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POTENTIAL IMPACTS OF SEISMIC ENERGY ON SNOW CRAB: AN UPDATE TO THE 2004 PEER REVIEW

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EXECUTIVE SUMMARY

A scientific study was conducted in December 2003 with indigenous mature female snow crab (*Chionoecetes opilio*) caged in an area off western Cape Breton Island (N.S.) to determine the potential effects of a seismic survey operation. A review of the results from this study concluded that the seismic survey did not result in mortality of snow crab, or the embryos they were carrying (DFO, 2004). However, a number of questions raised were judged to be worthy of further investigation. An effort was made to conduct additional experiments, extract as much information as possible from the original caging experiment, and to identify knowledge gaps. As a result of this initiative, a series of scientific papers was presented at the Gulf Fisheries Centre, Moncton (N.B.) in January 2007 (proceedings in Boudreau et al., 2009). The highlighted questions of concern and subsequent scientific findings were:

1. What sound pressure levels were actually encountered by the snow crab caged in the seismic survey area compared to snow crab caged at a reference site 23 km away?

Further analysis of RMS sound pressure levels collected before and during the December 2003 seismic survey showed that snow crab caged within the seismic survey area were exposed to values as high as 178 dB re µPa (compared to 118 dB re µPa at the reference site). Ambient noise levels at the seismic cage site had broadband sound levels up to 95 dB re µPa. Therefore, it is clear that snow crab caged within the ensonified area were subjected to higher sound levels than crab caged at the reference site.

2. Was the presence of foreign particles on the gills, antennules and statocysts (balance organs) of crab caged in the seismic area the result of cages having been dragged rather than exposure to seismic energy?

Snow crab recovered from cages in the ensonified area immediately following the December 2003 seismic survey were found to have higher concentations of sediment particles on their gills, antennules and in their statocysts (balance organs) than crab caged in the reference area. It was noted in the September 2004 review that sediment characteristics and oceanographic conditions differed between the cage deployment sites and that some crab cages within the ensonified area were dragged due to the interaction of surface ice with the mooring buoys. In May 2004, female snow crab in mesh bags were dragged for two hours over the bottom in the area of the former seismic survey. All snow crab survived this treatment and showed no ill effects including no fouling of the eyes, antennules, statocycts or gills. While these results do not support the hypothesis of organ fouling due to the dragging of cages in the ensonified area, a definitive conclusion could not be reached, since the two-hour duration of the study was considerably shorter than the 12-day caging period during the December 2003 seismic survey.

3. Did the seismic survey change the geographical distribution of snow crab and reduce abundance in the ensonifed area?

A bottom trawl survey carried out after the seismic survey (June 2004) provided no evidence of reduced abundance or changed geographic distribution of multiparous female snow crab compared with a bottom trawl survey conducted before the seismic survey (September 2003). However, there is considerable variation associated with the

abundance estimates, and it is noted that data obtained from bottom trawl surveys cannot provide the resolution to provide a definitive answer to this question.

4. Were the differences observed in the cellular structure of certain organs between the seismic and reference areas of concern, and the result of exposure to seismic energy?

Results of histological analyses, which revealed differences in the cellular structure of certain organs in these snow crabs, raised particular concern and questions as to the consequence of such abnormalities in terms of population level effects. Histological analyses carried out by DFO personnel suggested bruising of the hepatopancreas (liver equivalent) and ovaries of snow crab caged in the December 2003 experiment. To assess the significance of these observations and confirm that they were more common in the snow crab caged within the seismic area, samples of the tissues were read by an independent histopathologist. Abnormalities were found in all the groups examined, and there was not a higher degree of abnormalities in the seismic groups than reference groups. Multivariate analysis of the data showed significantly fewer abnormalities in snow crab caged at the seismic than reference site. Pathologies were more common in animals caged 5 months than 12 days suggesting an effect of caging. Overall, these analyses suggested that handling stress (including fishing and caging) produced the abnormalities observed. Therefore, it is not possible to draw any conclusion from the 2003 experiment because the protocol used (unknown status of the females before treatment) does not allow assessing the null hypothesis, i.e. a possible impact of seismic activity on snow crab females. It was recommended that such a study be carried out to provide a description of "normal" snow crab histology, against which future studies might be compared. Consequently, a two-and-a-half-year study, starting in July 2010, has been funded by the Offshore Energy Environmental Research Association (OEER) to improve understanding of the fundamental biological characteristics of snow crabs in their natural habitat and the physiological effects of handling.

5. Did exposure to seismic energy result in the snow crab losing their legs?

It was noted during the September 2004 peer-review meeting that among the snow crab caged in December 2003 and then sent to the Northwest Atlantic Fisheries Centre for analysis (45 from each of the seismic and reference site), a total of 26 legs were lost from the seismic group compared to only 3 legs from the reference group. Two lines of evidence suggest this difference was not the result of exposure to seismic energy. First, snow crab from the same test groups sent to the Gulf Fisheries Centre did not show a difference in rate of leg loss between seismic and reference sites. Second, further studies at the Northwest Atlantic Fisheries Centre failed to produce leg loss in snow crab exposed to sound levels up to 220 dB peak to peak, which is greater than sound levels encountered by crab in the 2003 survey. It was suggested that rough handling of the seismic group of crabs sent to Newfoundland (as test and reference site animals were sent in separate containers) may have been responsible for the elevated rate of leg loss.

6. Would methodological refinements alter the conclusion that snow crab embryos were unaffected by exposure to seismic energy?

Quantification of larvae hatching from females caged in December 2003 suggested no difference between the seismic site and reference site, therefore indicating

no effect of seismic energy on survival of embryos. However, reviewers at the September 2004 meeting suggested a number of methodological refinements to this analysis (DFO, 2004). Re-analysis confirmed the original conclusions: 1) exposure to seismic energy did not kill snow crab embryos; 2) female snow crab caged in the seismic area had a similar number of offspring to female snow crab caged in the reference area; and 3) rate of development was slower in seismic than in reference embryos. This could be related to seismic energy, to cooler temperatures at the seismic site than control site or other unmeasured parameters.

SOMMAIRE

Une étude scientifique sur le crabe des neiges (*Chionoecetes opilio*) a été réalisée en décembre 2003. Des femelles matures indigènes ont été placées en cage dans une zone située à l'ouest de l'île du Cap-Breton (Nouvelle-Écosse) afin de déterminer les effets possibles des activités de levés sismiques sur cette espèce. Après un examen par les pairs des résultats de cette étude, on a conclu que les levés sismiques n'avaient entraîné aucune mortalité et n'avaient eu aucune incidence sur les embryons portés par les femelles (MPO, 2004). Cependant, on a soulevé un certain nombre de questions jugées dignes d'un examen plus approfondi. On s'est efforcé de mener d'autres expériences, de soustraire toute l'information possible de l'étude préliminaire réalisée avec les cages et de repérer les lacunes en matière de connaissances. À l'issue de cette initiative, une série de documents scientifiques ont été présentés au Centre des pêches du Golfe, à Moncton (Nouveau-Brunswick) en janvier 2007 (compte rendu dans Boudreau et coll., 2009). Voici les principales questions d'intérêt et les constatations scientifiques s'y rattachant.

1. Quels étaient les niveaux de pression acoustique ressentis en réalité par les crabes des neiges encagés dans la zone de levés sismiques comparativement aux crabes encagés dans la zone témoin située à 23 kilomètres de distance?

Une analyse approfondie des niveaux de pression acoustique efficace recueillis avant et pendant les levés sismiques de décembre 2003 a indiqué que les crabes des neiges placés en cages dans la zone de levés sismiques étaient exposés à des valeurs allant jusqu'à 178 dB re µPa (comparativement à 118 dB re µPa pour la zone témoin). Les niveaux de bruit ambiant dans la zone d'essai où étaient placées les cages atteignaient un signal sonore de largeur de bande pouvant aller jusqu'à 95 dB re µPa. Par conséquent, il est évident que les crabes des neiges encagés dans la zone de levés sismiques étaient exposés à des niveaux acoustiques supérieurs par rapport aux crabes de la zone témoin.

2. La présence de particules étrangères sur les branchies, les antennules et dans les statocystes (organes de l'équilibre) des crabes encagés dans la zone d'essai était-elle attribuable au glissement des cages au fond de l'eau plutôt qu'à l'exposition à l'énergie sismique?

On a découvert que les crabes des neiges retirés des cages situées dans la zone d'essai immédiatement après les levés sismiques de décembre 2003 présentaient de plus grandes concentrations de particules de sédiments sur leurs branchies, leurs antennules et dans leurs statocystes (organes de l'équilibre) que les crabes encagés dans la zone témoin. On a noté dans l'examen des résultats réalisé en septembre 2004 que les caractéristiques des sédiments et les conditions océanographiques étaient différentes entre les sites de déploiement des cages et que certaines cages de la zone d'essai avaient été glissées au fond de l'eau en raison de l'interaction des glaces de surface et des bouées d'amarrage. En mai 2004, des crabes des neiges femelles placées dans des sacs-filets ont été glissées au fond de la zone des levés sismiques antérieurs pendant deux heures. Tous les crabes des neiges ont survécu à ce traitement et on n'a noté aucune conséquence désastreuse, donc pas de salissure marine dans les yeux, sur les antennules, dans les statocystes ou les branchies. Bien que ces constatations n'appuient en rien l'hypothèse de la salissure marine des organes en raison du glissement des cages au fond de la zone d'essai, il n'a

pas été possible de dégager une conclusion puisque la durée de l'étude (deux heures) était beaucoup plus courte que la période de placement en cages de 12 jours lors des activités de levés sismiques de décembre 2003.

3. Les levés sismiques ont-ils modifié la répartition géographique des crabes des neiges et ont-ils réduit l'abondance dans la zone d'essai?

Un relevé au chalut de fond mené après les levés sismiques (juin 2004) n'a fourni aucune preuve de réduction de l'abondance ou de modification de la répartition géographique des crabes des neiges femelles multipares comparativement aux résultats d'un tel relevé mené avant les activités de levés sismiques (septembre 2003). Cependant, on observe une importante variation sur le plan des estimations de l'abondance, et il est indiqué que les données obtenues à l'issue des relevés au chalut de fond ne peuvent tenir lieu de résolution pour offrir une réponse définitive à cette question.

4. Les différences observées dans la structure cellulaire de certains organes chez les crabes placés dans la zone d'essai par rapport à ceux de la zone témoin sont-elles préoccupantes, et sont-elles attribuables à leur exposition à l'énergie sismique?

Les analyses histologiques réalisées par le personnel du MPO ont suggéré la contusion de l'hépatopancréas (organe dont la fonction équivaut à celle du foie) et des ovaires des crabes des neiges encagés lors de l'expérience de décembre 2003. Afin d'évaluer l'importance de ces observations et de confirmer que de telles contusions étaient plus répandues chez les crabes encagés dans la zone d'essai, des échantillons des tissus ont été examinés par un histopathologiste indépendant. Des anomalies ont été découvertes chez tous les groupes faisant l'objet de l'examen, et le groupe présent dans la zone de levés sismiques ne présentait pas un degré plus élevé d'anomalies que les groupes témoins. Une analyse des données a révélé beaucoup moins d'anomalies chez les crabes des neiges encagés dans la zone d'essai que chez les crabes de la zone témoin. Les pathologies étaient plus répandues chez les spécimens encagés depuis cinq mois comparativement à ceux encagés durant 12 jours, ce qui suggère qu'elles sont attribuables à l'encagement. Dans l'ensemble, ces analyses suggèrent que le stress associé à la manipulation (pêche et encagement) a produit les anomalies observées. L'interprétation des tissus a été gênée par l'absence d'une description détaillée de l'histopathologie du crabe des neiges. Il a été recommandé qu'une telle étude soit réalisée dans le but de fournir une description de l'histologie d'un crabe des neiges « normal » à laquelle on pourrait se référer pour les études futures.

5. L'exposition à l'énergie sismique a-t-elle provoqué la perte de pattes chez le crabe des neiges?

Il a été noté lors de la réunion d'examen par les pairs tenue en septembre 2004 que, parmi les crabes des neiges encagés en décembre 2003 puis transférés au Centre des pêches de l'Atlantique nord-ouest aux fins d'analyse (45 spécimens de chacune des zones d'essai et témoin), un total de 26 pattes ont été perdues par les crabes du groupe de la zone d'essai comparativement à seulement trois pattes chez les crabes du groupe témoin. Deux sources de données suggèrent que cette différence n'était pas le fait de l'exposition à l'énergie sismique. D'une part, les crabes des neiges des mêmes groupes d'essai envoyés au Centre des pêches du Golfe ne présentaient pas de différence sur le plan de la

perte des pattes entre les deux zones. D'autre part, les études subséquentes réalisées au Centre des pêches de l'Atlantique nord-ouest n'ont pas réussi à provoquer de perte de pattes chez les crabes des neiges exposés à des niveaux acoustiques allant jusqu'à 220 dB crête à crête, soit un niveau sonore supérieur à celui auquel ont été exposés les crabes lors des levés sismiques de 2003. Il a été suggéré qu'une manipulation brutale du groupe des crabes des neiges de la zone d'essai lors de l'envoi à Terre-Neuve-et-Labrador (puisque les spécimens de la zone d'essai et de la zone témoin ont été envoyés dans des contenants distincts) pourrait avoir été responsable du taux élevé de perte de pattes.

6. Le perfectionnement de la méthodologie risquerait-il de modifier la conclusion voulant que les embryons des crabes des neiges n'aient pas été touchés par l'exposition à l'énergie sismique?

La quantification des larves issues des femelles encagées en décembre 2003 a suggéré qu'il n'y avait aucune différence entre la zone d'essai et la zone témoin, ne révélant, par ricochet, aucune incidence de l'énergie sismique sur la survie des embryons. Toutefois, les participants à la réunion d'examen par les pairs tenue en septembre 2004 ont suggéré un certain nombre de perfectionnements de la méthodologie de cette analyse. La réanalyse a permis de confirmer les conclusions initiales : 1) l'exposition à l'énergie sismique n'a pas tué les embryons des crabes des neiges; 2) les femelles encagées dans la zone de levés sismiques présentaient une progéniture semblable à celle des crabes femelles de la zone témoin; et 3) le taux de développement des embryons a été plus lent dans la zone d'essai par rapport aux embryons de la zone témoin, ce qui pourrait être lié à l'énergie sismique ou à la température plus basse dans la zone d'essai par rapport à la zone témoin.

BACKGROUND

In September 2000, the Environmental Studies Research Fund (ESRF) convened a workshop in Halifax (N.S.) to discuss research required to better understand the potential effects of seismic exploration on the fisheries of Atlantic Canada (Thomson et al., 2001). It was noted at this workshop that most research to date has focussed on marine mammals and fish that have sensitive hearing structures and air cavities in their bodies that might be adversely affected by sudden changes in pressure. Invertebrate species such as American lobster (*Homarus americanus*) and snow crab (*Chionoecetes opilio*), which support important fisheries in Atlantic Canada, had been largely ignored. During the 1990s, concern was expressed by commercial fishers in Cape Breton that seismic surveys might impact snow crab, and there were anecdotal suggestions in Newfoundland that seismic surveys might have affected catches (Christian et al., 2003). Therefore, one recommendation from the 2000 workshop was that studies be carried out on the effects of seismic air gun noise on snow crab and snow crab catches.

LGL Limited of St. John's Newfoundland was contracted by ESRF to carry out experimental exposures of snow crab to seismic air guns in the fall of 2002. No obvious effects were observed on behaviour, health, or catch rates of adults, but the eggs of one female showed significant developmental retardation after exposure at very close range (2 m). This study concluded: "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." An opportunity for pursuing this line of research arose one year later.

In December 2003, a seismic survey was conducted by Corridor Resources Inc. (CRI) 10 km off the west coast of Cape Breton. The seismic survey was carried out in a commercial snow crab fishing area: Western Cape Breton Snow Crab Area 19. Because of the lack of knowledge on the potential impacts of such a survey on local snow crab populations, an Environmental Effects Monitoring (EEM) research program, funded by the Environmental Studies Research Funds (ESRF) of the National Energy Board (NEB), was developed by the CRI, the Department of Fisheries and Oceans (DFO) and the Area 19 Snow Crab Fishermen's Association. The research program consisted of caging egg-bearing female snow crab on the bottom of the ensonified area. Egg-bearing females were also caged on the bottom, 27 km to the northeast of the seismic survey area, as a control group. Following the survey, crab were brought to the Moncton (N.B.) and St. John's (N.L.) DFO offices, and to the St. Francis Xavier University, Antigonish (N.S.), for analysis. The main objectives of the scientific study were to assess the effects of seismic exploration on the survival of adult female snow crab; survival, morphological development, and locomotion of snow crab larvae from exposed females; and to conduct a histopathological analysis of exposed females.

On September 29, 2004, a meeting was held to review the results of the scientific study (DFO, 2004). Results discussed at the meeting included the following major observations:

1) The seismic survey did not cause any acute or mid-term mortality of the crab, nor was there any evidence of changes to feeding in the laboratory;
2) Survival of embryos being carried by females crabs, and locomotion of the resulting larvae after hatch, were unaffected by the seismic survey; and
3) In the short-term, gills, antennules and statocysts (balance organs) were soiled in the test group, but were found to be completely cleaned of sediments when sampled five months later.

Supplemental observations discussed at the meeting included:

4) The hepatopancreas was found to be bruised in the test site;
5) Ovaries from animals at the test site were found to be bruised and had dilated oocytes with detached chorions;
6) In one test group, embryo hatch was delayed five days on average and resulting larvae were slightly smaller than controls; and
7) Orientation, as measured by the time an overturned crab needs to right itself, was different between test and control groups.

Further studies were recommended to help verify and resolve some of the major and supplemental observations discussed at the September 29 meeting.

On January 23, 2007, a meeting was held at the Gulf Fisheries Centre, Moncton (N.B.), to review the results of these supplementary studies. The supplementary studies included the following issues raised at the September 29 meeting:

1) Verification by an independent histopathologist of the interpretations made in September 2004 of the gonad and hepatopancreas tissue from snow crab caged within and outside the seismic survey area;
2) Investigation of the effect of dragging of cages on gill fouling;
3) Evaluation of current stock assessment data for evidence of reduced snow crab abundance within the December 2003 seismic survey area;
4) Investigation of the origin of histopathological abnormalities observed in the hepatopancreas and ovary of caged crab through an additional caging experiment;
5) Investigation of the hypothesis that exposure to seismic energy resulted in leg loss in caged crab;
6) Refinements of the survival estimates for embryos carried by crabs caged in the original experiment; and
7) Estimation of the sound pressure levels encountered by crabs in the December 2003 seismic survey and reference areas.

REPORT ORGANIZATION

Potential Impacts of Seismic Energy on Snow Crab is a collection of working papers presented at the meeting of January 23, 2007.

Chapter 1 discusses the acoustic measurements of the seismic survey (meeting of January 23, 2007, item 7). Chapter 2 discusses the investigation of the effect of the dragging of cages on gill fouling and the evaluation of current stock assessment data for evidence of reduced snow crab abundance within the December 2003 seismic survey area (meeting of January 23, 2007, items 2 and 3). Chapter 3 discusses the results of the verification by an independent histopathologist of the interpretations made in September 2004 of the gonad and hepatopancreas tissue from snow crab caged within and outside the seismic survey area (meeting of January 23, 2007, item 1). Chapter 4 addresses the origin of histopathological abnormalities observed in the hepatopancreas and ovary of caged crab through an additional caging experiment (meeting of January 23, 2007, item 4); the hypothesis that exposure to seismic energy resulted in leg loss in caged crab (meeting of January 23, 2007, item 5); and the refinements of the survival estimates for embryos carried by crabs caged in the original experiment (meeting of January 23, 2007, item 6). Finally, chapter 5 addresses three recommendations made at the meeting of January 23, 2007, including 1) examination of the replicability of histological readings made by Dr. L. Lee, 2) statistical analysis of histological data collected by Dr. L. Lee, and 3) estimation of sound pressure levels at the sites of the experimental cages. Chapter 5 also includes supplemental comparisons for the December 2004 experiment.

METHODOLOGY, APPROACH AND STUDY AREA

The December 2003 caging experiment was put together on short notice and with the resources available. It was recognized at the outset that its design would not address all questions that might arise, but it was seen as important to take advantage of an actual commercial seismic survey to address the most critical issues raised. Best available evidence indicated that the seismic survey would not result in any mortality or morbidity of snow crab in the area. The caging experiment was designed to confirm this prediction. Previous ESRF research on the effects of seismic energy on snow crab had recommended that future work should focus on reproductive stages and eggs. For this reason, the present study caged only female snow crab that were carrying eggs. Researchers experienced in working with marine crustaceans were engaged at the Gulf Fisheries Centre in Moncton, N.B. (Dr. Mikio Moriyasu), Saint Francis Xavier University in Antigonish, N.S. (Dr. Edwin Demont), and the Northwest Atlantic Fisheries Centre in St. John's, N.L. (Dr. Jerry Payne). Snow crab were captured in the area of the seismic survey, and some were caged in that area while others were caged in a reference area 41 km to the northeast (see Figure 1.2). Some crabs from each location were retrieved immediately after the seismic survey (short term; 12 days of caging time), and, to look for potential longer term effects, other crabs were retrieved after 5 months of caging time. Snow crab fishers worked with scientific staff on their commercial fishing vessels to obtain, cage, and retrieve the crab.

Multiparous female snow crab were installed on December 10-11, 2003, in 3 cm steel mesh cages fashioned from 122 cm x 91 cm x 41 cm lobster traps without doors. Crabs were caged at two sites about 18 km offshore: one at the intersection of a north-south and an east-west transect to be shot with seismic (lat. 46°33'N, long. 61°18'W; 70 m depth); and the other a reference site about 27 km to the northeast of the seismic survey area (lat. 46°48'N, long. 61°04'W; 108 m depth). At each site, 500 crabs were divided equally among 20 cages anchored on the bottom (see Moriyasu et al., 2004, for details). The seismic survey began on December 19, passed over the caged crabs at the seismic site on December 19 (NS transect) and December 20 (EW transect), and was completed on December 26.

CHAPTER 1. ACOUSTIC MEASUREMENTS

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1.1 Introduction

In December 2003, Geophysical Service Incorporated (GSI), on behalf of Corridor Resources Inc., conducted a 2-D marine seismic program in the waters off western Cape Breton (N.S.). The survey vessel, M/V GSI Admiral, employed a 1310 in.^{3} airgun array at a depth of 6 m, as the seismic source. A total of 506 line kilometres of data were obtained within an area that covered approximately 750 km^2.

The survey took place in a Corridor Resource Inc. licensed block within the Cheticamp natural gas prospect located approximately 20 km off the west coast of Cape Breton, in the waters of the southern Gulf of St. Lawrence. An abundant snow crab population in this region supports a lucrative local snow crab fishery. Furthermore, the southern Gulf of St. Lawrence is an important spawning and nursery region for snow crab. Therefore, consideration was taken to monitor any effects the seismic program might have had on the snow crab and other marine species in the region. The Department of Fisheries and Oceans (DFO) coordinated an observation study whereby the survival and health of caged snow crabs, placed at locations both inside and outside of the ensonified areas, were examined. In conjunction with the DFO crab observation study, an acoustic monitoring program was implemented to measure the sound levels that were generated by the airgun array. JASCO Research Ltd. was contracted to acquire acoustic measurements during the seismic survey. Data were acquired between December 20-23, 2003, in the near-field using ship deployed hydrophones. Additionally, ocean bottom seismometers (OBS) equipped with hydrophones were deployed from December 13-23, 2003, at both of the caged crab sites to measure sound levels experienced by the snow crabs. Two OBSs were placed inside the ensonified area and one about 20 NM north of this site outside the major extent of the ensonification.
The main objectives for JASCO's acoustic monitoring program were to:

- Establish the maximum ranges to which 180 dB and 190dB (RMS) sound levels were received by taking near-field measurements of the airgun events.
- Measure the sound pressure levels propagating horizontally away from the airgun arrays.
- Determine the sound levels measured at the caged snow crab holding locations.

1.2 Materials and methods

1.2.1 Seismic Survey Overview

Marine seismic airgun surveys are capable of producing high-resolution three-dimensional images of stratification within the Earth's crust, down to several kilometres depth, and have therefore become an essential tool for geophysicists studying the Earth's structure. Seismic airgun surveys may be a 2-D cross-section with a kilometre or more between survey lines or 3-D with denser line spacing in the order of a few hundred meters.

A typical airgun survey is operated from a single survey ship that tows both the seismic source and receiver apparatus. The seismic source is an airgun array consisting of many individual airguns that are fired simultaneously in order to project a high-amplitude seismo-acoustic pulse into the ocean bottom. The receiver equipment often consists of one or more streamers, often several kilometres in length, that contain hundreds of sensitive hydrophones for detecting echoes of the seismic pulse reflected from sub-bottom features. In other cases, the receiving equipment consists of seismometers placed on the ocean bottom.

The majority of the underwater sound field generated by a seismic survey is due to the airgun array and not the survey vessel itself. Airgun arrays are broadband acoustic sources that project energy from under 10 Hz to over 5 kHz. Most of the energy is concentrated below 200 Hz, which is useful for penetration into the sediment below the seabed. Generally, the frequency output of the array is inversely dependent on its volume. Conventional airguns are available with a wide range of chamber volumes, from under 5 $in.^3$ to over 2000 $in.^3$, and are used for many different applications from shallow-hazard surveys to deep crustal studies. The array consists of many airguns that are configured in such a way as to project the maximum amount of seismic energy vertically into the seafloor. However, a significant portion of the sound energy from the array is emitted at off-vertical angles and propagates into the surrounding environment. The frequency spectrum of the sound propagating near-horizontally can differ markedly from that of the sound directed downward. There can also be substantial differences in the amount and frequency spectrum of sound projected in different horizontal directions.

1.2.1.1 Airgun Operating Principals

An airgun is a pneumatic sound source that creates acoustic impulses by generating bubbles of compressed air in water. The rapid release of highly compressed air (typically at pressures of ~ 2000 psi) from the airgun chamber creates an oscillating air bubble in the water. The expansion and oscillation of this air bubble generates a strongly-peaked, high-amplitude acoustic impulse that is useful for seismic profiling below the seabed. The main features of the pressure signal generated by an airgun, as shown in Figure 1.1, are the strong initial peak and the subsequent bubble pulses. The amplitude of the initial peak depends primarily on the firing pressure and chamber volume of the airgun, whereas the period and amplitude of the bubble pulse depends on the volume and firing depth of

the airgun.

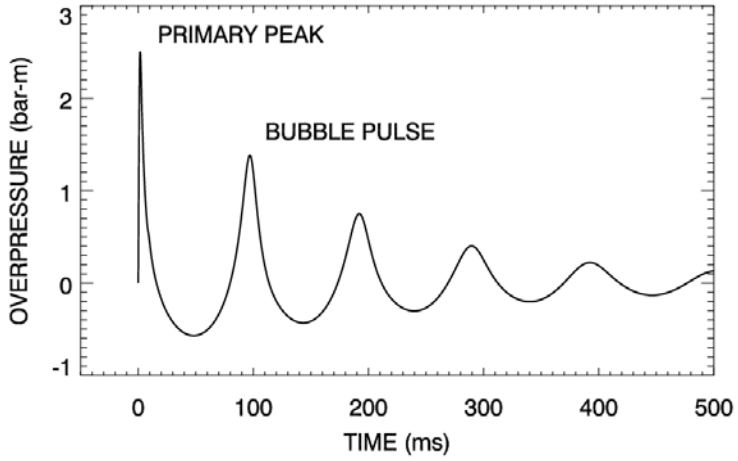

Figure 1.1: Overpressure signature for a single airgun, showing the primary peak and the bubble pulse.

Zero-to-peak source levels for individual airguns are typically between 220 and 235 dB re 1μPa @ 1m (0-p) (~ 1–6 bar · m)1, with larger airguns generating higher peak pressures than smaller ones. The peak pressure of an airgun, however, only increases with the cubic root of the chamber volume. Furthermore, the amplitude of the bubble pulse also increases with the volume of the airgun — and, for the geophysicist, the bubble pulse is an undesirable feature of the airgun signal since it smears out sub-bottom reflections. In order to increase the pulse amplitude (to "see" deeper into the Earth), geophysicists generally combine multiple airguns together into arrays. Airgun arrays provide several advantages over single airguns for deep geophysical surveying:

- The peak pressure of an airgun array in the vertical direction increases nearly linearly with the number of airguns;
- The geometric lay-out of airgun arrays can be optimized to project maximum peak levels toward the seabed (i.e., directly downward), whereas single airguns produce nearly omnidirectional sound; and
- By utilizing airguns of several different volumes, airgun arrays can be "tuned" to increase the amplitude of the primary peak and simultaneously decrease the relative amplitude of the bubble pulses.

1.2.1.2 Airgun Array Source Levels

The far-field pressure generated by a seismic airgun array is substantially greater than that of an individual airgun, but is also strongly angle dependent relative to the array axis. An array of 30 guns, for example, may have a zero-to-peak source level of 255 dB re 1μPa @ 1m (~56 bar · m) in the vertical direction. This apparently high value for the

[1] Source level in dB re 1 μPa @ 1 m = 20 log (pressure in bar · m) + 220

source level can lead to erroneous conclusions about the impact on marine mammals and fish for the following reasons:

Peak source levels for seismic survey sources are usually quoted relative to the vertical direction. However, due to the directional dependence of the radiated sound field, source levels off to the sides of the array are generally lower.

Far field source levels do not apply in the near field of the array where the individual airguns do not add coherently; sound levels in the near field are, in fact, lower than would be expected from far field estimates.

1.2.1.3 Survey Description

The seismic survey was carried out in the Corridor Resources Inc. licensed block EL2368, in the Southern Gulf of St. Lawrence off the west coast of Cape Breton (N.S.). The survey took place between December 3 and 25, 2003, covering 27 seismic lines. Figure 1.2 shows the survey region (outlined in green), the individual seismic tracks (red lines) and the caged snow crab locations (red stars).

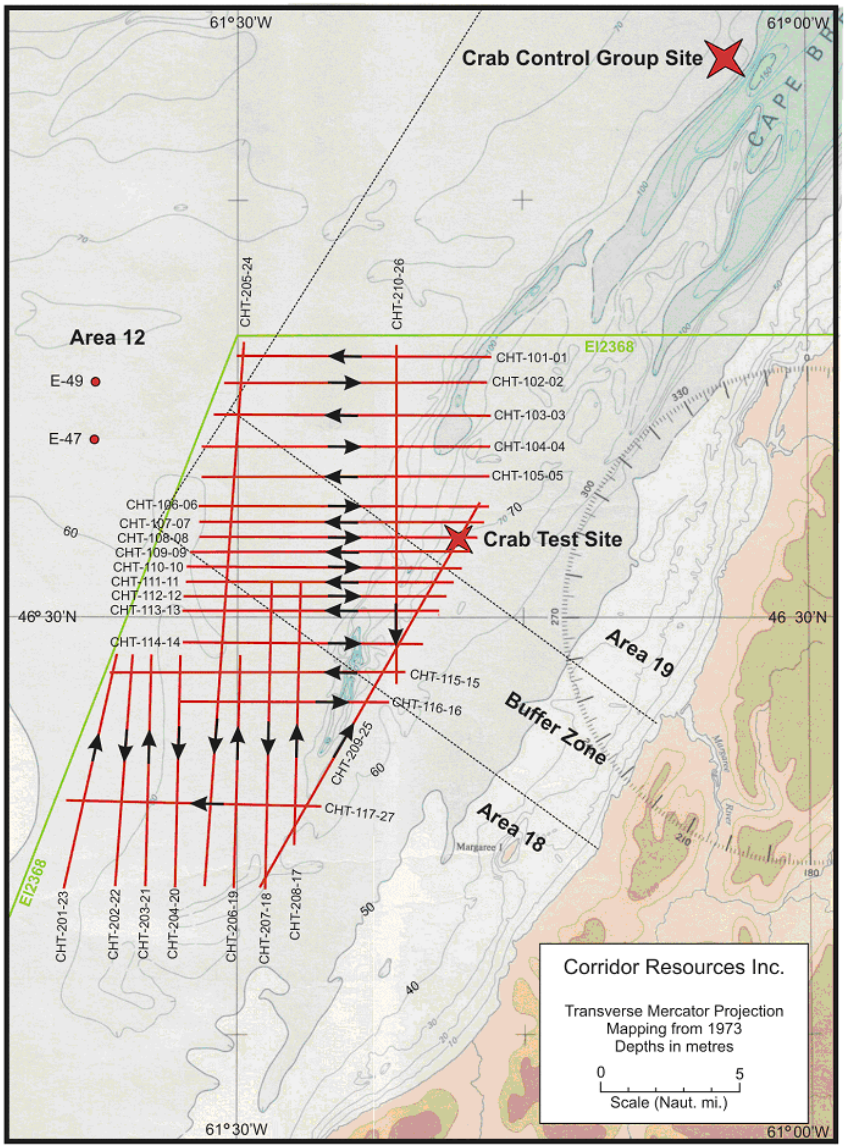

Figure 1.2: Map of the study area indicating the seismic block boundaries, the individual seismic tracks and the crab cage locations.

The survey took place in the waters off the coast of Cape Breton to the west of Margaree Harbour. CTD samples were collected by JASCO Research Ltd. on December 23, 2003, at locations to the east of seismic line CHT-113-13, from which a representative sound velocity profile was determined for the survey region and is presented in Figure 1.3. The upward refracting sound speed profile that is presented was taken from a cast at location lat. 46°33.603'N, long. 61°17.773'W.

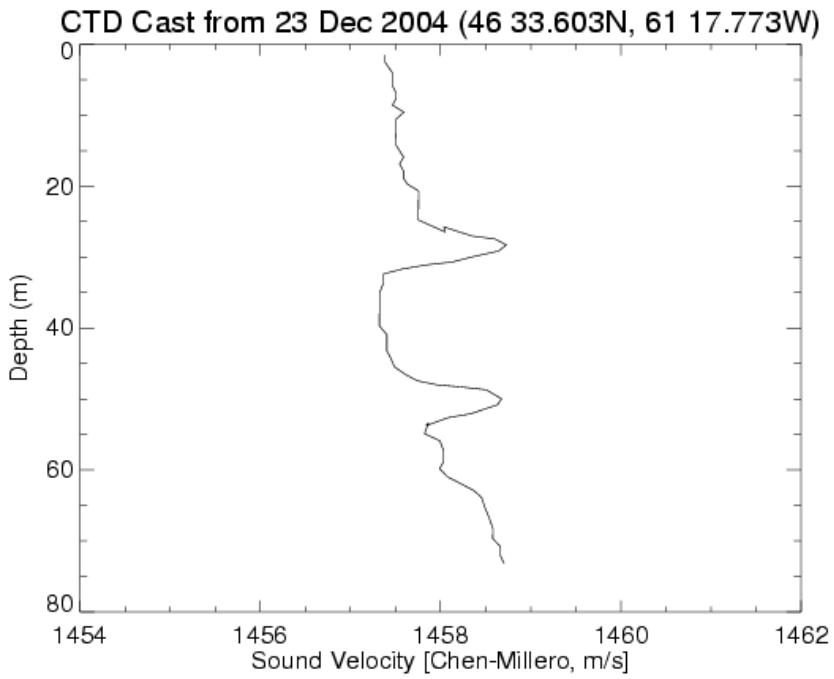

Figure 1.3: Sound speed profile from CTD cast performed on December 23, 2003, at location lat. 46°33.603'N, long. 61°17.773'W.

1.2.1.4 Airgun Array Layout

The airgun array, deployed at a depth of 6 m ±1 m, consisted of 10 guns (two single guns and four two-gun clusters) in a horizontal line array as shown in Figure 1.4. The total array volume was 1310 in.^{3}. The array operated with a firing pressure of 2000 psi with a nominal firing period of 10 seconds.

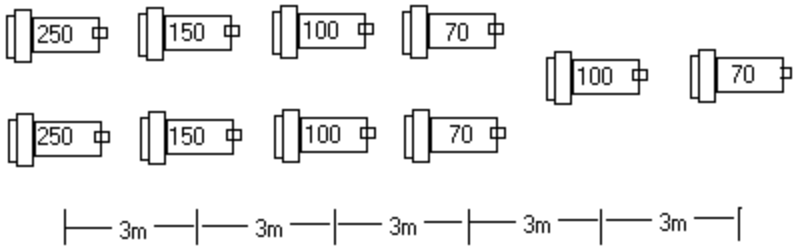

Figure 1.4: Airgun array layout.

1.2.2 Underwater Sound Metrics

The sound field is described in terms of a time-varying pressure *p(t)*. Several metrics are based on the root-mean-square, or "RMS", amplitude of the pressure p_{RMS}. For impulsive sounds, the RMS pressure depends strongly on the choice of the time window *T* over

which the RMS level is computed. A commonly used method to define the time window is based on the time over which 90% of the signal energy is received.

$$p_{\mathrm{RMS}} = \sqrt{\frac{1}{T}\int_T p(t)^2\, dt} \qquad (1)$$

The intensity of underwater sound is the flux of acoustic energy passing through a unit area per unit time. For plane waves it is computed by dividing the square instantaneous pressure by the acoustic impedance of the medium ρc, where ρ is the density of the medium and c is its sound speed.

$$I = \frac{p^2}{\rho c} \qquad [\mathrm{W/m^2}] \qquad (2)$$

The energy flux density of a signal is defined as the time-integral, taken over the duration of the pulse, of the instantaneous sound intensity.

$$E = \int_T I dt \qquad [\mathrm{J/m^2}] \qquad (3)$$

Since measured sound intensities typically vary over many orders of magnitude, sound levels are usually expressed in logarithmic decibel (dB) units. The decibel scale is a relative scale that indicates the sound level in relation to a pre-defined reference level.

$$\mathrm{SPL} = 10\log\left(\frac{I}{I_{ref}}\right) = 20\log\left(\frac{p}{p_{ref}}\right) \quad [\mathrm{dB}] \quad (4)$$

The reference most commonly used in underwater acoustics is $p_{ref} = 1$ μPa. The common reference intensity is 10^{-12} W/m^2.

In similar fashion, energy flux density is often expressed in decibels. The common reference energy flux density is defined as that produced by a plane wave of RMS amplitude 1 μPa acting over 1 second. Energy flux densities given in decibels are therefore commonly expressed in units referenced to μPa^2 s. This reference is equivalent to approximately 6.7 x 10^{-19} J/m^2.

Two commonly used metrics used for evaluating impacts on marine animals are the RMS sound pressure level, $\mathrm{SPL_{RMS}}$, and the sound exposure level, SEL. The RMS sound pressure level is a measure of the average sound level per second over the period of the pulse and is simply given by the decibel level of the RMS-pressure

$$\mathrm{SPL_{RMS}} = 20\log(p_{\mathrm{rms}}) \quad [\mathrm{dB\ re\ 1\mu Pa\ RMS}] \quad (5)$$

The sound exposure level, on the other hand, is a measure of the dosage of sound received over a time-period T. For impulsive sources, such as airguns, T should be the time period over which p_{RMS} was computed (see Equation 1). The SEL in this case is given by:

$$\mathrm{SEL} = \mathrm{SPL_{RMS}} + 10\log(T) \quad [\mathrm{dB\ re\ 1uPa^2\ s}] \quad (6)$$

The SEL value is numerically equal to energy flux density that is expressed in dB re μPa^{2}s.

Sometimes it is not sufficient to simply consider the broadband amplitude of the sound energy. The frequency distribution of the acoustic energy must also be taken into consideration because marine mammals are generally more sensitive to certain frequencies than others. For example, seismic noise at very low frequencies may be outside the audible frequency range of certain whales.

Band-limited levels are used for certain types of impact criterion, such as temporary

threshold shift (TTS), which is based on the maximum energy flux density in any 1/3-octave frequency band. The lower and upper bounds of the canonical 1/3-octave bands are given by the formulae

$$f_{\mathrm{l}} = 2^{-1/6} \times 10^{i/10}$$
$$f_{\mathrm{u}} = 2^{+1/6} \times 10^{i/10} \quad \textit{[Hz]} \quad \textit{(7, 8),}$$

where i is the band number. The corresponding 1/3-octave band center frequencies are $f_c = 10^{i/10}$ Hz.

1.2.3 Shallow Water Transmission Loss

Transmission loss in shallow water environments is strongly dependent upon interactions of the acoustic energy with the ocean's surface and bottom, and upon the properties of the water, particularly the water temperature and salinity. Marsh and Schulkin (1962) developed a set of commonly accepted, semi-empirical equations for computing transmission loss in defined shallow water environments. These equations take into account the source-receiver separation, the water and layer depths, the absorption properties of the water, the frequency, sea state, and bottom type, as well as attenuation and near field effects.

At short ranges Marsh and Schulkin define transmission loss with the following equation:

$$TL = 20\log r + \alpha r + 60 - k_L \qquad \mathrm{r} < \mathrm{H}.$$

Where α is an absorption coefficient, k_L is a "near-field" anomaly (that depends upon frequency, sea state and bottom type) and H is a function of the water depth (d) and layer depth (l) as defined as below:

$$H = \sqrt{\frac{1}{8}(d + l)}$$

At intermediate ranges, the equation is more accurately defined as:

$$TL = 15\log r + \alpha r + a_T\left(\frac{r}{H} - 1\right) + 5\log H + 60 - k_L \qquad \mathrm{H} \le \mathrm{r} \le 8\mathrm{H}$$

Where a_T is an attenuation factor, that depends upon frequency, sea state and bottom type.

Finally, at long ranges the equation is defined as:

$$TL = 10\log r + \alpha r + a_T\left(\frac{r}{H} - 1\right) + 10\log H + 64.5 - k_L \quad \mathrm{r} > 8\mathrm{H}$$

1.2.4 Recording Methodology

1.2.4.1 Recording Procedures

Near-field acoustic recordings were conducted out from a 42-ft. fishing vessel between December 20 and 23, 2003. During recordings the vessel lay at anchor with the engines shut down at locations adjacent to the Admiral's survey lines. Navigation and GPS data were used to compute ranges to the survey vessel as a function of time. Admiral's crew provided the survey vessel's navigation tracks and water depths. The total amount of

data collected was 4 h 44 min during five different seismic survey lines.

The ocean bottom seismometer (OBS) systems were deployed from a chartered Area 19 crab fishing 45-ft. dragger by GeoForce technical staff. One of the instruments (OBS-A) at the crab test site was at a depth of about 63 m at lat. 46°32.68'N, long. 61°17.57'W. The other device (OBS-P) was 716 m west-northwest of OBS-A at about 70 m depth at lat. 46°32.88'N, long. 61°18.05'W. OBS-T at the crab control site was at a depth of about 85 m at lat. 46°47.54'N, long. 61°04.38'W. A total of ten days (242 h 45 min) of acoustic data on each of the OBSs were obtained between December 13 and 23, 2003.

1.2.4.2 Recording Equipment

The system used for the ship based acoustic recordings portion of the survey consisted of the following components:

- One Reson TC4043 calibrated hydrophone, with nominal sensitivity -201 dB re V/µPa ±1 dB.
- One Reson TC4034 calibrated hydrophone, with nominal sensitivity -218 dB re V/µPa ±1 dB.
- Two 50-ohm, shielded hydrophone cable, 100 m lengths.
- Ithaco 451M programmable gain amplifiers (-10 dB to +80 dB in 1 dB steps) with built-in programmable high-pass filters (1 Hz to 1 kHz in decade steps).
- Marantz PMD690 digital audio recorders, sampling at 48 kHz on two channels with 16-bit resolution.

The hydrophones are individually calibrated prior to shipment, and the manufacturer provides spectral plots of each hydrophone's sensitivity in the frequency range 1 Hz to 100 kHz. They also provide directional calibration information, though directivity was negligible for the low frequencies considered in this work. TC4043 has nominal sensitivity –201 dB re V/µPa and manufacturer's specified uncertainty of ±3 dB over the bandwidth 2 Hz to 80 kHz. The uncertainty is ±1 dB in the 2 Hz to 20 kHz frequency range and the sensitivity there is extremely constant: ±0.5 dB over this band according to the calibration curves provided with the hydrophones. The TC4034 has nominal sensitivity –218 dB re V/µPa and manufacturer's specified uncertainty of ±2 dB over the bandwidth 15 Hz to 40 kHz, and ±2.5 dB over the bandwidth 15 Hz to 80 kHz. Both models of hydrophone have internal preamplifiers that are specified to drive up to 300 m of 50 ohm cable with less than 0.5 dB loss.

The three OBS systems used for acoustic measurements at the caged crab sites each contained an OAS E-4SD model hydrophone with a sensitivity of -187 dB re V/µPa ±1 dB. The hydrophones were omnidirectional and had a flat frequency response to within ±1 dB from 0 to 5 kHz. The standard setup had a preamplifier resulting in a total sensitivity of -45 dB re V/µPa ±1 dB. This type of system was deployed at the non-ensonified crab control site. The two ensonified crab test site OBSs were modified to a reduced sensitivity of -107.5 dB re V/µPa ±1 dB to avoid overloading of the systems.

1.2.5 Data Analysis Procedures

1.2.5.1 Pressure Waveform Data Analysis

Digital signal analyses of the airgun recordings were performed to obtain peak, RMS and energy flux density sound levels for all identified airgun pulses. The steps involved for processing data from each recording track were as follows:

- Airgun pulses were located in digital recordings by an automated peak-level detection algorithm.
- Signal amplitudes were translated to microPascals (μPa) by applying hydrophone sensitivity, preamplifier and amplifier gains and digital conversion gain.
- Maximum zero-to-peak sound pressure levels were calculated for each airgun pulse in dB re 1 μPa by reading the maximum pressure value.
- Cumulative energy flux density functions were computed by integrating square pressure through the pulse arrivals.
- 5% and 95% airgun energy density levels were extracted from the cumulative energy density function.
- RMS levels were computed by dividing the airgun energy flux density, received between the 5% and 95% times, by the corresponding time difference and taking the square root.

1.2.5.2 Spectral Level Data Analysis

Spectral levels for selected airgun pulses were computed in 1/3-octave bands. The steps involved for processing data from these recording were as follows:

- Pressure versus time waveforms were transformed to the frequency domain via Fourier Transform.
- 1/3-octave band energy flux density levels were calculated by numerically integrating the squared Fourier coefficients in the consecutive 1/3-octave bands with center frequencies between 10 Hz and 1000 Hz. The levels were converted to dB re 1 μPa2. Note that the presented band levels are not spectral levels (i.e. they are total band levels and are not referenced to 1 Hz).

1.2.5.3 Ambient Noise Data Analysis

While the OBHs were deployed and recording data, there were three periods totalling 83 hours during which the airguns were not firing. Data collected at the control site during these times were used to estimate background ambient noise levels in the area. The results are reported as broadband sound pressure levels in frequency bands from 10 Hz – 1 kHz, 10 Hz – 100 Hz, and 100 Hz – 1 kHz, as spectral levels over time and frequency, and as averaged 1/3-octave band spectral levels.

1.3 Results

1.3.1 Close Range Airgun Measurements

1.3.1.1 Measurements from Ship Deployed Hydrophones

Measurements at close ranges to the array using ship-deployed hydrophones were acquired on December 20, 2003 while the Admiral was acquiring data on Lines CHT-107-07 and CHT-109-09. The engine on the recording vessel was shut down for these recordings to minimize noise, and measurements were made as close to the seismic vessel as was safely and logistically possible without impeding the progress of the seismic survey. The recorded data was processed to determine the peak, RMS, and sound exposure levels of each airgun shot. Ranges as a function of time were determined using the Admiral's navigation records and the GPS logs from the recording vessel.

The levels received at various ranges from the airgun array as the seismic vessel acquired data along line CHT-107-07 are presented in Figure 1.5. The water depth measured 75.5 m at the recording location (lat. 46°33.496'N, long. 61°17.711'W) and the hydrophone was deployed to a depth of 34 m. The received levels were seen to decrease with range, as expected. The closest point of approach (CPA) was 485 m from the airgun array, where the observed sound pressure levels were 193 dB re μPa peak, 179 dB re μPa RMS and the sound exposure level was 171 dB re μPa. The figure presents data received as the seismic vessel approached and then departed from the CPA. The recording vessel was directly in front of the Admiral's track as it approached CPA, then was positioned to the side of the Admiral's track at the CPA, and times afterward; thus the recording vessel was exposed to levels representative of both "endfire" and "broadside" events throughout the recording.

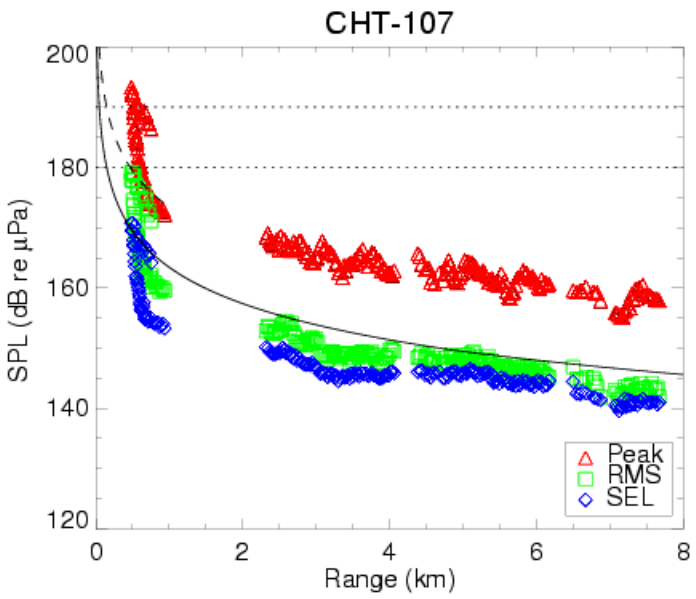

Figure 1.5: Peak, RMS, and SEL measurements as a functions of range at 34-m depth during seismic Line CHT-107-07 on Dec. 20, 2003. Solid black line indicates a fit of the Marsh and Schulkin transmission loss equations to the RMS data beyond 1 km range ("endfire" levels). Dotted black line indicates a fit of the Marsh and Schulkin transmission loss equations to the RMS data within 1 km range ("broadside" levels).

The maximum range of extent for levels of 180 dB re µPa RMS and 190 dB re µPa RMS were predicted by extrapolation along a fit to the measured RMS levels of a standard shallow water transmission loss curve based on the Marsh and Schulkin equations described in Section 4. The transmission loss curve indicated with a solid line in Figure 1.5 was fit so that most of the RMS data points lay below it, to provide a most conservative estimate of the 180 and 190 dB ranges. The curve and the measured data agree well at ranges beyond 1100 m, but at ranges less than 1100 m the measured levels exceed those predicted by the curve fit. This is assumed to be due to the array directivity; at closer ranges the levels were received near broadside of the line array (where the source level is maximum) and at longer ranges the levels were received from the array endfire. To provide an extrapolation of the "broadside" levels, a second transmission loss curve was fit to the data such that the RMS levels at ranges less than 1100 m would all lie below the curve. The expected ranges based on an extrapolation of the "broadside" levels were found to be 470 m and 134 m for the 180 dB re µPa RMS and 190 dB re µPa RMS levels, respectively. The expected ranges based on an extrapolation of the "endfire" levels were found to be 148 m and 47 m for the 180 dB re µPa RMS and 190 dB re µPa RMS levels, respectively. Also by extrapolation, the transmission loss curve fits provide an estimated broadband source level of 233 dB re µPa broadside and 223 dB re µPa endfire.

A horizontal, far-field directivity plot of received sound level as a function of angle "off-broadside" was generated from the sound level data by correcting the peak received levels for geometric spreading loss and normalizing to the broadside peak-maximum. Beam-angles were computed using logged GPS positions and the source-receiver

geometry. The resulting directivity pattern for the airgun line array is shown in Figure 1.6. Examining the figure, it is apparent that the airgun array is louder by a factor of 11-12 dB on the broadside axis of the array. This agrees with the difference between the estimated "broadside" and "endfire" levels as stated above.

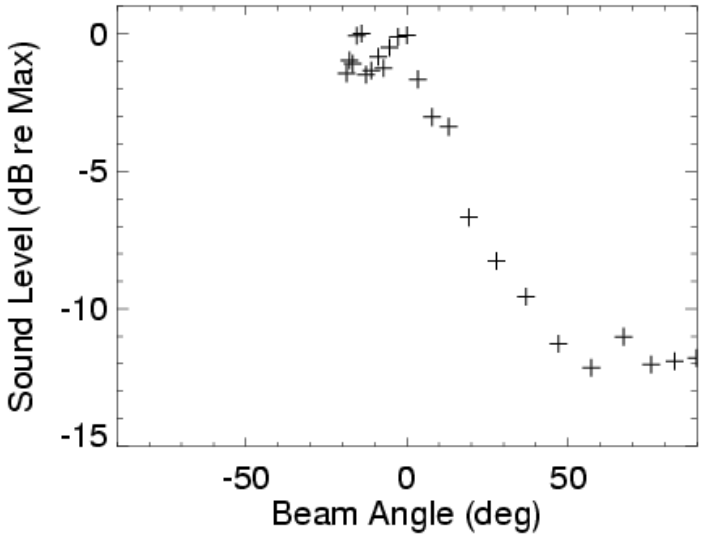

Figure 1.6: Airgun array directivity pattern. Sound levels are normalized to the maximum broadside levels (i.e., sound levels are 0 dB at the broadside peak) and corrected for spherical spreading loss.

