

Life history and reproductive ecology of a  
mid-winter spawner: the Burbot (*Lota lota*)

by

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of the requirement for the degree of  
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## Abstract

The apparent lack of external differences between sexes, coupled with their unusual reproductive habits, make Burbot (*Lota lota* Linnaeus 1758) a unique model for which to investigate life history and reproductive ecology traits. Burbot, a top level predator, are the only freshwater representative of the cod family (Gadidae). Despite being one of the most widely distributed freshwater fish species in the world, occurring throughout boreal and tundra ecosystems of the Northern Hemisphere, very little is known about their ecology. Theory suggests that life history traits develop as adaptive strategies for an organism to cope with environmental conditions. Reproduction is a key life history component, and requires tradeoffs between current reproductive investment, and adult survival and future reproductive investment. The objectives of this study were to investigate the life history and reproductive traits of Burbot through within and among population investigations of sound production and reception capabilities, sexual dimorphism and the circannual reproductive cycle, and life history variation across various environmental gradients. This study discovered that Burbot produced swim bladder generated sound and the calls were coincident with the spawning period, and similar to those made by other codfishes. There was an increase in female swim bladder mass towards the spawning season implying that calling is more prevalent during this time. However, swim bladder mass was not significantly different between the sexes, suggesting that both sexes of Burbot vocalize. Females generally had larger livers than males, and liver size increased with latitude for both sexes. Gonad sizes were not significantly different between sexes, an unusual finding for boreal fishes. While there was no relationship found between gonad size and environmental conditions, egg size

increased with lake size. The enormous gonadal investment by male Burbot suggests that sperm competition may be an important selective pressure in this species. Large testes size in Burbot may have co-evolved with their group spawning behaviour. In general, Burbot appear to use a bet-hedging periodic spawning strategy similar to other gadoids. This life history strategy, likely a holdover from its marine ancestry and the stability of their under-ice spawning environment, has allowed Burbot to occupy an enormous pan-Arctic distribution in a plethora of habitat types with very little genetic and phenotypic variation throughout their range.

## **Acknowledgements**

### *Dedication*

To all of the burbot and other fishes that were harmed in the making of this thesis.

I've had a lot of help from various people with various aspects of my research, my thanks to those of you who I may have missed below.

I am indebted to the guidance, encouragement, and mentorship I received from my committee Tom Johnston (OMNR/Laurentian University), John Gunn (LU), Jim Reist (Department of Fisheries and Oceans), and Jeff Hutchings (Dalhousie University). I couldn't imagine a better group of scientists to learn from. Thanks also to my external examiners Sherrylynn Rowe (Memorial University), and Gillian Crozier (LU).

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It is very rare for me to remember or pinpoint inspiration that had large influences on the direction of my life; but I can recall two such events that relate to my PhD. In the spring of 2006 I was snow bound at a diamond exploration camp on the tundra while doing a research project with David Mann of the University of South Florida. We were talking about how cool burbot are (obviously), and David said “you know burbot being a gadoid might be able to make sounds...”. In the spring of 2007 I was at the University of Guelph finishing my MSc. and I had just received a good chunk of external research funds to study burbot. I asked the opinion of a fellow grad student in my department Nikolaus (Klaus) Gantner, who was just finishing his PhD, whether or not I should peruse a doctorate. Klaus simply told me “You can’t not do it”. Thanks guys!

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#### **BURBOT**

This species is exceedingly rare in Great Bear lake. We took only two specimens and one of these by the unorthodox method of stabbing it with a hunting knife. Burbot are said to be plentiful in some of the small lakes around Great Bear.

Pete Cott

Yellowknife, Northwest Territories

2013



Sydney Withers-Cott, Yellowknife Bay, Great Slave Lake, August 2012

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## CHAPTER 1 - General Introduction

In order for a species to survive and successfully reproduce, the individuals must possess traits which allow them to cope with their environment. The process of natural selection favours traits that give an organism a high probability of survival and successful reproduction in its environment through genetic adaptations passed on from its parents (Dawkins 1976). Natural selection is classically illustrated through Darwin's finches (*Geospiza* sp.), a group of otherwise similar-looking and co-occurring birds that have evolved a variety of beak morphologies to exploit different niches and food resources (Grant and Grant 2002). Environmental conditions, either natural or anthropogenic, can influence the direction, magnitude and speed of these natural selection processes, at times dramatically (Grant and Grant 2002, Olsen et al. 2004, Hutchings 2005). The life history patterns of a species develop as adaptive strategies to persist in prevailing environmental conditions (Roff 1984, Reznick et al. 1990, Winemiller and Rose 1992).

Phenotypic changes observed in life history and reproductive traits are the result of both genetic and environmental variation, and can have individual and population level consequences (Hutchings et al. 2012). Such consequences are difficult to predict because of the infinite combination of life history traits and confounding environmental conditions (Cole 1954). The relative influence of either genetic or environmental factors depends on the species in question and the environmental conditions in which it lives. However, the study of life history traits is of practical importance because they link to the overall production of species. For example, certain species of fish are more economically

valuable at certain age classes, and it is desirable to maximize yield by having increased size at those age classes (Cole 1954).

Environmental conditions can influence life history traits such as growth or age at maturity (Rahel 1984, Persson et al. 1991, Holmgren and Appleberg 2000, Post et al. 2000). For example, in general, as latitude increases, growing seasons become shorter and winters become longer (Holmgren and Appleberg 2000). Fecundity (Leggett and Carscadden 1978), and the duration of the spawning period (Gotelli and Pyron 1991, Conover 1992) may be reduced with latitude. Conversely, age at maturity (Gotelli and Pyron 1991), variation in egg and larval survival, year-class strength, and recruitment (Leggett and Carscadden 1978) can increase with increasing latitude. The relative influence of these environmental conditions on fish life histories can vary between freshwater and marine systems (Winemiller and Rose 1992)

A species must have the reproductive capacity to replenish its population in order to survive; making reproductive traits central to the life history of a species (Cole 1954). Key life history traits include brood size (fecundity), size of young, age schedules (such as sexual maturity), mortality, and the interactions of these traits (Cole 1954, Stearns 1976). From a life history perspective, it is of benefit to a species to reduce mortality during the reproductive part of its life as much as possible (Cole 1954).

Slow-growing taxa, such as sharks, often have lower population densities, higher age at maturity, and larger body size, than fast growing organisms like the teleost fishes (Cole

1954, Hutchings et al. 2012). There needs to be a balance; if too much energy goes towards reproduction, it diminishes the ability for the individual to survive in unpredictable environments or compete with other species (Cole 1954, Stearns 1976). As the costs associated with reproduction increase, size and age at maturity decrease, and vice versa (Hutchings 2005). An organism that limits reproduction when environmental conditions are poor, stands a better chance of survival and being able to reproduce when conditions improve (Stearns 1976).

Fecundity is one way to increase the reproductive potential of a species. Producing many offspring provides an array of heritable variations for environmental conditions to impose selective pressure for the most favourable traits (Cole 1954, Van Winkle et al. 1993). High fecundity is common in organisms where the probability of progeny survival to a reproductive age is low, such as for parasites and many marine organisms (Cole 1954). Life history theory dictates that where adult mortality exceeds juvenile mortality, organisms should reproduce only once (Stearns 1976). This strategy is known as semelparity, and has evolved in many species, such as annual plants, most insects, bacteria, and the Pacific salmon (*Oncorhynchus* spp.) (Cole 1954). An alternative to semelparity is iteroparity, where a species has the ability to spread its reproductive potential over the course of its life in several reproductive events (Cole 1954). Iteroparity is selected for where juvenile mortality exceeds adult mortality (Stearns 1976). For iteroparous species, later reproduction increases adult survival and therefore longer life and later maturation would be selected for (Hutchings 2005). In some cases, a species can either be semelparous or iteroparous, depending on which is more advantageous under the

constraints of a particular environment (Leggett and Carscadden 1978). Regardless of the reproductive strategy adopted, it should tend towards maximizing the number of young surviving to sexual maturity summed over the life of the parent (Stearns 1976).

Fecundity is a critically important factor in an organism's life history, but one of many traits that contribute to individual fitness and population growth rate, and in many fishes, higher fecundity is not related to maximum population growth rate or additional population resilience (Hutchings et al. 2012). Individual fitness requires trade-offs between growth and survival at a given age (Hutchings 2005). In such cases where early mortality is high, maximum fecundity is selected for, great enough to ensure that some of the progeny will reach reproductive age, but not so much that adult survival is compromised (Cole 1954).

Many animals develop features whose function is not to help individuals survive, but help them to maximize their reproductive success; therefore, there can be asymmetry in the reproductive investment between the sexes which can lead to a variety of other sex-based differences. External cues can be used to signal good genes (Hamilton and Zuk 1982, Andersson 1994). Such cues can take many forms such as ornaments, behavioural displays, or calls. As opposed to monomorphic species, sexually dimorphic species have physical characteristics which differ significantly between the sexes. Sexual selection is like natural selection but, rather than environmental pressures, it is governed by competition for mates (Andersson 1994). From a reproductive standpoint, it is in the best interest of females to select a mate of the highest possible quality and for males to mate

with as many females as possible (Dawkins 1976, Howard 1978, Shapiro and Giraldeau 1996). Sexually dimorphic features contribute to sexual selection through mate selection, and intrasexual competition, allowing the female (in most cases) to choose the 'best' mate, and they can also be used to indicate sexual receptiveness or readiness (Andersson 1994, Taborsky 1998). Some species appear monomorphic to the human eye and have been assumed to not be governed directly by sexual selection, but have been found to have subtle traits apparent to the species that allow for mate choice (Andersson et al. 1998). Some fish species, for example, use non-visual cues, such as acoustic signalling, for courtship and to signal reproductive readiness (Hawkins 1993, Kasumyan 2009).

Sexes may also differ in terms of their energy allocation for reproduction (Andersson 1994). Successful reproduction is central to the viability of a species, but is also energetically taxing on the individual (Andersson 1994, Wong and Jennions 2003). The capacity of an organism to store energy can have implications for its reproductive success (Burton et al. 1997, Skjæraasen et al. 2006a). Energy storage, usually in the form of lipids, can often differ greatly between sexes (Henderson et al. 1996, Henderson et al. 2003). Because energy supplies are frequently limiting in natural ecosystems expenditure trade-offs are then required. For example, there can be trade-offs between attractiveness and individual maintenance requirements (Hamilton and Zuk 1982, Eliason and Shawkey 2011), or gonadal growth and defence of a territory or mate (Taborsky 1998, Blanchfield and Ridgway 1999).

A species that exhibits low levels of morphological variation across its range (Chen 1969) and limited or no apparent sexual dimorphism (McPhail and Lindsey 1970b, Weber 1976, McPhail 2007) is the Burbot (*Lota lota* Linnaeus 1758). These qualities, along with its unusual mid-winter spawning behaviour, and the fact that they are lightly exploited, make Burbot an excellent candidate for the study of life history traits and sexual selection.

Burbot are one of about 500 species in the diverse Order Gadiformes, and the only freshwater representative of Gadidae, a 53 species strong family of cod-like fishes. The closest relatives of Burbot are the three species within the Lotinae subfamily; the Ling (*Molva molva*), the Blue Ling (*Molva dypterygia*), and the Cusk (*Brosme brosme*) (Scott and Scott 1988, Cohen et al. 1990).

The Burbot is an elongate cod-like fish. A single barbel below the chin is a key identification characteristic as are the reduced pelvic fins with a tendril-like second ray located anterior to the large pectoral fins. The colouration varies, but usually includes shades of yellow, olive, or brown overlaid with a darker pattern of irregular shaped spots, and the ventral surface is often lighter in colour and un-patterned (Figure 1). The average size of Burbot collected in this study was approximately 500 mm and 1 kg, although this varies considerably among populations. Specimens of over 1500 mm (Mecklenburg et al. 2002) and 30 kg (McPhail and Paragamian 2000) have been reported.

Unusual among freshwater fishes, Burbot spawn in the winter between November and May (Scott and Crossman, 1973; McPhail 1997), when water temperatures are 1-4° C



(McPhail and Paragamian 2000). Like other gadoid fish, Burbot are an iteroparous broadcast spawner (McPhail 1997, Stewart and Watkinson 2004). In North America, Burbot tend to spawn in shallow (1.0 to 10.0 m) (McPhail 2007) water over sand or gravel shoals of lakes (Nelson and Paetz 1992, McPhail and Paragamian 2000, Holm et al. 2010), or in low velocity side channels or deposition areas of rivers (McPhail 2007). An unusual aspect of their reproduction is that they form writhing ‘spawning balls’. These aggregations may consist of dozens of Burbot (Cahn 1936, McCrimmon 1959, McPhail 2007, Holm et al. 2010). Because of their under-ice spawning behaviour the actual act of spawning in the wild has yet to be documented in detail. It is not known what the sex composition of the spawning balls is, or if there is any form of mate selection performed. Observations of gamete release during spawning ball behaviour have not been documented in the scientific literature.

Along with Northern Pike (*Esox lucius*), Burbot has the most expansive distribution of any freshwater fish species in the world (Van Houdt et al. 2005). Their Holarctic range occupies much of the boreal and tundra ecosystems in the Northern Hemisphere (Scott and Crossman 1973, Cohen et al. 1990, McPhail 1997, McPhail and Paragamian 2000, Stapanian et al. 2010) (Figure 2). Despite its enormous range Burbot has only two recognized subspecies. *L. l. lota* occurs throughout Eastern Europe, Asia, Alaska, and north-western Canada. *L. l. maculosa* occurs throughout North America, south-east of Great Slave Lake, Northwest Territories (NWT) and north of approximately 40° (Elmer et al. 2008) and Western Europe (Van Houdt et al. 2003). Phenotypically, *L. l. lota* has a narrower caudal peduncle and larger pectoral fins than *L. l. maculosa* (Chen 1969).

Regardless of location or subspecies, Burbot are a cold-stenothermal piscivorous species, belonging to the cool/cold thermal guild (Reist et al. 2006) that live in lakes, rivers, and cold streams (McPhail and Lindsey 1970a, Scott and Crossman 1973, Stewart and Watkinson 2004), with clean, well oxygenated water (Chambers et al. 2000, Van Houdt et al. 2005). Although Burbot are often associated with deep water habitats, they actually have the broadest depth distribution (e.g., 1-350 m in Lake Superior; Sitar et al. 2008) among boreal fishes and are known to make extensive movements between the profundal and the littoral zones (McPhail and Paragamian 2000, McPhail 2007).

Based on genetic data and fossil evidence, the genus *Lota* is thought to have evolved into a freshwater form between 5 and 15 million years ago (Van Houdt et al. 2005). The earliest known proto-Burbot (*Lota hulai*) was found in Pliocene-aged deposits in central Europe, and had very few morphological differences from modern Burbot (Van Houdt et al. 2003). Van Houdt et al. (2003) remarked on the lack of obvious morphological differentiation in Burbot across its circumpolar range, despite its split from the other gadoids during the Early Pliocene. The lack of morphological differentiation or ecological diversification may be a function of its life history.

A key to the universal success of Burbot may lie with its swim bladder. In post-glacial North America it is thought that two crustaceans, *Mysis* and its copepod prey (*Limnocalanus macrurus*), colonized post-Pleistocene North American lakes (Väinölä et al. 1994, Dooh et al. 2006), and provided a deepwater, lipid-rich food source for fishes

advancing on the front of retreating glaciers (Wilson and Hebert 1998, Wilson and Mandrak 2004, Dooh et al. 2006, Porter et al. 2007). The zooplanktivorous Ciscoes (*Coregonus* spp.) radiated into, and dominated (in terms of biomass) these deepwater pelagic habitats. These Ciscoes were followed by their primary predators, the Lake Trout (*Salvelinus namaycush*) and Burbot (Eshenroder et al. 1999). A problem that these fishes had to contend with in order to exploit deep water habitats was pressure.

Two strategies have evolved to aid in buoyancy compensation—hydrodynamic and hydrostatic lift (Eshenroder et al. 1999). Hydrodynamic lift is generated by swimming, where large fins act as hydrofoils and thrust is generated by the tail (Eshenroder et al. 1999). Hydrostatic lift is generated by incorporating low density compounds, such as lipids, into the body tissues and organs, or by reducing heavy tissue, thereby reducing the specific gravity of the fish and increasing buoyancy (Alexander 1972). Swim bladders are one of the most commonly employed strategies for maintaining neutral buoyancy among teleost fishes (Alexander 1993). On the basis of their swim bladder gas exchange system, teleost fishes can be divided into two groups; physostomous and physoclistous.

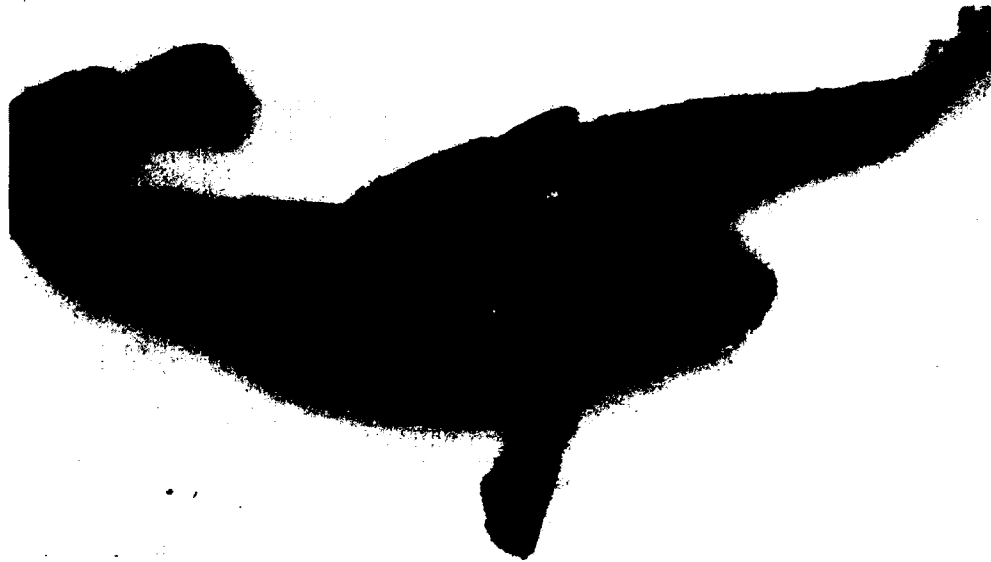
Physostomous swim bladders, such as those in salmonids, have a duct connecting the bladder to the digestive system, that permits the exchange of atmospheric air (Graham and Edelhauser 1968, Ingermann 1982) (Figure 3a). In contrast, physoclistous fishes, like the gadoids, do not possess an external connection to the swim bladder and rely on gas extracted from the circulatory system using a *rete mirabile* or gas gland (Figure 3b).

Negative buoyancy is advantageous for demersal species, such as the sculpins (Eshenroder et al. 1999) but restricts available habitat to the benthic zone, and prevents depth transitioning without expensive energy costs (Phleger 1998). Many epi-benthic fishes that rest and feed on the bottom have reduced swim bladders or lack a swim bladder altogether (Phleger 1998). Burbot spend much of the day on the bottom, often remaining motionless (Boyer et al. 1989, Edsall et al. 1993), yet unlike many benthic fish they have a well developed physoclistous swim bladder (Hawkins 1993). The question is why do they need such a well developed swim bladder?

