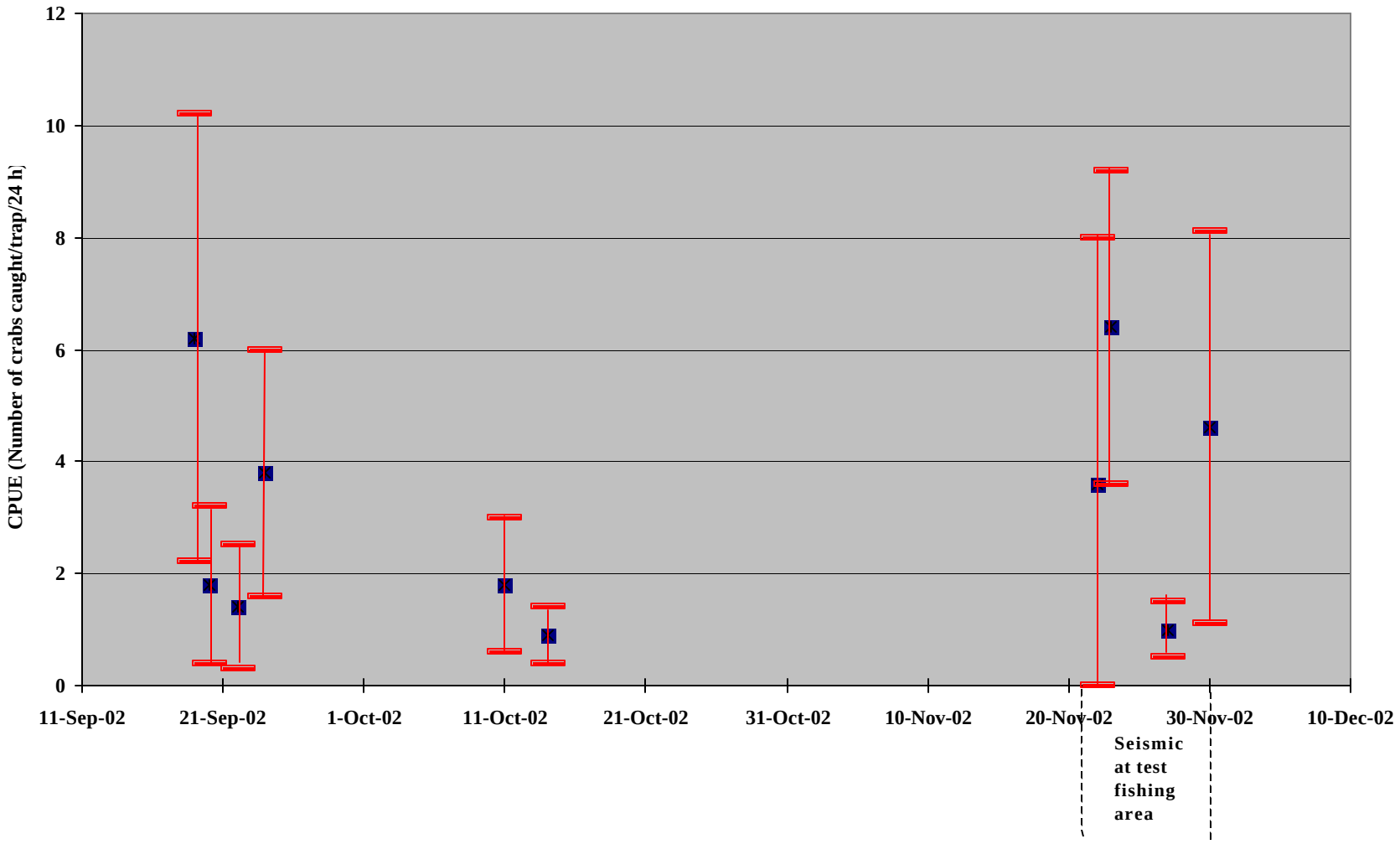

Figure 8. Mean standardized number of legal-sized snow crabs caught in the experimental commercial fishery trap fleet sets with soak times of 96 h or less. Upper and lower limits of standard deviation (red bars) are shown for each mean value (black box). Catch data are on fleet retrieval dates.

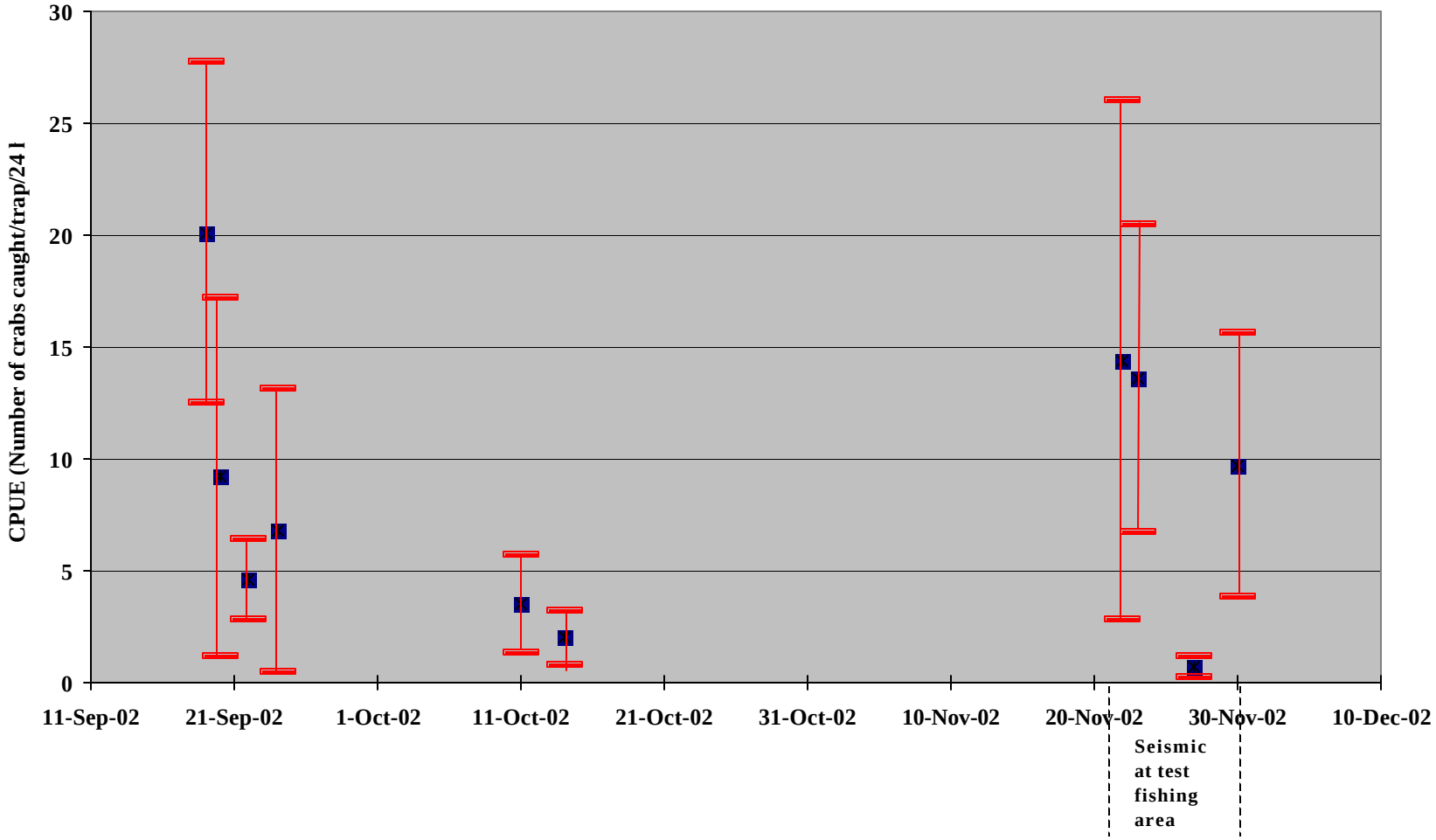

Figure 9. Mean standardized number of sublegal-sized snow crabs caught in the experimental commercial fishery trap fleet sets with soak times of 96 h or less. Upper and lower limits of standard deviation (red bars) are shown for each mean value (black box). Catch data are on fleet retrieval dates.

Table 11. Temporal and spatial relationships between seismic shooting and post-seismic experimental commercial fishing.

Date	Seismic Source	Location of Shooting	Number of Shots	Deployment and Haul Dates of Fishing Sets	Time Between End of Last Seismic Session and Set Deployment (h)	Time Between End of Last Seismic Session and Set Haul (h)
26 Oct	40-in^3	> 5-km	800[a]			
10 Nov	40-in^3	> 5-km	800[a]			
21 Nov	40-in^3	> 5-km	400[b]			
	200-in^3	Fishing site	200[c]	21-22 Nov	2	18
				22-23 Nov	20	45
				23-27 Nov	47	143
27 Nov	200-in^3	Fishing site	400[b]	29-30 Nov	40	60
30 Nov	200-in^3	Fishing site	400[b]	30 Nov-6 Dec	2	146
				6-12 Dec	148	292

5-km indicates that location of shooting was at least 5-km from experimental fishing location
[a] 4 shooting sessions
[b] 2 shooting sessions
[c] 1 shooting session

Geographic coordinates of fleet start and end points were recorded (see Figure 1 for locations). This provided a means of examining the potential effect of seismic shooting on catch rate from the perspective of distance from the seismic source.

Fishing gear soak times for the seven pre-seismic sets ranged from 19 to 141 hours, averaging about 55 hours per set (see Table 10). During the post-seismic experimental fishing period, the soak times ranged from 16 to 144 hours and averaged about 74 hours per set (see Table 10). The average number of legal-sized crabs caught per set (142) during the pre-seismic fishing was almost the same as for the post-seismic fishing (143). The average soak time during post-seismic fishing was almost 20 hours longer than during pre-seismic fishing due to poor weather conditions. The average number of traps fishing per set during post-seismic fishing (36.3) was slightly less than that used during pre-seismic fishing (39.7). When the data from all thirteen sets are considered, these differences are not significant but for the <96-h data, they are significant. Therefore, it was necessary to standardize the catch data to a 24-h period (known as catch-per-unit-effort or CPUE).

Initial Comparison of Pre- and Post-Seismic CPUE

Catch data have been standardized and presented as catch-per-unit-effort (CPUE), defined as the number of crabs caught per trap per 24-hour period. Only traps that were recovered and intact were used in the tables and analyses that follow. Traps that were set but not recovered or that were recovered in a damaged condition were not included. CPUE for legal-sized males during pre-seismic fishing ranged from 0.5 to 6.2 crabs/trap/24h compared to a range of 0.5 to 6.4 crabs/trap/24h during the post-seismic fishing (see Figure 8; Table 10). Similarly, CPUE for sublegal-sized males during pre-seismic fishing ranged from 0.8 to 20.1 crabs/trap/24h compared to a range of 0.4 to 16.0 crabs/trap/24h during post-seismic fishing (see Figure 9; Table 10). The smaller sublegal-sized crabs will leave the traps by crawling through the large mesh once the bait

has been fully eaten. Thus, these data are not as reliable as those for legal-sized crabs. Recently moulted soft-shelled crabs tend to disintegrate and so were not included in the analyses.

Only those sets with soak times less than or equal to 48 hours were plotted because as discussed below, data from longer soak times are probably much less reliable due to loss of bait effectiveness. Post-seismic CPUEs were higher in 25 of the 40 experimental crab traps (Figure 10). Considering the temporal differences between the pre-seismic and post-seismic sets, the mean number of legal-sized male crabs caught per 24 hour period was plotted for each of the 40 experimental traps during three periods: (1) 19 to 24 September (pre-seismic), (2) 11 October (pre-seismic), and (3) 22-30 November (post-seismic) (Figure 10).

Effect of Trap Soak Time on Pre- and Post-Seismic Fishing

Differences between pre-seismic and post-seismic CPUE were investigated using different soak times and crab trap inclusion criteria to determine if the difference in soak time could account for differences in CPUE. Crab fishermen indicated that the squid bait generally lasts no longer than four days and is probably most effective during the first two days of a set. Traps likely stop fishing after 96 h so CPUE statistics were based primarily on data from sets that were in the water no longer than 96 hours. CPUE for sets with soak times longer than 96 h would be affected more by soak time than those with shorter soak times. For the legal-sized male crabs, three soak times (=96 h, =48 h, and =30 h) were considered while for sublegal-sized male crabs, only the latter two soak times were used (Tables 12 and 13).

Under all soak time scenarios, the mean CPUE for legal-sized males was significantly greater during post-seismic fishing than during pre-seismic fishing (Table 12). For the sublegal-sized males, post-seismic mean CPUE was significantly greater than pre-seismic mean CPUE when using data from sets with soak times =48 hours. However, when only those sets with soak times less than or equal to 30 hours were considered, the pre-seismic and post-seismic mean CPUEs were essentially equal (Table 13).

Table 12. Statistical analyses of the legal-sized male catch-per-unit effort (CPUE) differences between pre-seismic commercial fishing and post-seismic commercial fishing (includes all traps that were retrieved intact).

Experimental Group	Soak Time (h)	Cumulative Number of Traps	CPUE (crabs/trap/ 24h)	p[a]	Significant Difference @ p<0.05
Pre-seismic	=96	239	2.7 ± 2.7	0.003	Yes
Post-seismic		147	4.0 ± 3.7		
Pre-seismic	=48	200	3.0 ± 2.9	< 0.0001	Yes
Post-seismic		111	4.9 ± 3.8		
Pre-seismic	=30	80	4.0 ± 3.7	0.046	Yes
Post-seismic		111	4.9 ± 3.8		

All CPUE data are presented as 'mean ± standard deviation'

[a] Calculated using Mann-Whitney Rank Sum Test

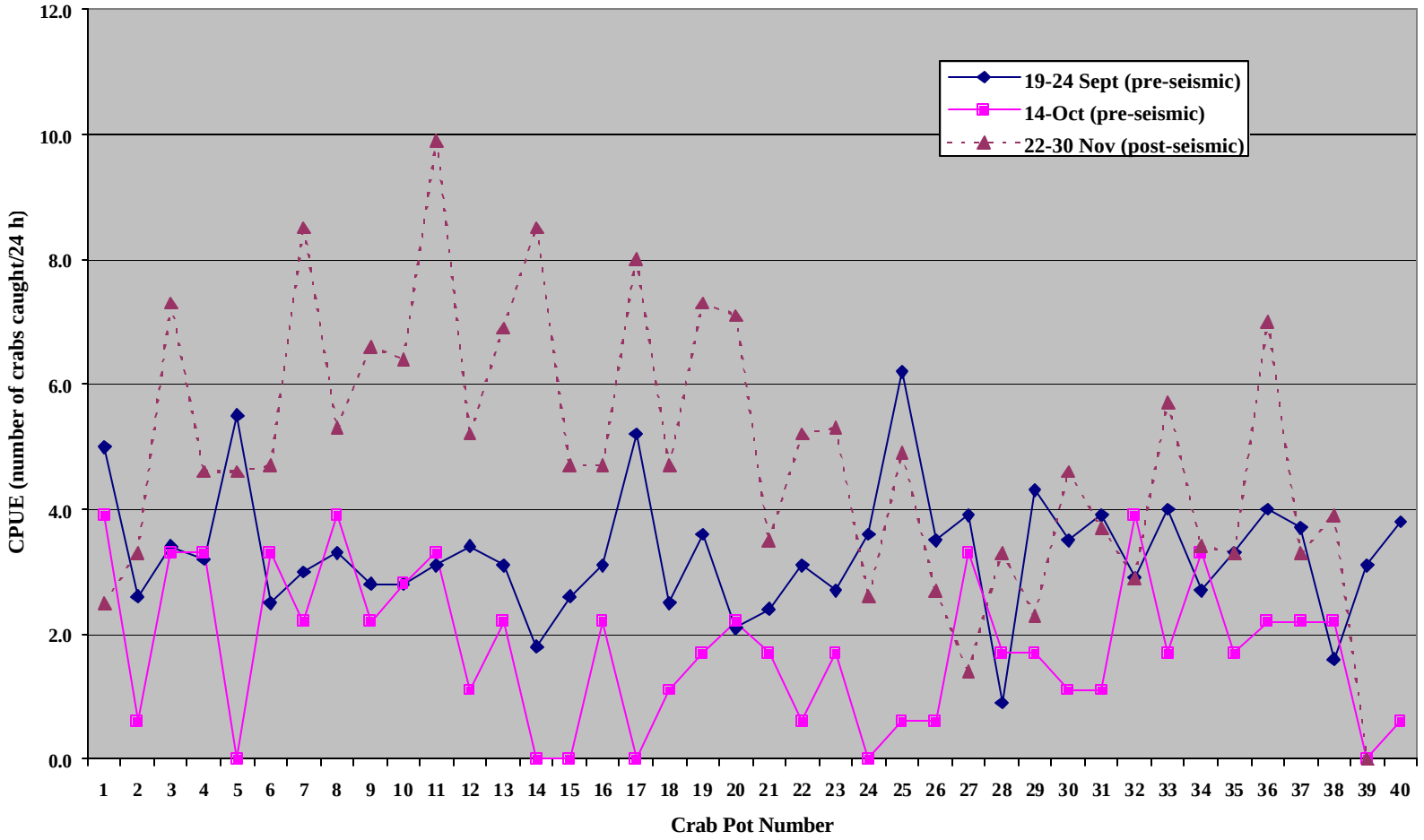

Figure 10. Mean standardized number of legal-sized snow crab caught in each trap for three different periods of the experimental commercial fishery. Only sets with soak times less than or equal to 48 hours were used (19-24 Sept: 4 sets; 11 Oct.: 1 set; 22-30 Nov: 3 sets).

Table 13. Statistical analyses of the sublegal-sized male catch-per-unit effort (CPUE) differences between pre-seismic commercial fishing and post-seismic commercial fishing (includes all traps that were retrieved intact).

Experimental Group	Soak Time (h)	Cumulative Number of Traps	CPUE (crabs/trap/ 24h)	p[a]	Significant Difference @ (p<0.05)
Pre-seismic	=48	200	8.8 ± 8.3	< 0.0001	Yes
Post-seismic		111	12.6 ± 8.7		
Pre-seismic	=30	80	14.7 ± 9.5	0.148	No
Post-seismic		111	12.6 ± 8.7		

All CPUE data are presented as 'mean ± standard deviation'
[a] Calculated using Mann-Whitney Rank Sum Test

Both the legal-sized and sublegal-sized crab CPUEs showed the largest differences between pre- and post-seismic fishing when sets with soak times =48 h were considered. Based on what fishermen said about the usual longevity of bait during fishing, there is a higher degree of confidence in data collected from sets with soak times =48 h than those of >48 h.

We are unable to explain why the post-exposure CPUE was significantly greater than the pre-exposure CPUE. Given the considerable variability in CPUE during both fishing periods and the six weeks that elapsed between the end of the pre-exposure fishing and the commencement of the post-exposure fishing, numerous biological and physical factors could be responsible for the increase in CPUE. These factors were more likely the sources of the observed differences than was the seismic source.

Effect of Distance Between Trap and Seismic Source on CPUE

The approximate angular distances between each crab trap and the seismic source were interpolated from the straight lines between the known locations of the end points of the fleet sets. Based on the median value of the distance data for traps of all fleet sets, each trap set was designated as either 'inner' (<365-m) or 'outer' (=365-m). The CPUE of the 'inner' and 'outer' groups of traps were then compared for both the pre-seismic and the post-seismic fishery sets with soak times =48-h. There were not any significant differences between the 'inner' and 'outer' CPUE in either case (Table 14).

Based on these analyses, no significant effect of distance between trap and seismic source on CPUE was detected for experimental fishing sets with the most bait-effective soak times.

The CPUE of the pre-seismic and post-seismic fishing sets with soak times =48-h were also compared for both the 'inner' and 'outer' groups of traps. In both cases, the post-seismic CPUE was significantly greater than the pre-seismic CPUE (Mann-Whitney Rank Sum Test, 'inner' p = 0.008 and 'outer' p = <0.0001). This not only confirms the previously discussed result that the overall post-seismic CPUE was significantly greater than the overall pre-seismic CPUE for sets with soak times =48-h (Table 12), but it also indicates that this significant difference occurred in traps both closest to and furthest from the seismic source.

Table 14. Statistical analyses of the legal-sized male CPUE differences between traps closest to the seismic source (inner) and traps furthest from the seismic source (outer). Analyses were performed separately for the pre- and post-seismic fishing with soak time =48 hours.

Fishing Period	Soak Time (h)	Trap Group	n	CPUE (crabs/trap/24 h)	p[a]	Significant Difference @ (p<0.05)
Pre	=48	Inner	70	3.0 ± 3.4	0.27	NO
		Outer	130	3.0 ± 2.5		
Post	=48	Inner	63	4.4 ± 3.8	0.05	NO
		Outer	48	5.6 ± 3.7		

All CPUE data are presented as 'mean ± standard deviation'
[a] Calculated using Unpaired t-Test on log transformed data

In summary, post-seismic catches were greater than pre-seismic catches, probably due to biological, behavioural, or physical factors unrelated to the seismic source. Statistical analyses of the catch data by distance from the seismic source indicated that there was no significant relationship between these variables.

Effects on Crab Behaviour

Telemetry

If the telemetry program had proceeded as planned, determinations of the locations of tagged crabs would have been coarse at best. It might have been possible to discriminate between an animal being in single hydrophone areas of coverage, in areas of overlap between two, three or four hydrophones, or entirely outside of the hydrophone array area of coverage. More precise locating of tagged animals might have been possible using a portable hydrophone system in combination with the four stationary hydrophone array system. Similar monitoring of decapod crustacean activity patterns has been successfully conducted in the past (Karnofsky et al. 1989; Christian 1995).

As it turned out, the abbreviated telemetry program allowed the coarse monitoring of only eight animals within the area of coverage of stationary Hydrophone No. 3. The other three hydrophones were lost due to extreme weather. The animals were tagged and deposited on bottom ten days before the onset of seismic shooting with the 200-in^3 sleeve gun array. During the 48-hour period before commencement of seismic shooting, six of the eight animals were within the area of coverage of Hydrophone No. 3 (~ 125,000-m^2 based on a 200-m radius). One of these transmitters was being decoded only intermittently. Forty-eight hours after the seismic shooting session, five of the six transmitters were still being strongly decoded by Hydrophone No. 3. Assuming that the transmitters were still attached to the crabs and that the crabs were still alive (probably a true assumption based on tag returns—see below), the animals did not leave the immediate area in response to the seismic shooting (Table 15). Given our success in holding, tagging, and monitoring the crab, we believe the tagged crab were alive during the experiment. They were present two days after shooting. Unfortunately, it was not possible to collect data on

the fine-scale activity patterns of the snow crab. Therefore, it was not possible with the telemetry program to even consider whether the seismic shooting caused the animals to become less active.