Close range recordings were also recorded as the Admiral acquired data along line CHT-109-09. Figure 1.7 presents the levels received at various ranges from the airgun array as the seismic vessel acquired data along this line. The water depth measured 67.7 m at the recording location (lat. 46°32.058'N, long. 61°24.073'W) and the hydrophone was deployed to a depth of 34 m. The received levels were seen to decrease with range, as expected. The closest range achieved was 767 m from the airgun array where the observed sound pressure levels were 190 dB re µPa peak, 179 dB re µPa RMS and the sound exposure level was 172 dB re µPa.

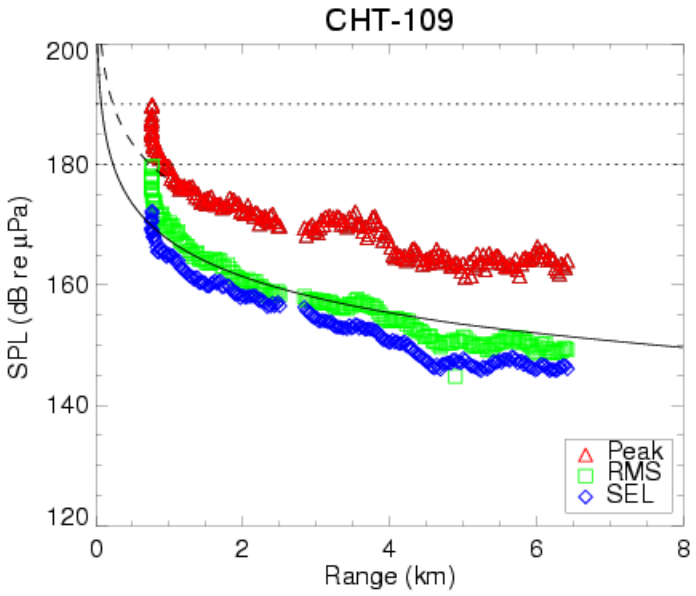

Figure 1.7: Peak, RMS, and sound exposure level measurements versus range at 34-m depth during seismic Line CHT-109-09 on Dec. 20, 2003. Solid black line indicates a fit of the transmission loss equations to the data beyond 1-km range ("endfire" levels). Dotted black line indicates a fit of the transmission loss equations to the data within 1 km range ("broadside" levels).

Again, transmission loss curves (based on the Marsh and Schulkin equations) were fit to the computed RMS "endfire" levels (solid line) and "broadside" levels (dashed line) as described above. Based on an extrapolation of the "broadside" levels of the line array, the 180 dB re µPa and 190 dB re µPa ranges were found to be 744 m and 236 m respectively. The ranges based on an extrapolation of the "endfire" levels were found to be 235 m and 75 m for the 180 dB re µPa and 190 dB re µPa levels, respectively (Table 1.1). The transmission loss curve fits provide, by extrapolation, an estimated broadband source level of 237 dB re µPa RMS broadside and 227 dB re µPa RMS endfire.

Table 1.1: Ranges to 180- and 190-dB levels based on extrapolations of measured broadside and endfire data.

	CHT-107-07		CHT-109-09	
	180 dB re µPa RMS	190 dB re µPa RMS	180 dB re µPa RMS	190 dB re µPa RMS
Broadside	470 m	134 m	744 m	236 m
Endfire	148 m	47 m	235 m	75 m

Figure 1.8 below shows the spectrograms of two individual airgun shot arrivals received with the ship deployed hydrophone at 765 m range (top) and at 1.9 km range (bottom) while the Admiral was acquiring data along line CHT-109-09. The plot for the airgun pulse received at 765-m range is representative of levels received "broadside" of the array, and the airgun pulse received at 1.9-km range is representative of levels received from the array "endfire".

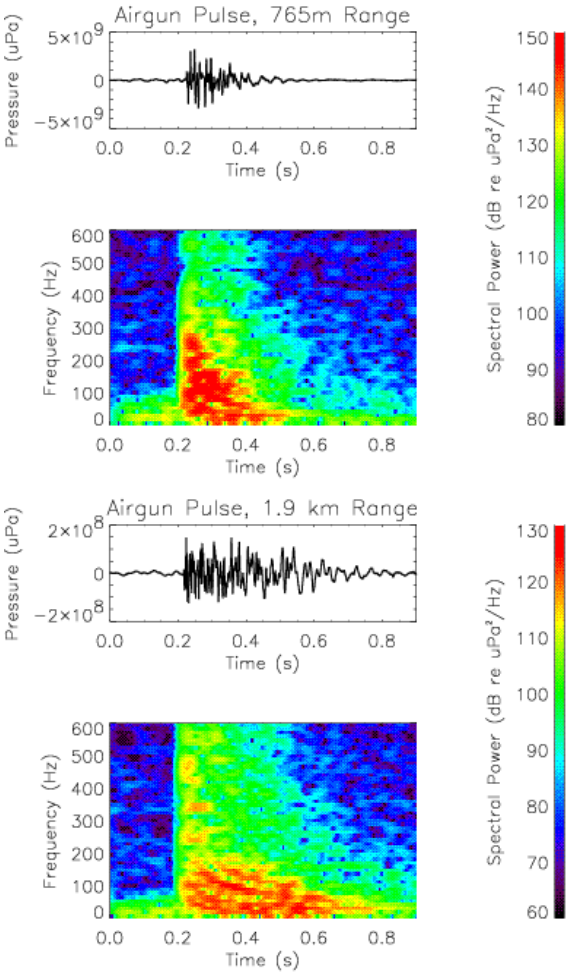

Figure 1.8: Plots of the pressure and spectral power for an individual airgun pulse measured on Dec. 20, 2003 at 1900-m range (right) and 765-m range (left). Admiral was acquiring data along line CHT-109-09 during these measurements.

1.3.1.2 Measurements from OBS Systems

Sound levels were recorded with the OBS systems located at the crab cage sites between December 15-21, 2003 while the Admiral was acquiring data on Lines CHT-101-01, CHT-209-25, CHT-108-08, CHT-107-07, CHT-109-09, CHT-105-05 and CHT-111-11. The data were processed to determine the peak, RMS, and sound exposure levels of each detected airgun shot. Ranges as a function of time were determined using the Admiral's navigation records, the drop locations of the seismometers, and the time information in the headers of the data files. The maximum sound levels recorded at each OBS for each line, and the corresponding ranges from the airgun array are given in Table 1.2.

Table 1.2: Maximum levels (peak, RMS, and SEL) measured on three ocean bottom hydrophones (OBS-A and OBS-P at the test site and OBS-T at the control site) during data acquisition on seven seismic lines.

Admiral Line	OBS	Range (km)	Peak (dB)	RMS (dB)	SEL (dB)
CHT-101-01	OBS-A	12.4	152	140	133
	OBS-P	11.9	157	143	139
	OBS-T	23.3	130	118	113
CHT-209-25	OBS-A	0.9	177	164	153
	OBS-P	0.2	191	177	166
	OBS-T	26.3	128	114	112
CHT-108-08	OBS-A	0.2	190	175	166
	OBS-P	0.1	192	178	167
	OBS-T	32.1	131	117	115
CHT-107-07	OBS-A	1.4	170	156	149
	OBS-P	1.0	176	163	154
	OBS-T	32.0	126	114	113
CHT-109-09	OBS-A	0.7	179	166	157
	OBS-P	1.1	174	161	152
	OBS-T	33.5	132	116	115
CHT-105-05	OBS-A	4.3	160	146	140
	OBS-P	3.9	165	151	146
	OBS-T	27.3	130	117	115
CHT-111-11	OBS-A	2.9	165	151	146
	OBS-P	3.2	162	150	143
	OBS-T	37.1	125	112	109

The Admiral passed through the crab test site at the closest range to both OBS-A and OBS-P while acquiring data along line CHT-108-08. Figure 1.9 presents the received levels as a function of range from the airgun array as the seismic vessel acquired data along this line at a heading of 269° on December 20, 2003. The CPA to OBS-A was 232 m where the observed sound pressure levels were 190 dB re μPa peak, 175 dB re μPa RMS and the sound exposure level was 166 dB re μPa. The CPA to OBS-P was 138 m where the observed sound pressure levels were 192 dB re μPa peak, 178 dB re μPa RMS and the sound exposure level was 167 dB re μPa.

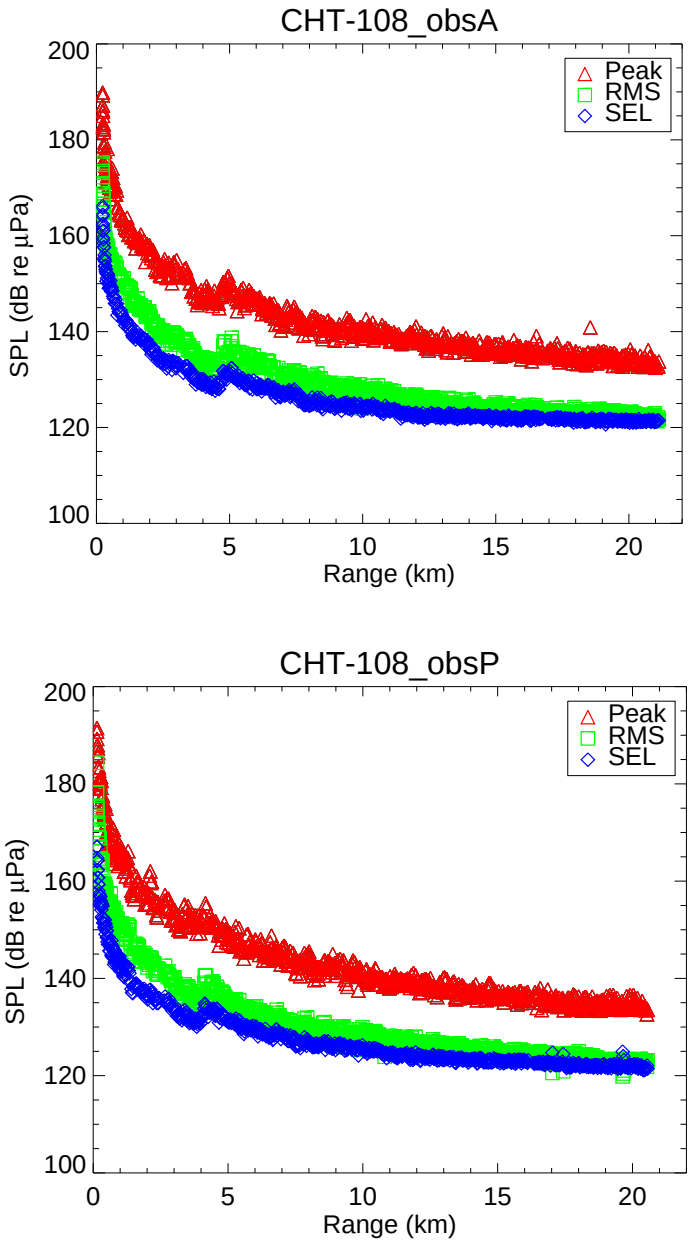

Figure 1.9: Peak, RMS, and sound exposure level measurements versus range obtained at OBS-A at 63-m depth (top panels) and OBS-P at 70- m depth (bottom panels) at the crab test site. Measurements were taken during seismic Line CHT-108-08 with a heading of 269 degrees through the test site on Dec. 20, 2003.

It is noted that the levels measured at the OBS locations are between 10 to 20 dB lower than the levels measured at corresponding ranges with the hydrophone deployed to 34 m depth. This is believed to be due to the upward refracting sound speed profile observed in the water column at this time of year. Also, the hydrophones at the OBS locations were situated about 1 m off the bottom of the ocean and, therefore, may have received lower sound levels due to increased refraction and absorption at the bottom sediment interface.

The 1/3-octave band spectral levels for an airgun event received at the CPA (232 m) to

OBS-A on Line CHT-108-08 is plotted in Figure 1.10. There is a peak of 157 dB re μPa2 in the band-spectral level at a center frequency of 200 Hz. The third-octave band-spectral level for an event received at the CPA (203 m) to OBS-P on Line CHT-209-25 is plotted in Figure 1.11. There is a peak of 162 dB re μPa2 in the band spectral level at a frequency of approximately 125 Hz.

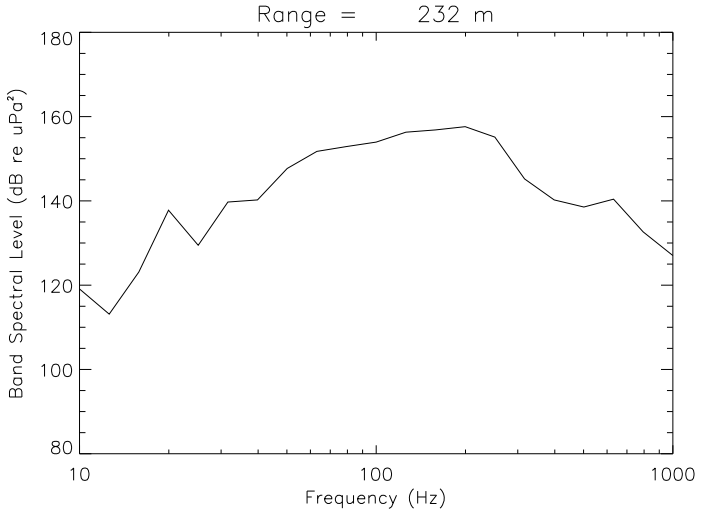

Figure 1.10: Third-octave band spectral levels as a function of frequency for an airgun event received at the CPA (232 m) to OBS-A on Dec. 20, 2003 while the Admiral acquired data along line CHT-108-08.

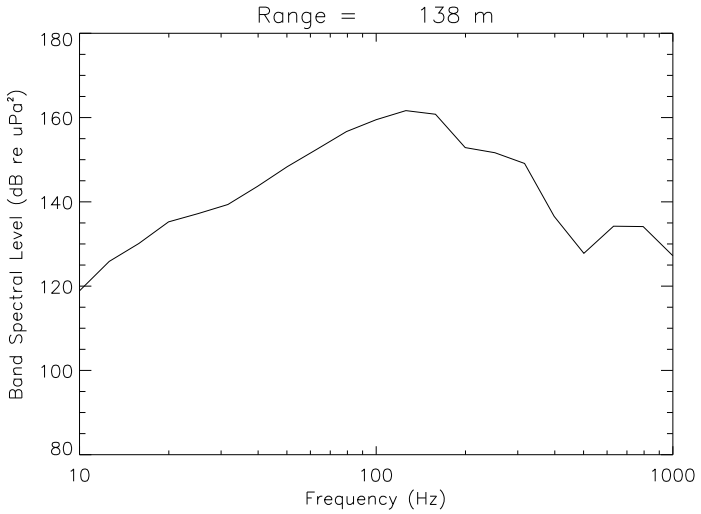

Figure 1.11: Third-octave band spectral levels as a function of frequency for an airgun event received at CPA (138 m) to OBS-P on Dec. 20, 2003, while the Admiral acquired data along line CHT-108-08.

1.3.2 Long Range Airgun Measurements

1.3.2.1 Measurements from OBS Systems

Sound pressure level measurements were obtained from the data recorded at the crab cage sites. The measurements at a range of about 23 km between the seismic vessel and the crab control site (OBS-T) while the Admiral acquired data along line CHT-101-01 on December 15, 2003, represented the highest sound levels measured at the control site, and are presented in Figure 1.12. The maximum observed sound pressure levels were 130 dB re µPa peak and 118 dB re µPa RMS and the sound exposure level was 113 dB re µPa.

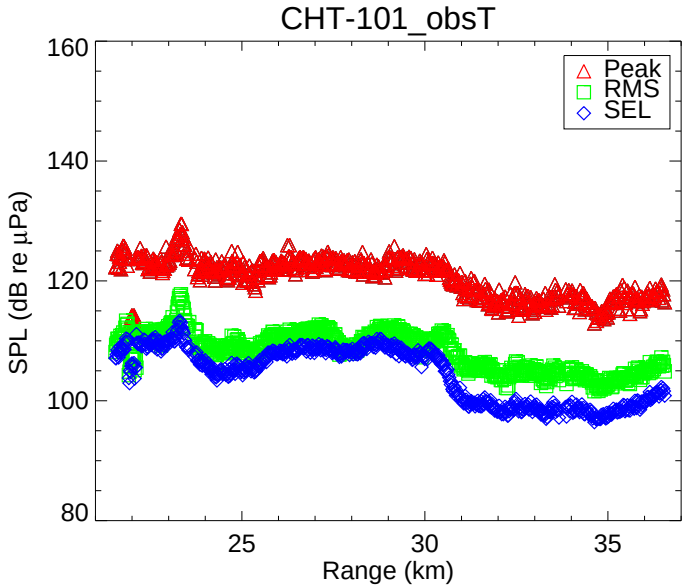

Figure 1.12: Peak, RMS, and sound exposure level measurements versus range obtained at OBS-T at 85-m depth at the control site. Measurements were taken during seismic Line CHT-101-01 with a heading of 89 degrees on the closest approach to the control site on Dec. 15, 2003.

Sound levels measured at the crab test site (OBS-A and OBS-P) while the Admiral acquired data along line CHT-101-01 on December 15, 2003, are shown in Figure 1.13. At a range of 20 km, the sound pressure levels measured at the test site were about 10 dB re µPa higher than those measured at 20-km range at the control site. This may have been due to topographical barriers or increased bottom absorption, as sound was transmitted from the airgun array north to the control site over varying topography. This may also have been due to the upward refracting sound speed profile and the fact that OBS-T was positioned 15-20 m deeper than OBS-P and OBS-A.

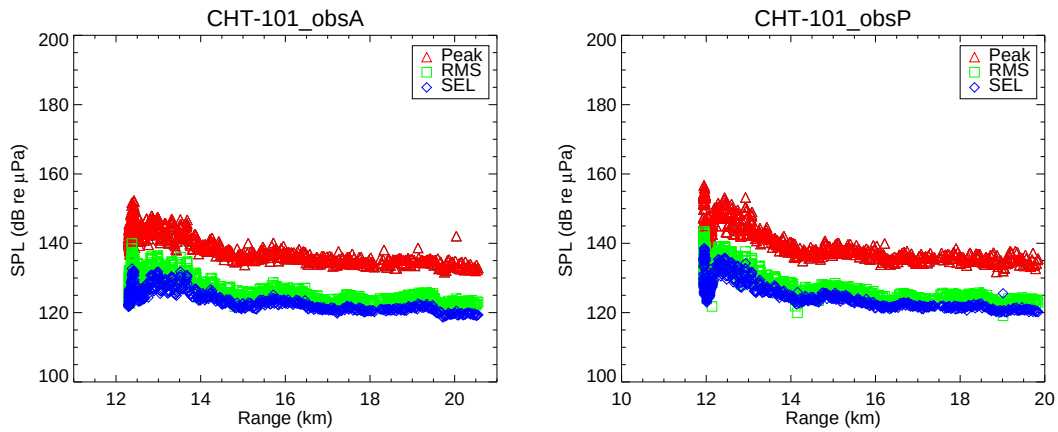

Figure 1.13: Peak, RMS, and sound exposure level measurements versus range obtained at OBS-A at 63-m depth (left) and OBS-P at 70-m depth (right). Measurements were taken during seismic Line CHT-101-01 with a heading of 89 degrees on the closest approach to the control site on Dec. 15, 2003.

1.3.3 Ambient Noise Measurements

One-minute sound pressure levels and spectral levels over frequency were calculated for the three time periods without airgun noise (Figure 1.14 to Figure 1.16). Ambient noise levels were around 75–95 dB re 1µPa in this area off Cape Breton in December 2003. The higher sound levels in Figure 1.14 resulted from what sounds like a tapping noise on or vibration of the hydrophone.

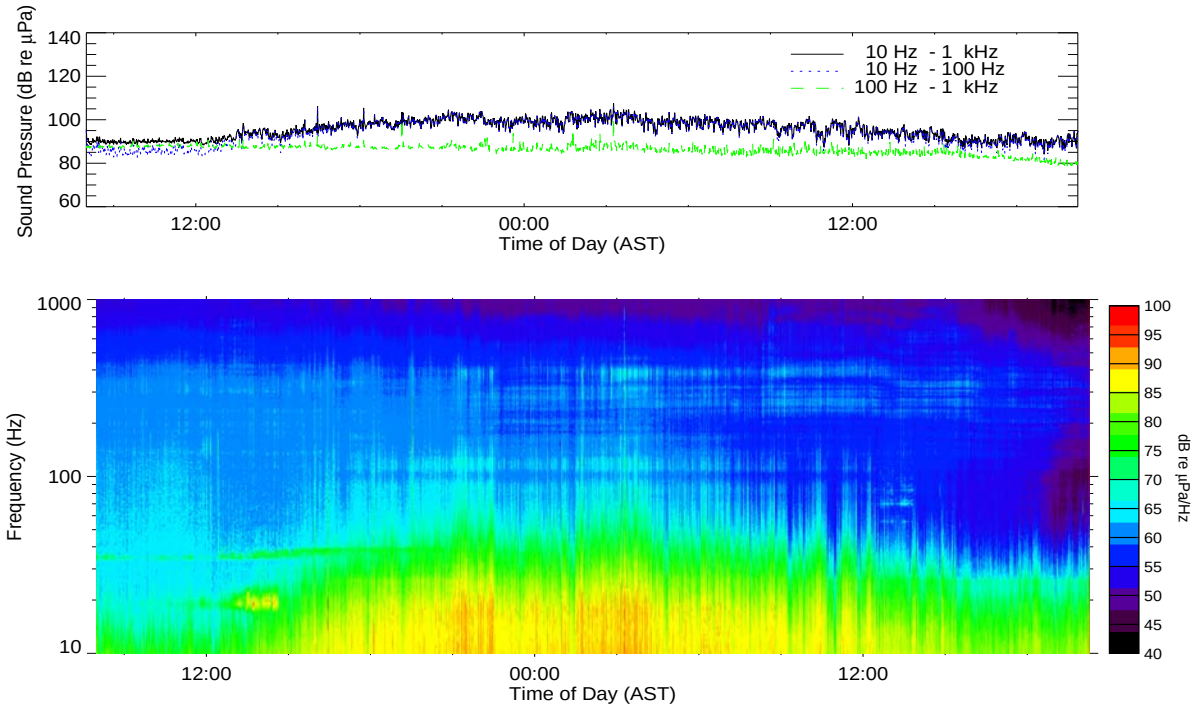

Figure 1.14: Ambient noise recording for 36 hours starting at 08:00:00 AST on December 13, 2003.

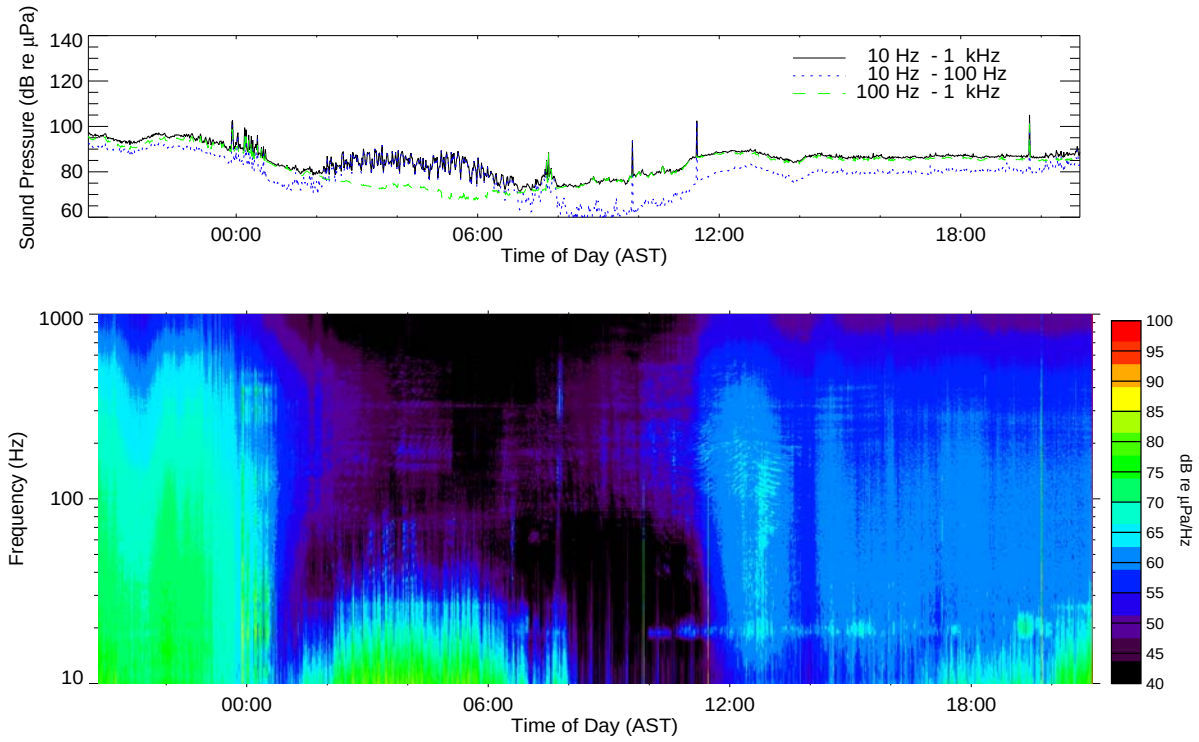

Figure 1.15: Ambient noise recording for 25 hours starting at 20:19:37 AST on December 15, 2003.

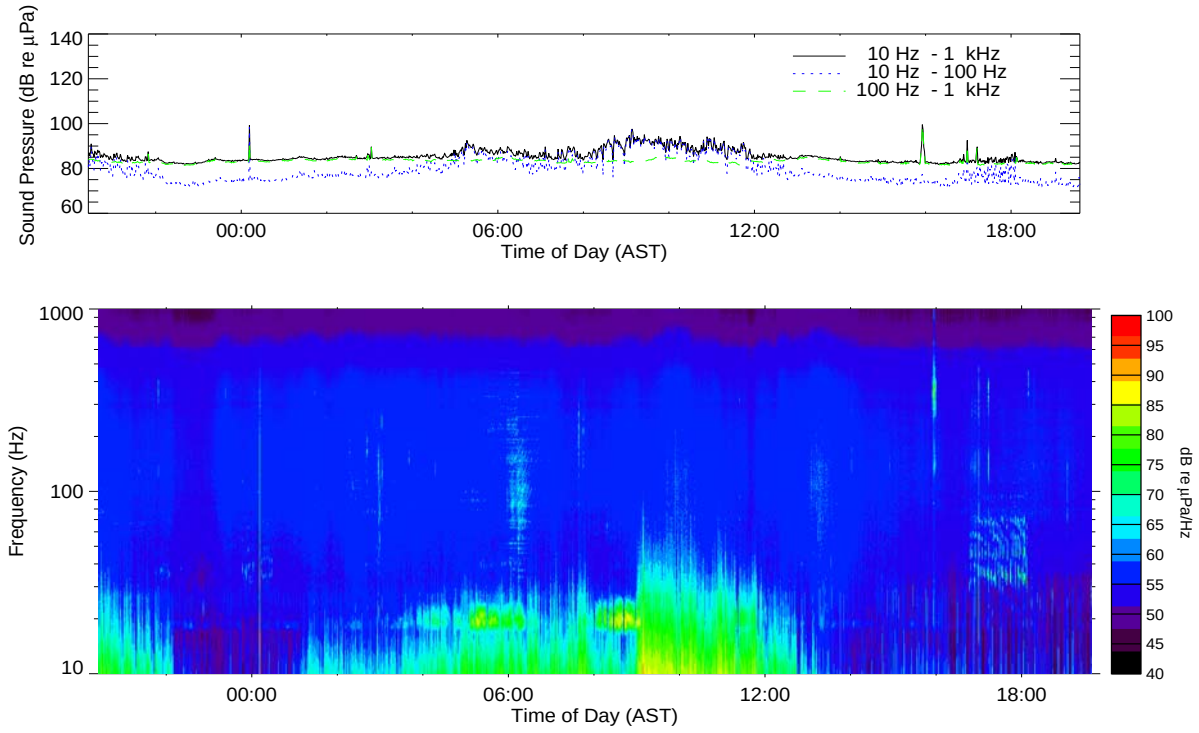

Figure 1.16: Ambient noise recording for 22 hours starting at 20:25:30 AST on December18, 2003.

The 1/3-octave band levels of ambient noise averaged for the entire 83-hour period (Figure 1.17) were the highest, up to 88.1 dB re μPa^2, below 20 Hz, and the lowest, about 74.6 dB re μPa^2, between 60 – 100 Hz. A smaller peak in sound level of 78.1 dB re μPa^2 also occurred at 400 Hz. The high levels below 20 Hz mainly occurred in the first 36 hours of recording and may have been from equipment self-noise or vibrations.

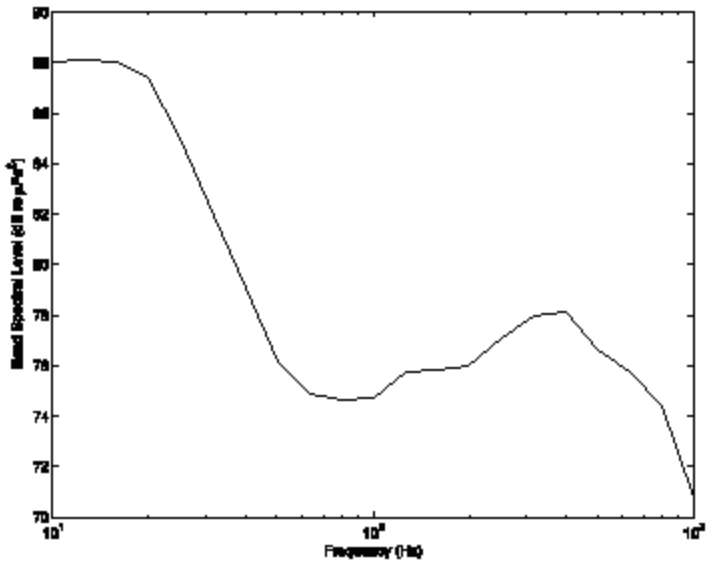

Figure 1.17: Average one-third octave band spectral levels of all 83 hours of ambient noise data collected starting from December 12–19, 2003.

1.4 Summary

Corridor Resources Ltd. implemented a field monitoring program as a means to document underwater sound production by their 2003 2-D seismic program off western Cape Breton (N.S.), to determine the resulting ensonification of the marine environment over a large area, and to monitor the exposure of caged snow crab for later analysis. To that end, JASCO Research Ltd. conducted an acoustic monitoring program that is detailed in this report. The following is a summary of the pertinent results.

1.4.1 Close range measurements of distance to 180- and 190-dB levels

Sound pressure measurements were made at close ranges to the airgun array. It was noted that maximum levels were received broadside of the array. The maximum ranges to which RMS levels of 180 dB re μPa and 190 dB re μPa were received were determined (by extrapolation of measured data) to be 744 m and 236 m, respectively.

1.4.2 Propagation of sound horizontally away from the array

Levels received broadside to the array were noted to be approximately 10 dB greater than levels received from the array endfire. The levels were seen to decrease with range in

accordance with a standard, empirical transmission loss curve.

1.4.3 Sound levels received at long ranges from the array

At long ranges from the airgun array it was noted that low frequency sound energy was decreased due to ocean bottom and surface interactions. Sound that propagated to the crab cage control site passed over varying bottom topography and resulted in lower received levels than those received at corresponding ranges at the crab test cage site where the sound traveled over a smoother bottom.

1.4.4 Sound levels measured at the caged snow crab holding locations

The maximum RMS sound pressure levels received at the caged snow crab test site measured 178 dB re μPa and occurred at OBS-P as the Admiral passed by along line CHT-108-08 at a range of 138 m. The 1/3-octave band spectral levels of this airgun event showed a peak of 162 dB re μPa^2 at the 125 Hz centered band. The maximum RMS sound pressure level received at the control site was observed at OBS-T as the Admiral passed by along line CHT-101-01 and measured approximately 118 dB re μPa at a range of 23 km.

The ambient noise levels at the caged crab control site OBS-T had broadband sound levels up to as high as about 95 dB re μPa. The 1/3-octave band spectral levels measured were about 68–75 dB re μPa^2 from 25–100 Hz and about 75–79 dB re μPa^2 at 300–400 Hz. These levels were 83–94 dB re μPa^2 less than the highest 1/3-octave band spectral level observed during the loudest airgun event experienced by the caged crabs.

1.5 Chapter summary

Snow crab caged within the area of the seismic survey were exposed to maximum RMS sound pressure levels of 178 dB re μPa at a range of 138 m. Snow crab caged at the reference site 23 km away from the nearest transect surveyed during the seismic survey were also exposed to sound pressure, but at the considerably lower level of 118 dB re μPa. Maximal airgun levels experience by caged crabs were 83–94 dB re μPa^2 above the highest 1/3-octave band spectral level observed as ambient sound. These levels are considered representative of a normal operational seismic survey. Snow crab were exposed to some level of sound, up to these levels, for the 23-day duration of the survey though cumulative exposure was not calculated.

CHAPTER 2. UPDATE OF THE STUDY ON THE EFFECTS OF SEISMIC NOISE ON FEMALE SNOW CRAB (*CHIONOECETES OPILIO*) EVALUATED BY A CAGING STUDY CONDUCTED OFF WESTERN CAPE BRETON ISLAND IN 2003-2004

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2.1 Abstract

Additional results to the original seismic study are presented herein, including experimental trawling (dragging) of female snow crabs (*Chionoecetes opilio*) in mesh bags on the ocean floor in order to assess the effect of drifting cages on eyes, antennules, gills and statocysts. This chapter also includes the comparison of the abundances and distributions of different categories of snow crab before (September 2003) and after (June 2004) the seismic testing.

There was no apparent effect of trawling on the antennules, gills and statocysts, suggesting that the possible drift of cages in the test site during the 2004-2005 study may not have been the cause of dirty gills, antennules and statocysts found in female snow crab. There was no clear evidence of decreases in abundance or changes in distribution patterns that might have been caused by seismic testing in any category of snow crab assessed. Our results suggest that a global comparison of abundance and distribution of snow crab may not be a robust tool to assess possible effects of seismic testing.

2.2 Introduction

In October 2003, a 2-D seismic test was announced to be conducted by Geophysical Service Inc., on behalf of Corridor Resources Inc. off western Cape Breton Island (Nova Scotia, Canada; Anonymous, 2001; Davis and Christian, 2002). In November 2003, the Department of Fisheries and Oceans (DFO), in collaboration with Corridor Resources Inc. and Area 19 Snow Crab Fishermen's Association, proposed a caging experiment to evaluate the possible impacts of seismic activity on snow crab. Due to time limitation, an abbreviated version (using only mature females) of the proposal was adopted (Figure 2.1). During a meeting in September 2004 (DFO, 2004), and following consultative meetings, additional information was requested including a comparative study on the abundance and geographic distribution of different categories of snow crab before (September 2003) and after (June 2004) seismic testing, as well as the results of experimental dragging of females conducted in spring 2004. This document presents the results of these two additional projects.

2.3 Materials and Methods

2.3.1 Female dragging experiment

In an attempt to examine the possible effects of cage drifting on the ocean bottom during the short-term caging portion of the seismic study, mesh bags containing multiparous females *Chionoecetes opilio* were artificially dragged on the ocean bottom to measure possible fouling of antennules, eyes, statocysts and gills. Additional crabs were collected with modified snow crab traps on May 19, 2004. Two lines of six traps each were baited with frozen mackerel (*Scomber scombrus*) and immersed for 24 hours near the December 2003 sampling locations (46º38.84' - 61º13.57'; 46º38.78' - 61º13.56' and 46º38.52' - 61º13.58'; 46º38.46' - 61º 13.63'). A total of 350 females were caught. All crabs were examined and sorted on board so that the females used for this study were as similar as possible in terms of their carapace condition (category-4, multiparous females), embryonic development stage (dark orange eggs), and number of missing appendages (no more than 2 missing legs prior to the catch and without newly lost appendages by handling). A total of 100 category-4 multiparous females were selected on board and 50 selected females were put in two leech bags (25 per bag) and secured with tie wraps (Figure 2.2). Both bags were lined and separated by 35 feet of rope. Two100-pound chains were added 15 feet in front of each leech bag to stir the bottom when dragging. Dragging was conducted for two hours on the fishing vessel *Fishful Thinking*. Ship speed varied between 1.0 and 3.0 knots, and water depths ranged between 41.1 and 43.7 fathoms. Start and end positions were 46º32.515' - 61º18.677' and 46º 33.750' - 61º 18.497', respectively. The dragged crabs were separated in chilled coolers by bag and brought to DFO Moncton. The remaining females (control specimens, n = 50) were also brought to Moncton for further examination. Complete biological analysis (tissue sampling, measurements and observations) was performed on 45 dragged and 45 control females.

2.3.2 Sample treatment, measurement and dissection

In the laboratory, missing appendages, carapace condition (CC), carapace width (CW), abdomen width, total weight, egg color (Moriyasu and Lanteigne, 1998), percentage of remaining eggs, and overall animal condition were recorded for all samples. Carapace and abdomen widths were measured with a modified electronic caliper to the nearest 0.01 mm. Total weight was recorded to the nearest 0.1 g. Internal examinations included various appendices and organs (eyes, antennules, statocysts, gills: Figure 2.3) that were dissected out for further observations by dissection microscope).

2.3.3 Statistical analyses

Appendix and organ (eyes, antennules, statocysts, gills) condition for test and control animals were classified into 3-5 categories depending on the appendix and organ. The comparison between test and control sites for condition of appendix or organ, excluding senile females, was made by the χ^2 test (2 x C contingency table test, Everitt, 1977). A 99% level of significance was used for all analyses.

2.3.4 Estimation of the abundance and geographic distribution of different biological categories of snow crab before and after seismic testing by bottom trawl survey

A regular annual bottom trawl survey was conducted in September 2003 in the southern Gulf of St. Lawrence including Area 19 and adjacent regions. In addition, an *ad hoc* bottom trawl survey was conducted in the area limited to Area 19 and adjacent regions in June 2004 (Figure 2.4 – 2.9) to assess crab abundance and geographic distribution after the seismic survey and prior to the 2004 fishing season. Both survey and data analyses were conducted by following the annual survey and data analysis protocols (Hébert et al., 2004). The geographic distribution and abundance index of the following biological categories of snow crab were estimated by point- and block-kriging techniques with external drift model, respectively, as described in Hébert *et al.* (in prep.): Adolescent males larger than 56 mm CW, Total adult males carapace larger than 95 mm CW, Pubescent females, Primiparous females, and Multiparous females, and All females.

2.4 Results

2.4.1 Morphometric characteristics of samples

The mean carapace width (CW: mm) and mean weight (W: g) of females used for the dragging experiment are summarized in Table 2.1 together with those dissected from test and control sites for short-term and mid-term in the original study (Moriyasu et al., unpublished).

Table 2.1: Summary of morphometric measurements of samples per site and per experiment.

	Short-term*		Mid-term*		Bottom dragging	
	Test	Control	Test	Control	Test	Control
Total samples retrieved	90	90	90	94	45	45
Date retrieved	2003-12-23		2004-05-11		2004-05-19	
Date Dissected/Observed	2003-12-23	2003-12-24	2004-05-12		2004-05-20	
Mean CW (mm)	69.78	72.29	71.94	72.91	67.57	73.79
Min CW (mm)	52.79	54.79	58.33	58.42	53.04	64.09
Max CW (mm)	87.72	89.84	86.45	91.65	97.60	118.59
Sample number	90	90	90	94	45	45
Mean W (g)	126.93	135.32	141.59	142.97	112.34	131.30
Min W (g)	57.40	62.40	79.00	78.80	59.10	95.6
Max W (g)	250.80	262.40	230.70	244.70	180.0	176.6
Sample size	90	90	90	94	45	45

* cited from Moriyasu et al. (unpublished)

2.4.2 Condition of appendices and organs dissected from test and control animals (Dragging experiment)

No external damage or mortality occurred in test crabs following the dragging experiment.

2.4.2.1 Eyes

Eye conditions: We followed the criteria used in the original study based on the observations made by dissection microscope, i.e., 1) clean and intact, 2) trace of sediment-like substance on the surface of the eyes and intact, 3) damaged, and 4) heavy epibiont coverage.

Comparison of the eye conditions between test and control samples: There were no significant differences in the eye condition between test and control animals after eliminating senile females ($\chi^2 = 9.392$, $p = 0.0245$).

Table 2.2: Number of individuals (without senile females) per eye condition in test and control groups.

	Clean Intact	Sediment trace	Damaged	Heavy epibionts	Total
Test	21	18	2	2	43
Control	11	8	1	9	29

2.4.2.2 Antennules

Condition of antennules: Condition of antennules was determined based on the criteria established in the original study, i.e., 1) clean and intact, 2) intermediate (some dirtiness on the aesthetasc hairs) and intact, 3) dirty, and 4) damaged outer flagellum and/or aesthetasc hairs. Contrary to eye condition, no accumulation of epibionts was found in any of the specimens observed.

Comparison of the antennule conditions between test and control samples: There was no significant difference between test and control animals ($\chi^2 = 10.428$, $p = 0.0153$).

Table 2.3: Number of individuals per condition of antennules for test and control groups.

	Clean Intact	Intermediate	Dirty	Damaged	Total
Test	7	10	22	4	43
Control	6	9	5	9	29

2.4.2.3 Statocyst

Condition of statocysts: Based on the original observations by dissection microscope, SEM and histology, the condition of statocysts (group hairs and statolith) was classified into four categories: 1) clean group hairs and intact statolith, 2) dirty group hairs and intact statolith, 3) clean group hairs and displaced statolith, and 4) dirty group hairs and displaced statolith.

Comparison of the statocyst conditions between test and control samples
For both samples, no animals were classified in category 3 (clean group hairs and displaced statolith) and category 4 (dirty group hairs and displaced statolith), therefore, comparisons were made with a 2x2 contingency table test. The results showed no significant difference between test and control animals ($\chi^2 = 2.922$, $p = 0.0874$).

Table 2.4: Number of individuals per condition of statocysts for females in test and control groups.

	Clean Intact	Dirty	Total
Test	41	0	41
Control	27	1	28

2.4.2.4 Gills

Condition of gills: Based on the observations by dissection microscope, SEM and histology, the condition of gills (degree of dirtiness among gill lamellae) was classified into three categories in the original study: 1) clean, 2) intermediate (with some sediment-like substances), and 3) dirty (filled with compacted sediment-like substances).

Comparison of the gill conditions between test and control samples: The condition of gills were classified into only two categories (Intermediate and dirty categories were not observed), therefore the comparison was made with a 2x2 contingency table between test and control groups. There was no significant difference ($\chi^2 = 1.204$, $p = 0.2726$).

Table 2.5: Number of individuals per condition of gills for females from test and control groups

	Clean	Intermediate	Total
Test	38	9	47
Control	21	7	28

2.4.2.5 Abundance index and geographic distribution of different biological categories of snow crab before and after the seismic survey

The abundance index of each biological category chosen was estimated before (September 2003) and after (June 2004) seismic testing. In both males (3% for adolescent ≥ 56mm CW -8% for adult ≥ 95mm CW) and females (9% for primiparous -34% for

adolescent with orange gonads), the abundance estimates have shown a decrease for all categories (Table 2.6).

Table 2.6: Estimation of population abundance of different biological categories of snow crab in Area 19 before (September 2003) and after (June 2004) seismic testing. Population abundance are accompanied by their 95% confidence intervals (95% CI).

Acronym	Year	Population	95% CI	95% CI
ADOM≥56	2003	21,400,000	15,630,602	28,610,329
	2004	17,545,800	13,009,478	22,082,122
ADUM≥95	2003	14,300,000	12,836,916	15,882,911
	2004	13,844,600	12,008,8251	15,680,375
ADOF	2003	6,017,850	4,611,072	7,719,287
	2004	3,959,620	1,927,276	5,991,964
PRIF	2003	3,801,470	2,428,396	5,677,986
	2004	3,457,230	1,891,537	5,022,923
MULF	2003	55,281,000	41,951,874	71,509,242
	2004	47,978,200	35,938,292	60,018,108
TF	2003	78,499,900	63,923,687	95,399,417
	2004	65,088,300	50,627,459	79,549,141

ADOM ≥ 56: Adolescent males larger than or equal to 56 mm CW, ADUM ≥ 95: Adult males larger than or equal to 95 mm CW, ADOF: Adolescent females with orange gonads, PRIF: Primiparous females, MULF: Multiparous females, TF: Total females.

The comparison of the geographic distribution of adolescent females with orange gonads prior to and post seismic testing showed a significant change (disappearance) in a high concentration spot observed in the northwestern border of Area 19, but no conspicuous change in the southern part of the Area (Figure 2.4-A). For primiparous females, the appearance of a high concentration spot was observed in the northwestern border of Area 19 in spring 2004. Also observed were the disappearance of a southernmost concentration patch and the growth of a concentration patch in the southwestern corner of Area 19 (Figure 2.4-B). No conspicuous change in the distribution pattern within Area 19 was observed in multiparous and total females (Figure 2.5-A, 2.5-B). For adolescent males ≥ 56 mm CW, the reduced concentration patches were observed within Area 19 after the seismic survey (Figure 2.6-A). No conspicuous change was discernible for adult males ≥ 95 mm CW except for the reduction of a concentration patch at the northwestern corner of Area 19 (Figure 2.6-B).

2.5 Discussion

2.5.1 Appendix and organ examination

The observation of eye, antennule, statocyst, and gill condition in multiparous females dragged on the ocean floor for a couple of hours did not show any significant difference compared to the control group. However, it is difficult to estimate how long the original test traps were dragged on the ocean floor. At least, we can conclude that a two-hour dragging of trap line on the ocean floor might not be enough to expect the accumulation of dirtiness on the gills and cause abnormal features in the statocysts.

As Moriyasu et al. (unpublished) pointed out, the eyes and antennules are not appropriate appendices to assess possible seismic effects; instead gills and statocysts may provide more useful information. It is important not to include senile female (that regularly show dirty and often damaged appendices) from the assessment, which may lead to erroneous conclusions.

2.5.2 Comparison of the abundance and geographic distribution of different categories of snow crab before and after the seismic survey based on the September 2003 and June 2004 trawl surveys

As a regular annual stock assessment of snow crab in the southern Gulf of St. Lawrence, a bottom trawl survey was conducted in September 2003 (Figure 2.7), which allowed us to compare the abundance estimates of multiparous females and their geographic distribution before (September 2003) and after (June 2004) the seismic testing of December 2003.

The comparison of results between two different seasons suggested that it is reasonable to assume that negative effects of seismic testing (e.g., massive mortality within the testing site or emigration towards outside of the site) were not discernible based on the abundance estimates and geographic changes in distribution patters of different categories of snow crab. In addition, various biological events such as seasonal migration, molting to the different category (adolescent to primiparous female) and human activity (commercial fishing started after the seismic survey and prior to the June survey) influenced the abundance and geographic distribution patterns. Furthermore, changes observed in a given category of crab do not necessarily mean the absolute decrease in abundance. For example, the decrease in adolescent females with orange gonads in June survey compared with the number in the September survey of the previous year suggests a transition of adolescent phase to primiparous female category over the winter.

This is also true for the change in distribution patterns. Comparison of geographic distributions suggests that despite some changes in geographic distribution of different categories of crab between the two surveys, it is reasonable to conclude that a negative effects of seismic testing (e.g. shifting geographic distribution or massive movement) was not discernible based on the density distribution changes in a given area.

2.6 Acknowledgement

We are indebted to B. Adams and the Area 19 Snow Crab Fishermen's Association executive members, and Dr. M. Chadwick (DFO, Gulf Region) for their continuous encouragement and support throughout the current project. Without the diligence of Captain Basil MacLean (CFV *Fishfull Thinking*) and his crew during the field sampling, this project would not have been realizable. Administrative support provided by Dr. S. Courtenay (DFO, Gulf Region), whom we thank for many helpful comments and suggestions.

2.7 Chapter summary

The hypothesis was tested that short-term fouling of snow crab eyes, antennules, gills and statocysts during the December 2003 caging experiment was caused by dragging of the cages through the sediment. Dragging of snow crab enclosed in mesh bags along the bottom in the area of the original caging failed to produce similar deposition of sediment on these organs. While this test failed to support the hypothesis, it was a much shorter duration of dragging than was experienced in the original experiment (2 h vs. 12 days) and did not exactly mimic the original conditions. Therefore it cannot be taken as a definitive disproof of the hypothesis.

The hypothesis was tested that the seismic survey resulted in a large localized decrease in snow crab density either because of mass mortalities or because crab moved away from the seismic area. Snow crab catches in bottom trawl surveys carried out before and after the seismic surveys did not show such a decrease though some differences in distribution were noted. It is not possible to say whether these differences in distribution are related to the seismic survey.

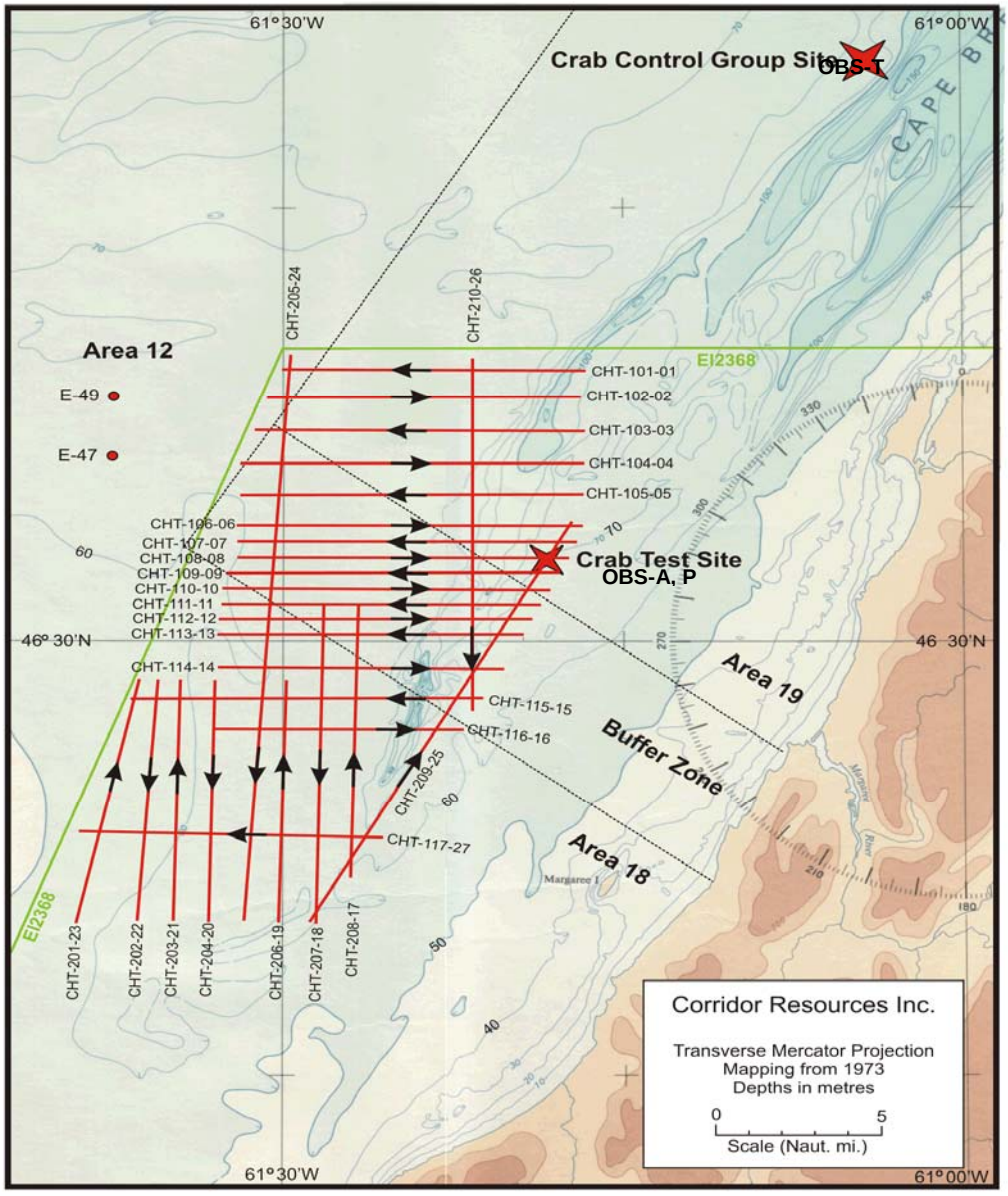


Figure 2.1: Seismic testing itinerary planned with the position of snow crab test and control sites. Before retrieval of short-term cages on December 23, 2003, all horizontal lines north of 46°30'N and three vertical lines (#205, 208 and 209) were covered by the seismic vessel. Ocean bottom seismometers were also immersed at test (OBS-A and P) and control site (OBS-T). (From Moriyasu et al., unpublished)

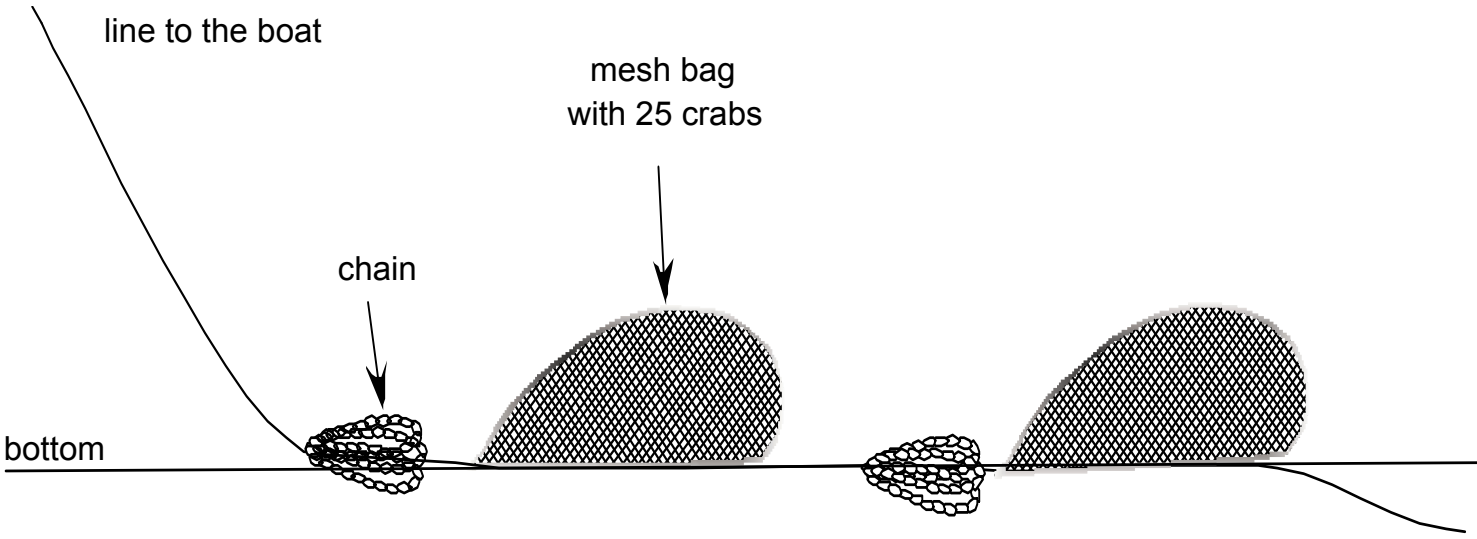


Figure 2.2: Schematic presentation of dragging bags containing female snow crabs.