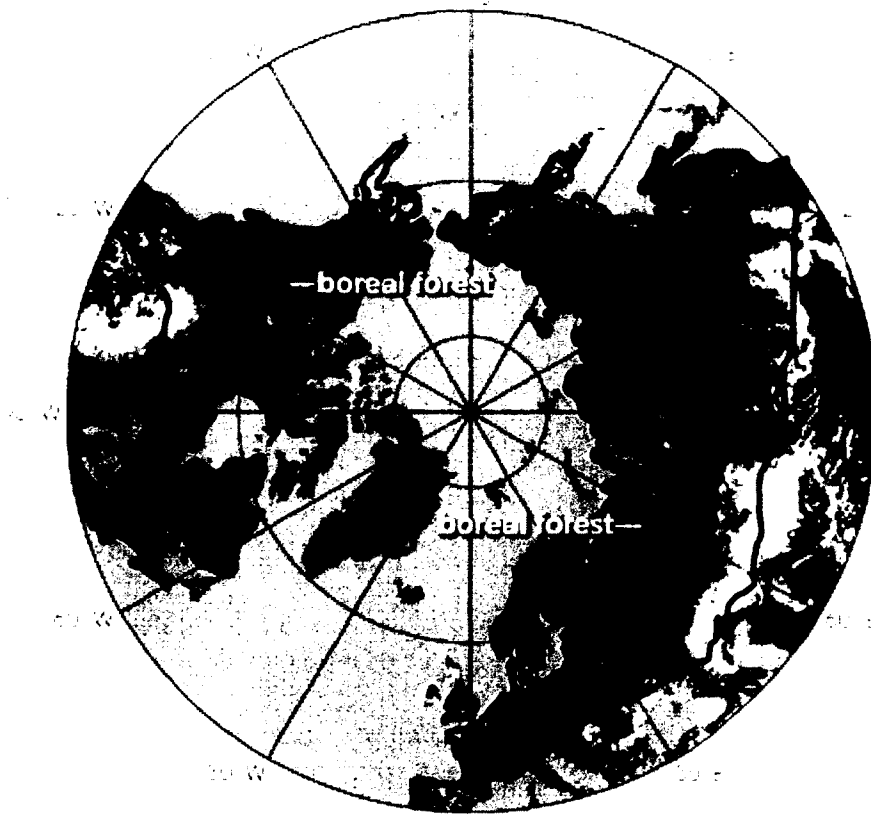
Some fishes produce sounds by drumming or contracting striated muscles that are found in association with the swim bladder (MacNeill and Brandt 1990, Connaughton et al. 2002, Mehner et al. 2007, Helland et al. 2009), producing sounds like knocks, hums, buzzes, or grunts (Hawkins 1993, Hawkins and Amorin 2000, Rowe and Hutchings 2006, Bass et al. 2008). Such sound producing abilities are often used by deep-dwelling species as a means to attract mates (Haedrich 1996). Perhaps sound production has evolved in some fishes spawning in light-limited environments as a non-visual adaptation for communication.

Burbot have an enormous range and occupy a variety of habitat types, but despite this, show little phenotypic variation. Also, Burbot do not appear to be sexually dimorphic, with no reported sex-based differences in morphology or behaviour. In this thesis, concepts of life history theory and sexual selection are investigated, using the Burbot as a model, a species that seems to show little apparent life history or reproductive variation.

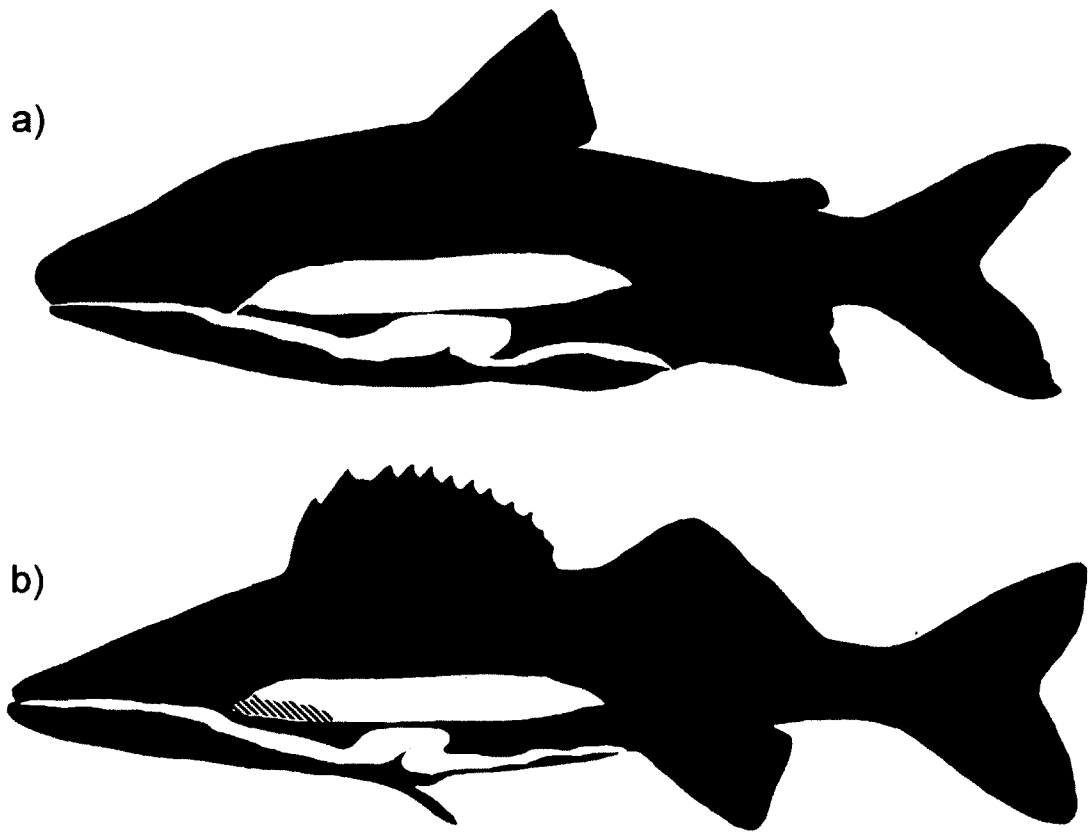
To better understand the mating system of an apparently monomorphic species, the potential for Burbot to exhibit sexual dimorphism is studied within a population (Chapter 2), as well as across populations (Chapter 4). The potential for Burbot to exhibit non-visual communication as an avenue for mate choice is investigated through experiments to assess their sound production and reception (Chapter 3). Trends in life history and reproductive traits of Burbot are examined along with its circannual cycle of gonadal development and energy allocation within a population (Chapter 2) and among populations, over the environmental gradients of latitude, productivity, and lake size (Chapter 4).



**Figure 1. Burbot from the Northwest Territories showing variability of colours and patterns. Top: Liard River Burbot with Mike Bryant, note the chin barbel and tendril-like pelvic fins, photo C. Bryant. Bottom: Chitty Lake Burbot used in this study.**



**Figure 2. The Holarctic distribution of Burbot (modified from Scott and Crossman 1973, McPhail and Paragamian 2000; Van Houdt et al. 2003; base map from [www.borealforest.org](http://www.borealforest.org)). Range of the two Burbot subspecies are delineated by the dashed line; *Llm* = *Lota lota maculosa*, *Lll* = *L. l. lota*, from Van Houdt et al. 2003. Note: Burbot are currently extirpated from the UK (Worthington et al. 2010).**



**Figure 3 a). A typical physostomous body plan in the Lake Whitefish (*Coregonus clupeaformis*). Note the connection (pneumatic duct) between the oesophagus and the swim bladder. b) A typical physoclistous body plan in the Walleye (*Sander vitreus*). Note that there is no connection between the swim bladder and the external environment, and there is a gas gland (red hashed area). Fish silhouettes courtesy of Doug Watkinson (Stewart and Watkinson, 2004).**



## **CHAPTER 2 - Sexual dimorphism and circannual reproductive development of Burbot (*Lota lota*): a mid-winter spawner**

### **Introduction**

Divergence in reproductive investment between the sexes can lead to a variety of other sex-based differences. Sexual dimorphism - physical characteristics that differ between the sexes - can arise from natural and/or sexual selection (Lande 1980), or environmental conditions (Shine 1989, 1991, Dunn et al. 2001). Sexually dimorphic features can signal the onset of reproduction, and can be used in mate selection, and intrasexual competition (Taborsky 1998). Females usually have the most invested in reproduction in terms of energy, and therefore are often the most discriminatory in mate selection (Andersson 1994). Females often prefer bigger males as they demonstrate fitness (Howard 1978). Larger males are often older and more experienced and are usually more successful in mating than smaller males (Howard 1978). It is in the reproductive best interest of the female to select a mate of the highest possible quality, whereas it is in the male's best interest to mate with as many females as possible (Dawkins 1976, Howard 1978, Shapiro and Giraldeau 1996). Consequently, sexes can differ in terms of behaviour, and energy allocation (Andersson 1994, Taborsky 1998).

Although externally-fertilizing taxa often lack any obvious sexual dimorphism (Levitan 2005), some display a wide variety of sexually dimorphic characteristics. Many fish approach the spawning period with a prominent breeding display, such as tubercles, bright colouration, or gross morphological features (e.g., humped back and hooked jaw of

Pacific salmon) to both indicate sex and signal reproductive readiness (Scott and Crossman 1973, Taborsky 1998). Sex-based differences may also be behavioural, such as the higher dispersal rates of male Brook Trout (*Salvelinus fontinalis*) (Hutchings and Gerber 2002), or male-biased spawning vocalizations of Atlantic Cod (Rowe and Hutchings 2006).

Reproduction is energetically expensive and trade-offs are required (Cole 1954, Stearns 1976, Wong and Jennions 2003, Hutchings 2005). Such trade-offs can be different between the sexes (Henderson et al. 2003) and can also result in sexual dimorphism (Schulte-Hostedde et al. 2001, Casselman and Schulte-Hostedde 2004). For example, there can be trade-offs between reproductive success in the current year with the potential to survive and reproduce in future years, between reproduction at an early age and long-term adult survival (Hutchings 1993, 2005), between attractiveness and individual maintenance requirements (Eliason and Shawkey 2011), between somatic growth and gonadal growth (Henderson et al. 2003), and between gonadal growth and defence of a territory or mate (Taborsky 1998). For some iteroparous fishes, lipids are the primary body constituent used to fuel the energetic demands of reproduction (Casselman and Schulte-Hostedde 2004). How energy is stored can have implications for the reproductive success of an individual (Burton et al. 1997, Skjæraasen et al. 2006a), and can vary between sexes (Henderson et al. 1996, Henderson et al. 2003). Liver lipids have been shown to be important for the reproductive success of lean fish such as the codfishes (Marshall et al. 1999, Skjæraasen et al. 2006a).

Although many species of boreal fishes have obvious primary sexual characteristics, such as the spawning colours and hooked jaw of male Brook Trout (Scott and Crossman 1973), in other fish species sexually dimorphic characteristics can be subtle or even cryptic to human eyes (Andersson 1994, Andersson et al. 1998). In these fishes, what mechanisms exist to allow for distinguishing between sexes or selecting mates? One such species that is not apparently dimorphic is the Burbot, the only freshwater representative of the cod family (Gadidae) (Scott and Crossman 1973, Cohen et al. 1990). Along with Northern Pike, Burbot are the most widely distributed of any freshwater fish species in the world (Van Houdt et al. 2005), living in cool/cold lakes, rivers, and streams throughout the boreal and tundra ecosystems in the Northern Hemisphere (Scott and Crossman 1973, Cohen et al. 1990, McPhail and Paragamian 2000, Stapanian et al. 2010). Like other gadoid fish, they are a broadcast spawner (McPhail 1997, Stewart and Watkinson 2004), and show no obvious external differences between the sexes (McPhail and Lindsey 1970b, Weber 1976, McPhail 2007). The reproductive ecology of this species has not been well studied (McPhail and Paragamian 2000) but the limited information available suggests it is quite distinct from other boreal freshwater fishes. First, Burbot usually spawn in mid-winter under ice, and usually at night (Scott and Crossman, 1973; McPhail 1997), whereas other boreal fishes spawn primarily in the spring after ice-out, as is the case with the esocids, during the early summer like the cyprinids, or in the fall before freeze-up as in most salmonids (Scott and Crossman 1973, Nelson and Paetz 1992, Stewart and Watkinson 2004, McPhail 2007, Holm et al. 2010). Second, some researchers have reported that spawning may consist of aggregations of a dozen (Cahn 1936, Holm et al. 2010) to upwards of 100 individuals in a writhing spawning ball (McPhail 2007).

Other species may congregate for spawning migrations, or stage together at spawning sites, such as the spawning runs of adfluvial Cisco (*Coregonus artedii*) (Blackie et al. 2012). Given that spawning of Burbot occurs under dark conditions (usually at night and under ice-cover), and that many individuals may be involved in a spawning group, it is possible that tactile, chemosensory, or even auditory cues (see Chapter 3 of this thesis) may be relatively more important than visual cues in the mate selection process. This could be associated with much more subtle differences between the sexes.

The apparent lack of external differences between sexes coupled with their unusual reproductive habits make Burbot a unique model in which to investigate the possibility of mate selection through subtle sexual dimorphic attributes. Also, investigating the reproductive ecology of Burbot will add to the growing body of information on the mating systems of gadoid fish in general, a group of fishes of tremendous economic importance (Cohen et al. 1990, Hutchings et al. 2012).

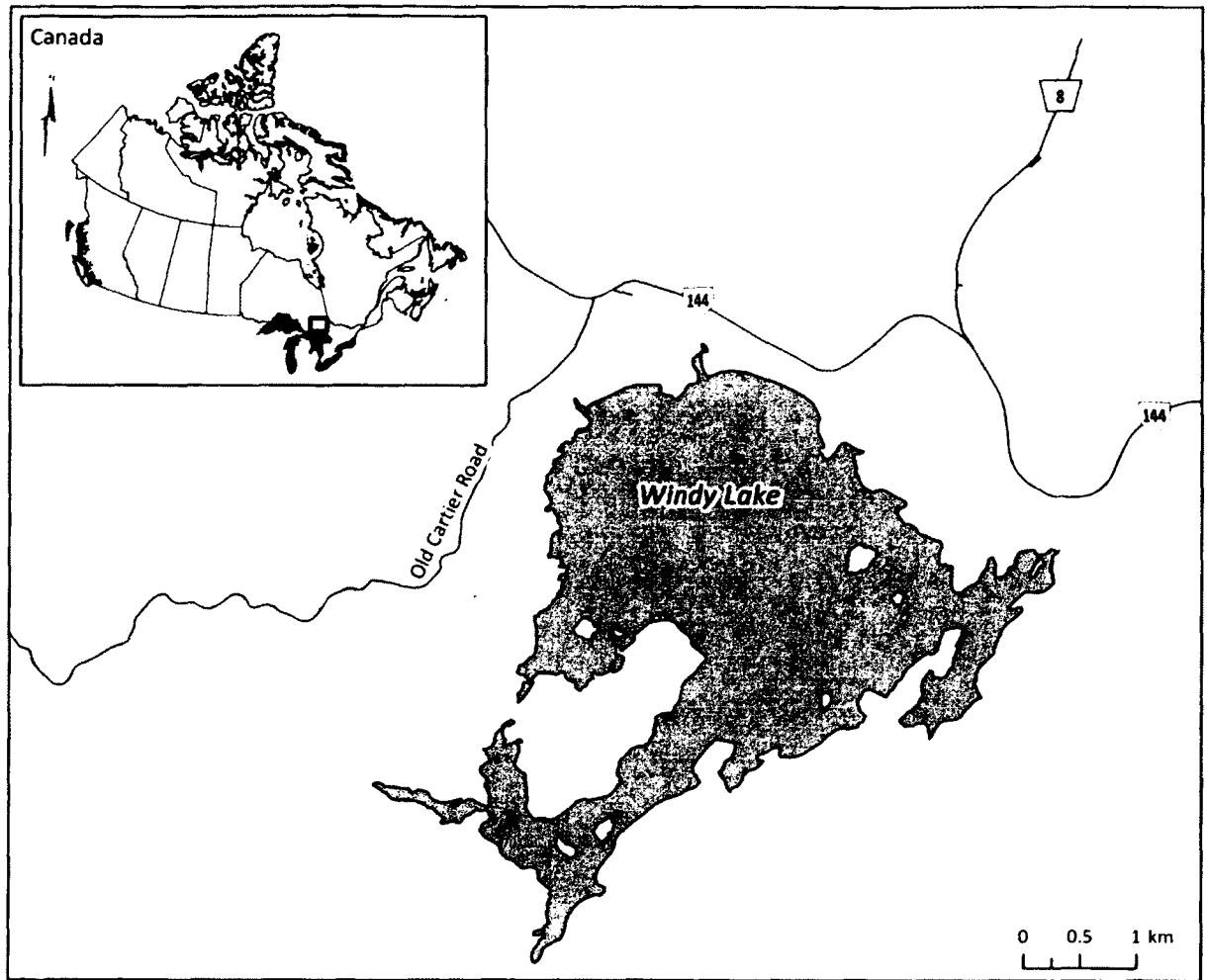
In this study, the potential for sexual dimorphism in Burbot is explored in a population sampled over a full year. This approach allowed the determination of the timing and duration of spawning, as well as the assessment of the seasonal pattern of sex-related differences. External phenotypic traits (body size, fin lengths), as well as internal traits (gonad size, liver size, swim bladder mass, gas gland mass, lipid storage), were compared between the sexes. Male-biased sexual dimorphism has been reported for the closely related Atlantic Cod for pelvic fin lengths (Skjæraasen et al. 2006b) and drumming muscle size (Rowe and Hutchings 2008). It is therefore predicted that mature male Burbot

would also have longer fins and more developed drumming muscles, as they are known to vocalize during the spawning period. Mature females were expected to have larger gonads, consistent with other boreal species, and both larger and more lipid-rich livers because of the role of the liver in vitellogenesis. Seasonal variation in liver size and lipid content was also expected to be more pronounced in females as they store and expend their energy on ovary formation. Males of many species have to actively seek out mates and this physical demand may be reflected by larger male size (Casselman and Schulte-Hostedde 2004). It is predicted that male Burbot will be larger than females, and that being physoclistous fish, males will have larger gas glands to facilitate roving for females across pressure gradients.

## **Methods**

### *Site description and field sampling*

Windy Lake (46°36' N, 81°27' W) is an oligotrophic boreal shield lake in north-eastern Ontario, Canada, near the City of Greater Sudbury (Figure 4). It is situated at 333 m above sea level, has a surface area of 1111 ha, a perimeter of 36 km, and mean and maximum depths of 10.7 and 65.6 m, respectively. The fish community includes Burbot, Lake Whitefish, Cisco (*C. artedi*), Yellow Perch (*Perca flavescens*), White Sucker (*Catostomus commersoni*), Lake Trout, Walleye, Smallmouth Bass (*Micropterus dolomieu*), Northern Pike, and Lake Chub (*Couesius plumbeus*), among others. Windy Lake supports a recreational fishery that primarily targets Lake Trout, Walleye, Smallmouth Bass, and Lake Whitefish. Burbot are rarely targeted by anglers but are frequently captured as by-catch during the winter.