As of the end of October 2003, five of the ten snow crabs that had been outfitted with acoustic transmitters during the 2002 study had been recaptured a number of months later by commercial fishermen during the 2003 snow crab fishery. Recapture locations during the May to July 2003 period ranged from 5 to 35-km from the tagging/release location off Cape St. Francis. All five recaptured crab were reported to be healthy and vigorous.

Based on the results of the limited telemetry program, the null sub-hypothesis "***Snow crabs remain in an area when exposed to seismic sounds regardless of received sound levels***" cannot be rejected. Unfortunately, it was not possible to test the null sub-hypothesis "***Snow crabs will not return to their original area within a few days of leaving that area in response to seismic sound***". However, it should be noted that based on the limited data, there was no evidence that crabs had moved out of the area in response to seismic and thus, there was no basis for testing whether they returned to the area.

Table 15. Results of limited telemetric monitoring of snow crab before and after exposure to energy from a 200-in^3 seven-sleeve gun array.

Date	Activity
11 Nov 2002	8 legal-sized male snow crab (CW 111 – 117-mm) tagged with acoustic transmitters. Transmitter codes were as follow: 124, 128, 136, 176, 192, 199, 207, and 208. The animals were released within the four hydrophone array at a depth of ~ 170-m.
19 Nov @ 16:00 to 21 Nov @ 13:00	All transmitters except for #s 124 and 176 were decoded by Hydrophone No. 3. Of the other six transmitters, all were decoded > 100 times by Hydrophone No. 3 except for #192. It was decoded only once. Six of the tagged crab are within the area of coverage (radius of ~ 200-m) of Hydrophone No. 3.
21 Nov @ 14:30	200 seismic shots (1 every 10 seconds) from 200-in^3 array positioned near Hydrophone No. 3.
21 Nov @ 15:30 to 22 Nov @ 10:30	Transmitters 128, 136, 199, 207 and 208 were frequently decoded by Hydrophone No. 3.
22 Nov @ 10:30 to 23 Nov @ 10:30	Transmitters 128, 136, 199, 207 and 208 were frequently decoded by Hydrophone No. 3.

Startle Response Determination

Prior to this study's field experimentation, snow crabs held in a tank facility at DFO were exposed to the sounds caused by striking two metal bars together beneath the water's surface. The snow crabs in the tank immediately exhibited behaviours that could be interpreted as being a response to the acoustic stimuli. These animals drew in their legs and tended to move away from the sound source.

Animals in the field were caged and lowered to the bottom at a depth of 50 m. They were then observed for 30 minutes in order to develop a sense of their pre-exposure behaviours. Generally,

the caged crabs exhibited minimal movement during the 30 minute pre-exposure period. After this pre-exposure period, the crabs were exposed to the energy from the 200-in^3 array located 50-m above them at surface. None of the three observers onboard the vessel could detect any obvious behavioural reponses to the onset of the seismic shooting. The crabs did not draw in their legs nor did they display any sudden increase in activity. No behavioural changes could be detected on subsequent viewings of the videotape. Any response from the animals would be expected to occur during the initial shots of the 200 shot session after which habituation could possibly occur. Peak received sound levels were 201 dB re 1 µPa and received energy was 150 dB. Based on the lack of responses demonstrated during the single session of videotaped observations of snow crabs exposed to seismic energy, the null sub-hypothesis that *"**Snow crabs do not exhibit observable reactions to sound, including those kinds of sounds emitted by seismic sources**"* cannot be rejected.

McCauley et al. (2000) reported startle responses by various fish species and squid when they received mean peak levels ranging between 168 and 173 dB re 1 µPa. Wardle et al. (2001) also reported 'startle response' behaviour by fish and some qualitative information on invertebrate behavioural responses when exposed to seismic energy. LaBella et al. (1996) briefly discussed clam behaviour in response to seismic.

Weather conditions made it difficult to stabilize the experimental cage that held the crabs. Initial attempts to videotape animals while the cage was suspended in the water column failed due to masking of the potential responses to seismic behaviours by the effects of cage movement.

Videotaping was then done in a more stable area with the experimental cage containing the crabs resting on bottom. Even under these conditions, the cage was jostled by water movement now and again.

Direct Effects on Crab Physiology

All treated crabs were exposed to 200 seismic shots at 10 sec intervals. Distance from the air gun or array was varied. Control animals were treated in a manner identical to that of the treated animals, but were not exposed to seismic energy.

Changes in chemical and biochemical parameters in the haemolymph or serum of crustaceans along with changes in cell components such as haemocytes, can be useful indicators of stress or physiological dysfunction (Paterson and Spanoghe 1997; Noga 2000).

Haemolymph Solutes

The refractive index of haemolymph provides a measure of total dissolved substances or solute that it contains. Results of the determination of refractive index of the haemolymph collected from control and treatment legal-sized (= 95-mm carapace width) and sublegal-sized crabs (<95-mm) are summarized below.

40-in^3 Gun – Immediate Post-Treatment Sampling

A significant difference in refractive index between the control and treatment groups was noted in the first replicate of legal-sized male crabs shot with the 40-in^3 sleeve gun at a two-metre distance. However, no significant differences between the control and treatment legal-sized males were found in the remaining treatments at 2, 10 and 15-m. Statistical analyses performed after combining the replicates at each distance also did not indicate any significant differences in refractive index between the control and treatment crabs (Table 16).

No significant differences in refractive index between control and treatment sublegal-sized male crabs were found at any of the three distances between the seismic source and crabs, whether the replicates were considered individually or combined (Table 16).

200-in^3 Array - Immediate Post-Treatment Sampling

There were no significant differences in refractive indices between control and treated groups of legal crabs held at various distances from the array of guns (200-in^3) (Table 17).

Changes in solute concentrations in haemolymph may indicate disturbances in osmoregulatory processes and solute concentrations were assessed in this study by refractometry. Refractive indices were similar between most control and exposed crabs. A small significant difference was noted between control and exposed legal crabs held at 2-m from the single gun. However no differences were observed with other legal crabs held under the same conditions during the second treatment at 2-m or when the two treatments were combined.

Serum Proteins

Protein concentrations in the serum samples collected immediately after legal-sized and sublegal-sized male crabs were exposed at various distances to either the single 40-in^3 sleeve gun or the 200-in^3 array were measured. Delayed changes in serum protein concentration were also monitored in legal-sized male crabs exposed to the 200-in^3 array at four metres. These animals were held in the DFO laboratory for two weeks before serum sampling was conducted. Serum protein results are summarised in Tables 18 to 20.

Differences in protein concentrations in serum may also be indicative of osmoregulatory or other physiological disturbances such as major tissue damage. No significant effects on protein concentrations were observed between control and exposed crabs either directly after exposure or after being held for four weeks.

Table 16. Comparison between control and treatment male snow crab haemolymph refractive index after exposure to 40-in^3 sleeve gun.

Crab Size	Experimental Group	Distance between sleeve gun and crabs								
		2-m			10-m			15-m		
		N	Refractive Index	p[a]	N	Refractive Index	p[a]	N	Refractive Index	p[a]
Legal-Sized	Treatment 1 [b]	4	1.053 ± 0.007	0.045 *	4	1.056 ± 0.008	0.403	4	1.055 ± 0.009	0.776
	Control 1 [c]	4	1.044 ± 0.003		4	1.053 ± 0.002		4	1.056 ± 0.007	
	Treatment 2 [d]	4	1.053 ± 0.003	0.202	4	1.048 ± 0.011	0.530	4	1.050 ± 0.009	0.578
	Control 2 [e]	4	1.060 ± 0.009		3	1.054 ± 0.011		4	1.053 ± 0.006	
	Treatment combined [f]	8	1.053 ± 0.005	0.765	8	1.052 ± 0.010	0.817	8	1.052 ± 0.009	0.529
	Control combined [g]	8	1.052 ± 0.010		7	1.053 ± 0.007		8	1.055 ± 0.006	
Sublegal-sized	Treatment1 [b]	4	1.051 ± 0.014	0.980	4	1.059 ± 0.009	0.425	4	1.053 ± 0.008	0.665
	Control 1 [c]	4	1.051 ± 0.013		4	1.051 ± 0.017		4	1.055 ± 0.005	
	Treatment 2 [d]	4	1.060 ± 0.013	0.558	3	1.042 ± 0.010	0.058	4	1.054 ± 0.007	0.719
	Control 2 [e]	4	1.064 ± 0.005		4	1.059 ± 0.007		4	1.051 ± 0.011	
	Treatment combined [f]	8	1.055 ± 0.014	0.744	7	1.052 ± 0.013	0.665	8	1.053 ± 0.007	0.975
	Control combined [g]	8	1.058 ± 0.012		8	1.055 ± 0.013		8	1.053 ± 0.008	

All data are expressed as mean ± standard deviation

[a] Calculated using Unpaired t-Test or Mann-Whitney Rank Sum Test
[b] First replicate of treated crabs
[c] First replicate of control crabs
[d] Second replicate of treated crabs
[e] Second replicate of control crabs
[f] Pooling of two treatment replicates
[g] Pooling of two control replicates
* Significantly different @ $p < 0.05$

Table 17. Comparison of control and treatment male snow crab haemolymph refractive index after exposure to 200-in^3 array.

Distance Between Seismic Source and Crabs (m)	Experimental Group	N	Refractive Index	p[a]
4	Treatment	4	1.050 ± 0.003	0.425
4	Control	4	1.055 ± 0.011	
50	Treatment	8	1.052 ± 0.012	0.353
50	Control	8	1.057 ± 0.011	
85	Treatment	4	1.060 ± 0.006	0.891
85	Control	4	1.059 ± 0.009	
170	Treatment	8	1.053 ± 0.007	0.683
170	Control	8	1.051 ± 0.009	

All refractive index data are expressed as 'mean ± standard deviation'
[a] Calculated using Unpaired t-Test

Table 18. Protein concentrations in the haemolymph serum of male crabs exposed to seismic energy from the 40-in^3 gun.

Crab Size	Experimental Group	Distance between air gun and crabs					
		2-m			10-m		
		N	Protein (mg/ml)	p[a]	N	Protein (mg/ml)	p[a]
Legal-sized	Treatment 1[b]	3	27.7 ± 7.2	0.659	3	27.0 ± 8.2	0.906
	Control 1[c]	4	24.9 ± 8.3		4	26.4 ± 3.0	
	Treatment 2[d]	4	26.5 ± 5.4	0.230			
	Control 2[e]	4	34.7 ± 11.0				
	Treatments 1 and 2 combined[f]	7	27.0 ± 5.7	0.543			
	Controls 1 and 2 combined[g]	8	29.8 ± 10.4				
Sublegal-sized	Treatment 1[b]	4	29.5 ± 12.8	0.968	4	31.5 ± 9.7	0.938
	Control 1[c]	4	29.1 ± 16.6		4	30.6 ± 20.5	
	Treatment 2[d]	4	35.2 ± 17.2	0.739			
	Control 2[e]	4	38.9 ± 12.0				
	Treatments 1 and 2 combined[f]	8	32.4 ± 14.3	0.825			
	Controls 1 and 2 combined[g]	8	34.0 ± 14.4				

All protein concentration data are expressed as 'mean ± standard deviation'
[a] Calculated using Unpaired t-Test or Mann-Whitney Rank Sum Test
[b] First replicate of treated crabs
[c] First replicate of control crabs
[d] Second replicate of treated crabs
[e] Second replicate of control crabs
[f] Pooling of two treatment replicates
[g] Pooling of two control replicates

Table 19. Protein concentrations in the haemolymph serum of male crabs exposed to seismic energy from the 200-in^3 array.

Experimental Group	N	Distance between array and crabs		
		4-m	50-m	170-m
Treatment	4	26.6 ± 3.4	19.7 ± 2.7	34.7 ± 12.3
Control	4	27.6 ± 14.5	31.2 ± 10.7	21.7 ± 10.7
p[a]		0.893	0.114	0.161

All protein concentration data are expressed as 'mean ± standard deviation'
[a] Calculated using Unpaired t-Test or Mann-Whitney Rank Sum Test

Table 20. Protein concentrations in the haemolymph serum of male crabs 2 weeks after exposure to seismic energy from the 200-in^3 array at 4-m.

Experimental Groups	N	Protein (mg/ml)	p[a]
Control	9	28.2 ± 7.1	0.550
Treated	9	26.4 ± 5.2	

All protein concentration data are expressed as 'mean ± standard deviation'
[a] Calculated using Unpaired t-Test or Mann-Whitney Rank Sum Test

Serum Enzymes

The API-ZYM system was used to assess enzymatic activity in the serum of legal-sized and sublegal-sized male crabs exposed to a 40-in^3 sleeve gun from distances of 2 and 10-m, and of legal-sized male crabs exposed to the 200-in^3 array from distances of 4, 50 and 170-m. Enzymatic activity was also assessed in the serum of legal-sized male crabs collected two weeks after the exposures to energy from the seven-gun array at four metres.

Over 1,600 enzyme reactions were recorded during the enzymatic activity assessment of 87 crabs. Of the 19 enzymes tested, six were active in all the samples analyzed. These included two esterases (esterase and esterase lipase), two phosphatases (acid phosphatase and naphtol-AS-BI-phosphohydrolase) and two glycosidases (α-glucosidase and N-acetyl-β-glucosaminidase). The enzyme profiles and intensities of coloured reactions were similar in the serum samples collected from control and treated animals in all exposure sessions (Table 21).

Serum enzymes are commonly used in human and veterinary medicine to diagnose damage to specific organs and tissues. Unlike human and other mammalian models (and fish to a certain extent) detailed studies are not available on enzyme profiles in either sera or tissues of crustaceans or other invertebrates. However, any major changes in enzyme levels in the sera of invertebrates could indicate that tissue damage had occurred. There was no indication of immediate or delayed major leakage of enzymes into haemolymph such as might be expected from significant damage to the hepatopancreas. However, it is important to note that the qualitative enzyme screening technique used (the API ZYM system) would not detect small enzyme changes. Examination for small enzyme changes would have involved lengthy quantitative studies on organs and sera, procedures well beyond the scope of this study.

Table 21. Results of API-ZYM tests for serum enzymatic activity.

Experimental Group	Crab #	Enzyme Analyte 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Legal-size 40 in^3 gun @ 10 m Exposed	1							1					1								
	2				2								1				2		1		
	3				2								1				1		1		
	4				2								1				1		1		
Legal-size 40 in^3 gun @ 10 m Control	5				2								1				2		1		
	6				2								1				1		1		
	7				2								1						1		
	8				2								1						1		
Legal-size 40 in^3 gun @ 2 m Exposed	49			2	2							.5	1						1		
	50	Test Failure																			
	51			1.5	2							.5	1				.5		1		
	52			1.5	2							.5	1						2		
Legal-size 40 in^3 gun @ 2 m Control	53			1.5	2							1	1				1		1		
	54			1.5	2							1	1				1		1		
	55			1.5	2							.5	1						1		
	56			1.5	2							.5	1						1		
Legal-size 40 in^3 gun @ 2 m Exposed	57			1.5	2								1				.5		1		
	58			1	1.5							.5	1				.5		2		
	59		3	.5	1							.5	1				.5		1		
	60			1.5	1.5							.5	1						1		
Legal-size 40 in^3 gun @ 2 m Control	61			1.5	2								1				.5		1		
	62			1.5	1.5							.5	1				1		1		
	63			1.5	1.5							.5	1						1		
	64			1.5	1.5							.5	1				.5		1		
Sublegal-size 40 in^3 gun @ 10 m Exposed	17				2		.5					.5	1						1		
	18			.5	2		.5					.5	1				.5		1		
	19			.5	2							.5	1				.5		1		
	20			.5	2							.5	1.5						1		
Sublegal-size 40 in^3 gun @ 10 m Control	21			.5	2							.5	1						.5		
	22			.5	2		.5					.5	1				.5		1		
	23			.5	2							.5	1						.5		
	24			.5	2							.5	1				.5		1		

Experimental Group	Crab #	Enzyme Analyte																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Sublegal-size 40 in^3 gun @ 2 m Exposed	33			1.5	2							.5	1				.5		.5		
	34			1.5	1.5							1	1				.5		2		
	35			1.5	2							1	1				1		.5		
	36	Test Failure																			
Sublegal-size 40 in^3 gun @ 2 m Control	37			1.5	1.5							.5	1				.5		1		
	38			1.5	1.5							.5	1				1		2		
	39	Test Failure																			
	40	Test Failure																			
Sublegal-size 40 in^3 gun @ 2 m Exposed	41		4	2	2							1	2				.5		1		
	42			1.5	2							.5	1						.5		
	43			1	1.5							.5	1				.5		1		
	44			.5	2							.5	1				.		1		
Sublegal-size 40 in^3 gun @ 2 m Control	45			1	1.5							.5	1				.5		1		
	46			1.5	1.5							.5	1				.5		1		
	47				1.5							.5	1						.5		
	48			1	1.5								.5						1		
Legal-size 200 in^3 array @ 4 m Exposed	121			.5	2		.5						1				.5		1		
	122			.5	2							1	1						1.5		
	123		4	.5	2		.5					3	2		.5		.5		2		
	124			.5	2		.5						2				.5		1.5		
Legal-size 200 in^3 array @ 4 m Control	125			.5	2		.5						1				2		1.5		
	126			.5	2		.5					.5	1				.5		1.5		
	127			.5	2		.5					.5	1				.5		1.5		
	128			.5	2		.5					.5	1				.5		1		
Legal-size 200 in^3 array @ 50 m Exposed	129		5	1	1					.5		4	4	.5	3		1.5		4		
	130			1	1							.5	1						1.5		
	131			1	1							.5	1						1.5		
	132			1	1							.5	1				.5		1.5		
Legal-size 200 in^3 array @ 50 m Control	133		5	1	1		.5					4	4				1		3		
	134			1	1							1	1						.5		
	135		5		1							4	4				.5		1		
	136		.5	.5	1		.5					1	1				.5		1		

Experimental Group	Crab #	Enzyme Analyte																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Legal-size 200 in^3 array @ 170 m Exposed	105			.5	1								1				.5				
	106			1	1							.5	1						1		
	107		.5	1	1							.5	1.5						.5		
	108			1.5	1.5								1				1		.5		
Legal-size 200 in^3 array @ 170 m Control	109			1	1								1				.5		1.5		
	110			.5	1								1				.5		1		
	111			1	1							.5	1				.5		1		
	112			1	1							.5	1				.5		2		
Legal-size 200 in^3 array @ 4 m Exposed 2 weeks before	E1			.5	1.5								1				.5		1		
	E2			1	1.5								1						1.5		
	E3			1	2							.5	1				.5		2		
	E4			1	2								1				.5		2		
	E5			1	1.5							.5	1				.5		.5		
	E6		5	1.5	2							4	5				.5		1		
	E7			.5	2							.5	1				2		1		
	E8			.5	1							.5	1				.5		1.5		
	E9		5	.5	2							4	5				1		2		
	E10				2								1				.5		.5		
Legal-size 200 in^3 array @ 4 m Control for exposure 2 weeks before	C1			.5	1.5							.5	1				.5		2		
	C2			1	2								1				1		1		
	C3			1	2								1						.5		
	C4			.5	1.5								1				.5		1		
	C5			.5	1.5							.5	1				.5		2		
	C6		5	.5	1.5							1	1				.5				
	C7			.5	1.5								1				.5		.5		
	C8			.5	1.5							.5	1				.5		2		
	C9			1	2							.5	1				1		1		

Reactions were graded visually based on colour intensity from 0 (blank cell) (no detectable enzyme activity) to 5 (maximum enzyme activity).

Analyte codes: 1=Control; 2=alkaline phosphatase; 3=esterase; 4=esterase lipase; 5=lipase; 6=leucine arylamidase; 7=valine arylamidase; 8=cystine arylamidase; 9=trypsin; 10=a-chymotrypsin; 11=acid phosphatase; 12=napthol-AS-BI-phosphohydrolase; 13= a-galactosidase; 14=ß-galactosidase; 15= ß-glucoronidase; 16= a-glucosidase; 17= ß-glucosidase; 18=N-acetryl- ß-glucosaminidase; 19= a-mannosidase; 20= a-fucosidase

Another phosphatase (alkaline phosphatase) and glycosidase (β-galactosidase) were detected in three of the control samples and two of the treatment samples. They are likely a result of contamination considering that they were not detected in any of the other 83 haemolymph samples that were analyzed. All five detections occurred in samples taken from different treatment sessions. Both alkaline phosphatase and β-galactosidase are enzymes found in the hepatopancreas or organelles such as haemocytes, possible sources of contamination during the haemolymph sampling or serum preparation in the field.

Relative Numbers of Haemocyte Types

Three different haemocyte types were identified in *"blood"* smears taken from crabs:

1. Hyalinocytes: size ~ 10-25 µm, round shape, dark blue or violet nucleus, cytoplasm void of granules

2. Granulocytes: size ~ 20-25 µm, round or irregular shape, clearly visible dark or pale blue nucleus, cytoplasm with eosinophilic granules

3. Semi-granulocytes: size ~ 18-21 µm, round or irregular shape, light to dark blue nucleus, a few colourless to pale blue cytoplasmic granules

Semi-granulocytes were the most abundant haemocyte type, followed by hyalinocytes and finally, granulocytes.

Differential haemocyte counts of granulocytes, semi-granulocytes and hyalinocytes were conducted on the haemolymph of legal-sized and sublegal-sized crabs collected immediately after exposure to the 40-in^3 sleeve gun at 2-m, on the haemolymph of legal-sized crabs collected immediately after exposure to the 200-in^3 array at 4-m, and on the haemolymph of legal-sized crabs collected two and four weeks after exposure to the 40-in^3 sleeve gun at 4-m, and the 200-in^3 seven gun array at 2-m, respectively.

There were no significant differences in the percentages of different haemocyte types between control and treatment groups of the legal-sized and sublegal-sized crabs that were sacrificed immediately after exposure (Table 22).

Table 22. Differential haemocyte counts in control and treatment crab haemolymph sampled immediately after exposure to seismic energy.

Crab Size/ Distance (m) /Seismic Source (in^3)	Experimental Group	N	Granulocyte Count	Semi-granulocyte Count	Hyalinocyte Count
Legal/2/40	Control	8	23.2 ± 2.9	43.2 ± 2.8	33.5 ± 4.0
	Treatment	8	24.8 ± 3.6	41.9 ± 3.6	33.4 ± 5.6
	p[a]		0.378	0.404	0.943
Sublegal/2/40	Control	8	24.3 ± 3.4	42.6 ± 3.3	33.1 ± 4.9
	Treatment	8	22.9 ± 2.6	43.5 ± 3.4	33.6 ± 4.7
	p[a]		0.385	0.610	0.843
Legal/4/200	Control	4	22.5 ± 2.7	41.7 ± 2.6	35.8 ± 4.6
	Treatment	4	24.8 ± 2.2	42.2 ± 3.8	33.0 ± 5.0
	p[a]		0.238	0.838	0.443

All haemocyte count data are expressed as 'mean percentage ± standard deviation'
[a] Calculated using Unpaired t-Test or Mann-Whitney Rank Sum Test after arcsin square root transformation of percentages

There were no significant differences in the percentages of different haemocyte types between control and treatment groups of the legal-sized crabs that were sacrificed two and four weeks after exposure to either the single gun or gun array (Table 23).