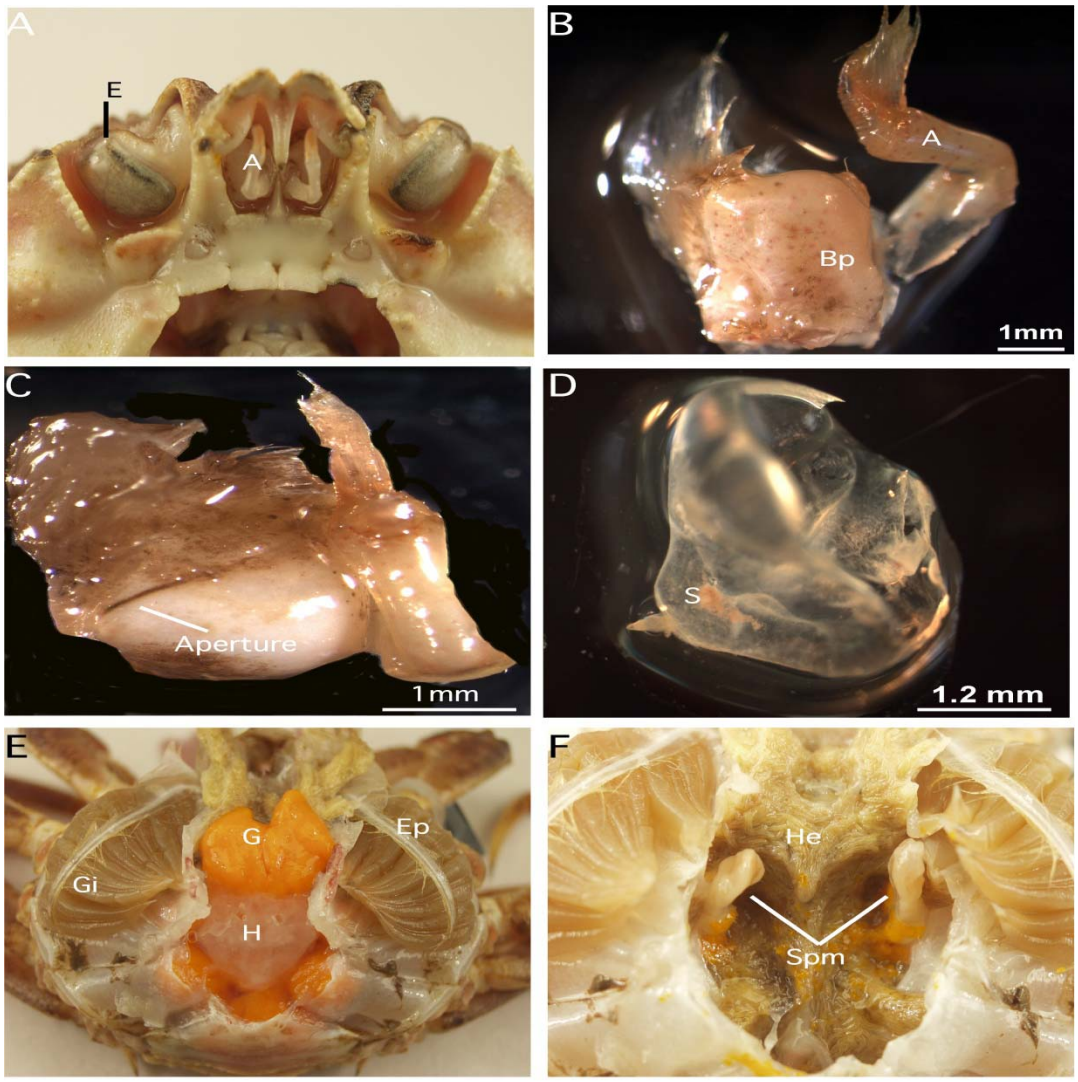


Figure 2.3: General view and internal anatomy of female snow crab (*Chionoecetes opilio*) showing the positions of studied appendices and organs. (A) Eyes and antennules; (B) Lateral view of the basal peduncle with antennule; (C) Aperture of the basal peduncle; (D) General view of statocyst dissected out from the basal peduncle showing the statolith; (E & F) Internal anatomy showing the positions of the appendices and organs. Abbreviations are as follows: A = antennule; Bp = basal peduncle; E = eye; Ep = epipodite of 1st maxilliped; G = gonad; Gi = gill; H = heart; He = hepatopancreas; Spm = spermathecae; S = statocyst.

(From Moriyasu et al., unpublished)

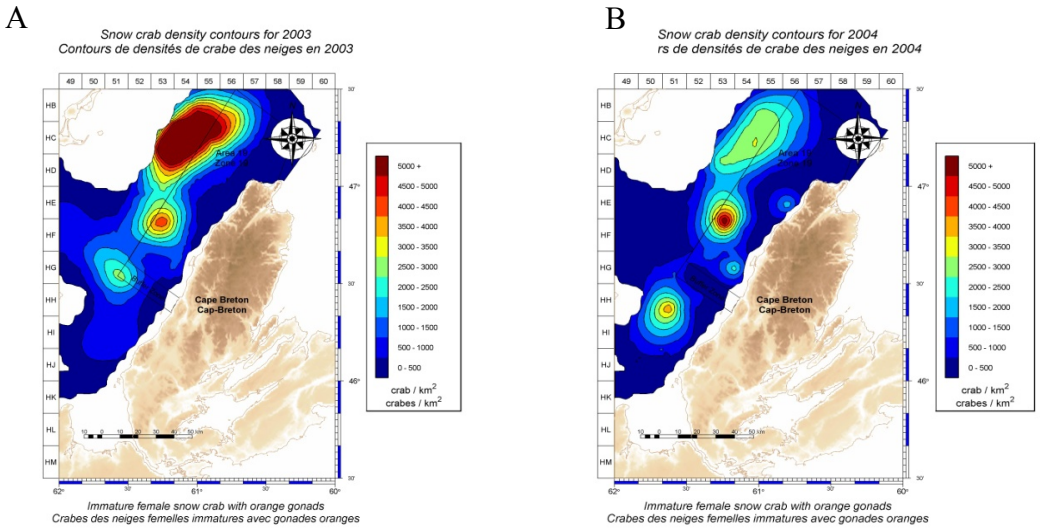


Figure 2.4: Geographic distribution of pubescent female snow crab (*Chionoecetes opilio*) based on the (A) September 2003 and (B) June 2004 surveys.

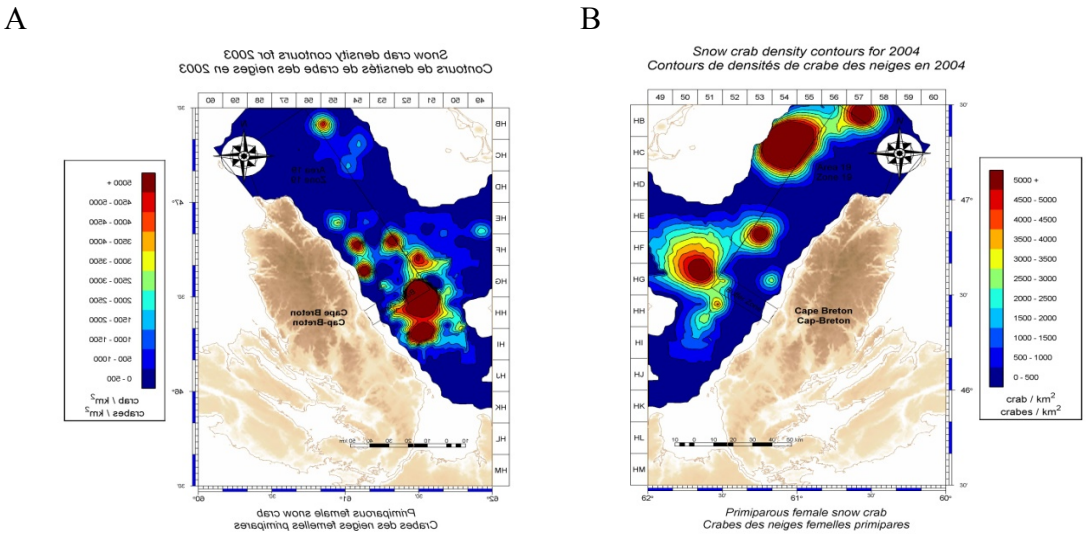
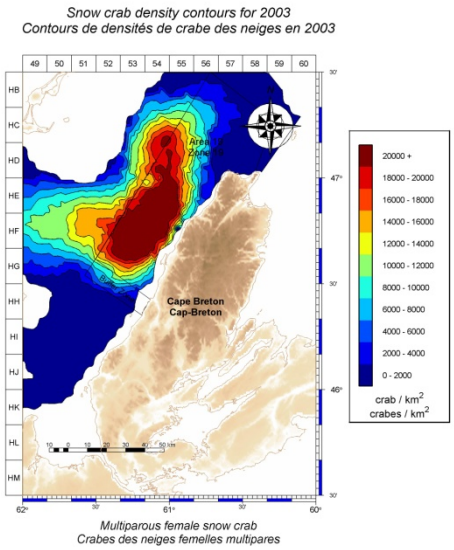


Figure 2.5: Geographic distribution of primiparous female snow crab (*Chionoecetes opilio*) based on the (A) September 2003 and (B) June 2004 surveys.

A



B

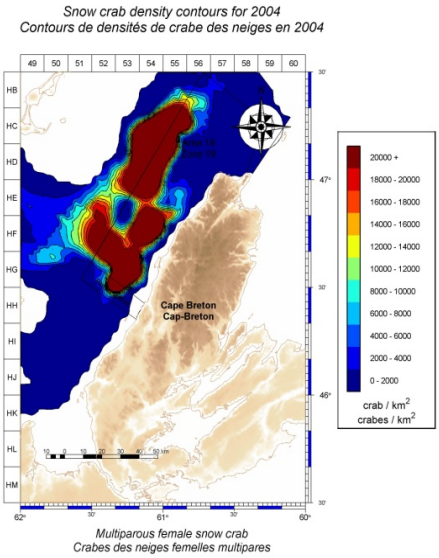
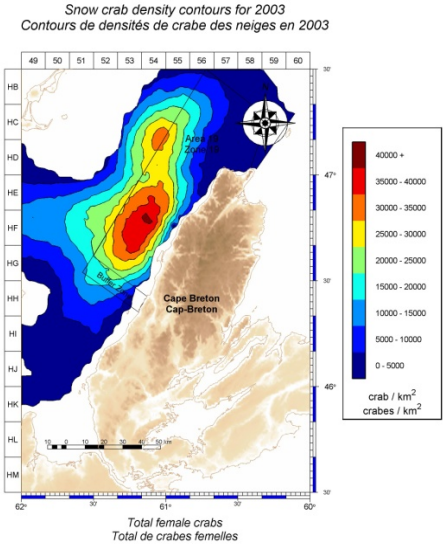


Figure 2.6: Geographic distribution of multiparous female snow crab (*Chionoecetes opilio*) based on the (A) September 2003 and (B) June 2004 surveys.

A



B

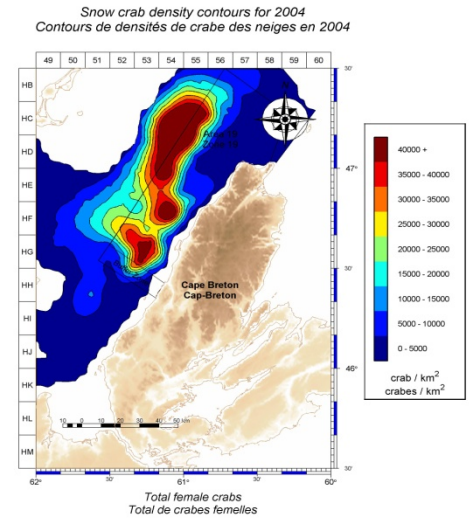
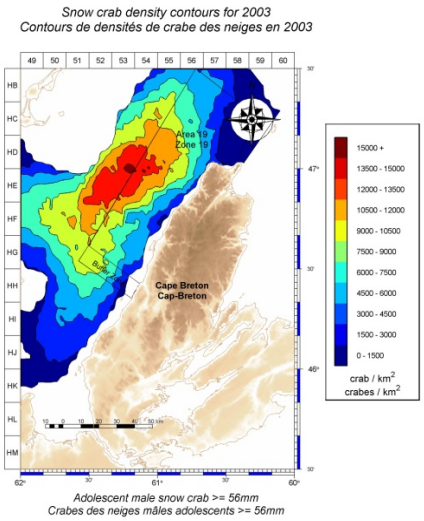


Figure 2.7: Geographic distribution of total female snow crab (*Chionoecetes opilio*) based on the (A) September 2003 and (B) June 2004 surveys.

A



B

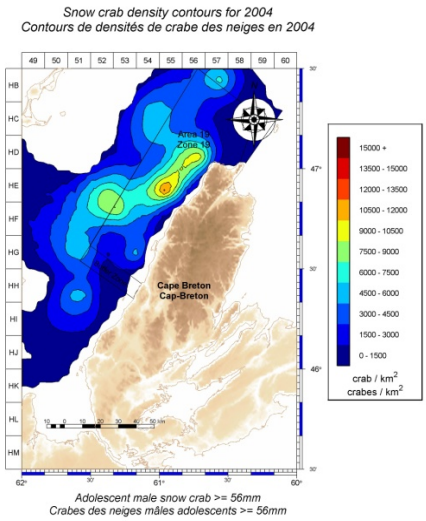
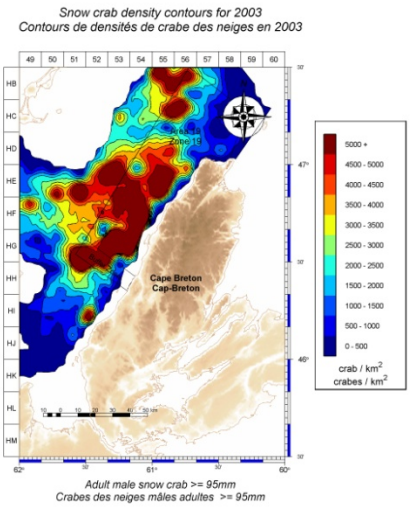


Figure 2.8: Geographic distribution of adolescent males > 56 mm CW snow crab (*Chionoecetes opilio*) based on the (A) September 2003 and (B) June 2004 surveys.

A



B

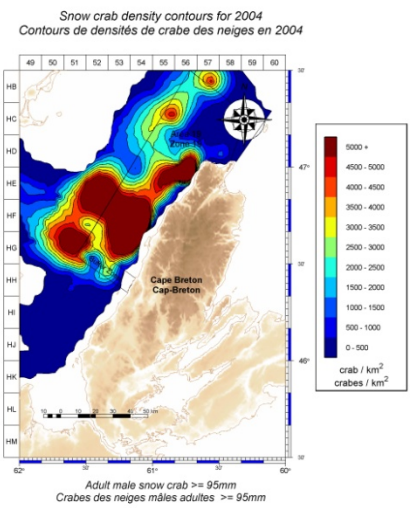


Figure 2.9: Geographic distribution of total adult male > 95 mm CW snow crab (*Chionoecetes opilio*) based on the (A) September 2003 and (B) June 2004 surveys.

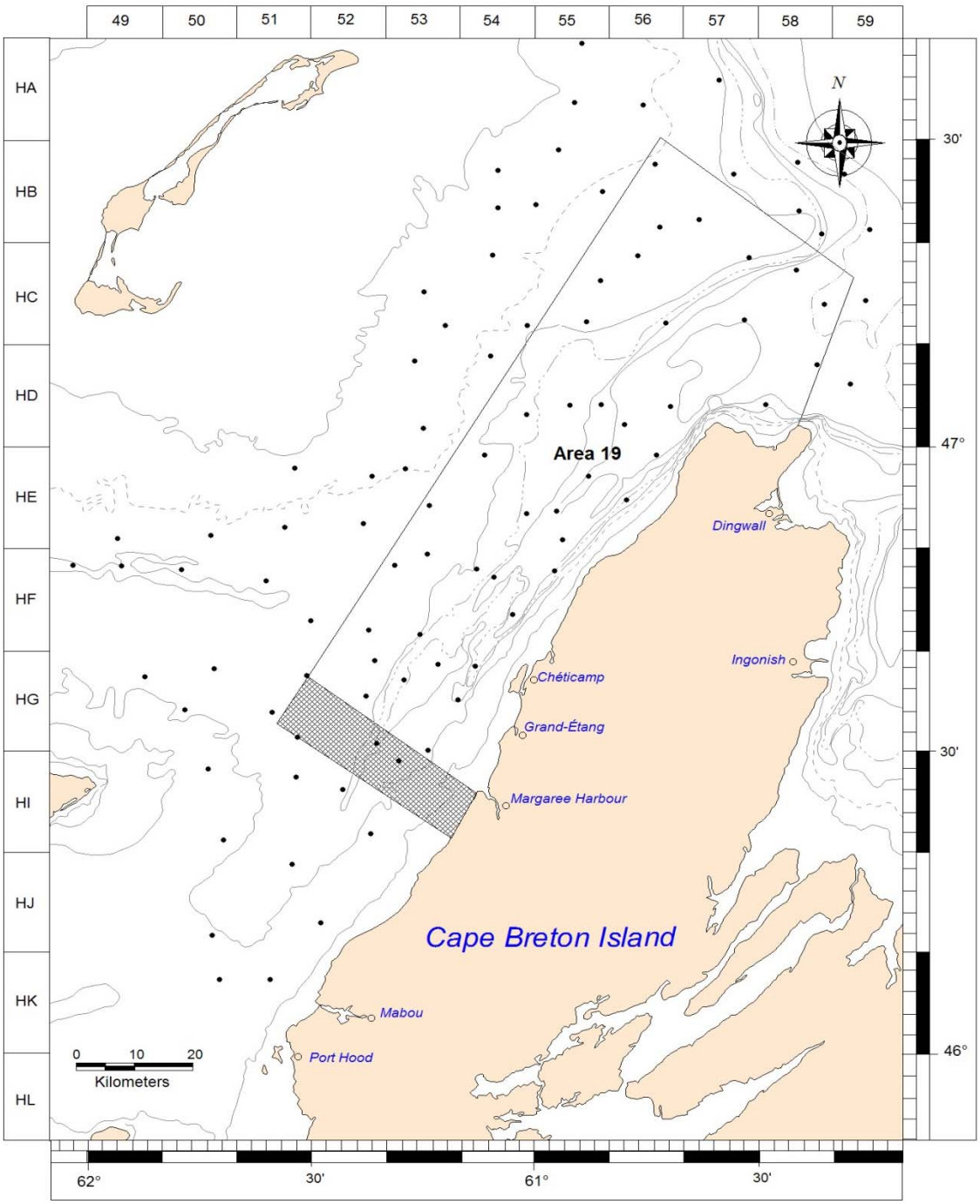


Figure 2.10: Trawl sampling stations visited during the September 2003 and June 2004 survey. Points indicate trawl survey sampling stations and the shaded area is a no snow crab commercial fishing zone. (From Moriyasu et al., unpublished)

CHAPTER 3. HISTOPATHOLOGICAL EVALUATION OF HEPATOPANCREAS AND OVARIES FROM CAGED SNOW CRABS(*CHIONOECETES OPILIO*) FAILS TO DISCERN CONTROL, AND SEISMIC EXPOSED SAMPLES DUE TO CONFOUNDING EFFECTS FROM CONFINEMENT, STARVATION AND/OR HANDLING STRESS

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3.1 Abstract

The aim of this study was to compare the histological appearance of hepatopancreas and ovaries of snow crabs held in cages at control and seismic exposed sites. Tissues were retrieved after short (12 days) or long (5 months) confinement, and processed for general histology. Four hundred and seven hepatopancreas slides and 352 ovarian slides were examined by light microscopy in a blind study. Tissues were assessed for trauma, hemocytic infiltration, edema, nuclear changes, detachment from basement membranes, intracellular and extracellular changes, etc., and subjectively given numerical values by an independent evaluator indicating the degree of abnormality. After the histological evaluation, the identity of the slides was revealed, and 407 and 352 hepatopancreas and ovary slides, respectively, were analyzed. Of these, 53.4% of the seismic exposed hepatopancreas slides correlated with the blind histological analysis of pathological findings, and 51.8% could be correlated with the seismic exposed ovaries. However, control slides did not correlate well and only 38.4% of the evaluated control hepatopancreas slides correlated with the key and 35.8% with the ovary control slides. When considering length of time that crabs were caged, a high degree of correlation could be discerned with the degree of pathological abnormalities. Crabs maintained in cages for five months following seismic exposure received higher pathologic indices than those caged for shorter times. Commonly observed abnormalities included inflammatory infiltration, scar tissue formation, disruption of basement membrane integrity, vacuolation of cells, which could have been brought about by physical impact as well as from confinement stress. It is concluded that caged crabs do not provide appropriate information following seismic exposure, and that perhaps, single large enclosures at control and seismic sites with crabs held for short periods following exposure would provide sufficient information as to the histological damage that may be sustained following seismic impacts.

3.2 Introduction

Marine organisms may be susceptible to damage when exposed to seismic waves (Hirst & Rodhouse, 2000; Malakoff, 2001), which are frequently used to map the ocean floors for oil deposits using high frequency array guns (Dragoset, 2000). Increasing evidence indicates that marine mammals are adversely affected by seismic surveys in terms of physiological and behavioral disruption (Gordon et al., 2004). Similar observations have been made with some fish species (Popper et al., 2004; Santulli et al., 1999). However,

in-depth studies are scarce and information is limited on the physiological effects on marine invertebrates (Parry & Gason, 2006). As a follow up to the studies by Christian et al. (2003) and DFO's (2004) report, this study evaluated histological changes on 407 hepatopancreas and 352 ovarian slides prepared from snow crabs (*Chionoecetes opilio*) held in cages off the coast of Cape Breton at control and seismic impacted sites. The slides were sent numerically coded and analyzed without any knowledge of which ones were seismic exposed, or if there were differences in exposure, or which ones were controls. Following the histological evaluation, abnormalities (mild, moderate and extreme) were observed in 234 out of 407 hepatopancreas slides, and 203 out of 352 ovarian slides, that could have been the result of some form of stress. These findings were compared against the decoded list, which was revealed following the histological report and the details of the experimental set-up. Qualitative and quantitative analysis with the statistical evaluation of the findings are reported here.

3.3 Materials and methods

3.3.1 Studied Specimens

Caged multiparous females (category 4) were collected from the control and seismic impacted sites and tissues prepared for histology as described elsewhere (Moriyasu et al., unpublished). The collection of crabs and experimental set-up is also described elsewhere (Moriyasu et al., unpublished). The hepatopancreatic and ovarian tissue sections were obtained from caged crabs held at sea for short (Dec. 2003 and Dec. 2004 collections, 12 days) and longer (May 2004, 5 months) duration that had been at control sites (about 27 km away from seismic testing site at 106-m depth) or exposed to seismic energy (directly beneath ensonified location at 78-m depth) during mid-December 2003 (for the December 2003 and May 2004 samples) and mid-December 2004 (for the December 2004 samples). Samples originated from multiple rectangular cages (48" x 36" x 16" with 1½" mesh size) each containing 25 female crabs (mean carapace width 70 mm and average weight 130 g; about the size of a large human hand).

3.3.2 Histological slides

Twelve boxes of hepatopancreas slides, with 820 slides in all, and eleven boxes of ovary slides, with 708 slides in all, were received at Wilfrid Laurier in May 2006. Each box contained duplicate slides (stained and unstained, some had triplicates) and only the stained slides were evaluated. Some slides were missing (e.g., hepatopancreas slide#233) or had wrong tissues. In all, 407 hepatopancreas and 352 ovarian slides stained with Masson's trichrome &/or Periodic Acid Schiff (PAS) were evaluated.

3.3.3 Histopathological Evaluation

A subjective score ranging from 1 to 5 (with 1 given to the normal appearance, and increasing values given to increasing abnormalities) was set for evaluating the slides. Specific parameters evaluated for hepatopancreas slides included: 1) M-cell numbers and positioning relative to the basal lamina. M cells are believed to increase with altered

physical states, especially with starvation (Al Mohanna et al. 1985; Al Mohanna & Nott 1987). For this parameter only, total absence of M cells was scored as 0 (but this may not necessarily reflect a "normal" state). 2) Nuclear morphology of the epithelial cells. Increased heterochromatin or nuclear breakage was scored as abnormal and given higher values. 3) Size of the epithelial cells' nuclei, with increasing scores given for those varying from the norm. 4) Thickness of the epithelial wall. 5) Level of vacuolation of the R-cells. 6) Activation of the fixed phagocytes of the interstitium. 7) Encapsulations and parasites. 8) Delamination of the basal lamina. 9) State of the peritrophic membranes. 10) Necrosis and/or autolysis of the tissue. 11) Abundance of collagenous connective tissue.

In contrast, nine parameters were evaluated for the ovary slides: 1) Delamination of the chorionic membrane. 2) Atresia of the follicles. 3) Packing density of oocytes. 4) Granulocytic infiltration of the tissue. 5) Overall "bruising' appearance (irregular shaped follicles, hemocyte abundance, debris, etc.). 6) Delayed maturation of the oocytes. 7) Peculiarity of the staining deviating from the norm. 8) Edema or homogenization of cellular components. 9) Encapsulations and/or presence of parasites.

3.3.4 Final analysis

The key list to the slides' identity as to treatment or control was received on September 9, 2006. Information regarding experimental set-up and variables were received November 1, 2006. Statistical analyses were performed with the pathological index values obtained from the histological findings or converted to a binary value and tested against the received key data. Statistical tests involved Spearman correlation analysis (non parametric) for comparing observed vs. actual values, unpaired t-test (Mann-Whitney) for comparing control and test samples, one-way ANOVA with Tukey's post test for mean box pathologic index comparisons, and multiple regression analysis with chi-squared tests.

3.4 Results

3.4.1 Histopathological analysis

The blind evaluation of the slides discerned histological changes that were grouped into 4 categories: a) normal, b) mild changes (that could be found with physiological variation within sampled population), c) moderate changes (that cannot be attributed to normal variability), and d) extreme changes (that were likely due to stressful conditions). The normal category (less than 17 and less than 13 subjective points for hepatopancreas and ovary slides respectively) were considered against the control pool for the post histological analysis, while the mild, moderate and extreme categories (≥ 17 & ≥ 13 points for hepatopancreas and ovary slides, respectively) were compared against the ensonified specimens, coding them in a binary system (1 = control, 2 = seismic) (see Appendix 1 & 2).

3.4.2 Statistical analysis

For the hepatopancreas slides, there was no significant correlation ($r = -0.08238$, $r^2 = 0.0067$, $p = 0.0970$) when comparing the binary coded histopathological findings with the coded (control or seismic) key. A slight negative correlation (inverse correlation) was observed for the ovary slides, with $r = -0.1298$, $r^2 = 0.1298$ & $p = 0.0148$), meaning that many slides coded as controls were seismic and vice versa.

Of 204 seismic exposed hepatopancreas slides, 109 slides were shown to correlate with the findings of the blind evaluation. This represents approximately 53.4% correlation. However, control hepatopancreas slides had low correlation at 38.7%.

Table 3.1: Correlation between classification of hepatopancreas slides in control and seismic exposed groups from blind evaluation and actual data. Included in this table is the number of slides that were classified properly (matched), the actual number of slides in each treatment group (actual) and the correlation between the match and actual values.

Hepatopancreas	matched	actual	% correlation
control	78	203	38.67
seismic	109	204	53.43
total	187	407	45.94

Of 186 seismic exposed ovary slides, 96 corresponded with the blind assessment, representing 51.6% correlation. However, control ovary correlated at only 35.5% and actually close to 65% was wrong, hence the inverse correlation.

Table 3.2: Correlation between classification of ovary slides in control and seismic exposed groups from blind evaluation and actual data. The following table indicates the number of slides that were classified properly (matched), the actual number of slides in each treatment group (actual) and the % correlation between the match and actual values.

Ovary slides	matched	actual	% correlation
control	59	166	35.54
seismic	96	186	51.61
total	155	352	44.03

On the other hand, analysis by length of caging correlated well to increasing pathogenic indices regardless of whether the crabs had been seismic exposed or not. Pathologic indices were averaged for each slide box, which contained samples of varying caging durations. Invariably, samples collected in Dec. 2004 (12 days) had the lowest average pathogenic indices, followed by samples collected in Dec. 2003 and the samples collected in May 2004 (5 months) had the highest mean pathologic indices (see Figures 3.1 & 3.2).

Table 3.3: Mean pathological score per slide box for hepatopancreas slides of snow crab caged for either 12 days or 5 months, regardless of treatment.

Hepatopancreas

slide box	mean score	collect date	caged time
1	18.912	Dec-03	12 days
2	16.908	Dec-04	12 days
3	20.574	May-04	5 months
4	17.242	Dec-03	12 days
5	18.471	May-04	5 months
6	16.77	Dec-04	12 days
7	19.559	May-04	5 months
8	18.03	Dec-03	12 days
9	20.324	May-04	5 months
10	16.917	Dec-03	12 days
11	19.85	May-04	5 months
12	20.523	May-04	5 months

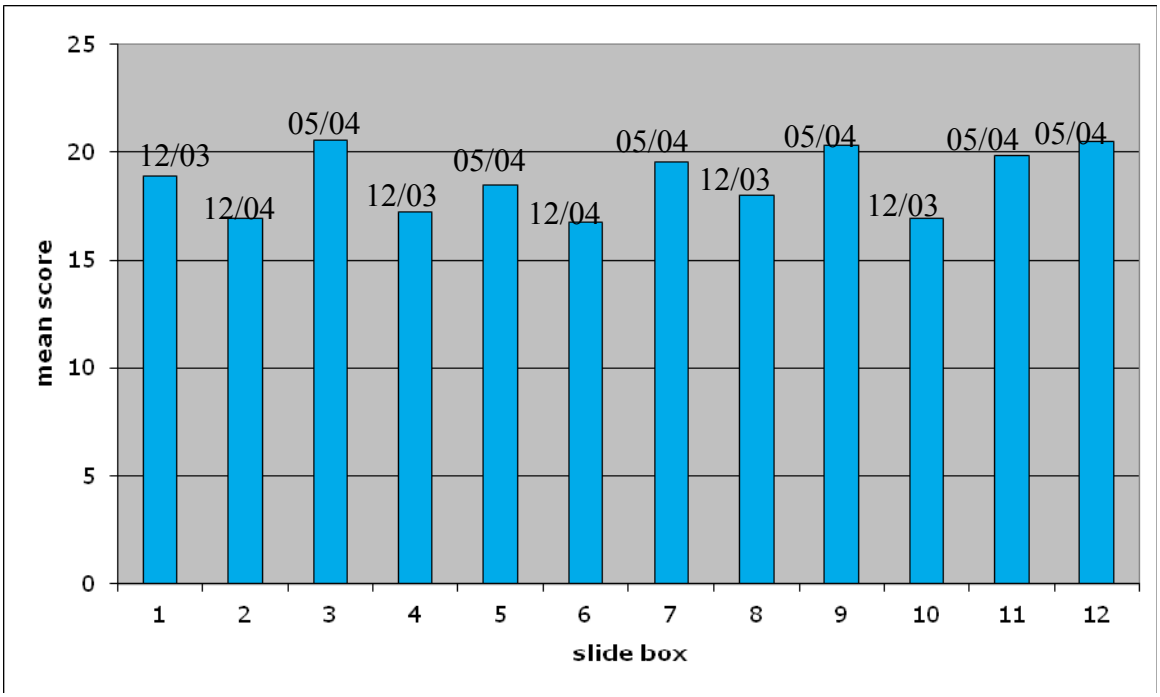

Figure 3. 1: Correlation of length of caging with pathologic index for hepatopancreas. Numbers above bars indicate collection dates. Boxes 3 & 9 are significantly different from all of the December collections ($P < 0.01$). Box 12 is also significantly different at $P < 0.05$.

Table 3.4: Mean pathological score per slide box for slides of ovaries of snow crab caged for either 12 days or 5 months, regardless of treatment.

Ovaries

slide box	mean score	collect date	caged time
1	12.3	Dec-04	12 days
2	16.13636	May-04	5 months
3	12.04	Dec-04	12 days
4	15.22414	Dec-03	12 days
5	16.42424	May-04	5 months
6	14.23529	Dec-03	12 days
7	17.55714	May-04	5 months
8	15.86364	May-04	5 months
9	15.92857	May-04	5 months
10	14.34091	May-04	5 months
11	11.96154	Dec-04	12 days

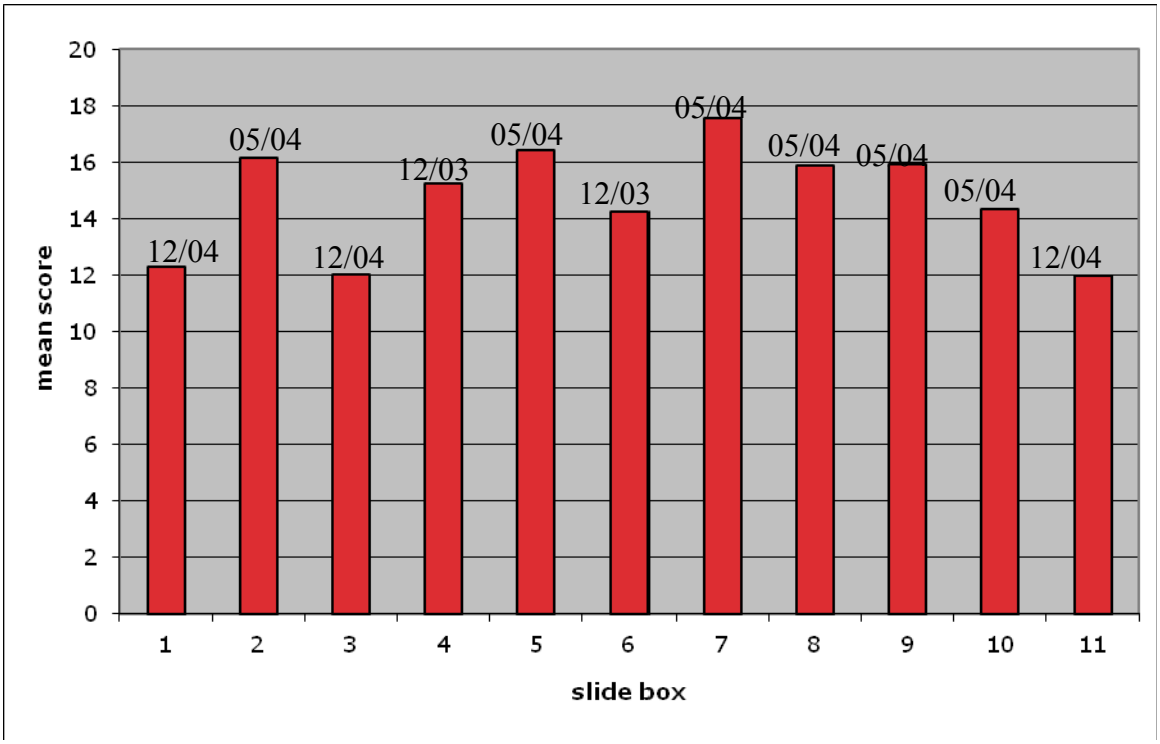

Figure 3.2: Correlation of length of caging with pathologic index for ovaries. Numbers above bars indicate collection dates. Boxes 2, 5, 7 & 8 are significantly different from the December 2004 collections ($P < 0.001$). Box 9 is also significantly different at $P < 0.01$.

Therefore, the observed pathologies appear to be mainly due to time in captivity rather than treatment effects. For both, hepatopancreas and ovary slides derived from crabs caged for 5 months showed higher degree of abnormalities than crabs caged for 12 days.

3.4.3 Morphological analysis

Histological re-analysis of slides' post-identification with the coded key did not reveal any common characteristics that could distinguish seismic exposed crab tissues from control tissues. Most tissues from crabs held in cages for two weeks, showed mild abnormalities regardless of whether they had been seismic exposed or not. Crabs caged for 5 months showed higher degree of abnormalities with higher incidence of secondary infections due to pathogenic invasion. In many cases, hepatopancreatic slides prepared from seismic exposed crabs appear to have damaged basement membranes, causing irregular tubular outlines. Similarly, ovarian slides from seismic exposed crabs appeared to have detached chorions and/or separation of follicular cells. However, these changes were not consistent across slides, and many seismic exposed tissue slides appeared healthy (see attached plates). Gonadal stage of development was taken into consideration when evaluating the ovarian slides as *C. opilio* has a limited (two reproductive seasons) and a long reproductive cycle of about two yrs (Comeau et al., 1999). In general, healthy ovaries could be observed at various growing, maturing and degenerating stages. Careful observations were made to discern damage from the post-spawning degenerating gonads that showed massive resorption and atretic follicles from seismic exposed gonads.

3.5 Discussion

This study could not establish with certainty that snow crabs exposed to seismic energy had detrimental histological effects on hepatopancreatic or ovarian tissues compared to control samples. Microscopic evaluation of blind coded slides demonstrated histopathological abnormalities close to 57.5% of the observed slides in both hepatopancreas and ovarian tissues. However, the abnormal-looking slides did not correlate well with seismic exposed crab slides, nor did the normal looking tissues to control crabs. Hence, it is not apparent that the observed histopathologies were due to seismic effects or to the stress of deployment (depressurization and repressurization), as well as to handling and confinement stress. Significant correlations to length of time in cages could be drawn for both hepatopancreas and ovary slides prepared from the long caged crabs (5 months), irrespective of treatment. A recent seismic experiment similar in scope and set-up to the present study was carried out off the coast of Norway with caged sandeel fish (*Ammodytes Marinus*; Hassel et al., 2004). Although the number of fish used was less than the present work, approximately 35% mortality for both experimental and control groups was reported. Hassel et al. (2004) attributed the equal mortalities to possible injuries due to handling and confinement of the fish although direct seismic effects could not be ruled out. Finally, rapid postmortem autolytic changes that can occur in marine invertebrate tissues were taken into consideration when interpreting these histological slides. Optimal fixation of marine invertebrate tissues is difficult and care must be taken in the interpretation of apparent damage seen in histological slides (Vogan

et al., 2001). Variation in the quality of histological integrity, such as peripheral areas of tissue blocks looking structurally different to tissues in the centre of the block, could be argued to be a marker of poor penetration of fixative, and this was observed in some slides.

For the present study, no significant correlations could be made with seismic exposure and observed histopathologies in the analyzed crab hepatopancreas and gonads. This lack of effect appears to be in agreement to other seismic exposure studies performed with marine invertebrates, but not with vertebrates. Pearson et al. (1994) investigated the effects of airgun discharges on survival and growth of Dungeness crab (*Cancer magister*) larvae. An array of seven airguns, discharging as close as 1 m away, from the larvae did not affect the crab larval survival. Parry and Gason (2006) studied catch rates of rock lobsters (*Panulirus interruptus*) following seismic surveys and reported that there was no evidence of a relationship between seismic surveys and long-term changes in catch rates. They suggest that perhaps because most invertebrates do not contain sound sensitive organs such as air bladders like those found in fish (Keevin & Hempen, 1997), invertebrates may be less vulnerable to adjacent loud sounds/explosions. However, conclusive evidence that seismic energy has no detrimental effects on invertebrate tissues had not been investigated other than the work by Moriyasu et al. within the scope of this study.

Histopathological observations reported in this study are in agreement with tissue damage observed in organisms under stress (Johnson, 1980). Whether the stress was from seismic waves, and/or confinement stress, starvation, handling and deployment will need to be further evaluated. As well, one cannot exclude some tissue peculiarities that may be unique to *C. opilio* and further histological studies should be performed at varying stages of their life cycle. The difficulty to assess histological changes in the snow crab tissues is mainly due to the lack of normal tissue histology studies on these species. Even though, the snow crab, *C. opilio*, has been proposed as a model for the Brachyurans (Elner & Beninger 1992), a comprehensive book on biological field techniques for Chionoecetes crabs is available (Jadamec et al., 1999) with detailed colour figures and description of anatomical features, and extensive literature exists on the fisheries, general biology, ecology and on some growth and reproductive aspects of *C. opilio* (Paul, 2000). The book by Johnson (1980) on the "Histology of the Blue Crab" is the closest histological treatise to study crab morphology and was used extensively to detail the aberrations observed in the present slides. Harrison and Hume's (1992) volume 10 on "Microscopic anatomy of invertebrates" was also a good resource to ascertain histological abnormalities.

Many invertebrates undergo ecdysis or molting, and *C. opilio* has been reported to undergo terminal molt at maturity, at 47-95 mm carapace width (CW) (Elner & Beninger, 1992). All crabs in this study were within that range, with smallest crab being 52.8 mm CW, thus confounding physiological effects from molting on the morphology of the tissues could be ruled out, but detailed histological studies are lacking (Harrison & Hume 1992). The CW range of the studied snow crabs, group them in the "multiparous" as opposed to "primiparous" females. However, *C. opilio* have been reported to have only

two spawning cycles (Elner & Beninger, 1992; Comeau et al., 1999), therefore the studied females were likely in their last spawning. Life expectancy of female *C. opilio* is about 5 years (Comeau et al., 1999) although others (Alunno-Bruscia & Sainte-Marie 1998) reported life expectancy up to 6.5 years depending on environmental conditions. Many of the observed abnormalities could be a direct response to senescence, an area that has not been investigated histologically (Harrison & Hume, 1992).

Although abnormality indices did not appear to be significantly different among control and seismic exposed crab tissues, the inverse correlation observed with gonadal morphology in the histological evaluation when correlated against the key is of interest. Perhaps, the initial acoustic stress may have mobilized energy reserves differently than in control female crabs, and seismic exposed crabs may have placed more effort on the fitness and survival of its eggs and protected these from subsequent stress damage, making their appearance more normal than those from control ovaries. This is in agreement with the findings of Moriyasu et al. as part of this study, in which oocytes from seismic exposed crabs were reported to be larger in size than in the controls. Damage to eggs could reflect in abnormal development and/or survival of future generations, as female reproductive cycles are long, approximately two years and females are thought to only hatch two broods in their lifetime (Comeau et al., 1999). Hence, seismic exposed crabs may quickly repair any damage and/or become more resistant to further damage. Alternatively, the seismic exposed crabs may have had better environmental conditions (food availability, higher temperatures) than the control crabs at their caged sites, which was indicated in the Moriyasu report. Further tests are needed to evaluate these possibilities.

Chronic damage/recovery is difficult to assess due to confounding events brought about by the crowded caging conditions and limited food supply. It is recommended that lesser density of crabs be used if the same traps are to be used for future experiments. Alternatively, larger covered enclosures built on the ocean floor directly beneath projected seismic paths and at various distances from the site up to several control sites within a set radius, but far removed, could be located for crabs to roam about and be collected at later times. Additionally, acute studies with shorter captivity times (< 1 week) may be needed to observe acute damage and discern morphological changes.

3.6 Chapter summary

Preserved samples of hepatopancreas (liver-equivalent) and ovary tissues taken from female snow crab caged at the seismic site and reference site were examined by an independent histopathologist to seek confirmation of an original interpretation that there was more tissue damage in the seismic than reference tissues. To avoid bias, the samples were read "blind," meaning that the independent histopathologist did not know their identity until after she had recorded her observations. This re-analysis failed to support the original interpretation of greater damage in the seismic crabs than reference crabs. Cell damage, consistent with physical trauma or stress from confinement or starvation, was observed in snow crab caged in both areas. A higher degree of cell damage was

observed in snow crab caged longer (5 months vs. 12 days), suggesting an effect of handling or caging. Reviewers of this work raised questions about the replicability of interpretations made in this study and requested a more rigorous statistical analysis of the data. These suggestions resulted in further studies, the results of which are reported in Chapter 5.

Correctly identified control slides.
Slides 267, 274 and 280.

Seismic exposed crab hepatopancreas.
Slides 269, 275 and 294 (all with mild abnormalities.

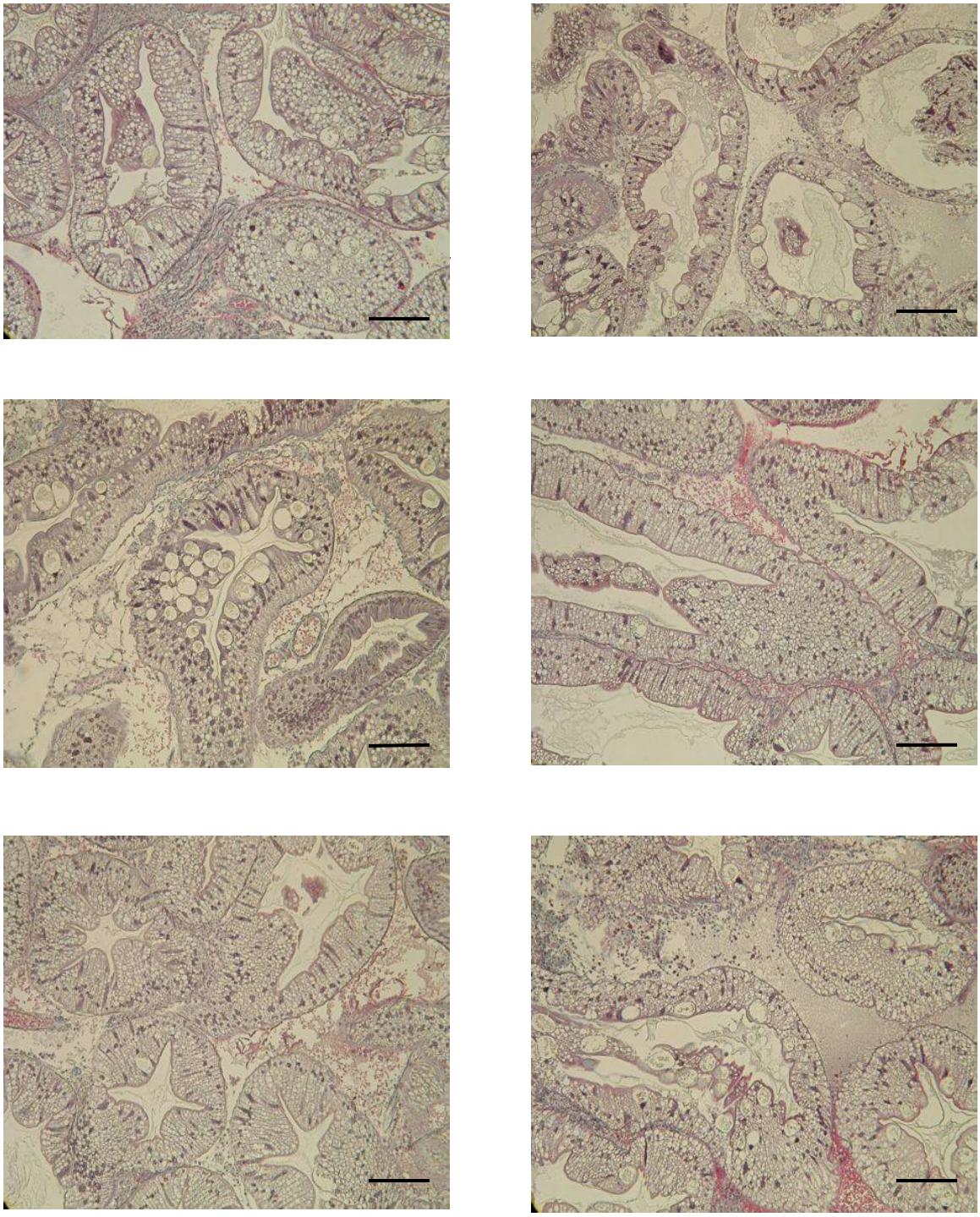

Figure 3.3: Hepatopancreas slides from snow crab caged for 12 days (Dec. 2003). 10x Obj. Bar = 250 um.

Correctly identified control slides of ovaries from caged crabs sampled Dec. 2003. Slides 137, 142 and 152.

Seismic exposed crab ovaries sampled Dec. 2003. Slides 138 (obviously pathological), 144 and 151 (apparently normal).

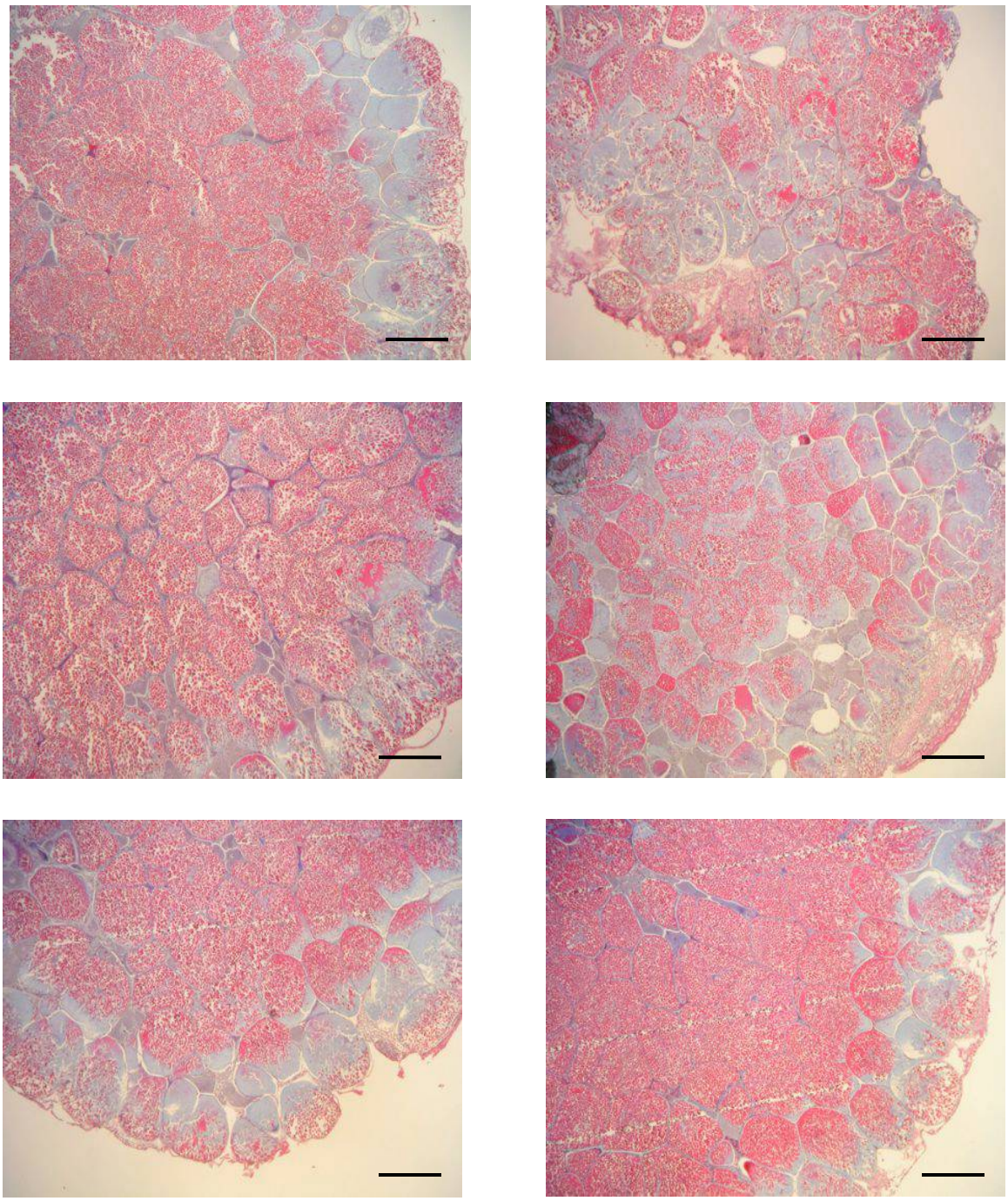

Figure 3.4: Ovarian slides from crabs caged for 12 days (Dec. 2003). 4x Obj. Bar = 500 um.

