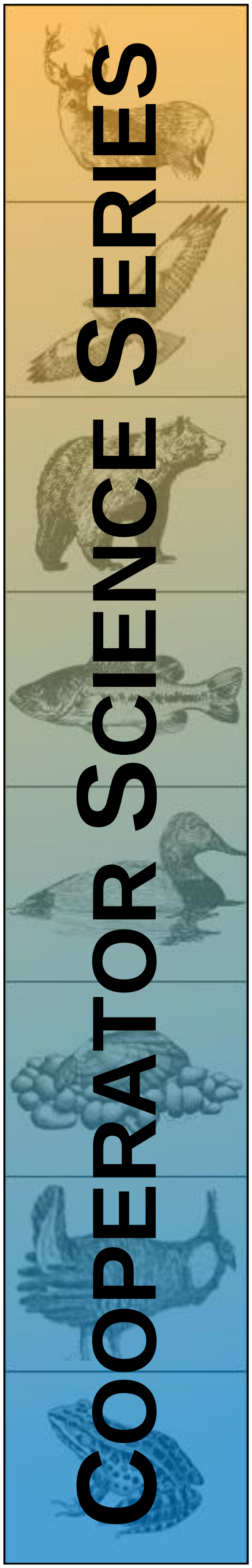

U.S. Fish and Wildlife Service

Assessing the feasibility of using acoustic monitoring for Burbot conservation, management, and production

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Final project report to the Kootenai Tribe of Idaho and the Bonneville Power Administration

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Abstract

Burbot *Lota lota* is the sole freshwater representative of the cod-like fishes and supports subsistence, commercial, and recreational fisheries worldwide above approximately 40° N. It is a difficult species to manage effectively due to its preference for deep-water habitats and spawning activity under the ice in winter. Like other gadiform fishes, Burbot use acoustic signaling as part of their mating system, and while the acoustic repertoire of the species has been characterized under artificial conditions (i.e., net pen suspended under ice in a natural lake), there has been no work to determine whether the species is as vocal in natural spawning aggregations. Our objective was to assess the feasibility of collecting and using acoustic data to characterize the spawning activity and locations of Burbot under field conditions. We recorded audio and video of Burbot spawning aggregations through holes drilled into the ice at known spawning grounds at Moyie Lake in British Columbia, Canada. Acoustic recordings (call counts and audiograms) were analyzed using Raven Pro v 1. 4 software. Acoustic behavior was also related to video data to determine how acoustic activity correlated to any observed spawning behavior. In general, wild Burbot spawning in Moyie Lake did not vocalize as frequently as counterparts spawning under artificial conditions. Further, Burbot vocalizations were not recorded in conjunction with spawning activity. While it may be feasible to use passive acoustic monitoring to locate Burbot spawning grounds and identify periods of activity, it does not seem to hold much promise for locating and quantifying spawning activity in real time.

Introduction

Burbot *Lota lota* is the sole freshwater representative of the gadoid fishes (order Gadiformes). It is an ecologically important species found worldwide at latitudes above approximately 40° N and supports important subsistence, commercial, or recreational fisheries in many locations. Burbot is relatively abundant throughout its range (Stapanian et al. 2010). However, some populations have been extirpated or suffered declines in abundance primarily due to habitat loss and alteration (McPhail and Paragamian 2000; Stapanian et al. 2010), shifts in temperature regime (Magnuson et al. 1990; Paragamian et al. 2000; Massol et al. 2007), and potentially overharvest (Hubert et al. 2008; Stapanian et al. 2010; Worthington et al. 2011). Burbot conservation, management, and recovery plans have been implemented in some jurisdictions and include harvest regulations and altered water releases to maintain thermal optima during Burbot migratory and reproductive periods (Stapanian et al. 2010).

Burbot are a difficult species to effectively manage due to its preference for deep-water habitats and spawning activity completed in the winter when most waterbodies in the Burbots' range are under ice-cover (Scott and Crossman 1973; McPhail and Paragamian 2000). This preference for spawning during winter creates a particular impediment to gauging the success of recovery actions, such as reintroduction efforts, or identifying habitat requiring special protection. However, assessing Burbot spawning using standard fisheries methods, such as capture surveys or visual monitoring, may be logistically difficult, ineffective or have undesirable effects (e.g., disturbing the spawning aggregation). Recently, Cott et al. (2014) described acoustic signaling by Burbot during their spawning period. Burbot produce a series of single and double biphasic calls at relatively low frequencies (> 1,000 Hz). The intervals between calls may decrease resulting in a "purring" or "humming" sound, similar to a revving motor (Cott et al. 2014). Burbot calls are similar to those reported for Haddock *Melanogrammus aeglefinus* (Hawkins and Rasmussen 1978; Hawkins and Amorin 2000; Bremner et al. 2002), and potentially serve the same functions during male-female and male-male interactions during the spawning season as in other gadoids (Bremner et al. 2002). This presents the possibility of using passive acoustic monitoring to evaluate Burbot spawning locations and their use. Further, this discovery could potentially relate acoustic activity to reproductive output. Passive acoustic monitoring has proven successful for mapping the location and timing of drum species spawning aggregations in estuaries along the Gulf and southeastern Atlantic coasts (Luczkovich and Sprague 2002; Lowerre-Barbieri et al. 2008; Walters et al. 2009). Furthermore, passive acoustic monitoring has been used to assess the timing of reproduction in other gadoid fishes, such as Atlantic Cod *Gadus morhua* (Nordeide and Kjellsby 1999) and Haddock (Casaretto et al. 2014).

While the acoustic repertoire of Burbot has been characterized (Cott et al. 2014), there has been no work to determine the function of individual call types or to understand the role of acoustic signaling in the Burbot mating system, particularly in wild spawning aggregations. In Haddock, acoustic signaling is related not only to courtship, but is also a component of male-male interactions (Bremner et al. 2002) and may be used outside of the spawning season (Hawkins and Rasmussen 1978). The development of a reliable protocol for using passive acoustic monitoring to locate Burbot spawning aggregations would provide a useful tool for non-invasively assessing the success of stocking and reintroduction efforts as well as surveying the spatial distribution and temporal use patterns of Burbot spawning habitat. Therefore, the purpose of this pilot study was to assess the feasibility of collecting and using acoustic data to characterize the spawning activity and locations of wild Burbot under field conditions. Secondarily, an attempt was made to create synchronized video and audio recordings of spawning Burbot to assess the role of acoustic signaling in their mating system.

Methods

Study area.—Moyie Lake is an approximately 850-ha lake of glacial origin located 20-km south of Cranbrook, British Columbia, Canada. The lake consists of two long, narrow basins connected by a 2.5-km river segment and drains into the Kootenay River by way of its outlet to the Moyie River (Figure 1). Burbot surveys were conducted along the southeastern shore of the north basin of Moyie Lake, approximately 2.5 km south of Cotton Creek. This location was selected because it was the only known Burbot spawning area in Moyie Lake where ice was thick enough to permit sampling activities in February 2015. The spawning ground was further divided for analysis purposes into a southern portion, characterized by a steep-sloped bottom and relatively deep water (5-10 m) with a retaining wall along the shoreline, and a northern portion consisting of a gentler slope and shallower water (2-5 m). Substrate in both locations was a mixture of small boulders, cobble, and gravel. At the time of sampling, ice thickness was 15-20 cm and water temperature was about 2.0°C. Sampling was conducted < 20 m from the shoreline.

Broodstock collection was being conducted jointly by the Kootenai Tribe of Idaho and the British Columbia Ministry of Forestry, Lands, and Natural Resource Operations during the acoustic surveys. These activities involved considerable amount of activity, such as biologists walking on the ice, drilling holes, and talking, which created noisy acoustic conditions on Moyie Lake. Range testing was conducted on Mineral Lake, a smaller (approx. 8 ha) lake located < 1.0 km west of the north basin of Moyie Lake (Figure 1) to evaluate the potential distance at which Burbot calls might be detectable. Ice cover was approximately 30 cm thick and water depth in the locations where range testing was conducted was approximately 18.3 m. The hydrophone was suspended approximately 6.1 m above the substrate. Water temperature at the time of testing was about 2.0°C.

