MINISTRY OF ENERGY AND MINES AND UNIVERSITY OF VICTORIA

Social Science and Science Partnerships

Final Report
September 2008

Environmental Impacts of air-gun surveys on Glass Sponges

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Executive summary

Air-gun surveys associated with the oil and gas exploration in the Queen Charlotte Charlotte (QC) Basin will insonify the seafloor with broadband, high intensity noise, thereby exposing the unique glass sponge reef systems of that area to acoustic impacts. The two most common species to the QC Basin are the same as those that form the Strait of Georgia reefs (*Aphrocallistes vastus* and *Heterochone calyx*). We used the opportunity offered by a research cruise funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) going to the Fraser Ridge glass sponge reef to conduct a brief, and preliminary, study of the immediate biological response of one of the major reef building glass sponges (*Aphrocallistes vastus*) to acoustic vibrations. The measure of response in these animals was reduction in their feeding currents. These currents were measured in the exit orifice by deployed instruments that had no physical contact with the animals. The sound source was a small, surface air-gun (164 cm^3) that generated average sound exposure levels of 151 dB re $\mu Pa^2 s^{-1}$ at the sponge location. The air-gun signal was distinct from background noise below 300 Hz.

The question of whether the sponge's excurrent flow responded to the pressures from the series of air-gun shots was addressed statistically. The sponge response to ambient conditions was compared to the excurrents measured at the air-gun shot times. For this single sample of 16 air-gun shots, the statistical analysis indicated that there was little or no evidence that the acoustic pressure from the air-gun influenced the physiological functions. The experimental work carried out here was very challenging, and many factors were difficult to control. First, since the ambient flow speed accounted for ~ 70% of the observed variation in the excurrent flow, it was impossible to separate this factor from the analysis. Second, the complexity of the approach constrained the sample size and duration of the observations. Daylight hours, marine mammal intrusion, preparation time for the Remote Operated Vehicle (ROV) and instrument malfunction all became confounding factors.

Nonetheless, the experimental study is novel and the statistical approach is a benchmark for future studies. Further laboratory work and field studies are necessary before meaningful conclusions can be drawn. New research should make use of more realistic sound levels, and study recovery times, habituation and longer term effects on tissue and skeletal integrity.

Background

Glass sponges are unusual animals. The sponge soft tissue forms a thin veneer over a rigid skeleton made of glass spicules that accounts for ~85% of the sponge mass. Their tissue is made of giant, multinucleated cells draped over a glass fiber skeleton. This body organization allows the sponge to transmit electrical signals over a body that otherwise lacks nerves (Leys and Mackie 1997) to coordinate the cessation of the feeding current in response to disturbance (Mackie et al. 1983a, Leys & Mackie 1997a, Leys et al. 1999b).

These animals form the subclass Hexactinellida in the Porifera (sponges). This group was once very extensive but is now relegated to the deep sea and a few unusual habitats. Worldwide today, living glass sponge reefs are known only from a few locations on the BC coast although more may be found. Reef-forming glass sponges are large animals over 1.5 m high. Their glass spicules are fused so the skeleton of a dead sponge remains intact and forms the foundation for settlement of new sponges. New generations overgrow the skeletons of their progenitors thus forming the glass sponge reef. Sediment infill consolidates the structure forming reefs up to 25 m high (Conway et al. 1991, Conway et al. 2001, Whitney et al. 2005).

Video transects indicate that the reefs provide a distinct benthic habitat within the Queen Charlotte (QC) Basin and are characterized by elevated biodiversity and a unique assemblage of associated fauna (Cook et al. unpublished data). Information and references
regarding glass sponge and glass sponge reefs was submitted to the BC Ministry of Energy, Mines and Petroleum Resources by Leys (Personal communication 2004). The discoveries of smaller (but similar) reefs on the Fraser River pro-delta (Conway et al. 2004) and on McCall Bank north of Vancouver provide a much easier access for a study system. The QC Sound sponge reefs are formed by three species of dictyonine sponges. The two most common species are those that form the Strait of Georgia reefs (*Aphrocallistes vastus* and *Heterochone calyx*).

Like other sponges, glass sponges feed by pumping large quantities of water through a specialized filtration system (Wyeth 1999). The first work to examine the food composition and metabolic indicators was conducted in situ in 2004 using the remotely operated vehicle ROPOS in Barkley Sound (Yahel et al. 2007). Their work developed the techniques that we have applied in this study. Additional work by Leys and Tompkins (2005) demonstrated that sponges will react to a stressor by cessation of water pumping. The consequence of pumping arrest is that feeding, respiration and other body functions are shut down. Another local glass sponge (*Rhabdocalyptus dawsoni)* will stop pumping when touched (Leys and Mackie 1999). Thus, measurements of arrests of pumping have served as a proxy for response to adverse stimuli.

Oil and gas exploration in the QC Basin will involve the use of air-gun surveys that will insonify the seafloor with broadband, high intensity noise. Air-gun arrays have dominant energy at low frequencies, where long-range propagation is likely. The ocean is an efficient medium for acoustic propagation with little attenuation at low frequencies.

Acoustic harassment through water propagated vibration can affect tissue integrity and behavior of marine mammals (Goold and Fish 1998, McCauley et al. 2003). Invertebrates have sensory structures also. For example, sea squirts stop feeding in response to vibrations (Mackie & Singla 2003).

Glass sponge reefs are unique to British Columbia and are poorly studied. Their significance and poor documentation in the QC Basin were recognized by the expert panels of both the British Columbia Scientific Review Panel (the "Strong Report") and the Royal Society of Canada (Hall et al. 2004). Our knowledge of the reefs is based on a few descriptive studies that identify the extraordinary nature of these animals that can build substantial structures over thousands of years. The glass sponge reef in the Strait of Georgia (Conway et al. 2004) provides an opportunity to study the ecology of glass sponge reefs under logistically feasible conditions.

We used the occasion of a research cruise funded by NSERC to examine briefly the effects of acoustic noise on the behaviour of glass sponges. Our objective was to test the hypothesis that the acoustic vibration produced by a small, surface operated air gun will not alter the normal pattern of sponge feeding activities. It is important to note that this work is a pilot study only. It was not possible to use a full seismic air-gun array in Strait of Georgia nor was there sufficient opportunity to replicate the work over several days.

Methods

The study was carried out onboard the Department of Fisheries and Oceans vessel CCGS *John .P Tully* using the ROV submersible ROPOS (www.ROPOS.com) during an eight-day research program (6-16 July 2005, co-PIs S. Leys and V. Tunnicliffe) to study glass sponge physiology and ecology. This work is not reported here.

Study site

The Fraser Ridge sponge reef is located in Strait of Georgia at 49º10'N 123º20'W (Conway et al. 2004). The Ridge substratum is compacted mud and clay. The majority of the sponges are densely packed in semi-circular mounds forming a crescent on the northeast crest in 185-160 m water. The reef complex is about 1 km long by about 400 m wide. Figure 2 illustrates the bathymetry and the location of the deployed instruments. Sponges can form dense thickets up to 1.5 m in height – two species dominate (Figure 3). Ambient water properties were recorded using an instrument package mounted on the ROV that included a pumping Conductivity, Temperature and Depth (CTD) probe (SBE 19plus, Seabird) with a CStar transmissometer (Wet Labs, 25 cm path length), a WETStar chlorophyll fluorometer (Wet Labs) and a Clark type oxygen sensor (SBE 43, Seabird). Discrete water samples were collected using Niskin bottles installed on the ROV and analyzed for dissolved inorganic nutrients, particulate organic carbon, particulate nitrogen and total suspended solids as described in Yahel et al. (2007).

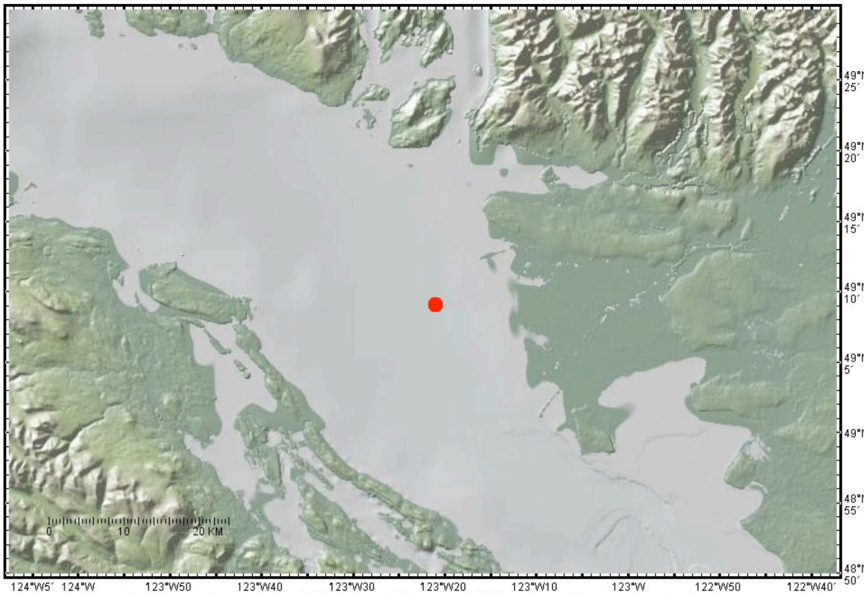

Figure 1. Location of Fraser Ridge (red circle) in Strait of Georgia.

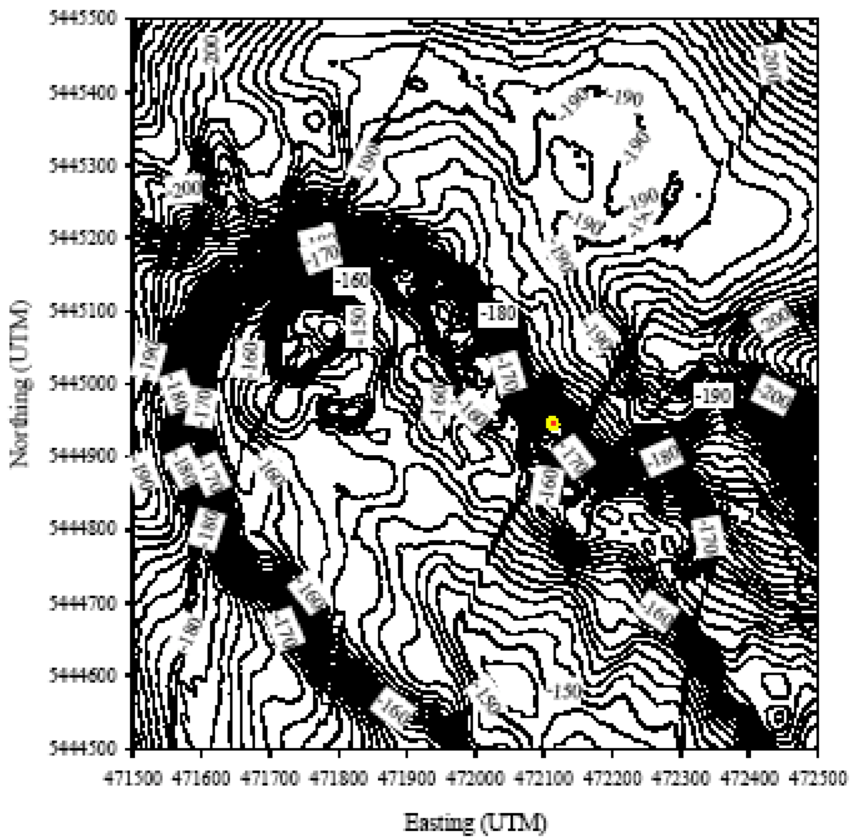

Figure 2. Bathymetry of the northern portion of Fraser Ridge (based on multibeam data, K. Conway, Geol. Survey Canada). The red dot indicates the location of the instrumented sponges and the current meters set to measure the ambient current. Axes are in meters.