Control slides 300, 301 and 302.

Seismic slides 303, 305 and 309.

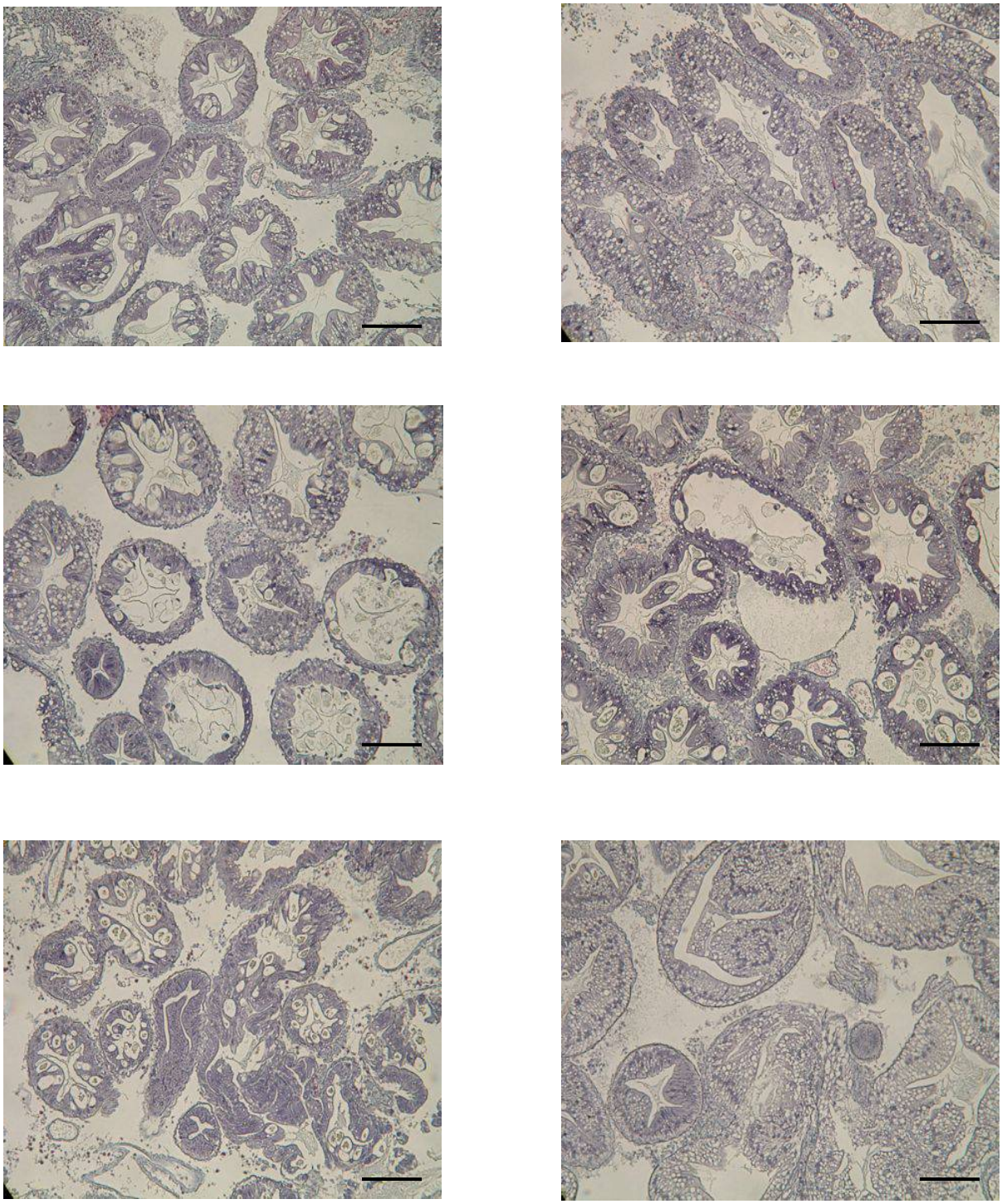

Figure 3.5**:** Hepatopancreas slides from crabs caged for 5 months (May 2004). 10x Obj. Bar = 250 um.

Correctly identified control slides of ovaries from caged crabs sampled May 2004. Slides 318, 326 and 335.

Correctly identified seismic exposed crab ovaries sampled. Slides 324, 329 and 351.

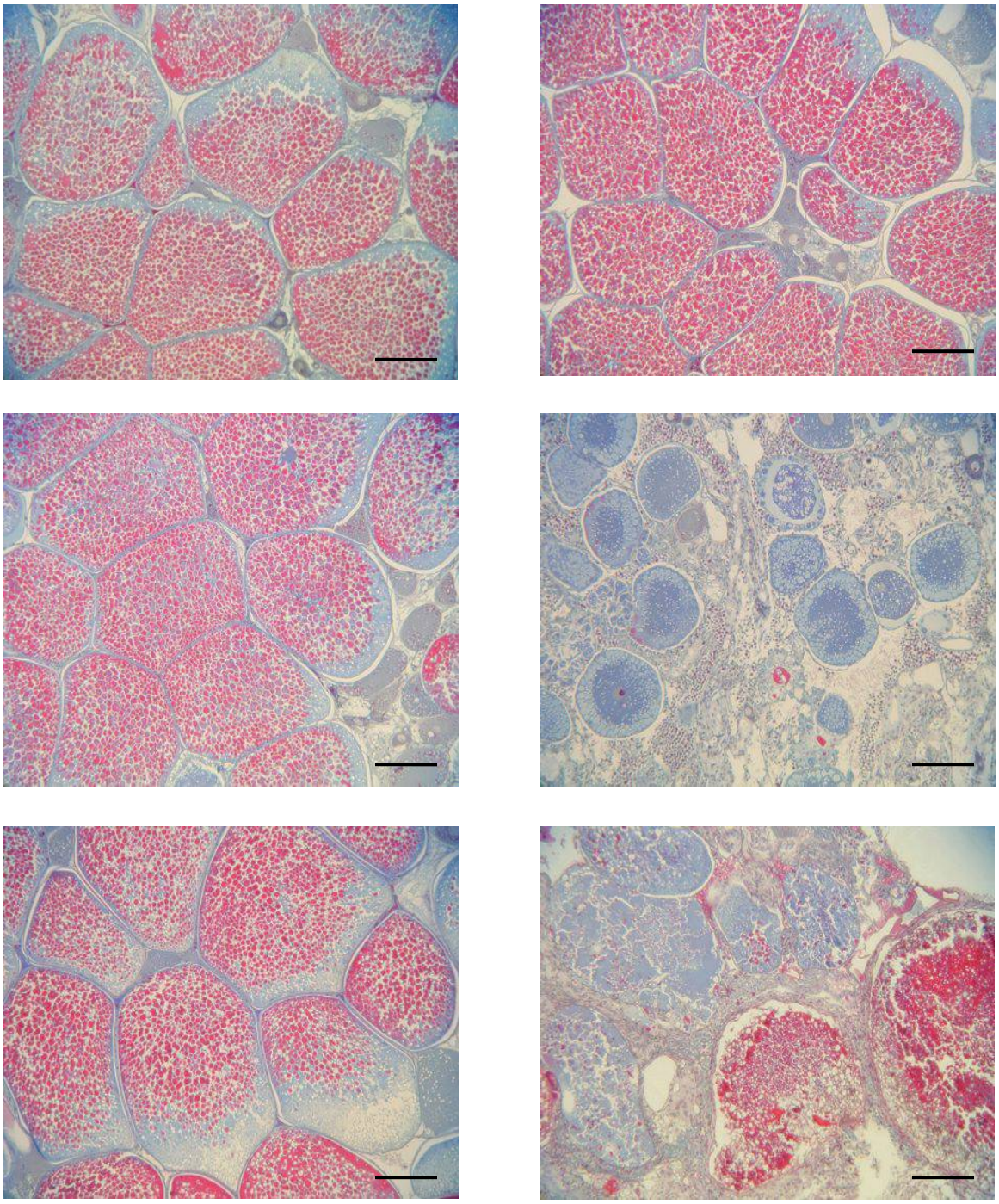

Figure 3.6: Ovarian slides from crabs caged for 5 months (May 2004). 10x Obj. Bar = 200 um.

CHAPTER 4. UPDATES ON RECOMMENDATIONS OF SEPTEMBER 2004 MEETING

This chapter reports progress on addressing three of the recommendations made during the September 2004 meeting (DFO, 2004). Included is the investigation of the origin of histopathological abnormalities observed in the hepatopancreas and ovary of caged crab through an additional caging experiment (4.1), the investigation of the hypothesis that exposure to seismic energy resulted in leg loss in caged crab (4.2) and the refinements of survival estimates for embryos carried by crabs caged in the original experiment (4.3).

4.1 External and histological observations of ovigerous snow crab female (*Chionoecetes opilio*) sampled in 2004 (from the wild and caged for 12 days)

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4.1.1 Background

Late in 2003 the Department of Fisheries and Oceans Canada (DFO) initiated a project to evaluate the possible impact of seismic activity on the physiology of multiparous snow crab female (*Chionoecetes opilio*). To carry out this project, animals were collected on the western coast of Cape Breton in December 2003 and caged at two sites: one within the area of seismic activity and a control. During the September 2004 meeting, results from the caging experiment for short- (12 days) and mid-term (five months) immersion periods showed that both the animals that were in the seismic testing site and the control site showed lesions of internal organs (DFO, 2004). Since the protocol did not account for an observation of multiparous females before they were caged, these lesions could not be attributed to the seismic activity. Hence it was decided to catch some snow crab off western Cape Breton in Dec. 2004, document the degree of histopathology of their hepatopancreas and ovary, and then cage some for a period of 12 days and see if this produced more abnormalities independent of any seismic exposure.

4.1.2 Materials and methods

4.1.2.1 Sampling

A total of 50 ovigerous female snow crab caught in the wild off western Cape Breton in the southern Gulf of St. Lawrence was brought to the GFC on December 01, 2004, for observations and dissections. On December 14, 2004, 100 females that were caged for 12 days, 50 females at the former seismic site and 50 females at the former control site, were brought to the laboratory for dissection.

The eyes, antennules, statocysts and gills from the 50 wild animals were dissected and observed under the stereomicroscope. The hepatopancreas of all females brought to the

laboratory (50 wild females and 100 caged females) were dissected for histological observations.

4.1.2.2 Histological techniques for the observations

The hepatopancreas was fixed in Bouin's fluid and stained with modified Masson's Trichrome (Gabe, 1968). For histological procedures, fixed tissues were dehydrated in a series of aqueous ethanol solutions, cleared in xylene agent, and then embedded in paraffin in a vacuum chamber. Blocks were sectioned serially at 5 μm on a rotary microtome. Two slides were prepared of each tissue (comprised of 5 serial sections), one was stained with Masson's Trichrome and the other was kept unstained. Final mounts were made with glass cover slips using a mounting resin. Light microscopic examinations were performed under an Olympus BX51 compound light microscope equipped with a color digital camera. The histological sections were photographed using bright field optics.

4.1.3 Results

4.1.3.1 External Observations

All the external observations were done on the appendices and organs dissected from wild animals collected in December 2004. For consistency, the same criteria used in the RAP report (Moriyasu et al., 2004) were used.

1) Eyes

Based on the stereomicroscopy observations of the eye (n = 50), the following external conditions were observed (Figure 4.1.1): 1) clean and intact (88%), 2) trace of sediment-like substance on the surface of eyes and intact, (6%), 3) one eye clean and other damaged (4%), and 4) eye with epibiont coverage (2%).

2) Antennules

The external condition of the antennules (i.e., the condition of the outer flagellum and aesthetasc hairs of the antennules) based on the stereomicroscopy observations (n = 49) were as follows (Figure 4.1.1): 1) clean and intact (96%), 2) intermediate with some dirtiness on the aesthetasc hairs and intact (0%), 3) clean and damaged (0%), and 4) damaged outer flagellum and/or aesthetasc hairs (4%)

3) Statocyst

The external condition of the statocyst (i.e., group hairs and statolith) based on the stereomicroscopy observations (n = 49) was classified into a single category (Figure 4.1.1): 1) clean (100%), 2) dirty (0%), 3) clean-absent (0%), and 4) dirty-absent (0%).

4) Gills

Based on the stereomicroscopy observations (n = 50), the external condition of the gills (i.e., degree of dirtiness among gill lamellae) was mainly classified into two of the three categories (Figure 4.1.2): 1) clean (36%), 2) intermediate (64%), and 3) dirty (0%).

4.1.3.2 Histological Observations of the Hepatopancreas

Histological observations were done on the hepatopancreas of the 50 females collected from the wild in Dec. 2004 (Figure 4.1.3) and 50 females caged for 12 days in Dec. 2004 in both the former control site (Figure 4.1.4) and the former seismic site (Figure 4.1.5). Based on the histological observations of the hepatopancreas, three distinct conditions were observed (Table 4.1.1):

1) Type-1 Epithelium of B-, R- and F-cells were uniform in shape and there was no apparent difference in the abundance of B- and R-cells (normal feature).
2) Type-2: Epithelium of B-, R-, and F-cells showing normal features, whereas some blood vessels showed light flow of blood.
3) Type-3. Epithelium of B-, R-, and F-cells showing normal features, whereas a massive hemorrhage was observed from a ruptured blood vessel.

4.1.4 Conclusion

The external observations of appendages for females collected in the wild in December 2004 showed that they were clean and in good condition. However, the histological observations of the hepatopancreas showed that the majority of those females had hemorrhage. Hence, the sample collected from the wild, with no treatment, already showed signs of internal anomalies with massive hemorrhage observed for 62% of those females. Their physiological condition (condition of the hepatopancreas in terms of hemorrhage) was not significantly different than those caged for 12 days at the former control and former seismic sites (Contingency table, $\chi^2_4 = 8.083$, $p = 0.089$). Also, females caged at the former control site showed the highest percentage of severe hemorrhage of the hepatopancreas. Therefore, it is not possible to draw any conclusion from the 2003 experiment because the protocol used (unknown status of the females before treatment) does not allow assessing the null hypothesis, i.e. a possible impact of seismic activity on snow crab females.

Table 4.1.1: Histological observations of the hepatopancreas of female snow crab (*Chionoecetes opilio*) collected in the wild in December 2004 (Wild), and caged for 12 days in Dec. 2004 in the same location as the 2003 seismic site (Caged – former seismic site) and its control (Caged - former control site). Type 1 characterized a normalhepatopancreas, while type 3 refers to a hepatopancreas with massive hemorrhage.

			Type	
Sample	N	1	2	3
Wild	47	10%	28%	62%
Caged – former seismic site	50	6%	20%	74%
Caged - former control site	49	0%	16%	84%

Contingency table test, $\chi^2_4 = 8.083$, $p = 0.089$

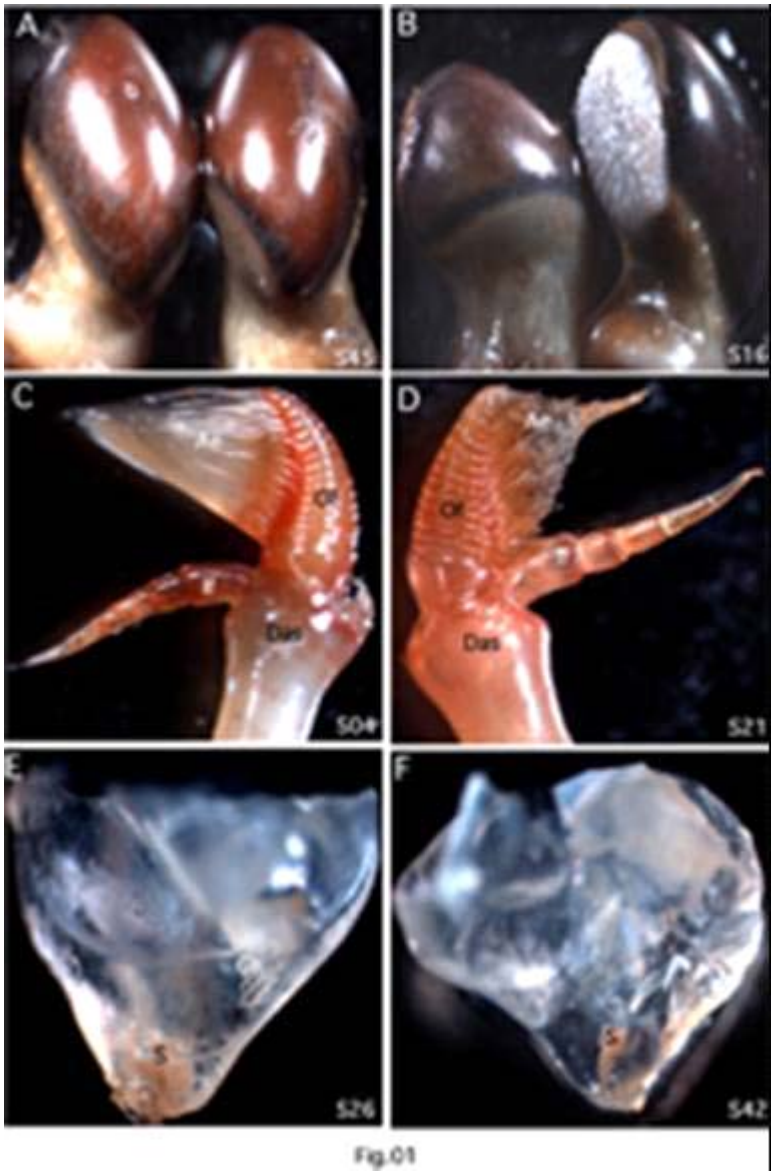

Figure 4.1.1: Stereomicroscopy micrographs showing the different conditions of eyes, antennules, and statocysts in female snow crab (*Chionoecetes opilio*) from the wild. (A) Clean surface of the eyes showing clean ommatidia and intact. (B) Surface of the eye covered by epibionts and one eye clean. (C) Clean antennules showing the distal segment with inner flagellum, and outer flagellum bearing aesthetasc hairs. (D) Dirty and broken damaged aesthetasc hairs of the antennules. (E & F) Clean statocyst with statolith.

Abbreviations are as follows: Ae =aesthetasc hair; Das = distal antennular segments; If = inner flagellum; Of = outer flagellum; Gh = Group hair; S = Statolith.

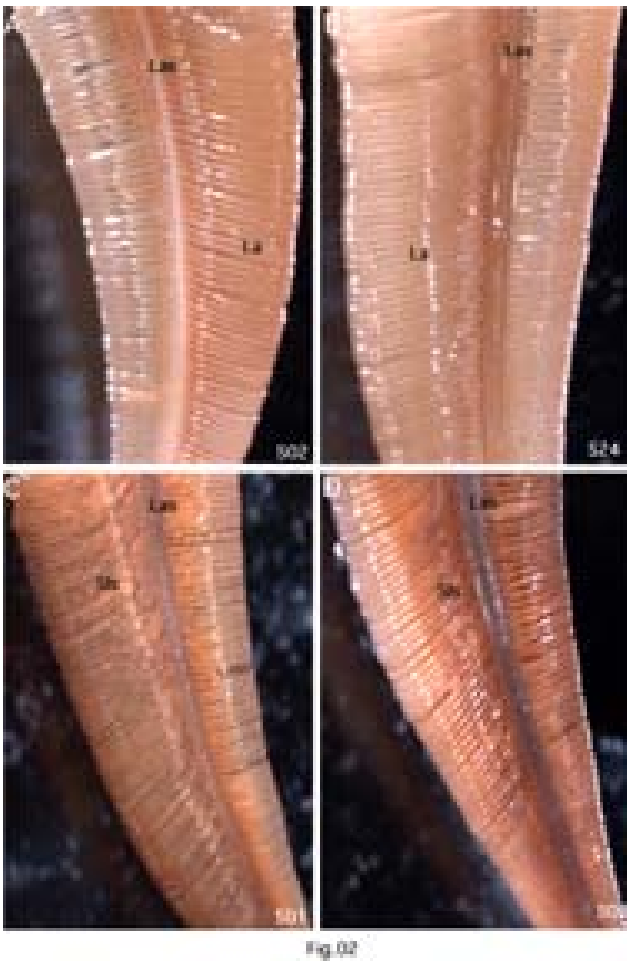

Figure 4.1.2: Stereomicroscopy micrographs of phyllobranchiate gills of female snow crab (*Chionoecetes opilio*) from the wild. (A & B) Clean lamellae. (C & D) Intermediate condition showing some dirtiness among gill lamellae.

Abbreviations are as follows: La = Lamella; Las = Lamellar septum; Sls = sediment-like substance.

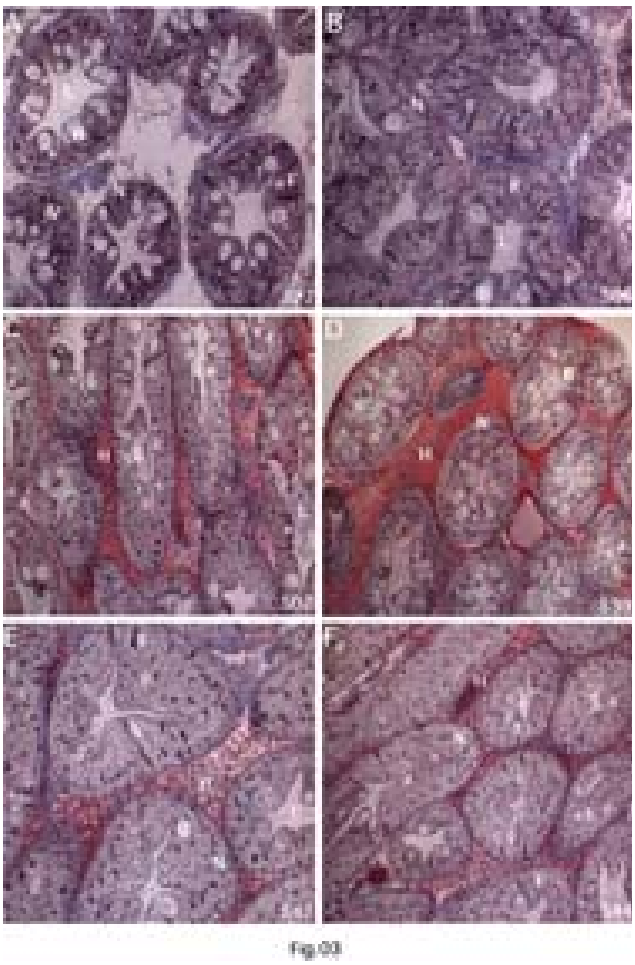

Figure 4.1.3: Light micrographs of a cross section of the hepatopancreas tubules for female snow crab (*Chionoecetes opilio*) collected from the wild. (A) Type-1, (normal feature): epithelium of B-, R-, and F-cells were uniform in shape. (B) Type-2, Blood vessel show light flow of blood. (C, D, E & F) Type-3, Massive hemorrhage is observed from a ruptured blood vessel.

Abbreviations are as follows: B = B-cells; F= F-cells; L= Lumen; N = Nuclei; R = R-cells; V = Vacuoles, H = Hemorrhages.

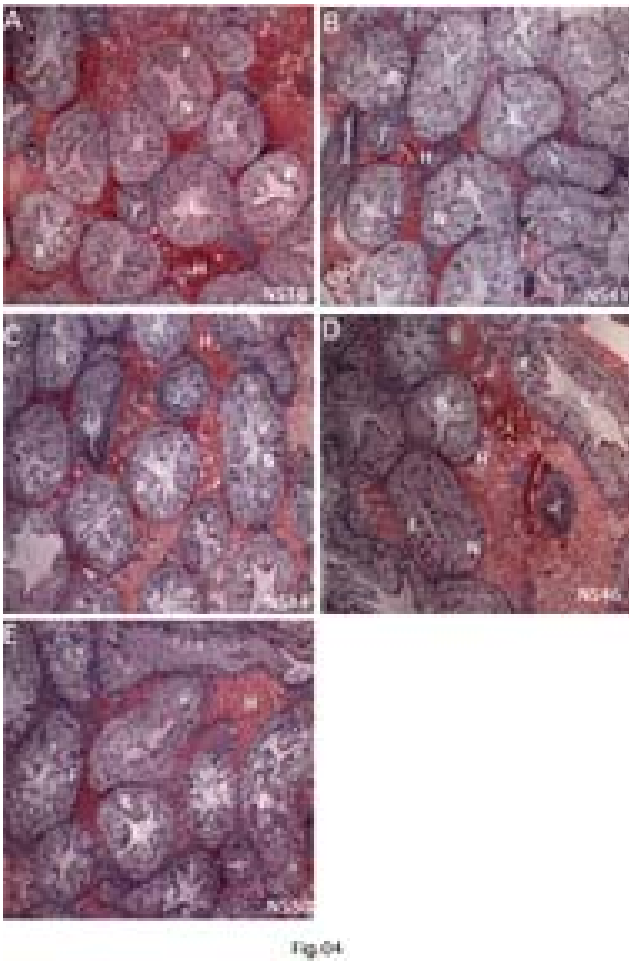

Figure 4.1.4: Light micrographs of a cross section of the hepatopancreas tubules for female snow crab (*Chionoecetes opilio*) caged 12 days in Dec. 2004 at the control site of the 2003 seismic study. (A, B, C, D & E) Type-3, massive hemorrhage is observed from a ruptured blood vessel.

Abbreviations are as follows: B = B-cells; F = F-cells; L = Lumen; N = Nuclei; R = R-cells; V = Vacuoles; H = Hemorrhages.

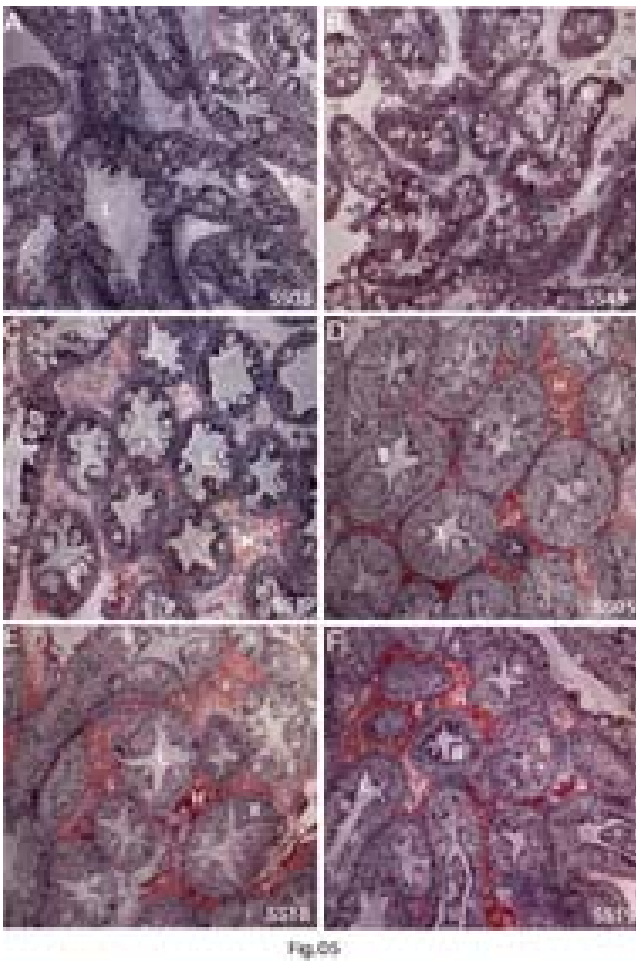

Figure 4.1.5: Light micrographs of a cross section of the hepatopancreas tubules for female snow crab (*Chionoecetes opilio*) caged for 12 days in Dec. 2004 in the seismic site of the 2003 seismic study. (A & B) Type-1, (normal feature): epithelium of B-, R-, and F-cells were uniform in shape and there was no apparent difference in the abundance of B- and R-cells. (C) Type-2, Blood vessel show light flow of blood. (D, E & F) Type-3, Massive hemorrhage is observed from a ruptured blood vessel.

Abbreviations are as follows: B = B-cells; F = F-cells; L = Lumen; N = Nuclei; R = R-cells; V = Vacuoles; H = Hemorrhages.

4.2 Investigation of leg loss in caged snow crabs following exposure to seismic energy

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4.2.1 Background

During the seismic testing of December 2003, egg-bearing female snow crabs were held in cages for 12 days in the seismic-exposed site and a control site 27 km northeast of the seismic testing. Some of these snow crabs were then sent to the Department of Fisheries and Oceans (DFO) in St. John's Newfoundland. They were held at the DFO facility from December 2003 to June 2004. During this period many of the 45 seismic-exposed snow crab lost one or more legs whereas the snow crab from the control site did not. The seismic exposed group lost a total of 26 legs during this period compared to only three in the control group. One of the recommendations of the September 2004 meeting was to submit information on snow crab leg loss for each of the facilities participating in the research program to determine if this occurred in all seismic-exposed groups or if it was an isolated incident in the crab sent to Newfoundland with an unrelated cause (DFO, 2004).

4.2.2. Materials and Methods

Following the seismic survey, the snow crab were sent to collaborating partners of the research program in three different research facilities, DFO facilities in St. John's (N.L.), Moncton (N.B.), and St. Francis Xavier University (SFX) in Antigonish (N.S.). No information was recorded on leg loss at SFX, therefore the comparison below is limited to N.L. and N.B. It is important to note that the N.L. and N.B. snow crabs were from different treatment groups. The ones housed at N.L. were caged for 12 days whereas the ones housed at N.B. were caged for 5 months.

4.2.3 Results and Discussion

There was no significant difference between crab caged at the seismic and control sites in number of missing legs upon arrival at N.B. (Table 4.2.1; Pearson Chi-square $\chi^2_3 = 5.602$; $p = 0.133$). Similarly, there was no significant difference between groups in number of missing legs at the end of the experiment at N.B. 47 days later ($\chi^2_3 = 4.695$; $p = 0.196$). Therefore, it is quite likely that leg loss in the seismic exposed group housed at N.L. was not caused by exposure to seismic sound, but was caused rather by something else such as rough handling of the seismic crabs in transit to N.L.

This hypothesis was supported in further studies done by Dr. J. Payne (N.L.), in which crabs were exposed to dB levels as high as 220dB, peak to peak, without leg loss difference. The most recent exposure was in December 2006 (200dB peak to peak), in which there was the loss of one leg in each of 34 control and 34 exposed crabs, one month post exposure.

Table 4.2.1: Number of leg loss in the reference and seismic-exposed crabs. Leg loss is presented as number of legs missing from an individual crab (from 0 to 3-4).

	At their arrival at GFC on May 12, 2004				
	0	1	2	3-4	Total
Reference	45	33	12	2	92
Seismic	37	27	22	5	91
Total	82	60	34	7	183
	At the end of the experiment on June 28, 2004				
	0	1	2	3	Total
Reference	37	35	15	4	91
Seismic	34	25	21	9	89
Total	71	60	36	13	180

4.3 Effects of seismic noise on survival, hatch and subsequent development of snow crab embryos

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4.3.1 Background

One of the main objectives of the December 2003 seismic research study was to address questions raised by Christian et al. (2003) about the effects of seismic energy on snow crab progeny. In December 2003, egg-bearing females were captured and caged within the seismic survey area and 27 km northeast of the survey to act as a control group. Some of the snow crabs were brought back 2 weeks (12 days) following the survey, while others stayed in their respective areas for 5 months, until May 2004. At this time, egg-bearing female snow crab were brought back to the Gulf Fisheries Center (DFO, Moncton, N.B.) and kept in the GFC aquarium facility during the hatch period, from May 16 to June 9. Eggs were collected and preserved for observation.

Initial results suggested that embryos from exposed females hatched later than control embryos, which resulted in smaller, less developed, embryos. During the September 2004 meeting (DFO, 2004), suggestions were made to refine the techniques employed to assess developmental stage. It was suggested that a better estimate of survival could be derived from distinguishing orange eggs from black eggs. Black eggs are eyed and survived to at least stage 11 of the 14 developmental stages described by Moriyasu and Lanteigne (1998). Orange eggs are less developed, may not have been fertilized, and are less likely to hatch. Also mentioned was that a better estimate of proportion of eggs, pre-zoea, and zoea in the control and seismic groups could be derived by taking subsamples: 1) from a shaken sample to keep the different stages mixed homogeneously (eggs sink); 2) using a 5 ml Stempel pipette (Van Guelpen et al., 1982) rather than simple disposable plastic pipette; 3) Obtain weights of each developmental stage for each of the seismic and control groups separately, and for orange and black eggs separately.

4.3.2 Materials and Methods

Samples were re-analyzed following these suggestions. The proportion of each developmental stage (orange eggs, black eggs, pre-zoea and zoea) was determined in all 17 samples collected, with a 5 ml Stempel pipette. The mean weight of each stage was determined by weighing a group of 100 individuals of each stage separately, for 5 samples.

4.3.3 Results

Results indicated that progeny from seismic-exposed females developed at a slower rate than the control group. In the seismic group, there was a smaller proportion of zoea larvae, a larger proportion of pre-zoea and unhatched eggs ($\chi^2_3 = 820$, $p < 0.0001$), and the pre-zoea and zoea were lighter (pre-zoea: $F_{1,8} = 5.43$, $p = 0.0482$; zoea: $F_{1,8} = 8.87$, p

= 0.0176; Table 4.3.1). The mean proportion of the different life stages (Table 4.3.1) for each group and the mean weight of each life stage (Table 4.3.1) indicates that an equal number of progeny from the two groups would be expected to differ in weight by 23% - close to the 22% difference in weight observed (Figure 4.3.1). Therefore it appears that there was little difference in total number of progeny from the two groups, but that rate of development was slower in seismic than control embryos (later hatch, more pre-zoea and fewer zoea in seismic than control group), which could be related to seismic energy or to cooler temperature at the seismic than control site. Interestingly, Christian et al. (2003) reported delayed embryonic development in snow crab eggs experimentally exposed at short range (2 m) to 200 shots from a 40 $in.^3$ air gun delivering sound levels of about 216 dB re 1 µPa. Overall, results indicate that exposure to seismic energy did not kill snow crab embryos (87% survival in the seismic group including black eggs, pre-zoea and zoea compared to 89% in controls).

Table 4.3.1: Comparison of the weight of egg and larval stages for the original and newly analyzed data. Stages compared include for the original data, eggs (both orange and black eggs), pre-zoea larvae and zoea larvae. For the new data, orange eggs (EggsO) and black eggs (EggsB), pre-zoea larvae and zoea larvae. Mean weight was calculated from 100 individuals of each developmental stage separately in 5 different samples. The mean proportion of each stage was determined for each of the 17 samples collected.

Stage	**Original data**						Difference	%
	Control			**Seismic**				
	Weight	Proportion	Product	**Weight**	Proportion	Product		
Eggs	**0.046**	13	0.0060	**0.046**	20	0.0092		
Pre-Z	**0.031**	45	0.0140	**0.031**	57	0.0177		
Zoea	**0.090**	42	0.0378	**0.090**	23	0.0207		
Sum			0.0577			0.0476	0.0102	**18**
Survival w/o Eggs		**87**			**80**			

	New data							
	Control			**Seismic**			Difference	%
	Weight	Proportion	Product	Weight	Proportion	Product		
EggsO	0.0135	11.2	0.0015	0.0124	13.2	0.0016		
EggsB	0.0150	4.6	0.0007	0.0163	8.1	0.0013		
Pre-Z	0.0255	35.7	0.0091	0.0225	48.4	0.0109		
Zoea	0.0314	48.6	0.0153	0.0217	30.3	0.0066		
Sum			0.0266			0.0204	0.0061	**23**
Survival w EggsB		**89**			**87**			

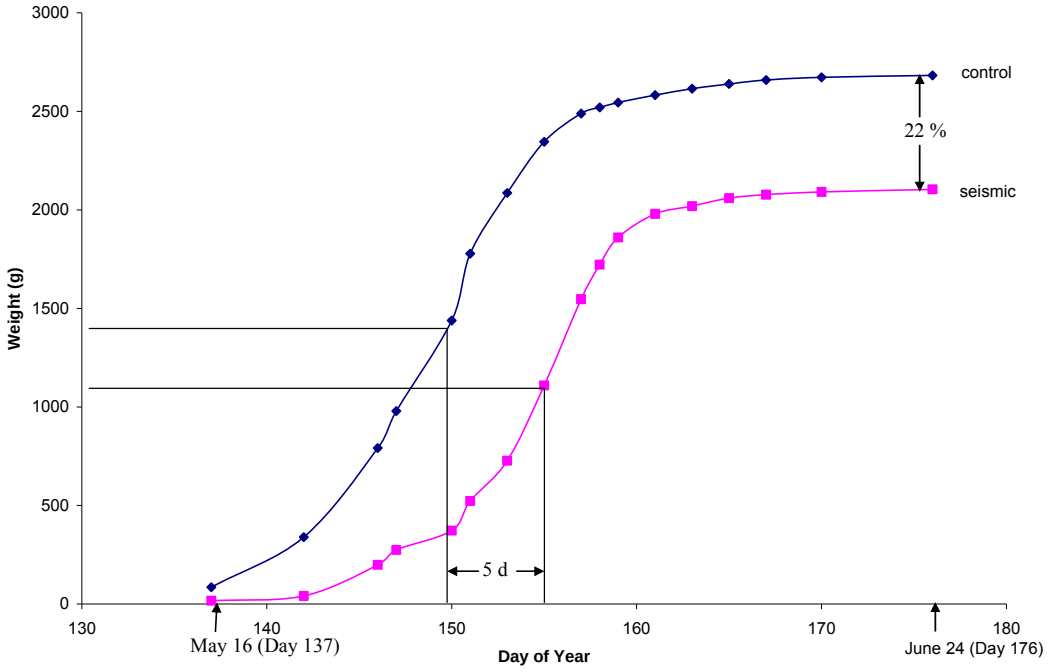

Figure 4.3.1: Cumulative weight of snow crab larvae, eggs, and embryos collected from filters of tanks holding multiparous female snow crab exposed to seismic energy (seismic) or not exposed (control). Filters were monitored between May 16 (Day 137) and June 24, 2004 (Day 176).

4.3.4 Conclusions

1. Exposure to seismic energy did not kill snow crab embryos (87% survival in the seismic group including black eggs, pre-zoea and zoea compared to 89% in controls).

2. Female snow crab caged in the seismic site had a similar number of offspring (eggs, pre-zoea and zoea) to female snow crab caged in the control site

3. Rate of development was slower in seismic than in control embryos (later hatch, more pre-zoea and fewer zoea in seismic than control group), which could be related to seismic energy or to cooler temperature at the seismic than control site.

4.4 Chapter summary

Mature female snow crab were captured off western Cape Breton Island and caged for 12 days in December 2004 - one year after the seismic survey - to test the hypothesis that the apparent bruising of the hepatopancreas observed in the 2003 caged animals was due to handling and caging rather than exposure to seismic energy. A similar hemorrhaging in the hepatopancreas was observed in the crab captured in 2004 even before they were caged and caging did not alter this condition. The origin of this condition is unknown. It was also noted that the snow crab collected for this experiment did not have sediment in their gills, eyes, antennules or statocysts, as had been observed in the snow crab caged in the seismic site in 2003.

The hypothesis that exposure to seismic energy caused a loss of legs in the crab transported to the Northwest Atlantic Fisheries Centre in 2003 was addressed by examining leg loss in crab transported to the Gulf Fisheries Centre. Leg loss among the Gulf Fisheries Centre crab was uncommon and not different between crab caged 12 days at the seismic site and reference site. In addition, subsequent experiments carried out at the Northwest Atlantic Fisheries Centre failed to produce leg loss in snow crab exposed to higher sound energy levels than had been encountered by crab in the 2003 caging experiment. Therefore it appeared that leg loss observed at the Northwest Atlantic Fisheries Centre was not related to seismic energy and may, instead, have been related to rough handling of some crab during transport.

Refinements to the quantification of snow crab larvae resulting from the embryos being carried by female snow crab caged in 2003 did not change the original interpretation, namely that there was no difference in the survival of embryos being carried by females caged in the seismic area versus reference area. Both groups showed a very high rate of survival (87-89%). However, the seismic embryos developed somewhat more slowly than control embryos, which could be related to seismic energy (as suggested in a previous experiment by Christian et al. 2003) or to cooler temperatures at the seismic than control site. Unfortunately, temperatures were not measured at the 2003 caging sites.

CHAPTER 5. UPDATE ON ACTION ITEMS - PROGRESS ON SNOW CRAB SEISMIC FILE SINCE THE JANUARY 23, 2007 MEETING AT GULF FISHERIES CENTRE

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The following section is an update on progress towards the action items identified at the 23 January meeting. There were three specific recommendations:

1) Examine replicability of histological readings done in Dr. Lucy Lee's lab
2) Carry out statistical analysis of histology data collected by Dr. Lucy Lee
3) Estimate sound pressure levels at the sites of the experimental cages, if possible

Also included are the results of supplemental comparisons of the hepatopancreas and ovary of female snow crab from a subsequent experiment in December 2004.

5.1. Recommendation 1: A statistically valid subsample of slides should be re-read by another trained observer to measure replicability of the measures recorded.

5.1.1 Progress

The original 407 hepatopancreas slides and 352 ovary slides were read by student Michael Wright under the supervision of Dr. Lucy Lee (Dept. Biology, Wilfrid Laurier University). Of these, slightly less than half (165 hepatopancreas and 151 ovary from 5 boxes of each tissue selected randomly from the 10 boxes available (box numbers 1, 3, 8, 9 and10 for hepatopancreas; box numbers 2, 4, 5, 6 and 10 for ovaries)) were reread by student Cheryl Lay under the supervision of Dr. Lucy Lee between February and April 2007. Both students analyzed the slides for the same histopathologies, of which there were 11 for hepatopancreas (number and positioning of M cells; nuclear shape; nuclear size; epithelial wall thickness; R-cells; phagocyte activation; encapsulation and parasites; delamination of basal membrane; peritrophic membrane / luminal contents; necrosis or autolysis; collagen) and 9 for ovaries (encapsulation; typical stain pattern; packing density; edema; atresia; bruising; granulocytic inflation; delayed maturity; delamination). For each slide, the severity of each pathology was recorded on a scale of 1-5 where 1 is normal and 5 abnormal. The scores for the 11 or 9 pathologies are then totaled to give an overall index of abnormality (sum).

Dr. Lee carried out a non-parametric Spearman rank correlation analysis between summed abnormality scores for the two datasets and Dr. Robert Gebotys (Department of Psychology, WLU) looked at correlation between readings for individual endpoints as well as the sum by Pearson correlation. Dr. Robert Gebotys' report is included in Appendix 3.

5.1.2 Ovary scores

The two datasets were significantly correlated ($r = 0.5618$, $p < 0.0001$, $n = 151$; Spearman rank correlation) with very similar mean total scores between the original (15.334) and reanalyzed (15.652) datasets. These means are not significantly different ($p = 0.1164$, Wilcoxon matched-pairs signed-ranks test). By Pearson correlation, all 9 endpoints correlated significantly between the two observers ($r = 0.3 – 0.8$; $p < 0.01$, summed endpoints $r = 0.8$, $p < 0.01$) except bruising ($r = 0$) (Appendix 3 m).

5.1.3 Hepatopancreas scores

The two datasets were significantly correlated ($r = 0.5354$, $p < 0.0001$, $n = 165$ Spearman rank correlation) though Cheryl's total abnormality scores were significantly higher than Mike's (24.194 vs. 19.006, $p < 0.0001$ Wilcoxon matched-pairs signed-ranks test). Dr. Gebotys' Pearson correlations of the 11 endpoints measured by the two observers varied from 0 – 0.7 with significant, positive correlations for 6 endpoints: epithelial wall thickness, R-cells, encapsulation and parasites, basal membrane delamination, necrosis or autolysis, collagen and the sum of all measures ($r = 0.6$) ($p < 0.01$ for all except $p < 0.05$ for epithelial wall thickness; Appendix 3 m).

Dr. Gebotys concluded that, in general, reliability of the data was acceptable and the ovarian data were more reliable than the hepatopancreas data.

5.1.4 Conclusions

Variance was noted between the histological readings of two different observers, but their readings were significantly correlated demonstrating acceptable replicability.

5.2. Recommendation 2: It is recommended that a statistician be engaged to conduct further quantitative analyses of these data before conclusions are drawn.

5.2.1 Progress

Dr. Robert Gebotys (Department of Psychology, Wilfrid Laurier University) was contracted by COOGER to carry out a multivariate analysis (MANOVA followed by univariate ANOVA on individual measures when warranted) of the original histopathology data including:

1) Ovary and hepatopancreas tissue from crab caged short-term (12 days in December 03) at the seismic site and control site
2) Ovary and hepatopancreas tissue from crab caged long-term (5 months, December 2003 – May 2004) at the seismic site and control site.

Dr. Robert Gebotys' report is included in Appendix 3.

5.2.2 Short-term caging

Hepatopancreatic results: There was a very significant difference between crab caged short-term at the seismic and control sites (MANOVA, Pillai's trace = 0.186, $F_{11, 118}$ = 2.448, P = 0.009; Appendix 3a). Three measures showed significantly higher means (i.e., more abnormality) in the control group than seismic group: epithelial wall thickness (seismic mean = 1.76 vs. control mean = 2.25), R cells (1.28 vs. 1.86) and necrosis (2.09 vs. 2.39) (univariate ANOVA; Appendix 3a).

Ovarian results: There was a very significant difference between the two groups (MANOVA, Pillai's trace = 0.451, $F_{9, 65}$ = 5.94, P < 0.001; Appendix 3b). Controls showed significantly more abnormality than seismic in six endpoints: atresia (seismic mean = 1.54 vs. control mean = 1.86), packing density (1.26 vs. 2.03), granularity (1.34 vs. 2.34), bruising (1.49 vs. 2.54), delayed maturation (1.25 vs. 2.23) and stain pattern (1.24 vs. 2.19) (univariate ANOVA; Appendix 3b).

5.2.3 Long-term caging

Hepatopancreatic results: There was a very significant difference between the two groups (MANOVA, Pillai's trace = 0.121, $F_{11, 166}$ = 2.08, p = 0.024; Appendix 3c). Four measures differed significantly between groups: nuclear shape (seismic mean = 1.51 vs. control mean = 1.34), R cells (2.06 vs. 2.55), membrane (1.99 vs. 2.39) and delamination (1.38 vs. 1.57) (univariate ANOVA; Appendix 3c). In all cases, except for nuclear shape, control crabs showed a higher degree of abnormality than seismic crabs.

Ovarian results: There was a very significant difference between the two groups (M, Pillai's trace = 0.173, $F_{9, 166}$ = 3.85, P < 0.001; Appendix 3d). Controls showed significantly more abnormality than seismics in delamination (seismic mean = 1.83 vs. control mean = 2.37) and stain pattern (1.37 vs. 1.65) (univariate ANOVA, Appendix 3d).

5.2.4 Conclusions

1. Crabs caged short-term (12 days in December 2003) at the seismic site showed fewer abnormalities of the hepatopancreas and ovary than crabs caged for the same time period at the control site.
2. This was also true for crab caged long-term (5 months from December 2003 – May 2004) except for one measure (nuclear shape) in one organ (hepatopancreas), which was more abnormal in seismic than control crab.

5.3. Recommendation 3: Sound pressure levels at the site of the experimental cages themselves should be estimated if possible.

5.3.1 Progress

This work was completed and incorporated into Chapter 1.

5.4. Supplemental Comparisons for the December 2004 experiment

5.4.1 Progress

Michael Wright was rehired in 2007 to analyze tissues provided by Kadra Benhalima, Michel Comeau and Mikio Moriyasu from a subsequent experiment. In this subsequent experiment, snow crab were caged for 12 days in the same two places exactly one year after the original experiment. The objective of this experiment was to determine whether differences between the two caging sites, rather than exposure to seismic energy, could produce differences in the condition of the hepatopancreas and ovaries. 150 crab were caught at the former seismic area and divided into three groups of 50 each. One group was sampled before the caging to assess tissue condition before caging and the other two groups were caged at the former seismic and control sites respectively. This experiment replicated only the short-term caging (12 days in December); the long-term 5 month caging was not replicated. Kadra's analyses of hepatopancreas tissue from these crabs revealed damage in all three groups, but no significant difference in type or degree of damage among the three groups (Chapter 3, section 3.1). Michael Wright analyzed the tissues for the same endpoints described for the original experiment.

Dr. Gebotys compared the data gathered from the three groups from the December 2004 experiment (caged at the former seismic site, caged at the former control site, and not caged). For each tissue, the summed abnormalities were compared among treatments by univariate ANOVA and the abnormalities were all examined in a MANOVA. In addition, Dr. Gebotys compared the three groups of the December 2004 experiment to the crabs in the original experiment (short and long-term caged at each of the seismic and control sites for a total of 4 groups) to determine whether the degree of abnormality was similar in both experiments.

5.4.2 Ovary comparisons

a) December 2004 Experiment

There was no difference in degree of abnormality among the three treatment groups (uncaged, caged at former seismic site, caged at former control site; n = 50 for each) when looking at the sum of all abnormalities (ANOVA $F_{2, 147} = 0.234$, $p = 0.791$. However, differences were found among treatments when the 9 types of abnormality measured were considered separately (MANOVA, Pillai's Trace = 0.208, $F_{18, 280} = 1.802$, $p = 0.025$). Univariate tests of between treatment effects showed a higher degree of encapsulation in crabs caged at the former seismic site than in crabs caged at the former control site or crabs not caged at all ($F_{2, 147} = 3.263$, $p = 0.041$).

b) December 2004 vs. original experiment (December 2003 - May 2004)

In general, crabs from the December 2004 experiment showed a significantly lower summed abnormality than crabs caged either short-term or long-term in the original 2003 experiment ($p < 0.001$, Mann-Whitney test on summed abnormalities – Lee; and

Pillai's trace = 0.778, $F_{54, 2346} = 6.477$, $p < 0.001$; MANOVA on individual endpoints – Appendix 31).

5.4.3 Hepatopancreas comparisons

a) December 2004 Experiment

There was no difference in degree of abnormality among the three treatment groups (uncaged, caged at former seismic site, caged at former control site; n = 50 for each) when looking at the sum of all abnormalities (ANOVA $F_{2, 193} = 0.234$, $p = 0.791$; the F and p are identical to those for the ovary analysis by coincidence). However, differences were found among treatments when the 11 types of abnormality measured were considered separately (MANOVA, Pillai's Trace = 0.282, $F_{22, 326} = 2.428$, $p < 0.001$). Univariate tests of between treatment effects showed a difference between crabs that were caged and not caged. Crabs that were caged at either of the two sites showed a higher degree of abnormality than uncaged crabs in nuclear shape ($F_{2, 172} = 4.023$, $p = 0.020$), nuclear size ($F_{2, 172} = 5.998$, $p = 0.003$) and peritrophic membrane / luminal contents ($F_{2, 172} = 3.522$, $p = 0.032$), but a lower degree of delamination of the basal membrane ($F_{2, 172} = 11.849$, $p < 0.001$).

One possible interpretation of these results may be that some abnormalities are produced by catching and handling crabs (delamination of basal membrane), which heal quickly, and are therefore less apparent in crabs caged for 12 days than in crabs examined immediately after capture. Other abnormalities though (nuclear size and shape, peritrophic membrane / luminal contents) may be produced or enhanced by caging, and are therefore more apparent in caged than uncaged crabs.

b) December 2004 vs. original experiment (December 2003 - May 2004)

In general, crabs from the December 2004 experiment showed a significantly lower summed abnormality than crabs caged either short-term ($p < 0.05$) or long-term ($p < 0.001$) in the original 2003 experiment (Mann-Whitney test on summed abnormalities – Lee; and Pillai's trace = 0.610, $F_{66, 2826} = 4.842$, $p < 0.001$; MANOVA on individual endpoints – Appendix 3k) .