Range testing in Mineral Lake. — The influence of distance on the detection of simulated Burbot calls and on their measured characteristics was assessed at Mineral Lake on 19 February 2015 (Figure 1). Burbot calls were simulated by tapping on a 355-mL plastic bottle wrapped in a woolen sock and submerged to a depth of approximately 0.75-1.00 m. We recorded simulated calls using a VLF-100 hydrophone (Vemco, Bedford, Nova Scotia, Canada) and DR-100 MK II 96k/24-bit portable stereo recorder (Tascam, Tokyo, Japan). A series of holes were drilled in the ice spaced 10, 25, 50, 75, and 100 m from the hole the hydrophone was deployed in (Figure 1). Simulated Burbot calls were produced in each hole by one researcher, who would tap on the plastic bottle, while the other researcher monitored the recording device.

Raven Pro v 1.5 software (Bioacoustics Research Program 2011) was used to visually and audibly identify simulated Burbot calls and to measure the characteristics of the simulated calls. We measured 14 variables describing the duration, amplitude, frequency, and power of the simulated calls (Table 1).

Audio and video recording of Burbot spawning activity.—Acoustic and video surveys of Burbot spawning activity were conducted in conjunction with annual broodstock collection efforts during 16-20 February 2015. Audio and video of Burbot spawning aggregations were recorded through holes drilled into the ice. Acoustic data were recorded using a VLF-100 hydrophone (Vemco, Bedford, Nova Scotia, Canada) and DR-100 MK II 96k/24-bit portable stereo recorder

(Tascam, Tokyo, Japan) Video data were recorded using an underwater camera attached to a portable digital video recorder.

Sampling was conducted along a series of holes drilled into the ice at varying distances from shore at known Burbot spawning areas in Moyie Lake. Acoustic and video recordings approximately 5-10 minutes in length were collected from each opening on a rotating basis. Additionally, a hydrophone and camera were deployed to the nearest hole in the ice whenever a group of Burbot was observed gathering into a potential spawning ball. Additional audio and video recordings were made at night from fixed stations established during daylight hours but monitored from shore after dark. The use of fixed stations monitored from shore was both a safety consideration and an attempt to minimize potential disturbances to the Burbot, such as the sound of walking on ice (Mann et al. 2009)

Raven Pro v 1.4 software (Bioacoustics Research Program 2011) was used to visually and audibly identify Burbot calls recorded in Moyie Lake and to measure their characteristics (Table 1). Recordings made by Cott et al. (2014) of known Burbot calls in Great Slave Lake, Northwest Territories, Canada, were processed in the same way and used as a reference to compare to the calls recorded in Moyie Lake. Acoustic behavior was related back to the video data collected to determine how acoustic activity correlates to any observed spawning behavior recorded.

*Data analysis.—*Discriminant function analysis was used to evaluate the characteristics between Burbot calls recorded in Moyie Lake, the simulated Burbot calls, and the reference Burbot calls recorded by Cott et al. (2014). A multivariate stepwise selection procedure (PROC STEPDISC) was used to identify the variables that possessed the greatest explanatory power. These variables were assessed for multicollinearity and parametric assumptions of normality and equality of variance; transformed as necessary using Box-Cox transformation series (Box and Cox 1964; Kutner et al. 2004). Once the transformations were applied, all variables were standardized to a standard deviation of one and a mean of zero. To prevent an arch effect (i.e., quadratic relationship amongst the first and second axes (Jongman et al. 1995)), correlation amongst all variables were assessed through Pearson's correlation analysis and any variables that were highly correlated ($r > 0.70$, $P < 0.01$; McGarigal et al. 2000) were considered for removal from the analysis to reduce multicollinearity (McGarigal et al. 2000). To decide which variables were to be eliminated, a Kruskal-Wallis test was conducted with each variable of a correlated pair as the dependent variable and the species abundance as the main effect (McGarigal et al. 2000). The variable with the greatest among-group variance (*F*-value) was ultimately kept for use in further analysis while the others were eliminated (Noon 1981). Multivariate analysis of variance (MANOVA) was used to assess whether the distance between the hydrophone and sound source influenced the parameters used to characterize the simulated Burbot calls.

All statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina) and a significance value of $P \leq 0.05$.

Results

Range testing.—A total of 219 simulated Burbot calls were identified from the 436 s of recordings made on Mineral Lake during the range testing experiment (Appendix A-1). There were no simulated calls detected at 100 m. Whilethe simulated calls detected at 0, 10, and 25 m were clear and distinct, those detected at 50 and 75 m were faint relative to the background noise, but could be discriminated visually in the spectrograph.

The characteristics of the simulated calls varied according to the distance between the hydrophone and the sound source (MANOVA: Wilk's $\lambda = 0.26$, $F_{13,205} = 45.67$, $P < 0.001$; Figure 2; Appendix A-1). As expected, the entropy, power, and amplitude of the simulated calls were inversely related to the distance between the hydrophone and sound source (Table 2). However, the duration of the simulated calls increased with increasing distance between the hydrophone and sound source (Table 2). This is likely an artefact of the simulated calls being less distinct and more difficult to separate from background noise with increasing distance.

The influence of distance between the hydrophone and sound source on the characteristics of the simulated Burbot calls is perhaps best illustrated by the relationship between distance, peak power, and the frequency at which peak power is reached (Figure 3). The higher frequency portions of a simulated Burbot call are rapidly lost with increasing distance making it appear that a great proportion of the energy of the call occurs at lower frequencies.

Characterization Burbot calls.—A total of 130 Burbot calls, consisting of the single and double biphasic pulses described by Cott et al. (2014; Figure 4), were identified from the 33 recordings (total time: 23,191 s) made during 16-20 February 2015 on Moyie Lake. However while there was significant background noise reported in Great Slave Lake during the recording of the reference calls (Martin and Cott 2015), there was a far greater amount of background noise present in the recordings made in this study relative to that of the single recording used from Cott et al. (2014). This background due to reduced and dynamic ice cover and the broodstock collection efforts noise is evident in the distorted waveforms of Burbot calls recorded from Moyie Lake and likely impaired the detection of Burbot calls when monitoring recordings in the field. Recordings containing Burbot calls were identified correctly as such in the field only twice. In contrast, there was a 66.7% false positive rate among the recordings identified in the field as containing Burbot calls and 21.2% of recordings contained Burbot calls that were not detected until the recordings were processed in the lab.

Many of the Burbot calls (n=52) recorded on Moyie Lake occurred as single, isolated pulses, with a mean (± SD) interval of 27.6 ± 93.7 s between them. However on twenty occasions, a series of 2-14 pulses were recorded within $\leq$ 2.0 s of each other. These extended calls accounted for the remaining 78 observed pulses and had a mean (± SD) pulse interval of 0.7 ± 0.5 s. Extended calls with decreasing pulse intervals leading to a rapid humming as described by Cott et al. (2014) were not observed.

Burbot calls recorded from Moyie Lake were qualitatively distinct from recordings of spawning Burbot made by Cott et al. (2014), hereafter referred to as reference calls, and from simulated Burbot calls recorded during range testing on Mineral Lake (Mahalanobis distance $\geq$ 4.95, $F_{6,489} \geq 79.96$, $P < 0.001$). Discriminant function analysis indicated that two canonical axes were necessary to separate the reference calls and simulated Burbot calls from those recorded on Moyie Lake, but the first axis accounted for 95% of the variability in the dataset (Table 3) and distinguished among the three categories primarily based upon the Q_{95} and maximum frequencies (Figure 5; Table 4). There were relatively few calls recorded on Moyie Lake that were incorrectly classified as reference calls (n=5) or simulated calls (n=11) by the discriminant functions and overall there was a low cross-validation error rate (12.34%).

The Burbot calls recorded during 08:00-12:00 were qualitatively similar to those recorded during 20:00-00:00, and both were distinct from those recorded during 12:00-20:00 (Figure 5a). The calls recorded during 08:00-12:00 and 20:00-00:00 exhibited lower Q_{95} frequencies, i.e., the frequency at which 95% of the energy of the call occurs above, than those recorded during other parts of the day. There was also evidence of differences in other parameters, such as duration, bandwidth, and peak frequency (Table A-2).

Discriminant function analysis supported the finding that the characteristics of the simulated calls varied by the distance between the sound source and the hydrophone (Figure 5b). Simulated calls tended to have a higher Q_{95} frequency than did the reference calls or the calls recorded in Moyie Lake. However, the simulated calls became more similar to the reference and Moyie Lake calls as the distance between the sound source and hydrophone increased (Figure 5b; Table A-2).