Haemocytes are believed to play a key role in crustacean immunology (Noga 2000) and changes in relative numbers of different haemocyte types are useful indicators of stress on immunological functions involved in disease defence (Jussila et al. 1997; Fotedar et al. 2001). In many crustacean species, three morphological haemocyte types can be distinguished, hyalinocytes, semi-granulocytes and granulocytes (Bauchau 1981; Soderhall and Cerenius 1992). To our knowledge, this is the first description of the presence of these three types of haemocytes in snow crabs.

Table 23. Differential haemocyte counts in control and treatment legal-sized crab haemolymph sampled two weeks after exposure to a 200-in^3 array and four weeks after exposure to a 40-in^3 air gun.

Distance (m)/ Seismic Source (in^3)	Experimental Group	N	Granulocyte Count	Semi-Granulocyte Count	Hyalinocyte Count
2/40	Control	8	23.9 ± 2.1	42.3 ± 2.4	33.9 ± 3.6
	Treatment	8	24.1 ± 3.3	42.9 ± 2.4	33.0 ± 4.3
	p[a]		0.872	0.662	0.688
4/200	Control	9	24.4 ± 3.0	43.4 ± 3.2	30.3 ± 3.0
	Treatment	9	23.0 ± 2.9	42.7 ± 3.4	34.3 ± 4.7
	p[a]		0.313	0.623	0.073

All haemocyte count data are expressed as 'mean percentage ± standard deviation'
[a] Calculated using Unpaired t-Test or Mann-Whitney Rank Sum Test after arcsin square root transformation of percentages

Organ and Tissue Pathology

There were no visible signs of external damage to the carapace, appendages or various internal organs in the 144 male crabs examined.

Pathology has been proposed to be one of the most reliable indicators for assessing health impairment in aquatic organisms including that caused by anthropogenic activities (Myers et al. 1987; Hinton et al. 1992). Although studies are limited, some pathological effects including haemorrhaging, swimbladder rupture, ear damage, eye damage and blindness have been observed in fish exposed at close range to high seismic energy (Turnpenny and Nedwell 1994; Hirst and Rhodhouse 2000). There appears to have been very few studies on the pathological effects of seismic emissions on invertebrates. Matishov (1992) reported shell splitting in scallops and spine damage in sea urchins upon exposure to an air gun at a distance of two metres, whereas Kosheleva (1992) reported no overt effects on mussels, periwinkles and crabs exposed at a distance of 0.5-m from a single air gun.

Statocysts

The snow crab statocyst is a round/oval-shaped sac-like epidermal invagination of cuticle containing a distinct hair field localized within the basolateral wall of the capsule (Figure 11). These hairs appeared to be analogous to sensory hairs reported in lobster (Cohen 1955; Atema and Voigt 1995). Individual hairs protrude laterally from recessed "pores" toward the outside of the statocyst (Figure 12). Most decapod statocysts contain cemented grains of sand called statoliths. These were not observed during the examination of the snow crab statocysts.

Lobster statocysts were also dissected and examined during this study. As with the snow crab, hairs within the lobster statocyst were readily visible using stereo microscopy at x63. These were the hairs that were examined for any alteration due to exposure to seismic energy.

Statocysts of all legal-sized and sublegal-sized male crabs exposed to energy from the 40-in^3 gun at distances of 2, 10 and 15-m, and legal-sized male crabs exposed to energy from the 200-in^3 array at distances of 4, 50, 85, and 170-m were examined using stereomicroscopy. Extremely close examination was made on the strap of setae of each of the 144 statocysts during the microscopy. There were no patches of hair ablation in either control or treatment samples. The occurrence of broken or twisted hairs was noted in a few samples but the prevalence appeared to be similar in both control and treatment animals. The altered hairs were most likely due to mechanical damage during dissection. Some representative samples were also analyzed using scanning electron microscopy. Based on the results of the examinations of snow crab statocysts and associated tissues, the null sub-hypothesis "***Seismic sounds do not cause damage to snow crab statocysts and associated tissues***" cannot be rejected. That is, there was no evidence of seismic-induced damage.

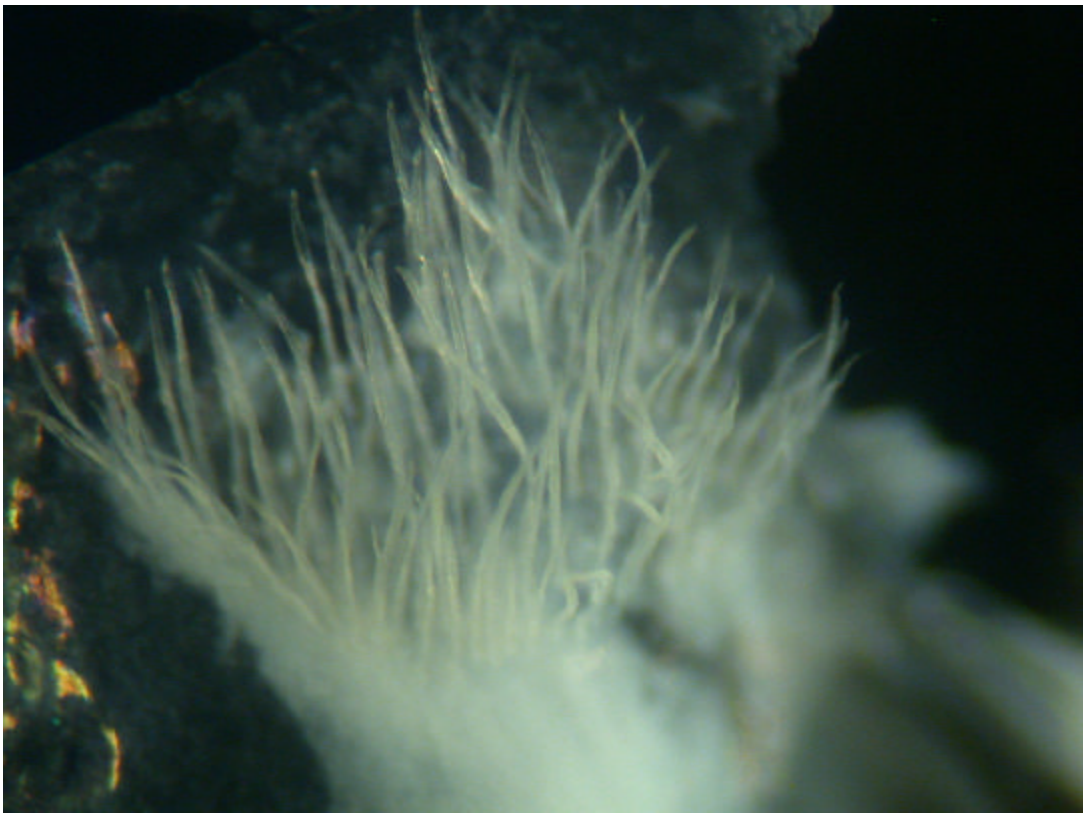

Figure 11. A field of sensory hairs in the snow crab statocyst (Stereo Microscope; x63).

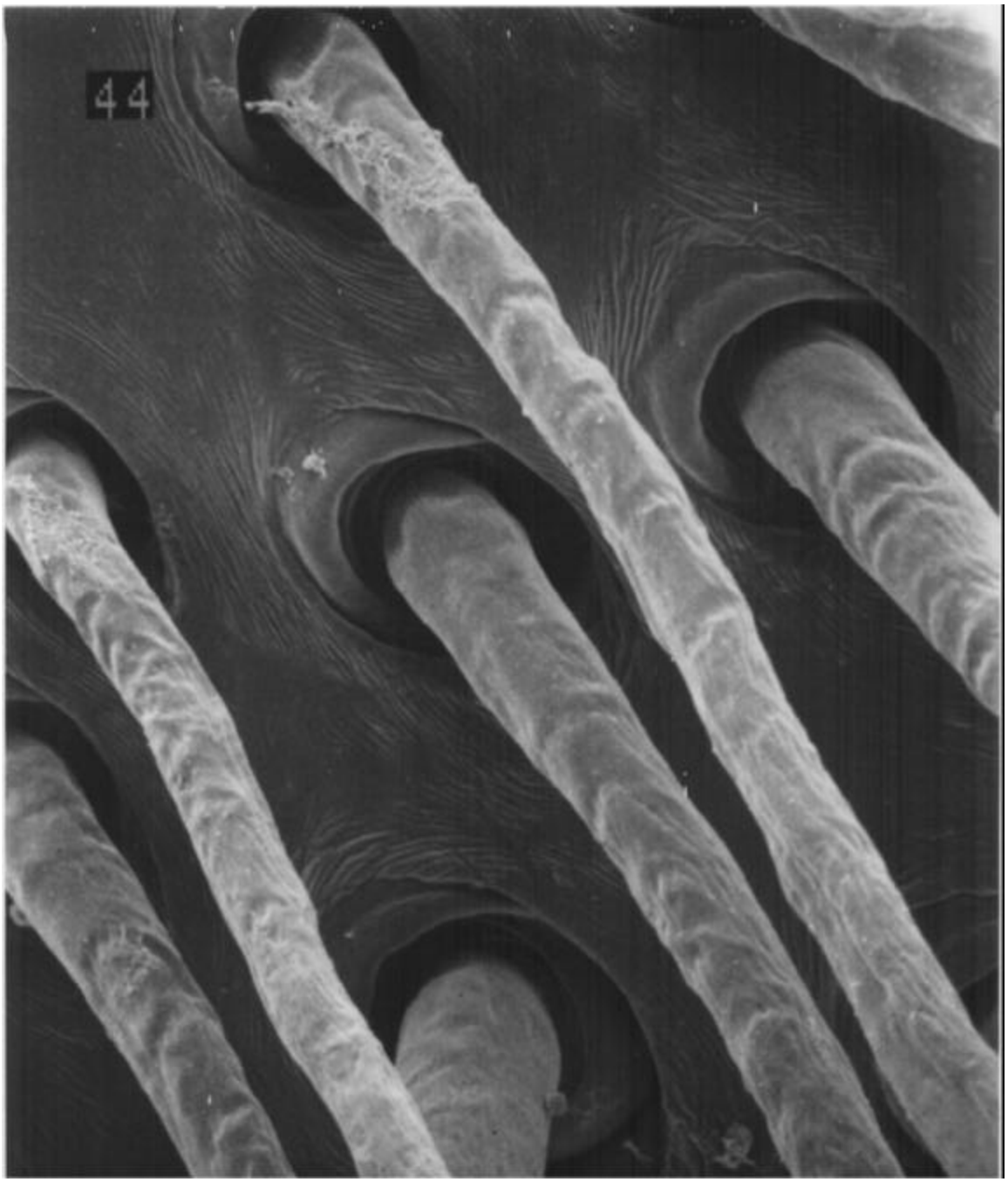

Figure 12. Sensory hairs protruding from recessed pores in the snow crab statocyst (Scanning Electron Microscope; x800).

The size of hairs in the sensory hair fields of crabs would have permitted easy detection of the kind of seismic-induced removal of clumps of sensory receptors reported by McCauley et al. (2003) in fish hearing organs that are similar in structure to statocysts.

There may be differences in sensitivity between fish hearing organs and statocysts. The sensory hairs (or kinocilia) found in the saccular epithelium of fish are small microtubules about 5-6 µm in length (Popper and Platt 1993). The equivalent sensory hairs in crabs are large chitinous hairs that are 300 µm or more in length. The anchoring and anatomical positioning of hair cells are also expected to vary in widely different species such as fish and crustaceans. We have noted distinct differences in this regard between crab and lobster. Further, unlike fish ears which commonly (but not always) contain relatively large and readily visible calcified otoliths associated with their sensory hairs, snow crabs were observed not to contain a large mass of sand grains called statoliths that are common in crustaceans. These were observed in lobsters during preliminary trials. The sensory cells of fish ears may be more prone to shearing stress from otolith movement than the sensory cells of snow crab statocysts that contain no moving hard parts. However, there is another variable which could be explored. Namely, McCauley et al. (2003) examined fish after several weeks of exposure, whereas crab statocysts were fixed in the field immediately after the exposure trials. Accordingly, it would be of interest to carry out studies for statocyst damage on the crabs that were exposed to seismic and are being held in the laboratory for observations of delayed effects.

Hepatopancreas and Heart

Hepatopancreatic and heart tissues of male crabs exposed to the single 40-in^3 air gun at distances of 2 and 10-m, and to the 200-in^3 array at 4, 50 and 170-m were examined for histopathological changes using light microscopy.

The hepatopancreas consists of many small blind tubules separated by haemal sinuses. No cases of tubule autolysis (possibly caused by bursting or release of digestive enzymes after cell rupture) or any other overt abnormalities were detected in the control and treatment samples (Figure 13).

The heart is composed of striated muscle tissue surrounded by a pericardium. There was no evidence of cell rupture or any other cellular damage in cardiac or pericardial tissues of either the control or treatment crabs (Figure 14).

Tissues have been fixed and archived should further studies be of interest.

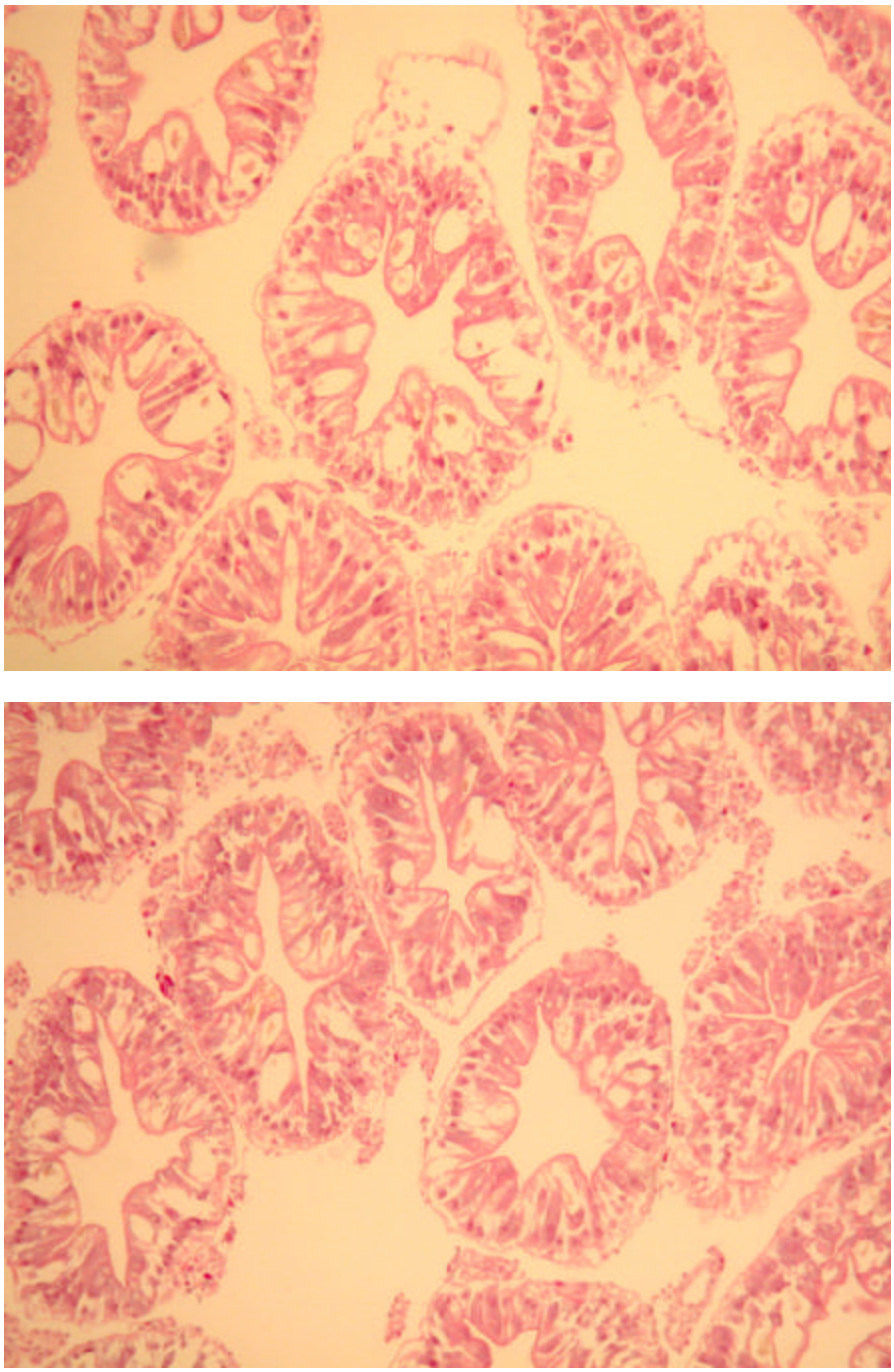

Figure 13. Representative transverse section of hepatopancreatic tubules from a control snow crab (top) and one exposed to the energy of a 40 in^3 air gun from a distance of 2-m (bottom) (Stain: hematoxylin and eosin) (x63).

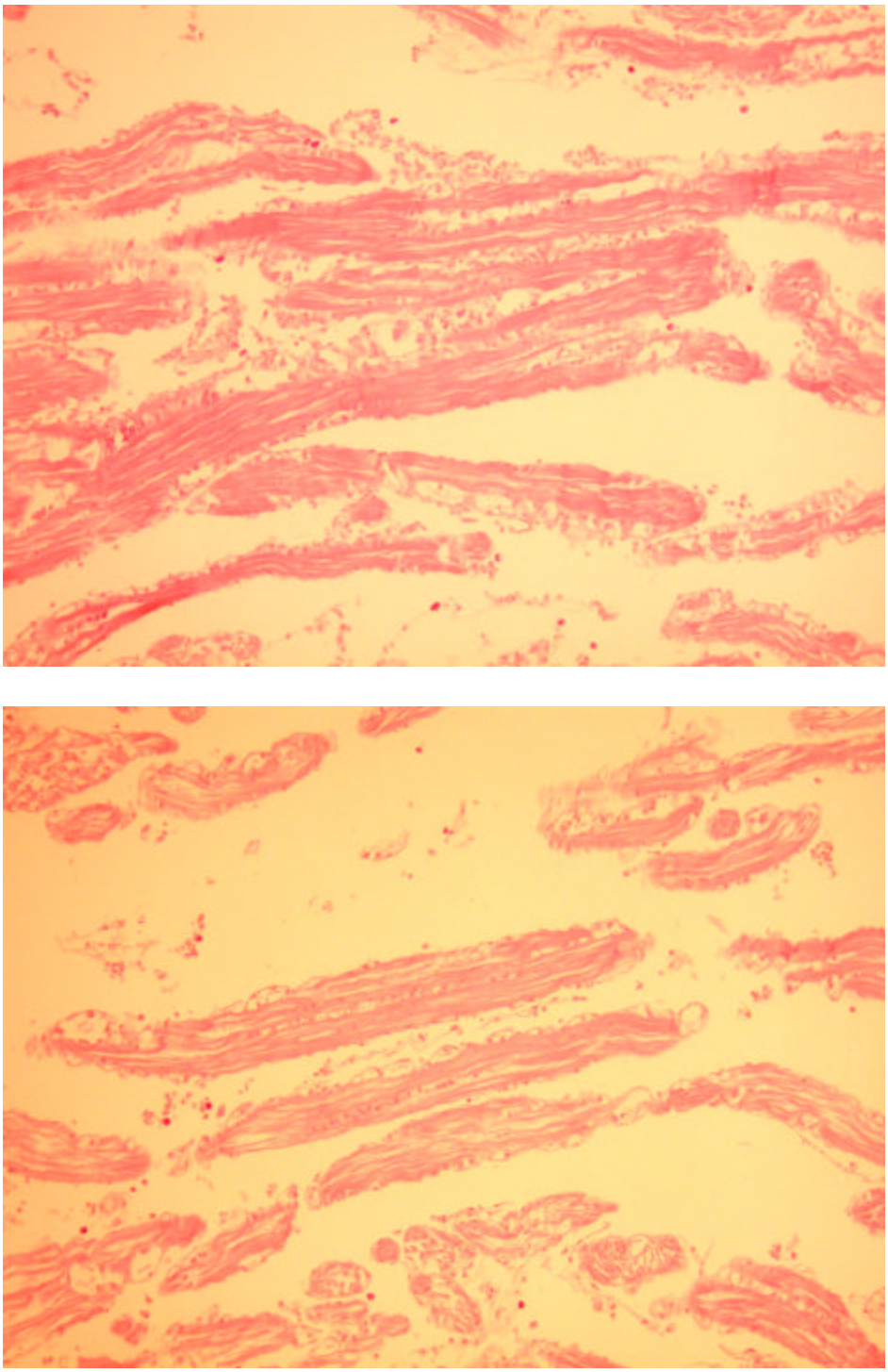

Figure 14. Representative longitudinal section of heart tissue fibers from a control snow crab (top) and one exposed to the energy of a 40 in^3 air gun from a distance of 2-m (bottom) (Stain: hematoxylin and eosin) (x63).

Immediate and Delayed Mortality

No crabs died during the experiments. In some cases, animals seemed more energetic immediately after exposure than the control animals. The three female crabs exposed to seismic energy appeared to take longer to right themselves immediately after exposure than the three control females. However, after 10 to 15 minutes, the treatment and control females righted themselves in about the same amount of time. The six female crabs were placed on their backs upon completion of the treatment with the single 40-in^3 air gun and observed until all had righted themselves.

As of 1 April 2003, none of the male and female crabs that were exposed to seismic energy and subsequently held in tanks at DFO had died. The females had been exposed to the single 40-in^3 gun at two metres on 2 November 2002, some of the males had been exposed to the 40-in^3 gun at two metres on 10 November, and the remainder of the males had been exposed to the 200-in^3 array at four metres on 30 November.

Therefore, there has not been any indication of immediate or delayed mortality of snow crabs exposed to seismic energy in this study. Matishov (1992) found that exposure of codfish at a distance of two to four metres from an air gun led to death within two days, whereas McCauley et al. (2003) did not observe delayed lethality in pink snapper 44 days after exposure to an air gun. Kosheleva (1992) reported no adverse effects in benthic invertebrates including crabs 30 days after exposure to a single air gun at a distance of 0.5-m.

Egg Development

Developing fertilized eggs that had been stripped from female crabs prior to the experiment were exposed to the 40-in^3 gun at a distance of 2-m for the standard exposure of 200 shots at 10 sec intervals. The treated and control eggs were then held in the laboratory for 12 weeks at which time they were examined with a stereomicroscope. Eggs were initially divided into two categories based on colour criteria: (1) "beige" eggs, and, (2) orange-brown eggs. It appeared that the beige eggs, although not entirely opaque, were dead, and the orange-brown eggs were alive. The orange-brown eggs were further divided into three categories based on eye size criteria: (1) no eyes, (2) small eyes, and, (3) big eyes according to a categorization system described by Moriyasu and Lanteigne (1998). Representative photographs of the different categories are given in Figures 15 and 16.