Figure 3. Typical scenes on the reef –dense thickets of glass sponges *Aphrocallistes vastus* (top image) and *Heterochone calyx* (bottom image). Images are about 2 m across.

<u>Sponge pumping rate</u>

Sponge pumping activity was documented using Acoustic Doppler Velocimeters (ADV) (6 MHz Vector, Nortek, and 5 MHz Hydra, Sontek). The Velocimeters are nonintrusive instruments that measure 3D velocities within a controllable sampling volume of few cm^3. Because all flow from these animals exits through a large ex-current aperture (osculum), measuring the exiting flow from the osculum of the sponge should provide a good proxy of its pumping behavior. A survey of the pumping behavior of eight individual *Aphrocallistes vastus* was conducted prior to the air gun experiments (see below) in three separate dives. The Vector velocimeter was attached to the portside manipulator of the ROV (Figure 4). The instrument was controlled in real-time (Table 1) and the data stream was visualized onboard using Vector 1.27 software (Nortek).

A target sponge and osculum were selected based on accessibility and minimized damage to nearby sponge thickets. At the beginning of each sampling session, the instrument was positioned next to the target osculum to measure the ambient flow. Then sponge pumping measurements were taken by carefully positioning the current meter into the excurrent flow with the ROPOS manipulator. A mechanical pointing device (Figure 4), controlled by the ROPOS operator, indicated the location of the sampling volume, and injection of fluorescein dye was used to visualize the excurrent flow (Figure 5, see also Yahel et al. 2007). Further verification of the exact position of the sampling volume with respect to the osculum walls was obtained by using the Probe Check utility (Vector 1.27, Nortek) to plot in real-time the acoustic signal received by each of the receivers versus the distance from the transmitter.

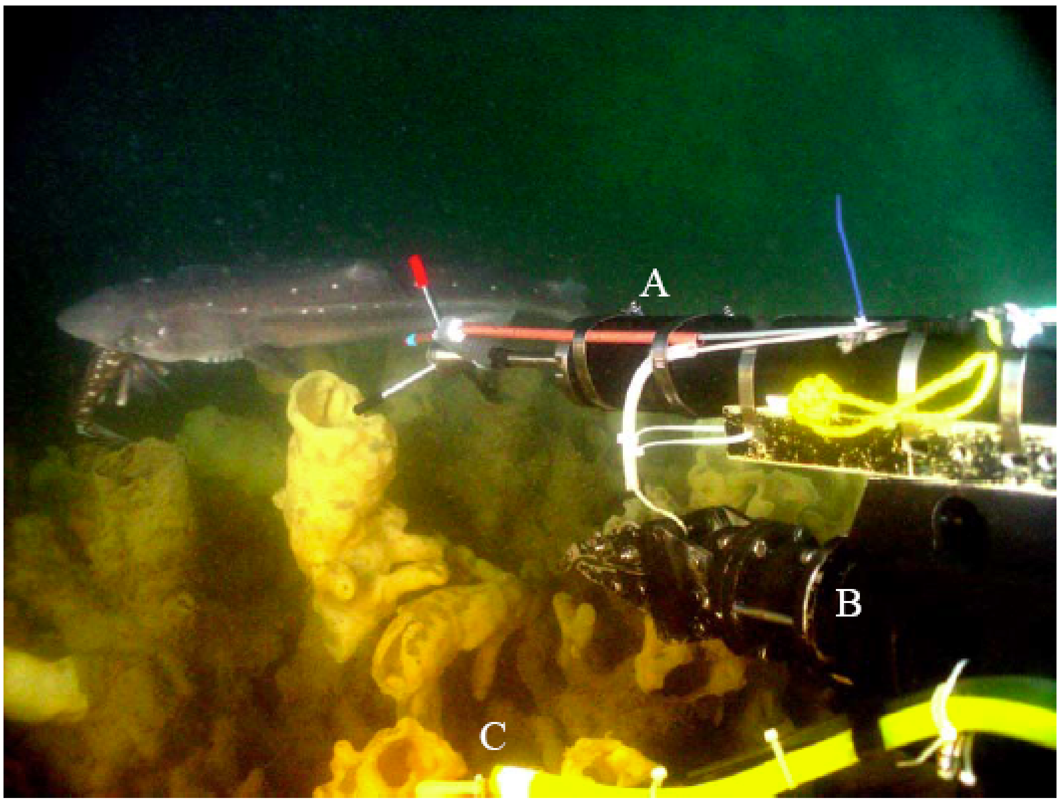

Figure 4. The Vector Acoustic Doppler Velocimeter (A) installed on the ROPOS's Magnum manipulator (B). The blue tip at the end of the copper tube attached to the Vector is a retractable sampling indicator. The three receivers are marked with red or black tips. The yellow tube (C) contains seawater dyed with sodium fluorescein used to visualize the sponge pumping activity. In the background is a small shark eating a ling cod. Picture was taken at ~170 m during dive R894 (7 July 2005).

To account for possible effects of the ROV presence, the tidal cycle phase, and other environmental factors on our snapshot measurements, we instrumented an individual sponge with an acoustic Doppler velocimeter for 6.1 days. These current meters were deployed in a self contained mode and, due to memory limitation, were programmed to record at a lower rate (0.1 Hz, Table 1). The proper orientation of the self contained current meters was challenging in the absence of real-time data stream. Indeed, a nine-day time-series from a third sponge proved useless upon retrieval as the sampling volume was positioned too deeply inside the osculum.

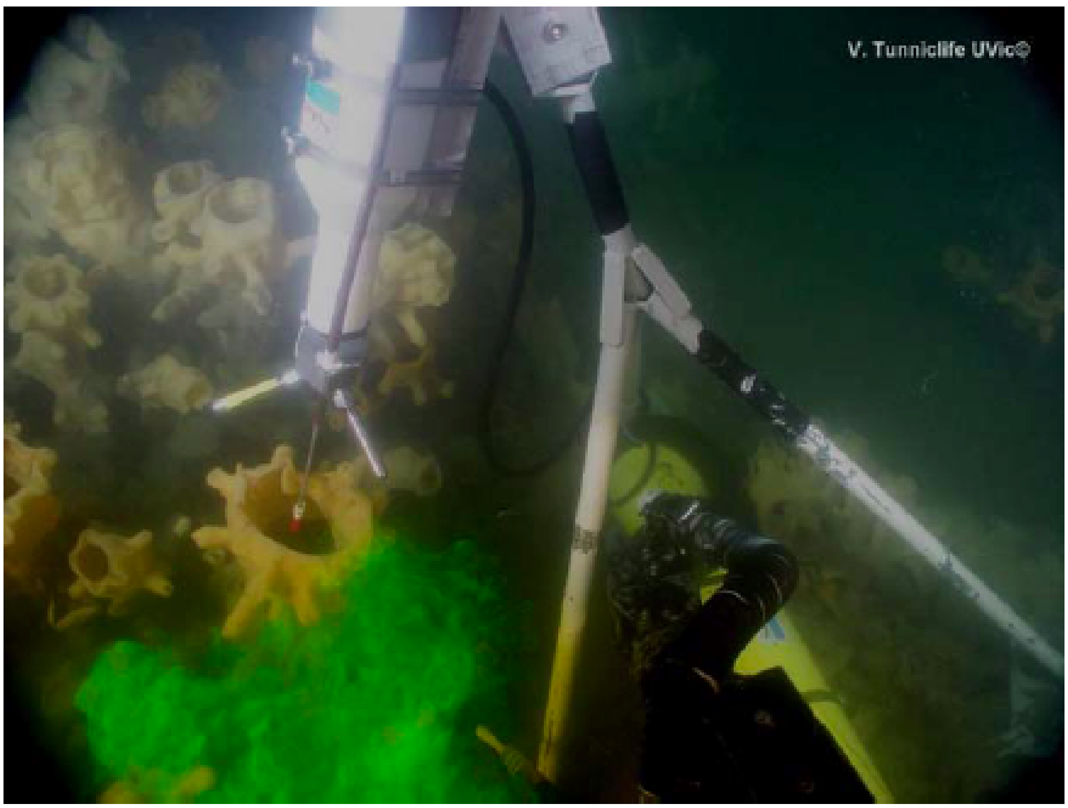

Figure 5. The 5 MHz Hydra acoustic Doppler velocimeter (Sontek) setup over the osculum of a glass sponge (*Aphrocallistes vastus*, Fraser Ridge, July 2005, water depth 162 m) for the long term time series of sponge pumping activity. The red tipped rod is a retractable indicator pointing to the location of the sampling volume. Yellow fluorescein dye is injected by the ROPOS to visualize the sponge pumping (no adverse response to the dye).

Table 1. Acoustic Doppler velocimeter settings for two instruments deployed over several days and the "hand-held" instrument used during the air-gun shots.

Parameter	Self contained Vector (Nortek)	Self contained Hydra (Sontek)	Real-time Vector (Nortek)
Sampling rate	2 Hz	1 Hz	2 Hz
Nominal velocity range	0.30 m s^{-1}	0.03 (±2 m s^{-1})	0.30 m s^{-1}
Burst interval	10 sec	10 sec	
Sample per burst	1	1	CONTINUOUS
Sampling volume	18.0 mm	2 ml	18.0 mm
Measurement load*	59%	100%	59%
Transmit length	4.0mm	NA	4.0mm
Receive length	0.01m	NA	0.01m
Velocity scaling	0.1mm	NA	0.1mm
Power level	HIGH	NA	HIGH
Coordinate system	ENU	ENU	XYZ
Sound speed	MEASURED	MEASURED	MEASURED

Acoustic effects on sponge pumping

Our primary sound source was a single, 10 in^3 (164 cm^3), Bolt air gun that was deployed from the stern of the Tully. Characterization of the emitted sound and its spatial propagation was made using an autonomous pop-up recorder anchored to the sea-bottom 162 m south-west of the instrumented glass sponges and from a surface system deployed from the stern of a separate recording vessel, *MV Strickland*. Appendix 1 gives a full description of air gun and hydrophone deployments and describes the methods used to extract the acoustic data. A total of 16 sequences of 3 or 5 air gun shots was executed between 1100h and 1700h (PDT) of 16 July 2006 (Table 2).