Spatial and temporal patterns of Burbot calls on Moyie Lake.—A greater proportion of Burbot calls were recorded from the northern portion of the Moyie Lake spawning grounds than from the southern portion (Figure 6). However, there was almost a three-fold difference in the length of recordings made in the northern portion of the spawning grounds. This discrepancy in effort is largely attributable to the southern portion of the spawning grounds not being safe to access after dark and receiving more disturbance from broodstock collection efforts for much of the study period.

There was a distinct increase in Burbot vocalizations recorded in Moyie Lake after sunset (Figure 7). While the highest proportion of Burbot calls were recorded during 20:00-00:00, a relatively large number of calls were also recorded during 16:00-20:00 particularly after factoring the relatively limited amount of recordings made during this period. Almost all of the calls recorded during 16:00-20:00 were recorded after sunset (approximately 18:00 during 16-20 February 2015). There were far fewer calls recorded during daylight hours, particularly during the four hours immediately after sunrise (approximately 07:45 during 16-20 February 2015).

In contrast, there did not seem to be any clear trend in distribution of Burbot calls recorded in Moyie Lake amongst the sampling days (Figure 8). However, it should be noted that the majority of the acoustic sampling on 16 February and 19 February was conducted after 16:00 while sampling on the other two days was primarily during 08:00-16:00.

Correlation of vocalizations to spawning behavior.—Large numbers (>20) of Burbot aggregated into a spawning ball were observed and recorded on video on three separate occasions during the morning of 20 February 2015 (Figure 9). These aggregations remained intact for 5-10 minutes and a synchronized audio recording was made each time. Even though the aggregations did not maintain a static position, the hydrophone was ≤ 10 m from the Burbot at all times. Only two Burbot calls were identified from the 3,006 s of audio recorded while observing a spawning ball. Further, there were an additional four occasions that at least a single Burbot was observed on the video monitor, usually passing through the observation area, while audio was being recorded. No Burbot calls were identified from the audio recordings made during these events.

Discussion

This study represents the second time that the use of acoustic signaling in Burbot has been reported and indicates that it is not an isolated occurrence. While the Burbot calls recorded in Moyie Lake are comparable to the single and double biphasic calls described by Cott et al. (2014), there were stark differences in acoustic repertoire and activity levels. Calls were largely isolated events in Moyie Lake, and only 130 pulses were recorded in 6.4 hrs of recording made across five days. In contrast, there was the same number of distinct pulses contained in the 54-s recording from Cott et al. (2014) used as a reference in this study. There are a number of potential explanations for this including thicker ice cover and lower levels of human activity on the ice above the spawning grounds. It is conceivable that the thicker ice cover reported by Cott et al. (2014), up to 110 cm, may have provided lower light levels than those experienced by Burbot in the present study and encouraged more acoustic signaling. There was also a higher level of human activity on the ice above the spawning grounds surveyed on Moyie Lake due to ongoing Burbot broodstock collection efforts (Stephenson and Evans 2015). The noise from this activity coupled with that from shifting and expanding ice likely influenced the ability to detect Burbot calls while monitoring the recordings in the field, and may account for some of the observed qualitative differences between the calls recorded in Moyie Lake and reference calls. Furthermore, the activity on the ice above the spawning Burbot may have depressed their acoustic signaling behavior either directly, through the disruption of removing individuals from the spawning grounds (Morgan et al. 1997; Dean et al. 2012), or indirectly, through increasing background noise that interfered with acoustic signaling (Finstad and Nordeide 2004; Mann et al. 2009). It is also possible that the present study was not conducted during a peak period of Burbot spawning in Moyie Lake. Cott et al. (2014) recorded the acoustic activity of a captive Burbot population continuously for 80 d and noted distinct peaks in activity coincident with spawning in wild Burbot in the same bay as the captive fish were being held. While reproductive Burbot were captured and ripe gametes successfully obtained from them (Stephenson and Evans 2015), there was not a particularly large number of Burbot spawning balls observed and overall Burbot densities on the spawning grounds seemed to be relatively low. The density of Burbot observed in Moyie Lake was not estimated, but was less than the 0.31 fish m^{-2} in the enclosure used by Cott et al. (2014) except during spawning ball formation when densities reached upwards of 30 Burbot m^{-2}.

The differences between Burbot calls recorded in Moyie Lake and reference calls recorded by Cott et al. (2014) could be attributable to several physical and biological factors. While the simulated calls were not a perfect proxy for Burbot calls, the range testing on Mineral Lake suggested that a maximum detection range of 50-75 m was likely for Burbot calls on Moyie Lake. In contrast, the Burbot observed by Cott et al. (2014) were confined in a 10 x 10 x 10 m enclosure suspended under ice and thus were physically unable to be further than 7.0-11.5 m (depending upon position in water column) from the hydrophone suspended 1.0 m from the bottom in the center of the enclosure. The potential variability between the fish producing the sound and the hydrophone can have a significant influence on the characteristics of the calls as demonstrated by the results of the range testing. Distance and difference in background noise could account for the observed separation between the Moyie Lake calls and the reference calls. However, there is another potential source of variability that has implications to the conservation, restoration, and management of Burbot populations. It is possible that Burbot populations differ in their acoustic repertoire and in the characteristics of the calls they produce. Similar differences have been observed in Atlantic Cod and have been proposed as a potential isolating mechanism between populations spawning in close proximity to one another (Rowe and Hutchings 2006), but this has not yet been thoroughly investigated under field conditions. Further evaluation of potential interpopulation variability in Burbot calls is warranted as it could be a factor to consider during the selection of source populations for recovery or restocking efforts, particularly if there is fine-scale population structure within a river basin or lake.

In other gadoids, such as Atlantic Cod and Haddock, spawning activity and acoustic signaling seem to be tightly linked and thought to primarily function to attract females to spawning aggregations and provide a basis for mate selection (Hawkins and Rasmussen 1978; Nordeide and Kjellsby 1999; Nordeide and Folstad 2000; Rowe et al. 2008; Rowe and Hutchings 2008; Burchard et al. 2014). However, the specific function of acoustic signaling in Burbot reproductive behavior is not clear. Cott et al. (2014) made no direct visual observations of the captive Burbot monitored in their study, so it is unclear whether the calls were made to establish dominance amongst males, attract receptive females, or signal to initiate or coordinate spawning activity, or were perturbed by prolonged captivity in a small enclosure with non-captive fish nearby. While the present study was successful in recording synchronized audio and video of Burbot spawning balls on three separate occasions, the almost complete absence of acoustic signaling during these events was unexpected. The presence of a diel pattern in acoustic signaling with the majority of activity occurring at night is consistent with what has previously reported for Burbot (Martin and Cott 2015). This nocturnal behavior is consistent with behavioral patterns observed in other gadoids, particularly Atlantic Cod (Brawn 1961; Hutchings et al. 1999; Nordeide and Kjellsby 1999; Rowe and Hutchings 2006) and Haddock (Burchard et al. 2014), as well as other taxa, most notably sciaenids (Holt et al. 1985; Lowerre-Barbieri et al. 2008; Walters et al. 2009). The results of the present study do not resolve the question of what is the role of acoustic signaling in Burbot reproduction, but do suggest that acoustic signaling is not a consistent component of Burbot behavior in Moyie Lake when actively engaged in spawning.

The lack of a specific link between acoustic signaling and spawning is one of several problems facing the development of using passive acoustic monitoring to quantitatively assess spawning behavior. Until the function of acoustic signaling in the mating system of Burbot is better understood, it will be impossible to draw meaningful conclusions regarding the potential population size, reproductive potential, or timing of spawning using passive acoustic monitoring. However, passive acoustic monitoring remains a potential tool for assessing Burbot spawning habitat use and occupation. The present study strongly suggests that using passive acoustic monitoring for real-time surveys of Burbot spawning habitat would not be cost-effective, as acoustic signaling may occur only infrequently or otherwise be highly variable over the course of a day, week, or spawning season. Further, environmental noise seems to have the potential to greatly reduce the detectability of Burbot calls during real-time monitoring. The majority of Burbot calls recorded in the present study were only identified when the capability to visualize the recordings was added during analysis in the lab. A potential solution to the issues of temporal variability and detectability would be to deploy automated recording devices in locations of interest. This would enable continuous monitoring over extended periods provided that issues with security and keeping the devices powered in remote locations could be resolved (Lobel 2002; Van Parijs et al 2009).