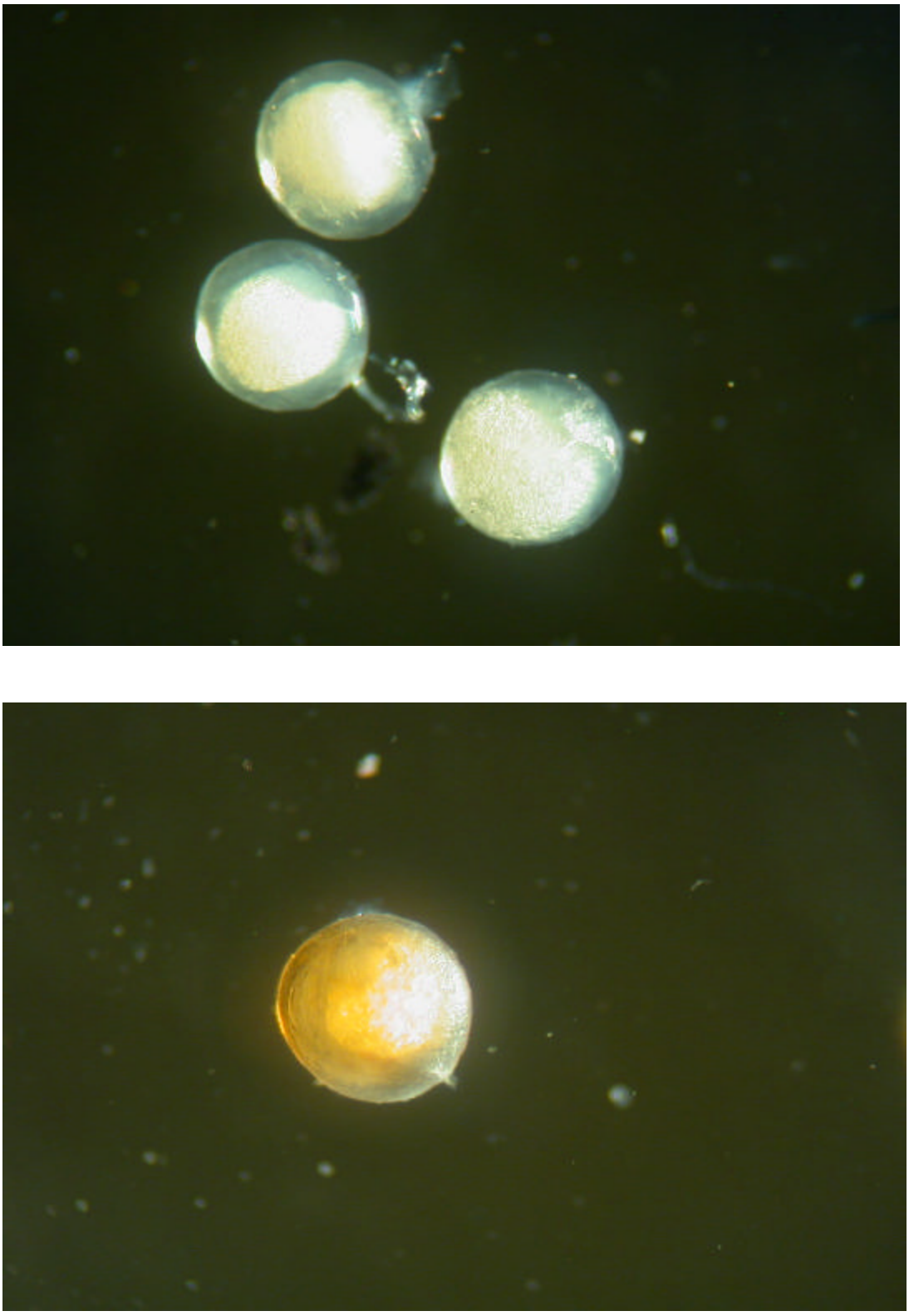

Figure 15. "Beige" or dead snow crab eggs (top); Orange uneyed egg (bottom) (x25).

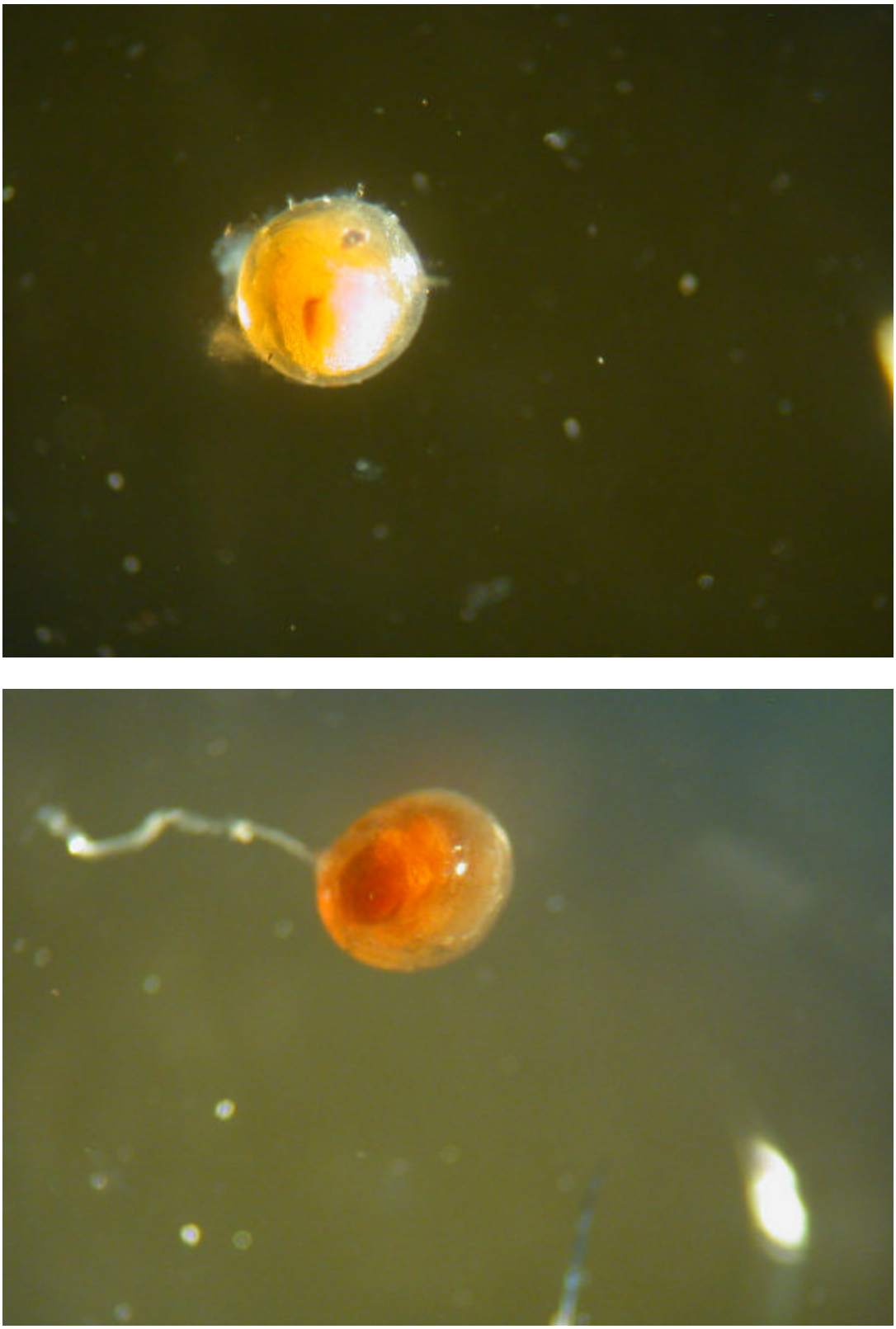

Figure 16. Orange small-eyed egg (top); Orange-brownish large-eyed egg (bottom) (x25).

Small-eyed eggs had lengths ranging from 140 to 240 µm and widths from 60 to 100 µm, Big-eyed eggs had lengths ranging from 240 to 400 µm and widths ranging from 100 to 180 µm.

Results of the examination of fertilized eggs are presented in the Table 24. The percentage of eggs in each category was calculated relative to the total of 2,095 control eggs and 2091 treatment eggs that were analyzed.

Table 24. Differences between untreated fertilized eggs and those exposed to the 40-in^3 gun at a distance of 2-m.

Colour Categories	Eye Size Categories	Control		Treatment		p[b]
		N	% [a]	N	% [a]	
Total Eggs Examined		2095		2091		
"Beige" eggs		99	4.7	135	6.3	0.034 *
Orange-brown eggs	Big-eyed	1479	70.6	939	44.9	<0.001 *
	Small-eyed	415	19.8	867	41.5	<0.001 *
	No eyes	102	4.9	150	7.2	0.002 *

[a] Percentage in relation to the total number of eggs examined
[b] Calculated using the z-Test
* Significant difference @ $p < 0.05$

Significant differences were found between the control and treatment eggs in all categories. The most significant differences between controls and treatments were in respect to the proportions of big-eyed eggs and small-eyed eggs. More than 70% of the untreated eggs were big-eyed while less than 45% of the treated eggs had big-eyes. This suggests that exposure to the seismic energy may have resulted in delayed embryonic development. There was also a statistically significant higher proportion of dead eggs in the treatment group (6.3%) than in the control group (4.7%), suggesting that exposure to seismic energy resulted in slightly increased mortality of fertilized eggs.

The early life stages of fish and invertebrates are often the most sensitive to harmful effects. Most studies of the effects of seismic energy on early life stages of marine organisms have been conducted on fish and they indicate that various effects are only produced within a few metres of an air gun and include immediate or delayed mortality (Banner and Hyatt 1973; Kostyuchenko 1973; Holliday et al. 1987; Kosheleva 1992; Booman et al. 1996), delamination of eye retina (Matishov 1992), brain pathology (Booman et al. 1996) and decrease in growth rate of larvae (Banner and Hyatt 1973). Survival, development and behaviour of Dungeness crabs were not affected by exposure to sound levels up to 231 dB re 1 µPa (Pearson et al. 1994).

In this study, over 2,000 eggs were exposed to 200 shots from a 40-in^3 air gun and received peak sound levels of about 216 dB re 1 µPa. These eggs and over 2,000 control eggs were then held for 12 weeks. Compared to the control eggs, the eggs exposed to seismic energy showed a 1.6% mortality of eggs and a 26% delay in development to the big-eyed stage. Close exposure to an air gun apparently resulted in these effects on embryonic growth and development in snow crabs. Such injury could result in premature death within the egg or loss of overall fitness upon hatching resulting in further mortality, or increased susceptibility to predation. Delayed development could lead to hatching out of phase with optimal environmental conditions. Tests

were only conducted at distances of 2-m from the source. The literature mentioned above indicates that these types of effects are evident at very close distances from air guns in several groups. It should be noted, however, that snow crab eggs in natural situations would never be this close to a seismic array because the eggs are held by the adult females on the bottom.

The effects on embryo development were obtained upon exposure of eggs to a single air gun at a distance of two metres. It is not known what distance would be required to produce no effect. There can be considerable heterogeneity in egg developmental stages in egg-bearing females. It would be difficult and time consuming to study effects by exposing females with eggs. Up to two years may be required for egg hatching in snow crabs. However, the approach used in this study of removing "undeveloped" eggs orange in colour from the pleopods of selected females and incubating them in the laboratory to obtain fairly homogeneous pools of developing embryos for exposure, was found to be a practical approach.

Summary of Direct Effects on Crab Physiology

Legal-sized and sublegal-sized male snow crabs were exposed to a single 40-in^3 air gun at distances of 2, 10 and 15-m, and to a 200-in^3 seven-gun array at distances of 4, 50, 85 and 170-m. Measured received peak broadband levels from the 40-in^3 air gun at 10 and 15-m ranged from 201.1 to 203.8 dB re 1 µPa and 203.1 to 203.2 dB re 1 µPa, respectively. The received peak broadband level at 2-m was interpolated as 221 dB re 1 µPa. Measured received peak broadband levels from the 200-in^3 seven-gun array at 50 to 85-m ranged from 197.4 to 202.8 dB re 1 µPa. The received peak broadband level at 170-m was extrapolated as 191 dB re 1 µPa. The received levels at 4-m could not be estimated because the hydrophone was located in the near field of the array and therefore received sound from individual guns rather than from the integrated array.

Haemolymph solute concentration was measured and expressed as a refractive index for each treated and control male snow crab involved in the crab health component of the study. No significant differences in refractive index were found between control and treated animal haemolymph that was withdrawn from the snow crabs immediately after exposure.

Protein concentrations in the control and treatment male crab serum samples collected immediately after exposure and 2 weeks after exposure did not differ significantly in any of the treatments. Protein concentration analyses were performed on legal-sized and sublegal-sized males exposed to the single gun at 2 and 10-m, and legal-sized male snow crabs exposed to the array at 4, 50, and 170-m.

Serum enzyme activity was assessed in the same animals used in the protein concentration analyses. According to the assessment with the qualitative API ZYM system, there were no clear differences in enzyme activity between the control and treatment snow crabs.

The relative numbers of haemocyte types were determined through 'smearing' of haemolymph withdrawn from legal-sized and sub-legal sized male snow crabs exposed to the single gun at 2-m and the seven-gun array at 4-m immediately after exposure. Smears were also prepared and

assessed using the haemolymph withdrawn from legal-sized snow crabs 4 weeks after exposure to the 40-in^3 air gun, and 2 weeks after exposure to the 200-in^3 array. No significant differences were found between control and treated snow crabs in any of the treatments.

It is important to note that generalized stress associated with experimentation, such as holding and catching, can also influence some haemolymph parameters (Jussila et al., 1997; Paterson and Spanoghe, 1997; Noga, 2000; Fotedar et al., 2001) and possibly mask the effects of potential stressors to a certain degree.

Snow crab organ and tissue pathology was also conducted during the 'crab health' component of the study. All statocysts were examined with stereomicroscope and a subsample was examined with electron microscopy. No differences between statocysts from control and treated snow crabs were noted. Hepatopancreatic and heart tissues were examined using light microscopy. Samples of these tissues removed from legal-sized snow crabs exposed to the single gun at 2 and 10-m, and to the array at 4, 50 and 170-m were examined along with the corresponding control animal tissues. No tubule autolysis or other abnormalities in the hepatopancreatic tissues nor cell rupture or other cellular damage in the cardiac and pericardial tissues were observed in either the treated or control animals.

Treated and control legal-sized male snow crabs are still being held at the DFO laboratory and observed for any chronic effects.

Neither acute nor delayed mortality of the adult crabs was observed during any of the treatments. The female crabs exposed to the 40-in^3 gun did appear to be temporarily slower to right themselves after being placed on their backs than the control female crabs. However, no difference in righting time was evident 45 minutes after exposure.

Twelve weeks after exposure to the 40-in^3 air gun at 2-m, fertilized eggs showed a 1.6% higher mortality than those 2,000+ eggs unexposed to seismic. In addition, 25.7% fewer of the 2,000+ exposed eggs had developed to the big-eye stage. Both of these differences were statistically significant. Treated and control egg-bearing female snow crabs are still being held at the DFO laboratory and observed for any chronic effects.

Based on the results of the physiological analyses of snow crabs exposed to seismic, the null sub-hypothesis *"**Seismic sounds do not cause other physiological damage to snow crabs**"* cannot be rejected. However, when applied to the developing fertilized eggs at close range, this null sub-hypothesis could very well be rejected.

CONCLUSIONS

Pilot studies that exposed legal-sized and sublegal-sized snow crabs to a single air gun and/or a seven-gun array did not indicate any immediate or delayed mortality or effects on a variety of haematological and histopathological indices, even in animals exposed to the seismic source at very close range (2 to 4-m). However, the pilot embryological study found major developmental differences between control and treatment groups of eggs exposed at close range (2-m) to a single air gun and examined after an additional 12 weeks incubation in the laboratory. This preliminary embryological study was carried out with only one pool of control and one pool of exposed eggs but careful attention was given to producing homogeneous pools of eggs for the study.

Any further studies on the effects of seismic on snow crabs might focus on the potential for egg damage with a view towards defining safe distance for normal egg development. A variety of tissues has also been archived or will be archived outside the framework of this study for possible further analyses if required (e.g., delayed effects on statocysts or eye pathology). Some consideration should also be given to the development of some approach to investigating the potential effects of seismic on snow crab larvae.

The study demonstrated that the catch-per-unit-effort (CPUE) during commercial snow crab fisheries was significantly higher during the post-seismic experimental fishery than during the pre-seismic fishery. Variability was quite high during both fisheries. Better weather conditions would certainly have enabled this aspect of the study to be completed in shorter time and thereby reduce some of the variability caused by other biological and physical factors. It is likely that the higher catches after the seismic airgun activity were due to physical, biological, or behavioural factors and not due to the seismic source. There was no relationship between catch and distance from the seismic source.

The limited telemetry work indicated that the few animals tagged with acoustic transmitters did not immediately leave the vicinity after exposure to seismic energy. Five of the 10 tagged animals were subsequently re-captured in the same general area during the following year's fishery. Caged experimental animals did not exhibit any immediate startle behaviour at the onset of seismic shooting.

Based on the results of this study, the overall null hypothesis tested during this study, ***"Sounds emitted during seismic exploration do not cause a significant decrease in the catch per unit effort of snow crabs"*** cannot be rejected. The fact that crabs did not react even when the seismic source was close indicates that the animals will not react to full-scale seismic when they are on the bottom in deeper water.

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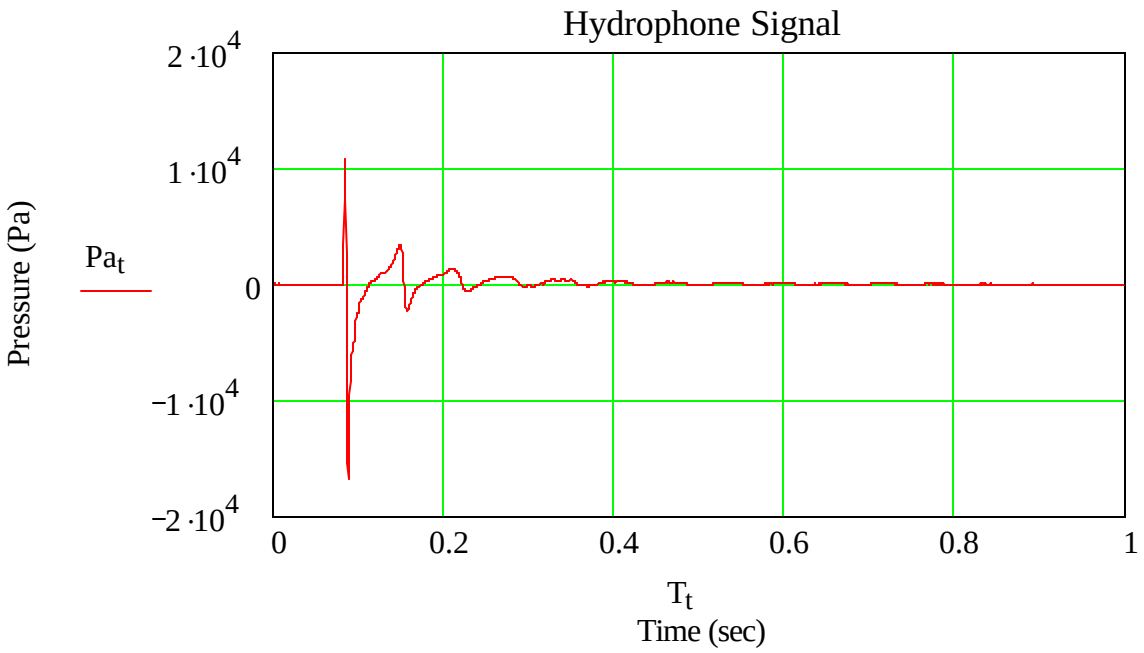
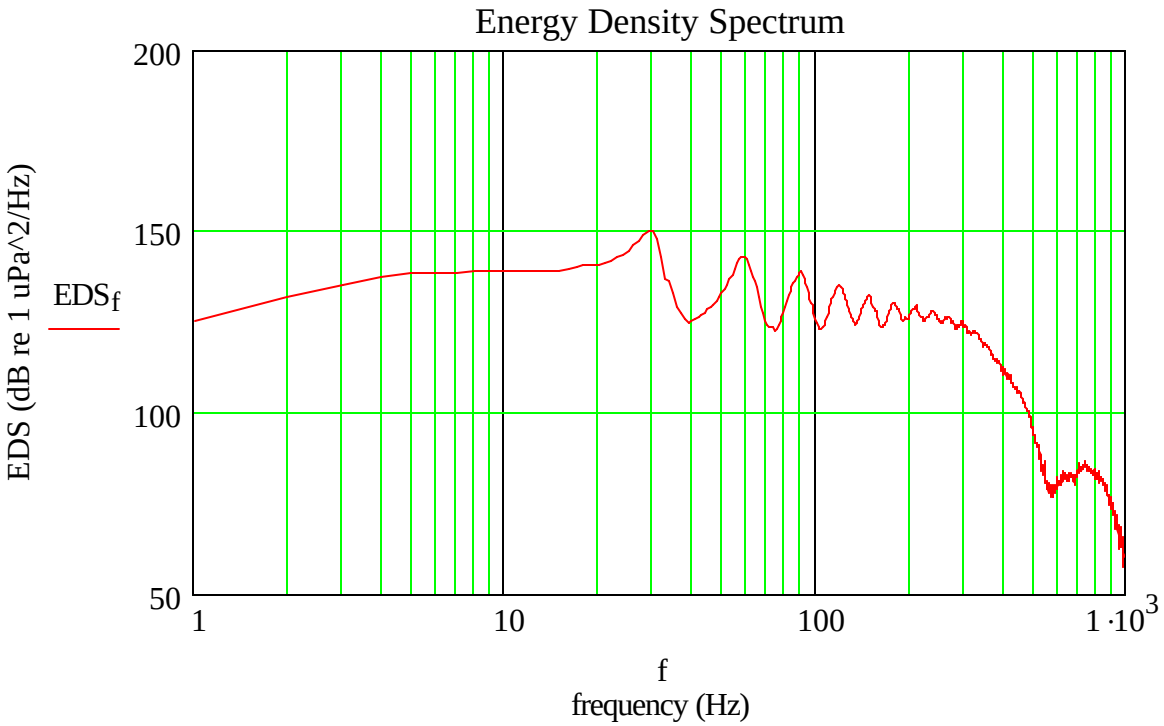
Appendix 1.