**Figure 4.** Study location, Windy Lake, Ontario, Canada.

Preliminary sampling was conducted in early February 2008. During the period of January 2009 to February 2010 sampling was carried out every two weeks in January and February, and approximately monthly during the rest of the year. Supplemental sampling was carried out in November 2010. No sampling was conducted during April and December due to unsafe ice conditions. Burbot were collected using long lines baited with pieces (~ 25 g) of salted Cisco as per Cott et al. (2011). Long lines were composed

of 45 m lengths of lead-core line, with 20 snelled hooks attached at 2 m intervals. Long lines were deployed from a boat in the open-water season, and beneath the ice using a jigger board in the winter. On each sampling date, long lines were set at each of five sites randomly selected from a grid superimposed over the lake area as per the Nordic protocol for experimental gillnetting (Morgan and Snucins 2005). Lines were retrieved the following day (set duration 18 – 24 h) and depths of the sampling sites and numbers of fish captured were recorded. Lines were deployed throughout depth strata from <5 m to >60 m. All Burbot were immediately killed with a sharp blow to the back of the head. The fish were transported back to the laboratory on wet ice and processed within 24 hours of capture.

#### *Laboratory analysis*

All Burbot were processed to obtain the following information: total length ( $\pm 1.0$  mm), total body mass ( $\pm 1.0$  g wet), whole gonad mass ( $\pm 0.1$  g wet), somatic mass (total mass minus gonads;  $\pm 1.0$  g wet), liver mass ( $\pm 0.1$  g wet), left and right pectoral fin lengths ( $\pm 1.0$  mm), left and right pelvic fin lengths ( $\pm 1.0$  mm), swim bladder mass ( $\pm 0.1$  g wet), gas gland mass ( $\pm 0.1$  g wet), sex, and state of maturity. Maturity was defined as: *immature* - gonad immature and not developing towards the next spawning period (i.e. fish not spawning that year); *green* - gonad developing towards next spawning event but gametes not yet free-flowing; *ripe* - mature gametes free-flowing; *spent* - gonads recently spawned-out; or *unknown* - unable to determine sexual maturity. Gut contents were removed from each fish before determination of body mass. Fin lengths were measured along a ruler placed in the fin-body insertion and held parallel to the longitudinal axis of

the fish body. Swim bladders were removed from the dorsal surface of the body cavity using a scalpel and weighed whole. The swim bladder was then everted (see Figure 5), and the gas gland was scraped from the inner surface and weighed separately. The drumming muscle in Burbot is well fused into the swim bladder (see Figure 6), wall making it difficult to extract, so swim bladder mass was used, with the gas gland scraped off, as a proxy for drumming muscle mass. Various tissues were collected and stored for further analyses. Sagittal otoliths were removed, rubbed clean, and placed in plastic vials to air dry. Whole livers were placed in small plastic bags and frozen at -20 °C. For sampling dates just prior to spawning (mid to late January), subsamples (~ 20 g) were cut from the mid-section of both mature gonads, placed in small plastic bags, and frozen at -20 °C.

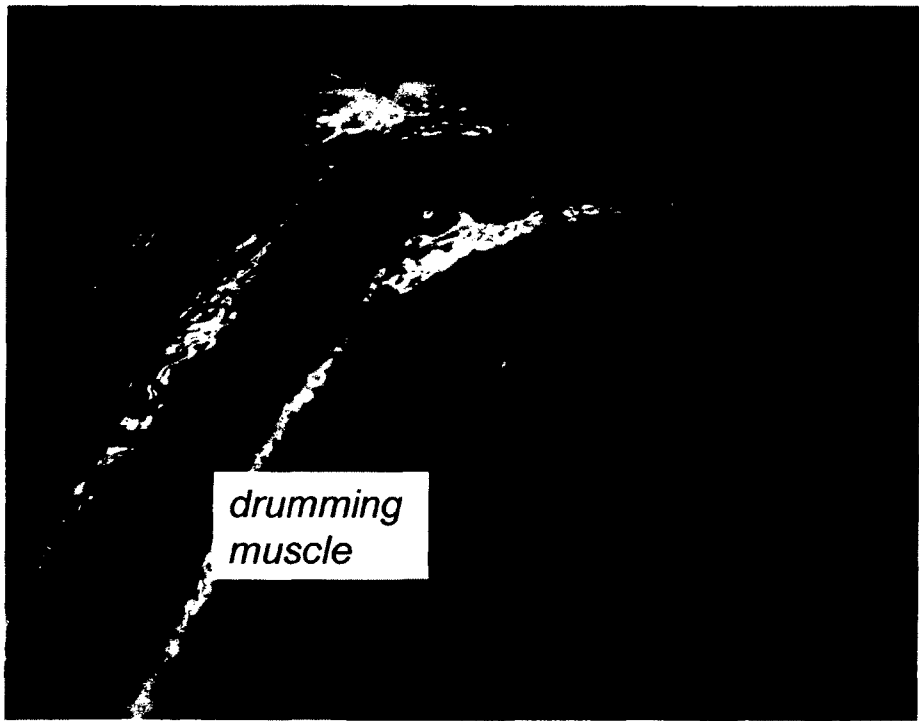
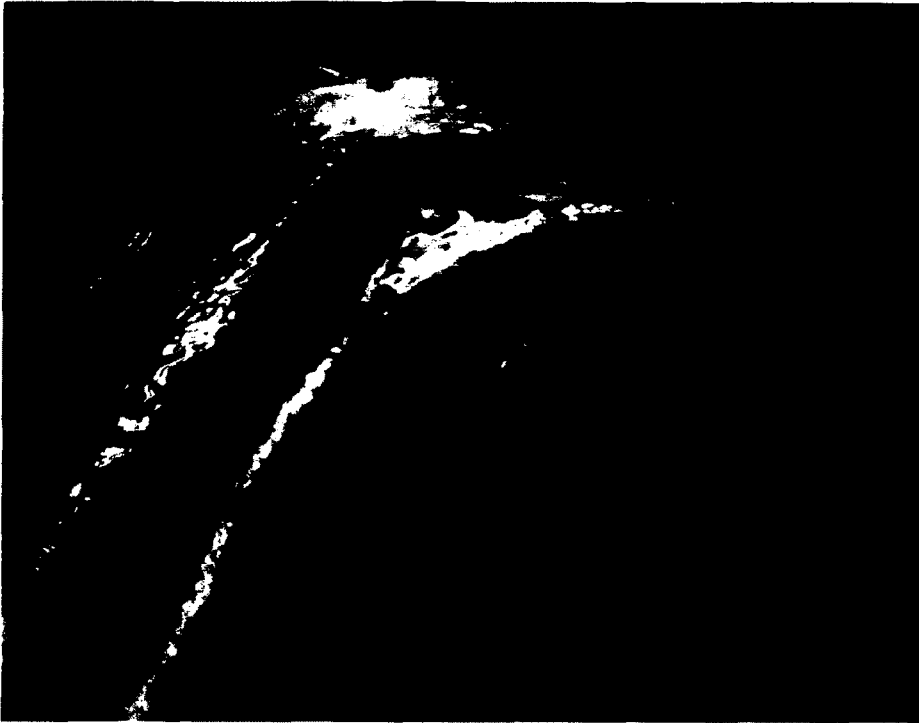
For consistency ages were determined only for fish sampled in January and February. Annuli were counted on sagittal otoliths prepared by the crack-and-burn technique (Edwards et al. 2011). Because these fish had been sampled near the time of spawning, the outer edge of the otolith was always counted as a complete annulus. Moisture and lipid contents were determined for liver and gonad samples. Tissues were thawed and homogenized using a hand-held food processor. Aliquots of the homogenate (~ 0.50 g) were weighed into pre-weighed glass centrifuge tubes, oven-dried (72 h at 40 °C), desiccated, and re-weighed to determine moisture content. The lipid content of the dried samples was then determined gravimetrically using a chloroform-methanol extraction procedure as per Moles et al. (2008). Moisture and lipid determinations were performed



in duplicate, and a third determination was carried out when the coefficient of variation of the first two determinations exceeded 15%.



**Figure 5. The swim bladder of a burbot a) inflated and still attached, and b) everted showing the gas gland.**



**Figure 6. The swim bladder of a burbot showing the drumming muscles.**

### *Data analysis*

Longline catch-per-unit-effort (CPUE) was expressed as fish caught per hook per night. Most morphometric and reproductive variables were expressed as proportions of body size. Gonadosomatic index (GSI) was estimated as the whole gonad mass divided by somatic mass, and hepatosomatic index (HSI) was estimated as liver mass divided by somatic mass. Similarly, swim bladder, and gas gland wet masses were expressed as proportions of somatic mass. Pectoral fin and pelvic fin lengths were expressed as proportions of total body length. There was no evidence of asymmetry between left and right lengths for either set of fins and thus, mean pelvic fin length and mean pectoral fin length were calculated for each fish. Variables were transformed as required to normalize residuals or linearize relationships. All variables expressed as proportions were arcsine-square-root transformed.

Statistical analyses were performed with SAS<sup>®</sup> version 9.2 (SAS 2009) procedures. Growth was examined by fitting size-at-age data to a von Bertalanffy model (Ricker 1975, Lester et al. 2004) using non-linear least-squares (NLIN procedure). For most other variables, data analysis followed a two step process. First, plots of size-adjusted mean values vs. sample date were examined to assess temporal trends. To test for seasonal effects, sampling dates were assigned to seasonal blocks defined as *spring* – March 1<sup>st</sup> to May 31<sup>st</sup>, *summer* – June 1<sup>st</sup> to August 31<sup>st</sup>, *fall* – September 1<sup>st</sup> to November 30<sup>th</sup>, and *winter* – December 1<sup>st</sup> to February 28<sup>th</sup>. This allowed for the use of equal time blocks that fit well with the reproductive cycle of the winter spawning Burbot. Seasonal differences in total CPUE were tested with ANOVA, while sex-based differences in

CPUE were tested using a paired-comparisons t-test (TTEST paired procedure) comparing sampling events.

Adjusted means (least-square means) of the dependent variables were estimated for each sex on each sampling date using an ANCOVA model (GLM procedure) with sex as the class variable and body size as the covariate. Means were adjusted to body sizes of 500 g when somatic mass was used as the covariate or 435 mm when total length was used as the covariate. These values approximated the overall mean sizes of fish captured during the study. For variables that showed no clear pattern of seasonal variation, sex-based differences were assessed by ANCOVA using the pooled data set. For variables that showed distinct patterns of seasonal variation, sex-based differences were tested either by conducting ANCOVA on restricted subsets of the seasonal data, or by conducting paired-comparisons t-tests (TTEST paired procedure) using the adjusted means for each sampling period, or age-class for size-at-age comparisons.

## **Results**

### *Sampling summary and CPUE*

A total of 472 Burbot were collected during the course of this study. The sex ratio of reproductively mature Burbot (green, ripe, and spent) collected was 1.26:1 females (n=202) to males (n=160), similar to the overall population of 1.23:1 (females n=260, males n=212). Only 5% of the Burbot collected were non-spawners. The average age of

spawning fish was 8.4 years (range 3 to 12) for females and 9.6 years (range 4 to 17) for males (Table 1).

The overall trend for total Burbot CPUE (Burbot/hook/night) was that the catch was lowest in the spring, increasing through the summer, peaking in the fall, and decreasing in the winter. More females than males were collected in the winter, with the inverse for the summer catch (Figure 7). However, none of these seasonal trends were statistically significant. There was no seasonal difference noted for total CPUE (ANOVA,  $F_{3,10} = 1.07$ ,  $P = 0.41$ ), female CPUE (ANOVA,  $F_{3,10} = 0.94$ ,  $P = 0.46$ ), or male CPUE (ANOVA,  $F_{3,10} = 1.65$ ,  $P = 0.24$ ), and there was no sex-based difference detected in CPUE (paired comparisons t-test,  $t = 0.88$ ,  $n = 14$ ,  $P = 0.40$ ).

#### *Growth and morphometry*

The von Bertalanffy model fit the growth trajectory well for both sexes of Burbot. Males appeared to grow faster than females before five years of age, and then slower than females after seven years of age (Figure 8). There was no difference in size at age between sexes with regards to mean total length (paired-sampled  $t$ -test,  $t = 1.00$ ,  $n = 8$ ,  $P = 0.34$ ) or mean somatic mass (paired comparisons t-test,  $t = 0.39$ ,  $n = 8$ ,  $P = 0.71$ ). The oldest fish collected were males.

Pectoral fin lengths did not show any clear pattern of seasonal variation. There was no body size (total length) by season interaction for either females (ANCOVA,  $F_{3,185} = 0.55$ ,  $P = 0.65$ ), or males (ANCOVA,  $F_{3,137} = 2.34$ ,  $P = 0.08$ ), and there was no seasonal difference in pectoral fin length for females (ANCOVA,  $F_{3,188} = 0.16$ ,  $P = 0.92$ ), or

males (ANCOVA,  $F_{3,137} = 2.25$ ,  $P = 0.09$ ). Furthermore, pectoral fin length did not differ between males and females (paired-comparisons t-test,  $t = -1.62$ ,  $n = 14$ ,  $P = 0.13$ ). Likewise, there was no seasonal trend evident for pelvic fin length. Body size by season interaction was not significant for either females (ANCOVA,  $F_{3,182} = 0.73$ ,  $P = 0.53$ ), or males (ANCOVA,  $F_{3,132} = 1.40$ ,  $P = 0.25$ ), and there was no significant seasonal effect on pelvic fin length in females (ANCOVA,  $F_{3,185} = 0.54$ ,  $P = 0.66$ ), or males (ANCOVA,  $F_{3,135} = 0.81$ ,  $P = 0.49$ ). There was no significant difference in pelvic fin lengths between the sexes (paired-comparisons t-test,  $t = -0.66$ ,  $n = 14$ ,  $P = 0.52$ ).

Swim bladder mass (gas gland removed) appeared to be lightest in the summer and heaviest in the spring, and this trend was most pronounced in females (Figure 9). There was no body size by season interaction for females (ANCOVA,  $F_{3,185} = 0.59$ ,  $P = 0.62$ ) or males (ANCOVA,  $F_{3,136} = 0.35$ ,  $P = 0.79$ ). The swim bladder mass was significantly different among seasons for females (ANCOVA,  $F_{3,188} = 4.83$ ,  $P = 0.003$ ) but not for males (ANCOVA,  $F_{3,139} = 1.52$ ,  $P = 0.21$ ). The swim bladder mass of female Burbot was significantly heavier in winter and spring than other seasons, but not significantly different between spring and winter (Tukey's test,  $P < 0.05$ ). Males had heavier swim bladders than females for most of the year (Figure 9), but differences between sexes were not significant when examined over the whole year (paired-comparison t-test,  $t = -1.20$ ,  $n = 13$ ,  $P = 0.25$ ). The seasonal trend for gas gland mass for both sexes appeared to be more complex with peaks in both winter and summer (Figure 9). There was no body size by season interaction for either sex (ANCOVA,  $F_{3,182} = 0.28$ ,  $P = 0.84$  [female];  $F_{3,134} = 2.28$ ,  $P = 0.09$  [male]), and significant seasonal differences in the gas gland mass for both

females (ANCOVA,  $F_{3,185} = 7.77$ ,  $P < 0.0001$ ) and males (ANCOVA,  $F_{3,137} = 2.77$ ,  $P = 0.04$ ). For females, gas gland mass was significantly heavier in winter than for other seasons, and for males gas gland mass was significantly heavier in spring than in other seasons (Tukey's test,  $P < 0.05$ ). Over the whole year, males had heavier gas glands than females (paired-comparisons  $t$ -test,  $t = -2.39$ ,  $n = 14$ ,  $P = 0.03$ ).

**Table 1. General characteristics of Burbot sampled from Windy Lake, Ontario during winter. G = green, R = ripe, S = spent, I = non-spawner, nd = no data.**

Sex (Maturity)	Total Length (mm)			Total Weight (g)			Age (yr)		
	n	mean ( $\pm$ std)	range	n	mean ( $\pm$ std)	range	n	mean ( $\pm$ std)	range
female (G, R, S)	203	430.6 (55.2)	283-621	203	511.9 (20.2)	142-1530	63	8.4 (2.1)	3-12
male (G, R, S)	160	437.3 (50.8)	304-590	160	523.6 (183.3)	166-1520	33	9.6 (3.1)	4-17
female (I)	10	377.1 (36.5)	331-439	10	329.1 (100.6)	219-519	6	7.3 (1.8)	5-10
male (I)	3	344.7 (35.0)	323-385	3	223.3 (41.1)	193-270	nd	nd	nd

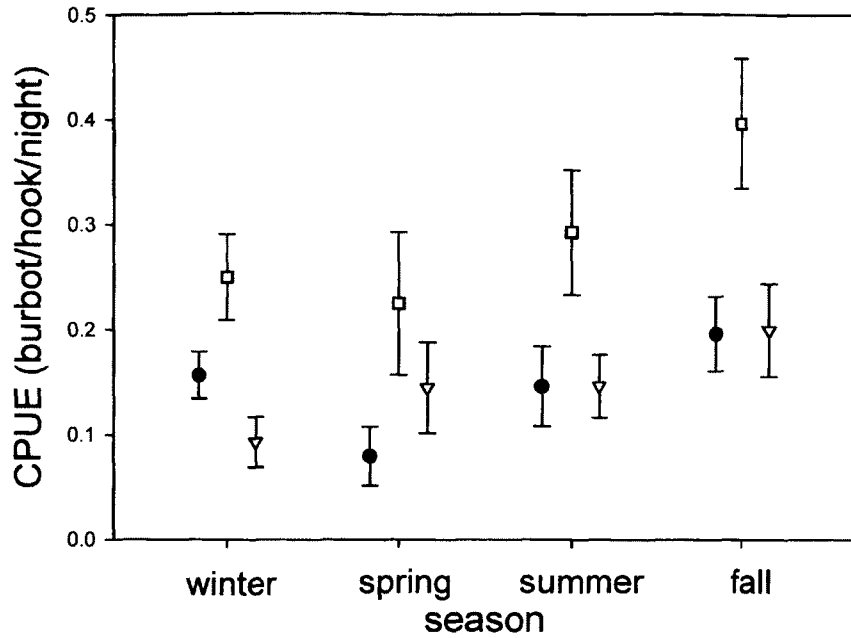


Figure 7. Catch-per-unit-effort (CPUE) vs. season for Burbot sampled by baited longlines from Windy Lake, Ontario. Symbols represent means ( $\pm$  SE) for females (black circles), males (open triangles), and both sexes combined (grey squares).