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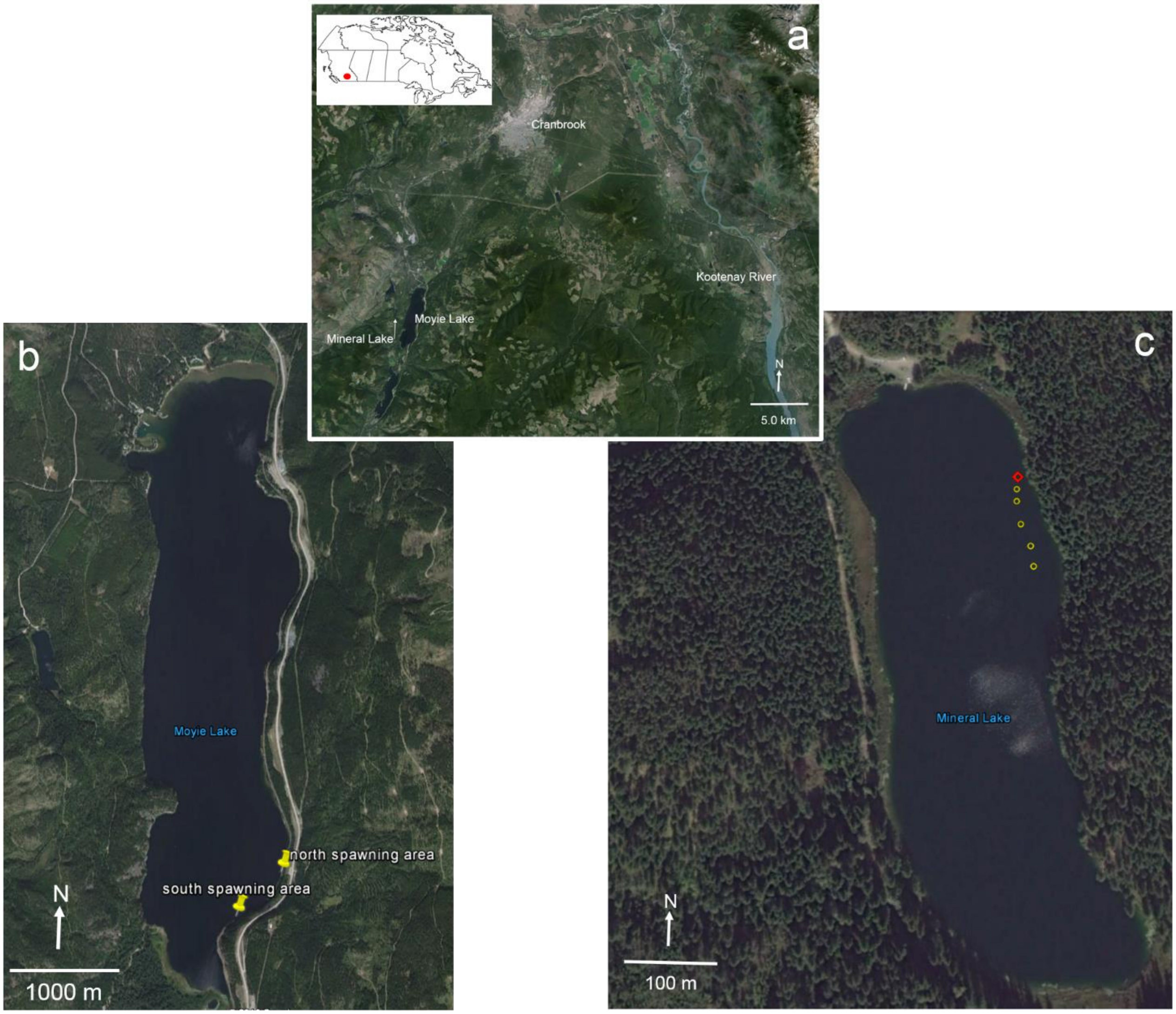

Figure 1. Location of the Moyie Lake and Mineral Lake study areas in British Columbia, Canada (a). Location of the north and south spawning areas surveyed in Moyie Lake during 16-20 February 2015 (b) and the location of the range testing experiment on Mineral Lake conducted on 19 February 2015 (c) are shown. In panel c, the hydrophone position is indicated by a red diamond while the locations of the holes drilled 10, 25, 50, 75, and 100 m for the production of simulated Burbot calls are indicated by yellow circles. Arial images courtesy of Province of British Columbia and Google Earth.

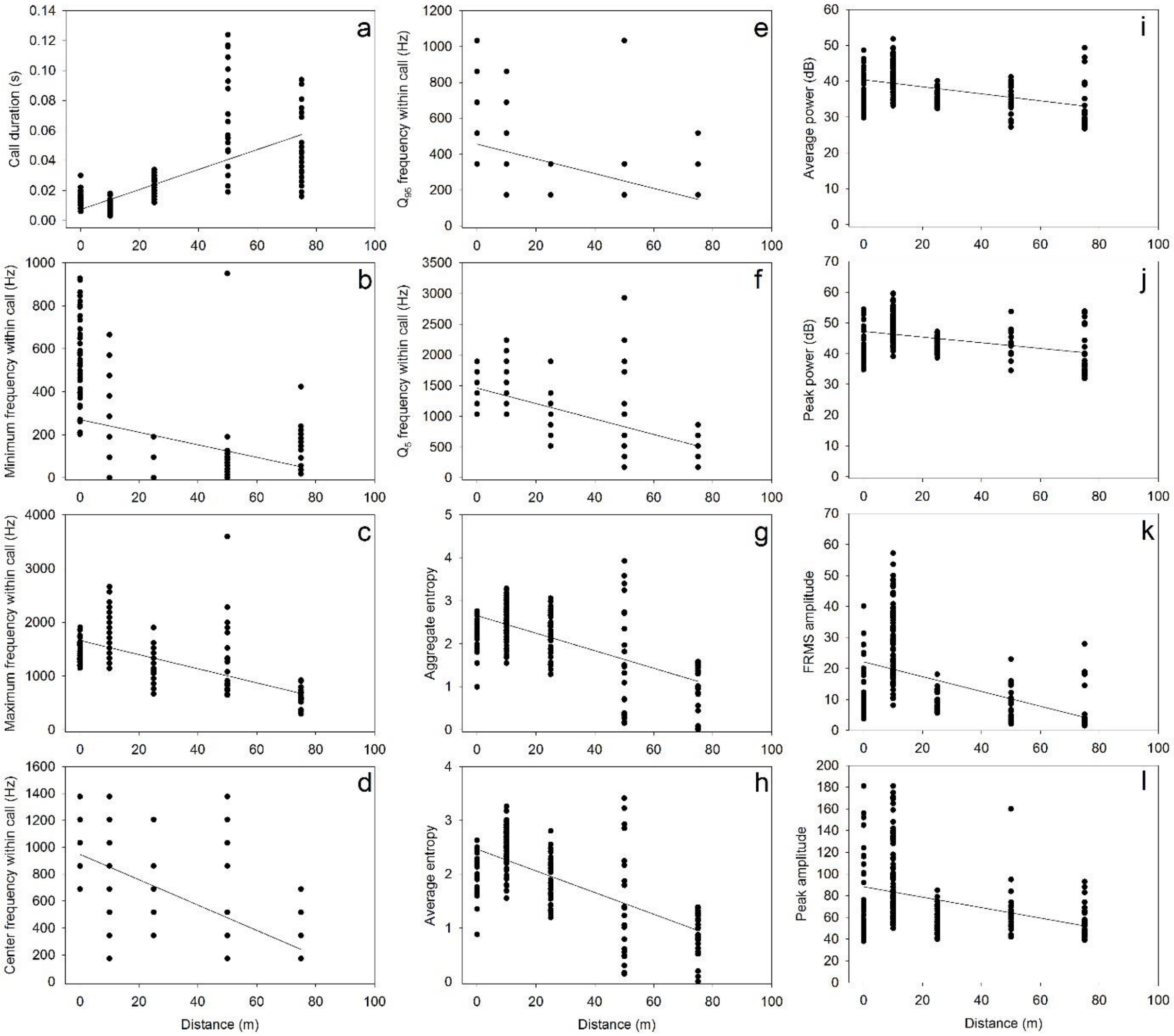

Figure 2. Duration (a); minimum (b), maximum (c) and center frequencies (d); the Q_{95} (e) and Q_5 frequency (f); aggregate (g) and average entropy (h); average (i) and peak power (j); and filtered root-mean-square (k) and peak amplitude (l) of simulated Burbot calls produced at varying distances from a hydrophone on Mineral Lake, British Columbia, Canada on 19 February 2015.