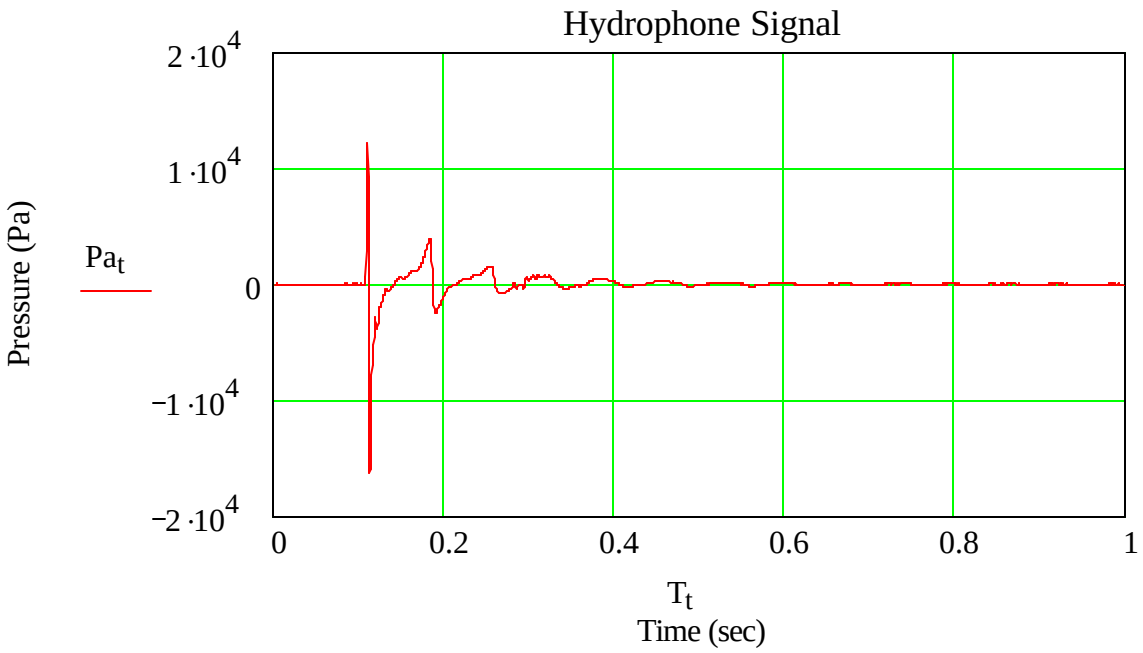
Graphs Showing Peak Levels and Sound Pressure (Energy) Density Spectra

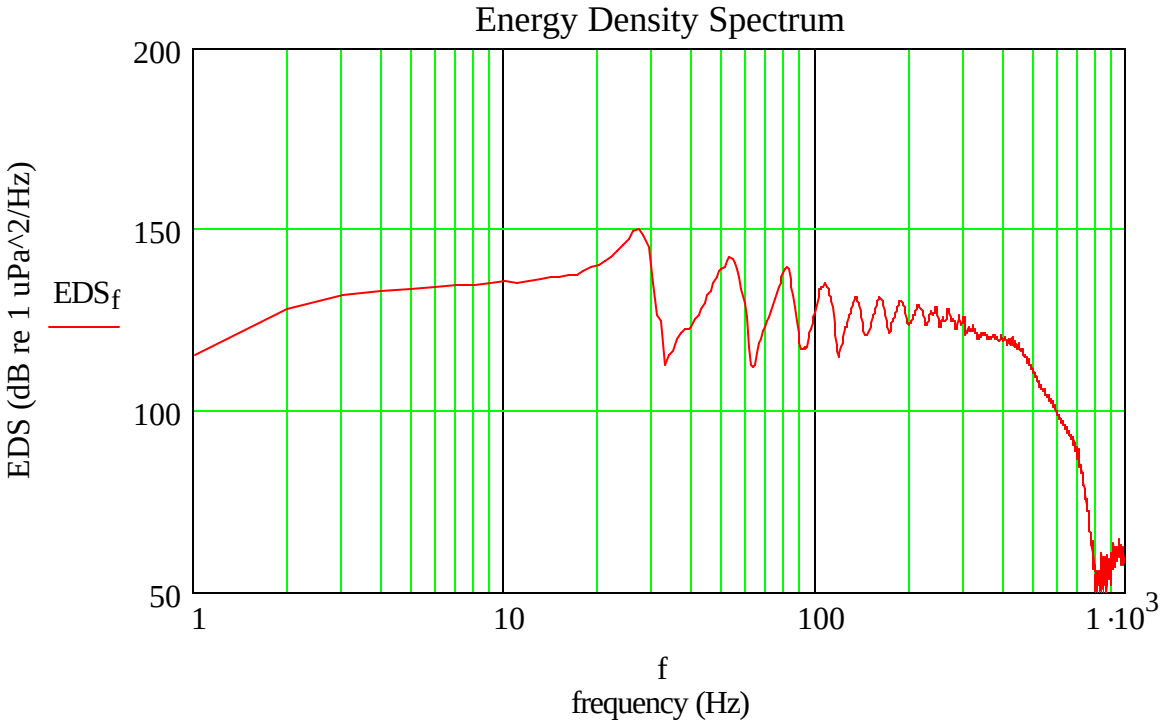
October 3 ,2002, Test 1, 40 cu. in. 10 meters





October 3 ,2002, Test 2, 40 cu. in. 10 meters





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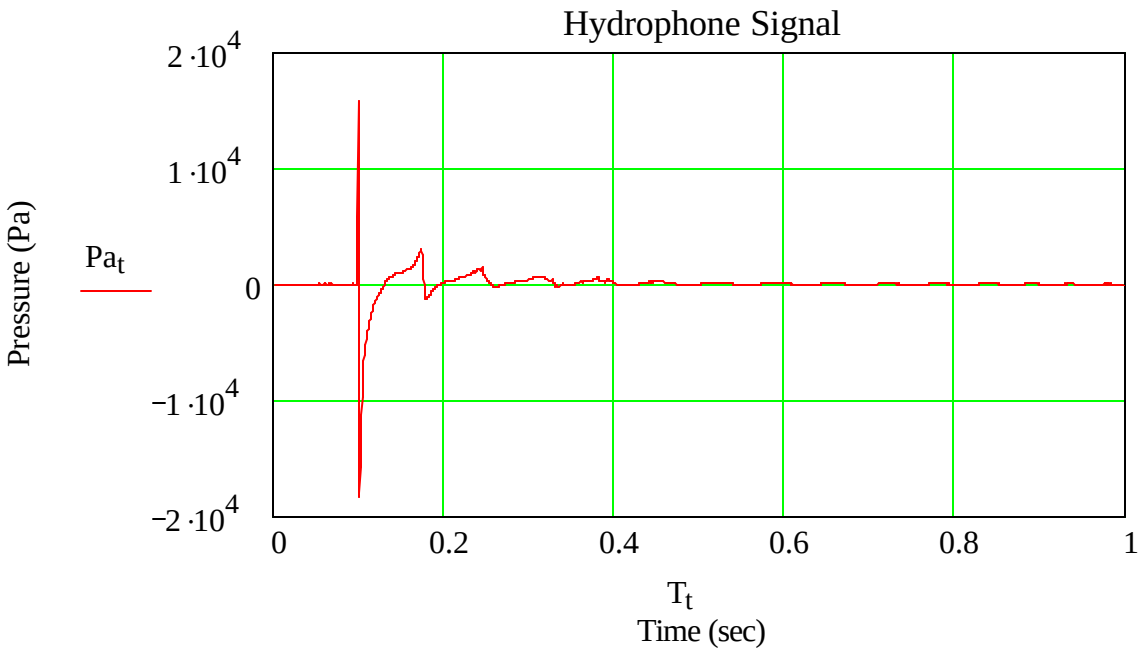

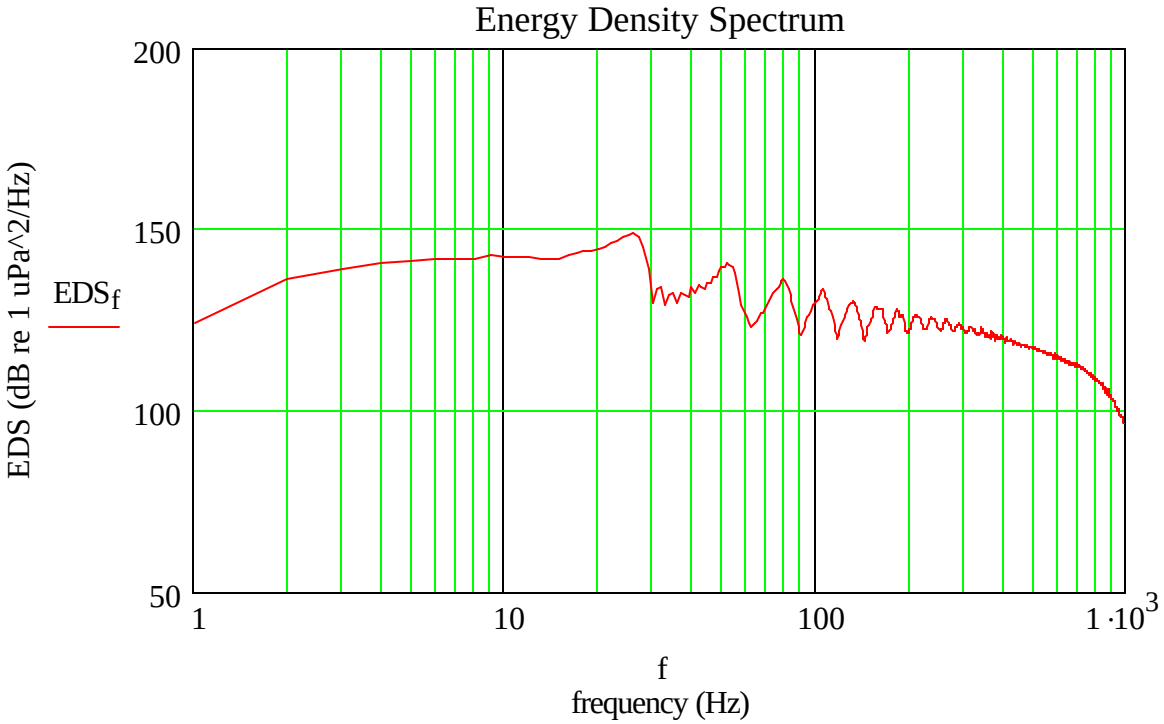

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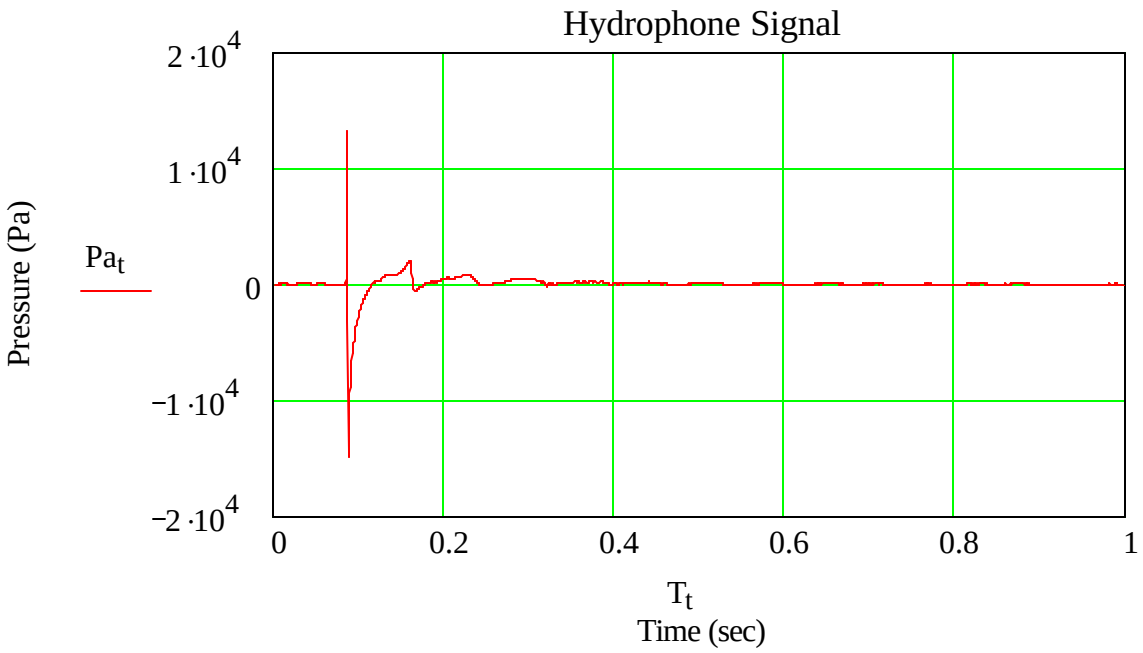
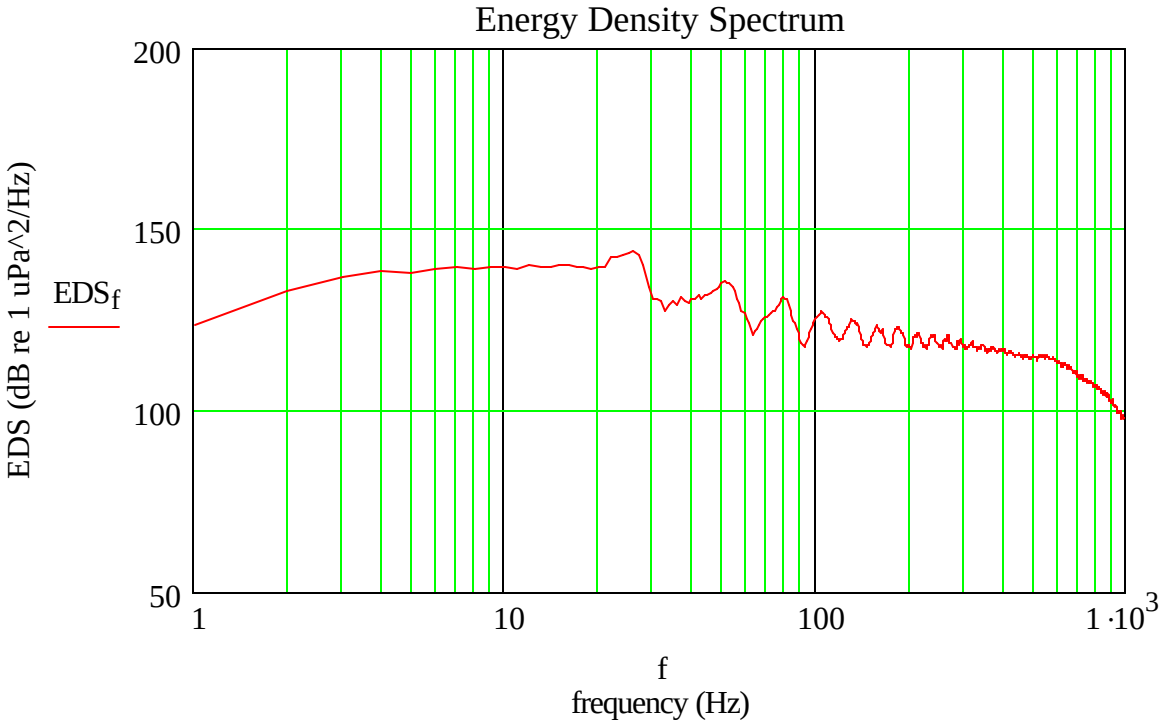
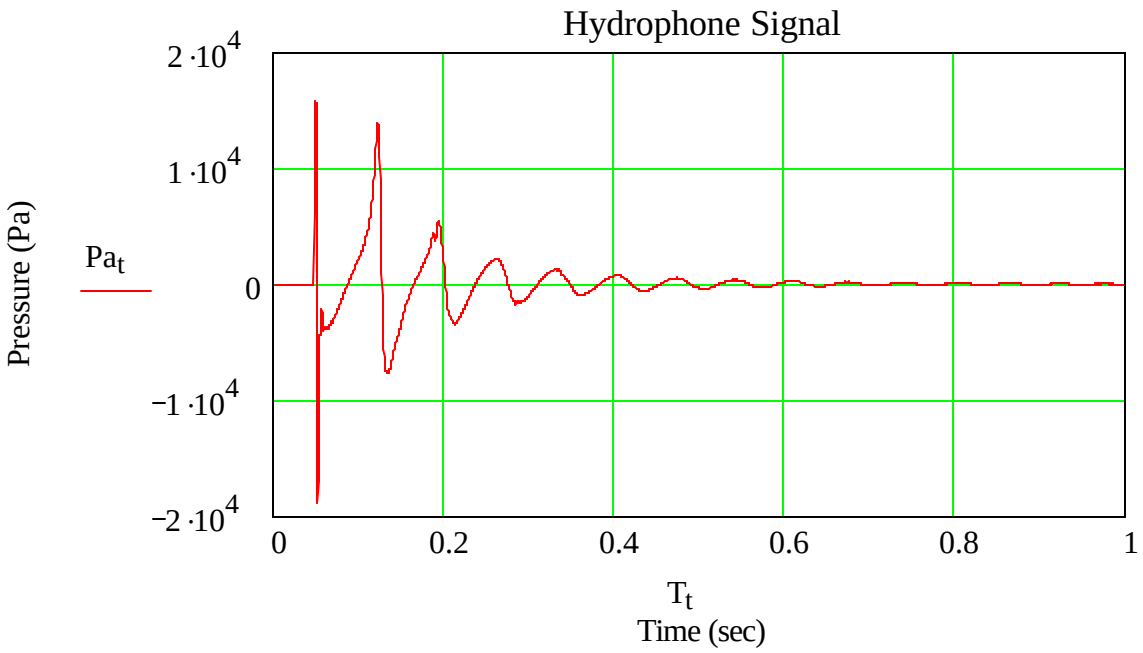
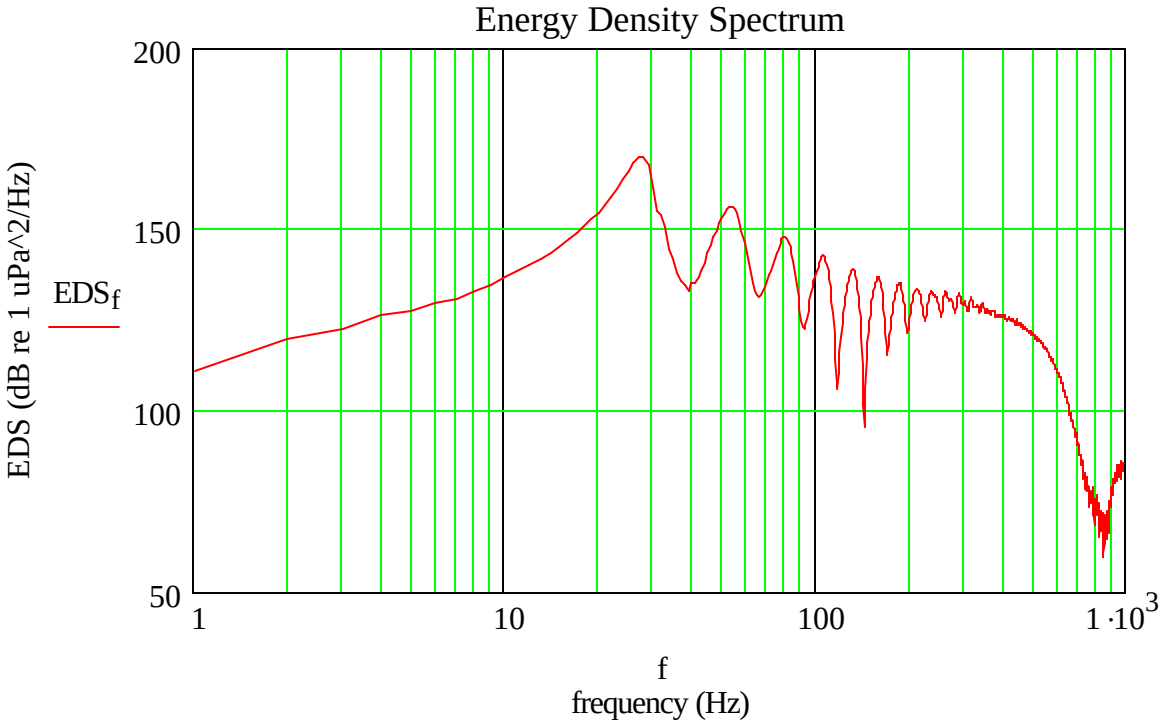


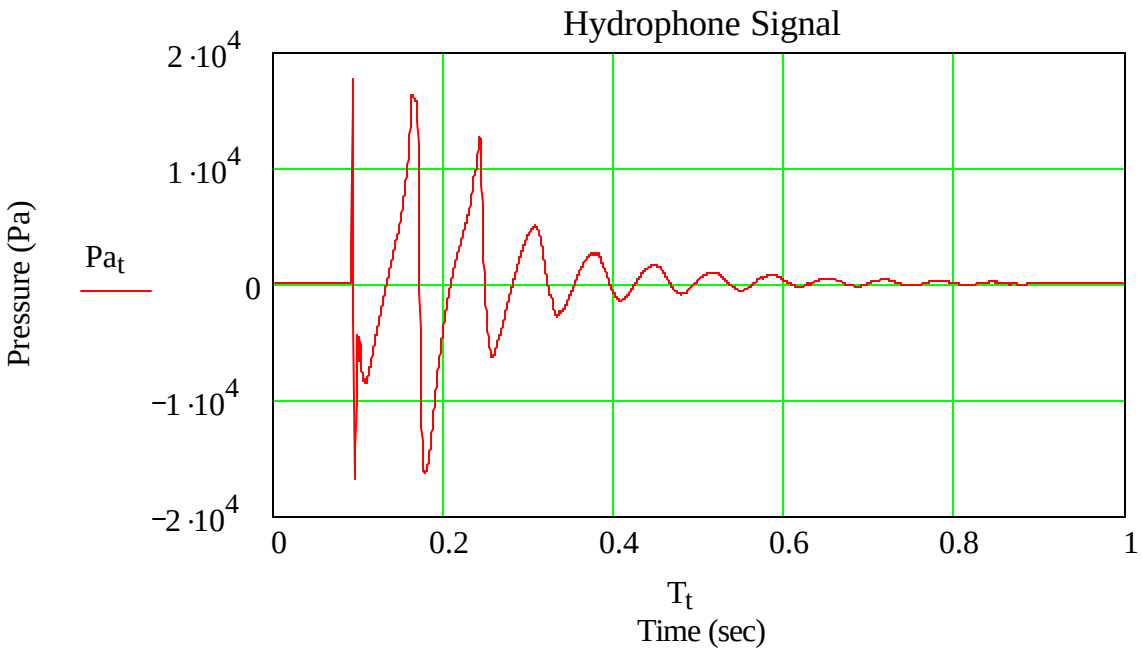


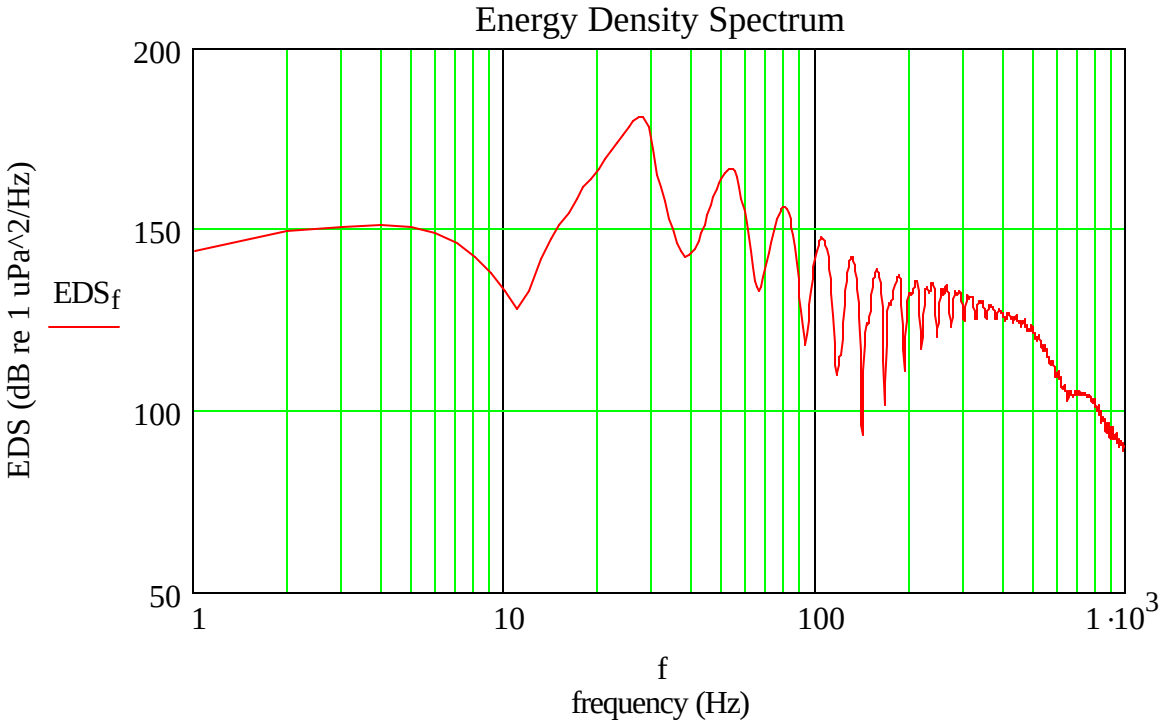
October 26 ,2002, 5 Meter Test, 40 cu. in. 5 meters