Before and during the air-gun operations, a trained mammal observer remained on the ship to ensure no shots occurred while mammals were in the area. As a result of a review of the planned air-gun operations conducted by the DFO prior to the experiment, a 300 m area around the air-gun was proscribed. Visual observations were made from the *Tully*'s monkey's island, the highest suitable vantage point on the ship. Firing was curtailed for 30 min after a mammal left the proscribed area. Four seals were sighted and one incursion reported. A full description is available in Appendix 2. It includes the statement: "Considering the relatively low noise levels expected to occur at the distances at which seals were observed, it is unlikely that any harbour seals were harmed by air-gun sounds during the study."

As hexactinellid sponges lack true neural system (Mackie 1980, Mackie et al. 1983b, Leys & Mackie 1997b, Leys et al. 1999a), we expect a slow response to external stimuli (Leys & Tompkins 2005). Moreover, *Aphrocallistes vastus* pumping is particularly robust to disturbance (Yahel et al. 2006). **Therefore, a "response" of the sponge was predefined as a 20% or 0.5 cm s^{-1} reduction of the excurrent flow initiated within 3 minutes from the last shot of a sequence and lasted for at least 2 min.** Due to logistical limitations, the real time recording with the *ROPOS*-held Vector had started only at the fifth sequence (Table 2). It is noteworthy that each time the air gun was fired, it would affect the entire sponge population, not only the individual we observed in real time. Therefore, any accumulative effect could be captured only by the (single) sponge that was monitored by the Hydra system for the long-term time series.

To test the hypothesis that the observed response pattern was a consequence of pure chance, we simulated the experimental design by projecting the timing of the air gun shots onto all of the observation times for the six days of the long term time series, and examined the signal for virtual "responses" as above.

Table 2. Air gun shots sequences 15 July 2005. The Hydra (Sontek) current meter was deployed to record a long term time series of the excurrent speed of an individual sponge in self contained mode while the Vector current meter was attached to the ROV *ROPOS* for real time observations of three different sponges (SP1-SP3). Times in this table are reported according to the shipboard computerized logging system that was used to synchronize the instruments and air gun shots.

Air gun Seq. #	**Time (UTC)**	**Time (PDT)**	**Air gun depth (m)**	**Interval between shots (s)**	**No. of shots**	**Recorded by**	**Sponge ID**
AG1	18:39:40	11:39:40	2	5	3	Hydra	Son
AG2	18:49:30	11:49:30	2	5	3	Hydra	Son
AG3	18:59:30	11:59:30	2	5	3	Hydra	Son
AG4	19:08:20	12:08:20	2	5	3	Hydra	Son
AG5	21:37:00	14:37:00	2	5	3	Hydra + Vector	Son + SP1
AG6	21:47:30	14:47:30	2	5	3	Hydra + Vector	Son + SP1
AG7	21:58:10	14:58:10	2	5	3	Hydra + Vector	Son + SP1
AG8	22:13:20	15:13:20	2	5	3	Hydra	Son
AG9	22:23:20	15:23:20	2	5	3	Hydra	Son
AG10	22:33:20	15:33:20	2	5	3	Hydra	Son
AG11	22:43:20	15:43:20	2	5	3	Hydra	Son
AG12	22:53:20	15:53:20	2	5	3	Hydra + Vector	Son + SP2
AG13	23:01:20	16:01:20	2	5	3	Hydra + Vector	Son + SP2
AG14	23:12:10	16:12:10	15	2	5	Hydra + Vector	Son + SP2
AG15	23:51:30	16:51:30	15	2	5	Hydra + Vector	Son + SP3
AG16	23:56:30	16:56:30	15	2	5	Hydra + Vector	Son + SP3

Results

Ambient conditions

During the study period (8-16 July 2005), the upper water column above the reef was heavily influenced by the Fraser river freshet (Figure 6), but at depths greater than 80 m, density and temperature profiles were quite uniform (Figure 6). This region of the Strait of Georgia is characterized by strong, mixed-semidiurnal tidal currents, dominated by northwardly floods. Detailed mapping of the northern section of the Fraser Ridge indicated that the majority of the sponges were concentrated at the upper third of the northeastern slopes.

Preliminary analysis suggests that during the study period (8-16 July 2005), a standing wave (cf. Dewey et al. 2005) formed over the ridge during the flood phase of the tide. During these periods, the strong (up to 92 cm s^{-1}) ambient near-bed current (1-2 m above bottom) accelerated down the slope, creating strong down-welling (vertical downward velocities of up to 39 cm s^{-1}!). In contrast, during the ebb phase, flows at the reef were low (5-15 cm s^{-1}), resembling slack conditions.

The general flow over the reef was predominantly across the ridge to the north northeast (mean progressive vector of ~15 km day^{-1}). Temperature was stable (9.74±0.09 ºC) throughout the study period. In contrast, oxygen, and turbidity concentrations showed high temporal and spatial dynamics. Turbidity was generally high with 680 nm transmissivity levels often falling below 30% m^{-1} at the benthic boundary layer (<10 m above bottom). Turbidity and oxygen showed sharp but opposite gradients within the benthic boundary layer so that during high turbidity periods, near bottom oxygen levels often dropped below 0.5 ml L^{-1}. As turbidity was modulated by the general rather than the local flow pattern, high turbidity (and low oxygen) periods were associated with both ebb and flood (low and high flow periods). Despite the location of the reef below the Fraser river freshet, total suspended solids concentrations were not extreme (max 8.25 mg L^{-1}) but rather comparable to the levels measured in other glass sponge habitats along the BC coast (Whitney et al. 2005, Yahel et al. 2006).

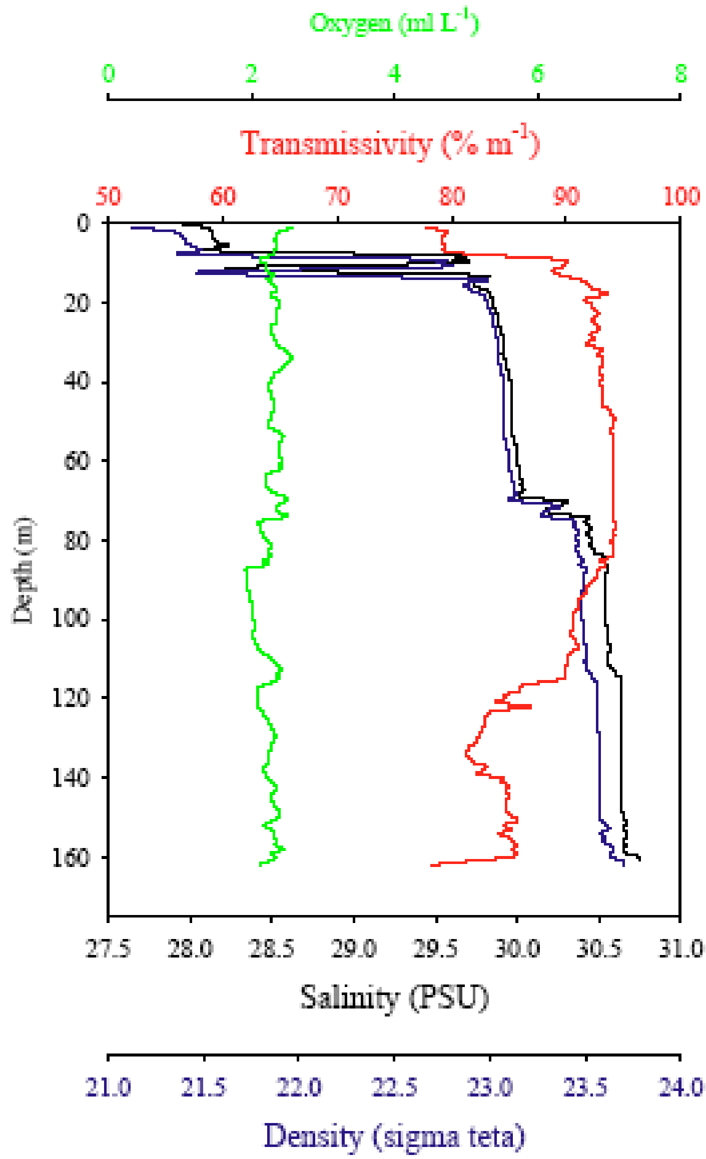

Figure 6. A typical hydrographic profile above the study site recorded with the online instrument package installed on the ROV (see methods).

Air gun shots

The average sound pressure level to which the sponges were exposed, in dB re μPa, was approximately 182 peak to peak, 177 zero to peak, 161.5 rms with an average sound exposure level of 151 dB re $\mu Pa^2 s^{-1}$ (see Appendix 1 for a full description of the air gun source levels measurements). The air-gun noise exceeds the background noise of the surrounding environment at frequencies below approximately 300 Hz, but above this frequency the received levels begin to approach the level of the background noise which contains noise from surrounding vessels, predominantly from the CCGS *John P Tully*.

Sponge pumping responses to air gun shots

The question whether the sponge excurrent speed is affected by the air-gun shots is addressed statistically. In a statistical test a null and an alternative hypothesis are defined (Milton 1999) and an experiment is carried out to determine the value of a test statistic. Then the statistical evidence against the null hypothesis is computed through the p-value, which is defined as the probability of getting an outcome (the test statistic) as extreme as or more extreme than the actual observed outcome if the null hypothesis is true. The smaller the p-value, the stronger is the evidence against the null hypothesis. A p-value greater than or equal to 0.1 is usually interpreted to mean there is little or no evidence against the null hypothesis in favor of the alternative hypothesis. The null hypothesis in this experiment is that the sponge excurrent speed is not affected by the air-gun shots and the alternative hypothesis is that the sponge excurrent speed is affected by the air-gun shots. Using the predefined definition of a "response" (page 8), we use the number of responses (R) as the test statistic.