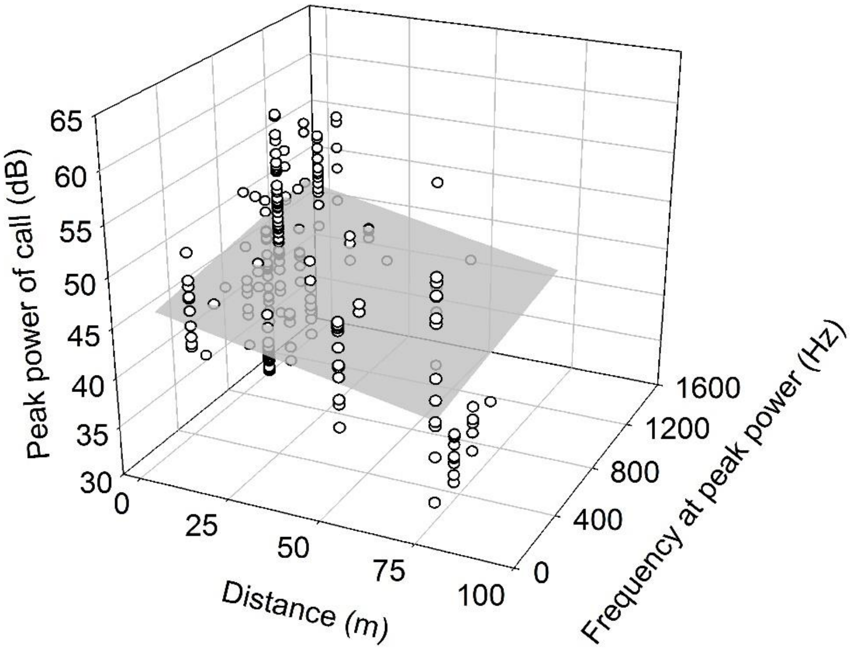

Figure 3. Effect of distance between the hydrophone and sound source on the relationship between the peak power and the frequency at which peak power occurred in simulated Burbot calls recorded in Mineral Lake, British Columbia, Canada on 19 February 2015.

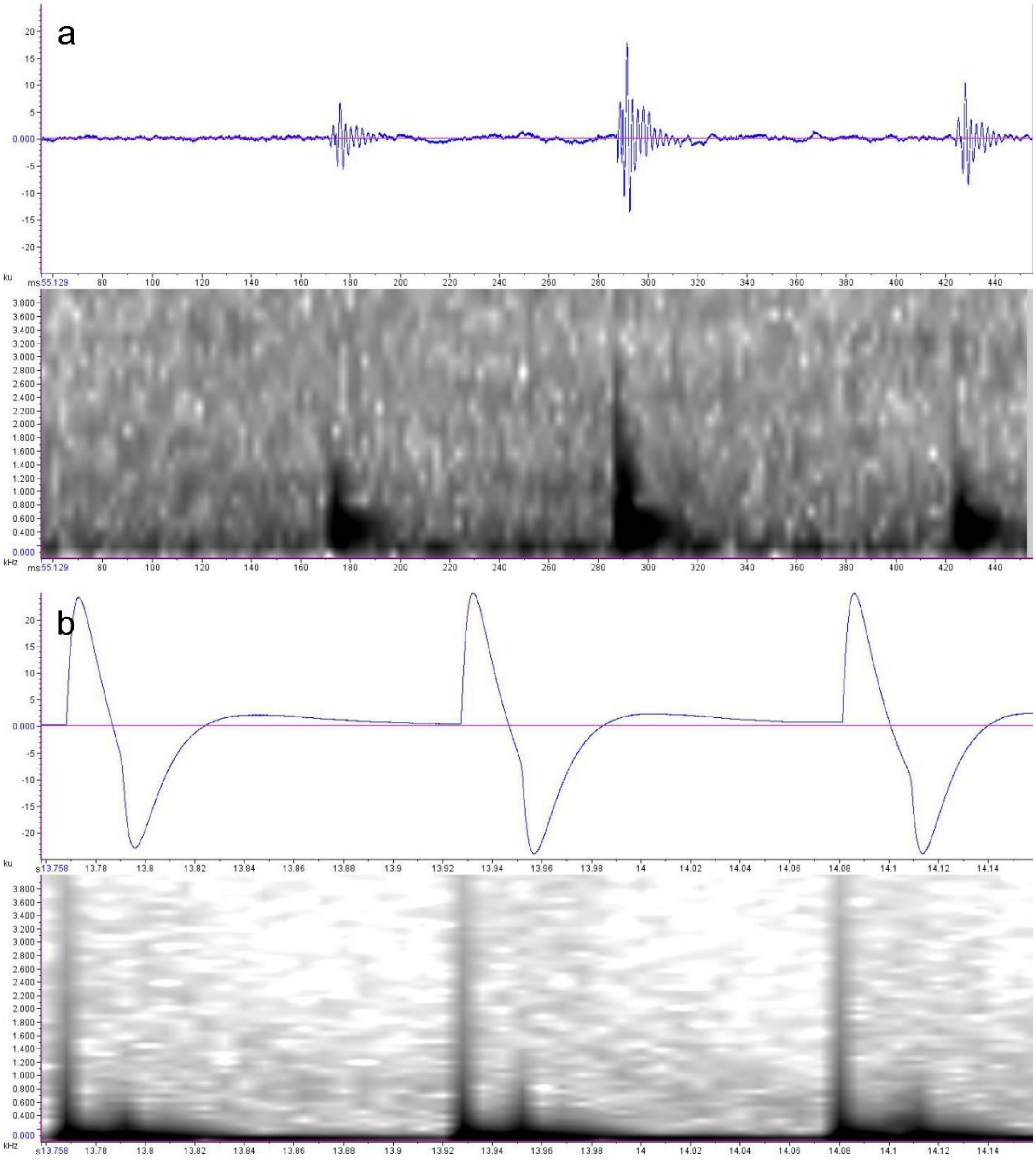

Figure 4. Representative waveform (top) and spectrogram (bottom) of Burbot calls recorded in Moyie Lake, British Columbia, Canada as part of the present study (a) and in Great Slave Lake, Northwest Territories, Canada (b) by Cott et al. (2014).