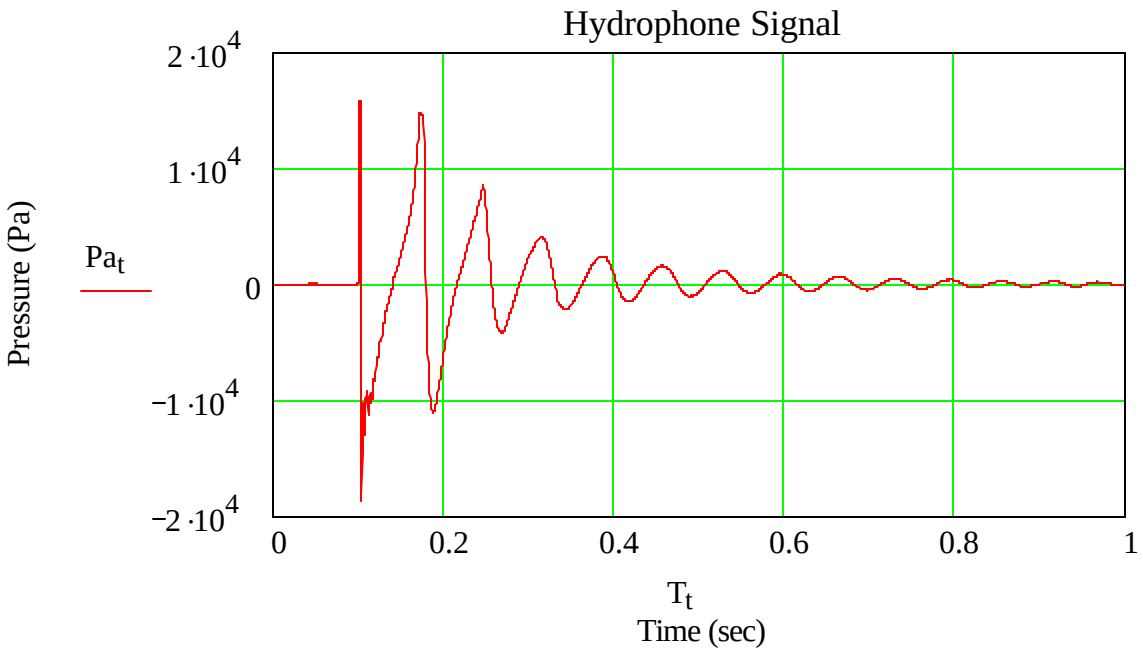


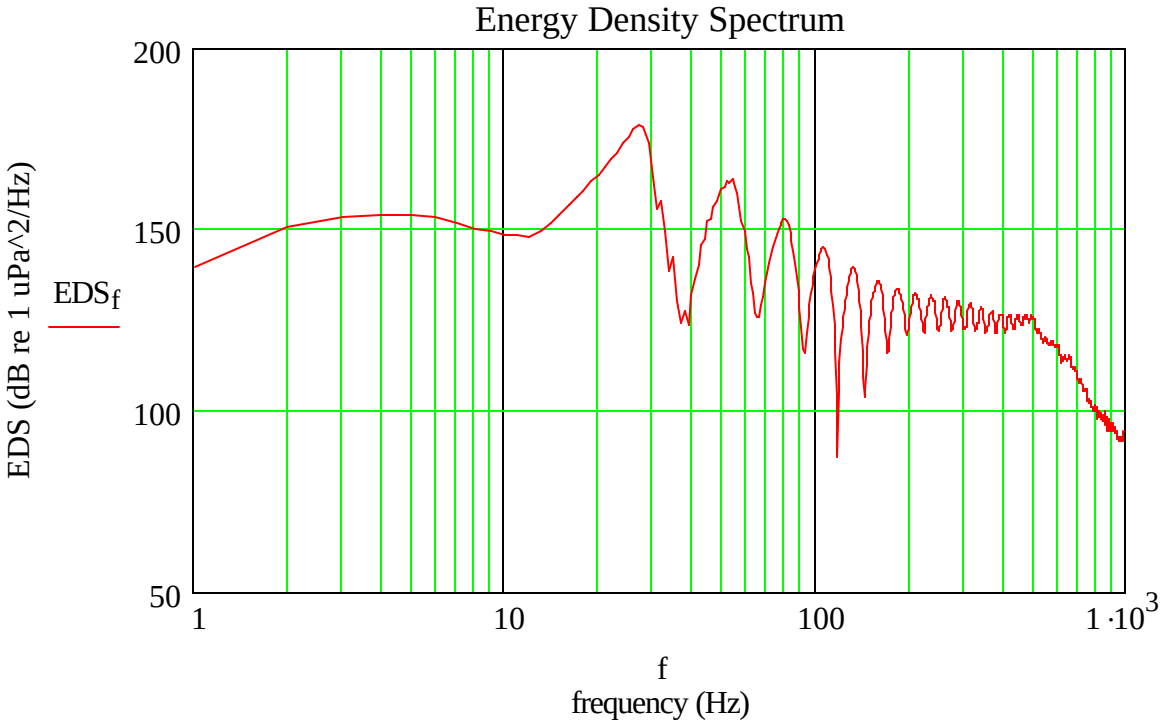
October 26 ,2002, 2 Meter Test, 40 cu. in. 2 meters



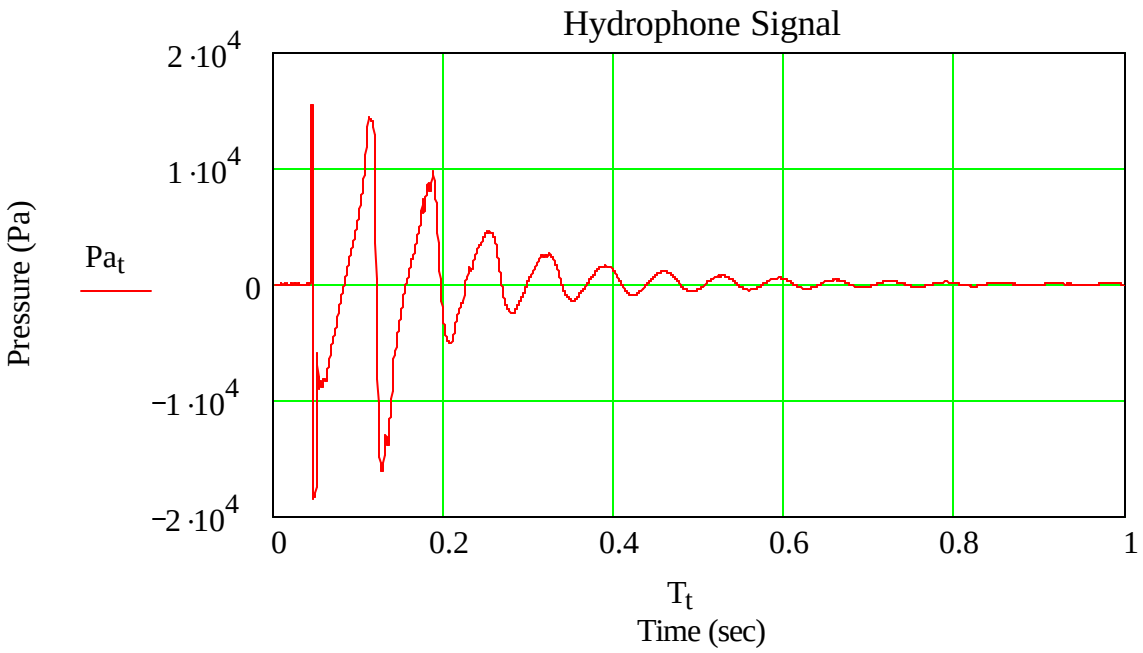


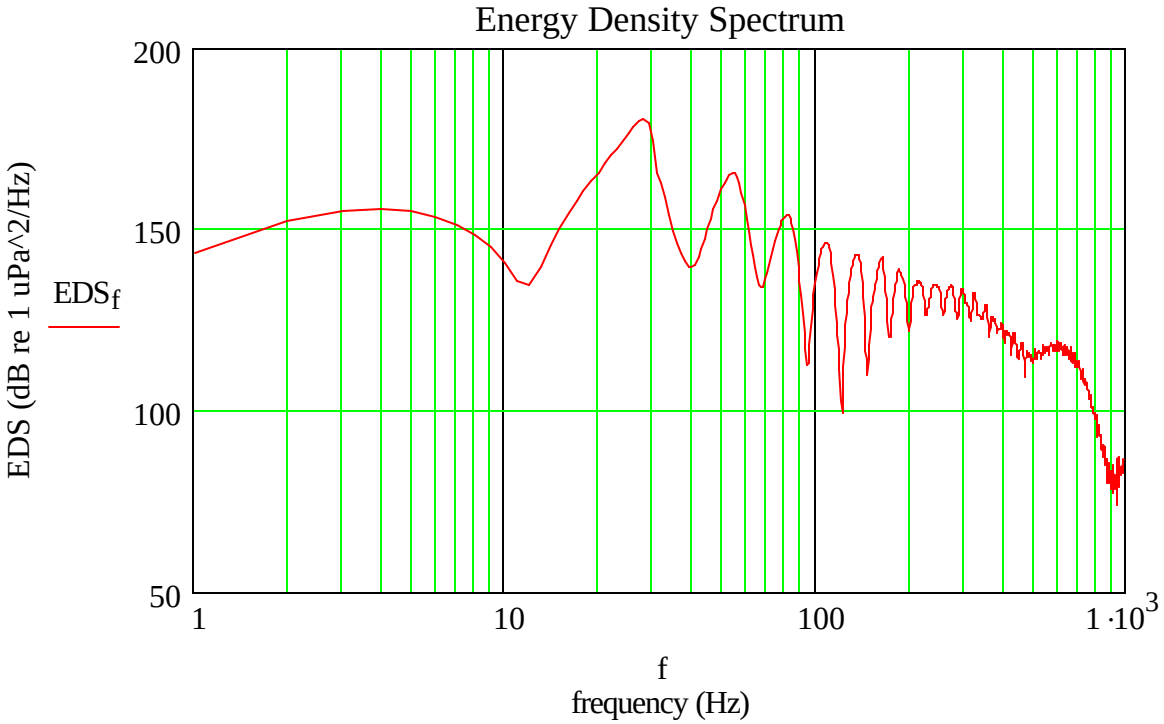
October 26 ,2002, Test 3, 40 cu. in. 2 meters



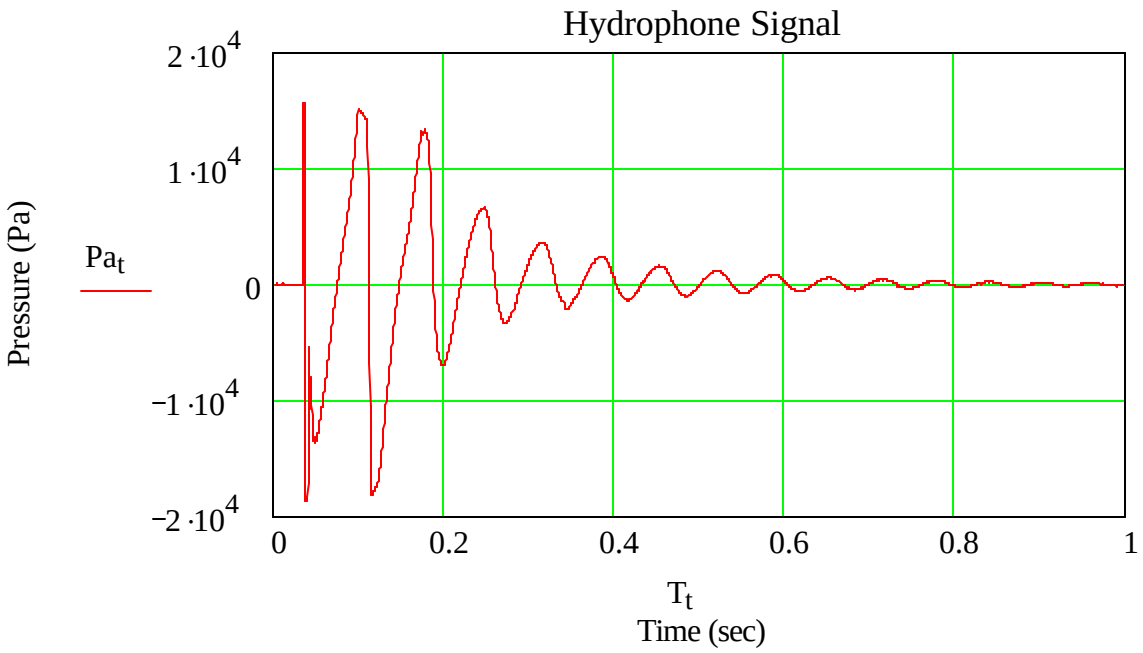


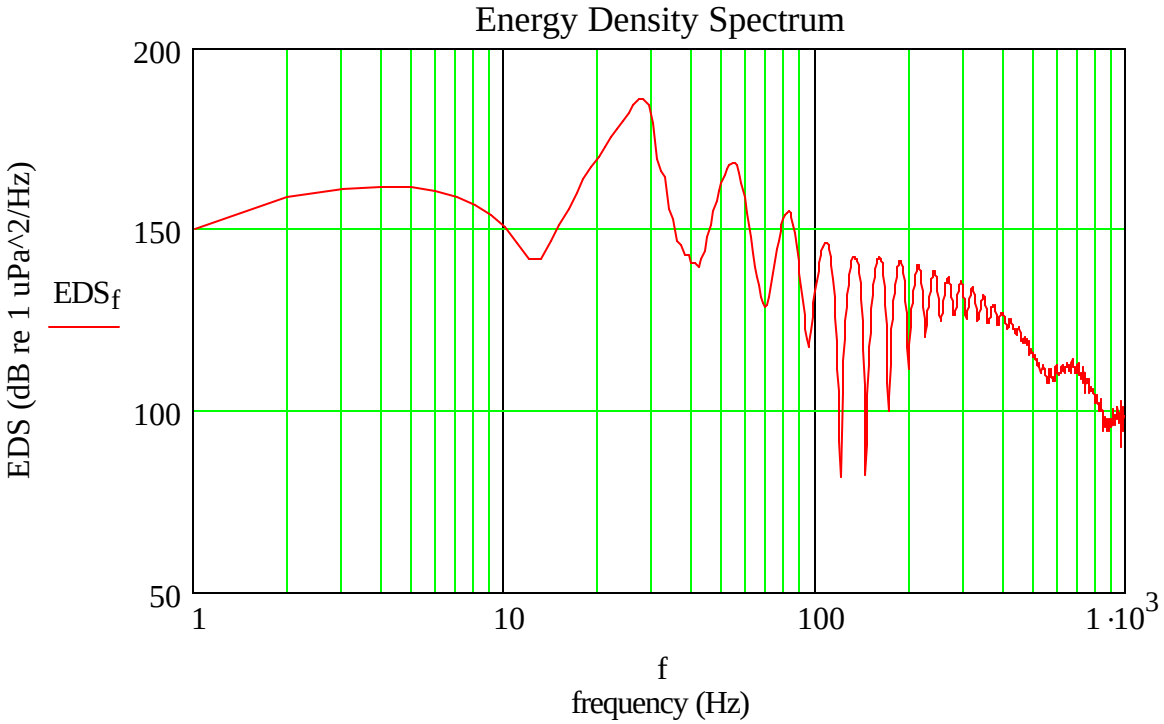
November 2 ,2002, Test 1, 40 cu. in. 2 meters



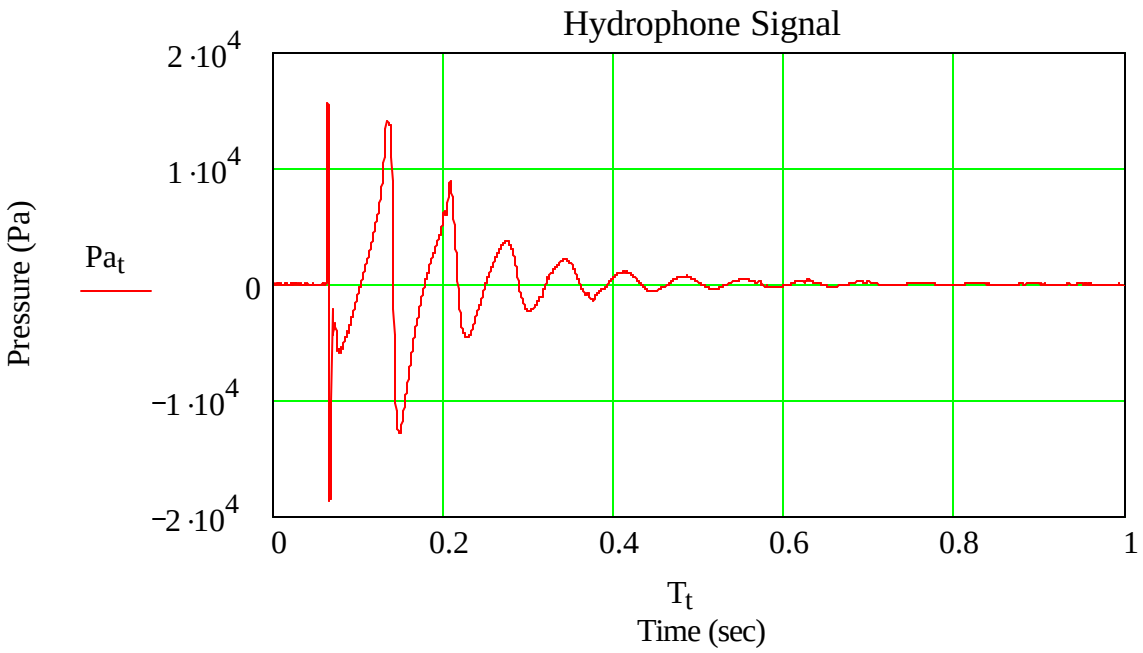


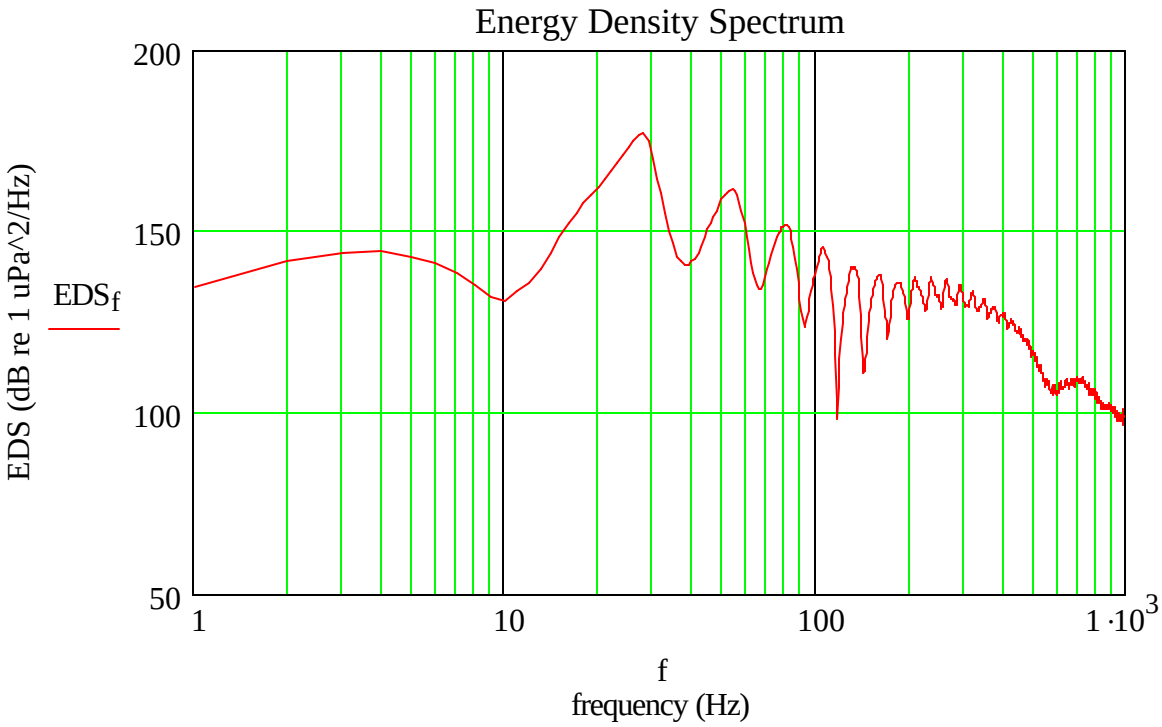
November 10 ,2002, Test 1, 40 cu. in. 2 meters