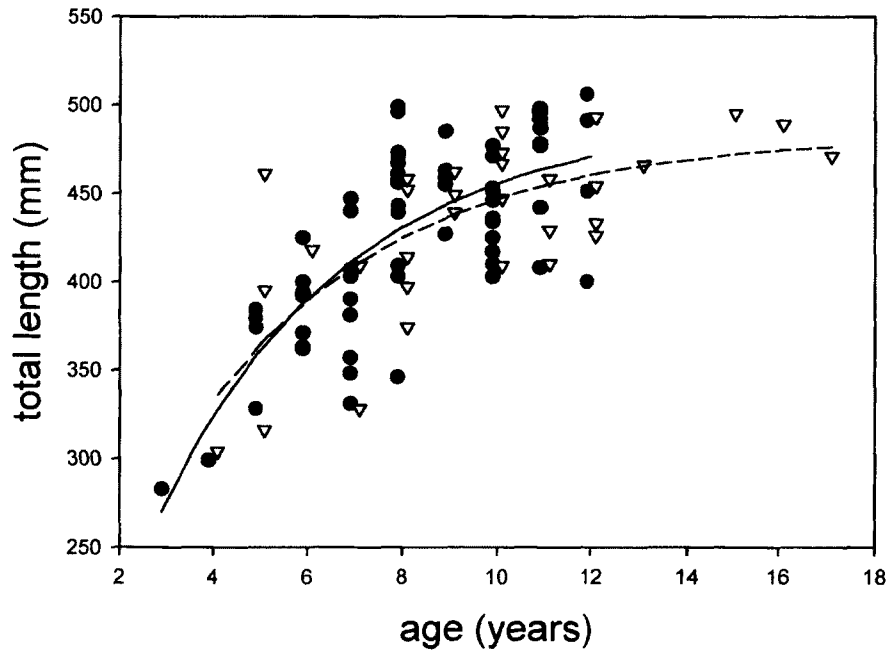
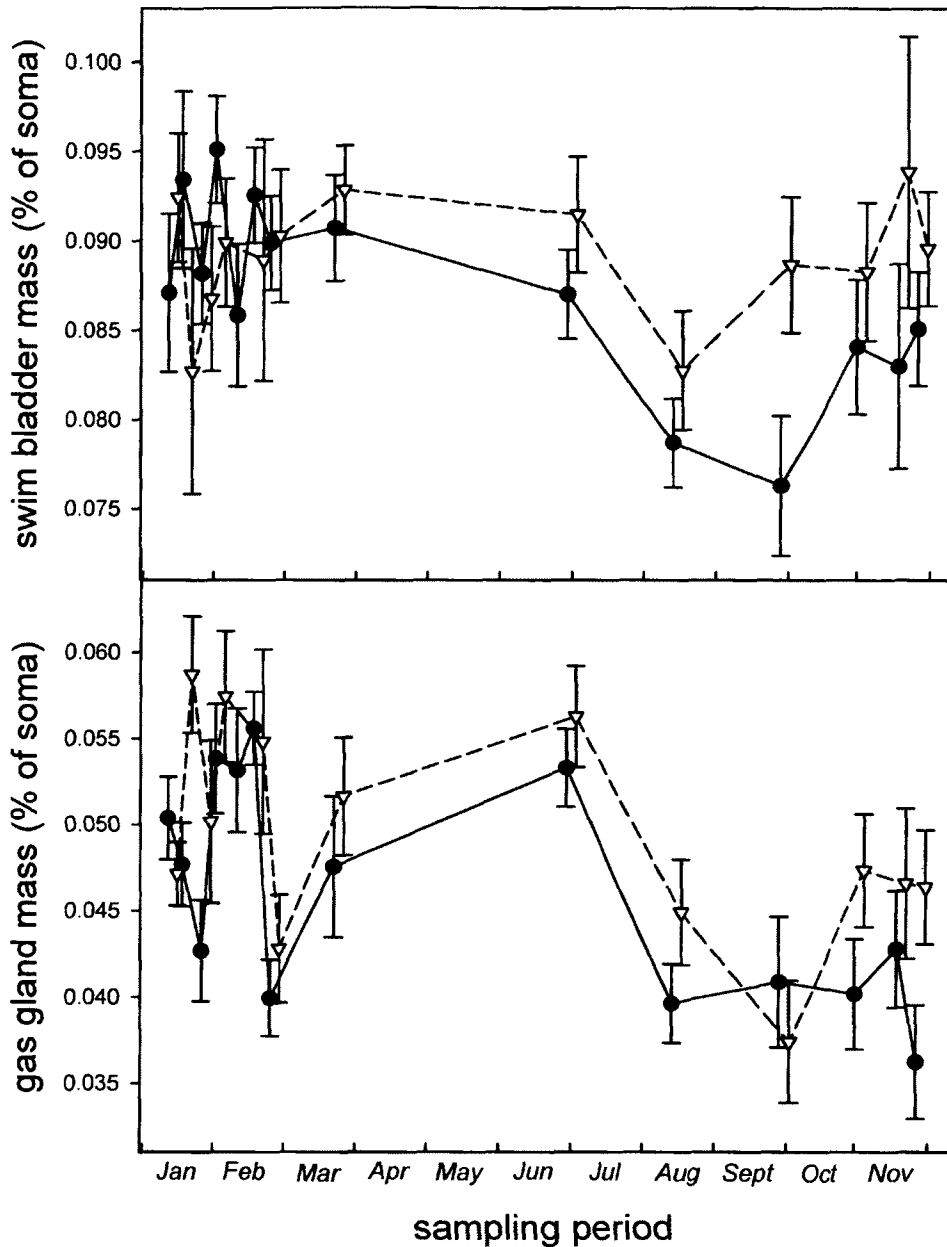


Figure 8. Total length vs. age (sagittal otolith) for Burbot collected from Windy Lake, Ontario. Black circles and solid line represent females, open triangles and dashed lines represent males. Ages of the two sexes are offset by 0.1 years to aid in presentation. Lines represent predicted size at age from fitted von Bertalanffy growth models.





**Figure 9.** Circannual variation in swim bladder mass (top) and gas gland mass (bottom) for female (black circles, solid line) and male (open triangles, dashed line) Burbot sampled from Windy Lake, ON. Symbols represent body size adjusted means ( $\pm$  SE). Sampling dates were offset by two days between the sexes to aid presentation.

### *Body condition*

Body condition, expressed as length adjusted somatic mass, exhibited slightly different seasonal trends for the two sexes. There was no interaction between total length and season for females (ANCOVA,  $F_{3,249} = 0.44$ ,  $P = 0.72$ ) or males (ANCOVA,  $F_{3,203} = 0.96$ ,  $P = 0.41$ ). Seasonal variation in female body condition was significant (ANCOVA,  $F_{3,252} = 6.57$ ,  $P = 0.0003$ ), with higher body condition in spring and winter than summer and fall, and significantly higher condition in spring than winter (Tukey's test,  $P < 0.05$ ). No significant seasonal difference was detected for males (ANCOVA,  $F_{3,206} = 2.31$ ,  $P = 0.08$ ). Overall, male Burbot had higher body condition than females (paired-comparisons t-test,  $t = 6.54$ ,  $n = 17$ ,  $P < 0.0001$ ).

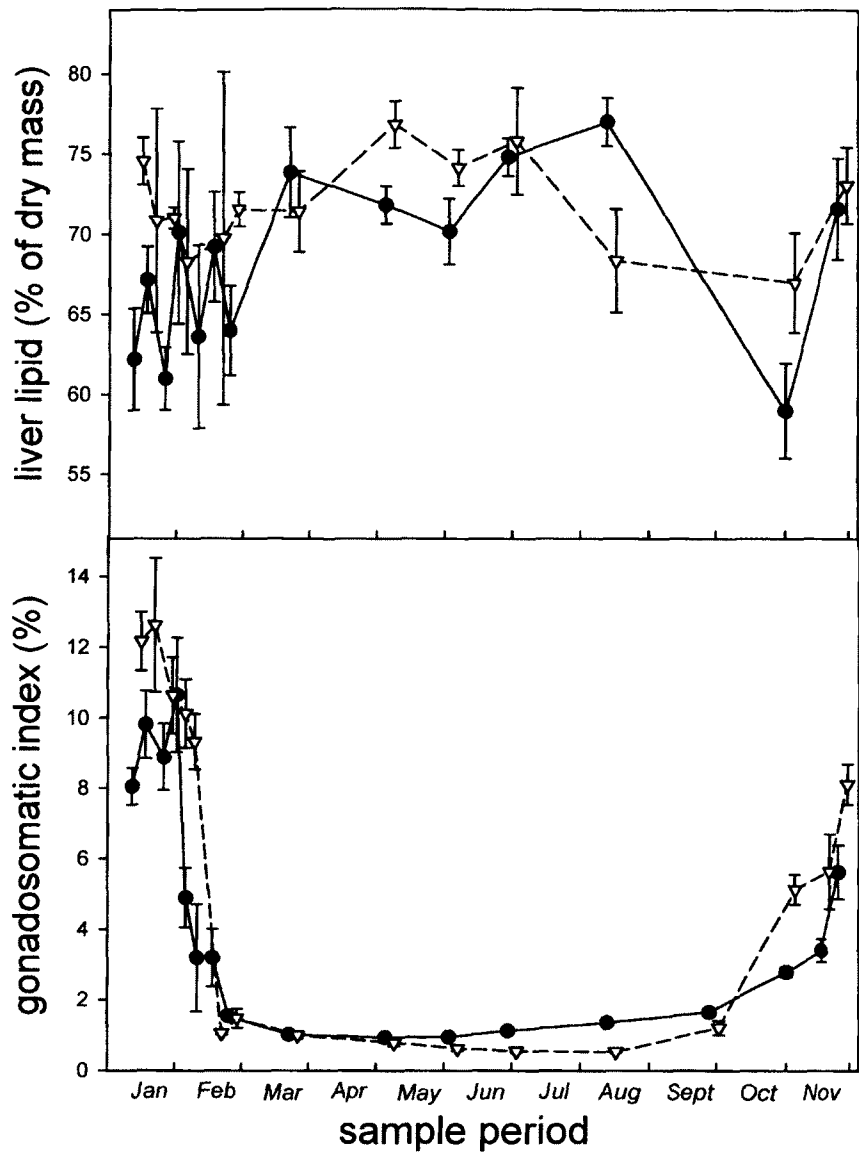
### *Energy storage and reproduction*

Liver size, expressed as HSI, was lowest in the fall and highest in the spring for both sexes. There was no body size by season interaction for either females (ANCOVA,  $F_{3,239} = 0.38$ ,  $P = 0.77$ ) or males (ANCOVA,  $F_{3,190} = 1.48$ ,  $P = 0.22$ ), and the observed seasonal trends were significant for females (ANCOVA,  $F_{3,242} = 10.40$ ,  $P < 0.0001$ ), and nearly significant for males (ANCOVA,  $F_{3,193} = 2.57$ ,  $P = 0.06$ ). Females had significantly higher HSI in both the spring and winter compared to the summer and fall (Tukey's test,  $P < 0.05$ ). Females had higher HSI than males (paired comparisons t-test,  $t = 5.65$ ,  $n = 17$ ,  $P < 0.0001$ ). Liver lipid concentrations in female Burbot appeared to peak in the summer, but no clear seasonal pattern was evident for male Burbot (Figure 10). There

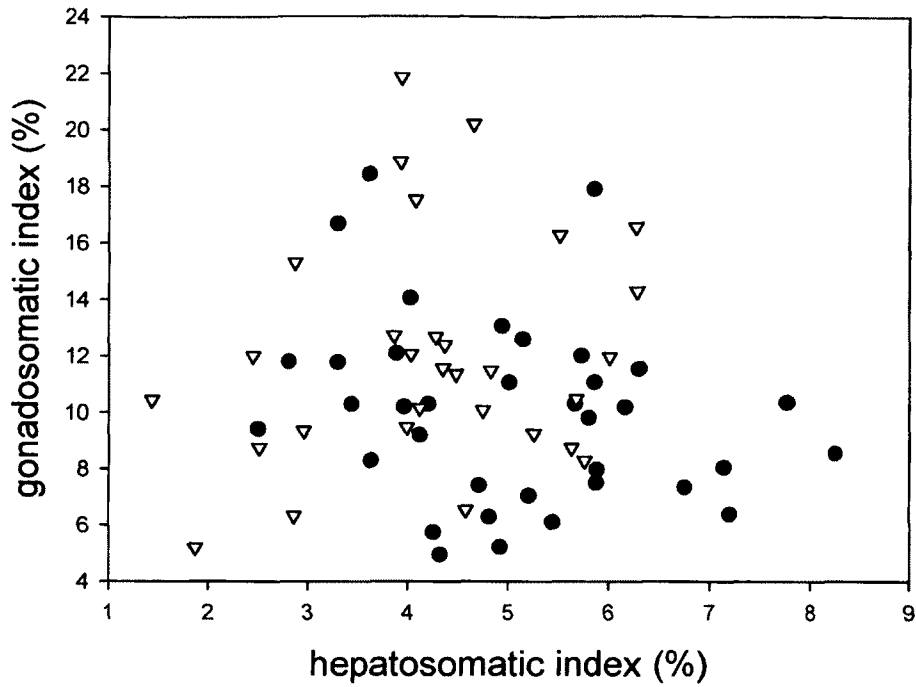
was no interaction between body size and season for females (ANCOVA,  $F_{3,129} = 1.53$ ,  $P = 0.21$ ) or males (ANCOVA,  $F_{3,105} = 1.01$ ,  $P = 0.39$ ). There was significant seasonal variation in female liver lipid concentration (ANCOVA,  $F_{3,132} = 4.77$ ,  $P = 0.003$ ) with winter concentrations lower than in other seasons (Tukey's test,  $P < 0.05$ ). There was no seasonal difference in lipid levels detected for male Burbot (ANCOVA,  $F_{3,108} = 0.12$ ,  $P = 0.95$ ). Males tended to have higher liver lipid concentrations than females, however this trend was not statistically significant (paired comparisons  $t$ -test,  $t = -2.09$ ,  $n = 14$ ,  $P = 0.06$ ).

Gonad size, expressed as GSI, increased rapidly in October and peaked in size by January in both sexes (Figure 11). Male Burbot in spawning condition (semen free-flowing) were first captured in mid-January, approximately two weeks before the first ovulated females were captured. Spent females first appeared in the catch in early February, whereas the first fully spent males were collected in the third week of February. The last unovulated females were captured in the fourth week of February. To compare gonad traits between the sexes, only Burbot captured from mid-January to early February that were in reproductive condition (i.e., green females, and green and ripe males) were used; gonads of fish captured at this time were deemed to be of terminal size and composition. There was no interactive effect of body mass and sex on GSI (ANCOVA,  $F_{1,116} = 0.03$ ,  $P = 0.86$ ) and males had significantly larger GSI than females (ANCOVA,  $F_{1,117} = 6.18$ ,  $P = 0.01$ ). For gonad lipid concentration, there was no interaction between body size and sex (ANCOVA,  $F_{1,31} = 0.51$ ,  $P = 0.48$ ), and female gonads had significantly higher lipid concentrations than males (ANCOVA,  $F_{1,32} = 1009.8$ ,  $P < 0.0001$ ).

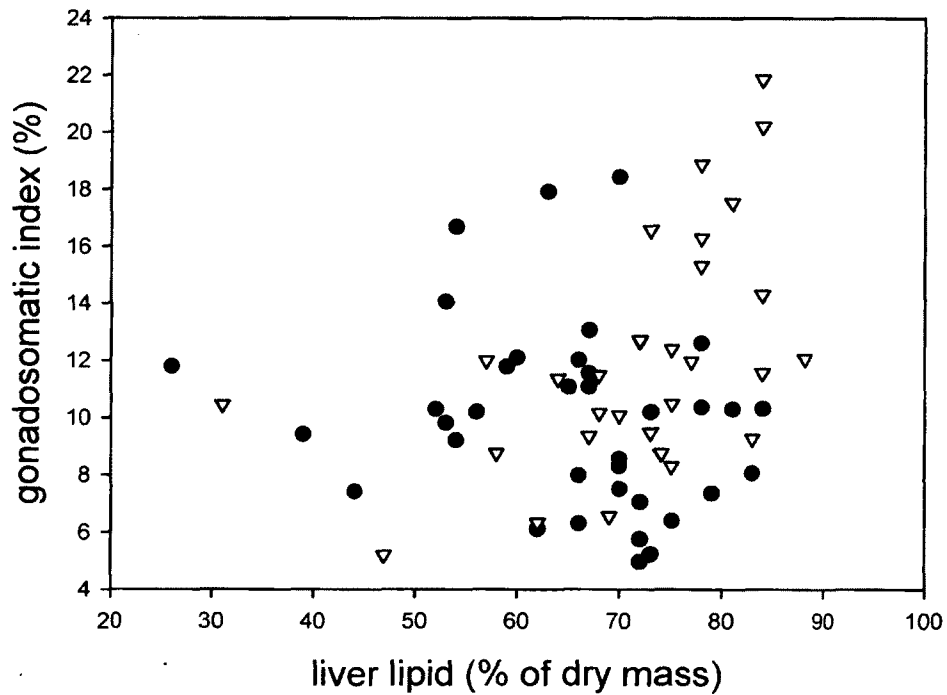
The relationships between GSI and HSI, and between GSI and liver lipid concentration for pre-spawn Burbot were not pronounced for either sex, with the exception of the relationship between liver lipids and GSI for male Burbot. As expected, for female Burbot HSI declined with increasing GSI but the relationship was weak (regression analysis,  $F_{1,34} = 2.18$ ,  $P = 0.15$ , slope = -0.58,  $r^2 = 0.06$ ). The opposite pattern was evident for males, but again the relationship was weak (regression analysis,  $F_{1,28} = 1.32$ ,  $P = 0.26$ , slope = 0.68,  $r^2 = 0.05$ ) (Figure 11). Similarly, the relationship between liver lipid concentrations and GSI for pre-spawn females was negative and weak (regression analysis,  $F_{1,34} = 1.56$ ,  $P = 0.21$ , slope = -0.17,  $r^2 = 0.04$ ). However, the relationship between liver lipid concentrations and GSI for pre-spawn males was positive, and stronger (regression analysis,  $F_{1,28} = 9.68$ ,  $P = 0.004$ , slope = 0.17,  $r^2 = 0.26$ ) (Figure 12).



**Figure 10. Circannual variation in liver lipid concentration (top) and gonadosomatic index (bottom) for female (black circles, solid line) and male (open triangles, dashed line) Burbot sampled from Windy Lake, ON. Symbols represent body size adjusted means ( $\pm$  SE). Sampling dates were offset by two days between the sexes to aid presentation.**



**Figure 11. Hepatosomatic index vs. gonadosomatic index for pre-spawn Burbot collected from Windy Lake, Ontario. Black circles represent female Burbot, open triangles represent male Burbot.**



**Figure 12. Liver lipid vs. gonadosomatic index for pre-spawn Burbot collected from Windy Lake, Ontario. Black circles represent female Burbot, open triangles represent male Burbot.**

## **Discussion**

Burbot in Windy Lake exhibited no sexual dimorphism in most of the traits investigated. Some notable exceptions were larger gonads and higher condition factor of males, and the larger liver size of females.

Contrary to what was predicted, there was no difference in catch (expressed as CPUE) between the sexes, nor was there any significant seasonal variation in CPUE within sexes. The sampling method using baited hooks confirmed that there was active feeding throughout the year. However, this method has potential biases, notably towards actively foraging fish, and those large enough to swallow a hook baited with a one half of a Cisco.

There were no significant differences in size at age between the sexes, consistent with earlier research (Chen 1969, Weber 1976, Kjellman 2003). There was also no evidence of precocious males in the Windy Lake population, however, small fish may not have been vulnerable to the long line gear due to gape limitations. In Chen's (1969) comprehensive work, it was noted that most of the Alaskan Burbot studied did not surpass age 14, and it was speculated that males reached senescence earlier than females. In Windy Lake, no females older than 12 were found, and although males up to 17 years old were collected, there were very few above 12 years old. The youngest reproductively mature female and male Burbot caught in Windy Lake were three and four years old, respectively. Windy Lake Burbot of reproductive age are small (low size-at-age) compared to other Burbot populations (see chapter 4 of this thesis) and (Berg 1949, Chen 1969, Magnin and Fradette 1977, Stapanian et al. 2011).

No evidence of sexual dimorphism with regard to pectoral fins, or pelvic fins was found. Fabricius (1954) commented that out of the four spawning pairs of Burbot he observed, pelvic fins of females were “considerably longer” than those of males, but this difference was not found in this study with a much larger sample size. However, this may be due to phenotypic differences between these populations. The pelvic fins of Burbot are filamentous and, like their chin barbel, they are equipped with chemosensory receptors (Hinkens and Cochran 1988) and they may be used as clasping appendages, as with other gadoids. Male Haddock (*Melanogrammus aeglefinus*) (Hawkins and Amorin 2000) and Atlantic Cod (Skjæraasen et al. 2006b) use their pelvic fins to clasp the pelvic fins of females to ensure vent-to-vent contact during spawning.