A two-minute moving average of excurrent speed versus time is shown in Figure 7 for the period of the 16 air-gun shots. Shots 2, 5 and 6 showed a response, for a total of R=3 responses over the 16 shots. To compute the p-value for the test, the distribution of R needs to be determined under the null hypothesis. An approximate distribution of R is obtained by observing the number of responses for ambient conditions (no air-gun shots) of the six-day experiment. From Table 2, the time differences between air-gun shot 1 and all the other shots were determined. The number of responses was then calculated for all possible 53,000 starting times in the experiment, i.e., assuming that air-gun 1 was fired at each observation time and it was followed by shots at the appropriate delays for all the other 15 shots. Figure 8 displays the histogram of R under the null hypothesis. Intuitively, since R ranges from 0 to 11 with the probabilities noted, under the no air-gun scenario a value of R equal to 3 when the air-gun shots occurred does not give support to the alternative hypothesis that the air-gun shots affect the excurrent feeding current.

To obtain a quantitative measure of the statistical evidence against the null hypothesis, we determine the p-value, the probability that R is greater than or equal to 3. From the data in Figure 8, the p-value (the area under the histogram for R from 3 to 16) equals 0.73. Since this p-value is much greater 0.1, there is little or no evidence against the null hypothesis in favor of the alternative hypothesis. That is, there is little or no evidence that the sponge is responding to the air-gun shots.

We also note the times of responses greater than two standard deviations above the mean as shown in Figure 9. These outliers appear randomly throughout the 6-day experiment time and are not related to the times of the true air-gun shots.

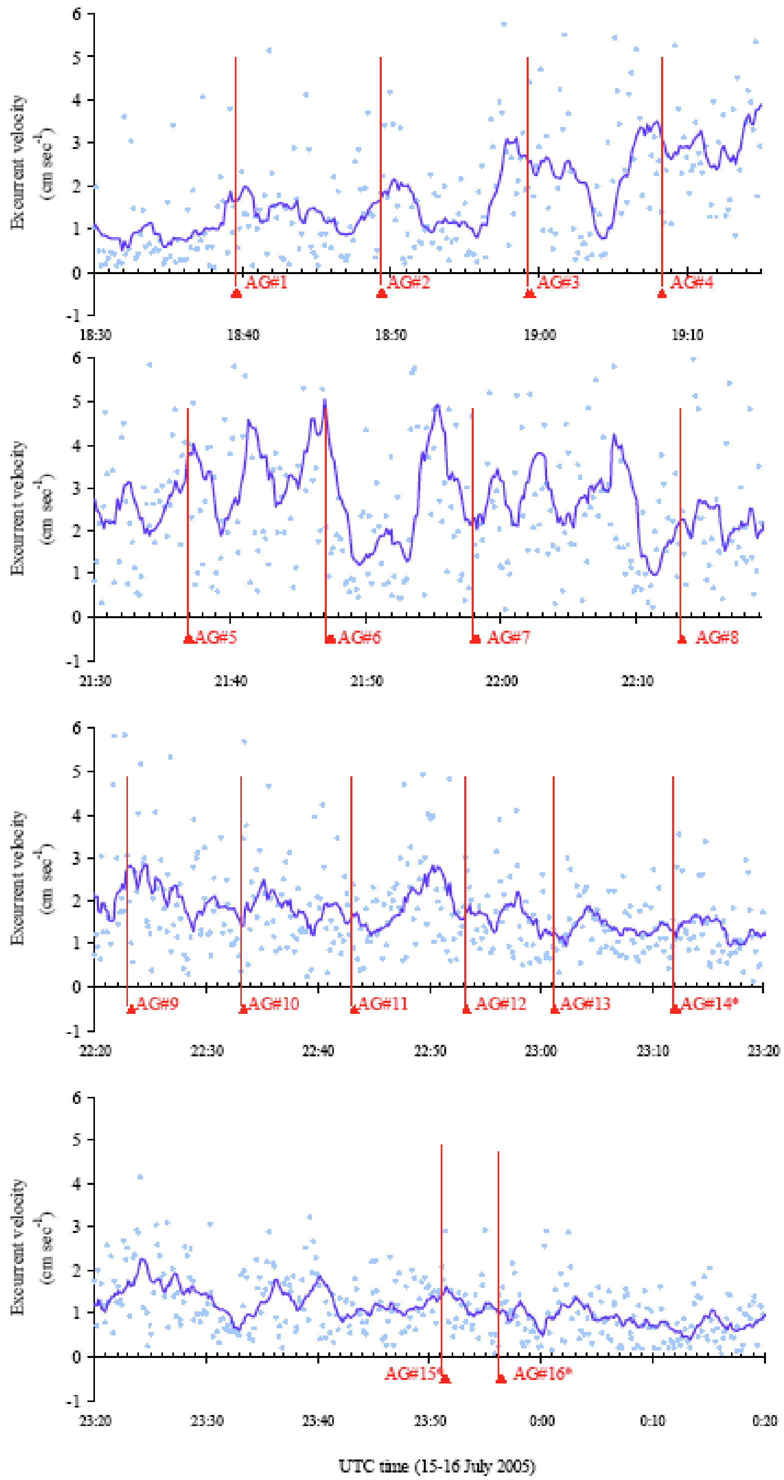

Figure 7. Zoom of the excurrent time series for the true air-gun shot times. Data points are at 10 s intervals. Solid line is two min moving average. Based on prior experimental work on nervous system responses in these animals, flow reduction of 20% within 3 min of stimulus is deemed a "response" (see Methods). Three responses are recorded, for shots 2, 5 and 6.

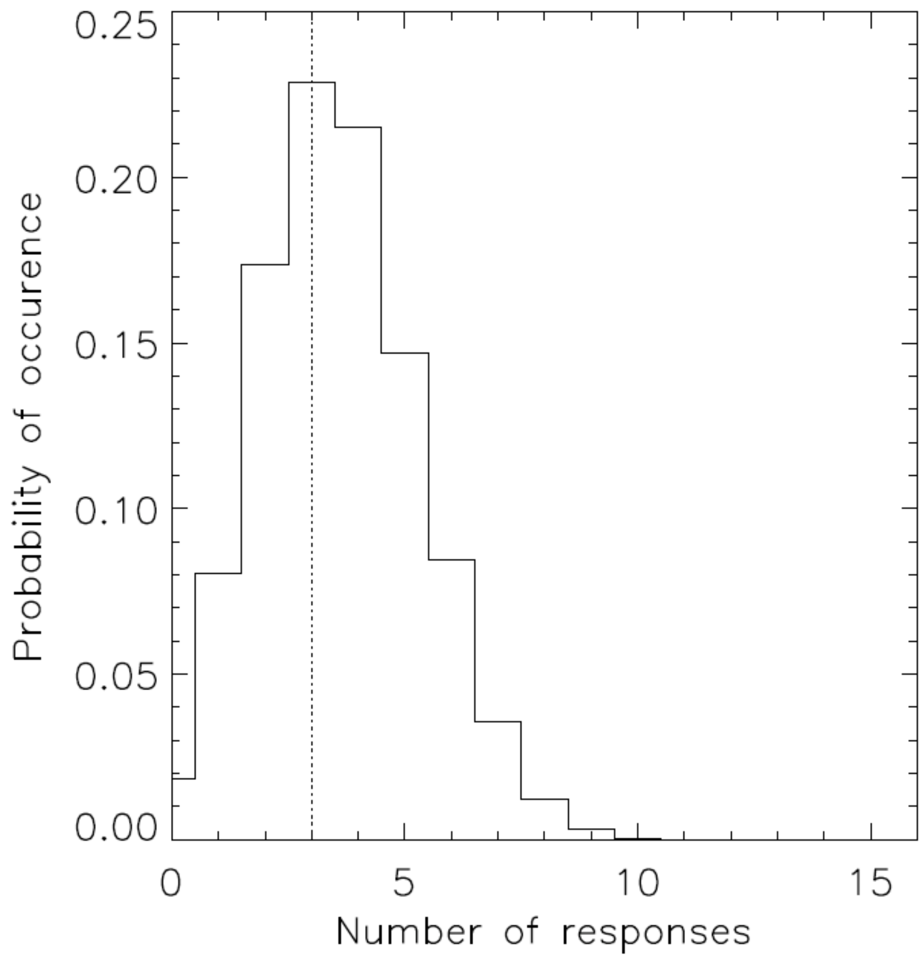

Figure 8. Frequency distribution of number of responses obtained in the first ten virtual shots in 53,000 simulations of the air-gun experiment scheme over the six-day time series of undisturbed pumping by an *Aphrocallistes vastus* . The vertical dotted line indicates the number of responses when the air gun shots occurred.

Correlation of Sponge Response and Ambient Current

The six hours of the air gun experiment (18:00 – 24:00 UTC) coincided with a flat tide starting roughly at 1815h UTC and gradually declining from 2000h UTC until the end of the experiment (Figure 10). The time series from the self contained current meter indicated that the sponge excurrent speed was highly dependant on the ambient flow speed (Figure 10, Yahel et al. in prep., Vogel 1974, 1977, 1978). The linear correlation coefficient of ambient and excurrent speeds over the six days was 0.85, for a 30-minute median data-smoothing filter. That is, a fraction equal to $0.85^2 = 0.72$ of the variation in the excurrent speed can be explained by the variation in the ambient speed. The p-value when testing the null hypothesis of zero correlation against the alternative hypothesis of a positive correlation was less than 0.001. However, while the ambient vertical velocity was predominantly downward, the excurrent flow was in the opposite direction (upward), indicating that we have indeed measured the sponge activity (Yahel et al. in prep.).

Sponge pumping measured by *ROPOS* with the real-time Vector ADV was in general agreement with the pattern and magnitudes measured by the Hydra ADV for the undisturbed sponge, suggesting that the ROV presence did not pose a significant disturbance to the sponge (data not shown). Unfortunately, we were able to accomplish these measurements only for shot sequences 5-7 and 12-16 (Figure 10; Table 2). The limited number of responses measured for these shots in three different sponges (Figure 11) agreed well with that measured by the Hydra for sponge ‘Son’. That is, as for the 'Son' sponge, responses were associated with air-gun sequence no. 5-7 and then again with sequence no. 14-16 (Figures 7 and 11).