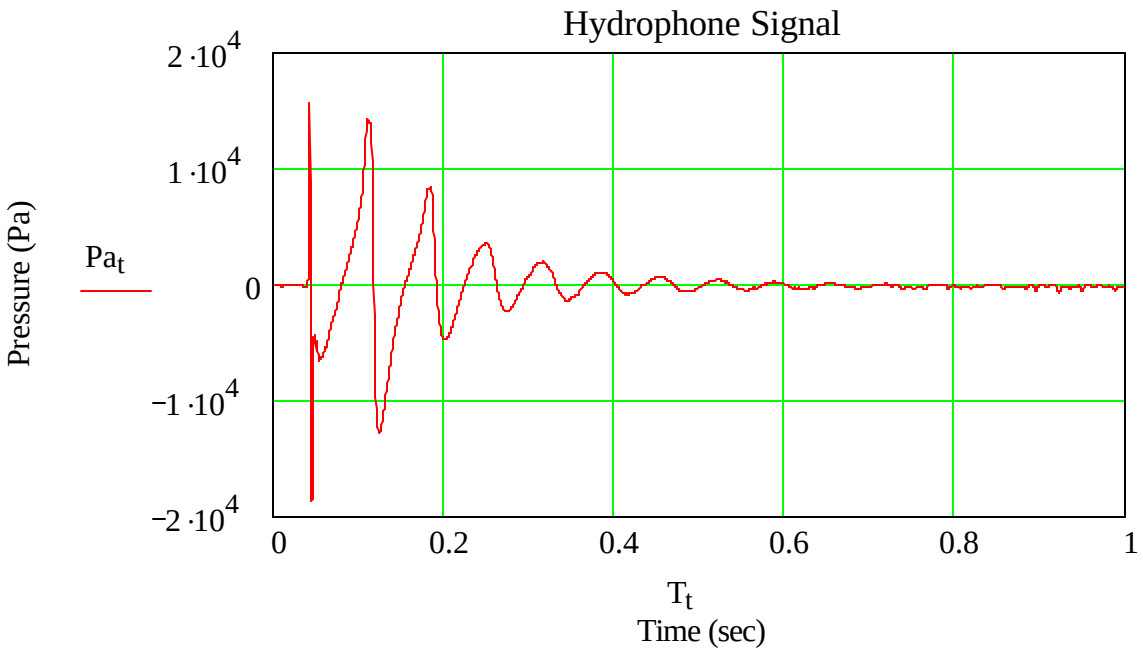


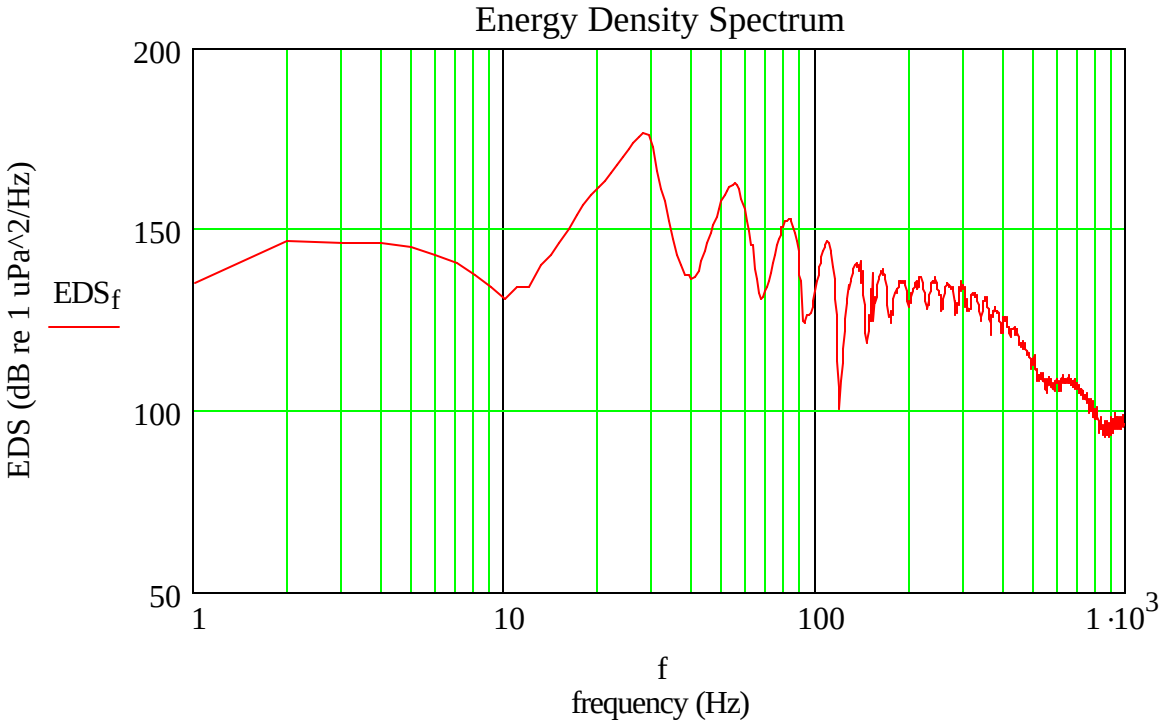
November 10 ,2002, Test 2, 40 cu. in. 2 meters



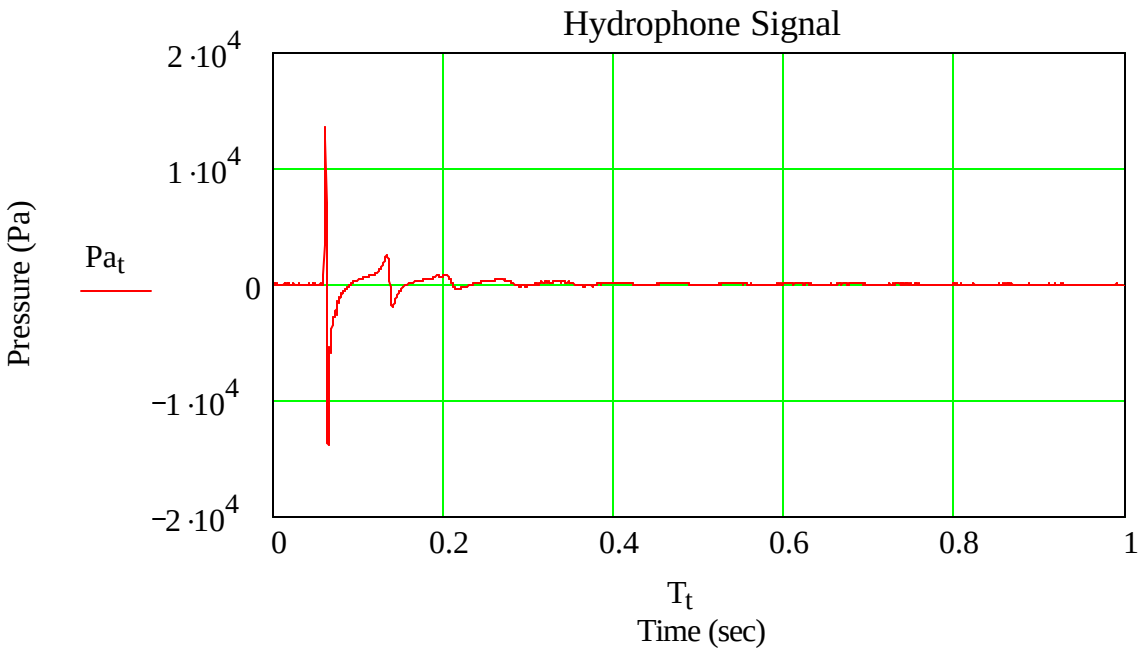


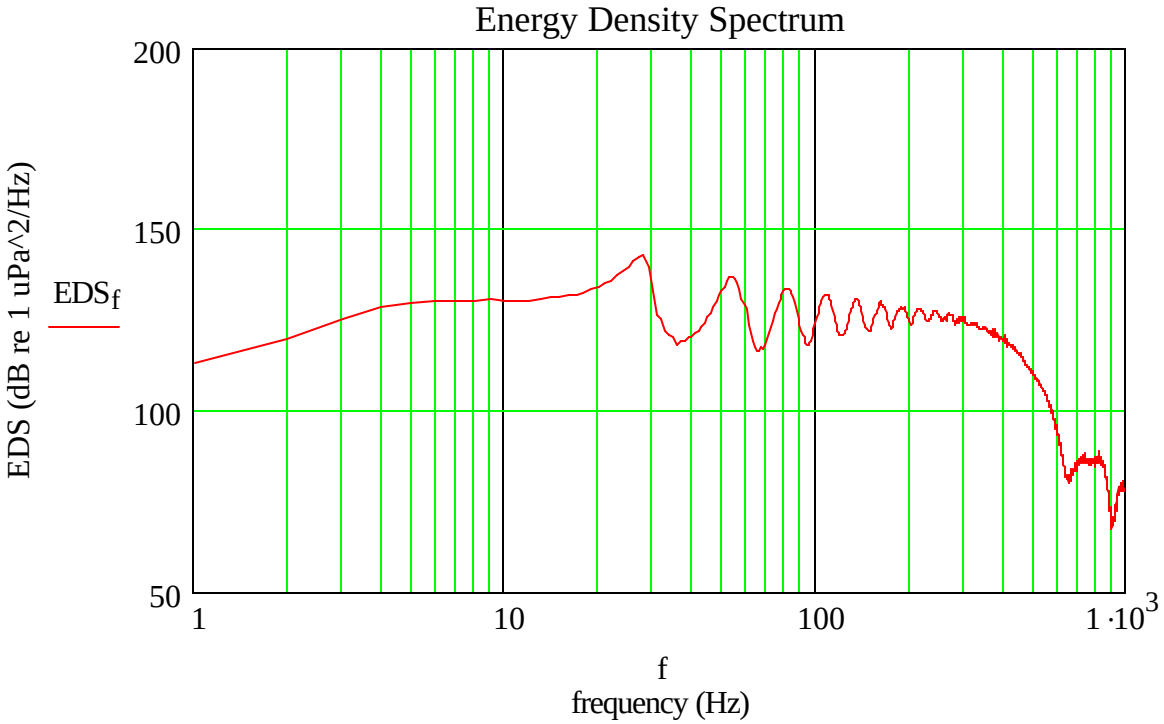
November 10 ,2002, Test 3, 40 cu. in. 2 meters



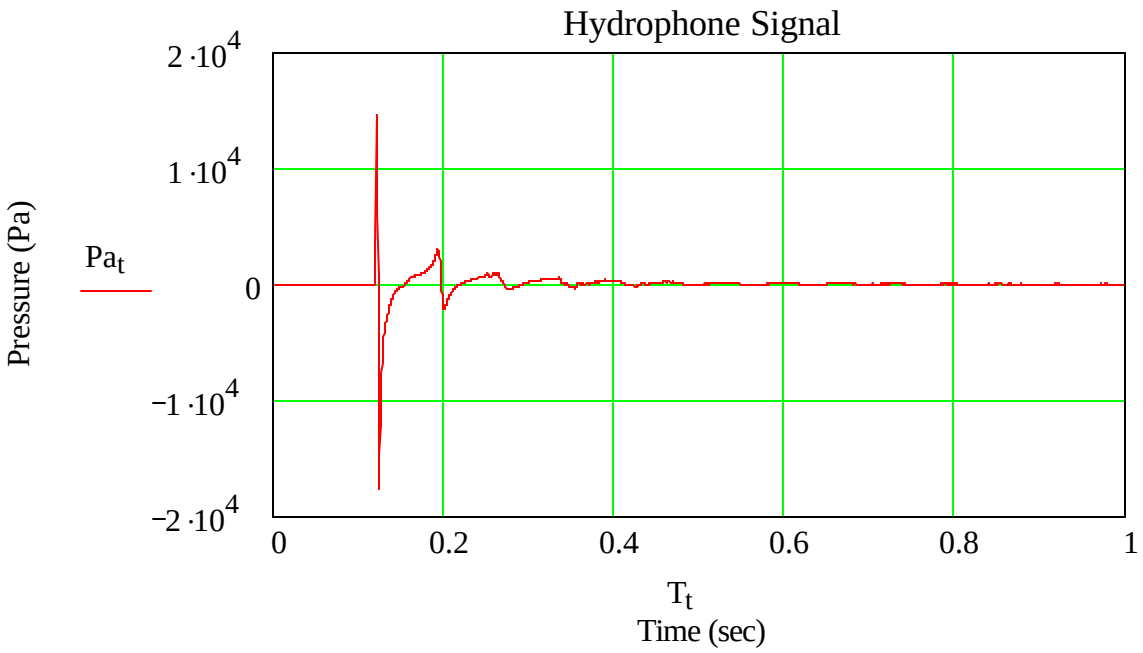


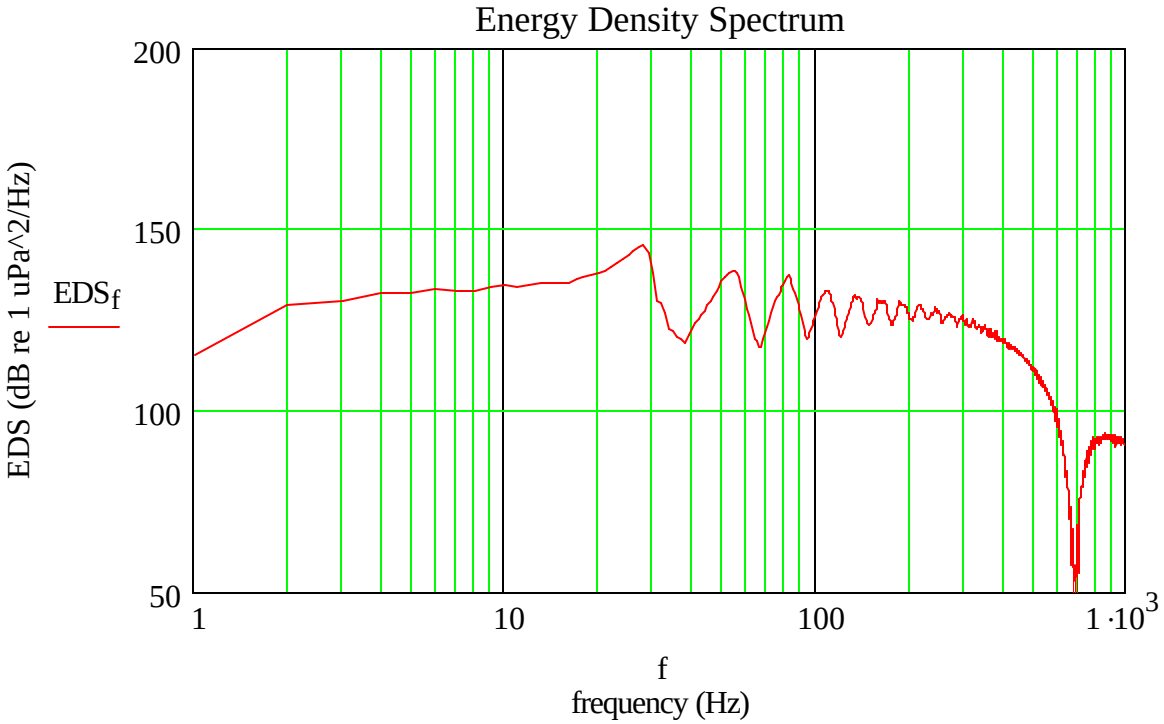
November 10 ,2002, Test 4, 40 cu. in. 15 meters



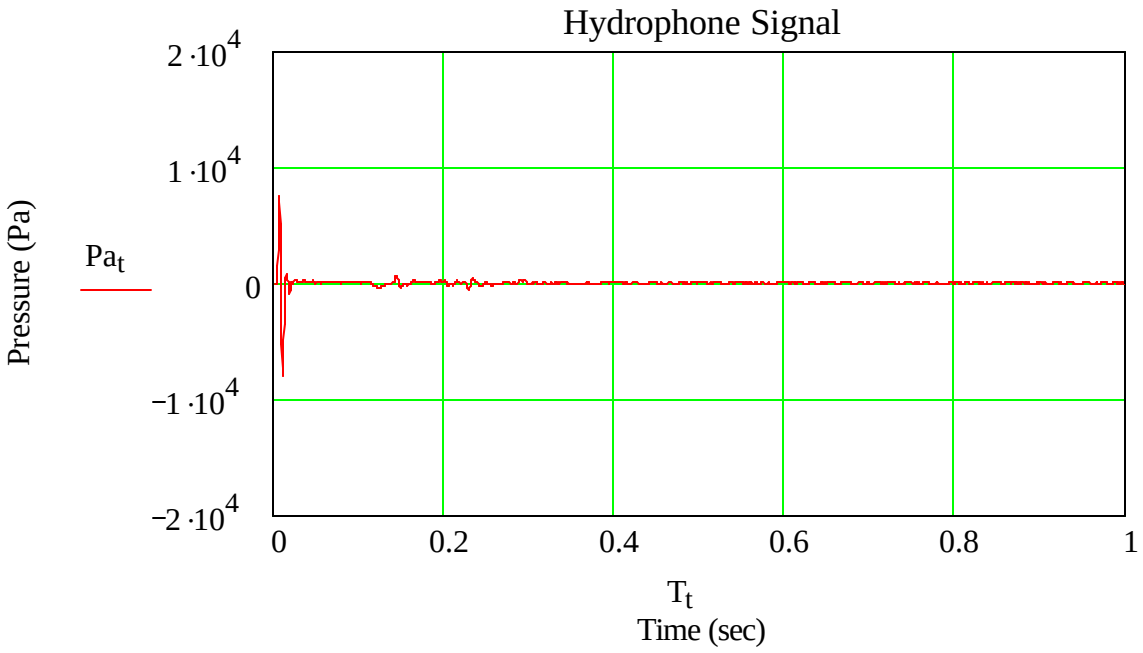


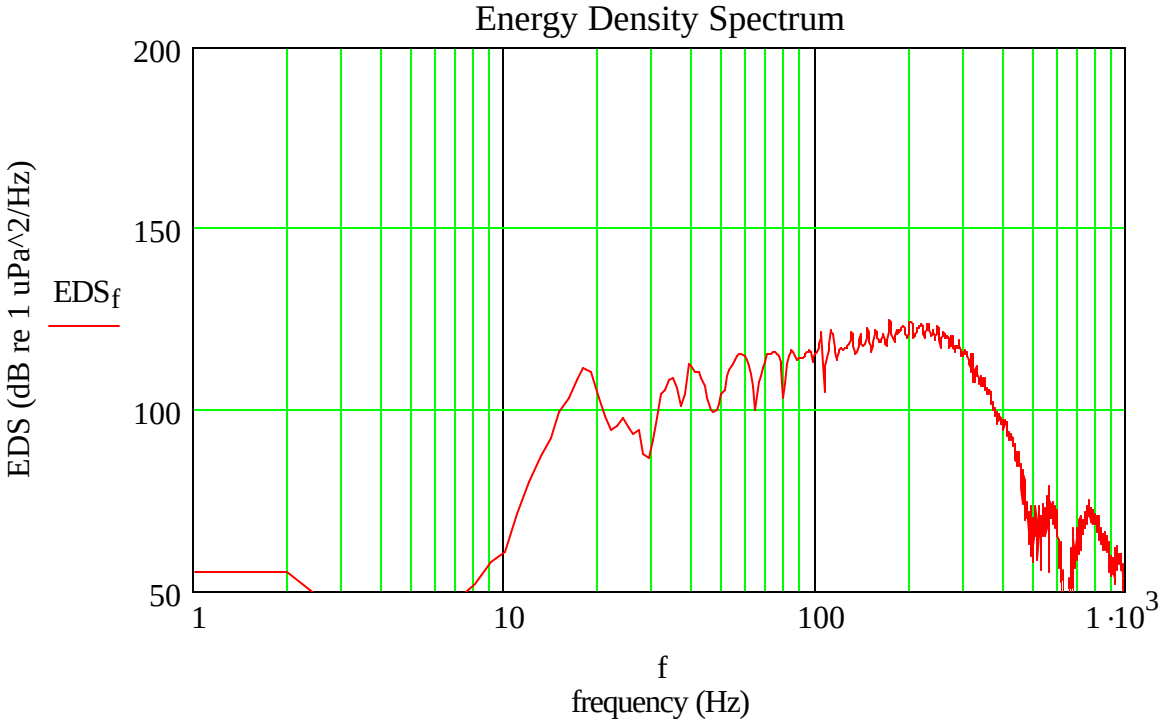
November 10 ,2002, Test 5, 40 cu. in. 15 meters