In Atlantic Cod, it has been shown that males with bigger drumming muscles are more successful at attracting mates, possibly because larger drumming muscles allow longer and louder vocalizations (Rowe and Hutchings 2004, Nordeide et al. 2008, Rowe and Hutchings 2008). Also, Atlantic Cod drumming muscle mass has been shown to increase with the onset of the reproductive period (Rowe and Hutchings 2004, Nordeide et al. 2008). A recent enclosure study has demonstrated that Burbot also vocalize during their spawning season (Chapter 3 of this thesis). In Windy Lake, Burbot swim bladder mass increased with the onset of the spawning period, but there were no differences between males and females. The similarity in swim bladder mass between the sexes during the spawning period suggests that calling may be used by both sexes during reproduction. The significance of heavier swim bladders in males at other times of the year is not clear,



but may be linked to swim bladder functions other than vocalization. Like other gadoids, calling may be used to find other Burbot, synchronize reproduction, and stage pre-spawning aggregations. Calling via drumming muscles is energetically expensive (Hawkins and Amorin 2000); however, the benefits of possible reduced intra-sexual competition, such as defending mates or territories, may outweigh the costs of calling. In the absence of visual cues, louder and longer drumming by male Burbot may show fitness and allow for mate choice by females. Female vocalizations may be associated with ovulation. Further examination under captivity would be required to test these predictions.

It is possible that the large congregations of Burbot in what have been described as 'spawning balls' (Cahn 1936, McCrimmon 1959, McPhail 2007, Holm et al. 2010) represent a type of lekking behaviour. A lek is an area used for courtship and display that is not tied to any particular resource (Emlen and Oring 1977, Howard 1978). Lekking is common in some birds and has been proposed as part of the mating system of Atlantic Cod (Nordeide and Folstad 2000) and Haddock (Hawkins and Amorin 2000). Such scenarios arise when individual males cannot monopolize females. Aggressive behaviour has not been reported for Burbot. In fact, in detailed aquarium-based observations of several spawning pairs of Burbot, Fabricius (1954) noted a complete absence of any type of intra-sexual antagonistic behaviour, with Burbot often sharing the same caves. Similarly, through the use of a remotely-operated underwater camera, Edsall et al. (1993) observed non-spawning Burbot showing a close affinity to one another, and occurring in close proximity to, and often touching, each other.

Burbot are physoclistous, meaning that they regulate buoyancy by moving gas in and out of the swim bladder with a gas gland (Alexander 1993). A relatively larger gas gland may facilitate vertical migrations through depth strata. The longline sampling of Windy Lake indicated that Burbot were present at depths from < 5 m to over 60 m. It was expected that gas gland mass would be greater for both sexes in the colder months when cold-water habitat is available at all depths, but would show a male-bias because males would presumably be actively seeking out females, where an increased ability for depth transitioning afforded by a larger gas gland would be beneficial. Gas gland mass did tend to be heaviest in winter or spring, as expected, it was also found that males had significantly heavier gas glands than females. An investigation of seasonal and sex-based habitat partitioning of Burbot would be an interesting topic for future research.

Burbot, like other codfishes, are very lean with very little lipid content (~ 1 %) in their muscle tissue (Wong 2008). The large, oil-rich liver appears to be the primary site of energy storage and allocation in Gadiformes. As predicted, female Burbot had larger livers than males, and there was a significant seasonal pattern in liver size and lipid concentration that was most pronounced in the females. It is likely that these seasonal cycles reflect energy storage and allocation patterns for gonad production. Feeding during reproduction would minimize the cost in terms of using stored energy, assuming that the energetic costs of foraging are less than using stored lipid reserves (Marshall et al. 1999, Binner et al. 2008). For an iteroparous spawner, life history theory predicts that the energy available for growth and reproduction is that which exceeds the energy needed for

basic functioning and survival of the individual (Skjæraasen et al. 2006a). The reproductive potential of females is significantly higher when food is abundant (Marshall et al. 1999). Some fishes, particularly semelparous species such as the Chinook Salmon (*Oncorhynchus tshawytscha*) do not eat during the most energetically taxing part of their reproductive process, being completely reliant on stored energy (Healey and Heard 1984). Unlike Chinook Salmon where reproduction is a terminal event, Burbot are iteroparous and must trade-off overall reproductive effort in a given year to some degree, in order to ensure survival until the next spawning season. However no strong relationship between liver lipids and GSI was found. The prevailing conditions within Windy Lake appear to offer Burbot of both sexes the luxury of maintaining relatively consistent energy stores, in the form of liver lipids, by actively feeding throughout the year including the spawning season. Lipids stored in the liver are accumulated throughout the year and are likely used for gonadal production by both sexes. Spawning females had lower lipid levels in their livers than males, but higher lipid levels in their gonads than males. Ovary development seems to require more lipids than does testis development, as the small, non-adhesive Burbot eggs contain a large oil globule (Cahn 1936, Chen 1969). This is similar to the closely related Cusk, but not Atlantic Cod (Scott and Scott 1988). Burbot eggs are only slightly negatively buoyant and remain in suspension after release (McCrimmon 1959), perhaps to assist in fertilisation or dispersion.

Burbot in Windy Lake began spawning in early February and concluded within a three week period. This was consistent over three years of sampling. There were male Burbot in reproductive condition through the spawning period, preceding the female Burbot by

two weeks, a strategy in males that appears to prepare them for spawning as soon as the first females ovulate (Howard 1978). Gonadal development of Windy Lake Burbot (expressed through GSI) began increasing in October, peaked about a month before the spawn in males and at the spawn time in females, and then dropped abruptly after the spawn. This is similar to the only other documented account of the annual cycle of gonadal development in Burbot (Chen 1969). Gonad size was significantly higher for male Burbot than female Burbot, which is very unusual among boreal fishes. For example, in Walleye and Lake Whitefish populations, female GSI is typically 5-10 times greater than male GSI (Moles et al. 2008, Johnston et al. 2012). Mean GSI for Windy Lake Burbot peaked at approximately 13% for males and 11% for females, but some individual males exceeded 20%. Male Atlantic Cod can also have GSI that equals or exceeds that of females (Burton et al. 1997). The enormous gonadal investment by male Burbot suggests that sperm competition may be an important selective pressure on testes size in this species. Sperm competition is important in externally fertilizing animals (Taborsky 1998) and may be quite intense in Burbot given their reported group spawning behaviour (Cahn 1936, McPhail 2007). There would be a trade-off of reproductive potential between increased body growth to attract mates and increased testes growth required to counter sperm competition. Both somatic growth and sperm production are energetically expensive (Shapiro and Giraldeau 1996, Henderson et al. 2003). For fish where sperm competition is important, males are typically larger than females (Parker 1992). There was no sex based difference in size at age of the Burbot investigated; however males had higher body condition (mass at length). The unusually large testes suggest that Burbot cope with sperm competition by investing enormously into sperm

production. In other types of sperm competition, energy is put into defending a mate against rival males rather than testes development (Andersson 1994, Blanchfield and Ridgway 1999). Gonad size tends to be inversely proportional to behavioural intrasexual competition such as territory defence (Taborsky 1998), but positively correlated with the intensity of direct sperm competition (Stockley et al. 1997).

There are several types of mating systems including monogamy, polygamy, and polyandry (Emlen and Oring 1977), and a variety of mating strategies and tactics such as territorialism, parasitism, and opportunism (Howard 1978, Taborsky 1998).

Differentiating among these systems is very difficult (Lande 1980), especially with a species like Burbot, which for the most part, has elusive nocturnal mating habits. With observations of spawning aggregations and the formation of writhing spawning balls (Cahn 1936, McPhail and Paragamian 2000), the mating system of Burbot appears to be aggregate, although pair-mating has been observed (Fabricius 1954). Cahn (1936) on the other hand suspected that the mating ball consisted of one or few females surrounded by many males, and Burbot may in fact be polyandrous. His idea is supported by the considerable gonadal investment of males suggesting intense sperm competition. With intense sperm competition there would be incentive for a male Burbot to obtain a prime position for gamete release. Female Burbot can produce several hundred thousand (Lawler 1963, Chen 1969, Morrow 1980), to well over a million eggs (Cahn 1936, Berg 1949), similar to marine gadoid fishes (Scott and Scott 1988, Cohen et al. 1990). In order to fertilize eggs as efficiently as possible, females may choose the “best” mate for vent-to-vent clasping, similar to other codfishes (Hutchings et al. 1999, Hawkins and Amorin

2000, Rowe and Hutchings 2008). This choice would allow the female's eggs maximum sperm coverage by the fittest male (Taborsky 1998). A rapid and simultaneous release of eggs and milt would be advantageous during close pair bonding, particularly when there is intense sperm competition. In addition, it is possible that males in the spawning ball may be somewhat opportunistic or parasitic spawners, and could also release their milt, further enhancing the fertilization potential. This type of group-release tactic has been observed with Atlantic Cod (Hutchings et al. 1999, Rowe and Hutchings 2003). It has been shown experimentally that the sperm production of fish with a repeat (within season) spawning tactic is positively correlated with the number of females it is likely to encounter (Shapiro and Giraldeau 1996). If male Burbot spawn repeatedly with several females within the short spawning season, then having large testes would be advantageous. However, this opportunistic strategy may partially circumvent female choice (Howard 1978, Shapiro and Giraldeau 1996). Burbot appear to spawn within a very short time window, as it was unusual to encounter a female with free running eggs during the spawning period, in this and other populations studied (see Chapter 4 of this thesis). In the only recorded observation of the actual act of spawning by Burbot, Fabricius (1954) observed multiple spawnings within the same individual mating pairs; these spawning events occurred in quick succession (5-20 minutes) and the female was completely spent within 3 hours. The evidence from this study indicates that, similar to other gadoids (Hutchings et al. 1999, Rowe and Hutchings 2008), Burbot may use some amount of mate choice despite being an aggregate spawner without obvious external sexually dimorphic characteristics.

This study has demonstrated that Burbot exhibit a low level of sexual dimorphism. The low light conditions of their under-ice spawning environment would make visual mating cues less useful than other cues such as acoustic signalling (see Chapter 3 of this thesis), a mechanism used in the mating systems of other codfishes (Hawkins and Amorin 2000, Rowe and Hutchings 2006, 2008). As mating systems and tactics are more of a continuum than an absolute (Taborsky 1998) variation among Burbot populations is likely. Likewise, the circannual pattern of variation in reproductive development, and particularly energy storage, will likely differ among populations in relation to prevailing environmental conditions. However, Burbot display very little phenotypic or genetic variation throughout their range (Van Houdt et al. 2005, Elmer et al. 2008), little variation in life history traits, and no evidence of sexual dimorphism among populations (Chapter 4 of this thesis). As such, the patterns described herein are likely indicative of Burbot and gadoid reproductive ecology in general.

### **CHAPTER 3 - Song of the Burbot: sound production and reception by *Lota lota***

#### **Introduction**

Sound, as it relates to animals, can be separated into two broad categories; sounds that are of interest are typically called ‘signals’, and sounds that interfere with signal detection are generally referred to as ‘noise’ (Fay 2012). The effects of noise on biota in aquatic environments is a growing concern around the world as anthropogenic noise sources continue to increase (Popper and Hawkins 2012). Aquatic animals, including many fish rely on sound production and reception to interact with conspecifics and their environment (Popper and Hawkins 2012). Fish produce sounds mainly in social contexts (Hawkins 1993), and like other fauna, sound can be an important component of their reproductive ecology (Andersson 1994) as it is used for courtship, to signal reproductive readiness, as well as for agonistic, territorial, and alarm signals (Hawkins 1993, Kasumyan 2009). Investigations of sound production and reception are therefore vital to fully understand the reproductive ecology of many fish species and are critical to understanding the potential impacts of anthropogenic sounds on fish ecology.

One mechanism fish use to produce sound is the rapid contraction of striated muscles located dorso-laterally on the swim bladder (Hawkins and Amorin 2000, Parmentier et al. 2006). The swim bladders of fishes are the earliest vocalization organ in vertebrates, existing before the evolution of the larynx (amphibians, reptiles, and mammals) and the syrinx (birds) (Bass et al. 2008). Fishes in the family Gadidae (the codfishes), a highly important family commercially (Cohen et al. 1990), are known to possess these swim



bladder muscles (hereafter referred to as drumming muscles) and can use them to produce sounds during the mating season (Hawkins 1993, Kasumyan 2009). They may have a simple call, as in the case of Atlantic Cod, which is limited to a simple low frequency grunt (Brawn 1961b, Hawkins and Rasmussen 1978, Rowe and Hutchings 2006) or hum (Rowe and Hutchings 2006); while others, such as Haddock (*Melanogrammus aeglefinus*), produce a more complex mixture of grunts, knocks, hums, and buzzes (Hawkins and Rasmussen 1978, Hawkins and Amorin 2000). Not all codfishes possess drumming muscles, nor are all codfishes with drumming muscles known to vocalize. However, all the gadoid fish known to vocalize do possess drumming muscles, making the presence of drumming muscles a good indicator of potential vocal capability (Hawkins and Rasmussen 1978).

The Burbot is one of four species within the Lotinae subfamily of the Gadidae, along with the Ling, the Blue Ling, and the Cusk (Cohen et al. 1990). All Lotinae species possess drumming muscles (Hawkins and Rasmussen 1978)(see Figure 13) and in burbot they are well-fused into the swim bladder wall (Figure 6). The Burbot is the only freshwater member of the cod family (Cohen et al. 1990), and is one of the most widely distributed freshwater fish species in the world (Van Houdt et al. 2005), occurring in lakes, rivers, and streams throughout the Northern Hemisphere (Scott and Crossman 1973, Cohen et al. 1990, McPhail and Paragamian 2000, Stapanian et al. 2010). Very little is known about the reproductive ecology of Burbot (McPhail and Paragamian 2000), largely because they have the unusual trait of spawning in mid-winter at night, often under ice cover (Scott and Crossman 1973, McPhail and Paragamian 2000). They are known to form spawning

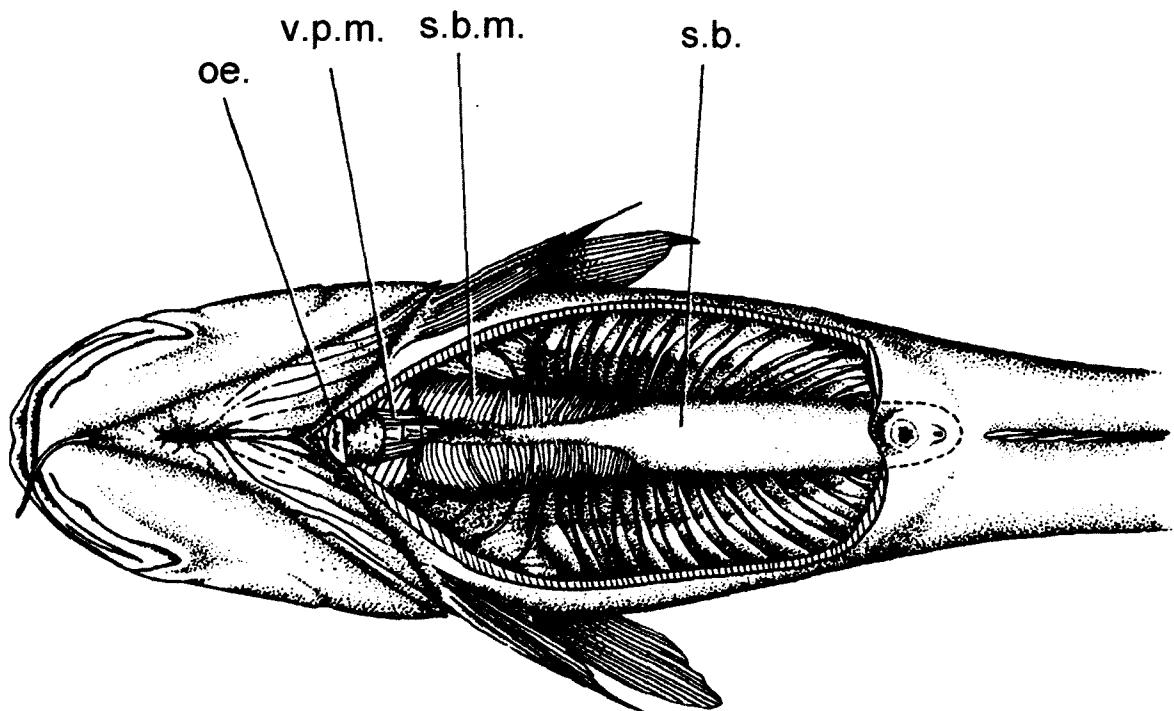
aggregations that can consist of dozens of individuals, often writhing together (Cahn 1936, McPhail 2007, Holm et al. 2010). It is not known if spawning actually occurs during these aggregations, as observations of gamete release in wild Burbot have yet to be documented. The fact that Burbot are not outwardly sexually dimorphic (McPhail and Lindsey 1970b, Weber 1976, McPhail 2007) (also see chapter 2 of this thesis), possess drumming muscles, and spawn at night in an otherwise low-light environment potentially makes sound production and reception a prime sensory system for transmitting mating cues. It is hypothesised herein that Burbot vocalize and that their calls are associated with reproduction.

The current study investigates the acoustic ecology of Burbot with the novel approach of using the deployment of a sound recorder to assess fish vocalizations in a large lake under ice-cover. This approach allows for seasonal assessment, as well as having the advantage of assessing sound production in a natural environment without the acoustical difficulties (e.g., reflection) associated with recording fish sounds in aquaria (Hawkins 1993).

Further, sound reception of Burbot is investigated by directly assessing their hearing ability in a laboratory setting. Different size-classes of Burbot are assessed to investigate ontogenetic shifts in hearing ability. As acoustic signalling may be a part of the mating system of Burbot, it is predicted that there will be an ontogenetic shift in hearing sensitivity; increasing from juvenile to adult.

In northern regions, resource development is often conducted in the winter using ice roads to reach areas that would be otherwise inaccessible (Cott et al. 2008). Construction of,

and transportation across these ice roads can produce substantial noise in underwater environments (Mann et al. 2009a). The combination of the under-ice spawning period of Burbot and anthropogenic noise prevalent in their habitat at that time of year makes it critical to better understand the sensory ecology of this species. Gaining a better understanding of the reproductive ecology of Burbot will also further our understanding of the mating systems of the codfishes in general, many of which - like that of the Burbot - are poorly studied.