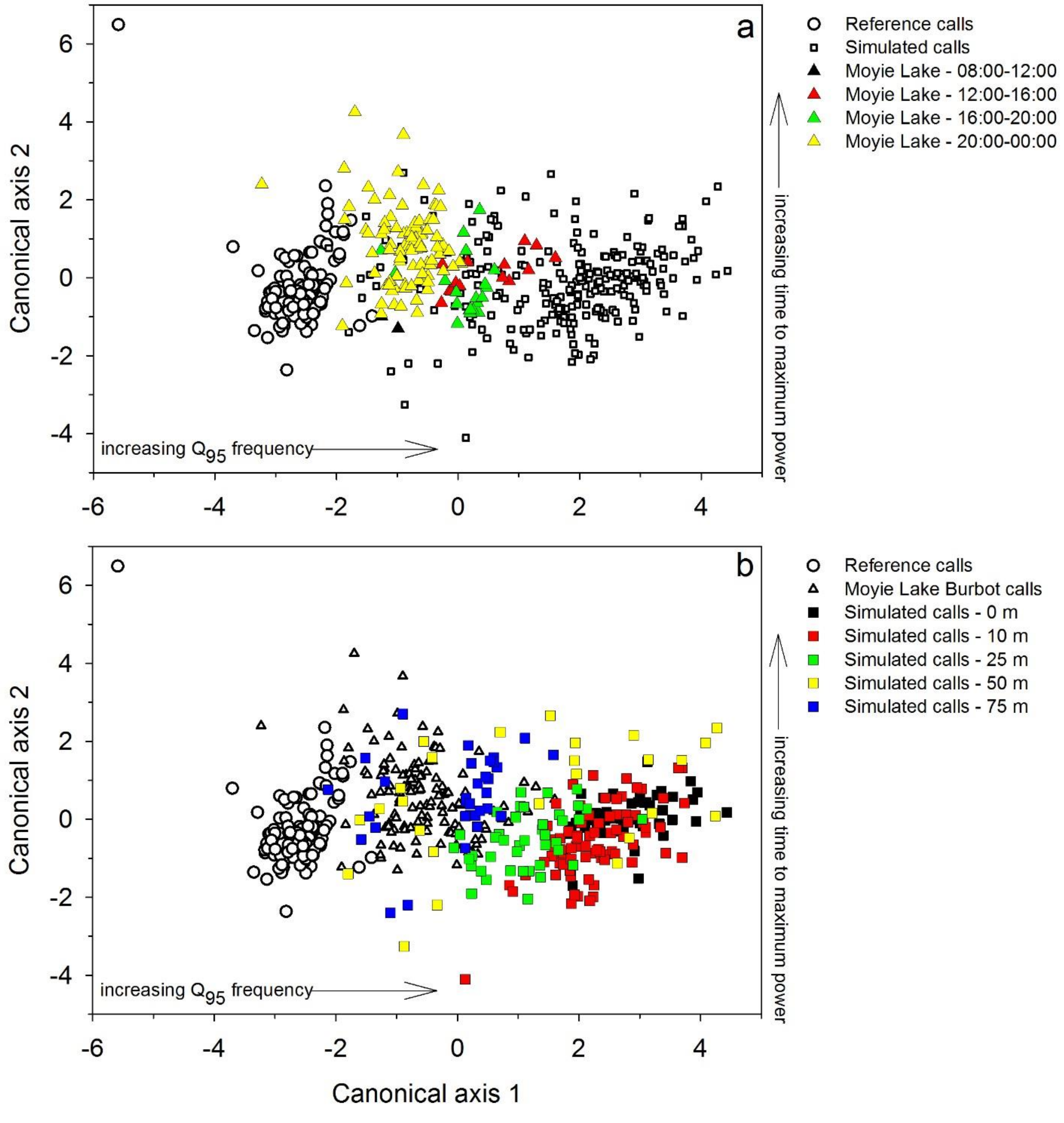

Figure 5. Discriminant function analysis ordination diagram of the two canonical axes separating Burbot calls recorded in Moyie Lake, British Columbia, Canada 16-20 February 2015, simulated Burbot calls, and reference Burbot calls recorded in Great Slave Lake, Northwest Territories, Canada by Cott et al. (2014). In panel a, Burbot calls recorded in Moyie Lake at different times of day are highlighted, showing a separation between calls recorded at night (20:00-00:00) and morning (08:00-12:00) from those recorded during the rest of the day. The effect of distance from the recording hydrophone on the characteristics of simulated Burbot calls is illustrated in panel b.

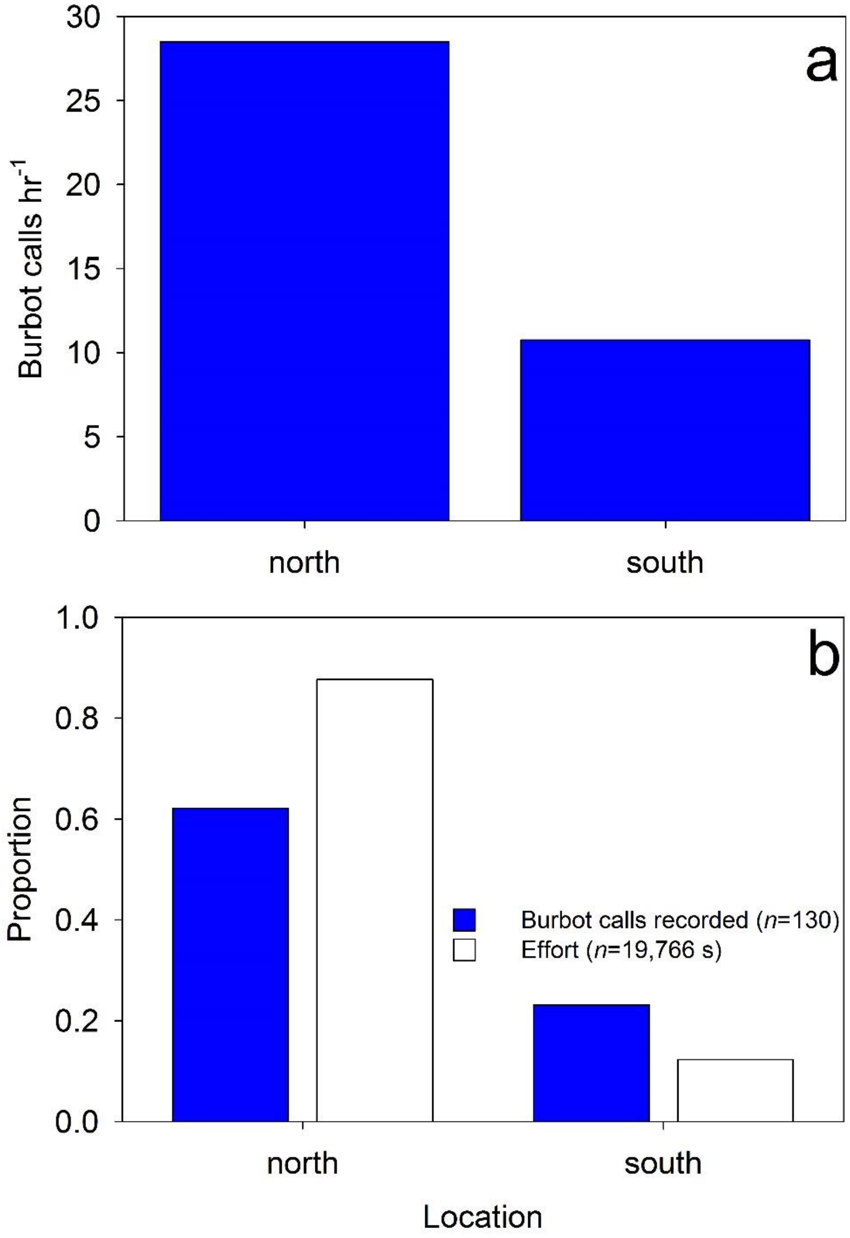

Figure 6. Spatial distribution of Burbot calls recorded from the northern portion and southern portion of the surveyed spawning grounds in Moyie Lake, British Columbia, Canada 16-20 February 2015 displayed as calls recorded per hour (a) and as proportion of total calls and recording time (b).