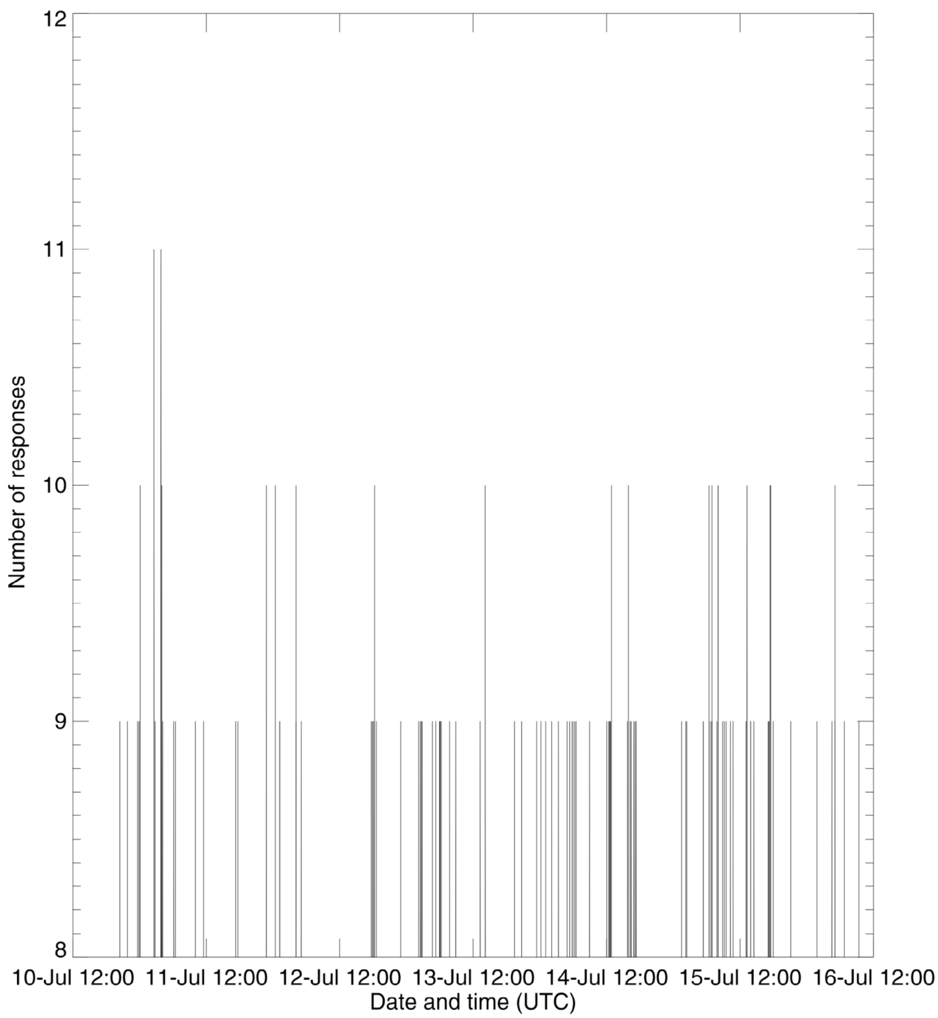

Figure 9. Number of responses greater than or equal to eight (two standard deviations above the mean) for the assumed air-gun shot sequence for all time samples in the experiment. These outliers occur at random times.

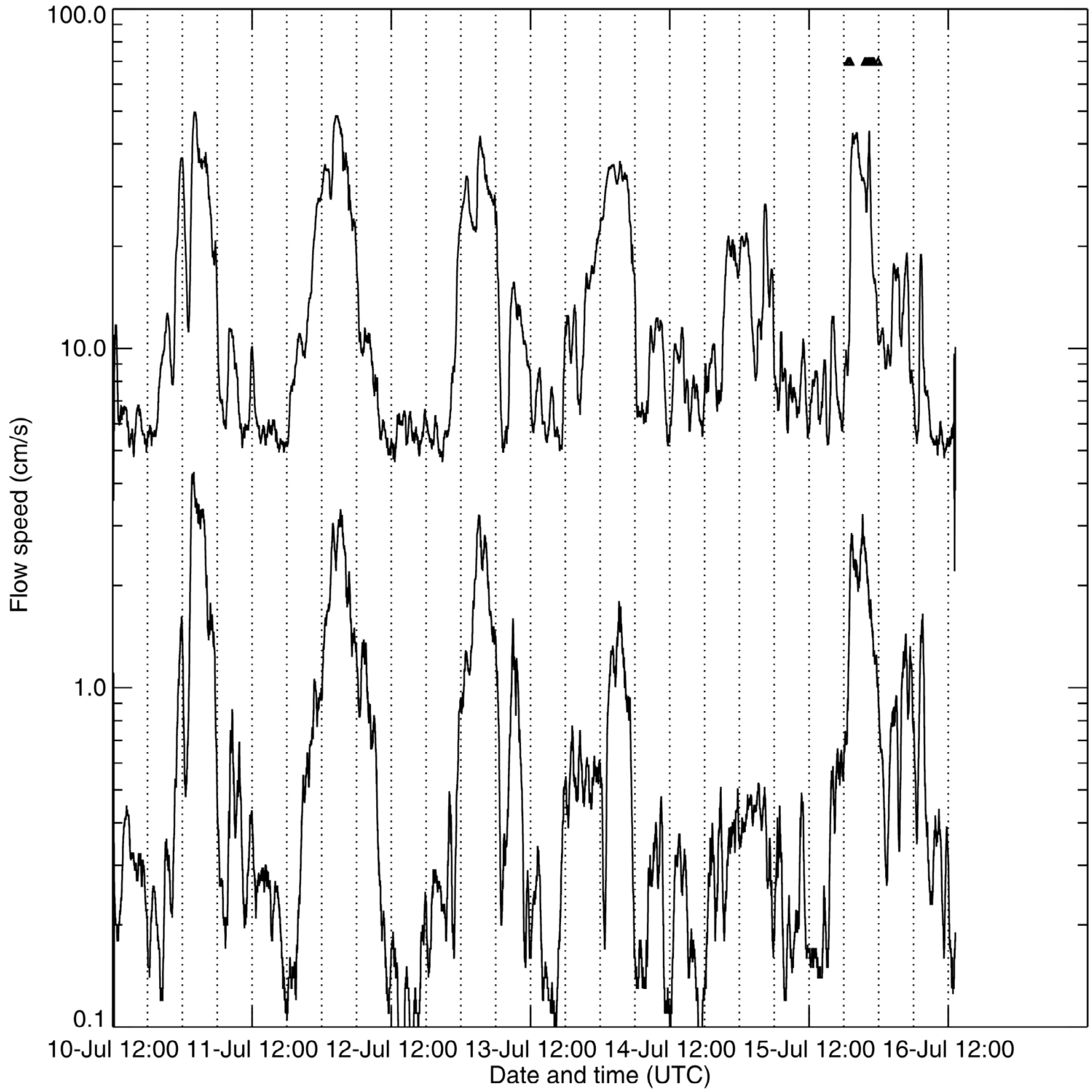

Figure 10. Upper curve depicts the ambient currents; the lower curve represents the flow from osculum of sponge 'Son' in Table 2. Air gun shots occurred on the last day. Excurrent speed was measured by a Hydra ADV looking down into an *Aphrocallistes vastus* osculum at 161 m depth (Figure 5). Ambient flow was measured at the same depth 65 m to the north, 4 m above bottom by a 300 KHz WH ADCP (RDI). Data points were recorded at 10 s intervals, solid line is 30 min moving median.

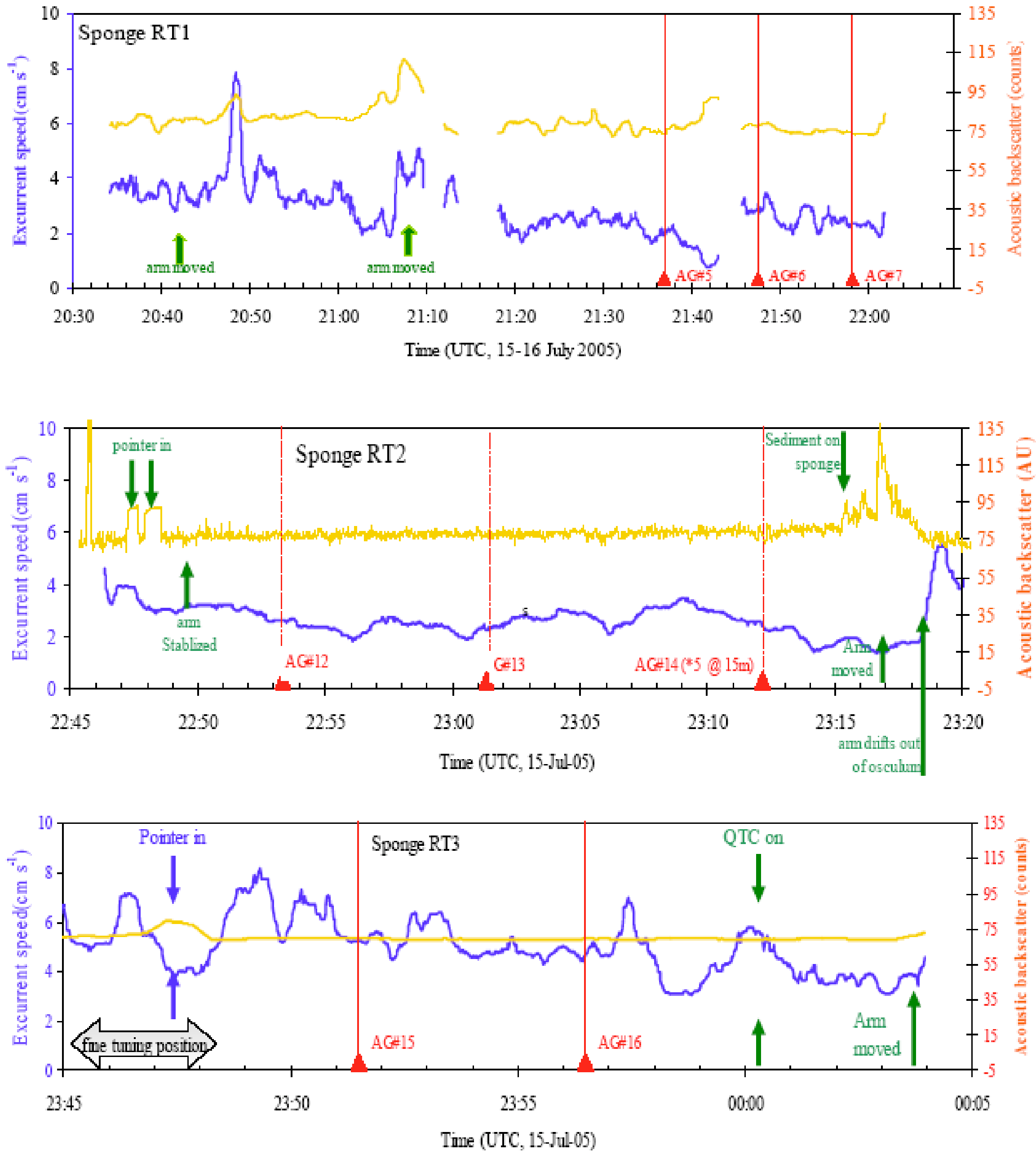

Figure 11. Time series of the excurrent flow speed (blue) and acoustic backscatter intensity (yellow) from three sponges recorded with the 5 MHz Vector held by the ROV manipulator during the air gun experiment. Data were collected at 1 Hz; solid line is one min moving median. Backscatter is a measure of suspended particle concentration.