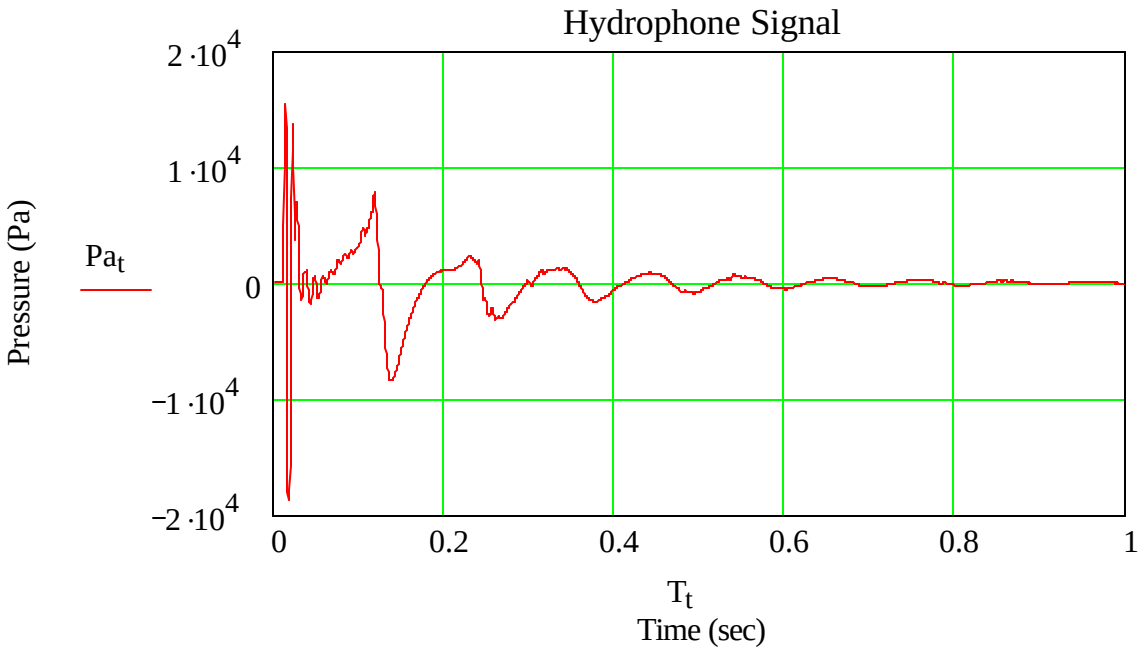


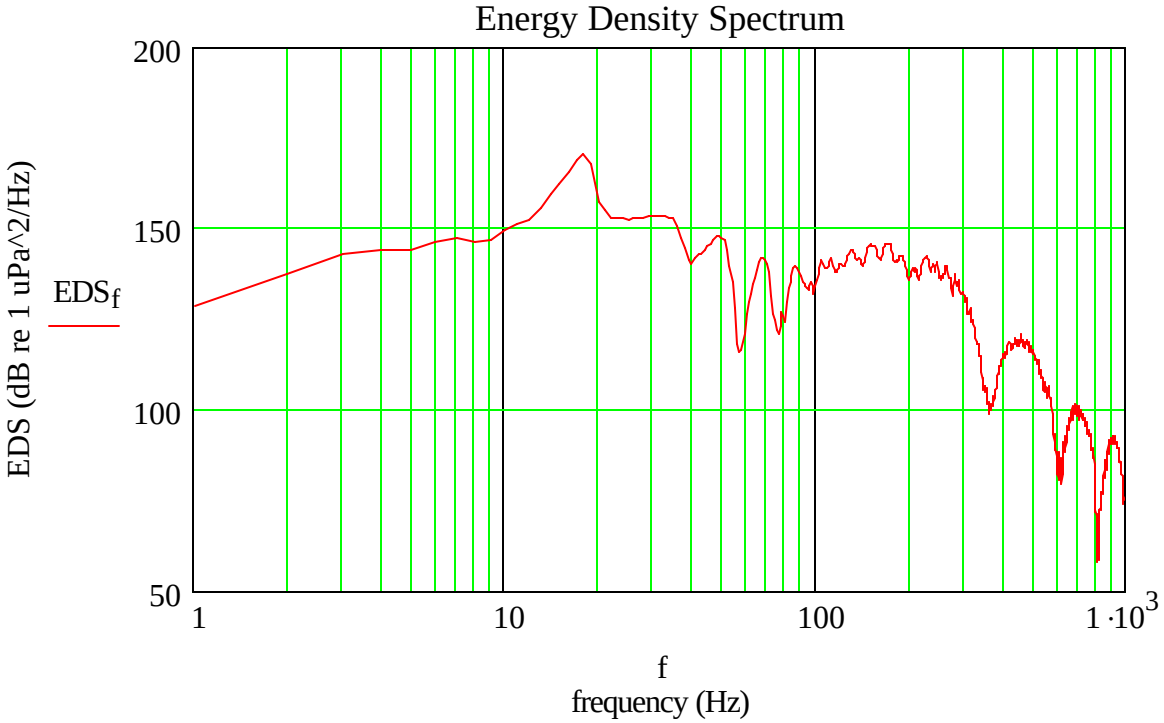
November 27 ,2002, Test 1, 200 cu. in, 85 meters



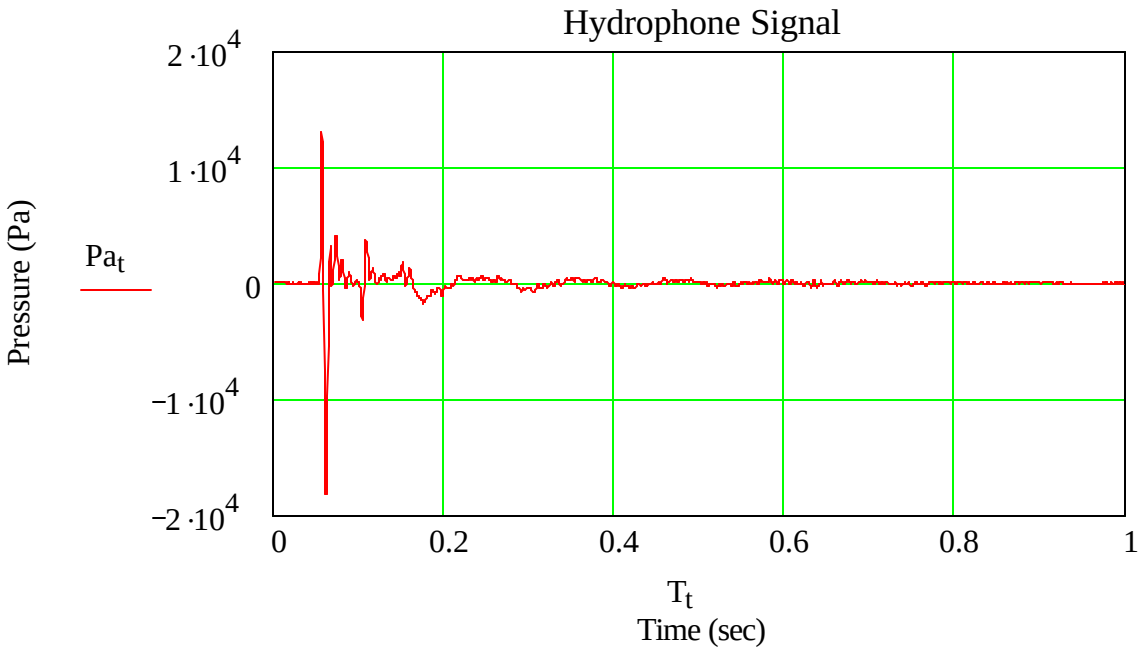


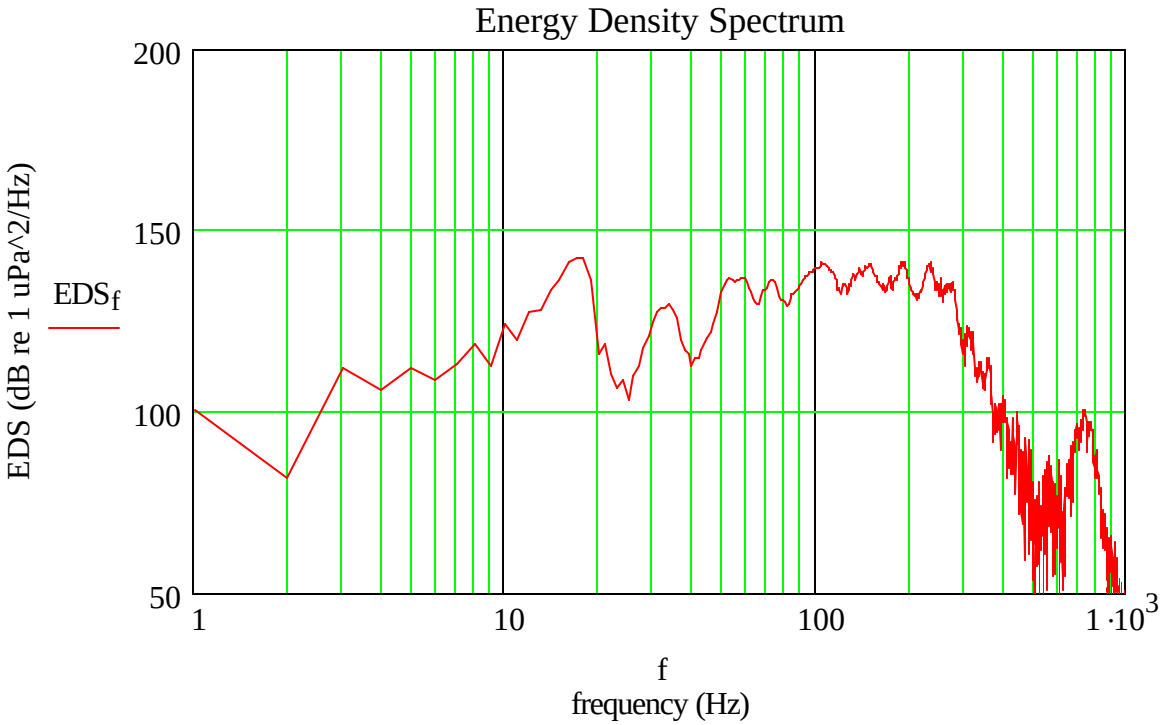
November 30 ,2002, Test 1, 200 cu. in, 4 meters



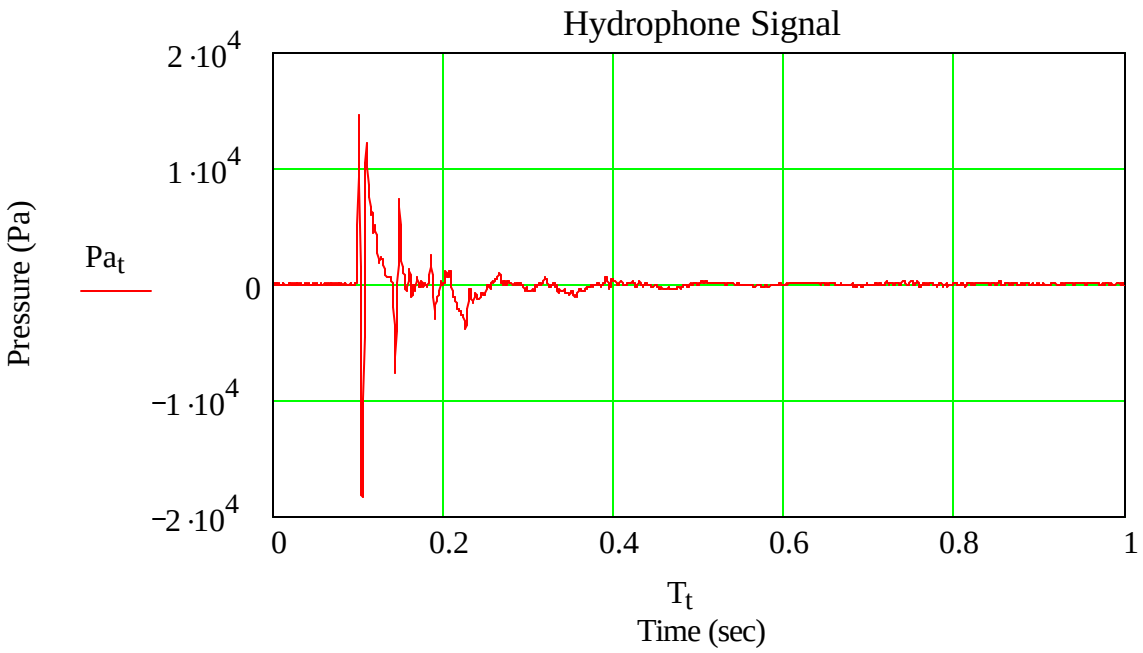


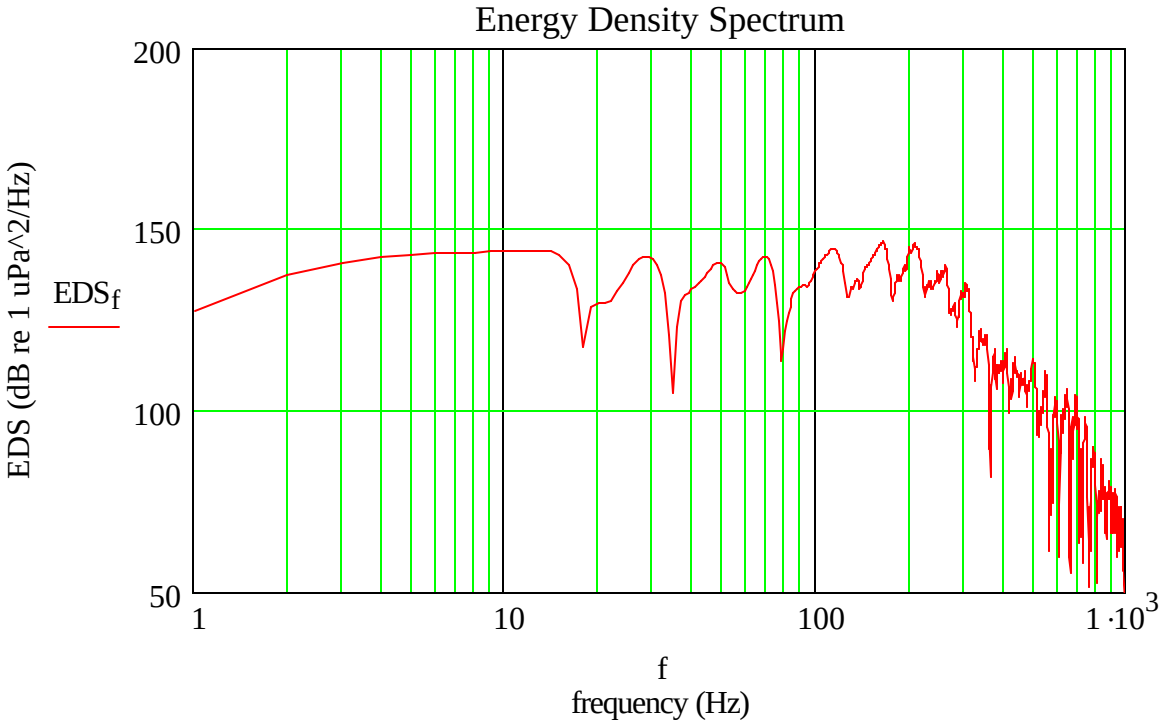
December 1 ,2002, Test 1, 200 cu. in, 50 meters Low Frequency





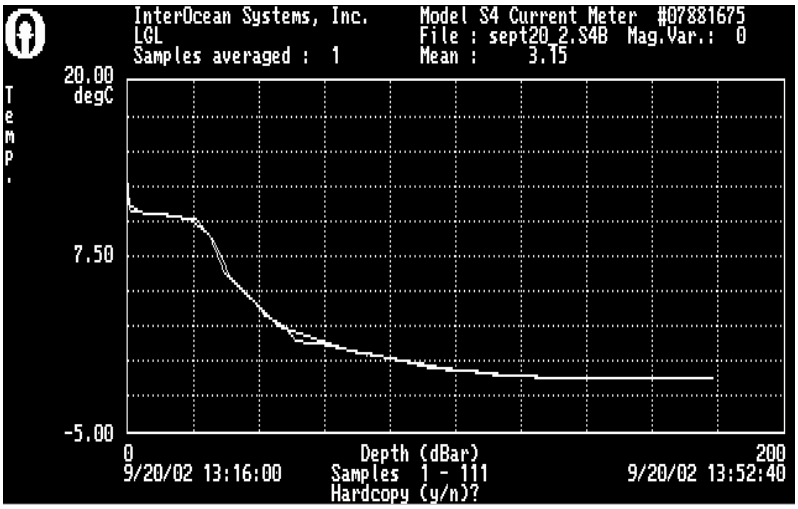
December 1 ,2002, Test 1, 200 cu. in, 50 meters High Frequency



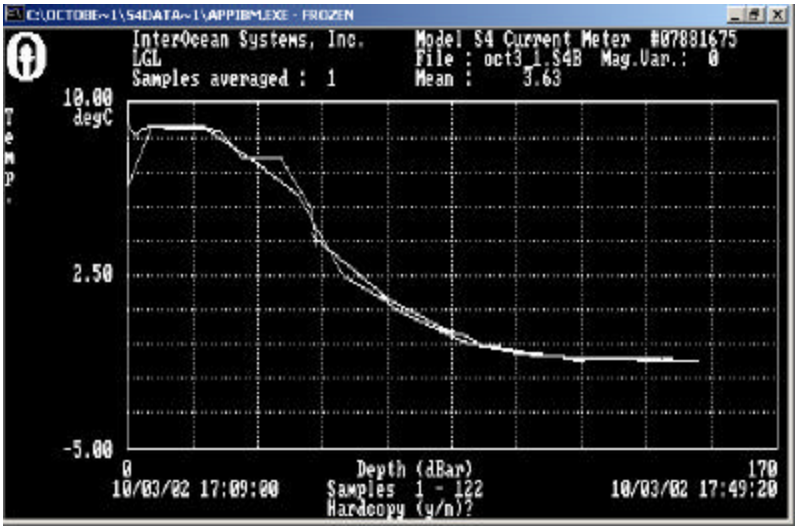


Appendix 2.

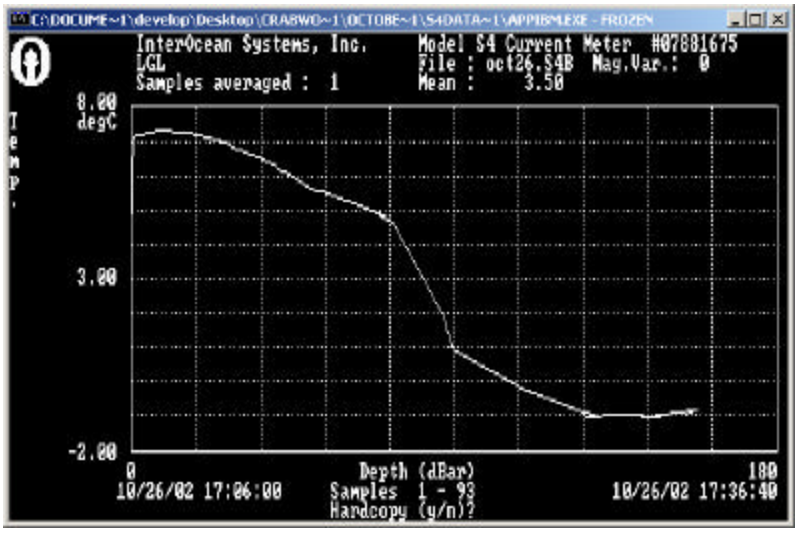
Water Column Profiles



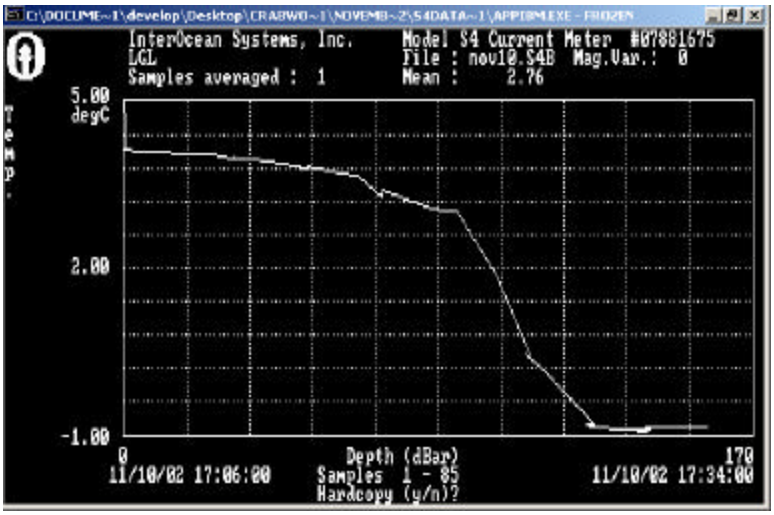
September 20, 2002



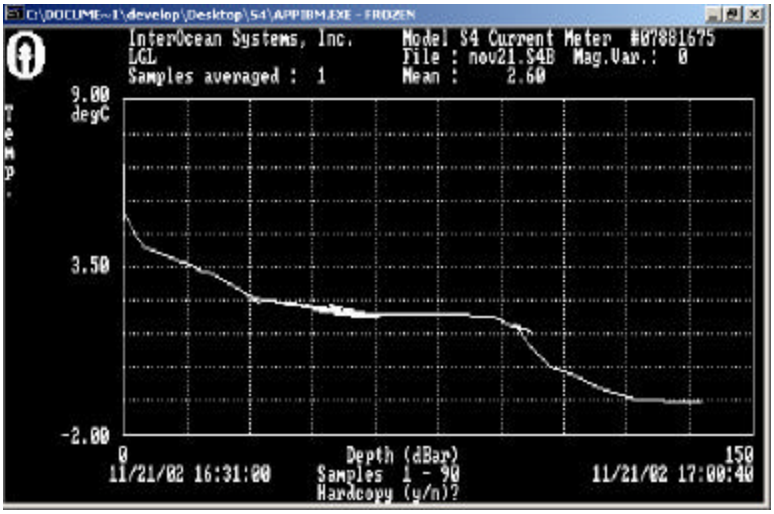
October 3, 2002



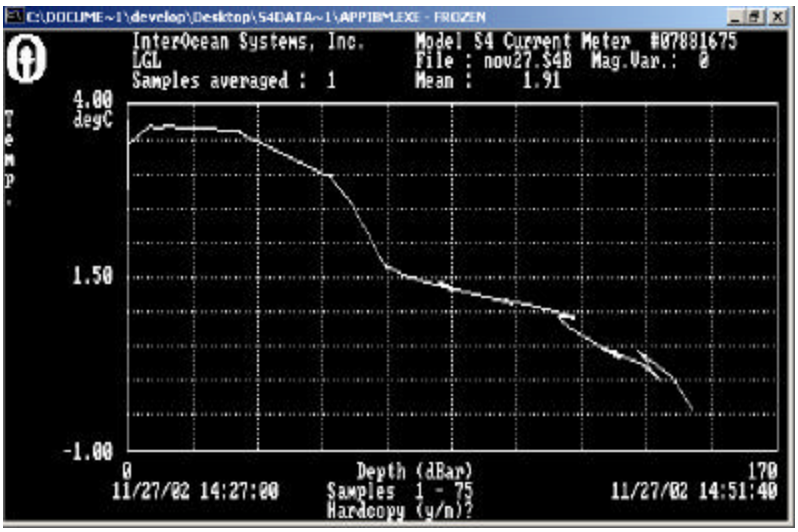
October 26, 2002



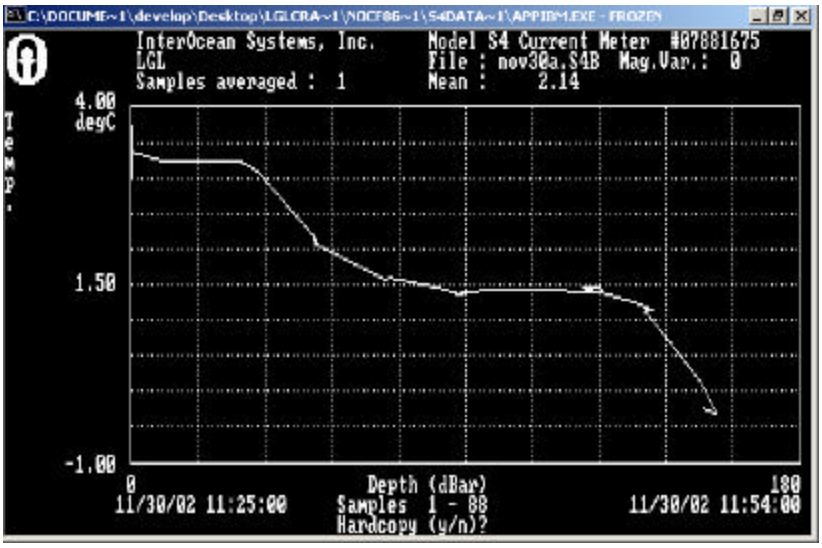
November 10, 2002



November 21, 2002



November 27, 2002



November 30, 2002