5.4.4 Conclusions

1. With one exception (encapsulation in ovary tissue) crabs caged at the former seismic site did not show increased abnormalities of the hepatopancreas or ovary relative to crabs caged at the former control site, suggesting that any differences observed between these sites in the original experiment were not due to characteristics of the sites themselves such as depth, sediments, currents etc.
2. Some abnormalities of the hepatopancreas may be produced or enhanced by catching or handling of crabs (delamination of basal membrane) and others may be produced or enhanced by caging (nuclear size and shape, peritrophic membrane / luminal contents)
3. Wild and caged crabs from the December 2004 experiment showed a lower degree of abnormalities than crabs caged in the original December 2003 experiment, suggesting that the latter were subjected to greater stress. Furthermore, the moving of the crabs from the collection site (seismic) to the control site caused further stress, reflected in the

significantly different pathologic conditions seen in the crab caged both short-term and long-term at the control site than seismic site in the original experiment.
4. Overall, it would appear that handling stress (including fishing and caging) produced the abnormalities observed
5. If exposure to seismic energy produced any abnormalities in the hepatopancreas or ovary, it was not detectable over and above the effects of handling stress

5.4.5 Postscript - Jerry Payne - St. John's Newfoundland - December 2008

Payne et al (2008) recently carried out an update on the effects of seismic on fish and shellfish. The update was carried out for a workshop on seismic held by DFO in March, 2007. Regarding the Cape Breton study on snow crab, the following was stated: "evidence supporting a hypothesis that the various effects observed were due to normal variability (and not due to seismic)" has also recently been obtained from an ESRF supported study in Newfoundland (DFO, N.L. Region, unpublished). Female crab were exposed to higher sound levels than those measured at the test site in the Cape Breton study and maintained in the lab for several months. No difference was observed with respect to mortality, leg loss, egg loss or hepatopancreas and ovary histopathology. The results support the earlier preliminary study on snow crab carried out by Christian et al. (2003).

5.5 Chapter summary

Subsequent to the January 23, 2007, review meeting held at the Gulf Fisheries Centre additional work was done to address three suggestions made by reviewers. The first was to confirm the replicability of histological interpretations made by the independent histopathologist. Re-reading of a subset of samples by another observer demonstrated acceptable replicability. The second suggestion of reviewers was that the data reported by the independent histopathologist be subjected to rigorous statistical analysis to support interpretation. This analysis was carried out, and confirmed the interpretation made by the independent histopathologist (Chapter 3). While snow crab caged at both the seismic site and reference site in 2003 showed abnormalities of the hepatopancreas and ovary, there were actually fewer abnormalities observed at the seismic site. These results are inconsistent with the abnormalities having been caused by exposure to seismic energy. The third suggestion of reviewers was that sound levels actually encountered by crab caged during the December 2003 seismic survey be estimated. This work was done and was reported in Chapter 1.

In addition, hepatopancreas and ovary tissues from crab caged in the seismic area and reference area one year after the seismic survey (i.e., caged in December 2004) were reanalyzed by an independent histopathologist and the data were subjected to rigorous statistical analysis. This reanalysis confirmed abnormalities in the hepatopancreas and ovaries of snow crab caged at the two sites in 2004, and in crabs sampled before caging. Overall, it would appear that handling stress (including fishing and caging) produced the abnormalities observed.

Adapted from Boudreau et al. 2009. Proceedings Tech Report.

CHAPTER 6. CONCLUSIONS, QUESTIONS REMAINING AND NEXT STEPS

RESPONSES TO THE TOPICS OF CONCERN

1. Despite the distance between sites (23 km), snow crab caged outside of the seismic survey area (i.e., controls) were still exposed to some degree of seismic sound pressure (approximate maximum root-mean-square (RMS) sound pressure received was 118 dB re µPa, compared with 178 dB re µPa within the seismic area).
2. The cause of sediment fouling of gills, eyes, antennules and statocycts in crab caged for 12 days in the seismic survey area is not known. An experiment conducted in May 2004 showed that this effect was not produced by short-term (two hours) dragging of snow crab in mesh bags, suggesting that dragging of the cages in the original 2003 experiment might not have been the cause. Moreover, this fouling appeared to be short-term in nature and did not appear to impact long-term survival. It was not observed in snow crab recovered from cages five months after the 2003 survey, and was not observed in snow crab caught in the area one year after the survey.
3. No changes in snow crab abundance or distribution due to the seismic survey could be resolved through analysis of current stock assessment data. However, current stock assessment methodologies do not have the resolution to show statistically significant changes in the levels of snow crab distribution or abundance from the seismic survey operations above that of natural variation.
4. Independent, blind, verification by an external histopathologist confirmed the presence of abnormalities in the hepatopancreas (liver equivalent) and ovary of female snow crab caged during the December 2003 experiment. However, these abnormalities did not appear to have been caused by exposure to seismic energy. Analyses of the data by a statistician indicated that abnormalities were no more common at the seismic site than reference site, and in most cases were actually less common. The fact that abnormalities were more prevalent in crab caged for 5 months than 12 days suggested that they might be related to stress of handling and caging. Similar abnormalities observed in crab caught off western Cape Breton Island one year after the seismic survey suggest that this may have been a pre-existing condition in female snow crab of this population.
5. It does not appear that exposure to seismic energy resulted in leg loss initially reported by DFO-NFLD scientists after the December 2003 caging experiment. While snow crab sent to DFO-NFLD showed a higher rate of leg loss among the seismic-exposed than reference group, this was not observed in snow crab from the same field study sent to DFO-NB. Furthermore, a subsequent experiment at DFO-NFLD failed to produce leg loss in snow crab exposed to dB levels as high as 220 dB. Instead, it was suggested that rough handling of the box containing the

6. Exposure to seismic energy did not kill snow crab embryos (87% survival in the seismic group including black eggs, pre-zoea and zoea compared to 89% in controls). Larvae carried by crab caged at the seismic site were less heavy and less developed (smaller proportion of zoea larvae, a larger proportion of pre-zoea and unhatched eggs) than larvae carried by crab caged at the control site, which can have important consequences upon long-term survival. However, temperature differences are known to have occurred at these two sites, and slower development may have resulted from lower incubation temperatures during caging at the seismic site than the control site.

SOURCES OF UNCERTAINTY

During the initial 2004 analysis of study results, a number of constraints associated with the study design were identified. These constraints are equally applicable to the analysis presented here and are reiterated.

Test and control sites were quite different in temperature, substrate and food availability, and these factors made it difficult to clearly interpret the results. The test site was colder, shallower and may have had sediments with elevated levels of organic material as compared to the control site. Temperature is an important variable controlling development, metabolism and healing of marine animals. In addition, due to the order of crab capture, caging, and redeployment (i.e. the samples were not randomized) in light of weather conditions and safety of operations during the experimental period, the snow crab placed at the control site were slightly larger than crab at the test site despite both groups originating from the same place and time.

Logistical and safety constraints limited the 2003 study to one test and one control site. Furthermore, based on the results of a previous study by LGL Limited (Christian et al. 2003) that raised concerns about reproduction, the study was focused on mature female snow crab. The LGL study found no effects of seismic energy on behaviour, health or catch rates of adult snow crab, but the eggs of one female showed significant developmental retardation after experimental exposure at very close range (2 m).

No pre-seismic data were collected on the condition of snow crab in Area 19 to compare to post-seismic crabs. This lack of baseline information made it difficult to determine whether the sublethal pathologies observed post-seismic were the result of exposure to seismic energy, or were already present in the population. However, the study did provide histopathological data upon which to build and showed the way for development of a more detailed study, which has since been proposed.

While sound pressure levels were measured near the crabs caged within the seismic and control areas, particle motion produced by the seismic device, which may also affect animals, was not measured. In addition, animals in the short- and medium-term

experiments received different levels of exposures of seismic energy. Forty-two hours of seismic testing were done after retrieving animals for the short-term experiments. It should be noted that the seismic array was a comparatively small one, and that the control site received some seismic exposure, though considerably less than the experimental site.

CONCLUSIONS

The 2003 caging study provided some definitive findings on the potential effects of seismic energy on snow crab (e.g., no immediate mortality). Nevertheless, some questions remain due to confounding factors such as differing environmental condition and handling/caging procedures that may account for the differences observed between snow crab that were caged in close proximity to a seismic survey and snow crab that were caged at a "control" location. Subsequent studies and analysis have been unable to separate the influence of these confounding factors from the potential impacts resulting from exposure to seismic noise. Study design limitations (e.g., stress from capture and caging animals) suggest that further analysis of our experimental results obtained to date is unlikely to provide additional information or insight. The 2003 study clearly demonstrated the importance of careful experimental design, the need for the development of environmental effects monitoring test protocols, and the need for pre-seismic baseline data. Several additional studies have been recommended to assess the impact of seismic surveys on invertebrate species of concern in Atlantic Canada. These include:

- Investigation of the natural histopathology of snow crab hepatopancreas and ovaries and further exploration of the reason(s) for observed "abnormalities."
- Investigation of the use of spatial analytical tools for detecting impacts of non-fisheries related activities using fisheries and DFO survey information.
- Investigation of the influence of incubation temperature on larval development of snow crab.

It is also recommended that future experiments related to the effects of seismic noise on invertebrates include measurement of particle motion in addition to sound pressure levels.

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APPENDIX 1. SCORES OF THE HISTOPATHOLOGICAL ANALYSIS OF SNOW CRAB HEPATOPANCREAS FROM CONTROL OR SEISMIC EXPOSED SITES, AND MATCH BETWEEN THE BINARY (BLIND) CODING AND THE CODED KEY (ORIGINAL CODING).

Table 1: Scores of the histopathological analysis of the hepatopancreas of snow crab from control or seismic exposed sites. Pathological scores lower than 17 are considered normal (green), 17 to lower than 20 represent mild changes (yellow), 20 to 24 illustrate moderate changes (orange) and scores greater than 24 indicate extreme changes (red). The binary coding is the coding that was assigned to each slide during the blind evaluation. Also included are the coded key (original or real code) and the match between the binary coding and coded key.

Slide #	Pathological score	Binary coding	Coded key	Match	Box #	Date sampled				
8	10.5	1	1	1	1	2003-Dec				
53	11	1	2	0	2	2004-Dec	normal=1	abnormal=2	total	
57	11	1	1	1	2	2004-Dec	173	234	407	
82	11	1	2	0	2	2004-Dec	42.5061	57.493857		
385	11	1	2	0	12	2004-May				
10	11.5	1	1	1	1	2003-Dec				
127	11.5	1	2	0	4	2003-Dec				
348	11.5	1	2	0	10	2003-Dec				
360	11.5	1	1	1	10	2003-Dec				
16	12	1	1	1	1	2003-Dec				
18	12	1	2	0	1	2003-Dec				
39	12	1	2	0	2	2004-Dec				
60	12	1	1	1	2	2004-Dec				
77	12	1	2	0	2	2004-Dec				
139	12	1	2	0	4	2003-Dec				
168	12	1	2	0	5	2004-May				
176	12	1	2	0	5	2004-May				
289	12	1	1	1	8	2003-Dec				
349	12	1	1	1	10	2003-Dec				
399	12	1	1	1	12	2004-May				
46	12.5	1	1	1	2	2004-Dec				
52	12.5	1	2	0	2	2004-Dec				
78	12.5	1	2	0	2	2004-Dec				
81	12.5	1	2	0	2	2004-Dec				
140	12.5	1	2	0	4	2003-Dec				
227	12.5	1	2	0	6	2004-Dec				
283	12.5	1	1	1	8	2003-Dec				
352	12.5	1	2	0	10	2003-Dec				

400	12.5	1	2	0	12 2004-May
12	13	1	1	1	1 2003-Dec
50	13	1	2	0	2 2004-Dec
124	13	1	2	0	4 2003-Dec
125	13	1	2	0	4 2003-Dec
135	13	1	2	0	4 2003-Dec
175	13	1	2	0	5 2004-May
189	13	1	1	1	6 2004-Dec
242	13	1	1	1	7 2004-May
251	13	1	1	1	7 2004-May
295	13	1	1	1	8 2003-Dec
330	13	1	1	1	9 2004-May
337	13	1	2	0	10 2003-Dec
343	13	1	2	0	10 2003-Dec
381	13	1	1	1	11 2004-May
395	13	1	2	0	12 2004-May
409	13	1	2	0	4 2003-Dec
47	13.5	1	2	0	2 2004-Dec
65	13.5	1	2	0	2 2004-Dec
68	13.5	1	2	0	2 2004-Dec
72	13.5	1	2	0	2 2004-Dec
117	13.5	1	1	1	3 2004-May
199	13.5	1	1	1	6 2004-Dec
213	13.5	1	1	1	6 2004-Dec
235	13.5	1	2	0	7 2004-May
334	13.5	1	2	0	10 2003-Dec
365	13.5	1	2	0	11 2004-May
21	14	1	2	0	1 2003-Dec
80	14	1	1	1	2 2004-Dec
86	14	1	1	1	3 2004-May
134	14	1	2	0	4 2003-Dec
154	14	1	2	0	5 2004-May
157	14	1	1	1	5 2004-May
163	14	1	2	0	5 2004-May
234	14	1	2	0	7 2004-May
260	14	1	2	0	7 2004-May
265	14	1	1	1	7 2004-May
280	14	1	1	1	8 2003-Dec
290	14	1	1	1	8 2003-Dec
320	14	1	2	0	9 2004-May

327	14	1	1	1	9	2004-May
341	14	1	2	0	10	2003-Dec
22	14.5	1	1	1	1	2003-Dec
24	14.5	1	1	1	1	2003-Dec
70	14.5	1	2	0	2	2004-Dec
119	14.5	1	2	0	4	2003-Dec
133	14.5	1	1	1	4	2003-Dec
138	14.5	1	2	0	4	2003-Dec
162	14.5	1	2	0	5	2004-May
166	14.5	1	2	0	5	2004-May
179	14.5	1	2	0	5	2004-May
180	14.5	1	2	0	5	2004-May
188	14.5	1	1	1	6	2004-Dec
197	14.5	1	1	1	6	2004-Dec
205	14.5	1	1	1	6	2004-Dec
222	14.5	1	1	1	6	2004-Dec
225	14.5	1	1	1	6	2004-Dec
245	14.5	1	2	0	7	2004-May
252	14.5	1	2	0	7	2004-May
340	14.5	1	2	0	10	2003-Dec
347	14.5	1	2	0	10	2003-Dec
361	14.5	1	1	1	10	2003-Dec
375	14.5	1	1	1	11	2004-May
384	14.5	1	2	0	12	2004-May
408	14.5	1	2	0	4	2003-Dec
31	15	1	2	0	1	2003-Dec
49	15	1	2	0	2	2004-Dec
56	15	1	2	0	2	2004-Dec
59	15	1	2	0	2	2004-Dec
79	15	1	2	0	2	2004-Dec
101	15	1	2	0	3	2004-May
110	15	1	1	1	3	2004-May
137	15	1	2	0	4	2003-Dec
152	15	1	2	0	5	2004-May
191	15	1	1	1	6	2004-Dec
192	15	1	1	1	6	2004-Dec
193	15	1	2	0	6	2004-Dec
203	15	1	1	1	6	2004-Dec
208	15	1	1	1	6	2004-Dec
218	15	1	1	1	6	2004-Dec

238	15	1	1	1	7 2004-May
250	15	1	2	0	7 2004-May
257	15	1	2	0	7 2004-May
274	15	1	1	1	8 2003-Dec
368	15	1	2	0	11 2004-May
410	15	1	2	0	4 2003-Dec
17	15.5	1	1	1	1 2003-Dec
38	15.5	1	2	0	2 2004-Dec
61	15.5	1	2	0	2 2004-Dec
63	15.5	1	2	0	2 2004-Dec
83	15.5	1	1	1	2 2004-Dec
115	15.5	1	1	1	3 2004-May
136	15.5	1	1	1	4 2003-Dec
165	15.5	1	1	1	5 2004-May
172	15.5	1	2	0	5 2004-May
221	15.5	1	1	1	6 2004-Dec
284	15.5	1	2	0	8 2003-Dec
291	15.5	1	2	0	8 2003-Dec
307	15.5	1	1	1	9 2004-May
325	15.5	1	2	0	9 2004-May
328	15.5	1	1	1	9 2004-May
329	15.5	1	1	1	9 2004-May
358	15.5	1	2	0	10 2003-Dec
359	15.5	1	1	1	10 2003-Dec
406	15.5	1	2	0	12 2004-May
5	16	1	1	1	1 2003-Dec
14	16	1	1	1	1 2003-Dec
54	16	1	1	1	2 2004-Dec
62	16	1	2	0	2 2004-Dec
97	16	1	1	1	3 2004-May
98	16	1	1	1	3 2004-May
100	16	1	2	0	3 2004-May
155	16	1	1	1	5 2004-May
161	16	1	2	0	5 2004-May
169	16	1	1	1	5 2004-May
187	16	1	1	1	6 2004-Dec
194	16	1	1	1	6 2004-Dec
198	16	1	1	1	6 2004-Dec
202	16	1	1	1	6 2004-Dec
204	16	1	2	0	6 2004-Dec

267	16	1	1	1	8	2003-Dec
271	16	1	2	0	8	2003-Dec
303	16	1	2	0	9	2004-May
310	16	1	1	1	9	2004-May
339	16	1	2	0	10	2003-Dec
342	16	1	1	1	10	2003-Dec
109	16.5	1	1	1	3	2004-May
116	16.5	1	1	1	3	2004-May
123	16.5	1	2	0	4	2003-Dec
126	16.5	1	2	0	4	2003-Dec
132	16.5	1	2	0	4	2003-Dec
142	16.5	1	2	0	4	2003-Dec
148	16.5	1	2	0	5	2004-May
150	16.5	1	2	0	5	2004-May
159	16.5	1	2	0	5	2004-May
200	16.5	1	1	1	6	2004-Dec
207	16.5	1	2	0	6	2004-Dec
210	16.5	1	1	1	6	2004-Dec
212	16.5	1	1	1	6	2004-Dec
220	16.5	1	2	0	6	2004-Dec
229	16.5	1	1	1	6	2004-Dec
231	16.5	1	1	1	6	2004-Dec
254	16.5	1	2	0	7	2004-May
306	16.5	1	1	1	9	2004-May
322	16.5	1	2	0	9	2004-May
41	17	2	2	1	2	2004-Dec
43	17	2	2	1	2	2004-Dec
48	17	2	2	1	2	2004-Dec
55	17	2	2	1	2	2004-Dec
84	17	2	2	1	2	2004-Dec
153	17	2	2	1	5	2004-May
174	17	2	2	1	5	2004-May
184	17	2	1	0	6	2004-Dec
201	17	2	2	1	6	2004-Dec
209	17	2	1	0	6	2004-Dec
224	17	2	1	0	6	2004-Dec
255	17	2	1	0	7	2004-May
276	17	2	1	0	8	2003-Dec
285	17	2	1	0	8	2003-Dec
294	17	2	2	1	8	2003-Dec

296	17	2	1	0	8	2003-Dec
309	17	2	2	1	9	2004-May
336	17	2	2	1	10	2003-Dec
354	17	2	1	0	10	2003-Dec
357	17	2	2	1	10	2003-Dec
397	17	2	1	0	12	2004-May
19	17.5	2	1	0	1	2003-Dec
26	17.5	2	1	0	1	2003-Dec
104	17.5	2	1	0	3	2004-May
118	17.5	2	2	1	4	2003-Dec
211	17.5	2	2	1	6	2004-Dec
219	17.5	2	1	0	6	2004-Dec
266	17.5	2	2	1	7	2004-May
275	17.5	2	2	1	8	2003-Dec
299	17.5	2	2	1	8	2003-Dec
304	17.5	2	1	0	9	2004-May
305	17.5	2	2	1	9	2004-May
388	17.5	2	2	1	12	2004-May
404	17.5	2	2	1	12	2004-May
23	18	2	2	1	1	2003-Dec
30	18	2	2	1	1	2003-Dec
94	18	2	1	0	3	2004-May
141	18	2	1	0	4	2003-Dec
144	18	2	2	1	4	2003-Dec
160	18	2	2	1	5	2004-May
182	18	2	2	1	6	2004-Dec
190	18	2	1	0	6	2004-Dec
196	18	2	1	0	6	2004-Dec
215	18	2	1	0	6	2004-Dec
216	18	2	1	0	6	2004-Dec
217	18	2	1	0	6	2004-Dec
223	18	2	2	1	6	2004-Dec
226	18	2	1	0	6	2004-Dec
228	18	2	1	0	6	2004-Dec
236	18	2	1	0	7	2004-May
259	18	2	2	1	7	2004-May
272	18	2	1	0	8	2003-Dec
286	18	2	1	0	8	2003-Dec
292	18	2	2	1	8	2003-Dec
344	18	2	2	1	10	2003-Dec

353	18	2	2	1	10	2003-Dec
373	18	2	1	0	11	2004-May
4	18.5	2	1	0	1	2003-Dec
9	18.5	2	1	0	1	2003-Dec
32	18.5	2	1	0	1	2003-Dec
69	18.5	2	2	1	2	2004-Dec
87	18.5	2	1	0	3	2004-May
99	18.5	2	1	0	3	2004-May
106	18.5	2	1	0	3	2004-May
120	18.5	2	2	1	4	2003-Dec
173	18.5	2	2	1	5	2004-May
237	18.5	2	2	1	7	2004-May
244	18.5	2	1	0	7	2004-May
262	18.5	2	2	1	7	2004-May
281	18.5	2	2	1	8	2003-Dec
287	18.5	2	1	0	8	2003-Dec
297	18.5	2	1	0	8	2003-Dec
298	18.5	2	1	0	8	2003-Dec
312	18.5	2	1	0	9	2004-May
314	18.5	2	2	1	9	2004-May
369	18.5	2	1	0	11	2004-May
370	18.5	2	2	1	11	2004-May
376	18.5	2	2	1	11	2004-May
402	18.5	2	2	1	12	2004-May
44	19	2	2	1	2	2004-Dec
71	19	2	2	1	2	2004-Dec
108	19	2	1	0	3	2004-May
130	19	2	2	1	4	2003-Dec
185	19	2	1	0	6	2004-Dec
195	19	2	1	0	6	2004-Dec
206	19	2	1	0	6	2004-Dec
261	19	2	2	1	7	2004-May
301	19	2	1	0	9	2004-May
331	19	2	2	1	9	2004-May
338	19	2	2	1	10	2003-Dec
363	19	2	1	0	10	2003-Dec
377	19	2	1	0	11	2004-May
380	19	2	2	1	11	2004-May
2	19.5	2	1	0	1	2003-Dec
11	19.5	2	1	0	1	2003-Dec

58	19.5	2	2	1	2 2004-Dec
178	19.5	2	1	0	5 2004-May
264	19.5	2	2	1	7 2004-May
346	19.5	2	2	1	10 2003-Dec
364	19.5	2	1	0	11 2004-May
393	19.5	2	1	0	12 2004-May
405	19.5	2	1	0	12 2004-May
407	19.5	2	1	0	3 2004-May
7	20	2	1	0	1 2003-Dec
20	20	2	1	0	1 2003-Dec
42	20	2	2	1	2 2004-Dec
145	20	2	1	0	4 2003-Dec
277	20	2	1	0	8 2003-Dec
288	20	2	1	0	8 2003-Dec
315	20	2	1	0	9 2004-May
378	20	2	2	1	11 2004-May
15	20.5	2	1	0	1 2003-Dec
45	20.5	2	2	1	2 2004-Dec
75	20.5	2	2	1	2 2004-Dec
96	20.5	2	1	0	3 2004-May
107	20.5	2	2	1	3 2004-May
121	20.5	2	2	1	4 2003-Dec
131	20.5	2	2	1	4 2003-Dec
214	20.5	2	1	0	6 2004-Dec
362	20.5	2	2	1	10 2003-Dec
371	20.5	2	2	1	11 2004-May
382	20.5	2	2	1	11 2004-May
403	20.5	2	1	0	12 2004-May
3	21	2	1	0	1 2003-Dec
13	21	2	1	0	1 2003-Dec
76	21	2	1	0	2 2004-Dec
128	21	2	2	1	4 2003-Dec
183	21	2	1	0	6 2004-Dec
243	21	2	2	1	7 2004-May
321	21	2	1	0	9 2004-May
398	21	2	2	1	12 2004-May
51	21.5	2	2	1	2 2004-Dec
85	21.5	2	2	1	3 2004-May
93	21.5	2	1	0	3 2004-May
143	21.5	2	2	1	4 2003-Dec

186	21.5	2	1	0	6 2004-Dec
232	21.5	2	2	1	7 2004-May
240	21.5	2	2	1	7 2004-May
269	21.5	2	2	1	8 2003-Dec
270	21.5	2	1	0	8 2003-Dec
293	21.5	2	1	0	8 2003-Dec
350	21.5	2	2	1	10 2003-Dec
356	21.5	2	2	1	10 2003-Dec
383	21.5	2	1	0	11 2004-May
392	21.5	2	1	0	12 2004-May
36	22	2	2	1	2 2004-Dec
89	22	2	1	0	3 2004-May
95	22	2	1	0	3 2004-May
170	22	2	2	1	5 2004-May
181	22	2	2	1	5 2004-May
278	22	2	1	0	8 2003-Dec
326	22	2	1	0	9 2004-May
351	22	2	2	1	10 2003-Dec
355	22	2	1	0	10 2003-Dec
64	22.5	2	1	0	2 2004-Dec
105	22.5	2	2	1	3 2004-May
149	22.5	2	1	0	5 2004-May
273	22.5	2	1	0	8 2003-Dec
282	22.5	2	1	0	8 2003-Dec
379	22.5	2	1	0	11 2004-May
33	23	2	1	0	1 2003-Dec
67	23	2	2	1	2 2004-Dec
311	23	2	2	1	9 2004-May
332	23	2	1	0	9 2004-May
335	23	2	2	1	10 2003-Dec
6	23.5	2	1	0	1 2003-Dec
37	23.5	2	2	1	2 2004-Dec
90	23.5	2	2	1	3 2004-May
113	23.5	2	2	1	3 2004-May
241	23.5	2	2	1	7 2004-May
256	23.5	2	2	1	7 2004-May
372	23.5	2	1	0	11 2004-May
396	23.5	2	2	1	12 2004-May
1	24	2	1	0	1 2003-Dec
122	24	2	2	1	4 2003-Dec

151	24	2	2	1	5 2004-May
300	24	2	1	0	9 2004-May
316	24	2	1	0	9 2004-May
333	24	2	1	0	9 2004-May
28	24.5	2	1	0	1 2003-Dec
73	24.5	2	1	0	2 2004-Dec
171	24.5	2	1	0	5 2004-May
247	24.5	2	1	0	7 2004-May
249	24.5	2	2	1	7 2004-May
268	24.5	2	1	0	8 2003-Dec
279	24.5	2	1	0	8 2003-Dec
319	24.5	2	1	0	9 2004-May
367	24.5	2	1	0	11 2004-May
111	25	2	1	0	3 2004-May
114	25	2	2	1	3 2004-May
129	25	2	2	1	4 2003-Dec
146	25	2	1	0	4 2003-Dec
324	25	2	1	0	9 2004-May
345	25	2	1	0	10 2003-Dec
74	25.5	2	2	1	2 2004-Dec
103	25.5	2	1	0	3 2004-May
167	25.5	2	2	1	5 2004-May
401	25.5	2	1	0	12 2004-May
34	26	2	1	0	1 2003-Dec
40	26	2	2	1	2 2004-Dec
302	26	2	1	0	9 2004-May
374	26	2	2	1	11 2004-May
92	26.5	2	2	1	3 2004-May
246	26.5	2	2	1	7 2004-May
253	26.5	2	1	0	7 2004-May
263	26.5	2	2	1	7 2004-May
29	27	2	1	0	1 2003-Dec
156	27	2	2	1	5 2004-May
313	27	2	1	0	9 2004-May
318	27	2	2	1	9 2004-May
390	27	2	1	0	12 2004-May
391	27	2	2	1	12 2004-May
230	27.5	2	2	1	6 2004-Dec
386	27.5	2	1	0	12 2004-May
88	28	2	1	0	3 2004-May

112	28	2	1	0	3	2004-May
158	28	2	2	1	5	2004-May
248	28	2	2	1	7	2004-May
323	28	2	1	0	9	2004-May
66	28.5	2	2	1	2	2004-Dec
91	28.5	2	1	0	3	2004-May
317	28.5	2	1	0	9	2004-May
147	29	2	2	1	4	2003-Dec
308	29	2	1	0	9	2004-May
258	29.5	2	1	0	7	2004-May
25	30	2	1	0	1	2003-Dec
164	31	2	1	0	5	2004-May
177	31	2	1	0	5	2004-May
366	31	2	1	0	11	2004-May
239	31.5	2	2	1	7	2004-May
102	32	2	1	0	3	2004-May
27	32.5	2	2	1	1	2003-Dec
389	34	2	2	1	12	2004-May
394	36	2	2	1	12	2004-May

Table 2: Average pathological scores corresponding to each box of hepatopancreas slides from snow crab from control and seismic exposed sites, along with the standard deviation, number of slides, collection date, and length of time the snow crab were caged.

Box	Average	SD	n	Date	Caged time
1	18.912	5.227	34	36494	12 days
2	16.91	4.407	49	36860	12 days
3	20.606	4.731	34	36646	5 months
4	17.55	4.265	33	36494	12 days
5	18.471	5.329	34	36646	5 months
6	16.77	2.485	50	36860	12 days
7	19	5.235	34	36646	5 months
8	18.03	3.279	33	36494	12 days
9	20.324	4.769	34	36646	5 months
10	16.917	3.728	30	36494	12 days
11	19.85	4.338	20	36646	5 months
12	20.523	6.801	22	36646	5 months
			407		

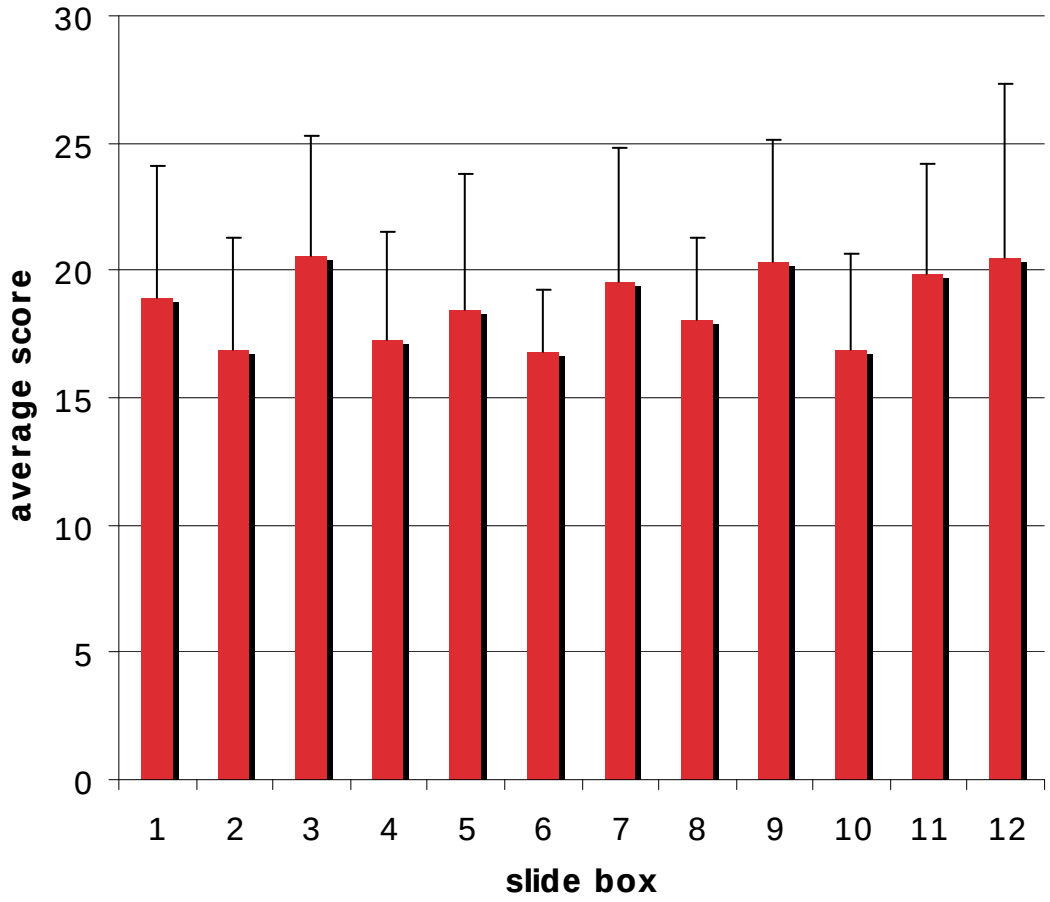


Figure 1: Average pathological score (and standard deviation) of snow crab hepatopancreas corresponding to each slide box. Each box contained a mixture of slides from the control and the seismic exposed snow crab.

Table 3: Matches between blind coding and actual coding for each box of slides of snow crab hepatopancreas. Percent matches are specified for both the control and seismic groups, and also for overall match.

Box#	#Slides	Matches	Code	Match	Total	% Match	Overall match
box1	34	12	control	9	28	32%	35.2941
			seismic	3	6	50%	
box2	49	25	control	6	9	67%	51.0204
			seismic	19	40	48%	
box 3	34	15	control	8	25	32%	44.1176
			seismic	7	9	78%	
box 4	33	13	control	2	5	40%	39.3939
			seismic	11	28	39%	
box 5	34	14	control	4	9	44%	41.1765
			seismic	10	25	40%	
box 6	50	28	control	23	40	58%	56
			seismic	5	10	50%	
box 7	34	20	control	4	10	40%	58.8235
			seismic	16	24	67%	
box 8	33	13	control	7	24	29%	39.3939
			seismic	6	9	67%	
box 9	34	13	control	7	24	29%	38.2353
			seismic	6	10	60%	
box 10	30	16	control	5	9	56%	53.3333
			seismic	11	21	52%	
box 11	20	9	control	2	11	18%	45
			seismic	7	9	78%	
box 12	22	9	control	1	9	11%	40.9091
			seismic	8	13	62%	
total	407	187		187	407	45.9459	45.9459

Hepatopancreas	Matched	Actual	%
Control	78	203	38.42
Seismic	109	204	53.43
Total	187	407	45.95

APPENDIX 2. SCORES OF THE HISTOPATHOLOGICAL ANALYSIS OF SNOW CRAB OVARIES FROM CONTROL OR SEISMIC EXPOSED SITES, AND MATCH BETWEEN THE BINARY (BLIND) CODING AND THE CODED KEY (ORIGINAL CODING).

Table 1: Scores of the histopathological analysis of snow crab ovaries from control or seismic exposed sites. Pathological scores from 9 to < 13 are considered normal (green), 13 to 16 represent mild changes (yellow), > 16 to < 20 illustrate moderate changes (orange), and scores 20 and greater indicate extreme changes (red).

Slide #	Path. score	Binary	Coded	Match	Box #	Date sampled
19	9	1	2	0	1	2004-Dec
21	9	1	1	1	1	2004-Dec
22	9	1	1	1	1	2004-Dec
26	9	1	1	1	1	2004-Dec
36	9	1	1	1	1	2004-Dec
41	9	1	1	1	1	2004-Dec
46	9	1	1	1	1	2004-Dec
48	9	1	1	1	1	2004-Dec
90	9	1	2	0	3	2004-Dec
91	9	1	1	1	3	2004-Dec
93	9	1	2	0	3	2004-Dec
101	9	1	2	0	3	2004-Dec
102	9	1	1	1	3	2004-Dec
105	9	1	2	0	3	2004-Dec
106	9	1	2	0	3	2004-Dec
108	9	1	1	1	3	2004-Dec
112	9	1	1	1	3	2004-Dec
131	9	1	2	0	3	2004-Dec
152	9	1	1	1	4	2003-Dec
162	9	1	1	1	4	2003-Dec
214	9	1	2	0	6	2003-Dec
342	9	1	2	0	11	2003-Dec
353	9	1	2	0	11	2003-Dec
88	9.5	1	1	1	3	2004-Dec
354	9.5	1	2	0	11	2003-Dec
10	10	1	1	1	1	2004-Dec
11	10	1	1	1	1	2004-Dec
17	10	1	1	1	1	2004-Dec
18	10	1	1	1	1	2004-Dec
25	10	1	2	0	1	2004-Dec
28	10	1	1	1	1	2004-Dec
29	10	1	2	0	1	2004-Dec
30	10	1	1	1	1	2004-Dec
55	10	1	2	0	2	2004-May
87	10	1	2	0	3	2004-Dec

99	10	1	2	0	3	2004-Dec
113	10	1	2	0	3	2004-Dec
117	10	1	2	0	3	2004-Dec
120	10	1	2	0	3	2004-Dec
136	10	1	2	0	4	2003-Dec
142	10	1	1	1	4	2003-Dec
144	10	1	2	0	4	2003-Dec
151	10	1	2	0	4	2003-Dec
153	10	1	1	1	4	2003-Dec
203	10	1	2	0	6	2003-Dec
206	10	1	2	0	6	2003-Dec
213	10	1	2	0	6	2003-Dec
220	10	1	2	0	6	2003-Dec
249	10	1	1	1	7	2004-May
343	10	1	2	0	11	2003-Dec
344	10	1	2	0	11	2003-Dec
345	10	1	2	0	11	2003-Dec
346	10	1	2	0	11	2003-Dec
347	10	1	2	0	11	2003-Dec
350	10	1	2	0	11	2003-Dec
20	10.5	1	1	1	1	2004-Dec
23	10.5	1	1	1	1	2004-Dec
32	10.5	1	1	1	1	2004-Dec
34	10.5	1	1	1	1	2004-Dec
86	10.5	1	1	1	3	2004-Dec
92	10.5	1	2	0	3	2004-Dec
98	10.5	1	1	1	3	2004-Dec
100	10.5	1	2	0	3	2004-Dec
109	10.5	1	2	0	3	2004-Dec
124	10.5	1	2	0	3	2004-Dec
128	10.5	1	2	0	3	2004-Dec
129	10.5	1	2	0	3	2004-Dec
137	10.5	1	1	1	4	2003-Dec
161	10.5	1	1	1	4	2003-Dec
185	10.5	1	1	1	5	2004-May
208	10.5	1	2	0	6	2003-Dec
209	10.5	1	2	0	6	2003-Dec
228	10.5	1	2	0	6	2003-Dec
337	10.5	1	2	0	10	2004-May
2	11	1	1	1	1	2004-Dec
5	11	1	2	0	1	2004-Dec
13	11	1	1	1	1	2004-Dec
35	11	1	1	1	1	2004-Dec
43	11	1	1	1	1	2004-Dec
45	11	1	1	1	1	2004-Dec
89	11	1	2	0	3	2004-Dec

94	11	1	2	0	3	2004-Dec
96	11	1	2	0	3	2004-Dec
97	11	1	2	0	3	2004-Dec
107	11	1	2	0	3	2004-Dec
111	11	1	2	0	3	2004-Dec
141	11	1	2	0	4	2003-Dec
150	11	1	1	1	4	2003-Dec
197	11	1	2	0	6	2003-Dec
200	11	1	2	0	6	2003-Dec
268	11	1	2	0	8	2004-May
291	11	1	2	0	8	2004-May
326	11	1	1	1	10	2004-May
341	11	1	2	0	10	2004-May
349	11	1	2	0	11	2003-Dec
50	11.5	1	1	1	1	2004-Dec
83	11.5	1	2	0	2	2004-May
119	11.5	1	2	0	3	2004-Dec
121	11.5	1	2	0	3	2004-Dec
126	11.5	1	2	0	3	2004-Dec
127	11.5	1	1	1	3	2004-Dec
196	11.5	1	2	0	6	2003-Dec
207	11.5	1	2	0	6	2003-Dec
226	11.5	1	2	0	6	2003-Dec
247	11.5	1	2	0	7	2004-May
257	11.5	1	1	1	7	2004-May
312	11.5	1	2	0	9	2004-May
335	11.5	1	1	1	10	2004-May
338	11.5	1	1	1	10	2004-May
6	12	1	1	1	1	2004-Dec
37	12	1	1	1	1	2004-Dec
40	12	1	1	1	1	2004-Dec
61	12	1	2	0	2	2004-May
70	12	1	2	0	2	2004-May
78	12	1	2	0	2	2004-May
130	12	1	2	0	3	2004-Dec
140	12	1	1	1	4	2003-Dec
145	12	1	2	0	4	2003-Dec
148	12	1	2	0	4	2003-Dec
223	12	1	2	0	6	2003-Dec
294	12	1	2	0	8	2004-May
317	12	1	2	0	9	2004-May
327	12	1	1	1	10	2004-May
12	12.5	1	2	0	1	2004-Dec
33	12.5	1	2	0	1	2004-Dec
39	12.5	1	2	0	1	2004-Dec
42	12.5	1	2	0	1	2004-Dec

68	12.5	1	2	0	2	2004-May
69	12.5	1	1	1	2	2004-May
81	12.5	1	2	0	2	2004-May
110	12.5	1	2	0	3	2004-Dec
114	12.5	1	2	0	3	2004-Dec
125	12.5	1	1	1	3	2004-Dec
135	12.5	1	1	1	4	2003-Dec
149	12.5	1	1	1	4	2003-Dec
155	12.5	1	1	1	4	2003-Dec
172	12.5	1	2	0	5	2004-May
188	12.5	1	1	1	5	2004-May
193	12.5	1	1	1	5	2004-May
194	12.5	1	1	1	5	2004-May
229	12.5	1	2	0	6	2003-Dec
240	12.5	1	2	0	7	2004-May
250	12.5	1	2	0	7	2004-May
300	12.5	1	2	0	9	2004-May
318	12.5	1	1	1	9	2004-May
332	12.5	1	2	0	10	2004-May
334	12.5	1	1	1	10	2004-May
336	12.5	1	2	0	10	2004-May
348	12.5	1	2	0	11	2003-Dec
4	13	2	1	0	1	2004-Dec
8	13	2	2	1	1	2004-Dec
62	13	2	2	1	2	2004-May
63	13	2	2	1	2	2004-May
65	13	2	2	1	2	2004-May
85	13	2	2	1	3	2004-Dec
173	13	2	1	0	5	2004-May
176	13	2	1	0	5	2004-May
179	13	2	2	1	5	2004-May
180	13	2	1	0	5	2004-May
191	13	2	1	0	5	2004-May
195	13	2	2	1	5	2004-May
198	13	2	1	0	6	2003-Dec
212	13	2	2	1	6	2003-Dec
217	13	2	2	1	6	2003-Dec
255	13	2	1	0	7	2004-May
260	13	2	1	0	7	2004-May
273	13	2	1	0	8	2004-May
277	13	2	1	0	8	2004-May
278	13	2	2	1	8	2004-May
279	13	2	2	1	8	2004-May
292	13	2	1	0	8	2004-May
305	13	2	1	0	9	2004-May
319	13	2	2	1	10	2004-May

320	13	2	2	1	10	2004-May
325	13	2	2	1	10	2004-May
328	13	2	2	1	10	2004-May
15	13.5	2	2	1	1	2004-Dec
16	13.5	2	1	0	1	2004-Dec
27	13.5	2	1	0	1	2004-Dec
67	13.5	2	2	1	2	2004-May
72	13.5	2	2	1	2	2004-May
82	13.5	2	1	0	2	2004-May
115	13.5	2	2	1	3	2004-Dec
139	13.5	2	1	0	4	2003-Dec
166	13.5	2	2	1	5	2004-May
187	13.5	2	2	1	5	2004-May
205	13.5	2	2	1	6	2003-Dec
224	13.5	2	2	1	6	2003-Dec
230	13.5	2	1	0	7	2004-May
333	13.5	2	1	0	10	2004-May
339	13.5	2	2	1	10	2004-May
14	14	2	1	0	1	2004-Dec
38	14	2	1	0	1	2004-Dec
77	14	2	2	1	2	2004-May
138	14	2	2	1	4	2003-Dec
146	14	2	1	0	4	2003-Dec
183	14	2	1	0	5	2004-May
189	14	2	2	1	5	2004-May
192	14	2	2	1	5	2004-May
202	14	2	2	1	6	2003-Dec
267	14	2	1	0	8	2004-May
274	14	2	1	0	8	2004-May
284	14	2	2	1	8	2004-May
288	14	2	2	1	8	2004-May
295	14	2	1	0	8	2004-May
306	14	2	1	0	9	2004-May
60	14.5	2	1	0	2	2004-May
76	14.5	2	1	0	2	2004-May
80	14.5	2	2	1	2	2004-May
84	14.5	2	2	1	3	2004-Dec
103	14.5	2	2	1	3	2004-Dec
118	14.5	2	2	1	3	2004-Dec
132	14.5	2	1	0	3	2004-Dec
160	14.5	2	1	0	4	2003-Dec
163	14.5	2	2	1	5	2004-May
174	14.5	2	1	0	5	2004-May
204	14.5	2	2	1	6	2003-Dec
211	14.5	2	2	1	6	2003-Dec
237	14.5	2	1	0	7	2004-May

243	14.5	2	1	0	7	2004-May
252	14.5	2	2	1	7	2004-May
253	14.5	2	1	0	7	2004-May
258	14.5	2	2	1	7	2004-May
265	14.5	2	2	1	8	2004-May
281	14.5	2	2	1	8	2004-May
286	14.5	2	2	1	8	2004-May
310	14.5	2	2	1	9	2004-May
330	14.5	2	2	1	10	2004-May
7	15	2	1	0	1	2004-Dec
54	15	2	1	0	2	2004-May
64	15	2	1	0	2	2004-May
181	15	2	1	0	5	2004-May
184	15	2	1	0	5	2004-May
218	15	2	2	1	6	2003-Dec
219	15	2	2	1	6	2003-Dec
241	15	2	2	1	7	2004-May
254	15	2	2	1	7	2004-May
259	15	2	1	0	7	2004-May
264	15	2	1	0	7	2004-May
275	15	2	1	0	8	2004-May
276	15	2	2	1	8	2004-May
301	15	2	1	0	9	2004-May
302	15	2	1	0	9	2004-May
311	15	2	2	1	9	2004-May
315	15	2	2	1	9	2004-May
316	15	2	1	0	9	2004-May
340	15	2	1	0	10	2004-May
66	15.5	2	1	0	2	2004-May
225	15.5	2	1	0	6	2003-Dec
231	15.5	2	2	1	7	2004-May
266	15.5	2	2	1	8	2004-May
283	15.5	2	1	0	8	2004-May
307	15.5	2	1	0	9	2004-May
313	15.5	2	1	0	9	2004-May
321	15.5	2	2	1	10	2004-May
331	15.5	2	1	0	10	2004-May
31	16	2	1	0	1	2004-Dec
47	16	2	1	0	1	2004-Dec
52	16	2	2	1	2	2004-May
57	16	2	2	1	2	2004-May
74	16	2	2	1	2	2004-May
75	16	2	2	1	2	2004-May
95	16	2	1	0	3	2004-Dec
143	16	2	1	0	4	2003-Dec
235	16	2	1	0	7	2004-May

242	16	2	2	1	7	2004-May
271	16	2	1	0	8	2004-May
272	16	2	2	1	8	2004-May
297	16	2	2	1	8	2004-May
298	16	2	1	0	9	2004-May
304	16	2	2	1	9	2004-May
309	16	2	1	0	9	2004-May
165	16.5	2	1	0	5	2004-May
227	16.5	2	2	1	6	2003-Dec
239	16.5	2	1	0	7	2004-May
245	16.5	2	1	0	7	2004-May
262	16.5	2	1	0	7	2004-May
296	16.5	2	2	1	8	2004-May
324	16.5	2	2	1	10	2004-May
3	17	2	2	1	1	2004-Dec
44	17	2	2	1	1	2004-Dec
171	17	2	1	0	5	2004-May
175	17	2	1	0	5	2004-May
210	17	2	1	0	6	2003-Dec
234	17	2	2	1	7	2004-May
248	17	2	1	0	7	2004-May
263	17	2	1	0	7	2004-May
122	17.5	2	1	0	3	2004-Dec
182	17.5	2	2	1	5	2004-May
238	17.5	2	2	1	7	2004-May
282	17.5	2	2	1	8	2004-May
293	17.5	2	2	1	8	2004-May
352	17.5	2	2	1	11	2003-Dec
24	18	2	1	0	1	2004-Dec
116	18	2	1	0	3	2004-Dec
133	18	2	2	1	3	2004-Dec
199	18	2	2	1	6	2003-Dec
270	18	2	2	1	8	2004-May
289	18	2	2	1	8	2004-May
1	18.5	2	1	0	1	2004-Dec
9	18.5	2	1	0	1	2004-Dec
51	18.5	2	1	0	2	2004-May
156	18.5	2	1	0	4	2003-Dec
159	18.5	2	1	0	4	2003-Dec
233	18.5	2	1	0	7	2004-May
290	18.5	2	2	1	8	2004-May
303	19	2	2	1	9	2004-May
169	19.5	2	1	0	5	2004-May
222	19.5	2	2	1	6	2003-Dec
59	20	2	2	1	2	2004-May
186	20	2	1	0	5	2004-May

215	20	2	1	0	6	2003-Dec
53	20.5	2	2	1	2	2004-May
104	20.5	2	2	1	3	2004-Dec
168	21	2	2	1	5	2004-May
177	21	2	2	1	5	2004-May
232	21	2	1	0	7	2004-May
73	21.5	2	1	0	2	2004-May
178	22	2	1	0	5	2004-May
269	22.5	2	2	1	8	2004-May
201	23	2	1	0	6	2003-Dec
190	23.5	2	1	0	5	2004-May
285	23.5	2	1	0	8	2004-May
71	24	2	2	1	2	2004-May
280	24	2	1	0	8	2004-May
246	24.5	2	1	0	7	2004-May
164	25	2	1	0	5	2004-May
157	25.5	2	1	0	4	2003-Dec
236	25.5	2	1	0	7	2004-May
56	26	2	1	0	2	2004-May
216	26	2	1	0	6	2003-Dec
287	26	2	1	0	8	2004-May
329	26	2	2	1	10	2004-May
158	26.5	2	1	0	4	2003-Dec
49	27	2	2	1	1	2004-Dec
351	27	2	2	1	11	2003-Dec
170	27.5	2	1	0	5	2004-May
167	28	2	1	0	5	2004-May
221	28	2	1	0	6	2003-Dec
58	28.5	2	2	1	2	2004-May
323	28.5	2	1	0	10	2004-May
134	29	2	1	0	4	2003-Dec
154	29	2	1	0	4	2003-Dec
256	29	2	1	0	7	2004-May
308	29.5	2	2	1	9	2004-May
314	30	2	1	0	9	2004-May
79	32	2	1	0	2	2004-May
244	32	2	2	1	7	2004-May
123	32.5	2	2	1	3	2004-Dec
251	33	2	1	0	7	2004-May
261	35.5	2	2	1	7	2004-May
147	38	2	1	0	4	2003-Dec

	Normal = 1	Abnormal = 2	Total
	149	203	352
%	42.329545	57.670455	

Table 2: Mean pathological score corresponding to each box of ovary slides, along with the standard deviation, number of slides, collection date and the length of time the snow crabs were caged.

Slide box	Mean score	SD	n	Collect date	Caged time
1	12.3	3.408	50	36860	12 days
2	16.136364	5.148	33	36646	5 months
3	12.04	3.992	50	36860	12 days
4	15.224138	7.337	29	36494	12 days
5	16.424242	4.645	33	36646	5 months
6	14.235294	4.585	34	36494	12 days
7	17.557143	6.301	35	36646	5 months
8	15.863636	3.628	33	36646	5 months
9	15.928571	4.923	20	36646	5 months
10	14.340909	4.481	22	36646	5 months
11	11.961538	5.039	13	36860	12 days
			352		

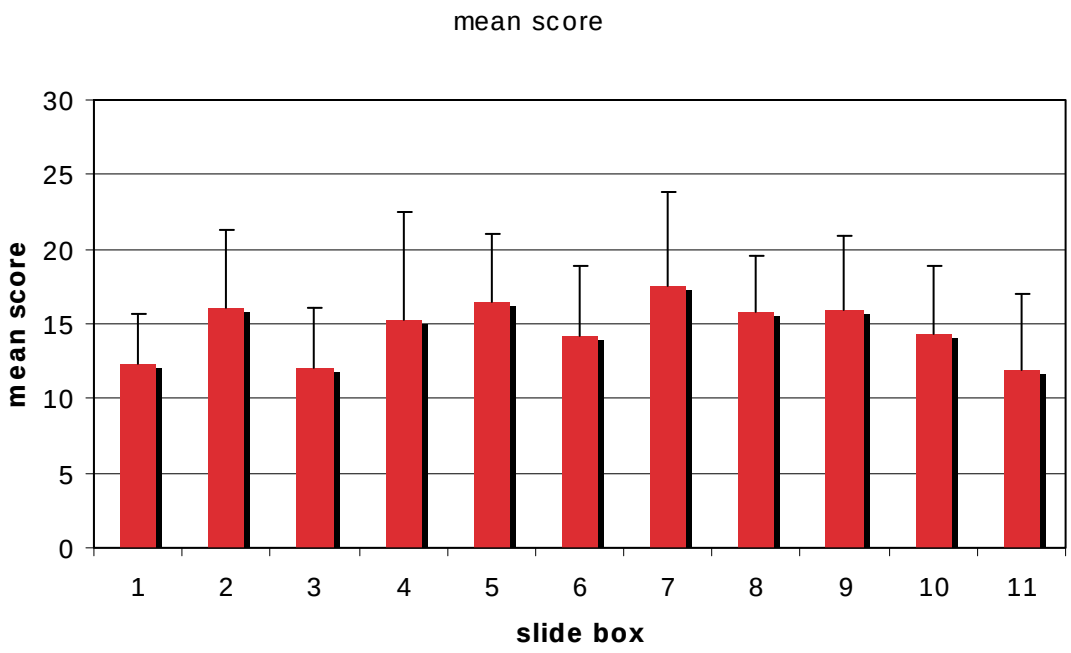


Figure 1: Mean pathological score (and standard deviation) of snow crab ovaries corresponding to each slide box. Each box contained a mixture of slides from snow crabs from the control and the seismic exposed site.