Discussion

The exact sound level (or pressure wave) experienced by the sponge during a full scale survey will depends on the sound array design and height above the bottom. However, it is likely that such a survey will produce a much higher signal (10-15 dB) in comparison to the air-gun shots we used.

Immediate response to seismic acoustic waves is well known in many marine organisms – especially marine mammals. Whether effects on non-mammals are deleterious or sustained are poorly investigated. This report does provide additional information in two areas highlighted as gaps by a national review (DFO 2004): characterizing sound received on the seafloor and observing in situ response by an invertebrate species. As far as the authors can determine, it is the first such study on sponges.

Sponges have a narrow range of behavioral responses – they cannot swim away, change shape, move appendages or alter blood flow. The only response we can measure with non-invasive means is flow through the animal. This flow through the walls and out a central "mouth" is necessary for respiration and for feeding. Cessation for sustained periods is likely to affect the animal's health. Because signals are conducted through the animal in a very slow manner, response to any stimulus will also be slow. The Fraser Ridge sponges live in an environment of high currents, high suspended matter, strong tidal modulation and ambient noise from shipping. So a response to disturbance such as a small air gun was not expected.

Laboratory experiments with *Aphrocallistes vastus* have indicated that undisturbed sponges will pump continually for many hours. However, arrests in feeding current were recorded in the absence of experimental stimuli (S. Leys, personal communication). Arrest-recovery cycles (dips in the flow record, typically lasting 1-3 min) with no apparent pattern occurred on an irregular basis both in the field and the laboratory (Yahel et al. unpublished, Leys & Tompkins 2005). Repeated electrical stimuli eventually fail to cause arrests of the feeding current in *Rhabdocalyptus dawsoni* (Leys et al. 1999b). In contrast, when exposed to suspended sediment, sponges responded with pumping arrests that lasted for 0.5 to 6 min (S. Leys pers. comm.). In the lab, sponges ceased to response to repeated application of low sediment doses. However, increased disturbance by suspended sediment caused prolonged arrest-recovery cycles associated with a marked reduction in pumping for several hours (S. Leys pers. comm.). Continuous, steady pumping was rarely observed in the field where flow and suspended loads are highly variable.

In the *in situ* pumping records reported here, a rhythmic pumping activity resembling that reported by Leys & Tompkins (2005) appeared with the initiation of air gun shots (Figures 7, 10) but it was superimposed on the general trend induced by the ambient flow (Figure 10). While this response resembles sponge response for increased sediment load (Leys & Tompkins 2005), ambient flow speed accounted for ~70% of the observed variation. This high dependency of sponge excurrent speed on the ambient conditions and the unfortunate coincidence of ambient flow reduction with the air-gun experiment render impossible the separation of these putative factors.

The question of whether the sponge's excurrent flow responded to the pressure from a series of air-gun shots was addressed by a statistical analysis over all the excurrent data from the experiment. The sponge's response to ambient conditions was compared to the response at the true air gun shot times. For this single sample of 16 air-gun shots, there were only three responses according to the working definition of response. The statistical analysis showed that this number of responses was not significant. For this experiment, there is little or no evidence that the acoustic pressure from the shots influenced the physiological functions of the sponge.

The work described here is difficult to carry out in the field, as other external factors are difficult to control. The complexity of the approach constrained sample size and duration of observation. Daylight hours, marine mammal intrusion, ROV setup, instrument malfunction and tidal timing all became confounding factors. Nonetheless, the study is novel and the techniques can be refined. Further laboratory studies are necessary. They should focus on more realistic sound levels, recovery times, habituation and longer term effects (e.g., on tissue and skeletal integrity) before final conclusions are drawn.

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Appendix 1

Airgun Source Level Measurements
UVIC Sponge Reef Study

Report prepared by Melanie Austin

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Version 1.3
Date August 11, 2005

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1. INTRODUCTION

On July 15, 2005 a study into the effects of the noise from airguns on the feeding water flow rates of glass sponges was carried out at Fraser Ridge in the Strait of Georgia, British Columbia. The sponges were instrumented with ADCP equipment for monitoring the water pumping activity and video observations of the sponges were recorded using a remotely operated vehicle, ROPOS, operated from the Canadian Coast Guard vessel John P. Tully. The sound source was a single, 10 in^3 , Bolt airgun that was deployed from the stern of the Tully. Acoustic measurements were made using an autonomous pop-up recorder (anchored with an acoustic release to the sea-bottom near the instrumented glass sponges) and from a 'surface' system deployed from the stern of a separate recording vessel, MV Strickland. This report describes the results of analysis of the acoustic recordings that were measured during these exposure tests. Estimates of the airgun source levels and the corresponding sponge exposure levels are provided.

2. MEASUREMENT APPARATUS

The surface deployed system consisted of the following components:

1. Two Reson TC4043 calibrated hydrophones, with nominal sensitivity -201 dB (re V/µPa ± 1 dB) and flat frequency response between 2 Hz and 100 kHz.
2. JASCO Research AIM2000 depth and temperature sensor, operated and monitored through serial communications with a PDA.
3. One shielded, faired, five-conductor sub-sea cable (70-metres total length), capable of powering and transmitting data from the two hydrophones and the depth sensor unit simultaneously.
4. Two Ithaco 541M 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).
5. Marantz PMD690 digital stereo audio recorder, capable of sampling at 48-kHz on two channels with 16-bit resolution.

* The positions of both ships were used to convert each measured sound level to the source level at 1 m.

The sub-sea cable was deployed over the A-frame off stern of the Strickland and the recording equipment was set-up inside the Strickland laboratory.

The bottom-mounted pop-up recording system consisted of the following components:

1. Reson TC4043 calibrated hydrophone, with nominal sensitivity -201 dB (re V/µPa ± 1 dB) and flat frequency response between 2 Hz and 100 kHz.
2. Reson TC4032 calibrated hydrophone, with nominal sensitivity –170 dB (re V/µPa± 1 dB) and flat frequency response between 15 Hz and 100 kHz
3. Marantz PMD660 digital stereo audio recorder, sampling at 44.1 kHz on two channels with 16-bit resolution.

4. Cylindrical Aluminum pressure case rated to 1000 m depth, with inner diameter of 5.75″ and total length 20″. The pressure case was affixed to a steel frame for attaching to the acoustic release system and mounting the hydrophones.
5. InterOcean shallow water acoustic release system, including 111-D acoustic release, 1100E surface unit and TR2000 acoustic transponder, both in custom cases rated to 300m.
6. Four 11-inch diameter spherical deep trawl floats rated to 1500m.
7. Two 40 lb concrete blocks for anchoring.

Figure 1 shows a photograph of the pop-up system after retrieval.

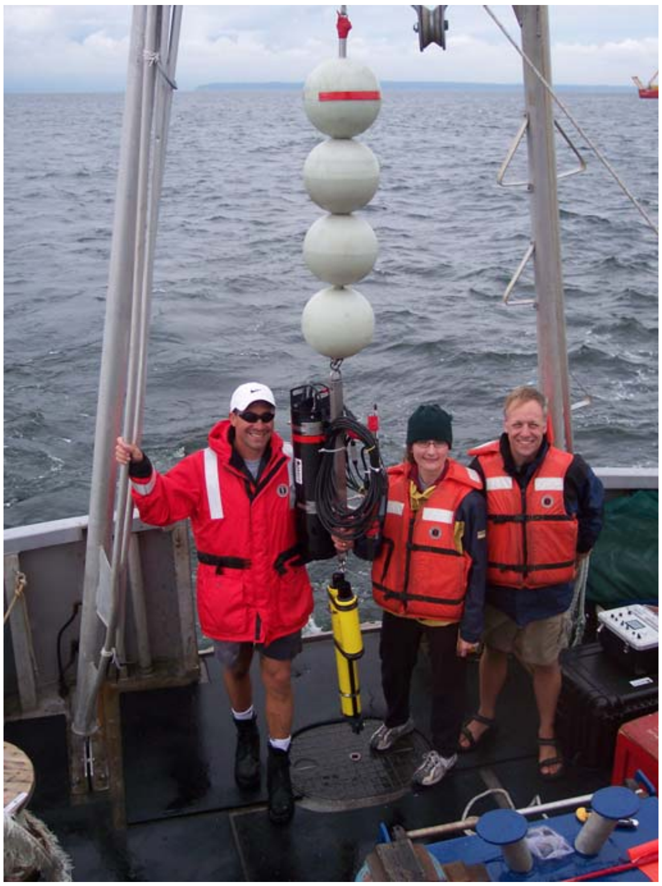

Figure 1: Pop-up acoustic recorder. Hydrophones are mounted opposite the black pressure case which houses the digital recorder and batteries. The yellow instruments at bottom are the acoustic release and transponder.

3.OBSERVATIONS

Acoustic recordings of the airgun shots were obtained from both the bottom-mounted and surface deployed systems. The bottom-mounted system was deployed just before ROPOS was prepared to enter the water and remained at the sea-bottom until the conclusion of the experiments, a total time of approximately 4.5 hours. The system was deployed at position

49° 9.359'N 123° 23.049'W and sank to the bottom under its own weight. The water depth was 160 m at this location. Upon completion of the experiments, a release command was transmitted to the unit to release it from its concrete anchor.

The surface hydrophones were deployed over the A-frame at the stern of the recording vessel and the hydrophone depth was monitored throughout the recordings.

The acoustic data were processed to determine the following metrics:

- Zero to peak pressure amplitude (0-p)
- Peak to peak pressure amplitude (p-p)
- Root-mean-square pressure value within the time window containing 90% of the airgun pressure signature (RMS)
- Sound exposure level (SEL), which is the integral of the squared sound pressure over the duration of the pulse.

The data were low pass filtered at 3kHz prior to computing the metrics. This cut-off frequency was found to be sufficient to filter out the background noise levels.

3.1. TEST SHOTS

A series of three-shot sequences was performed prior to the deployment of the ROV to record information from the instrumented sponges when exposed to airgun noise, without contributions from ROPOS itself. The surface-deployed hydrophone system was deployed off the stern of the Strickland during these test shots and data recording began in time to capture the final of the three-shot sequences. The bottom-mounted recording system was not yet in the water. The surface hydrophones were deployed at a depth of 32.7m and the Strickland was located 816 m from the Tully. The levels received during these test shots are shown in the following table. The received sound pressure levels (SPL) were consistent to within approximately 1 dB re μPa between the shots.

Table 1 Received levels recorded on the surface deployed hydrophones (32.7m receiver depth, 2m source depth) during the 'test' exposures. Measurement range is 816m.