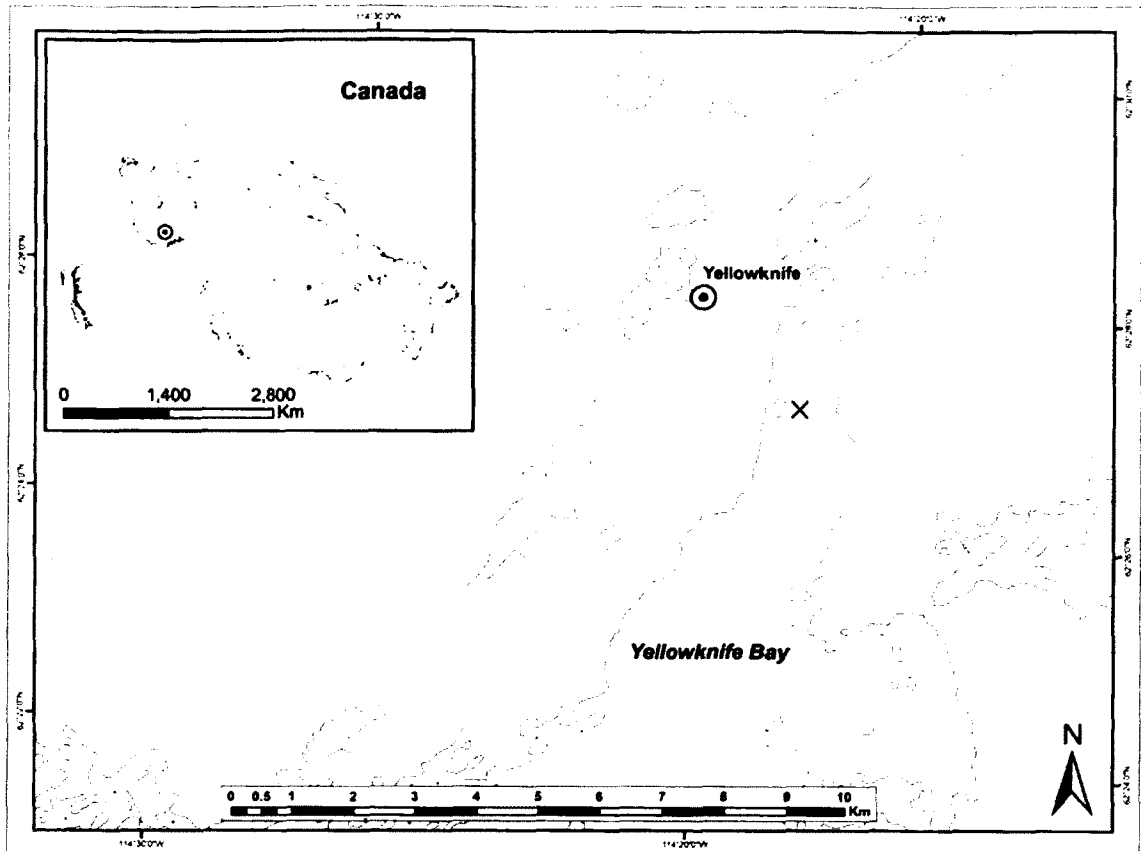


**Figure 13. The swim bladder of a Burbot (ventral view), showing drumming muscles; oe. = oesophagus, v.p.m. = vertebro-pharyngeal muscles, s.b.m. = swim bladder (drumming) muscle, s.b. = swim bladder. Illustration courtesy of A.D. Hawkins.**

## **Methods**

### *Sound production*

To assess if Burbot vocalize in association with reproduction, an experimental enclosure was populated with pre-spawn Burbot and monitored for sounds. The enclosure (hereafter the Lota-tron) was placed under the ice in Yellowknife Bay, Great Slave Lake near the City of Yellowknife, Northwest Territories, Canada (Figure 14) (Cott et al. 2012). The enclosure was a 10 m x 10 m x 10 m net pen constructed of acoustically transparent, 1” nylon mesh, that allowed for free mixing of the surrounding lake water. In Canada, Burbot typically spawn between January and March, in shallow water (0-10 m), over a variety of substrates from silt and sand to coarse gravel and cobble (Scott and Crossman 1973, McPhail and Paragamian 2000, McPhail 2007). The Lota-tron was deployed on December 8<sup>th</sup> 2009, prior to the expected spawning period, and positioned in a mean water depth of 9.5 m over a firm sandy-silt substrate intermixed with gravel and cobble. This allowed the mesh bottom to be resting directly on the substrate. The bottom of the Lota-tron was anchored in place by concrete blocks attached to the outside corners. The top corners of the Lota-tron were pulled tight and anchored in place above the ice with ice-screws. The top edge of each wall was equipped with floats that came into direct contact with the ice. This allowed the ice to freeze the tops of the walls in place, with the ice serving as a lid thus forming a 900 m<sup>3</sup> enclosure and eliminating the opportunity for Burbot to escape during the experiment. The ice thickness at Lota-tron deployment was approximately 50 cm, attaining a maximum thickness of 110 cm by the conclusion of the study.



**Figure 14. Location of the Lota-tron experiment (denoted by the “X”) in Yellowknife Bay, Great Slave Lake, NWT, Canada.**

An automated multi-channel acoustic recorder (AMAR; JASCO Applied Sciences, Victoria, BC, Canada) was deployed in the center of the Lota-tron, 1 m from the lake bottom. The AMAR was suspended from the surface and anchored to the bottom using rope to minimize mooring-related noise. A M15B hydrophone (GeoSpectrum Technologies Inc., Dartmouth, NS, Canada) with a sensitivity of -160 dBV per  $\mu\text{Pa}$  was fitted to the AMAR. A continuous recording was made at a sampling rate of 16 kHz. This configuration had a broadband noise floor of 81 dB re 1  $\mu\text{Pa}$  and a spectral noise floor of 42 dB re 1  $\mu\text{Pa}^2/\text{Hz}$  at 100 Hz. The AMAR recorded from December 15<sup>th</sup>, 2009 until retrieval on March 6<sup>th</sup>, 2010, spanning the expected spawning period for this Burbot

population. This time period was selected because sampling of Burbot from Yellowknife Bay in previous years determined that spawning had concluded by early March (Chapter 4 of this thesis).

The purpose of the Lota-tron was to house a group of captive Burbot with the expectation that these Burbot would vocalize and be recorded. In addition, it was hoped that the captive Burbot would attract other Burbot from the immediate area, as has been demonstrated previously (Paragamian et al. 2002). Burbot on the outside of the Lota-tron would be able to vocalize independently of any influence the structure may have on their behaviour. A total of 31 Burbot were collected from Yellowknife Bay near the Lota-tron using a combination of baited long lines and gillnets as per Cott et al. (2011), and deposited immediately into the Lota-tron. To minimize the risk of barotrauma to Burbot, all fishing gear was set in water depths less than 10 m (Bruesewitz et al. 1993). Thirteen of the captured Burbot were placed in the Lota-tron from December 19<sup>th</sup>, 2009 to January 13<sup>th</sup>, 2010, and the remaining 18 were collected and added in the period from February 9<sup>th</sup> to February 18<sup>th</sup>. Burbot in the Lota-tron were fed periodically with cut-up Cisco (*Coregonus artedi*). In addition, natural food sources such as amphipods and schools of ninespine stickleback (*Pungitius pungitius*) were observed (with an underwater camera) within the Lota-tron. Burbot are known to have a seasonal preference for ninespine stickleback, and feed upon them heavily during the winter (Lawler 1963). The following fish species are also known to frequent Yellowknife Bay; Lake Whitefish (*Coregonus clupeaformis*), Inconnu (*Stenodus leucichthys*), walleye (*Sander vitreus*), Northern Pike,

White Sucker (*Catostomus commersoni*), Longnose Sucker (*Catostomus catostomus*), Lake Trout, Slimy Sculpin (*Cottus cognatus*), and others (Sawatzky et al. 2007).

Post-processing of acoustic data employed a combination of manual and automated detection. The spectral density as a function of time (spectrogram) was obtained for the entirety of the recording duration. Where the spectrogram indicated periods of increased acoustic energy, manual analysis (listening to the recordings) was performed for samples of increased acoustic energy and for samples with lower acoustic energy. The initial manual analysis identified dominant sound sources, primarily cracking ice (but also noise from snowmobiles, ice-road traffic, and air traffic), and their characteristics, such as frequency, content and duration. The analysis also identified sounds of biological origin, presumed to be Burbot, and their characteristics. From this knowledge, a software zero-crossing detector was implemented to automate detection of likely Burbot vocalizations. For positive Burbot detection, a zero-crossing (time-domain waveform going from a positive value to negative or vice versa) of longer than 0.005 s and shorter than 0.3 s (and exceeding a minimum voltage) was found to detect likely Burbot vocalization while rejecting typical ice-cracking events. Follow-up manual analysis was then performed to spot-check detected events.

#### *Spawning population characteristics*

During the collection of Burbot for the Lota-tron, fish that were hooked badly or otherwise injured were sacrificed and used to assess reproductive condition of the local population and to obtain associated biological information. This included: total length ( $\pm$

1.0 mm), total body mass (total mass minus gut contents  $\pm$  1.0 g wet), sex, and state of maturity. Maturity was defined as: *green* - gonad developing or developed but gametes not free-flowing; *ripe* - mature gametes free-flowing; *spent* - gonads recently spawned-out; or *immature* - gonad immature and not developed (i.e. a fish not spawning that spawning season).

### *Sound reception*

Hearing ability of burbot was assessed in the laboratory based on auditory evoked potentials (AEP), a technique used to measure the whole brain electrical response to auditory stimuli (Corwin et al. 1982; Mann et al. 2007). Adult burbot were collected in September 2009 from Windy Lake in north-eastern Ontario (46°36' N, 81°27' W) using baited long-lines as per Cott et al. (2011). Juvenile burbot were collected from shallow water along rocky shorelines in Windy Lake using a Smith-Root Type VII backpack electrofishing unit. All fish were transported to the laboratory in aerated holding tanks (adults and juveniles separated) filled with lake water.

Auditory testing was conducted in a 1.7 m long tank placed in a sound attenuating chamber (vocalbooths.com) with ambient noise levels below 85 dB re 1  $\mu$ Pa (Belanger et al. 2010; Wright et al. 2005) with fish completely submerged at least 5 cm under the water surface. Sound stimuli (tone bursts) were delivered via an underwater speaker (UW-30 Lubell Labs) situated 75 cm from the fish. A stainless steel electrode (Rochester Electromedical) was inserted under the skin on the dorsal midline of the head above the brainstem, with reference and ground electrodes positioned as per Mann et al. (2007).



Tone bursts were generated in SigGen (Tucker-Davis Technologies, TDT) software and presented through a TDT System 3 evoked potential workstation. Each tone burst was 10 ms in duration with a 2 ms rise/fall time gated through a Hanning window. Tone bursts were presented in alternate phases (90 and 270°) with 200 presentations of each phase and responses averaged to reduce stimulus artefact, resulting in 400 traces averaged for each combination of sound level and frequency. Responses were collected and averaged in BioSig (TDT) through the TDT evoked potential workstation and stored for offline assessment of auditory thresholds for each frequency. Burbot were subject to sounds at frequencies of 100, 200, 400, 800, and 1600 Hz with an initial sound pressure level (SPL) of 140 dB re 1  $\mu$ Pa rms. Auditory threshold was determined using visual analysis of the resultant auditory waveforms as the decibel level at which the neural response was not above the background level (Wright et al. 2005, Mann et al. 2007).

To assess the potential for ontogenetic shifts in hearing ability, AEP was conducted on three different size-classes of burbot (Table 3), with the hearing ability analyzed at each frequency as per Popper et al. (2005). To facilitate comparisons with previous work, data are presented in both pressure units, and in acceleration units. Acceleration values were obtained by placing a triaxial accelerometer (Brüel & Kjær – type 4524 modified for underwater use) on the fish testing platform after trials were completed. Statistical analyses were performed with SAS<sup>®</sup> version 9.2 (SAS 2009) procedures, using analysis of variance (ANOVA), with size-class as the class variable, and SPL at a given frequency as the dependant variable, followed by a Tukey's HSD comparison of means (GLM procedure),  $\alpha = 0.5$ .

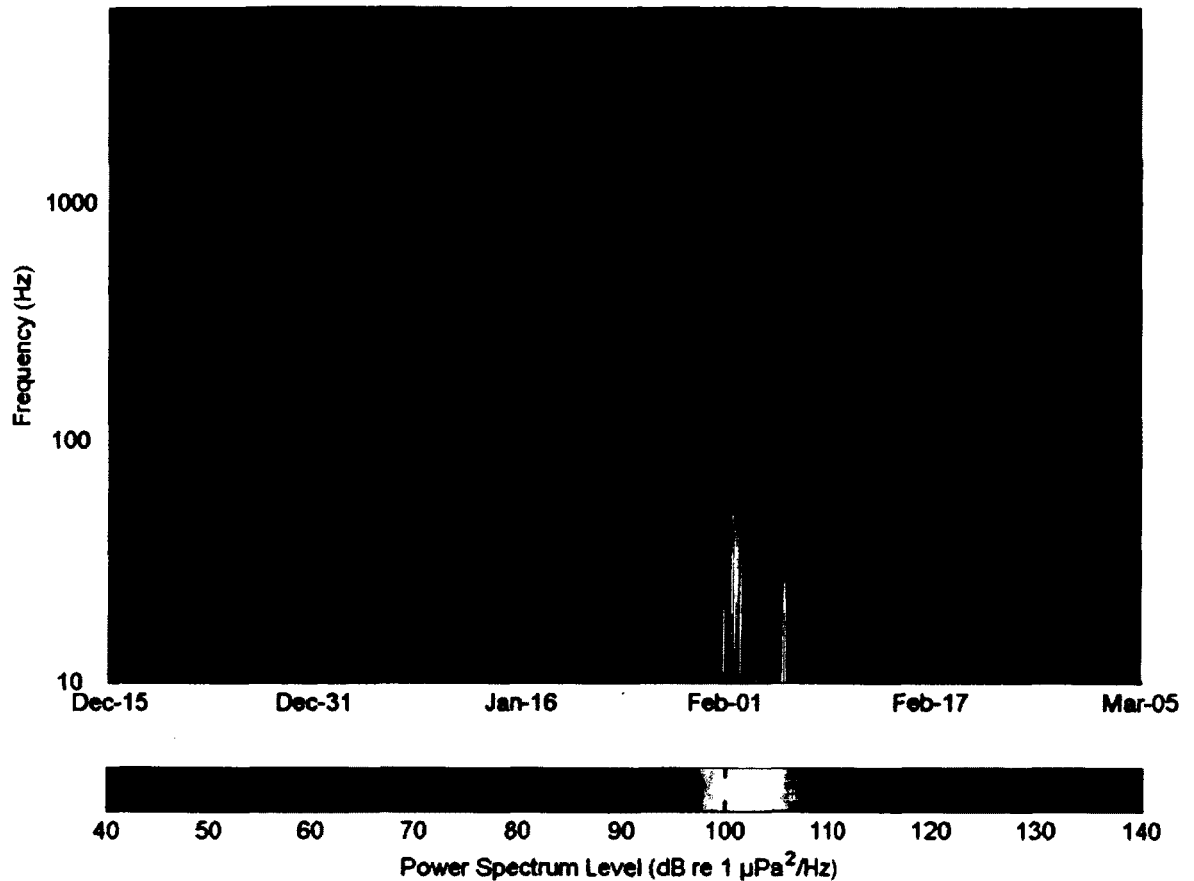
**Table 2. Summary statistics for Burbot used in auditory evoked hearing potential assessment.**

size class	n	total length (mm)			total weight (g)			age (year)		
		mean	std dev	range	mean	std dev	range	mean	std dev	range
< 10 g	5	92.0	8.4	80-102	4.5	1.2	2.6-5.8	0		0
10 - 40 g	3	160.3	26.2	136-188	23.4	10.6	14.5-35.2	1		1
> 500 g	3	495.0	38.5	457-534	709.5	197.7	566.4-935.1	12.7	3.5	9-16

## **Results**

### *Sound production in the Lota-tron*

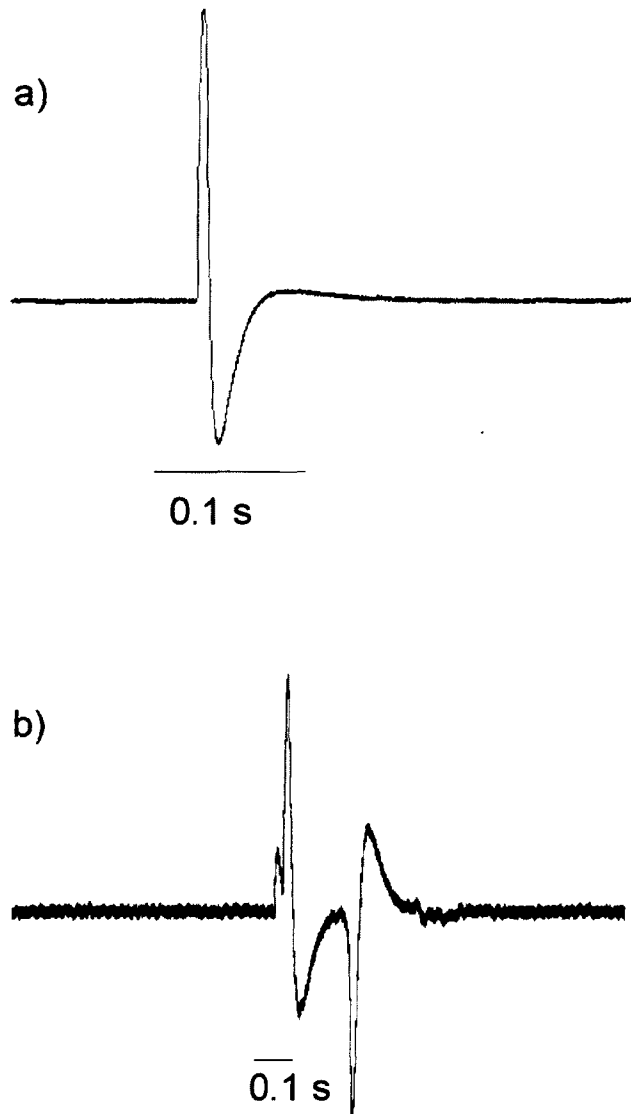
A spectrogram showing the acoustic energy throughout the recording period in the enclosure indicates a few periods of increased sound levels in early and mid February (Figure 15). Manual evaluation of these periods of increased energy found much of the acoustic energy in the brief, but intense, bout of February 1<sup>st</sup> and 2<sup>nd</sup> to clearly be of biological origin. Because the only species capable of vocalization inside the Lota-tron (and in Great Slave Lake) were Burbot, these sounds were ascribed to Burbot vocalization.



**Figure 15.** Sound energy recorded within an experimental enclosure in Yellowknife Bay, Great Slave Lake, NWT Canada, from December 15<sup>th</sup>, 2009, to March 6<sup>th</sup>, 2010.