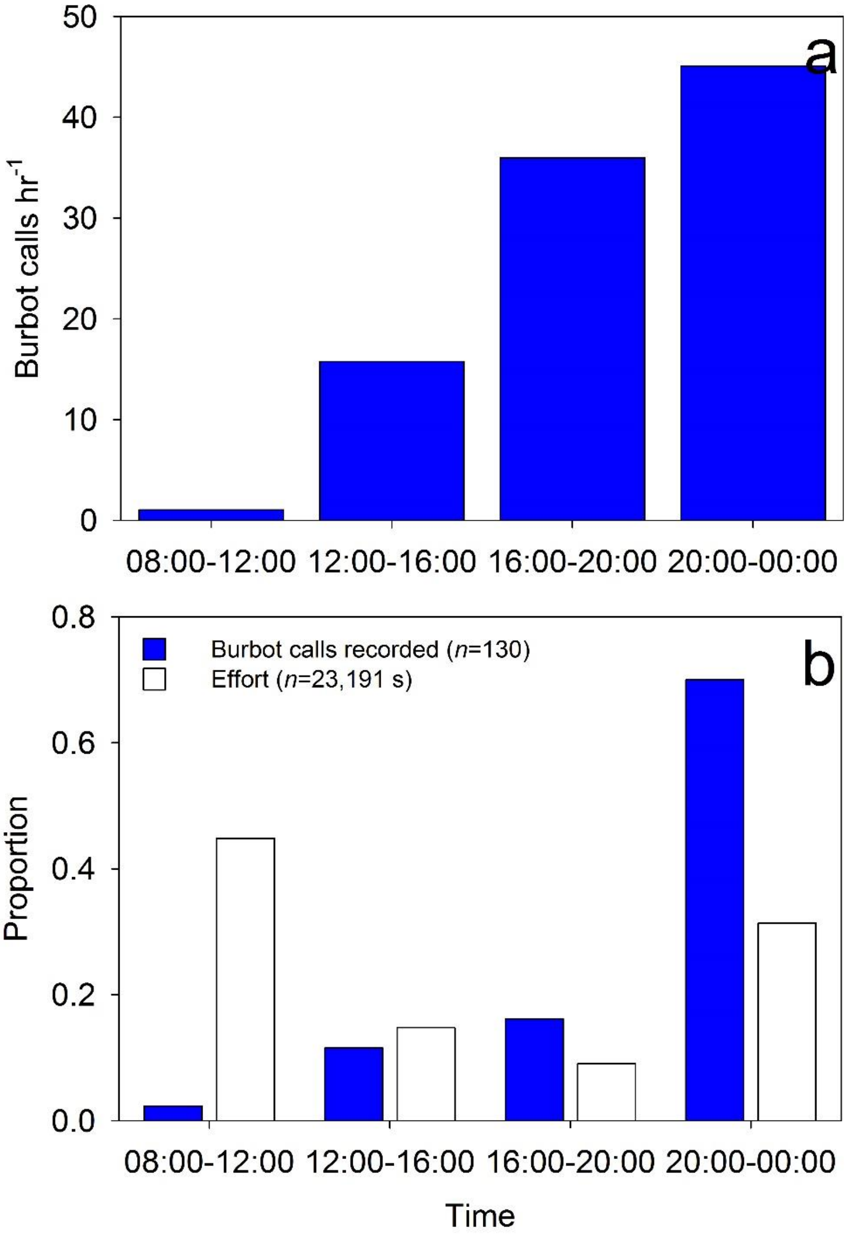

Figure 7. Temporal distribution of Burbot calls recorded from the surveyed spawning grounds in Moyie Lake, British Columbia, Canada 16-20 February 2015 displayed as calls recorded per hour (a) and as proportion of total calls and recording time (b).

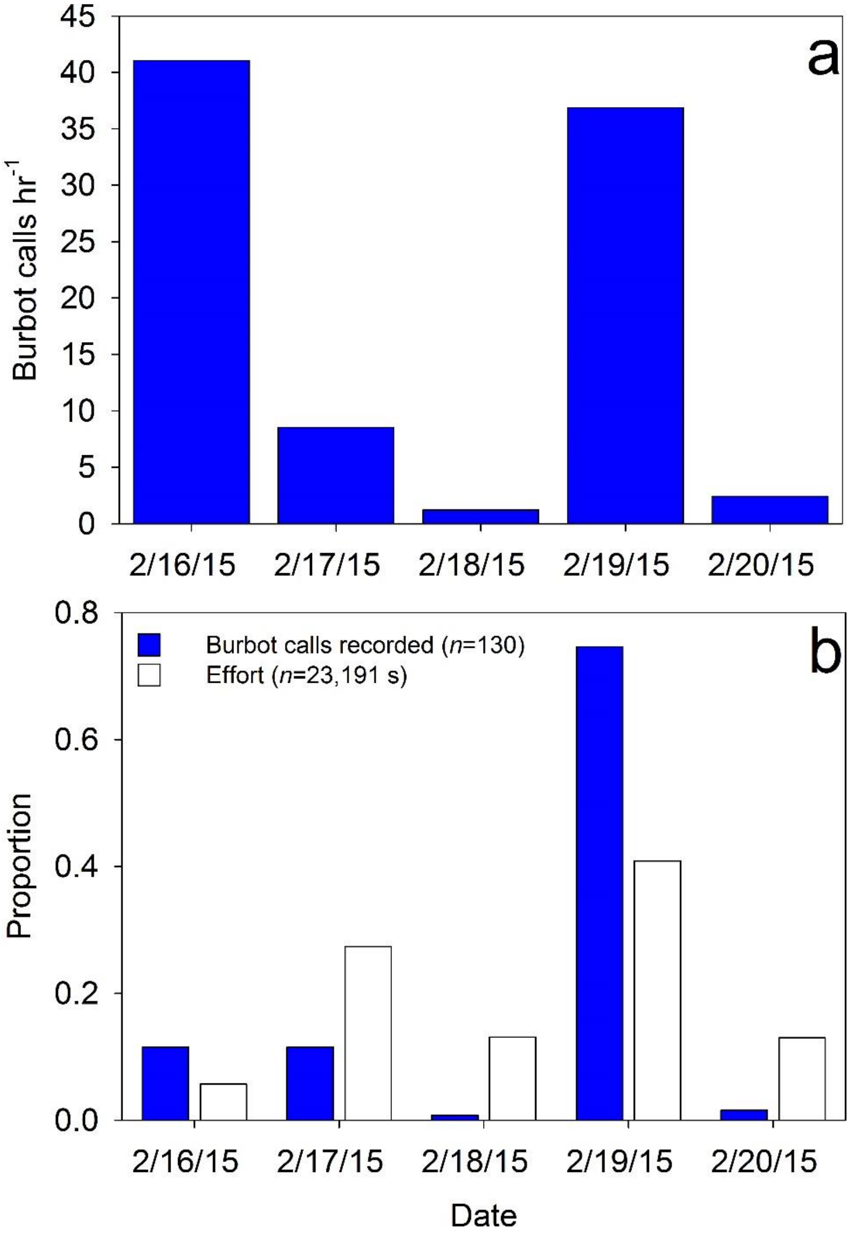

Figure 8. Distribution of Burbot calls recorded from the surveyed spawning grounds in Moyie Lake, British Columbia, Canada amongst the 16-20 February 2015 sampling dates displayed as calls recorded per hour (a) and as proportion of total calls and recording time (b).

Figure 9. Images of a Burbot aggregated into a spawning ball in Moyie Lake, British Columbia, Canada on 20 February 2015 at approximately 10:00. No Burbot calls were identified from the audio file recorded during this event.

Table 1. Description of variables measured in Raven Pro v. 1.4 and used to characterize Burbot calls and simulated Burbot calls recorded in Moyie Lake and Mineral Lake, British Columbia, Canada during 16-20 February 2015. Descriptions of the variables are summarized from those provided in the Raven Pro v. 1.4 software manual (Bioacoustics Research Program 2011).

Variable	Description	Units
Low frequency	Lowest frequency recorded within a call	Hz
High frequency	Highest frequency recorded within a call	Hz
Center frequency	Median frequency; frequency that divides the call into two intervals of equal energy	Hz
Q_5 frequency	Frequency at which the energy in the call reaches the 95th percentile, i.e., 5% of the energy of the call occurs in frequencies above this one	Hz
Q_{95} frequency	Frequency at which the energy in the call reaches the 5th percentile, i.e., 95% of the energy of the call occurs in frequencies above this one	Hz
Range	Difference between high and low frequency	Hz
BW90	Bandwidth 90%; i.e., difference between Q_5 and Q_{95} frequencies	Hz
Aggregate entropy	Measure of the distribution of energy across the frequencies within a call summed across the duration of the call; this is a measure of disorder within a sound with a pure tone having a value of 0	—
Average entropy	Measure of the distribution of energy across the frequencies within a call averaged across the duration of the call; this is a measure of disorder within a sound with a pure tone having a value of 0	—
Average power	Average of power spectral density across the duration of the call	dB
Peak power	Maximum power value recorded within a call	dB
Max time	Time taken within a call to reach peak power, standardized to proportion of call elapsed	—
FMRS amplitude	Filtered mean-root-square (FMRS) amplitude or effective amplitude	—
Peak amplitude	The greatest absolute value of amplitude	—

Table 2. Results of individual linear regressions performed within a multivariate analysis of variance evaluating the effect of distance between a source of simulated Burbot calls and hydrophone and the characteristics of the simulated calls as measured in Raven Pro v 1.4. Experiment was performed on Mineral Lake in British Columbia, Canada on 19 February 2015.