Shot Number	Time	Mean Received SPL			
		0-p (dB re μPa)	p-p (dB re μPa)	RMS (dB re μPa)	SEL (dB re $\mu Pa^2/s$)
1	12:10:01	161.37	165.77	144.16	137.44
2	12:10:04	159.38	164.77	143.38	136.71
3	12:10:07	159.96	165.46	144.12	137.54

Average Received Levels	160.24	165.33	143.89	137.23

3.2. SHOTS DURING SPONGE OBSERVATIONS

Eight separate experiments were carried out while three sponges (Sponge #1, #2 and #3) were under video observation by ROPOS. Three experiments were performed at each of the first two sponge locations and two experiments were performed at the third sponge location. In each experiment, the airgun was fired either three or five times consecutively at an interval of approximately 2-3 seconds. Acoustic recordings of the airgun shots were obtained from both the bottom-mounted and surface deployed systems throughout these sponge observation experiments. The surface deployed hydrophones were at a depth of 35.8m throughout the experiments and the bottom-mounted hydrophones were at approximately 159m depth.

The source depth was 2m and the airgun fired three times for the first five sponge observation experiments. The source depth was changed to 15m and the airgun fired 5 times for the final three observation experiments.

Analysis of the data from the bottom-mounted system found that pressure levels received on the more sensitive hydrophone (Model TC4032), as expected, exceeded the saturation level of the recorder and the data were clipped at the maximum record level. These data consequently could not be used to provide estimates of the peak pressure levels. The data from the less sensitive hydrophone (Model TC4043) were not clipped, however this channel was found to be experiencing an input impedance problem that reduced the amplitude of the recorded signal. In order to recover this data, a calibration of the recorder was performed after the experiments to provide a frequency dependent correction factor for the damaged channel. The Reson hydrophones provide a calibration insert line that allowed calibration through the hydrophone's own preamplifier using a function generator. The calibration was performed through the frequency range of interest: 10 Hz – 15 kHz. This correction factor was then applied in the frequency domain to correct the data from the right channel prior to computing the noise metrics. The following plot shows an example of the results of this correction procedure.

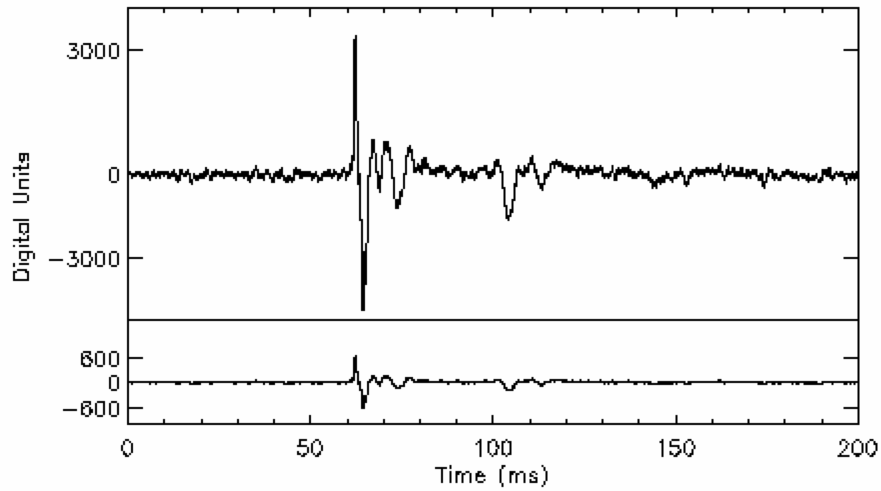

Figure 2 Pressure-time signature of one airgun shot received from the Right channel of the bottom-mounted hydrophone recording system. Bottom: raw data prior to correction. Top: corrected data.

The data in Table 2 present the mean levels received at the surface hydrophones, averaged over the number of shots in each experiment series. There were two experiments during which the airgun shots were not recorded with the surface system and so the values from the right channel of the bottom-mounted system are presented. The highlighted rows are levels taken from the right channel of the bottom-mounted system, with the correction factor applied. As was observed in the test trial, the levels received within a given experiment were observed to be consistent between shots to within ± 1 dB re µPa.

Table 2 Mean levels recorded during the glass sponge observations. Highlighted rows indicate data from the bottom mounted hydrophone system. All other data are from the surface deployed system. Levels are averaged over all shots in each experiment.

Time of first shot	Measure-ment Range (m)	No. of Shots	Source Depth (m)	Mean Received SPL			
				0-p (dB re μPa)	p-p (dB re μPa)	RMS (dB re μPa)	SEL (dB re $\mu Pa^2/s$)
14:37:07	391	3	2	167.31	170.64	150.21	141.56
14:47:51	185	3	2	175.01	179.84	158.52	145.76
14:58:12	448	3	2	161.95	166.57	146.97	138.46
15:54:18	283	3	2	162.64	168.21	143.85	139.85
16:02:15	272	3	2	161.09	166.78	147.19	140.02
16:13:03	295	5	15	172.66	177.5	158.02	150.82
16:51:59	170	5	15	171.19	175.90	160.34	149.65
16:57:01	258	5	15	173.14	177.02	152.63	146.53

The intensity of underwater sound decreases with increasing distance from the sound source. At close ranges, the rate of decrease is well described by spherical spreading loss, defined as 20log(range). The spherical spreading loss assumption neglects bottom and surface reflections. Spherical spreading was used in this analysis to estimate the airgun source levels from the received levels.

Analysis of the data from the surface hydrophones indicated that when the source was at 2m depth, the direct path signal was attenuated due to destructive interference with the surface ghost. This can be observed in the waveforms in Figure 4. As the horizontal range from the source increases, the direct path and the surface reflected signals arrive at the receiver nearly simultaneously. Source levels computed based on these received levels would underestimate the true airgun source level.

The data recorded when the source was at 15m depth and all the data recorded on the bottom-mounted system were suitable for computing source levels since the temporal separation between the arrival of the direct path and the surface reflection was sufficient that the surface reflection did not interfere destructively with the peak pulse of direct path signal. Source levels were therefore computed by adding 20log(range) to the levels received with the bottom-mounted hydrophone system. Similarly source levels were estimated from the received levels on the surface hydrophones when the source was at 15 m depth. The source-receiver separations were computed from GPS logs from the Tully and the Strickland, and using the relative source and receiver depths. It was found that the mean airgun source levels were as shown in Table 3.

Table 3 Average airgun source levels during the sponge observation experiments.

Average Airgun Source Levels (dB re μPa at 1m)			
0-p	**p-p**	**RMS**	**SEL**
222 ± 1	226 ± 1	206 ± 4	195 ± 3

The average source levels above were used to estimate the sound levels to which the observed sponges were exposed. Table 4 presents the computed sponge exposure levels.

Table 4. Mean sound pressure levels received at the sponge locations.

Time of first shot	Sponge Depth (m)	Sponge 'ID'	No. of Shots	Source Depth (m)	Mean Sound Pressure Levels at Sponge			
					0-p (dB re μPa)	p-p (dB re μPa)	RMS (dB re μPa)	SEL (dB re $\mu Pa^2/s$)
14:37:07	165	#1	3	2	177.36	182.01	161.45	151.07
14:47:51	165	#1	3	2	177.36	182.01	161.45	151.07
14:58:12	165	#1	3	2	177.36	182.01	161.45	151.07
15:54:18	168	#2	3	2	177.21	181.86	161.29	150.91
16:02:15	168	#2	3	2	177.21	181.86	161.29	150.91
16:13:03	168	#2	5	15	177.91	182.56	162.00	151.62
16:51:59	168	#3	5	15	177.91	182.56	162.00	151.62
16:57:01	168	#3	5	15	177.91	182.56	162.00	151.62

Figure 3 presents the spectral level of a representative airgun event to illustrate the frequency content of the airgun shots and the background noise. The top plot shows the pressure-time signature of a single airgun shot received on the surface deployed system at 99m range. The airgun noise exceeds the background noise of the surrounding environment at frequencies below approximately 300Hz, but above this frequency the received levels begin to approach the level of the background noise which contains noise from surrounding vessels, predominantly from the Tully.

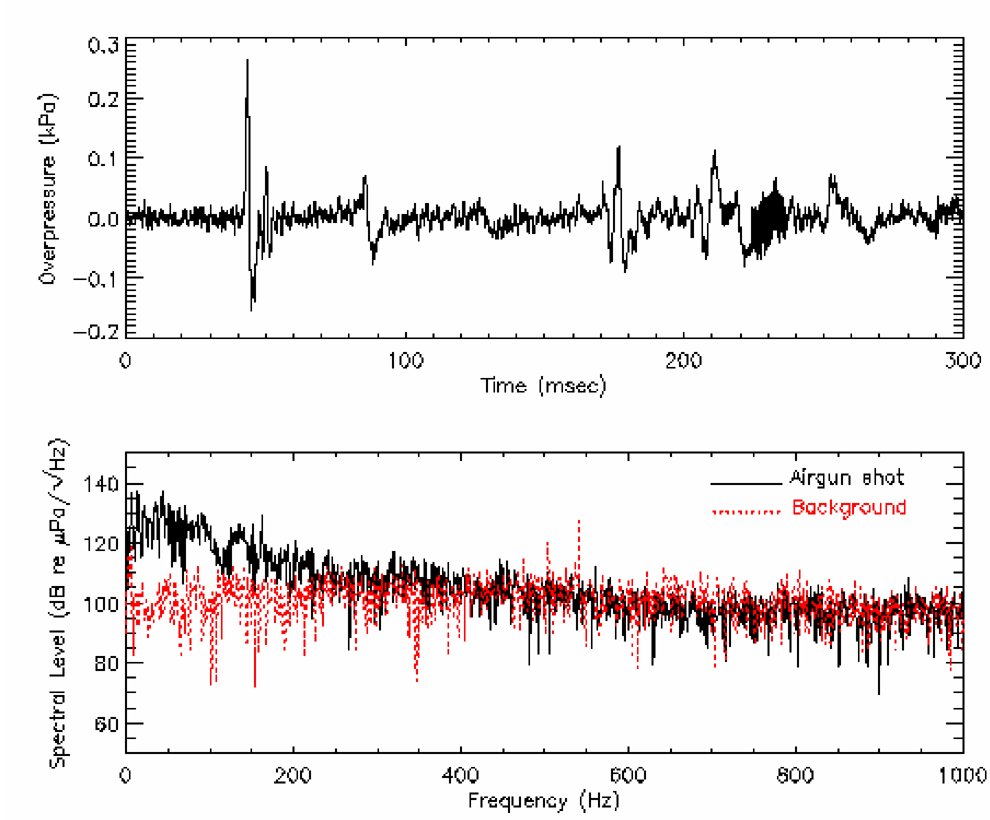

Figure 3 Sample of one airgun shot received at the surface deployed hydrophone system. Top: pressure-time waveform of airgun shot. Bottom: spectral decomposition of the above airgun pulse (solid, black line) and of the background noise one second before the airgun shot (dotted, red line).