Table 3: Matches between blind coding and actual coding for each box of slides of snow crab ovaries. Percent matches are specified for both the control and the seismic groups, and also for overall match.

Box#	#Slides	Matches	Code	Match	Total	% Match	Overall match
box1	50	31	control	26	37	70.2703	62
			seismic	5	13	38.4615	
box2	33	16	control	1	11	9.09091	48.4848
			seismic	15	22	68.1818	
box 3	50	17	control	9	13	69.2308	34
			seismic	8	37	21.6216	
box 4	29	12	control	11	22	50	41.3793
			seismic	1	7	14.2857	
box 5	33	14	control	4	22	18.1818	42.4242
			seismic	10	11	90.9091	
box 6	34	12	control	2	7	28.5714	35.2941
			seismic	10	27	37.037	
box 7	35	12	control	2	22	9.09091	34.2857
			seismic	10	13	76.9231	
box 8	33	18	control	0	12	0	54.5455
			seismic	18	21	85.7143	
box 9	20	7	control	1	11	9.09091	35
			seismic	6	9	66.6667	
box 10	22	14	control	5	9	55.5556	63.6364
			seismic	9	13	69.2308	
box 11	13	2	control	0	0		15.3846
			seismic	2	13	15.3846	
Total	352	155		155	352	44.0341	44.0341

Ovary	Matched	Total	%
control	59	166	35.542
seismic	96	186	51.613
total	155	352	44.034

APPENDIX 3. DR. GEBOTYS' STATISTICAL EVALUATION OF THE HISTOPATHOLOGICAL DATA COLLECTED BY DR. LUCY LEE AND ASSOCIATES

Blind Data Scoring

The data was scored blind to prevent bias. The researcher received the data without knowledge of which of the seven treatments the slide was a member. Only after the data was scored was the researcher sent a key that matched a slide with a treatment (i.e., treatment key given as 1 = control dec03, 2 = seismic dec03, 3 = control may04, 4 = seismic may04, 5 = control site dec04, 6 = seismic site dec04, and 7 = wild site). The data is therefore unbiased.

Comparison of December 2003 data of control vs. seismic groups

Multivariate analysis of variance was used to compare the seismic and the control groups on two sets of dependent measures 1) hepatopancreatic and 2) ovarian.

Hepatopancreatic results
MANOVA analysis revealed a very significant difference between the two groups, Pillias trace = 0.186, $F_{11,118} = 2.448$, $p = 0.009$. (Please see Appendix 3a for details on the multivariate tests.) Three dependent measures differed significantly between groups using univariate ANOVA results: epithelial wall thickness (seismic mean = 1.76, control mean = 2.25), R cells (1.28, 1.86), and necrosis (2.09, 2.39). (Please see Appendix 3a for means.) Note the control means are higher than the seismic.

Ovarian results
MANOVA analysis revealed a very significant difference between the two groups, Pillias trace = 0.451, $F_{9,65} = 5.94$, $p < 0.001$. (Please see Appendix 3b for details on the multivariate tests.) Six dependent measures differed significantly between groups using univariate ANOVA results: artesia (seismic mean = 1.54, control mean = 1.86), packing density (1.26, 2.03) and granularity (1.35, 2.34), bruising (1.49, 2.53), delayed maturation (1.13, 2.07), and stain pattern (1.25, 2.19). (Please see Appendix 3b for means.)

Comparison of May 2004 data of control vs. seismic groups

Multivariate analysis of variance was used to compare the seismic and the control groups on two sets of dependent measures: 1) hepatopancreatic and 2) ovarian.

Hepatopancreatic results
MANOVA analysis revealed a very significant difference between the two groups, Pillias trace = 0.121, $F_{11,166} = 2.08$, $p = 0.024$. (Please see Appendix 3c for details on the multivariate tests.) Four dependent measures differed significantly between groups using univariate ANOVA results, nuclear shape (seismic mean = 1.51, control mean = 1.34), R cells (2.06, 2.55), membrane (1.99, 2.39) and delamination (1.38, 1.57). (Please see

Appendix 3c for means.) Note the control means are higher than the seismic except for nuclear shape.

Ovarian results

MANOVA analysis revealed a very significant difference between the two groups, Pillias trace = 0.173, $F_{9,166} = 3.85$, $p < 0.001$. (Please see Appendix 3d for details on the multivariate tests.) Two dependent measures differed significantly between groups using univariate ANOVA results, delamination (seismic mean = 1.83, control mean = 2.37) and stain pattern (1.38, 1.65). (Please see Appendix 3d for means.)

Comparison of December 2004 data of control vs. seismic groups

Multivariate analysis of variance was used to compare the seismic and the control groups on two sets of dependent measures: 1) hepatopancreatic and 2) ovarian.

Hepatopancreatic results

MANOVA analysis revealed no significant difference between the two groups, Pillias trace = 0.083, $F_{11,87} = 0.71$, $p = 0.725$. (Please see Appendix 3e for details on the multivariate tests.)

Ovarian results

MANOVA analysis revealed no significant difference between the two groups, Pillias trace = 0.07, $F_{9,90} = 0.813$, $p = 0.605$. (Please see Appendix 3f for details on the multivariate tests.)

Comparison of D03MAY (time levels- December 2003 and May 2004 data) and CVSS (intervention levels- control vs. seismic groups)

Hepatopancreatic results

A two factor multivariate analysis of variance was performed with time factor at two levels, Dec. 2003 and May 2004, and intervention factor with the levels control and seismic. There was a significant two-way interaction of both factors, $p = 0.039$. (Please see Appendix 3g for details on the multivariate tests.) Means are reported in this appendix as well as graphs of significant interactions.

Ovarian results

A two factor multivariate analysis of variance was performed with time factor at two levels, Dec. 03 and May 04, and intervention factor with the levels control and seismic. There was a significant two-way interaction of both factors $p = 0.003$. (Please see Appendix 3h for details on the multivariate tests.) Means are reported in this appendix as well as graphs of significant interactions.

Comparison of D03vs4 (time levels- December 2003 and December 2004 data) and CVSS (intervention levels- control vs. seismic groups)

Hepatopancreatic results

A two factor multivariate analysis of variance was performed with time factor at two levels- Dec. 03 and Dec. 04, and intervention factor with the levels control and seismic. There was a significant two-way interaction of both factors $p = 0.027$. (Please see Appendix 3i for details on the multivariate tests.) Means are reported in this appendix as well as graphs of significant interactions.

Ovarian results

A two factor multivariate analysis of variance was performed with time factor at two levels, Dec. 03 and Dec. 04, and intervention factor with the levels control and seismic. There was a significant two-way interaction of both factors $p = 0.001$. (Please see Appendix 3j for more multivariate tests.) Means are reported in this appendix as well as graphs of significant interactions.

Comparison of wild vs. all other treatments

Multivariate ANOVA results comparing the seven treatments (treatment key given as 1 = control dec03, 2 = seismic dec03, 3 = control may04, 4 = seismic may04, 5 = control site dec04, 6 = seismic site dec04, and 7 = wild site) were very significant,
Pillias trace for both hepatopancreatic and ovarian data was $p < 0.001$. (Please see Appendix 3k and 3l for multivariate tests and graphs of means.)

Inter-rater reliability

Two raters independently evaluated slides for both the hepatopancreatic (a random selection of n = 165 slides from the total dataset) and ovarian (a random selection of n = 151 slides from the total dataset) dependent variables. Pearson product moment correlations were computed for both raters on the above variables and the sum score. In general, the reliability was acceptable (some correlations were zero whereas others were over 0.7). The ovarian data was more reliable than the hepatopancreatic. Please see Appendix 3 m for the actual values and significance, where the null hypothesis is the correlation is equal to zero.

Appendix 3a
- Hepatopancreatic dependent variables
- Comparison of December 2003 data of control vs. seismic groups

Multivariate Tests (b)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.963	281.092(a)	11.000	118.000	.000	.963
	Wilks' Lambda	.037	281.092(a)	11.000	118.000	.000	.963
	Hotelling's Trace	26.203	281.092(a)	11.000	118.000	.000	.963
	Roy's Largest Root	26.203	281.092(a)	11.000	118.000	.000	.963
dec03in	Pillai's Trace	.186	2.448(a)	11.000	118.000	.009	.186
	Wilks' Lambda	.814	2.448(a)	11.000	118.000	.009	.186
	Hotelling's Trace	.228	2.448(a)	11.000	118.000	.009	.186
	Roy's Largest Root	.228	2.448(a)	11.000	118.000	.009	.186

(a) Exact statistic
(b) Design: Intercept + dec03in

.

Descriptive Statistics

	1 control 0 seismic	Mean	Std. Deviation	N
M Cells (number and positioning)	.00	1.05	.469	64
	1.00	1.19	.593	66
	Total	1.12	.538	130
Nuclear Shape	.00	1.26	.445	64
	1.00	1.27	.474	66
	Total	1.27	.458	130
Nuclear Size	.00	1.69	1.787	64
	1.00	1.39	.515	66
	Total	1.54	1.310	130
Epithelial Wall Thickness	.00	1.76	.859	64
	1.00	2.25	1.107	66
	Total	2.01	1.019	130
R-cells	.00	1.281	.7760	64
	1.00	1.864	1.1009	66
	Total	1.577	.9951	130
Phagocyte Activation	.00	2.07	.734	64
	1.00	2.24	.781	66
	Total	2.16	.760	130
Encapsulations and Parasites	.00	1.27	.462	64
	1.00	1.24	.577	66
	Total	1.26	.522	130
Delamination of BM	.00	1.22	.407	64
	1.00	1.22	.465	66
	Total	1.22	.436	130
Peritrophic Membrane/Luminal Contents	.00	2.08	.818	64
	1.00	2.16	1.078	66
	Total	2.12	.956	130
Necrosis or Autolysis	.00	2.09	.934	64
	1.00	2.39	.988	66
	Total	2.24	.969	130
Collagen	.00	1.38	.815	64
	1.00	1.22	.534	66
	Total	1.30	.689	130

Appendix 3b
- Ovarian dependent variables
- Comparison of December 2003 data of control vs. seismic groups

Multivariate Tests(c)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Pillai's Trace	.947	128.572(b)	9.000	65.000	.000	.947	1157.146	1.000
	Wilks' Lambda	.053	128.572(b)	9.000	65.000	.000	.947	1157.146	1.000
	Hotelling's Trace	17.802	128.572(b)	9.000	65.000	.000	.947	1157.146	1.000
	Roy's Largest Root	17.802	128.572(b)	9.000	65.000	.000	.947	1157.146	1.000
dec03	Pillai's Trace	.451	5.942(b)	9.000	65.000	.000	.451	53.476	1.000
	Wilks' Lambda	.549	5.942(b)	9.000	65.000	.000	.451	53.476	1.000
	Hotelling's Trace	.823	5.942(b)	9.000	65.000	.000	.451	53.476	1.000
	Roy's Largest Root	.823	5.942(b)	9.000	65.000	.000	.451	53.476	1.000

(a) Computed using alpha = .05
(b) Exact statistic
(c) Design: Intercept + dec03

Descriptive Statistics

	1 control 0 seismic	Mean	Std. Deviation	N
Delamination	.00	1.11	.277	46
	1.00	1.22	.435	29
	Total	1.15	.348	75
Atresia	.00	1.54	.585	46
	1.00	1.86	.731	29
	Total	1.67	.659	75
Packing Density	.00	1.261	.6123	46
	1.00	2.034	.9904	29
	Total	1.560	.8620	75
Granulocytic Infiltration of CT	.00	1.35	.482	46
	1.00	2.34	1.111	29
	Total	1.73	.920	75
"Bruising"	.00	1.49	.572	46
	1.00	2.53	1.149	29
	Total	1.89	.981	75
Delayed Maturation	.00	1.13	.324	46
	1.00	2.07	1.374	29
	Total	1.49	.995	75
Typical Staining Pattern	.00	1.25	.621	46
	1.00	2.19	1.543	29
	Total	1.61	1.161	75
Edema/Homogenization	.00	1.58	.722	46
	1.00	1.84	.803	29
	Total	1.68	.761	75
Encapsulation	.00	1.42	.836	46
	1.00	1.31	1.039	29
	Total	1.38	.915	75

Appendix 3c
- Hepatopancreatic dependent variables
- Comparison of May 2004 data of control vs. seismic groups

Multivariate Tests (b)							
Effect		**Value**	**F**	**Hypothesis df**	**Error df**	**Sig.**	**Partial Eta Squared**
Intercept	**Pillai's Trace**	.962	386.338(a)	11.000	166.000	.000	.962
	Wilks' Lambda	.038	386.338(a)	11.000	166.000	.000	.962
	Hotelling's Trace	25.601	386.338(a)	11.000	166.000	.000	.962
	Roy's Largest Root	25.601	386.338(a)	11.000	166.000	.000	.962
may04in	**Pillai's Trace**	.121	2.081(a)	11.000	166.000	.024	.121
	Wilks' Lambda	.879	2.081(a)	11.000	166.000	.024	.121
	Hotelling's Trace	.138	2.081(a)	11.000	166.000	.024	.121
	Roy's Largest Root	.138	2.081(a)	11.000	166.000	.024	.121
(a) Exact statistic							
(b) Design: Intercept + may04in							

1 control 0 seismic					
Dependent Variable	**1 control 0 seismic**	**Mean**	**Std. Error**	**95% Confidence Interval**	
		Lower Bound	**Upper Bound**	**Lower Bound**	**Upper Bound**
M Cells (number and positioning)	**.00**	1.500	.092	1.318	1.682
	1.00	1.699	.093	1.515	1.883
Nuclear Shape	**.00**	1.517	.060	1.399	1.634
	1.00	1.347	.060	1.227	1.466
Nuclear Size	**.00**	1.778	.079	1.621	1.934
	1.00	1.830	.080	1.671	1.988
Epithelial Wall Thickness	**.00**	2.244	.105	2.037	2.452
	1.00	2.426	.106	2.216	2.636
R-cells	**.00**	2.061	.160	1.745	2.378
	1.00	2.551	.162	2.231	2.871
Phagocyte Activation	**.00**	1.850	.071	1.709	1.991
	1.00	1.778	.072	1.636	1.921
Encapsulations and Parasites	**.00**	1.383	.079	1.228	1.538
	1.00	1.392	.079	1.235	1.549
Delamination of BM	**.00**	1.383	.067	1.252	1.515
	1.00	1.574	.067	1.441	1.707
Peritrophic Membrane/Luminal Contents	**.00**	1.989	.086	1.820	2.158
	1.00	2.386	.086	2.216	2.557
Necrosis or Autolysis	**.00**	2.156	.094	1.970	2.341
	1.00	2.273	.095	2.085	2.461
Collagen	**.00**	1.306	.073	1.161	1.450
	1.00	1.278	.074	1.132	1.424

Appendix 3d
- Ovarian dependent variables
- Comparison of May 2004 data of control vs. seismic groups

Multivariate Tests(c)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Pillai's Trace	.961	451.339(b)	9.000	166.000	.000	.961	4062.050	1.000
	Wilks' Lambda	.039	451.339(b)	9.000	166.000	.000	.961	4062.050	1.000
	Hotelling's Trace	24.470	451.339(b)	9.000	166.000	.000	.961	4062.050	1.000
	Roy's Largest Root	24.470	451.339(b)	9.000	166.000	.000	.961	4062.050	1.000
may04in	Pillai's Trace	.173	3.855(b)	9.000	166.000	.000	.173	34.696	.993
	Wilks' Lambda	.827	3.855(b)	9.000	166.000	.000	.173	34.696	.993
	Hotelling's Trace	.209	3.855(b)	9.000	166.000	.000	.173	34.696	.993
	Roy's Largest Root	.209	3.855(b)	9.000	166.000	.000	.173	34.696	.993

(a) Computed using alpha = .05
(b) Exact statistic
(c) Design: Intercept + may04in

Descriptive Statistics

	1 control 0 seismic	Mean	Std. Deviation	N
Delamination	.00	1.83	.626	89
	1.00	2.37	1.132	87
	Total	2.10	.949	176
Atresia	.00	2.29	.648	89
	1.00	2.17	.769	87
	Total	2.23	.711	176
Packing Density	.00	2.213	2.3047	89
	1.00	2.046	.9106	87
	Total	2.131	1.7566	176
Granulocytic Infiltration of CT	.00	1.35	.716	89
	1.00	1.55	.931	87
	Total	1.45	.833	176
"Bruising"	.00	1.87	.782	89
	1.00	2.09	1.066	87
	Total	1.98	.937	176
Delayed Maturation	.00	1.35	.743	89
	1.00	1.57	1.036	87
	Total	1.46	.904	176
Typical Staining Pattern	.00	1.38	.840	89
	1.00	1.65	1.103	87
	Total	1.51	.986	176
Edema/Homogenization	.00	1.94	.721	89
	1.00	1.97	.729	87
	Total	1.96	.723	176
Encapsulation	.00	1.30	.891	89
	1.00	1.45	1.068	87
	Total	1.37	.982	176

Appendix 3e
- Hepatopancreatic dependent variables
- Comparison of December 2004 data of control vs. seismic groups

Multivariate Tests (b)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.977	336.029(a)	11.000	87.000	.000	.977
	Wilks' Lambda	.023	336.029(a)	11.000	87.000	.000	.977
	Hotelling's Trace	42.486	336.029(a)	11.000	87.000	.000	.977
	Roy's Largest Root	42.486	336.029(a)	11.000	87.000	.000	.977
dec04in	Pillai's Trace	.083	.711(a)	11.000	87.000	.725	.083
	Wilks' Lambda	.917	.711(a)	11.000	87.000	.725	.083
	Hotelling's Trace	.090	.711(a)	11.000	87.000	.725	.083
	Roy's Largest Root	.090	.711(a)	11.000	87.000	.725	.083

(a) Exact statistic
(b) Design: Intercept + dec04in

Appendix 3f
- Ovarian dependent variables
- Comparison of December 2004 data of control vs. seismic groups

Multivariate Tests(c)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Pillai's Trace	.968	301.603(b)	9.000	90.000	.000	.968	2714.427	1.000
	Wilks' Lambda	.032	301.603(b)	9.000	90.000	.000	.968	2714.427	1.000
	Hotelling's Trace	30.160	301.603(b)	9.000	90.000	.000	.968	2714.427	1.000
	Roy's Largest Root	30.160	301.603(b)	9.000	90.000	.000	.968	2714.427	1.000
dec04in	Pillai's Trace	.075	.813(b)	9.000	90.000	.605	.075	7.321	.379
	Wilks' Lambda	.925	.813(b)	9.000	90.000	.605	.075	7.321	.379
	Hotelling's Trace	.081	.813(b)	9.000	90.000	.605	.075	7.321	.379
	Roy's Largest Root	.081	.813(b)	9.000	90.000	.605	.075	7.321	.379

(a) Computed using alpha = .05
(b) Exact statistic
(c) Design: Intercept + dec04in

Appendix 3g
- Hepatopancreatic dependent variables
- Comparison of D03MAY (time levels- December 2003 and May 2004 data) and CVSS (intervention levels- control vs. seismic groups)

Multivariate Tests(b)

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.956	582.117(a)	11.000	294.000	.000
	Wilks' Lambda	.044	582.117(a)	11.000	294.000	.000
	Hotelling's Trace	21.780	582.117(a)	11.000	294.000	.000
	Roy's Largest Root	21.780	582.117(a)	11.000	294.000	.000
cvss	Pillai's Trace	.088	2.594(a)	11.000	294.000	.004
	Wilks' Lambda	.912	2.594(a)	11.000	294.000	.004
	Hotelling's Trace	.097	2.594(a)	11.000	294.000	.004
	Roy's Largest Root	.097	2.594(a)	11.000	294.000	.004
d03may	Pillai's Trace	.241	8.464(a)	11.000	294.000	.000
	Wilks' Lambda	.759	8.464(a)	11.000	294.000	.000
	Hotelling's Trace	.317	8.464(a)	11.000	294.000	.000
	Roy's Largest Root	.317	8.464(a)	11.000	294.000	.000
cvss * d03may	Pillai's Trace	.066	1.902(a)	11.000	294.000	.039
	Wilks' Lambda	.934	1.902(a)	11.000	294.000	.039
	Hotelling's Trace	.071	1.902(a)	11.000	294.000	.039
	Roy's Largest Root	.071	1.902(a)	11.000	294.000	.039

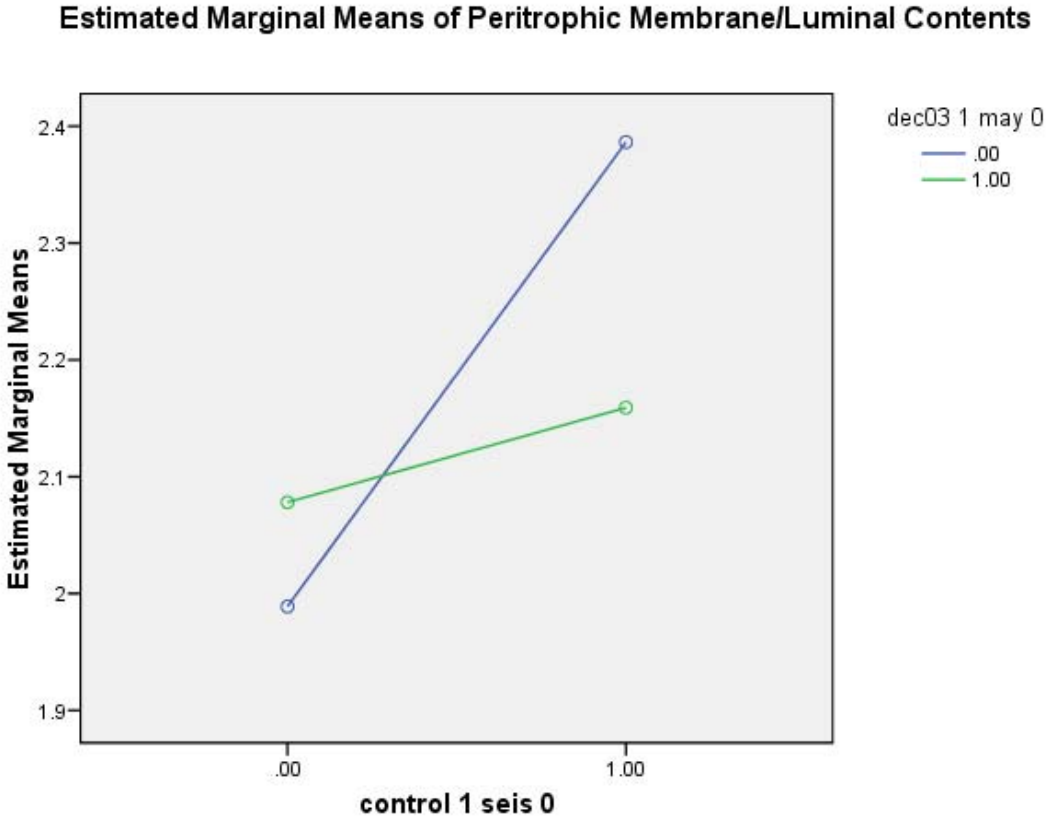
(a) Exact statistic
(b) Design: Intercept + cvss + d03may + cvss * d03may

Descriptive Statistics

	control 1 seis 0	dec03 1 may 0	Mean	Std. Deviation	N
M Cells (number and positioning)	.00	.00	1.50	.828	90
		1.00	1.05	.469	64
		Total	1.31	.734	154
	1.00	.00	1.70	.921	88
		1.00	1.19	.593	66
		Total	1.48	.834	154
	Total	.00	1.60	.878	178
		1.00	1.12	.538	130
		Total	1.40	.789	308
Nuclear Shape	.00	.00	1.52	.578	90
		1.00	1.26	.445	64
		Total	1.41	.541	154
	1.00	.00	1.35	.554	88
		1.00	1.27	.474	66
		Total	1.31	.521	154
	Total	.00	1.43	.571	178
		1.00	1.27	.458	130
		Total	1.36	.532	308
Nuclear Size	.00	.00	1.78	.667	90
		1.00	1.69	1.787	64
		Total	1.74	1.255	154
	1.00	.00	1.83	.830	88
		1.00	1.39	.515	66
		Total	1.64	.743	154
	Total	.00	1.80	.750	178
		1.00	1.54	1.310	130
		Total	1.69	1.031	308
Epithelial Wall Thickness	.00	.00	2.24	.940	90
		1.00	1.76	.859	64
		Total	2.04	.936	154
	1.00	.00	2.43	1.052	88
		1.00	2.25	1.107	66
		Total	2.35	1.076	154
	Total	.00	2.33	.998	178
		1.00	2.01	1.019	130
		Total	2.20	1.018	308
R-cells	.00	.00	2.061	1.4414	90
		1.00	1.281	.7760	64
		Total	1.737	1.2670	154
	1.00	.00	2.551	1.5993	88
		1.00	1.864	1.1009	66
		Total	2.256	1.4442	154
	Total	.00	2.303	1.5370	178
		1.00	1.577	.9951	130

		Total	1.997	1.3810	308
Phagocyte Activation	.00	.00	1.85	.685	90
		1.00	2.07	.734	64
		Total	1.94	.712	154
	1.00	.00	1.78	.669	88
		1.00	2.24	.781	66
		Total	1.98	.753	154
	Total	.00	1.81	.676	178
		1.00	2.16	.760	130
		Total	1.96	.731	308
Encapsulations and Parasites	.00	.00	1.38	.662	90
		1.00	1.27	.462	64
		Total	1.34	.588	154
	1.00	.00	1.39	.822	88
		1.00	1.24	.577	66
		Total	1.33	.728	154
	Total	.00	1.39	.743	178
		1.00	1.26	.522	130
		Total	1.33	.661	308
Delamination of BM	.00	.00	1.38	.600	90
		1.00	1.22	.407	64
		Total	1.31	.533	154
	1.00	.00	1.57	.663	88
		1.00	1.22	.465	66
		Total	1.42	.611	154
	Total	.00	1.48	.637	178
		1.00	1.22	.436	130
		Total	1.37	.575	308
Peritrophic Membrane/Luminal Contents	.00	.00	1.99	.811	90
		1.00	2.08	.818	64
		Total	2.03	.812	154
	1.00	.00	2.39	.812	88
		1.00	2.16	1.078	66
		Total	2.29	.939	154
	Total	.00	2.19	.833	178
		1.00	2.12	.956	130
		Total	2.16	.886	308
Necrosis or Autolysis	.00	.00	2.16	.923	90
		1.00	2.09	.934	64
		Total	2.13	.925	154
	1.00	.00	2.27	.861	88
		1.00	2.39	.988	66
		Total	2.32	.916	154
	Total	.00	2.21	.892	178
		1.00	2.24	.969	130

		Total	2.23	.924	308
Collagen	.00	.00	1.31	.760	90
		1.00	1.38	.815	64
		Total	1.34	.781	154
	1.00	.00	1.28	.620	88
		1.00	1.22	.534	66
		Total	1.25	.584	154
	Total	.00	1.29	.692	178
		1.00	1.30	.689	130
		Total	1.30	.690	308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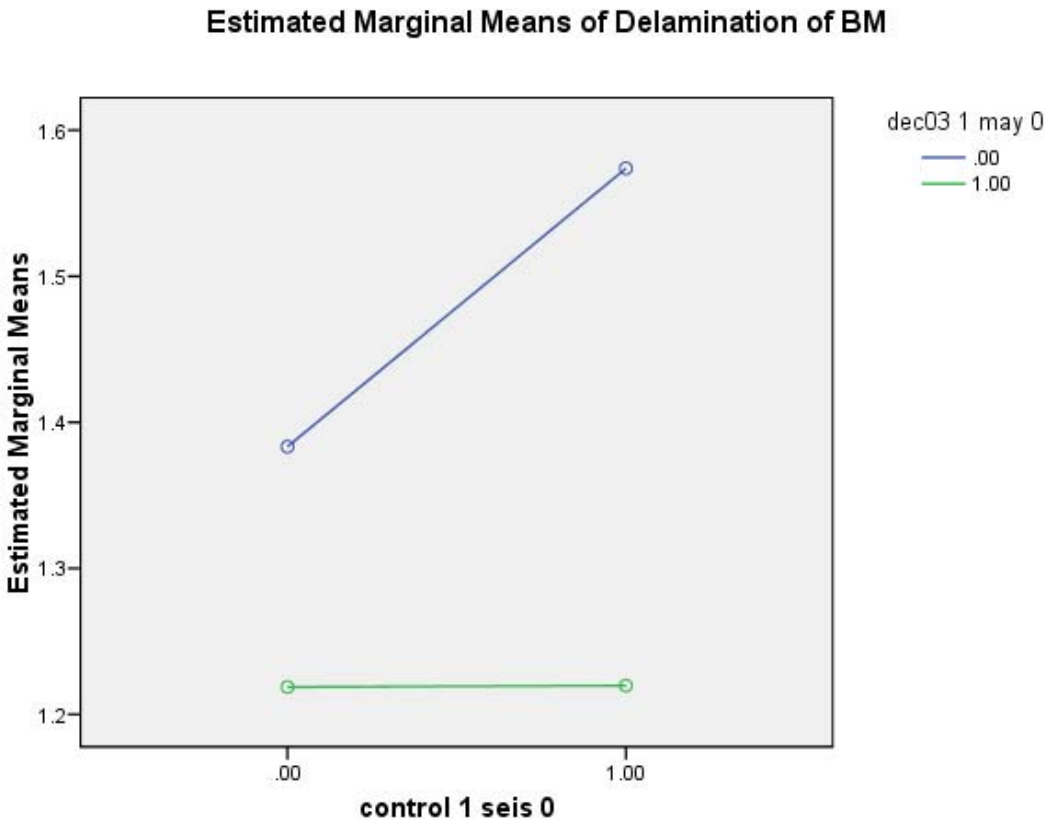
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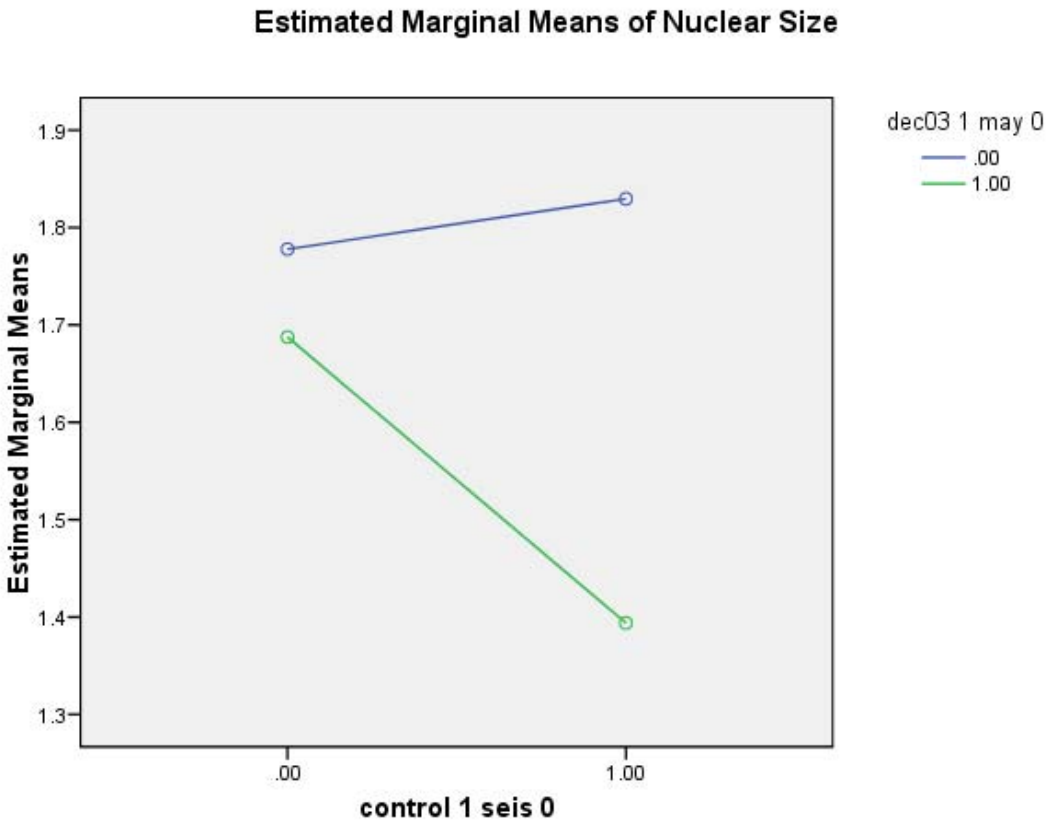
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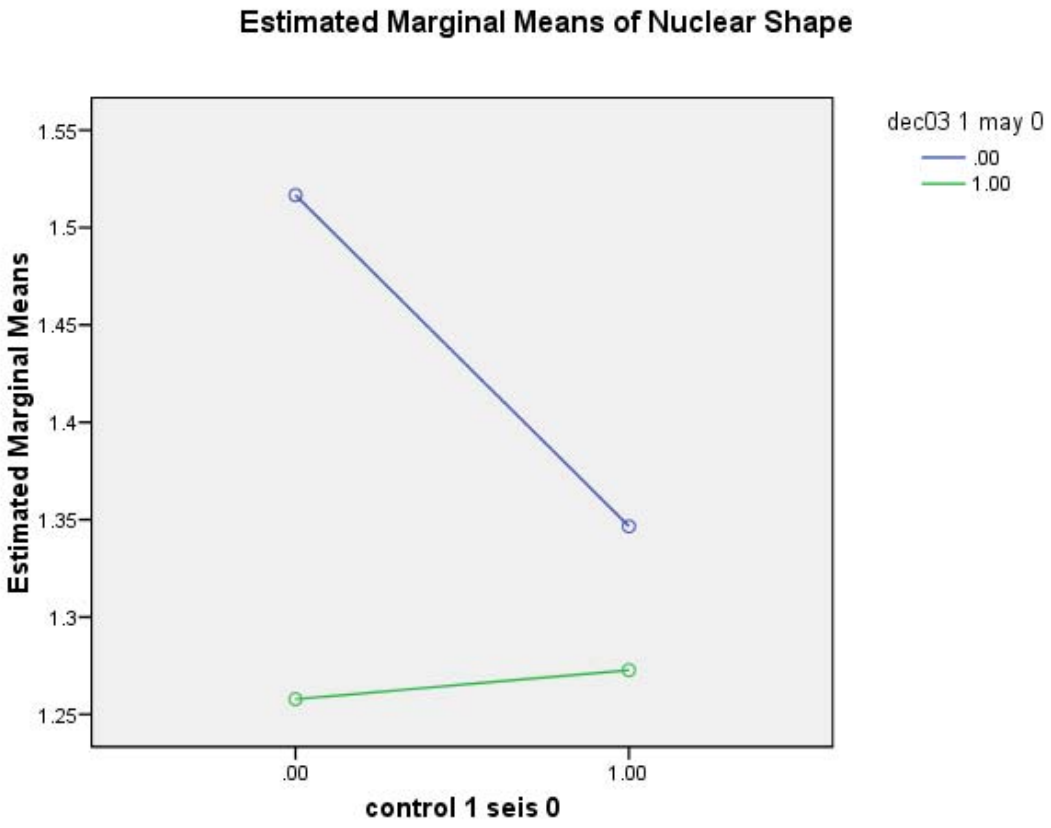
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Appendix 3h
- Ovarian data

Multivariate Tests (b)

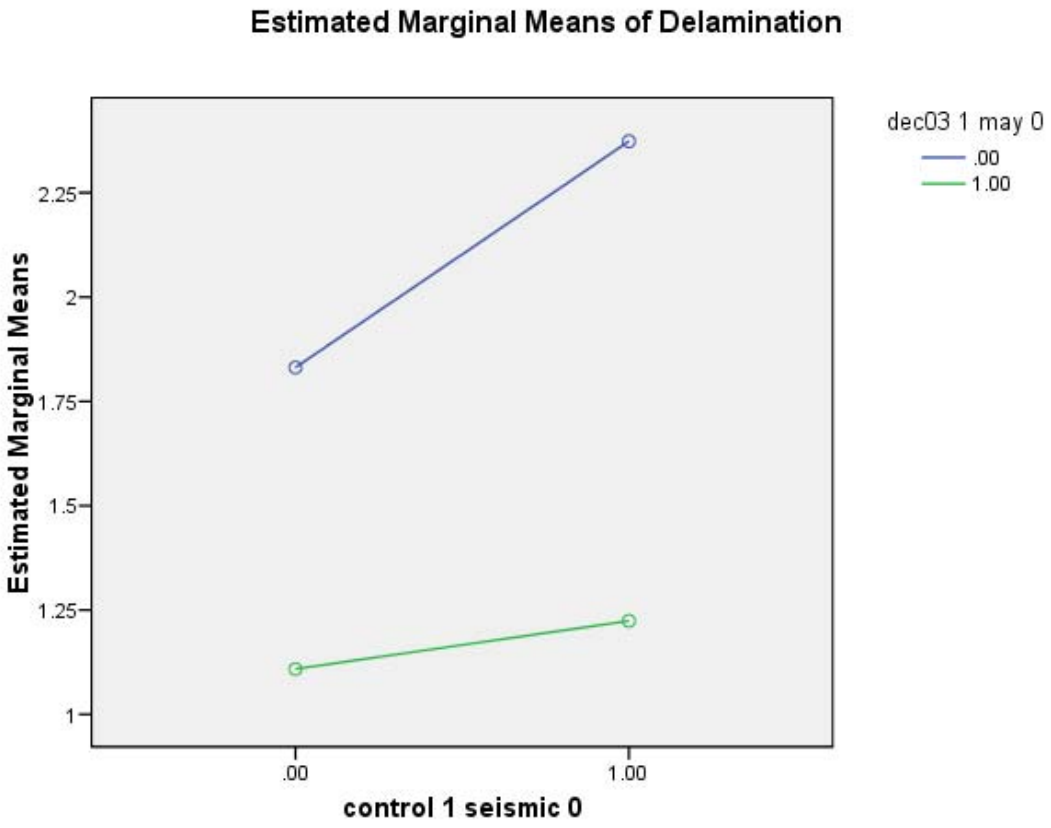
Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.939	406.673(a)	9.000	239.000	.000
	Wilks' Lambda	.061	406.673(a)	9.000	239.000	.000
	Hotelling's Trace	15.314	406.673(a)	9.000	239.000	.000
	Roy's Largest Root	15.314	406.673(a)	9.000	239.000	.000
d03may	Pillai's Trace	.333	13.280(a)	9.000	239.000	.000
	Wilks' Lambda	.667	13.280(a)	9.000	239.000	.000
	Hotelling's Trace	.500	13.280(a)	9.000	239.000	.000
	Roy's Largest Root	.500	13.280(a)	9.000	239.000	.000
cvss	Pillai's Trace	.210	7.062(a)	9.000	239.000	.000
	Wilks' Lambda	.790	7.062(a)	9.000	239.000	.000
	Hotelling's Trace	.266	7.062(a)	9.000	239.000	.000
	Roy's Largest Root	.266	7.062(a)	9.000	239.000	.000
cvss * d03may	Pillai's Trace	.098	2.888(a)	9.000	239.000	.003
	Wilks' Lambda	.902	2.888(a)	9.000	239.000	.003
	Hotelling's Trace	.109	2.888(a)	9.000	239.000	.003
	Roy's Largest Root	.109	2.888(a)	9.000	239.000	.003

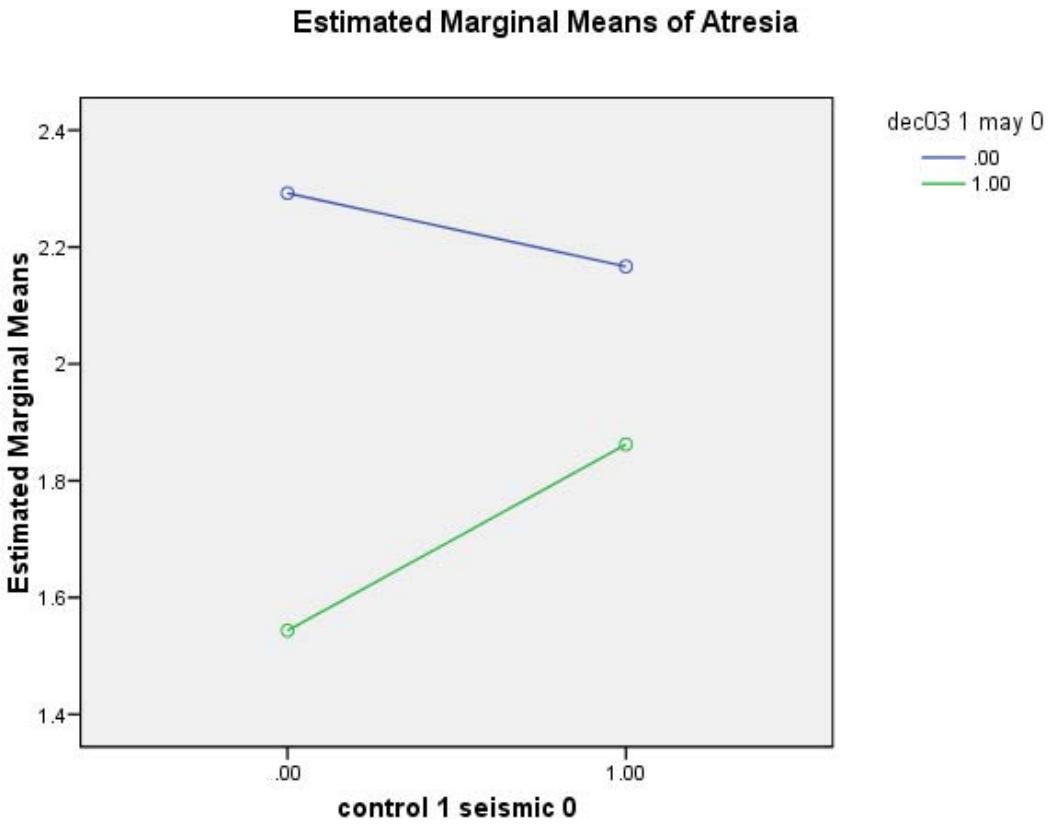
(a) Exact statistic
(b) Design: Intercept + d03may + cvss + cvss * d03may

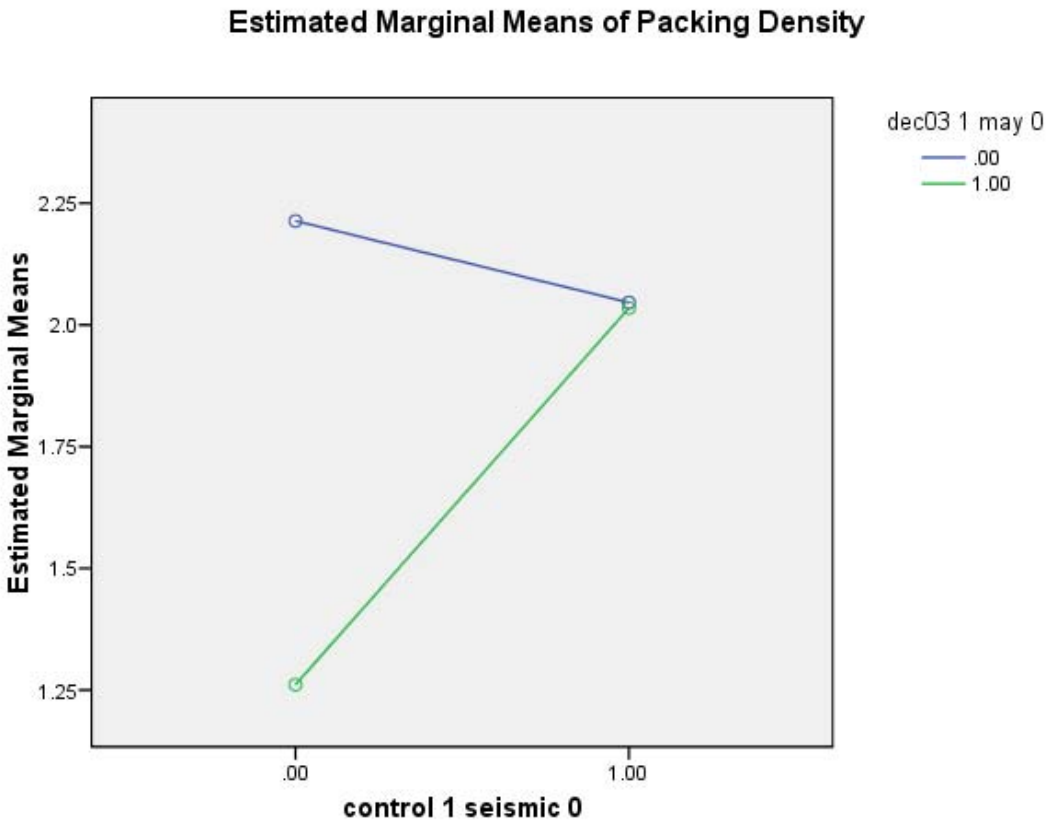
Descriptive Statistics

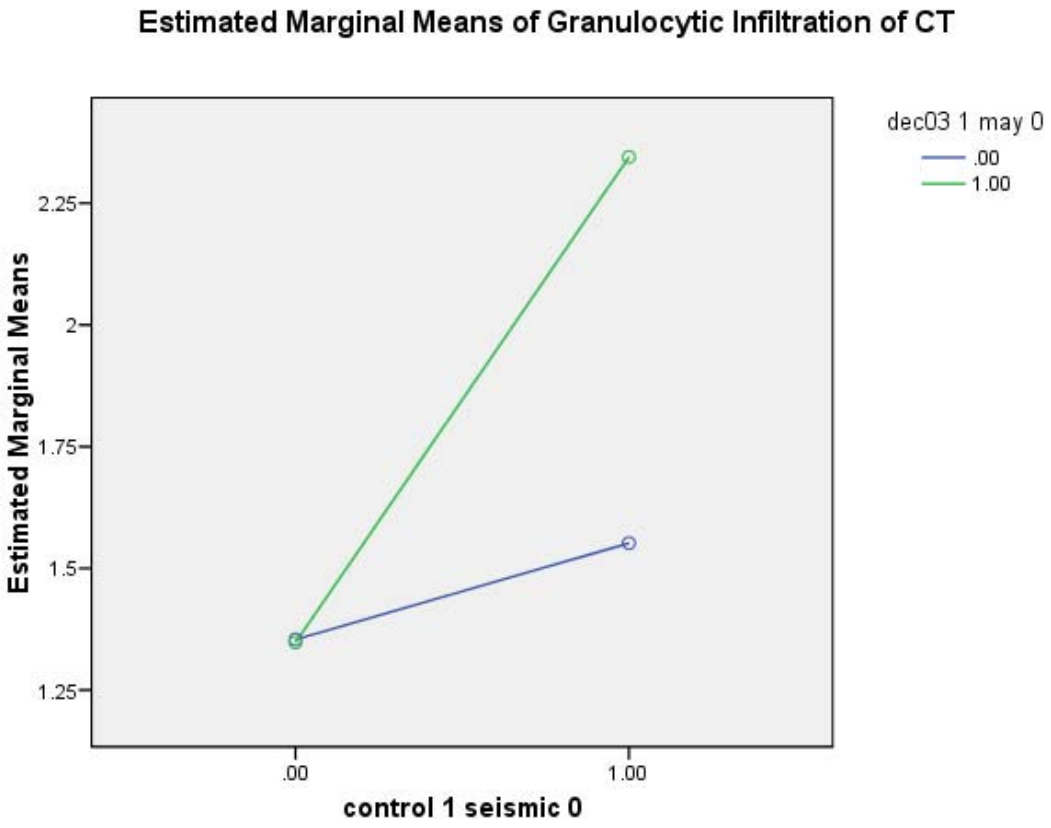
	control 1 seismic 0	dec03 1 may 0	Mean	Std. Deviation	N
Delamination	.00	.00	1.83	.626	89
		1.00	1.11	.277	46
		Total	1.59	.633	135
	1.00	.00	2.37	1.132	87
		1.00	1.22	.435	29
		Total	2.09	1.120	116
	Total	.00	2.10	.949	176
		1.00	1.15	.348	75
		Total	1.82	.924	251
Atresia	.00	.00	2.29	.648	89
		1.00	1.54	.585	46
		Total	2.04	.719	135
	1.00	.00	2.17	.769	87
		1.00	1.86	.731	29
		Total	2.09	.768	116
	Total	.00	2.23	.711	176
		1.00	1.67	.659	75
		Total	2.06	.741	251
Packing Density	.00	.00	2.213	2.3047	89
		1.00	1.261	.6123	46
		Total	1.889	1.9544	135
	1.00	.00	2.046	.9106	87
		1.00	2.034	.9904	29
		Total	2.043	.9268	116
	Total	.00	2.131	1.7566	176
		1.00	1.560	.8620	75
		Total	1.960	1.5647	251
Granulocytic Infiltration of CT	.00	.00	1.35	.716	89
		1.00	1.35	.482	46
		Total	1.35	.644	135
	1.00	.00	1.55	.931	87
		1.00	2.34	1.111	29
		Total	1.75	1.033	116
	Total	.00	1.45	.833	176
		1.00	1.73	.920	75
		Total	1.54	.868	251
"Bruising"	.00	.00	1.87	.782	89
		1.00	1.49	.572	46
		Total	1.74	.738	135
	1.00	.00	2.09	1.066	87
		1.00	2.53	1.149	29
		Total	2.20	1.099	116
	Total	.00	1.98	.937	176
		1.00	1.89	.981	75

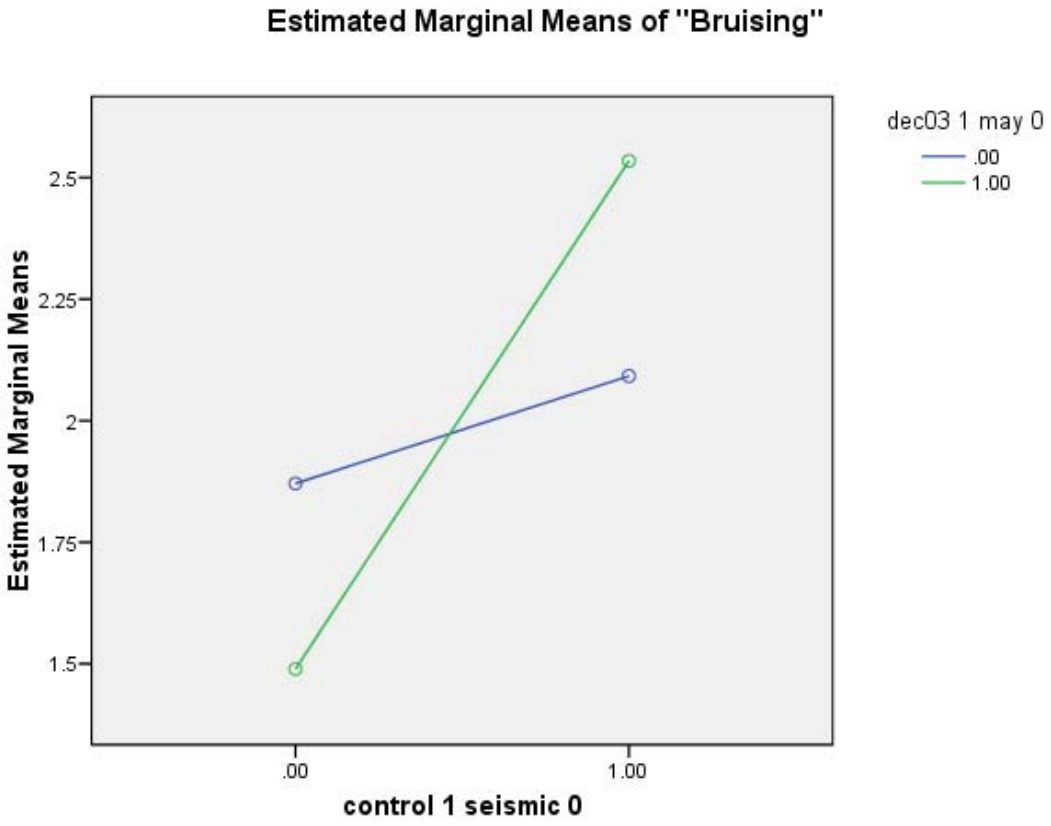
		Total	1.95	.949	251
Delayed Maturation	.00	.00	1.35	.743	89
		1.00	1.13	.324	46
		Total	1.28	.640	135
	1.00	.00	1.57	1.036	87
		1.00	2.07	1.374	29
		Total	1.70	1.144	116
	Total	.00	1.46	.904	176
		1.00	1.49	.995	75
		Total	1.47	.930	251
Typical Staining Pattern	.00	.00	1.38	.840	89
		1.00	1.25	.621	46
		Total	1.33	.773	135
	1.00	.00	1.65	1.103	87
		1.00	2.19	1.543	29
		Total	1.78	1.243	116
	Total	.00	1.51	.986	176
		1.00	1.61	1.161	75
		Total	1.54	1.040	251
Edema/Homogenization	.00	.00	1.94	.721	89
		1.00	1.58	.722	46
		Total	1.82	.740	135
	1.00	.00	1.97	.729	87
		1.00	1.84	.803	29
		Total	1.94	.746	116
	Total	.00	1.96	.723	176
		1.00	1.68	.761	75
		Total	1.87	.744	251
Encapsulation	.00	.00	1.30	.891	89
		1.00	1.42	.836	46
		Total	1.34	.872	135
	1.00	.00	1.45	1.068	87
		1.00	1.31	1.039	29
		Total	1.41	1.058	116
	Total	.00	1.37	.982	176
		1.00	1.38	.915	75
		Total	1.37	.961	251