The basic unit of sound production appeared to be a stereotyped biphasic pulse (Figure 16a). These biphasic pulses were often found singularly, with no other pulse preceding or following within a few seconds, or as double pulses with two of the stereotyped pulses closely spaced (<1s), and the second pulse (typically) inverted in phase from the first (Figure 16b). Biphasic pulses are characteristic of sounds made with a gas-filled swim bladder, such as those of Haddock (Hawkins and Amorin 2000). A sequence of these pulses constituted an individual call. Calls ranged in duration from a few seconds to close to a minute long. The duration of time between calls for an individual fish could not be

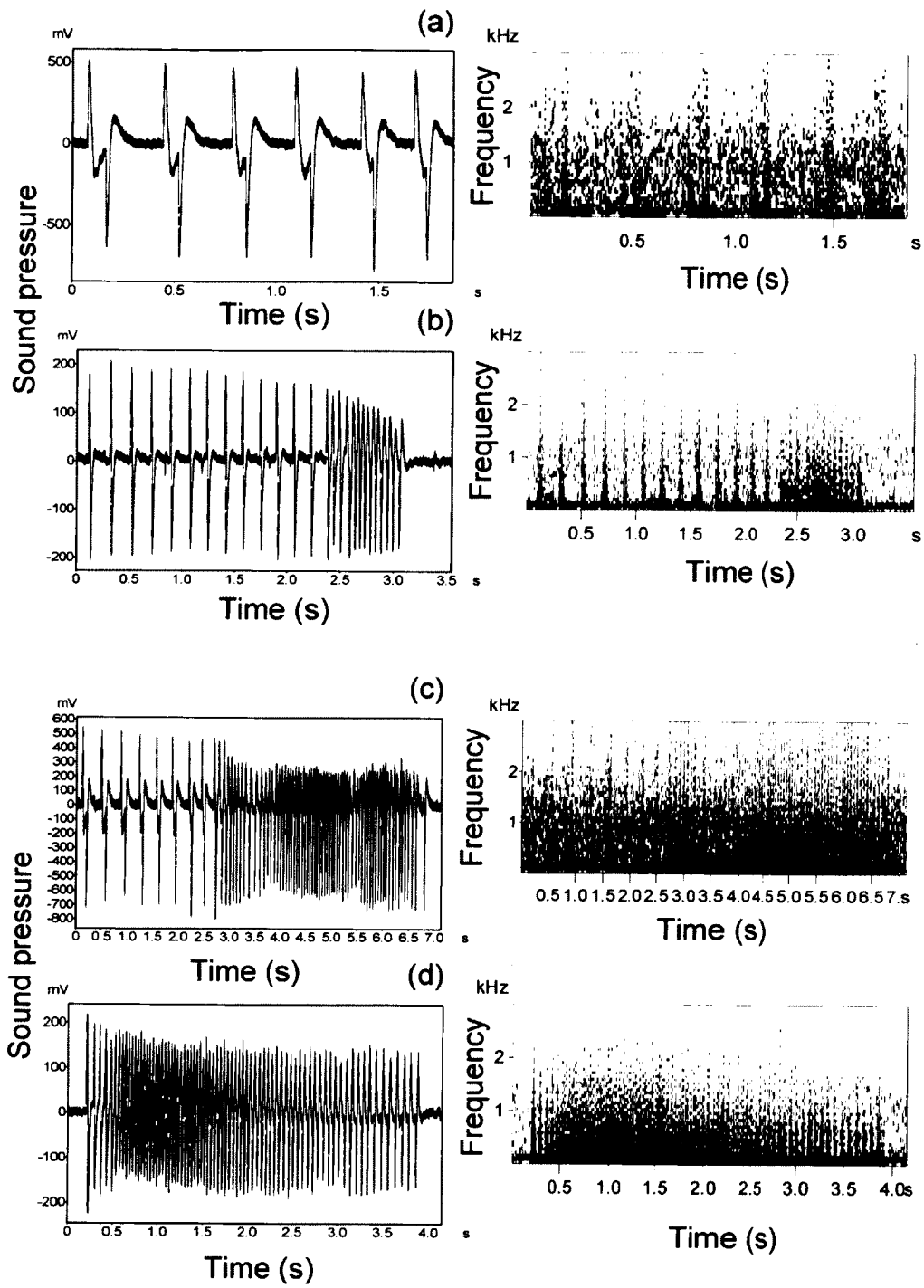
determined, as it appeared that several Burbot were calling at the same time, often with calls overlapping. Distinct, individual calls are described below and capture the variety of call types observed.



**Figure 16. The base unit of a Burbot call is a biphasic pulse (expressed as sound pressure over time) which can occur a) singly or b) doubly, with the second pulse inverted in phase from the first.**

Complex calls were observed during the bout of February 1<sup>st</sup> and 2<sup>nd</sup>. Biphasic pulses combined to form slow knocks (Figure 17a), sounding like a series of individual drum beats. During the knocks, the interpulse interval decreased briefly (increased frequency) (Figure 17b), or in a sustained manner ending in a hum (Figure 17c) until the call sounded like an engine running. In some cases the hum was present from the beginning of the call (Figure 17d). Complex buzz-like calls of longer duration with frequency modulation and sustained periods of low interpulse interval were also recorded (Figure 18). The higher-frequency portions consisted of nearly sinusoidal waveforms (Figure 18, insets) of up to 140 Hz. Burbot calls were strikingly similar to the calls of Haddock (Figure 19). For example, the call shown in Figure 18a) would be similar to a male Haddock calling alone, whereas the calls shown in Figures 18b) and c) are analogous to calls made by a male Haddock as a female or rival male approaches. The call shown in Figure 18d) is similar to that of a Haddock just prior to actual spawning (A.D. Hawkins, *pers. comm.*).

A zero-crossing auto-detector was used to search for potential Burbot vocalizations in the recorded files (see Methods). The detector rejected dominating sounds associated with typical ice cracking while detecting sounds that were manually identified as Burbot vocalization. The simple biphasic pulses, however, were difficult to distinguish from some forms of apparent ice cracking and anthropogenic sounds such as airplanes, trucks, and footsteps. Follow-up manual analysis failed to find strong evidence of Burbot calls outside of the February 1<sup>st</sup>-2<sup>nd</sup> bout, or any evidence of a diurnal pattern in calling. So, it is not clear if Burbot outside of the Lota-tron were silent or simply vocalized at a much reduced rate (and possibly amplitude) during the remainder of the recording period.



**Figure 17. A variety of Burbot vocalizations, including (a) slow knocks, (b) slow knocks with frequency upsweep, (c) slow knocks increasing in pulse speed and changing to a hum, (d) a hum call.**

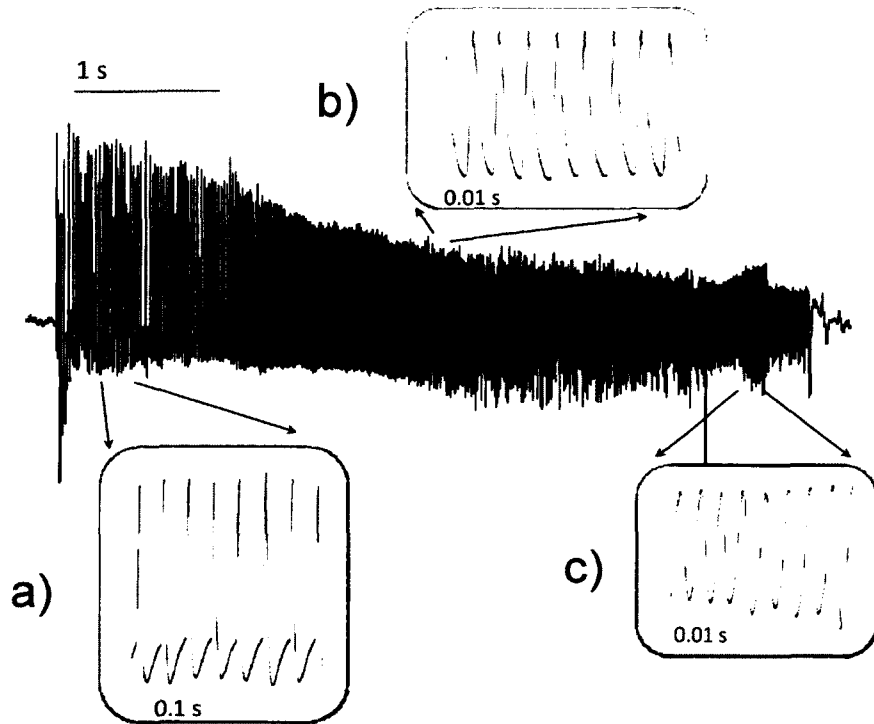


Figure 18. A Burbot buzz call, made up of a variety of different pulse types a) low frequency, b) mid-frequency, and c) nearly sinusoidal high frequency. Calls expressed as sound pressure over time.

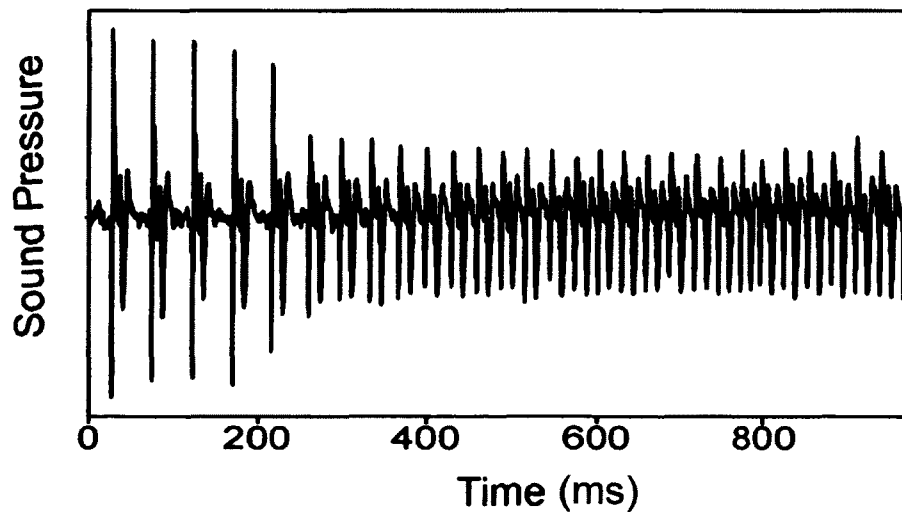
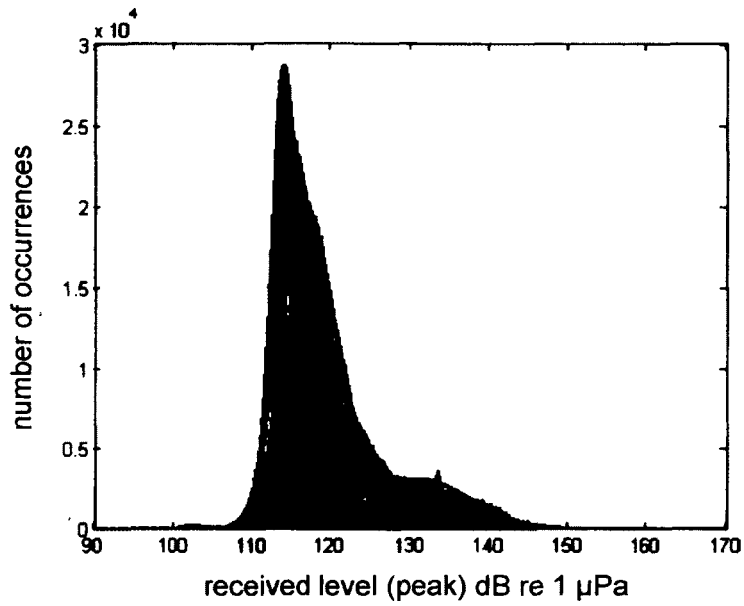


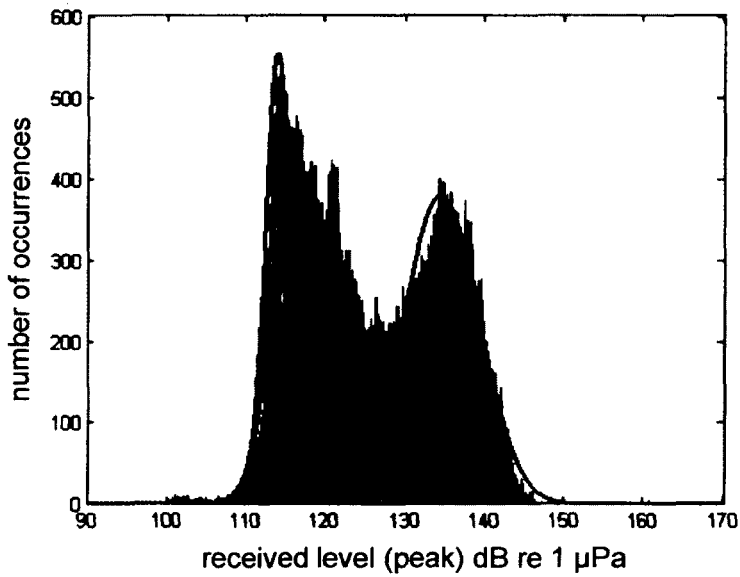
Figure 19. A typical call of a male Haddock, beginning as separate knocks then increasing in pulse speed to a hum when another male approached. This call is very similar to that of Burbot (compare to Figure 15b). Haddock waveform courtesy of A.D. Hawkins.

In an effort to determine the source level of Burbot calls, the received level of detected events was plotted as a histogram (Figure 20). It was found that three normal (Gaussian) distributions were needed to (manually) fit the received level data. The highest level mode was found at  $131 \pm 2.60$  (SD) dB re 1  $\mu$ Pa SPL, with lower level modes of  $117.15 \pm 1.84$  (SD) and  $113.65 \pm 1.09$  (SD) dB re 1  $\mu$ Pa SPL. Because it is not clear which of the auto-detected events should be ascribed to Burbot vocalizations, another histogram of received levels was plotted for the period of February 1<sup>st</sup> and 2<sup>nd</sup>, during the time that Burbot were clearly vocalizing. Again, the received level data were fit with three normal distributions (Figure 21). The same fitting parameters were used for the lower two modes,  $117.15 \pm 1.84$  (SD) and  $113.65 \pm 1.09$  (SD) dB re 1  $\mu$ Pa SPL (but scaled for the number of observations). A slightly higher SPL with a smaller standard deviation ( $134.5 \pm 2.18$  (SD) dB re 1  $\mu$ Pa SPL) was fit to the higher mode manual analysis of the February 1<sup>st</sup> and 2<sup>nd</sup> data. This was used to better determine if sounds were produced by Burbot or from some other source. Manual analysis found that higher SPL events registered by the auto-detector were Burbot, but that lower SPL events could not be distinguished from other possible sources. A conservative approach is to eliminate the lower SPL modes from consideration and only use the highest SPL mode in estimating the source level of Burbot vocalization. In this case, the received levels for Burbot calls were at  $131 \pm 2.60$  (SD) and  $134.5 \pm 2.18$  (SD) dB re 1  $\mu$ Pa SPL for the entire recording and the February 1<sup>st</sup> and 2<sup>nd</sup> period, respectively.





**Figure 20.** Frequency distribution of received levels for detected events during under-ice recording in Great Slave Lake, NWT for the period of December 15<sup>th</sup>, 2009 to March 6<sup>th</sup>, 2010. These data are well fitted by three normal distributions. White lines indicate normal distributions for individual modes, red line is combined distribution of the three modes.



**Figure 21.** Frequency distribution of received levels for detected events during under-ice recording in Great Slave Lake, NWT for the period of February 1<sup>st</sup> and 2<sup>nd</sup>, 2010. White lines indicate normal distributions for individual modes, red line is combined distribution of the three modes.

To determine the source level from the received level, transmission loss must be taken into account. With the recorder centered in the Lota-tron at 1 m from the lake bottom, and assuming the Burbot were randomly distributed, the mean distance to the recorder was 5.48 m. Using spherical spreading ( $20 \cdot \log(r)$ ) to correct for transmission loss, 14.78 dB should be added to the received level to obtain source levels of 145.78 and 149.28 dB re 1  $\mu$ Pa SPL for the entire recording and the February 1<sup>st</sup> and 2<sup>nd</sup> period, respectively. However, the cinderblocks anchoring the AMAR recorder were the only form of structure in the Lota-tron, and Burbot are known to show a high affinity for structure and each other (Edsall et al. 1993), as well as to form writhing spawning balls (Cahn 1936, McPhail and Paragamian 2000). Therefore, it is unlikely that the Burbot within the Lota-tron were randomly distributed. If, as is likely, the Burbot congregated near the anchor structure, they would have been approximately 1 m from the recorder and no transmission loss correction would be applied. From these assumptions, the source levels of Burbot vocalization recorded on February 1<sup>st</sup> and 2<sup>nd</sup> are estimated to be between  $131 \pm 2.60$  (SD) and  $134.5 \pm 2.18$  (SD) dB re 1  $\mu$ Pa SPL.

### *Spawning population characteristics*

A total of 64 Burbot captured near the enclosure site were dissected to obtain information on reproductive development and biological characteristics. Ripe Burbot were collected from mid-January to the second week of March, with spent fish appearing in the catch at the start of March. This timing was consistent for both sexes. The ratio of spawning to non-spawning Burbot was 1.1:1 which is similar to other northern Burbot populations (see Chapter 4 of this thesis). The timing of Burbot calls within the Lota-tron

corresponded to the reproductive development of the Yellowknife Bay Burbot population at large. Two fish (one male and one female) recovered from the Lota-tron on April 20<sup>th</sup>, were both spent. The Lota-tron was upset by shifting ice at spring break-up and when it was recovered on May 24<sup>th</sup>, one wall was dragged well below the water thereby liberating the remaining Burbot.

### *Sound reception*

There was evidence of an ontogenetic shift in hearing ability of Burbot; however, the direction of the shift was contrary to what was predicted. The smallest Burbot had the greatest hearing sensitivity. The hearing for all Burbot was most sensitive in the lower frequency range, with decreasing sensitivities as frequencies increased (Table 3; Figure 22). At 100, 200, and 400 Hz, the smallest size class of Burbot (juveniles, < 10 g) showed the best hearing sensitivity. At 100 and 200 Hz, adult Burbot had the second most sensitive hearing (ANOVA,  $F_{2,8} = 12.6$ ,  $P = 0.003$  and  $F_{2,8} = 16.0$ ,  $P = 0.002$ , respectively; Tukey's test,  $P < 0.05$ ). At 400 Hz, hearing sensitivity decreased with increasing size-class (ANOVA,  $F_{2,7} = 32.2$ ,  $P = 0.0003$ ; Tukey's test,  $P < 0.05$ ). There were no significant differences in auditory sensitivity among Burbot size-classes at 800 or 1600 Hz (ANOVA,  $F_{2,7} = 0.02$ ,  $P = 0.98$  and  $F_{1,2} = 0.48$ ,  $P = 0.56$ , respectively). Hearing ability for the smallest Burbot was not detected at 1600 Hz. Adult (>500 g) hearing thresholds were most sensitive at 100 and 200 Hz (mean = 131.7 dB re 1  $\mu$ Pa) (Table 3), corresponding with the vocal spectrum range of call pulses (<10 – 120 Hz). When the pulses coalesce into a buzz, the fundamental frequency ranges up to about 120 - 140 Hz. The sound produced by Burbot was < 300 Hz (Figure 15), within the most

sensitive hearing range of the species, which is also similar to the hearing sensitivities of co-occurring freshwater species (Mann et al. 2007), as well as other gadoid fishes (Chapman 1973, Chapman and Hawkins 1973, Mann et al. 2009b).