3.3. ADDITIONAL SHOTS BETWEEN SPONGE OBSERVATIONS

Additional airgun shots were fired while the ROPOS was repositioning to the location of Sponge #2. These shots were fired to allow the recording vessel to maneuver to a selection of positions at varying ranges from the source for a more complete description of the transmission loss in the water surrounding the sponge observation sites. The levels received at the surface hydrophones during these experiments are presented in Table 5.

Table 5 Mean levels received at the surface deployed hydrophone system while the sponges were not under video observations. Values shown are averaged over all shots in each experiment.

Time of first shot	Measurement Range	No. of Shots	Source Depth (m)	Mean Received Sound Pressure Levels			
				0-p (dB re μPa)	p-p (dB re μPa)	RMS (dB re μPa)	SEL (dB re μPa2/s)
15:13:31	99	3	2	168.20	172.28	150.09	144.53
15:24:23	215	3	2	164.10	168.22	146.21	139.66
15:34:44	275	3	2	163.07	167.64	145.59	139.26
15:44:25	616	3	2	160.15	165.29	146.13	135.80

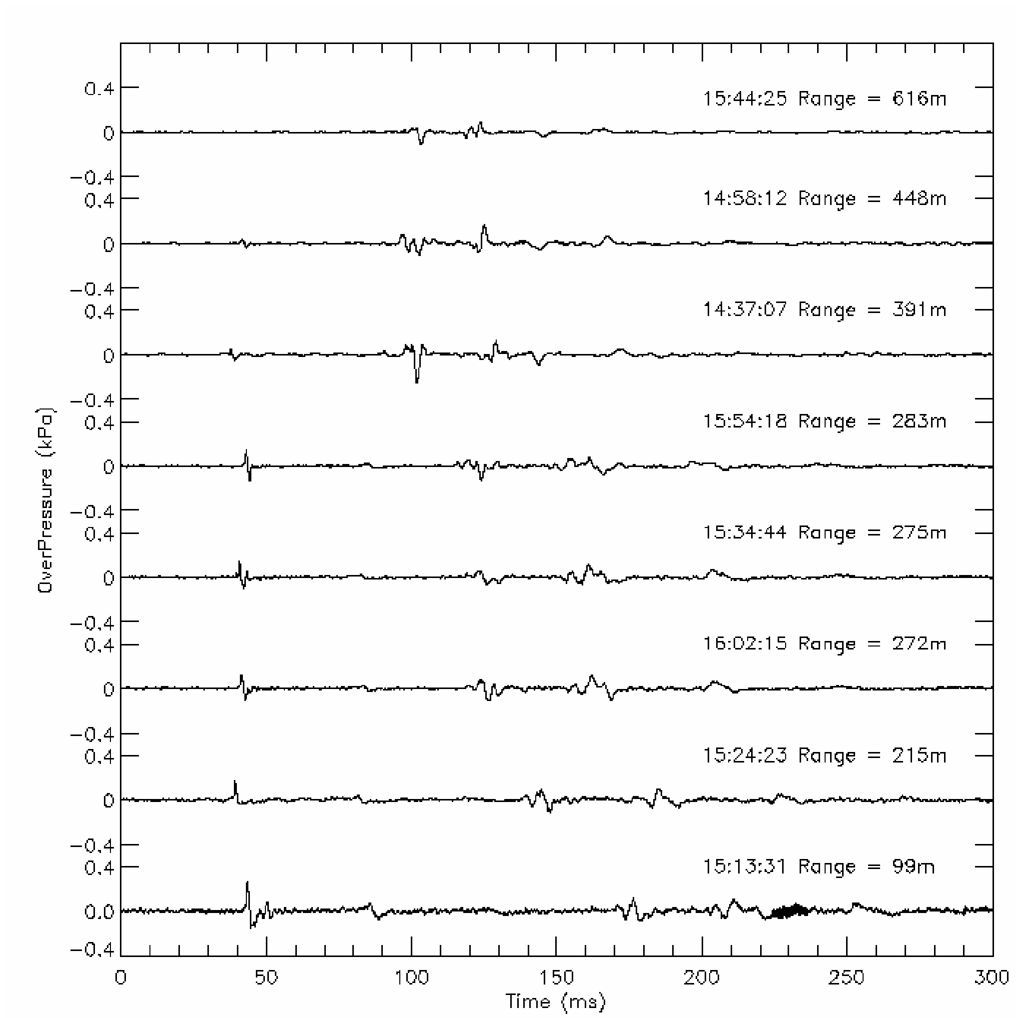

Figure 4 Pressure-time waveforms of a selection of airgun shots with varying range from the source.

Figure 4 shows the pressure waveforms of the first airgun event from several of the three-shot series with the source at 2 m depth. These waveforms are all taken from the surface deployed hydrophone data. The time of the first shot, and the source-receiver ranges, are indicated above each waveform in the figure. As the range from the source increased, the direct path signal was attenuated due to destructive interference with the surface reflected path.

4. SUMMARY

Acoustic data recordings obtained during an experiment to observe the effects of airgun noise on glass sponges have been processed to provide peak pressure levels, rms pressure levels and sound exposure levels at various ranges from the acoustic source. These received levels have been used to compute source levels at the airgun and exposure levels at the sponge locations. The average peak source level observed, in dB re μPa at 1m, was 226 p-p (222 0-p). The average rms source level and sound exposure level were 206 dB re μPa at 1m and 195 dB re $\mu Pa^2/s$ at 1m respectively. The source level variations among shots were observed to be very consistent (within ± 1 dB re μPa). The average sound pressure level to which the sponges were exposed, in dB re μPa, was approximately 182 peak to peak, 177 zero to peak, 161.5 rms with an average sound exposure level of 151 dB re $\mu Pa^2/s$. The noise from the airgun exceeded background noise levels at low frequencies but contributed minimally to the overall background noise (including noise from the Tully) at frequencies greater than approximately 300Hz.

Marine Mammal Monitoring and Mitigation during a Seismic Experiment in the Strait of Georgia, British Columbia

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Description of Airgun Activity

During the study of potential effects of airgun sounds on glass sponges at Fraser Ridge in the Strait of Georgia, British Columbia, a single 10 in^3 airgun was deployed behind the CCGS *Tully* in ~165 m of water. The airgun was suspended from the starboard stern, 2–15 m below the water surface. The airgun was operated intermittently, starting at ~11:40 and ending at 16:57 Pacific Standard Time (PST) on 15 July 2005. A series of 3–5 consecutive shots (spaced 5 s apart) was fired, typically followed by 10–30 min of no airgun activity (although some periods of no operations were longer). In total, approximately 47 shots were fired. During the entire study, the vessel was more or less stationary (using power to stay in place) at 49º10 N and 123º23 W. None of the activities were carried out near pinniped (in particular, harbour seal) haul-outs.

Visual Monitoring Methods

During the study conducted from the *Tully*, one observer trained in marine mammal identification and observation methods was on board. Visual watches took place throughout most of the study, but particularly when the airgun was firing. Visual observations were made from the *Tully*'s monkey's island, the highest suitable vantage point on the ship, where the observer's eye level was ~15.9 m above sea level (asl). The monkey's island afforded a view of ~320º centered on the front of the *Tully*, with partial obstructions to the stern.

Visual watches aboard the *Tully* were primarily conducted by the observer, but the ship crew also kept watch for marine mammals from the bridge (12.8 m asl) when the observer was unavailable (i.e., meal breaks, etc.). Observations were conducted from 09:00 to 17:30 PST. The observer scanned around the vessel, alternating between unaided eyes and 7×50 Fujinon binoculars. The binoculars were equipped with reticles on the ocular lens to measure depression angles relative to the horizon, an indicator of distance. Observations of marine mammals were made from 30 min prior to the start up of the airgun until 30 min after the airgun was shut down.

For each sighting, the following information was recorded: species, number of individuals seen, direction of movement relative to the vessel, vessel position and activity, sighting cue, behavior when first sighted, behavior after initial sighting, heading (relative to vessel), bearing (relative to vessel), distance, behavioral pace, species identification reliability, and environmental conditions.

Mitigation

The mitigation measure that was used during the study was to delay airgun operation (or cease airgun operation) when a marine mammal was sighted within a 300-m safety radius. This safety radius was in effect for all marine mammals (i.e., pinnipeds and cetaceans), even though the draft guidelines for seismic in Canadian waters do not call for the mitigation of effects on pinnipeds. A precautionary approach was taken in regard to pinnipeds due to agency (DFO) concerns about adverse effects on harbour seals that might be whelping nearby. The airgun could not be operated for 30 min after the sighting or until the animal was seen outside of the safety radius. A hand-held radio was used by the observer to communicate the presence of a marine mammal to the science party and bridge, and to initiate a shut down, if required.

Results

The environmental conditions during the study were variable, ranging from overcast to light drizzle, and Beaufort Wind Force of 1–3. A total of 8.5 h of observations were conducted for marine mammals. During that time, four sightings of individual harbor seals were made. The first seal was seen at 13:31, ~1 h 20 min after a series of airgun shots. The seal surfaced four times within the 300 m safety radius, over a period of 1 h, during which time the airgun operation was suspended. Airgun operations were delayed for a total of 1 h, until the seal had not been seen within the safety radius for 30 min. The seal was initially seen ~180 m from the *Tully* and did not show any overt reaction. Rather, it was seen swimming slowly at the surface, occasionally looking at the vessel, and diving several times.

The second sighting of a seal occurred at 14:50, ~3 min after a series of airgun shots were fired. The seal swam at a moderate pace, approximately 500 m from the *Tully*. It dove, and was seen again several minutes later, at a distance of 430 m. Three airgun shots were fired at 14:58, and the seal was not sighted again.

The third harbor seal was sighted at 15:17, ~3 min after a series of three shots were fired. The seal was initially at a distance of 310 m, swimming away from the *Tully* at a moderate pace. Three more airgun shots were fired at 15:24, but the seal was not resighted.

The fourth harbor seal was seen at 16:07, ~4 min after a series of 3 shots. The seal was seen swimming slowly across the bow at a distance of 250 m from vessel (the seal was located >300 m from the airgun at the stern of the vessel). Five shots were fired at 16:13, and the seal was not seen again.

Summary

Harbour seal was the only species observed during the marine mammal monitoring conducted during a 1-day study of the effects of a single 10 in^3 airgun on glass sponges in the Strait of Georgia. One harbour seal entered the 300-m safety radius, prompting an airgun shut down. Considering the relatively low noise levels expected to occur at the distances at which seals were observed, it is unlikely that any harbour seals were harmed by airgun sounds during the study.