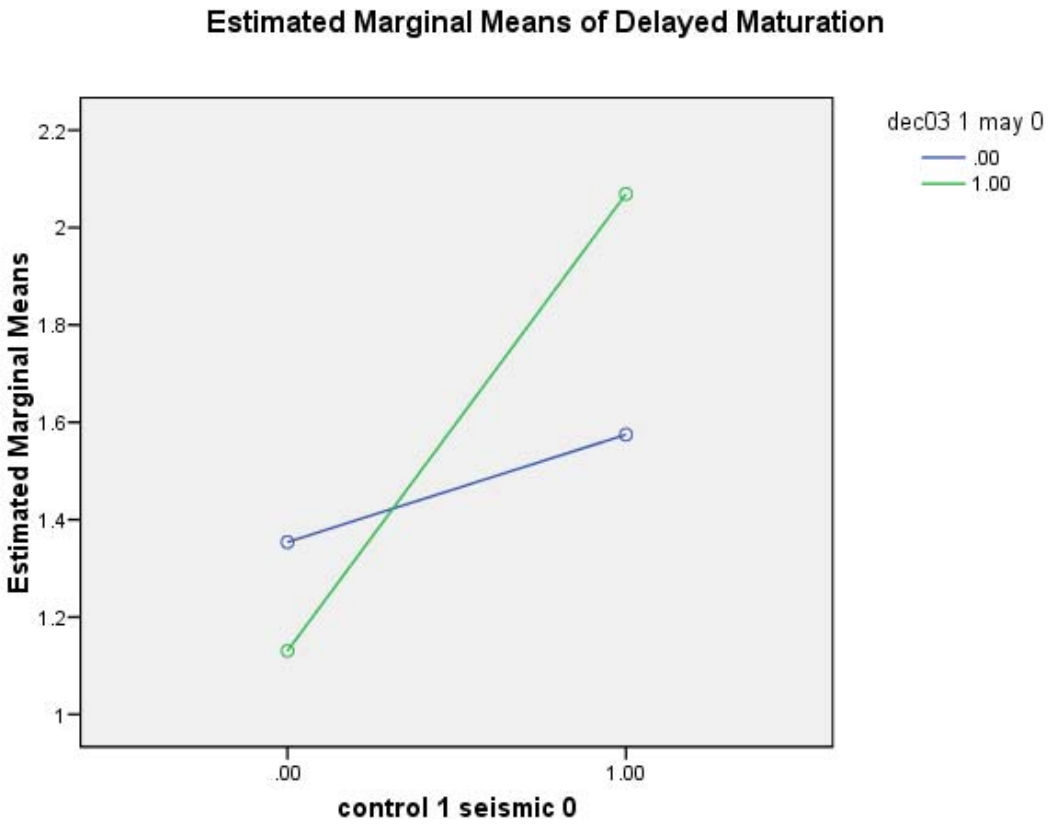

Estimated Marginal Means of Delamination
dec03 1 may 0
.00
1.00
Estimated Marginal Means
2.25
2
1.75
1.5
1.25
1
.00
1.00
control 1 seismic 0

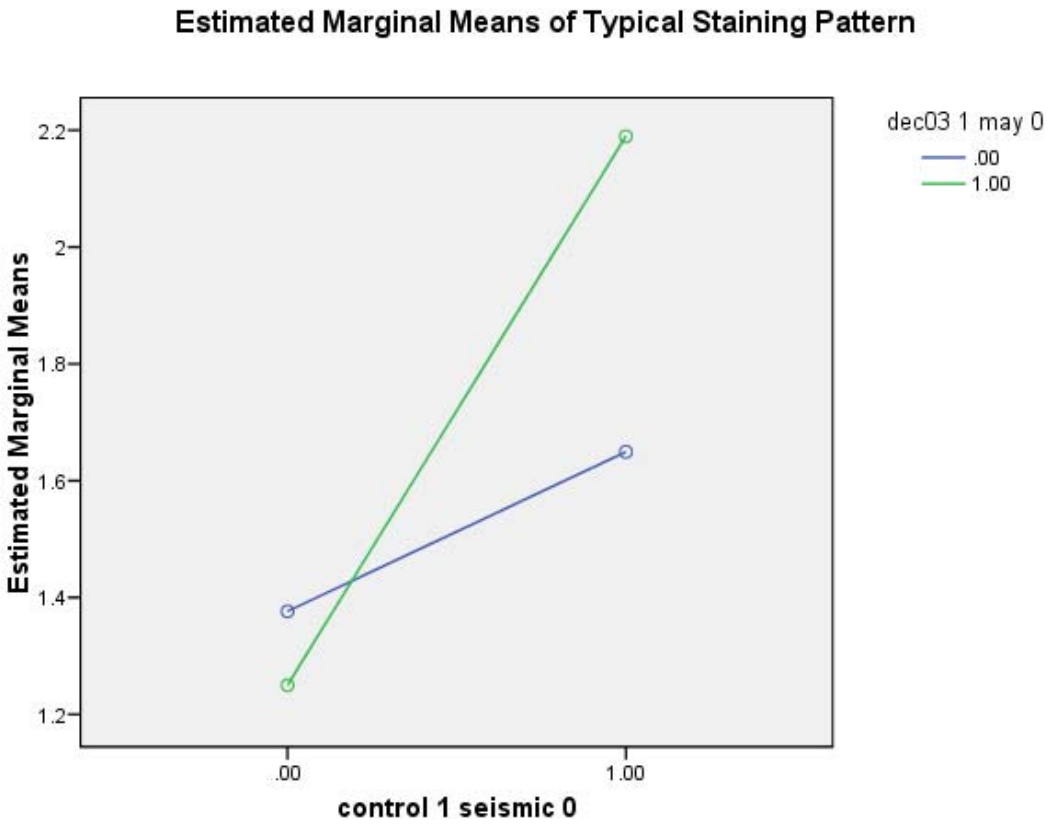

Estimated Marginal Means of Atresia
Estimated Marginal Means
2.4
2.2
2
1.8
1.6
1.4
.00
1.00
control 1 seismic 0
dec03 1 may 0
.00
1.00


Estimated Marginal Means of Packing Density
dec03 1 may 0
.00
1.00
Estimated Marginal Means
2.25
2.0
1.75
1.5
1.25
.00
1.00
control 1 seismic 0


Estimated Marginal Means of Granulocytic Infiltration of CT
dec03 1 may 0
.00
1.00
Estimated Marginal Means
2.25
2
1.75
1.5
1.25
.00
1.00
control 1 seismic 0


Estimated Marginal Means of "Bruising"
dec03 1 may 0
.00
1.00
Estimated Marginal Means
2.5
2.25
2
1.75
1.5
.00
1.00
control 1 seismic 0


Estimated Marginal Means of Delayed Maturation
Estimated Marginal Means
2.2
2
1.8
1.6
1.4
1.2
1
.00
1.00
control 1 seismic 0
dec03 1 may 0
.00
1.00


Estimated Marginal Means of Typical Staining Pattern
Estimated Marginal Means
2.2
2
1.8
1.6
1.4
1.2
.00
1.00
control 1 seismic 0
dec03 1 may 0
.00
1.00

Appendix 3i

- Comparison of D03vs4 (time levels - December 2003 and December 2004 data) and CVSS (intervention levels- control vs. seismic groups)

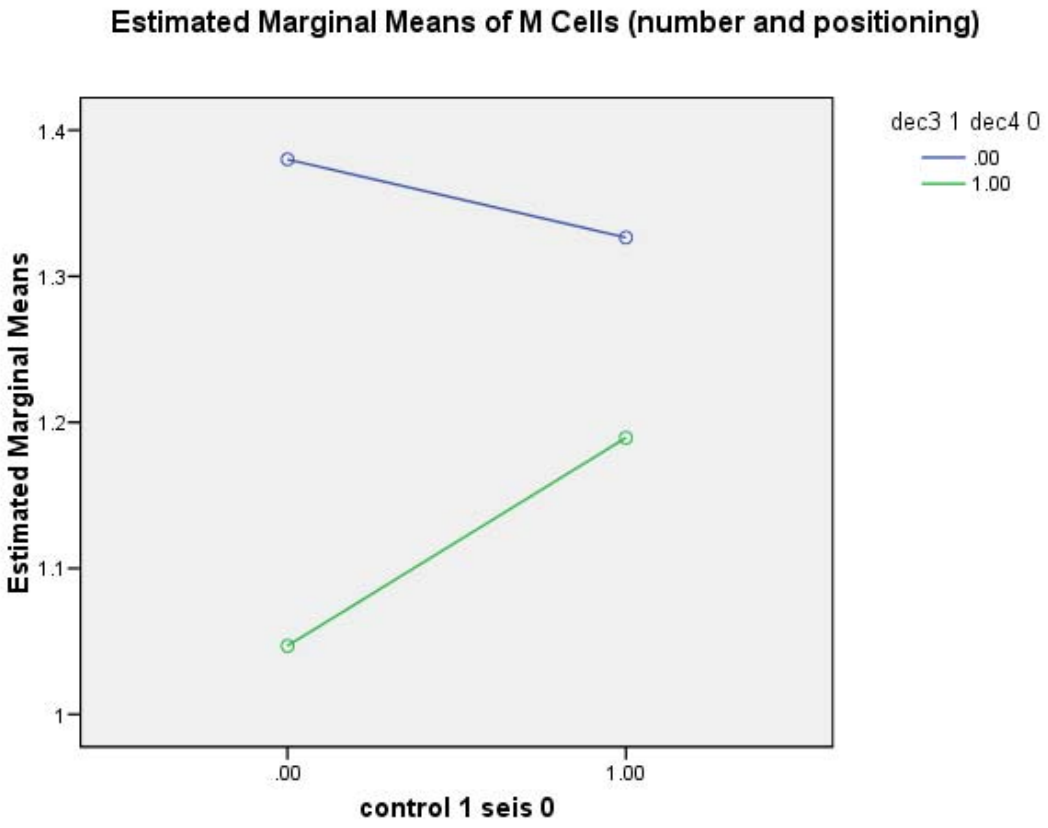
- Hepatopancreatic results multivariate tests and significant interactions

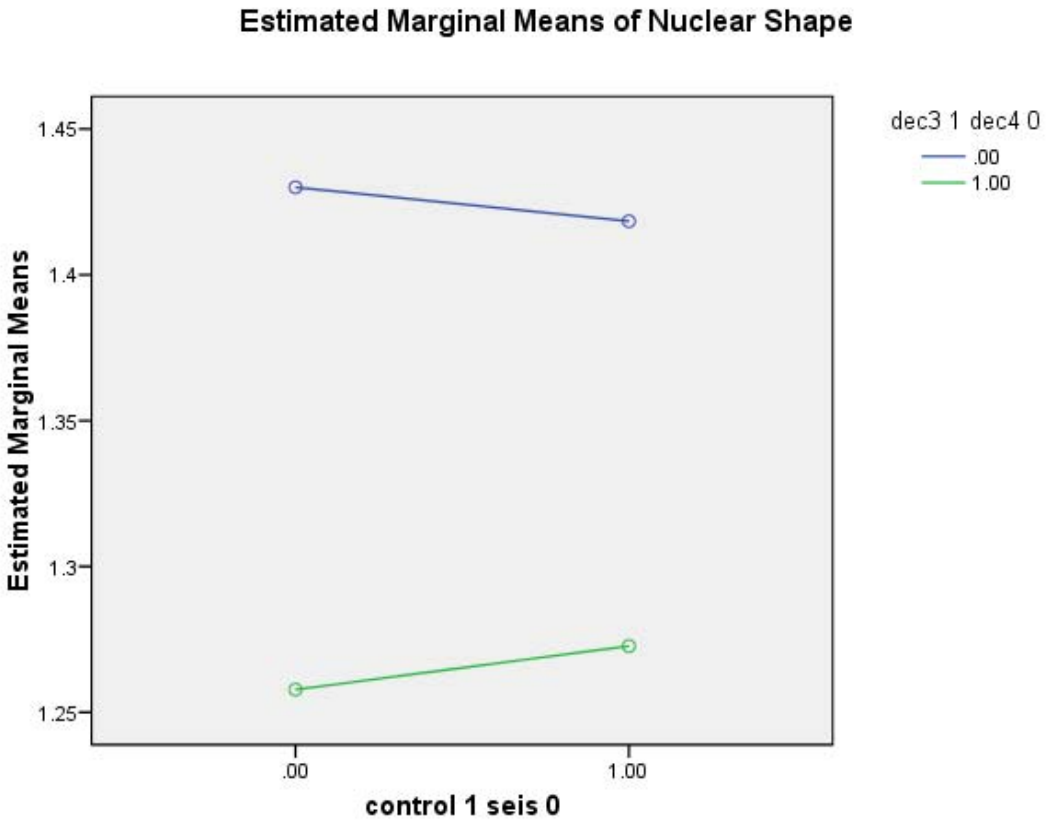
Multivariate Tests (b)

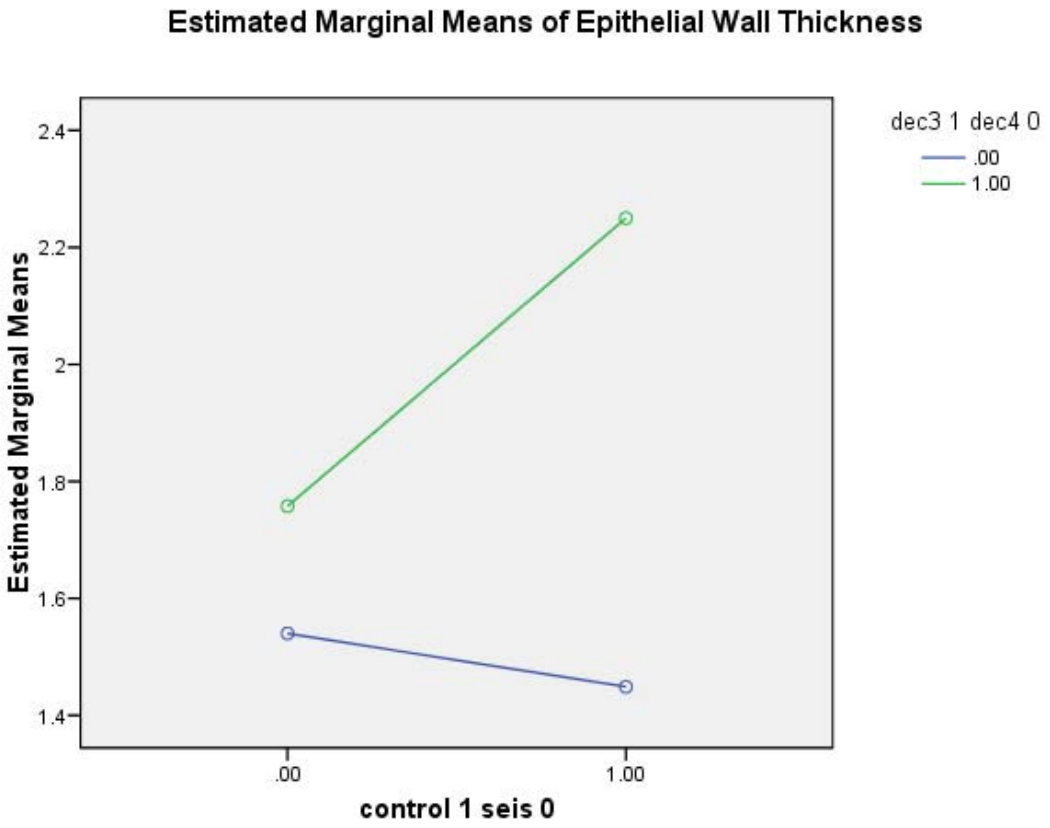
Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.965	541.841(a)	11.000	215.000	.000
	Wilks' Lambda	.035	541.841(a)	11.000	215.000	.000
	Hotelling's Trace	27.722	541.841(a)	11.000	215.000	.000
	Roy's Largest Root	27.722	541.841(a)	11.000	215.000	.000
cvss1	Pillai's Trace	.054	1.114(a)	11.000	215.000	.352
	Wilks' Lambda	.946	1.114(a)	11.000	215.000	.352
	Hotelling's Trace	.057	1.114(a)	11.000	215.000	.352
	Roy's Largest Root	.057	1.114(a)	11.000	215.000	.352
dec3vs4	Pillai's Trace	.215	5.354(a)	11.000	215.000	.000
	Wilks' Lambda	.785	5.354(a)	11.000	215.000	.000
	Hotelling's Trace	.274	5.354(a)	11.000	215.000	.000
	Roy's Largest Root	.274	5.354(a)	11.000	215.000	.000
cvss1 * dec3vs4	Pillai's Trace	.094	2.029(a)	11.000	215.000	.027
	Wilks' Lambda	.906	2.029(a)	11.000	215.000	.027
	Hotelling's Trace	.104	2.029(a)	11.000	215.000	.027
	Roy's Largest Root	.104	2.029(a)	11.000	215.000	.027

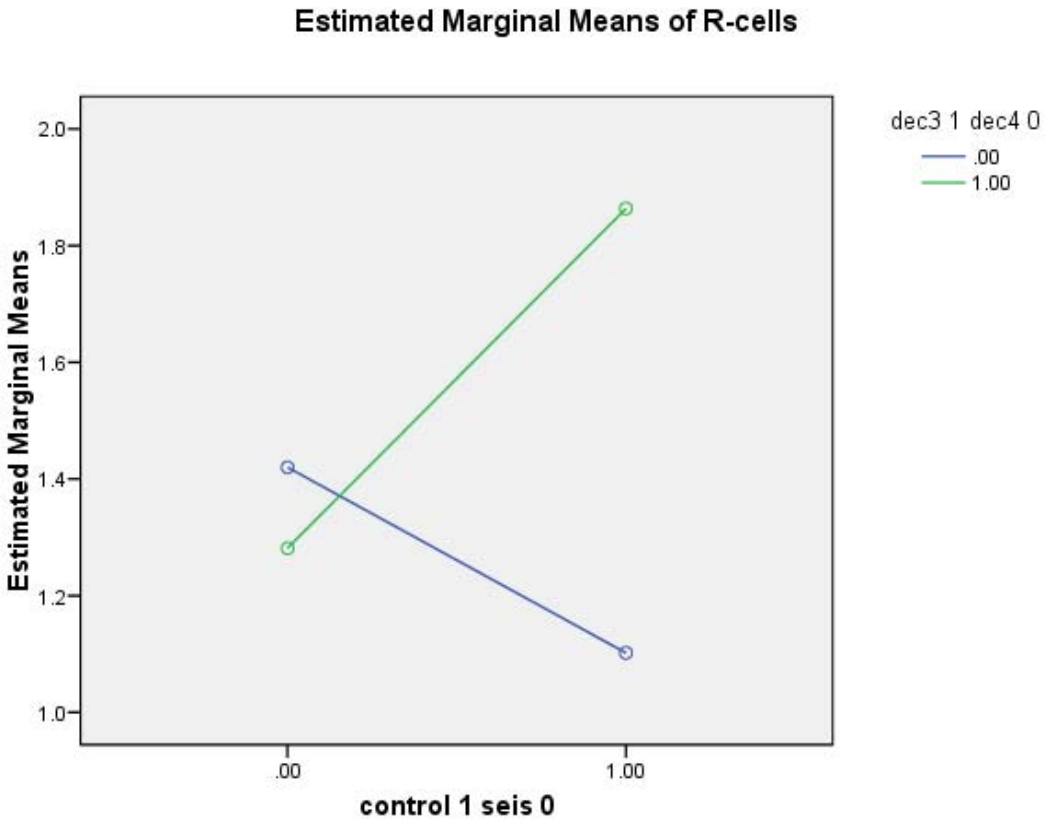
(a) Exact statistic

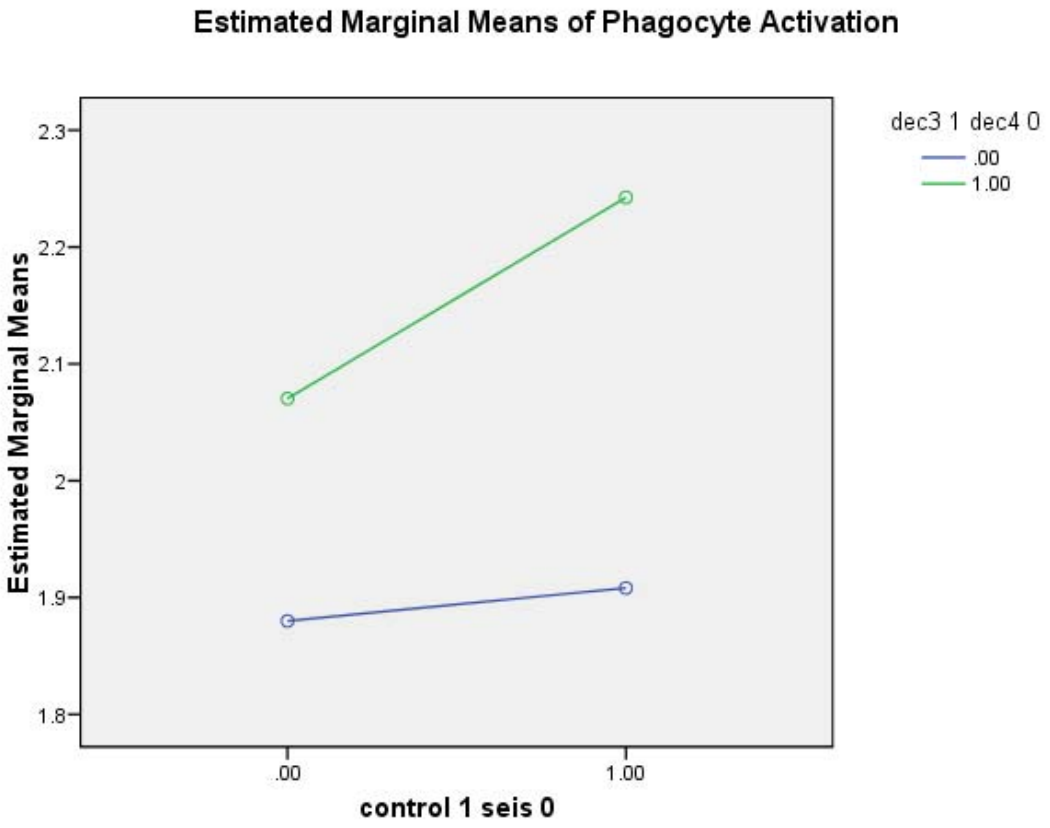
(b) Design: Intercept + cvss1 + dec3vs4 + cvss1 * dec3vs4


Estimated Marginal Means of M Cells (number and positioning)
Estimated Marginal Means
1.4
1.3
1.2
1.1
1
.00
1.00
control 1 seis 0
dec3 1 dec4 0
.00
1.00


Estimated Marginal Means of Nuclear Shape
Estimated Marginal Means
1.45
1.4
1.35
1.3
1.25
.00
1.00
control 1 seis 0
dec3 1 dec4 0
.00
1.00


Estimated Marginal Means of Epithelial Wall Thickness
Estimated Marginal Means
2.4
2.2
2
1.8
1.6
1.4
.00
1.00
control 1 seis 0
dec3 1 dec4 0
.00
1.00


Estimated Marginal Means of R-cells
Estimated Marginal Means
2.0
1.8
1.6
1.4
1.2
1.0
.00
1.00
control 1 seis 0
dec3 1 dec4 0
.00
1.00

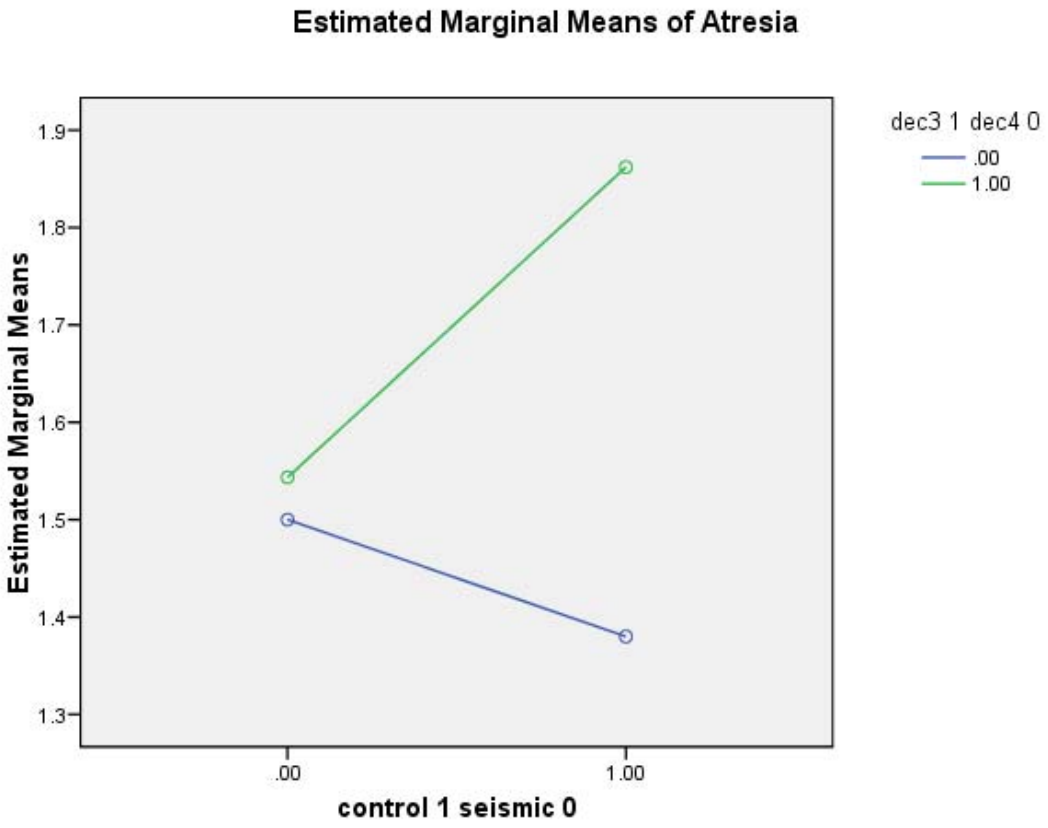

Estimated Marginal Means of Phagocyte Activation
Estimated Marginal Means
2.3
2.2
2.1
2
1.9
1.8
.00
1.00
control 1 seis 0
dec3 1 dec4 0
.00
1.00

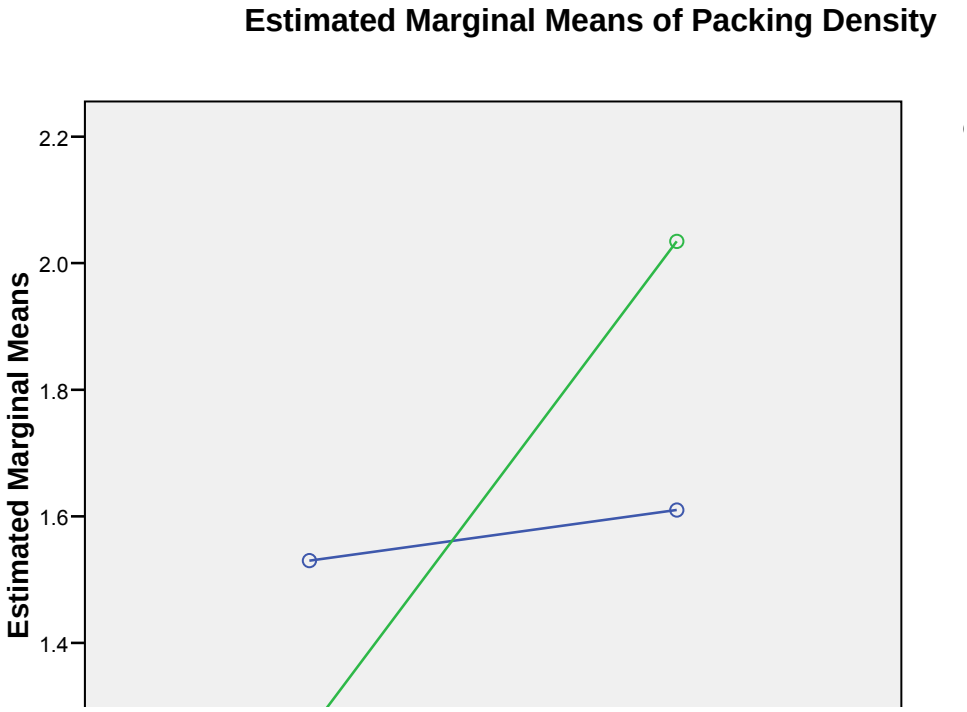
Appendix 3j
Multivariate tests and significant interactions for ovarian data

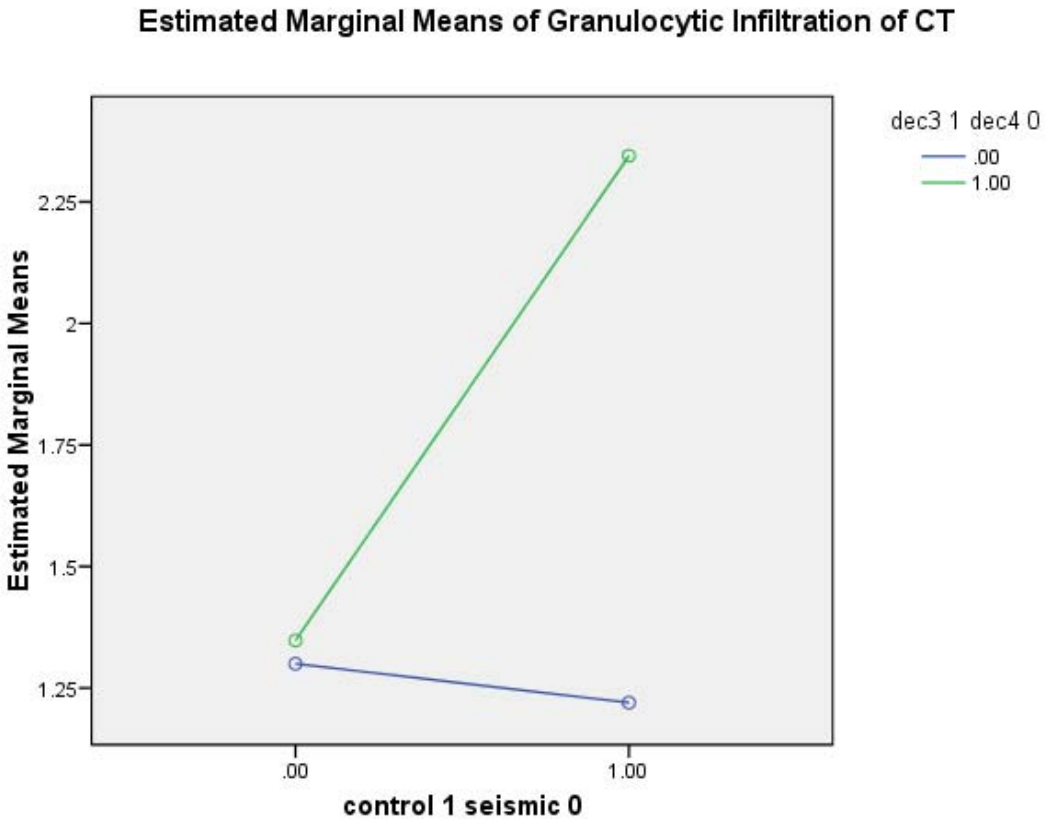
Multivariate Tests (b)

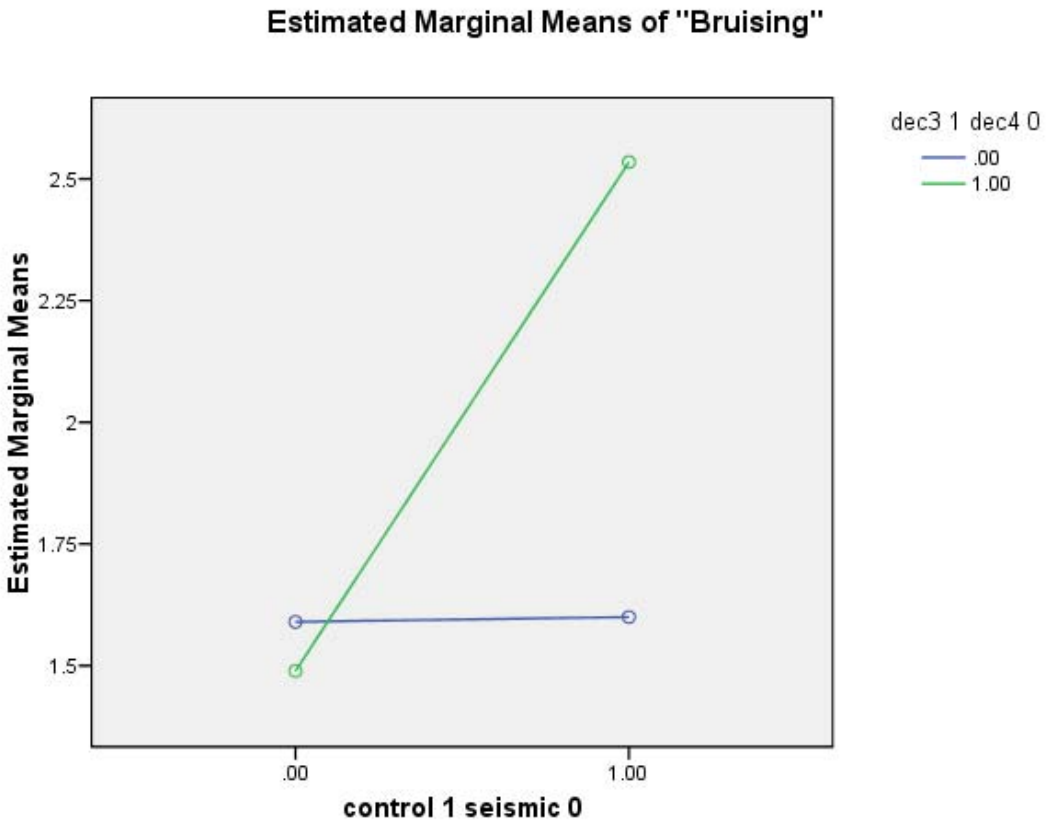
Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.951	353.764(a)	9.000	163.000	.000
	Wilks' Lambda	.049	353.764(a)	9.000	163.000	.000
	Hotelling's Trace	19.533	353.764(a)	9.000	163.000	.000
	Roy's Largest Root	19.533	353.764(a)	9.000	163.000	.000
cvss1	Pillai's Trace	.164	3.547(a)	9.000	163.000	.000
	Wilks' Lambda	.836	3.547(a)	9.000	163.000	.000
	Hotelling's Trace	.196	3.547(a)	9.000	163.000	.000
	Roy's Largest Root	.196	3.547(a)	9.000	163.000	.000
dec3vs4	Pillai's Trace	.208	4.745(a)	9.000	163.000	.000
	Wilks' Lambda	.792	4.745(a)	9.000	163.000	.000
	Hotelling's Trace	.262	4.745(a)	9.000	163.000	.000
	Roy's Largest Root	.262	4.745(a)	9.000	163.000	.000
cvss1 * dec3vs4	Pillai's Trace	.155	3.327(a)	9.000	163.000	.001
	Wilks' Lambda	.845	3.327(a)	9.000	163.000	.001
	Hotelling's Trace	.184	3.327(a)	9.000	163.000	.001
	Roy's Largest Root	.184	3.327(a)	9.000	163.000	.001

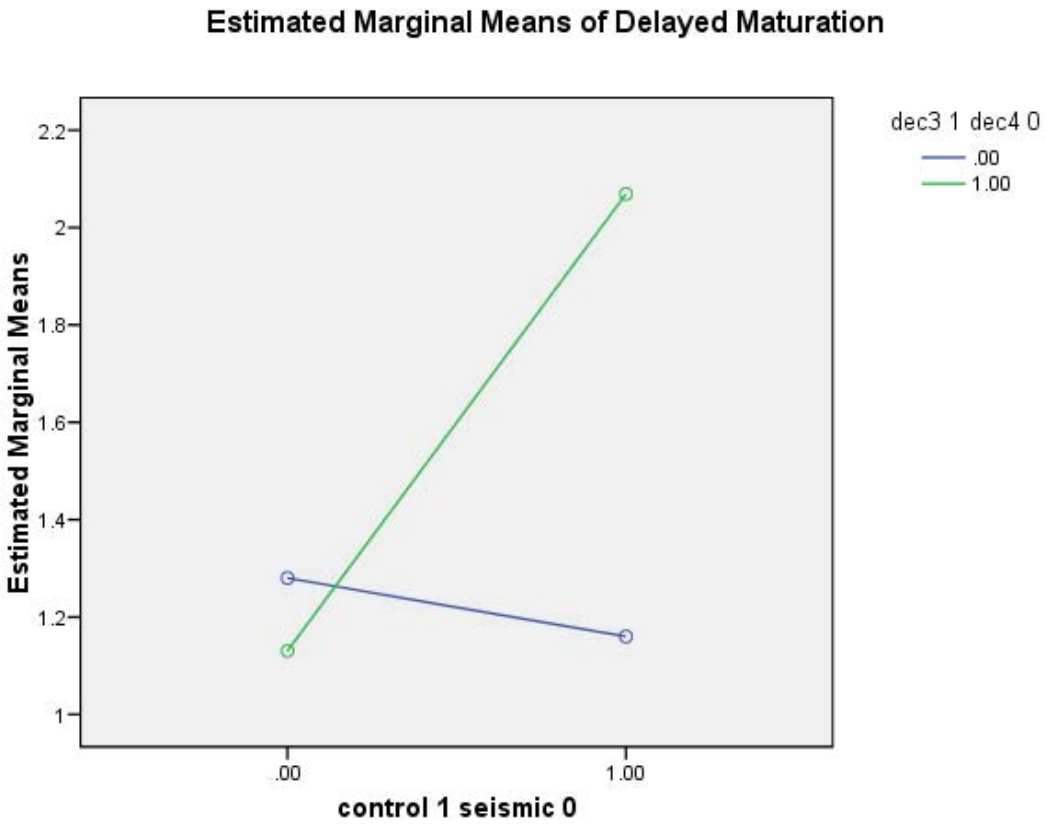
(a) Exact statistic
(b) Design: Intercept + cvss1 + dec3vs4 + cvss1 * dec3vs4

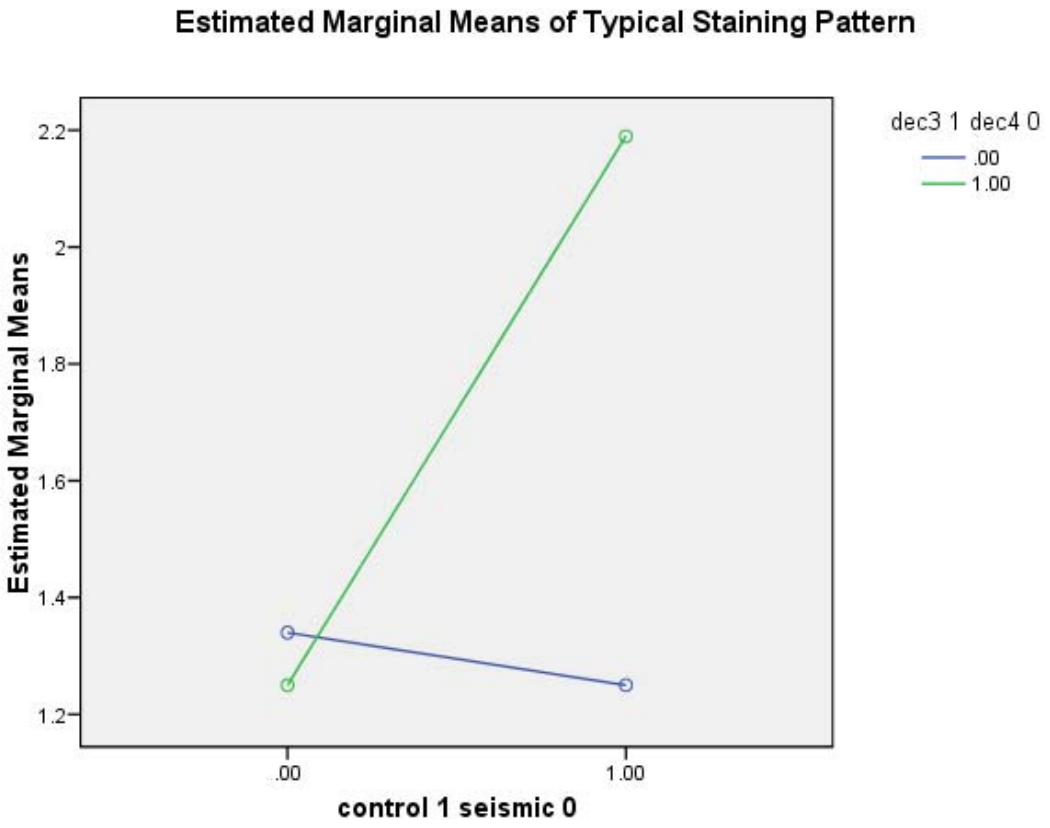

Estimated Marginal Means of Atresia
Estimated Marginal Means
1.9
1.8
1.7
1.6
1.5
1.4
1.3
.00
1.00
control 1 seismic 0
dec3 1 dec4 0
.00
1.00


Estimated Marginal Means of Packing Density
Estimated Marginal Means
2.2
2.0
1.8
1.6
1.4


Estimated Marginal Means of Granulocytic Infiltration of CT
dec3 1 dec4 0
.00
1.00
Estimated Marginal Means
2.25
2
1.75
1.5
1.25
.00
1.00
control 1 seismic 0


Estimated Marginal Means of "Bruising"
Estimated Marginal Means
2.5
2.25
2
1.75
1.5
.00
1.00
control 1 seismic 0
dec3 1 dec4 0
.00
1.00


Estimated Marginal Means of Delayed Maturation
Estimated Marginal Means
2.2
2
1.8
1.6
1.4
1.2
1
.00
1.00
control 1 seismic 0
dec3 1 dec4 0
.00
1.00


Estimated Marginal Means of Typical Staining Pattern
Estimated Marginal Means
2.2
2
1.8
1.6
1.4
1.2
.00
1.00
control 1 seismic 0
dec3 1 dec4 0
.00
1.00

Appendix 3k

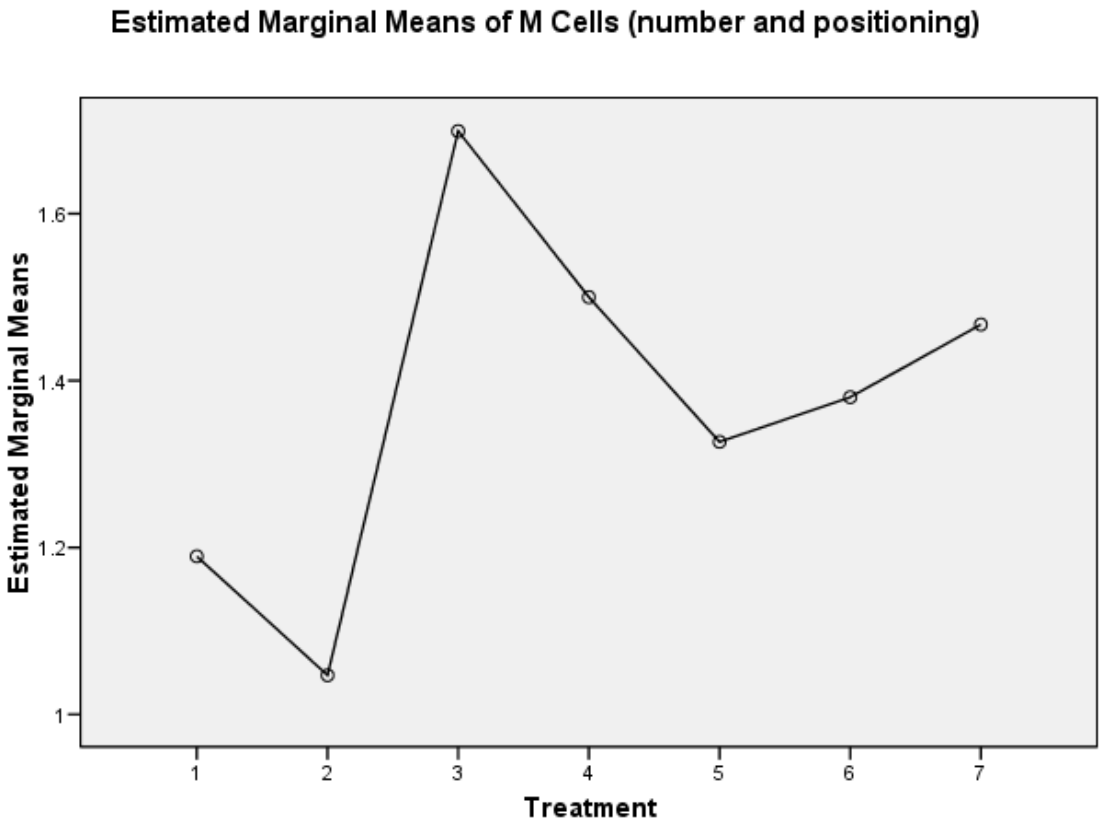
Wild vs. others - hepatopancreatic variables

treatment key given as 1 = control dec03, 2 = seismic dec03, 3 = control may04, 4 = seismic
may04, 5 = control site dec04, 6 = seismic site dec04, and 7 = wild site.