Variable	β_1 (± SE)	$F_{1,217}$	P	R^2
Duration	0.027 ± 0.002	171.50	<0.001	0.44
Low frequency	-0.013 ± 0.002	24.21	<0.001	0.10
High frequency	-0.026 ± 0.002	155.55	<0.001	0.42
Center frequency	-0.028 ± 0.002	190.56	<0.001	0.47
Q_5 frequency	-0.019 ± 0.002	56.43	<0.001	0.21
Q_{95} frequency	-0.028 ± 0.002	162.99	<0.001	0.43
Aggregate entropy	-0.026 ± 0.002	147.92	<0.001	0.41
Average entropy	-0.028 ± 0.002	184.38	<0.001	0.46
Average power	-0.017 ± 0.003	47.94	<0.001	0.18
Peak power	-0.016 ± 0.002	39.03	<0.001	0.15
FMRS amplitude	-0.018 ± 0.002	54.91	<0.001	0.20
Peak amplitude	-0.014 ± 0.002	29.43	<0.001	0.12

Table 3. Tests of canonical correspondence dimensions used to discriminate between Burbot calls recorded in Moyie Lake, British Columbia 16-20 February 2015, simulated Burbot calls recorded in Mineral Lake, British Columbia, Canada on 19 February 2015 and Burbot calls recorded in Great Slave Lake, Northwest Territories, Canada by Cott et al. (2014).

Canonical axis	**Canonical correlation**	**Eigenvalue**	**Proportion of variance**	***F***	**df1**	**df2**	***P***
1	0.83	2.15	0.95	85.24	10	982	< 0.001
2	0.31	0.11	0.05	13.43	4	492	< 0.001

Table 4. Standardized canonical discriminant function coefficients for variables used to discriminate between simulated Burbot calls recorded in Mineral Lake, British Columbia, Canada on 19 February 2015 and reference Burbot calls recorded by Cott et al. (2014).

Variable	Dimension 1	Dimension 2
Maximum frequency	0.88	-0.35
Q_{95} frequency	0.97	-0.14
Maximum Time	0.62	0.68
Low frequency	0.60	-0.50
Duration	-0.41	0.06

Appendix

Table A-1. Mean (± SE) characteristics related to duration, frequency, power, and amplitude of Burbot calls recorded during different time periods in Moyie Lake, British Columbia, Canada 16-20 February 2015 and reference Burbot calls recorded by Cott et al. (2014) in Great Slave Lake, Northwest Territories, Canada.

Variable	08:00-12:00	12:00-16:00	16:00-20:00	20:00-00:00	Reference calls
Count	3	15	21	91	130
Recording duration (s)	10,392	3,429	2,102	7,268	54
Duration (s)	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.16 ± 0.02	0.17 ± 0.03
Range (Hz)	962.57 ± 190.61	859.47 ± 84.77	1,183.70 ± 108.33	596.63 ± 20.35	3806 ± 111.26
Low frequency (Hz)	0 ± 0	17.72 ± 6.41	5.10 ± 2.21	1.15 ± 0.55	3.80 ± 2.00
Center frequency (Hz)	172.3 ± 0	275.63 ± 28.12	344.52 ± 26.579	172.3 ± 0	64.90 ± 1.43
High frequency (Hz)	962.57 ± 190.61	877.19 ± 84.50	1188.80 ± 108.51	597.78 ± 20.46	3809.81 ± 111.46
Q5 frequency (Hz)	172.3 ± 0	172.3 ± 0	172.3 ± 0	172.3 ± 0	64.90 ± 1.43
Q95 frequency (Hz)	344.5 ± 0	735.00 ± 84.84	623.46 ± 25.16	338.83 ± 6.84	97.12 ± 3.92
Peak frequency (Hz)	172.3 ± 0	218.22 ± 20.35	336.32 ± 27.82	174.19 ± 1.89	64.90 ± 1.43
Aggregate entropy (dB)	0.92 ± 0.17	1.62 ± 0.12	1.69 ± 0.08	0.78 ± 0.03	0.51 ± 0.01
Average entropy (dB)	0.79 ± 0.13	1.47 ± 0.10	1.53 ± 0.07	0.77 ± 0.02	0.48 ± 0.03
Average power (dB)	54.57 ± 4.25	59.81 ± 0.42	63.70 ± 1.79	59.03 ± 1.05	81.10 ± 0.38
Maximum power (dB)	68.67 ± 3.41	70.62 ± 0.59	76.90 ± 1.81	72.76 ± 1.24	105.72 ± 0.33
BW90	172.3 ± 0	562.74 ± 84.84	451.17 ± 25.15	166.61 ± 6.84	32.21 ± 3.07
F-MRS amplitude	154.7 ± 30.1	173.53 ± 5.94	403.06 ± 67.86	285.09 ± 37.48	8619.43 ± 174.95
Maximum amplitude	307 ± 132	681 ± 48.69	1743.33 ± 318.65	1,266.95 ± 186.65	2,3683.15 ± 440.62
Peak amplitude	625.5 ± 186.5	770.2 ± 65.35	1765.1 ± 315.62	1,378.62 ± 201.25	2,4078.31 ± 399.13

Table A-2. Mean (± SE) characteristics related to duration, frequency, power, and amplitude of simulated Burbot calls recorded in Mineral Lake, British Columbia, Canada on 19 February 2015 and reference Burbot calls recorded by Cott et al. (2014).

Variable	0 m	10 m	25 m	50 m	75 m	Reference calls
Count	40	87	41	22	29	130
Recording duration (s)	130	41	34	42	97	54
Duration (s)	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.07 ± 0.01	0.04 ± 0.00	0.17 ± 0.03
Range (Hz)	937.15 ± 34.86	1571.45 ± 37.81	1011.86 ± 49.13	1142.13 ± 152.26	439.92 ± 20.02	3806 ± 111.26
Low frequency (Hz)	550.79 ± 28.45	132.84 ± 17.48	88.08 ± 12.15	112.87 ± 41.31	151.96 ± 14.16	3.80 ± 2.00
Center frequency (Hz)	1021.58 ± 27.13	873.07 ± 25.94	567.22 ± 29.01	462 ± 91.65	308.89 ± 26.18	64.90 ± 1.43
High frequency (Hz)	1487.94 ± 36.50	1704.29 ± 33.30	1099.94 ± 44.89	1255 ± 151.08	591.88 ± 25.58	3809.81 ± 111.46
Q5 frequency (Hz)	669.04 ± 30.98	348.46 ± 19.68	252.1 ± 13.58	219.28 ± 39.56	237.62 ± 17.96	64.90 ± 1.43
Q95 frequency (Hz)	1378.14 ± 33.91	1430.99 ± 29.38	991.59 ± 45.73	970.97 ± 164.07	481.16 ± 30.08	97.12 ± 3.92
Peak frequency (Hz)	1013.57 ± 27.62	871.11 ± 33.22	600.83 ± 49.29	399.36 ± 85.11	314.83 ± 27.13	64.90 ± 1.43
Aggregate entropy (dB)	2.22 ± 0.06	2.67 ± 0.04	2.22 ± 0.08	1.57 ± 0.27	1.04 ± 0.1	0.51 ± 0.01
Average entropy (dB)	2.02 ± 0.05	2.54 ± 0.04	1.9 ± 0.06	1.43 ± 0.22	0.89 ± 0.08	0.48 ± 0.03
Average power (dB)	37.59 ± 0.94	42.36 ± 0.45	35.58 ± 0.29	35.22 ± 0.85	33.12 ± 1.23	81.10 ± 0.38
Maximum power (dB)	44.63 ± 0.98	49.07 ± 0.47	42.42 ± 0.36	44.65 ± 0.93	39.40 ± 1.31	105.72 ± 0.33
BW90	709.09 ± 30.45	1082.54 ± 32.70	739.48 ± 47.08	751.71 ± 158.09	243.54 ± 24.95	32.21 ± 3.07
F-MRS amplitude	16.31 ± 3.70	28.34 ± 1.28	9.18 ± 0.44	8.21 ± 1.15	5.18 ± 1.17	8619.43 ± 174.95
Maximum amplitude	84.51 ± 13.00	84.1 ± 4.17	49.32 ± 1.42	57.55 ± 5.92	47.76 ± 2.93	2,3683.15 ± 440.62
Peak amplitude	93.47 ± 13.30	99.26 ± 3.86	55.73 ± 1.71	67.23 ± 5.11	54.48 ± 2.74	2,4078.31 ± 399.13