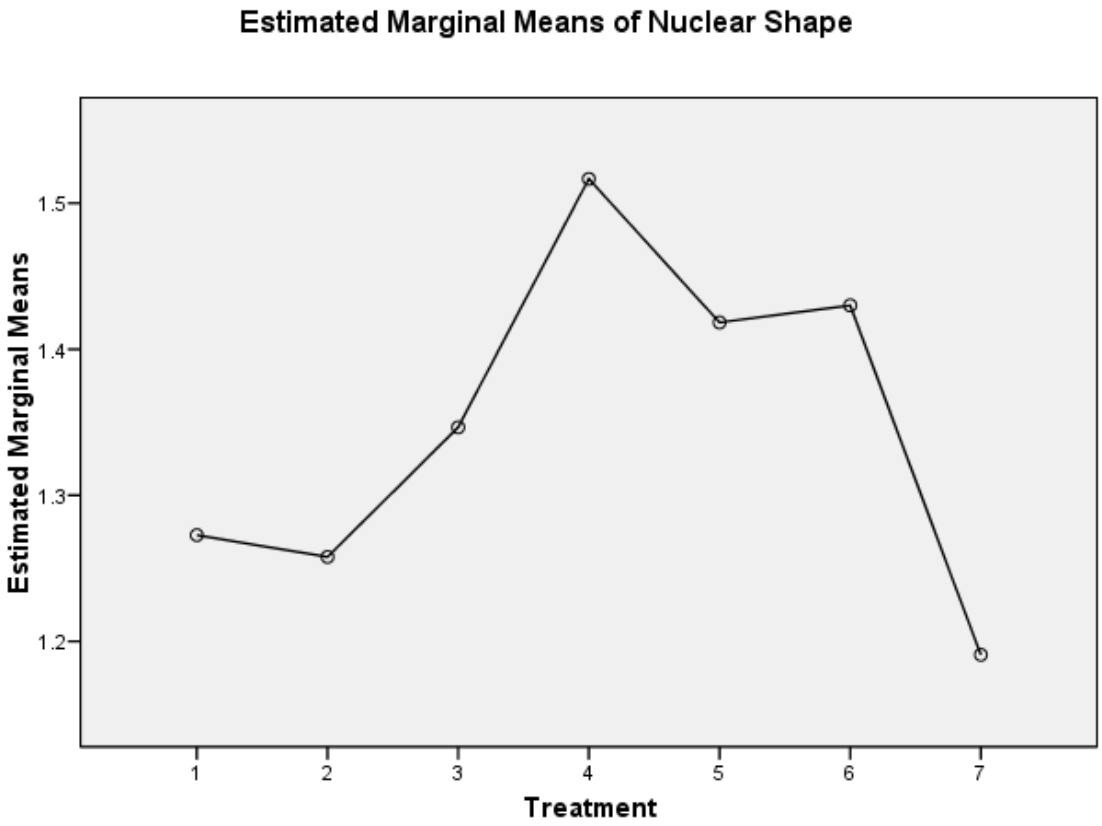
Multivariate Tests(c)

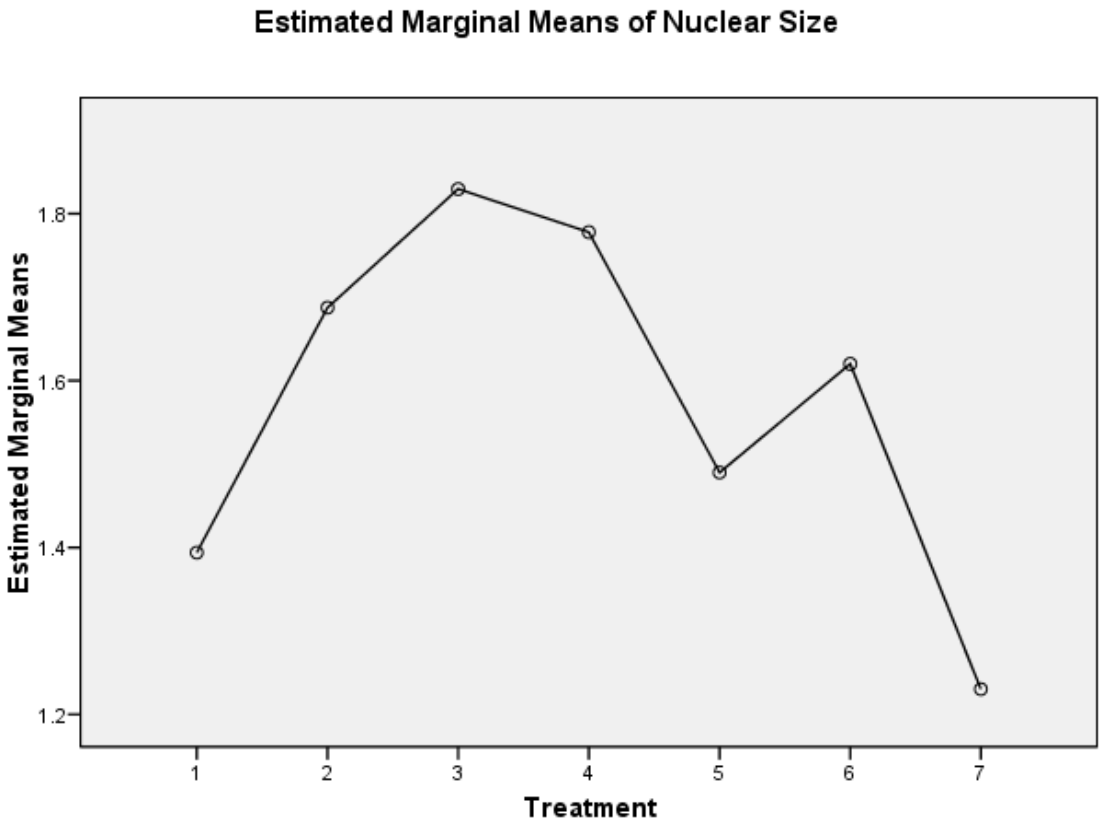
Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.957	943.945(a)	11.000	466.000	.000
	Wilks' Lambda	.043	943.945(a)	11.000	466.000	.000
	Hotelling's Trace	22.282	943.945(a)	11.000	466.000	.000
	Roy's Largest Root	22.282	943.945(a)	11.000	466.000	.000
Treatment	Pillai's Trace	.610	4.842	66.000	2826.000	.000
	Wilks' Lambda	.507	5.122	66.000	2498.951	.000
	Hotelling's Trace	.762	5.357	66.000	2786.000	.000
	Roy's Largest Root	.345	14.791(b)	11.000	471.000	.000

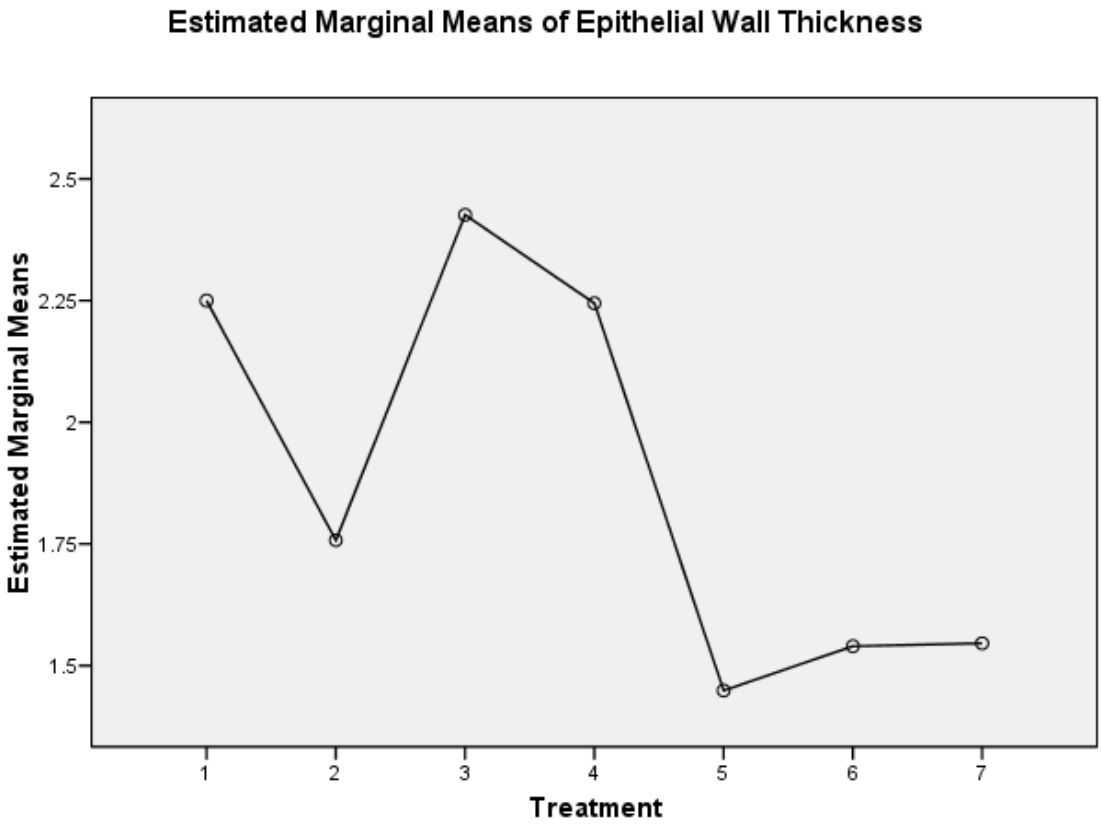
(a) Exact statistic
(b) The statistic is an upper bound on F that yields a lower bound on the significance level.
(c) Design: Intercept + Treatment

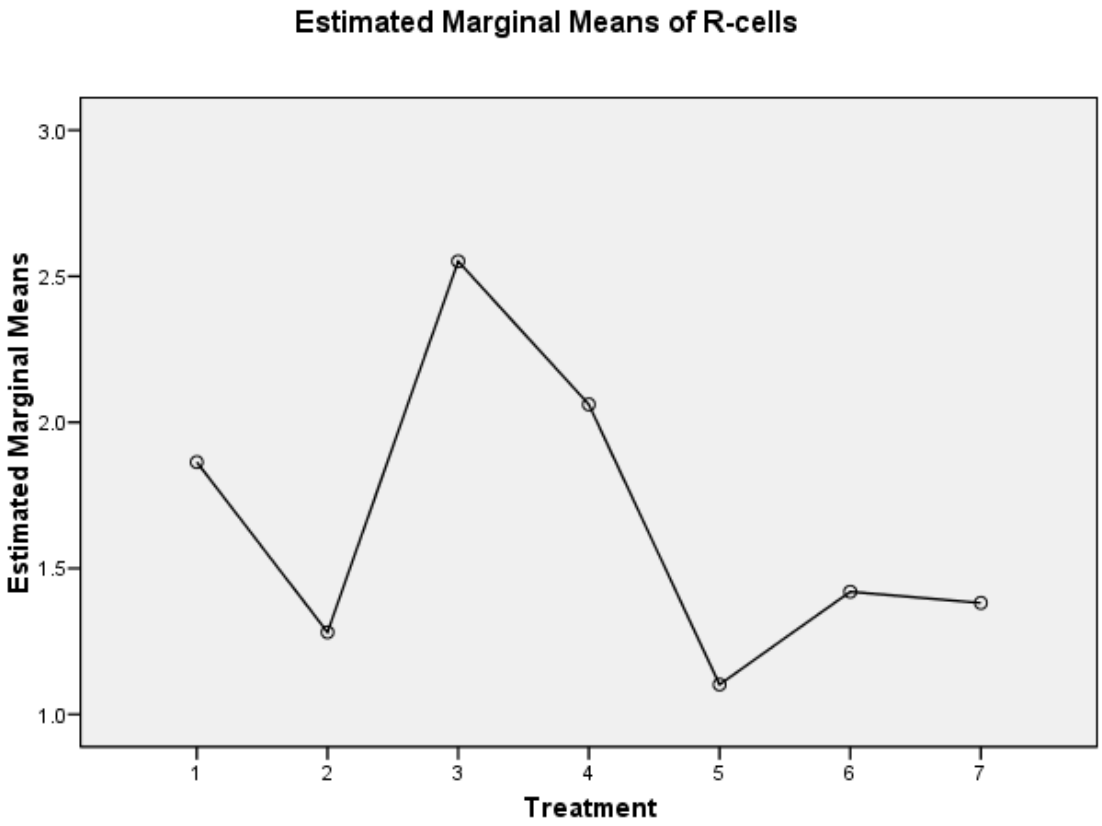


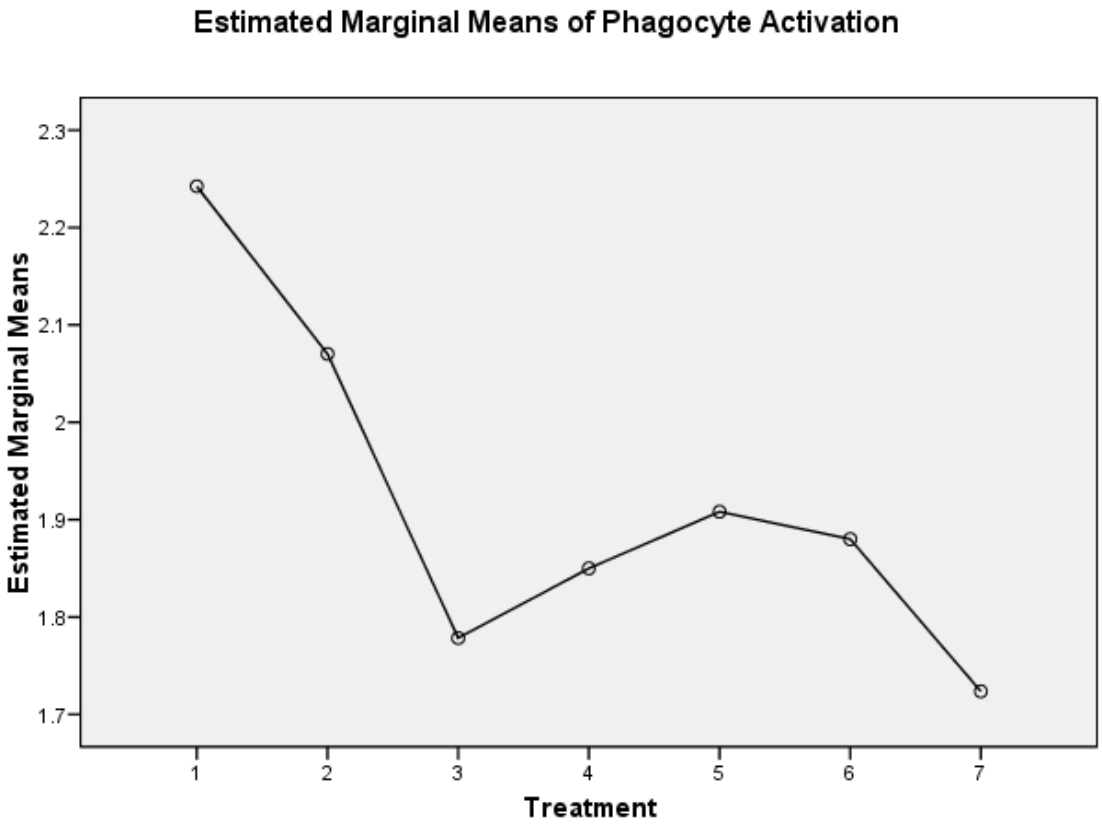
treatment key
given as 1 = control dec03, 2 = seismic dec03, 3 = control may04, 4 = seismic may04, 5 = control site dec04, 6 = seismic site dec04, and 7 = wild site.

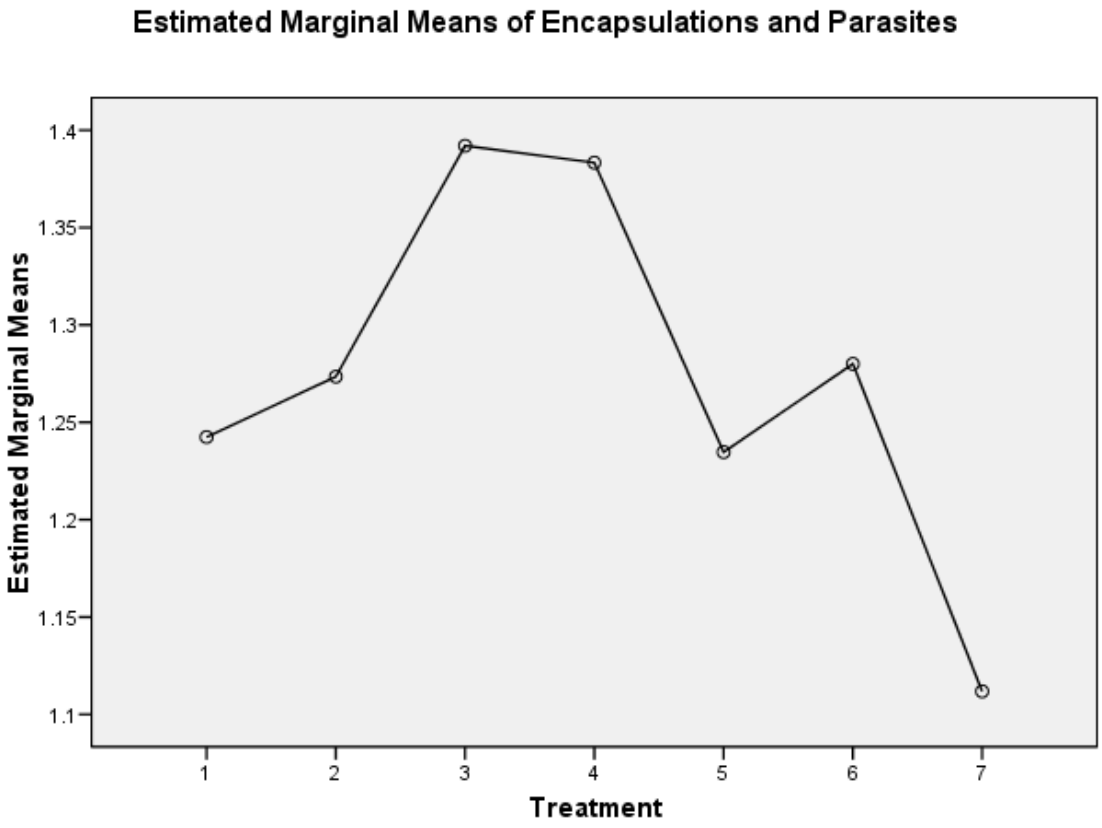

Estimated Marginal Means of Nuclear Shape
Estimated Marginal Means
1.5
1.4
1.3
1.2
1
2
3
4
5
6
7
Treatment

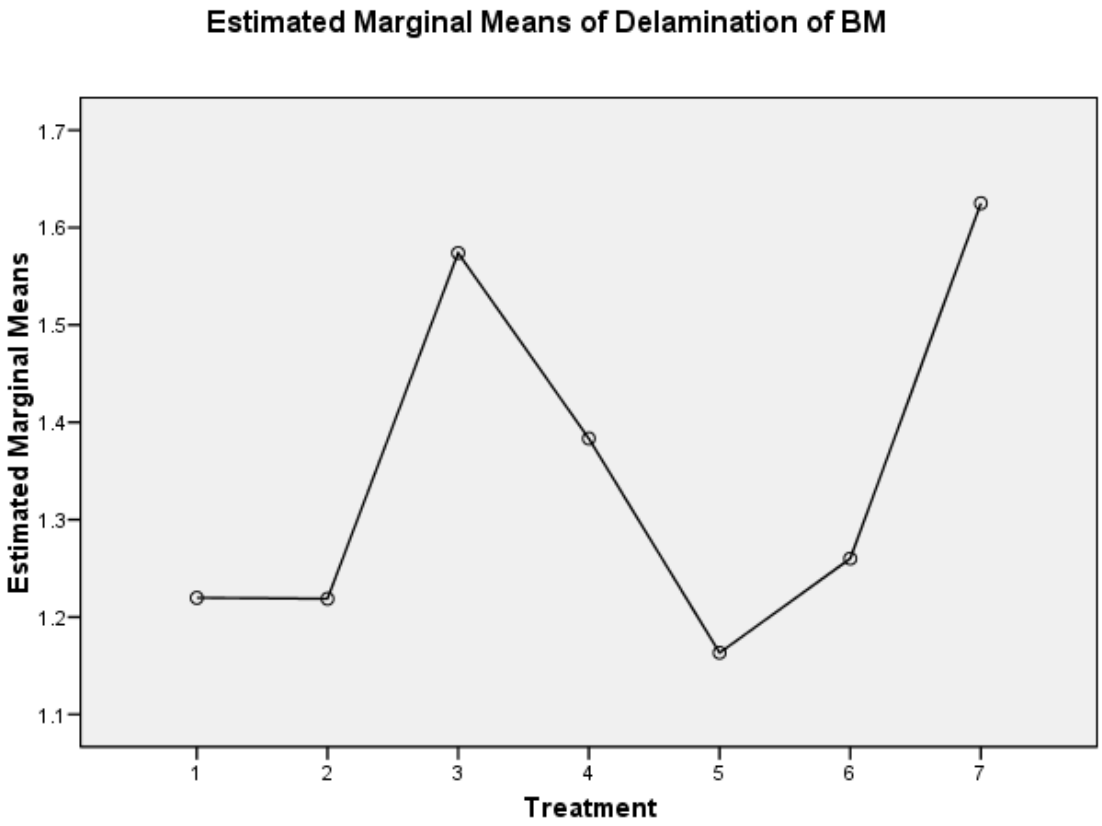

Estimated Marginal Means of Nuclear Size
Estimated Marginal Means
1.8
1.6
1.4
1.2
1
2
3
4
5
6
7
Treatment

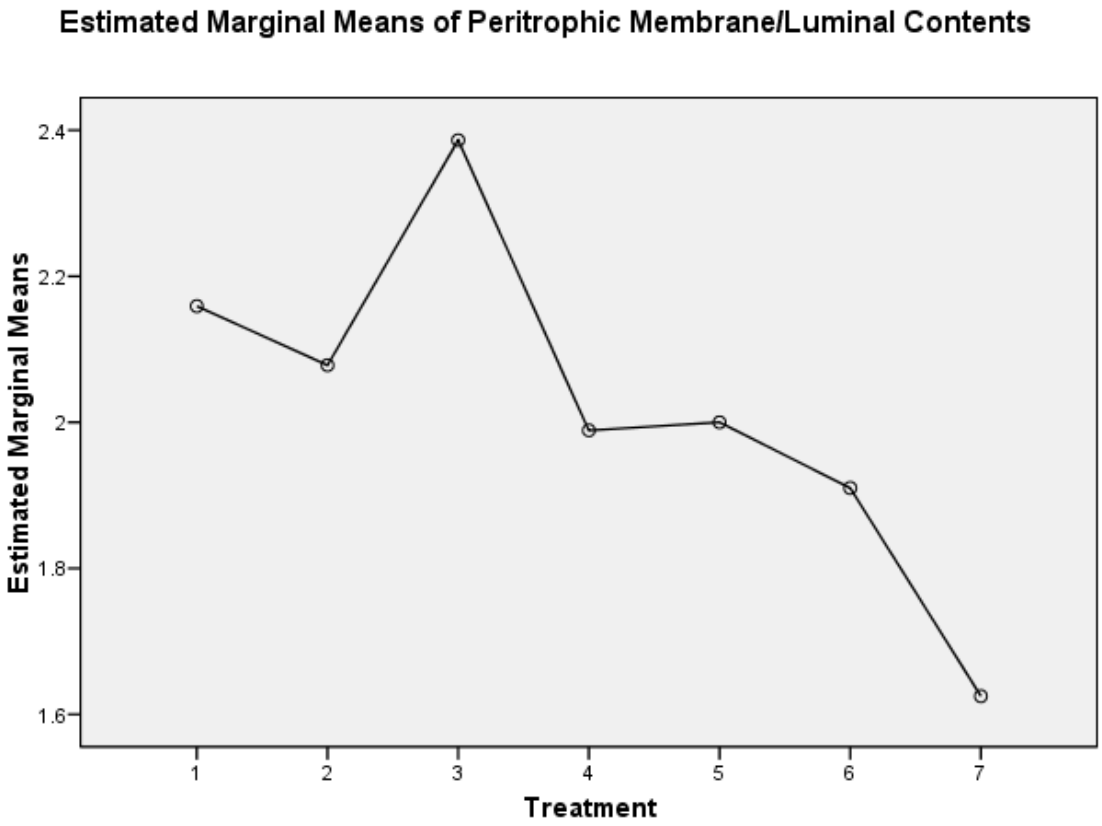

Estimated Marginal Means of Epithelial Wall Thickness
Estimated Marginal Means
2.5
2.25
2
1.75
1.5
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of R-cells
Estimated Marginal Means
3.0
2.5
2.0
1.5
1.0
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Phagocyte Activation
Estimated Marginal Means
2.3
2.2
2.1
2
1.9
1.8
1.7
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Encapsulations and Parasites
Estimated Marginal Means
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Delamination of BM
Estimated Marginal Means
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Peritrophic Membrane/Luminal Contents
Estimated Marginal Means
2.4
2.2
2
1.8
1.6
1
2
3
4
5
6
7
Treatment

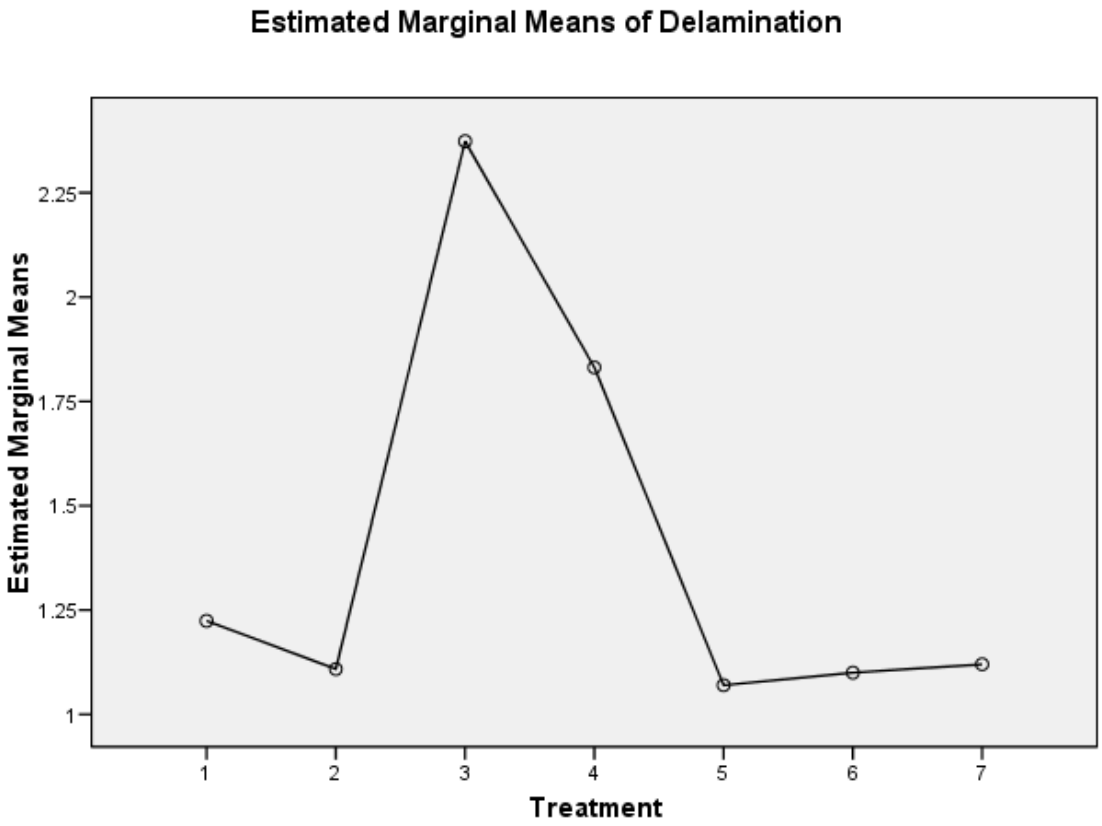
Appendix 3l
Ovarian wild vs. other treatments

treatment key
given as 1 = control dec03, 2 = seismic dec03, 3 = control may04, 4 = seismic may04, 5 = control site dec04, 6 = seismic site dec04, and 7 = wild site.

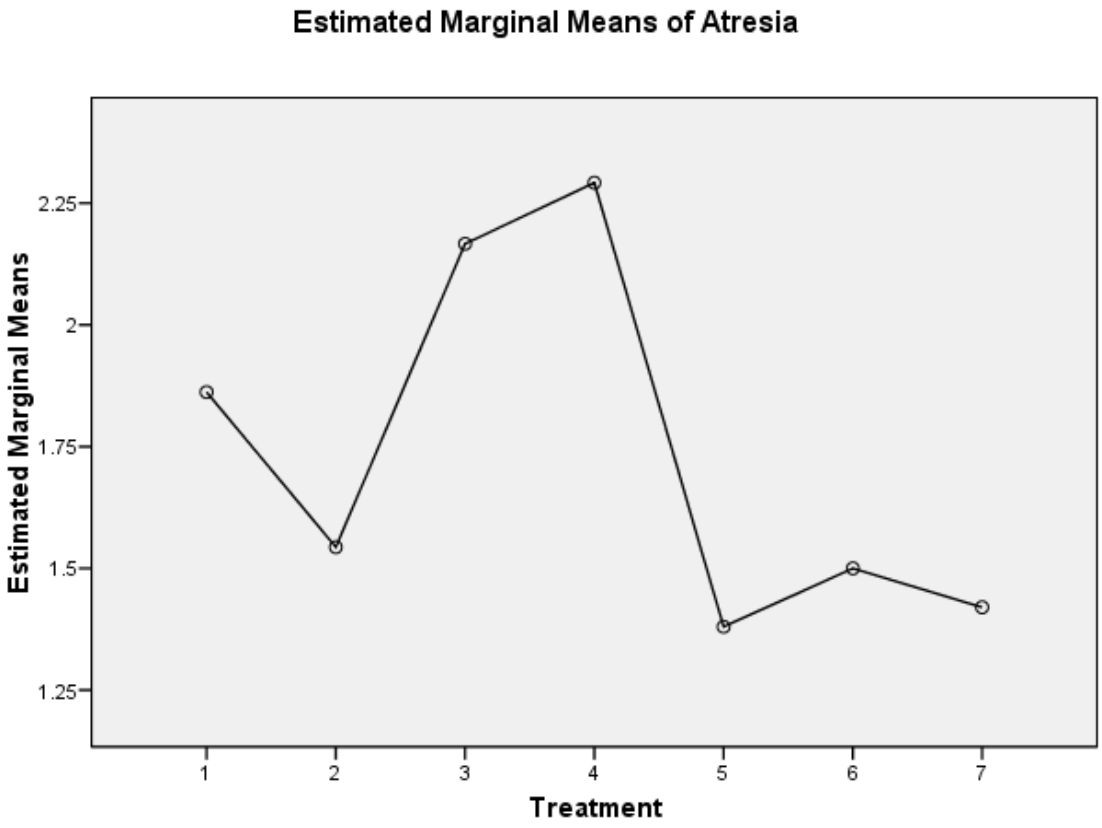
Multivariate Tests (d)

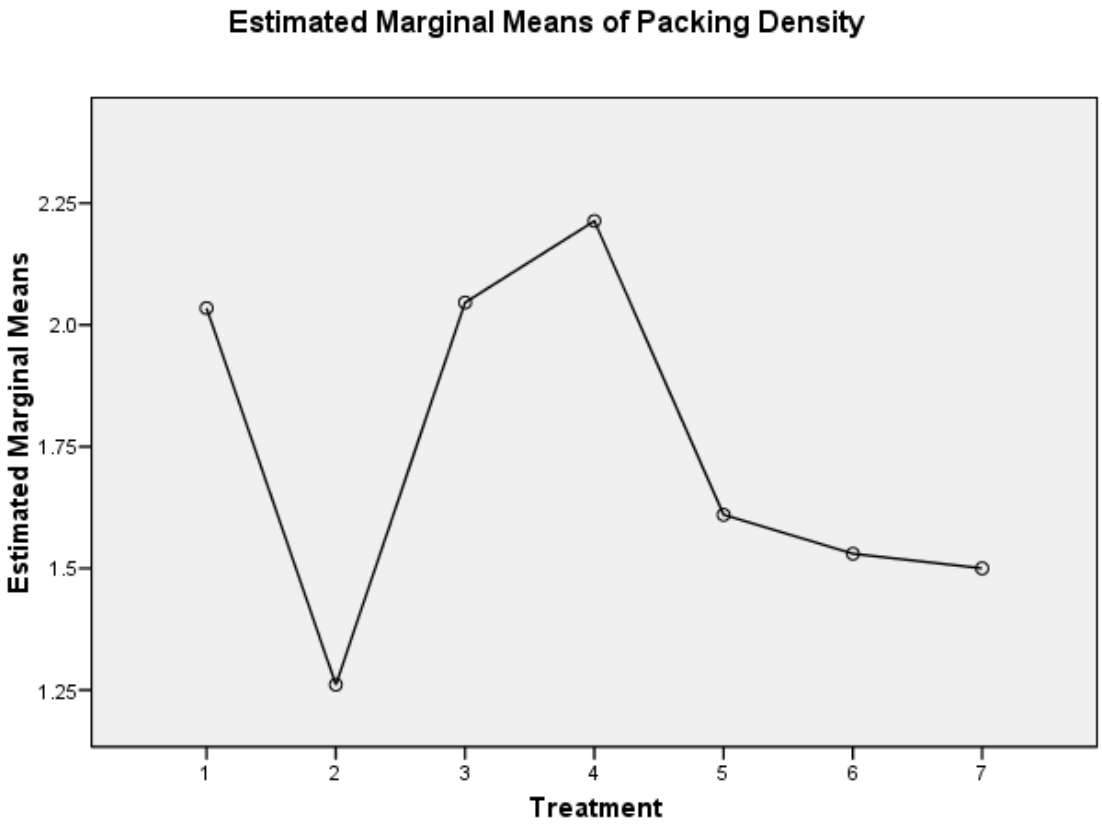
Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Pillai's Trace	.936	623.320(b)	9.000	386.000	.000	.936	5609.878	1.000
	Wilks' Lambda	.064	623.320(b)	9.000	386.000	.000	.936	5609.878	1.000
	Hotelling's Trace	14.533	623.320(b)	9.000	386.000	.000	.936	5609.878	1.000
	Roy's Largest Root	14.533	623.320(b)	9.000	386.000	.000	.936	5609.878	1.000
Treatment	Pillai's Trace	.778	6.477	54.000	2346.000	.000	.130	349.756	1.000
	Wilks' Lambda	.374	7.773	54.000	1972.816	.000	.151	351.451	1.000
	Hotelling's Trace	1.300	9.255	54.000	2306.000	.000	.178	499.759	1.000
	Roy's Largest Root	.985	42.791(c)	9.000	391.000	.000	.496	385.123	1.000

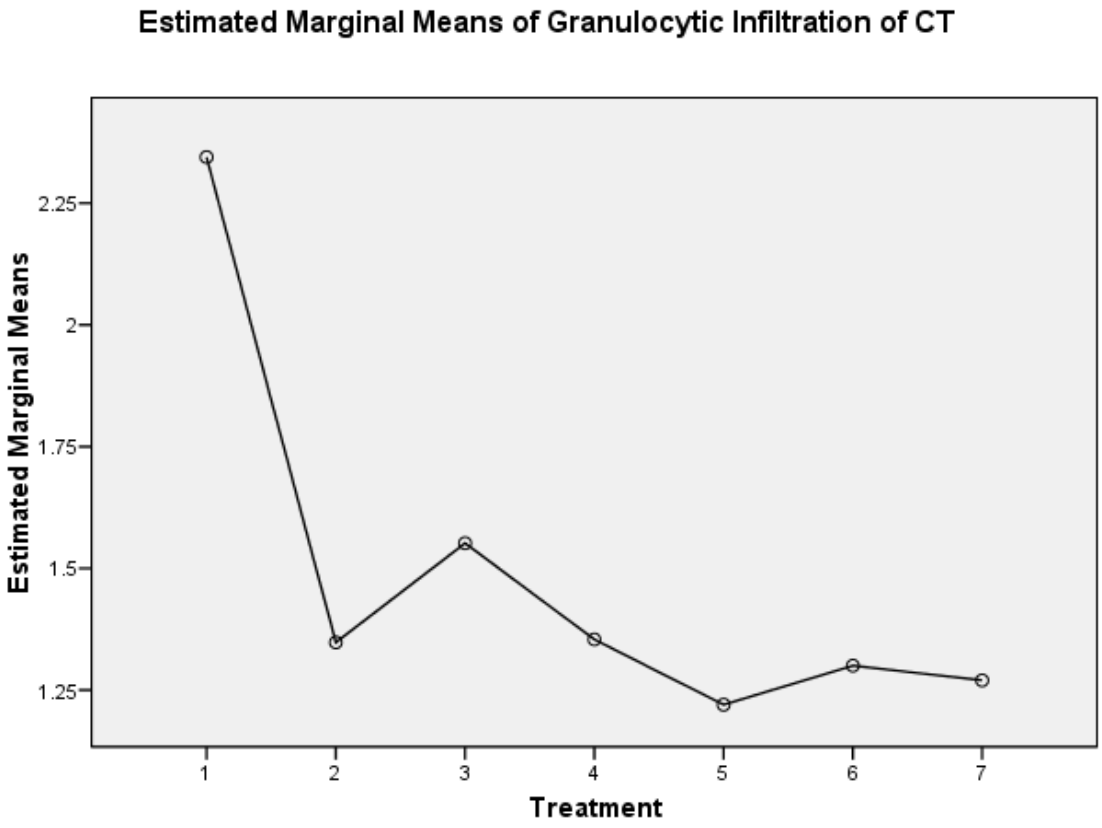
(a) Computed using alpha = .05
(b) Exact statistic
(c) The statistic is an upper bound on F that yields a lower bound on the significance level.
(d) Design: Intercept + Treatment

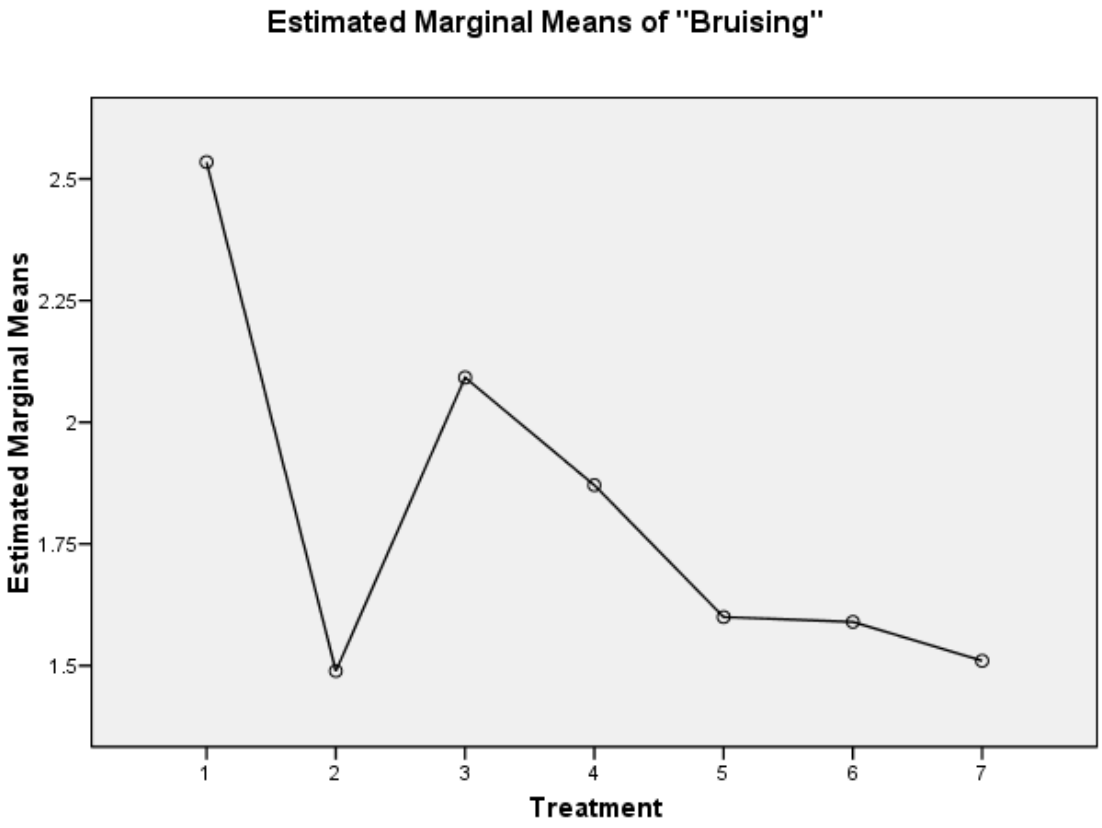


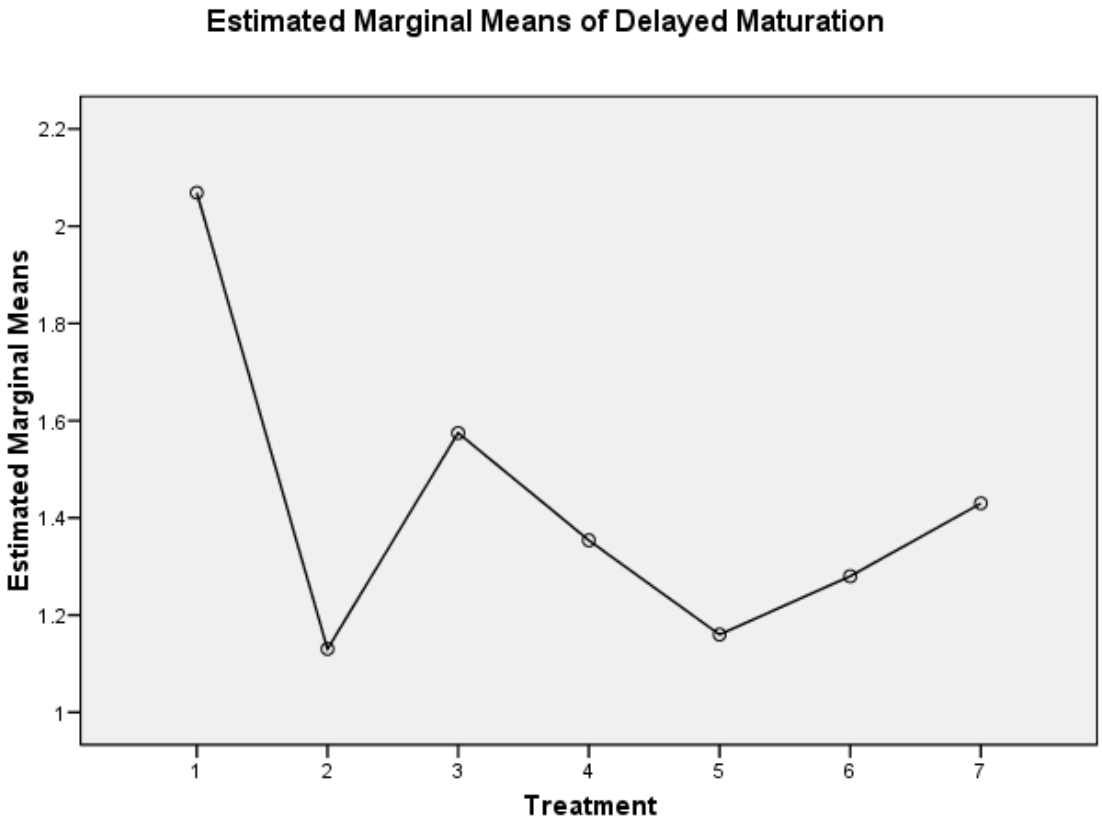
treatment key
given as 1 = control dec03, 2 = seismic dec03, 3 = control may04, 4 = seismic may04, 5 = control site dec04, 6 = seismic site dec04, and 7 = wild site.

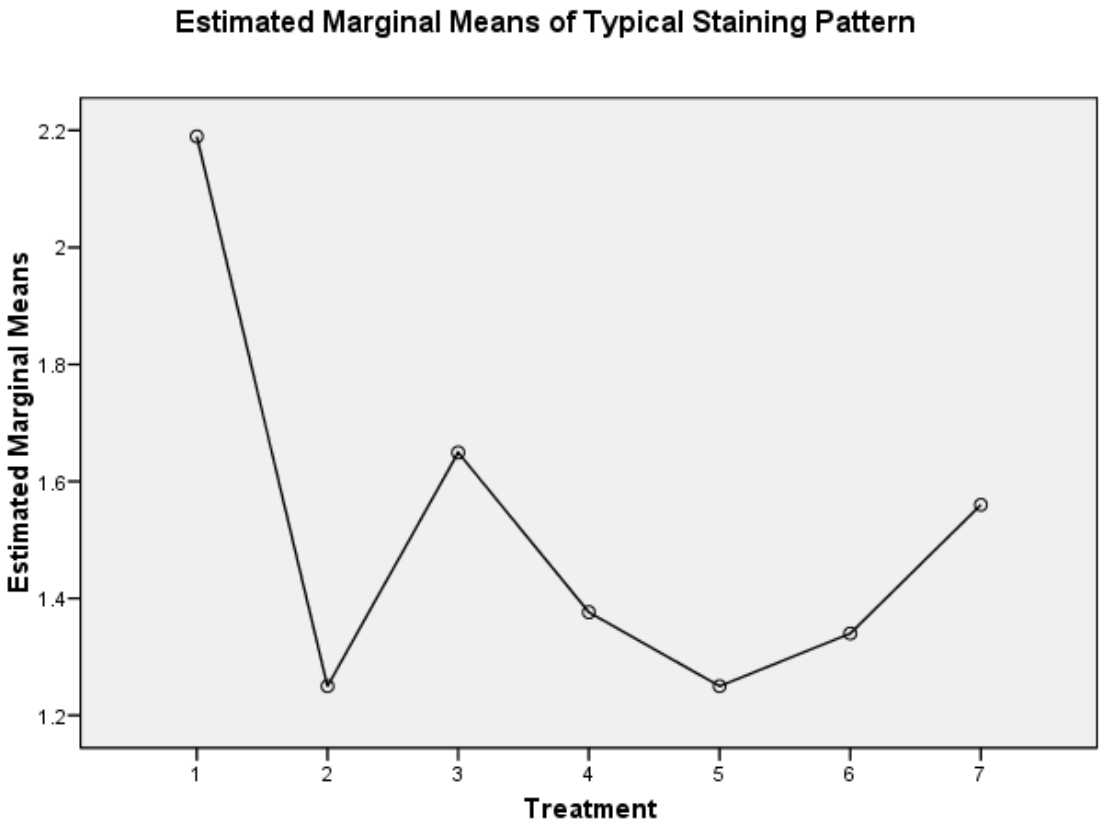

Estimated Marginal Means of Atresia
Estimated Marginal Means
2.25
2
1.75
1.5
1.25
1
2
3
4
5
6
7
Treatment

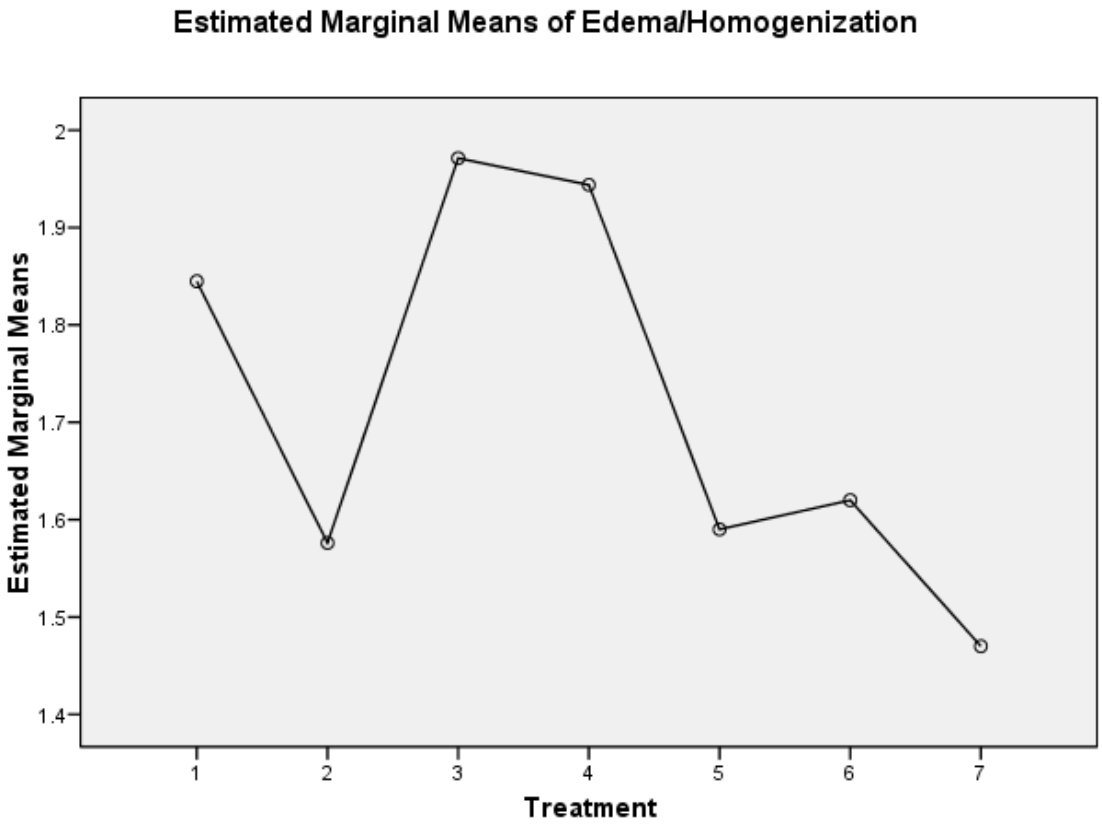

Estimated Marginal Means of Packing Density
Estimated Marginal Means
2.25
2.0
1.75
1.5
1.25
1
2
3
4
5
6
7
Treatment

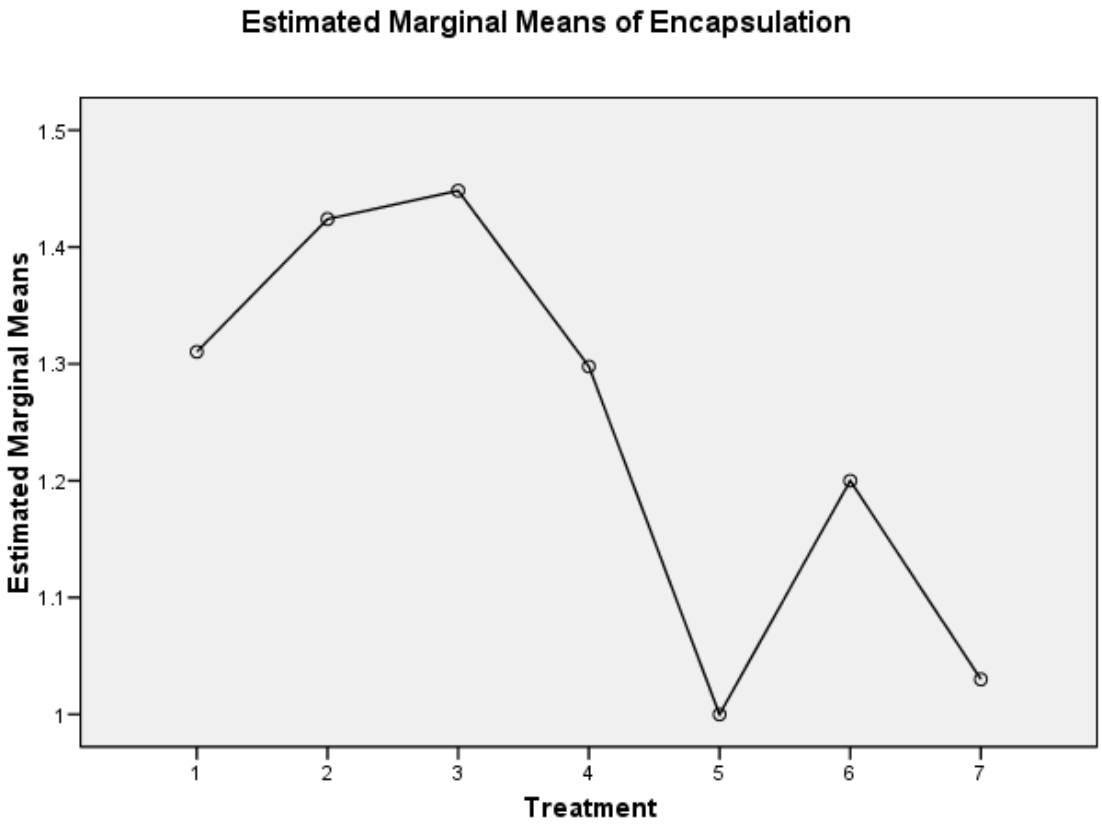

Estimated Marginal Means of Granulocytic Infiltration of CT
Estimated Marginal Means
2.25
2
1.75
1.5
1.25
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of "Bruising"
Estimated Marginal Means
2.5
2.25
2
1.75
1.5
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Delayed Maturation
Estimated Marginal Means
2.2
2
1.8
1.6
1.4
1.2
1
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Typical Staining Pattern
Estimated Marginal Means
2.2
2
1.8
1.6
1.4
1.2
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Edema/Homogenization
Estimated Marginal Means
2
1.9
1.8
1.7
1.6
1.5
1.4
1
2
3
4
5
6
7
Treatment


Estimated Marginal Means of Encapsulation
Estimated Marginal Means
1.5
1.4
1.3
1.2
1.1
1
1
2
3
4
5
6
7
Treatment

Appendix 3m
Inter-rater reliability -Pearson Product moment Correlation

Hepatopancreatic variables

Correlations

		M Cells (number and positioning)	M Cells (number and positioning)
M Cells (number and positioning)	Pearson Correlation	1	.028
	Sig. (two-tailed)		.720
	N	165	165
M Cells (number and positioning)	Pearson Correlation	.028	1
	Sig. (two-tailed)	.720	
	N	165	165

Correlations

		Nuclear Shape	Nuclear Shape
Nuclear Shape	Pearson Correlation	1	.080
	Sig. (two-tailed)		.306
	N	165	165
Nuclear Shape	Pearson Correlation	.080	1
	Sig. (two-tailed)	.306	
	N	165	165

Correlations

		Nuclear Size	Nuclear Size
Nuclear Size	Pearson Correlation	1	.149
	Sig. (two-tailed)		.056
	N	164	164
Nuclear Size	Pearson Correlation	.149	1
	Sig. (two-tailed)	.056	
	N	164	165

Correlations

		Epithelial Wall Thickness	Epithelial Wall Thickness
Epithelial Wall Thickness	Pearson Correlation	1	.165(*)
	Sig. (two-tailed)		.035
	N	164	164
Epithelial Wall Thickness	Pearson Correlation	.165(*)	1
	Sig. (two-tailed)	.035	
	N	164	165

* Correlation is significant at the 0.05 level (two-tailed).

Correlations

		R-cells	R-cells
R-cells	Pearson Correlation	1	.669(**)
	Sig. (two-tailed)		.000
	N	165	165
R-cells	Pearson Correlation	.669(**)	1
	Sig. (two-tailed)	.000	
	N	165	165

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Phagocyte Activation	Phagocyte Activation
Phagocyte Activation	Pearson Correlation	1	-.091
	Sig. (two-tailed)		.247
	N	165	165
Phagocyte Activation	Pearson Correlation	-.091	1
	Sig. (two-tailed)	.247	
	N	165	165

Correlations

		Encapsulations and Parasites	Encapsulations and Parasites
Encapsulations and Parasites	Pearson Correlation	1	.253(**)
	Sig. (two-tailed)		.001
	N	165	165
Encapsulations and Parasites	Pearson Correlation	.253(**)	1
	Sig. (two-tailed)	.001	
	N	165	165

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Delamination of BM	Delamination of BM
Delamination of BM	Pearson Correlation	1	.347(**)
	Sig. (two-tailed)		.000
	N	165	165
Delamination of BM	Pearson Correlation	.347(**)	1
	Sig. (two-tailed)	.000	
	N	165	165

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Peritrophic Membrane/Luminal Contents	Peritrophic Membrane/Luminal Contents
Peritrophic Membrane/Luminal Contents	Pearson Correlation	1	.007
	Sig. (two-tailed)		.927
	N	165	165
Peritrophic Membrane/Luminal Contents	Pearson Correlation	.007	1
	Sig. (two-tailed)	.927	
	N	165	165

Correlations

		Necrosis or Autolysis	Necrosis or Autolysis
Necrosis or Autolysis	Pearson Correlation	1	.589(**)
	Sig. (two-tailed)		.000
	N	165	165
Necrosis or Autolysis	Pearson Correlation	.589(**)	1
	Sig. (two-tailed)	.000	
	N	165	165

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Collagen	Collagen
Collagen	Pearson Correlation	1	.383(**)
	Sig. (two-tailed)		.000
	N	161	161
Collagen	Pearson Correlation	.383(**)	1
	Sig. (two-tailed)	.000	
	N	161	165

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Sum	Mike'sum
Sum	Pearson Correlation	1	.611(**)
	Sig. (two-tailed)		.000
	N	165	165
Mike'sum	Pearson Correlation	.611(**)	1
	Sig. (two-tailed)	.000	
	N	165	165

** Correlation is significant at the 0.01 level (two-tailed).

Ovarian data

Correlations

		Delamination	Delamination
Delamination	Pearson Correlation	1	.417(**)
	Sig. (two-tailed)		.000
	N	151	151
Delamination	Pearson Correlation	.417(**)	1
	Sig. (two-tailed)	.000	
	N	151	151

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Atresia	Atresia
Atresia	Pearson Correlation	1	.304(**)
	Sig. (two-tailed)		.000
	N	151	151
Atresia	Pearson Correlation	.304(**)	1
	Sig. (two-tailed)	.000	
	N	151	151

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Packing density	Packing Density
Packing density	Pearson Correlation	1	.795(**)
	Sig. (two-tailed)		.000
	N	151	151
Packing Density	Pearson Correlation	.795(**)	1
	Sig. (two-tailed)	.000	
	N	151	151

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Granulocytic inflation	Granulocytic Infiltration of CT
Granulocytic inflation	Pearson Correlation	1	.547(**)
	Sig. (two-tailed)		.000
	N	151	151
Granulocytic Infiltration of CT	Pearson Correlation	.547(**)	1
	Sig. (two-tailed)	.000	
	N	151	151

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Bruising	"Bruising"
Bruising	Pearson Correlation	1	-.055
	Sig. (two-tailed)		.503
	N	151	151
"Bruising"	Pearson Correlation	-.055	1
	Sig. (two-tailed)	.503	
	N	151	151

Correlations

		Delayed Maturity	Delayed Maturation
Delayed Maturity	Pearson Correlation	1	.772(**)
	Sig. (two-tailed)		.000
	N	151	151
Delayed Maturation	Pearson Correlation	.772(**)	1
	Sig. (two-tailed)	.000	
	N	151	151

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Typical Staining Pattern	Typical Stain Pattern
Typical Staining Pattern	Pearson Correlation	1	.676(**)
	Sig. (two-tailed)		.000
	N	151	151
Typical Stain Pattern	Pearson Correlation	.676(**)	1
	Sig. (two-tailed)	.000	
	N	151	151

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Edema	Edema/Homogenization
Edema	Pearson Correlation	1	.310(**)
	Sig. (two-tailed)		.000
	N	151	150
Edema/Homogenization	Pearson Correlation	.310(**)	1
	Sig. (two-tailed)	.000	
	N	150	150

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		Encapsulation	Encapsulation
Encapsulation	Pearson Correlation	1	.305(**)
	Sig. (two-tailed)		.000
	N	151	151
Encapsulation	Pearson Correlation	.305(**)	1
	Sig. (two-tailed)	.000	
	N	151	151

** Correlation is significant at the 0.01 level (two-tailed).

Correlations

		sum	Total score
sum	Pearson Correlation	1	.765(**)
	Sig. (two-tailed)		.000
	N	151	151
Total score	Pearson Correlation	.765(**)	1
	Sig. (two-tailed)	.000	
	N	151	152

** Correlation is significant at the 0.01 level (two-tailed).