**Table 3. Auditory evoked potential measured for three size-classes of Burbot. SPL = sound pressure level. PA = particle acceleration.**

size class	n	frequency (Hz)	SPL (dB re 1 $\mu$ Pa)		PA (dB re 1 $m \cdot s^{-2}$ )	
			mean (std $\pm$ )	range	mean (std $\pm$ )	range
< 10 g	5	100	114 (7.4)	105–125	-59.3 (7.2)	-68.0–-48.6
< 10 g	5	200	118 (4.5)	115–125	-58.8 (4.3)	-61.7–-52.2
< 10 g	5	400	128 (4.5)	125–135	-50.6 (3.9)	-53.3–-44.5
< 10 g	5	800	147 (2.7)	145–150	-33.7 (2.1)	-35.3–-31.4
< 10 g	4	1600	NR	NR	NR	NR
10 - 40 g	3	100	138.3 (7.6)	130–145	-35.6 (7.4)	-43.7–-29.2
10 - 40 g	3	200	138.3 (5.8)	135–145	-39.5 (5.5)	-42.7–-33.2
10 - 40 g	3	400	143.3 (2.9)	140–145	-37.2 (2.5)	-40.1–-35.7
10 - 40 g	3	800	146.7 (2.9)	145–150	-34.0 (2.3)	-35.3–-31.4
10 - 40 g	3	1600	146.7 (10.4)	135–155	-30.0 (9.7)	-40.9–-22.3
> 500 g	3	100	131.7 (5.8)	125–135	-42.1 (5.6)	-48.6–-38.9
> 500 g	3	200	131.7 (5.8)	125–135	-45.8 (5.5)	-52.2–-42.7
> 500 g	3*	400	152.5 (3.5)	150–NR	-29.1 (3.1)	-31.3–NR
> 500 g	3*	800	147.5 (10.6)	140–NR	-33.3 (8.3)	-39.2–NR
> 500 g	3**	1600	155–NR	155–NR	-22.3	-22.3–NR

NR = no response detected, \* = no response detected from one Burbot, \*\* = no response detected from two Burbot

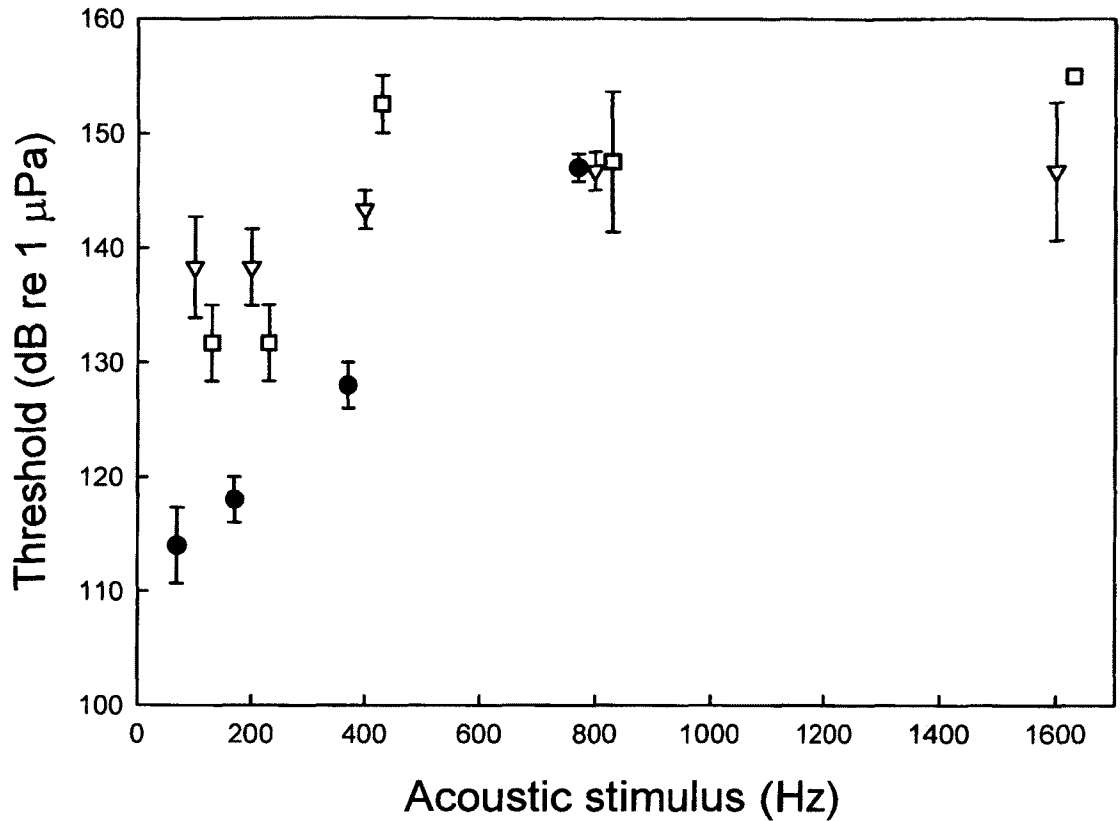


Figure 22. Auditory evoked potential (AEP) for three size classes of Burbot (Mean  $\pm$  SE). Black circles represent juvenile Burbot < 10 g. Open triangles represent juvenile Burbot from 10 to 20 g. Grey squares represent adult Burbot.

## Discussion

This study is the first to document and describe Burbot vocalizations, and the first to document calls produced by any fish species under ice-cover. Although observations of spawning behaviour were not part of this study, the bouts of calls manually identified as Burbot match the reproductive period for the population investigated. This strongly suggests that sound production is linked to Burbot reproduction, and may be an important part of their mating system. In one Burbot population investigated (Chapter 2 of this thesis), swim bladder mass (including drumming muscles) increased towards the spawning period, but there was no significant difference in swim bladder mass between

the sexes. This suggests that both sexes of Burbot vocalize, and that calling is more prevalent during the spawning season. Other codfishes use vocalizations to synchronize spawning and elicit gamete release (Hawkins 1993). In Atlantic Cod, males are the principal vocalists (Rowe and Hutchings 2008). Of the gadoid calls known, results of this study indicate that Burbot vocalizations are very similar to those of Haddock. The ability for Burbot to vocalize may be a result of needing to signal in poor light conditions under ice-cover. Acoustic signalling is used with other codfishes that spawn at depth where ambient light is limited (Hawkins and Amorin 2000). Haddock may produce the widest array of sounds among gadoid fishes (Hawkins and Amorin 2000). Burbot also appear to be loquacious with different combinations of call types identified; however, the full extent of their vocal repertoire could not be examined in the present study as the individual fish were not under direct observation.

Although little is known regarding the courtship behaviour of Burbot, with actual spawning in the wild yet to be described, it is probable that Burbot exhibit similar spawning behaviours as other codfishes. The courtship and spawning behaviour is quite stereotypical among many gadoid fish, including their postures, movements, and pigmentation changes. This holds true regardless of whether the species vocalizes or not (Hawkins and Rasmussen 1978), and seems to be the case for Burbot. For example, the only documented observation of Burbot spawning was of four pairs of captive burbot in an aquarium by Fabricius (1954). He remarked on the striking similarity of spawning courtship between Burbot and cod, particularly the manner in which males mount the females ventrally for vent to vent contact. Upon gamete release, Haddock make vigorous

tail movements presumably to assist with milt and egg mixing (Hawkins and Amorin 2000), which Burbot have also been observed to do (Fabricius 1954). For those species that do vocalize, vocalizations were associated with specific behaviours such as ventral mounting in Haddock (Hawkins and Amorin 2000), and Atlantic Cod (Brawn 1961b, Rowe and Hutchings 2006).

It has long been speculated that the reproductive activities of Burbot (e.g., mid-winter spawning) may be inherited from their marine ancestors (Cahn 1936). If so, Burbot, like other gadoids, may use vocalizations for mate selection. It has been demonstrated that mate selection is part of the mating system of Atlantic Cod (Hutchings et al. 1999, Rowe and Hutchings 2006, Skjæraasen et al. 2006b, Rowe and Hutchings 2008), and it has been speculated to be the case for Haddock as well (Hawkins and Amorin 2000). Calling may be a means to assess the size and fitness of a prospective mate. Further, Burbot are known to congregate at spawning sites, often in large numbers (Cahn 1936, Fabricius 1954, Weber 1976, McPhail 2007, Holm et al. 2010). If Burbot reproductive behaviour is consistent with other gadoids, these spawning congregations may be a form of lekking that may allow females to discriminate between males. Lekking has been proposed as part of the mating system of Atlantic Cod (Nordeide and Folstad 2000) and Haddock (Hawkins and Amorin 2000).

Vocalizations associated with agonistic behaviour are prevalent in Atlantic Cod (Brawn 1961a, Rowe and Hutchings 2006) and Haddock (Hawkins and Amorin 2000). These calls are characteristically short, low, grunts in Atlantic Cod (Rowe and Hutchings 2006)

and knocks or humming in Haddock (Hawkins and Amorin 2000). In contrast, of the few observations of Burbot behaviour, it has been noted that adult Burbot are not aggressive towards one another. In observations of free swimming Burbot (outside of the spawning season), Edsall et al. (1993) remarked that Burbot were often found in pairs or small groups, and usually in physical contact with one another. This affinity towards other Burbot is pronounced in the onset of spawning season when Burbot have been observed to form writhing spawning balls of many individuals (Cahn 1936, McPhail and Paragamian 2000, Holm et al. 2010). In observations of captive spawning Burbot, Fabricius (1954) was surprised by the complete lack of aggressive behaviour among males in the aquarium, even when in the presence of reproductively receptive females. Also, the enormous gonadal investment by males, at times exceeding that of females, (Chapters 2 and 4 of this thesis) is suggestive of intense sperm competition (Stockley et al. 1997) but low intrasexual competition (Taborsky 1998) as there would have to be a trade-off between testes production and aggressive behaviour.

The current study is the first to document and describe the hearing ability of adult Burbot and one of only two (see Mann et al. 2007) that have examined hearing in this species at all. The hearing thresholds measured for adult Burbot were most sensitive at the frequencies of their sound production (Cott 2013), and similar to the hearing abilities of other gadoid fishes (Chapman 1973; Chapman and Hawkins 1973; Mann et al. 2009b). Auditory tests (such as in this study) that are performed in controlled laboratories where ambient noise levels are lower than that of a natural environment, and as such, physiological hearing thresholds - compared to behavioural trials - should be seen as an



estimate of the potential hearing ability for the species, with actual hearing abilities of fish varying among environments with differing ambient conditions (Belanger et al. 2010). The hearing measured for Ling, and other codfishes by Chapman (1973) and Chapman and Hawkins (1973) was more sensitive than that found for Burbot. This may be a result of differences in hearing ability among these fishes or differences in the experimental approach or technology used among the studies. However, caution should be exercised when comparing the hearing sensitivities for fishes among studies.

What was unexpected was that the juvenile Burbot had more sensitive hearing than adults. It has been speculated that juvenile fish may have less sensitive hearing than adult fish because their auditory systems develop during maturation (Popper et al. 2005). However, for species with well developed hearing abilities, such as Round Gobies *Neogobius melanostomus*, hearing can be most sensitive as young and decrease with size (Belanger et al. 2010), as was the case with Burbot in this study. Ontogenetic differences in habitat use between juveniles and adults offer a possible explanation for the direction of the ontogenetic shift in hearing ability. Juveniles are found in shallow water near the shoreline and are highly associated with cover (Ryder and Pesendorfer 1992; Stewart and Watkinson 2004), as they are readily preyed upon by a variety of piscivorous fishes (Amundsen et al. 2003; Ryder and Pesendorfer 1992). Perhaps young Burbot need more sensitive hearing to avoid predation. In contrast, adult burbot generally reside in the offshore benthic zone (Scott and Crossman 1973; Stewart and Watkinson 2004), and would be at little risk of predation. Fabricius (1954) observed in aquaria, young-of-the-year Burbot “fighting vigorously” over their respective hiding spots. Such aggressive

intraspecific behaviour may be accompanied with agonistic calls. Some codfishes, such as the Lythe *Pollachius pollachius*, are known to vocalize as juveniles and adults, whereas some species may only vocalize as juveniles. For instance, the Saithe *Pollachius virens* has drumming muscles as juveniles but these are absent as adults (Hawkins and Rasmussen 1978). The assessing if young Burbot vocalize is a topic for future study.

The only other documented hearing threshold for Burbot was reported by Mann et al. (2007), where the hearing potential for a single 38 mm, (0.3 g) young-of-the-year Burbot was assessed. Given its size (smaller than the smallest Burbot assessed in this study), this Burbot would probably have only recently switched from a pelagic to benthic existence (McPhail and Paragamian 2000; Ryder and Pesendorfer 1992). The AEP of the Burbot in Mann et al. (2007) was more sensitive than the Burbot examined in the current study. This ontogenetic trend of decreased hearing sensitivity is not unprecedented among the gadoid fishes. Walleye Pollock *Theragra chalcogramma* have decreased hearing sensitivity with increasing age, despite having larger inner ear size, and like Burbot, all age classes had better hearing at lower frequencies (Mann et al. 2009b).

The under-ice soundscape at the time of Burbot spawning vocalizations is relatively quiet. With the absence of wave and storm noises, ice cracks are among the only forms of natural noise (Mann et al. 2009a). Northern resource developments are often conducted during winter over frozen lakes and rivers because of the ability to make ice roads to access otherwise inaccessible and remote areas (Cott et al. 2008, Mann et al. 2009a). These activities generate sounds that infiltrate under-ice environments changing the

soundscapes of aquatic ecosystems. Such sounds may have the potential to disturb fishes (Mann et al. 2009a). Ice-cracks can be quite loud, but the difference between natural noises like ice cracks and anthropogenic noises is that the former are transient and the latter persist (Mann et al. 2009a). Persistent anthropogenic noises that are above the normal ambient conditions of the environment can raise the hearing threshold requirement for signal detection by a given species, and may render some low level sounds undetectable (Hawkins 1993, Fay 2012). Because Burbot spawn in the winter, under-ice noise produced by industrial activities may disturb them directly or may mask their spawning vocalizations, reducing their ability to find each other, and possibly disrupt their spawning activities. Finstad and Nordeide (2004) suggested that noise from commercial fishing activities may disturb spawning vocalizations and behaviour of Atlantic Cod. Such disruptions may interfere with mate selection, spawning success, or egg viability (Rowe and Hutchings 2006). Anthropogenic noise also has the potential to physically harm fishes (Popper and Hawkins 2012). For example, noise from air-gun generated seismic activities have been shown to cause temporary (Popper et al. 2005) or permanent (McCauley et al. 2003) hearing loss in fishes depending on species and severity of exposure.

The current study adds considerable new information but still underscores how little is known about the sensory ecology of fishes, particularly for those species - such as Burbot – that have peculiar life histories, or live in remote areas. Such fishes may be vulnerable to anthropogenic noise impacts in ways that are not anticipated. For example, with the warming trend in the Arctic, the rates of shipping activity and oil and gas exploration will

continue to increase (Hassol 2004). Increased anthropogenic noise caused by industrial activity is likely to alter the under-ice soundscapes of marine environments that would otherwise be relatively quiet (Blackwell et al. 2004). Arctic Cod (*Boreogadus saida*) and Greenland Cod (*Gadus ogac*) are known to possess drumming muscles (Hawkins and Rasmussen 1978), and it is possible that other Arctic-dwelling cods do also. These cods may rely on acoustic signalling and may be negatively impacted by changes in their underwater soundscapes. These arctic dwelling cods, Arctic Cod in particular, are a critical component of Arctic marine food webs. Arctic cod are the primary consumer of plankton in Arctic waters, and in turn they are consumed by a variety of animals from sea birds to whales (Scott and Scott 1988). Disruption to the reproductive success of Arctic Cod could have disastrous effects on delicate Arctic ecosystems.

The current study confirms that Burbot vocalize and that the timing of their vocalizations is within their spawning period. It is therefore likely that acoustic signalling is part of the mating systems of Burbot as it is for Atlantic Cod and Haddock. Among-population variation in sound production of Atlantic Cod has been documented (Rowe and Hutchings 2006), and it is possible that this would be the case for Burbot as well. This information has implications for understanding the reproductive and sensory ecology of other gadoid fishes. If the mating systems for Burbot, Atlantic Cod, and Haddock are used as a model for gadoid fishes in general, then it may be useful in predicting the reproductive ecology of other elusive cod species such as those in the Arctic. As Burbot call in association with reproduction, like some other species of codfishes, passive acoustics may be a useful, cost effective, and non-lethal means for determining the timing and location of spawning of

vocal codfishes that would otherwise be difficult to sample by conventional means. The under-ice spawning habits of Burbot make direct observation of spawning behaviour difficult, but this coupled with associated sound production, warrants further investigation. Understanding the sensory abilities quantified in the current study are vital to assessing the impacts of anthropogenic noise issues in light of increasing northern ice-based development, particularly for a winter spawning fish.

## **CHAPTER 4 - Life history stability among Burbot populations across environmental gradients**

### **Introduction**

The study of life history traits is of fundamental ecological interest (Cole 1954). Theory suggests that life history patterns develop as adaptive strategies to environmental conditions (Cole 1954, Stearns 1976, Roff 1984, Reznick et al. 1990, Winemiller and Rose 1992). Variation in environmental conditions, be it natural or anthropogenic, can influence fish life history traits such as age or size at maturity, and growth, and this can translate into broad shifts in population structure and dynamics (Rahel 1984, Persson et al. 1991, Holmgren and Appleberg 2000, Post et al. 2000, Olsen et al. 2004, Hutchings et al. 2012). Where an animal resides along an environmental gradient can have profound influences on key life history attributes such as sex determination (Lagomarsino and Conover 1993) and overall reproductive strategy (Leggett and Carscadden 1978). Successful reproduction is central to the viability of species, but is also energetically taxing on the individual (Cole 1954, Stearns 1976, Andersson 1994).

For fish, reproduction requires trade-offs between spawner survival and gamete production (Cole 1954, Stearns 1976, Leggett and Carscadden 1978, Roff 1984, Winemiller and Rose 1992, Hutchings 1993, 2005). Species with large clutches often reproduce in fewer but more synchronous periodic episodes (i.e., spawning occurring at the same time within a given year, but with individuals spawning over several years) (Winemiller and Rose 1992), as a means to optimize maternal fitness in the face of variable environmental conditions (Cole 1954, Stearns 1976, Van Winkle et al. 1993).

The higher costs of reproduction trade-off with adult survival and result in earlier maturity, higher mortality, and shorter life span (Hutchings 2005). Periodic spawning allows adult survival over suboptimal conditions (Vila-Gipert et al. 2002) by spreading reproductive effort over many years to compensate for bad years of poor larval or juvenile success (Winemiller and Rose 1992).

Environmental gradients that are known to influence life history patterns and reproductive ecology of fishes include latitude, waterbody productivity, and ecosystem size (e.g., lake size). Water temperature and photoperiod change dramatically with latitude, and at higher latitudes temperature and photoperiod increase in importance (Pankhurst and Porter 2003), making latitude an important determinant of reproductive ecology. As latitude increases growing seasons become shorter, spring and summer photoperiods increase, winters become longer and fish communities become less diverse (Holmgren and Appleberg 2000). These changes may be associated with reductions in both fecundity (Leggett and Carscadden 1978) and the duration of the spawning period (Gotelli and Pyron 1991, Conover 1992), as well as increased age at maturity (Gotelli and Pyron 1991). It has been suggested that variation in egg and larval survival, year-class strength, and recruitment also increase with increasing latitude (Leggett and Carscadden 1978). The effects of photoperiod and temperature on these life history traits are well documented for many teleost species, but the mechanisms remain uncertain (Pankhurst and Porter 2003).

With increased productivity, water clarity can be reduced and can influence the habitat availability and foraging ability of fishes, and the overall community composition of the system (Bergman 1991, Persson et al. 1991). The general relationship between lake area and depth is linear (Vadeboncoeur et al. 2008), and lake size is inversely correlated with water clarity (Fee et al. 1996, Vadeboncoeur et al. 2008), so trends seen with productivity gradients will often be opposite of those seen in lake-size gradients.

In order to better assess the generality of environmental trends in life history strategies, a more complete understanding of the reproductive ecology for a wider range of species is required (Vila-Gipert et al. 2002). It is well known that latitude influences the reproductive activities of many spring and fall-spawning fishes occurring in temperate and arctic North America (Scott and Crossman 1973), but what influence does latitude have on fishes that spawn in the middle of winter? And what other environmental conditions may influence the life history traits of a mid-winter spawner? Unlike spring- and fall-spawning freshwater fish species, the influence that environmental gradients have on a mid-winter spawner has yet to be explored. In addition, few studies have examined the influence of multiple environmental variables on several life history and reproductive traits (Conover et al. 2009).

Winter is a difficult time for many lake-dwelling fishes due to reduced food availability, light, oxygen, and temperature. However, despite its harshness, the under-ice environment of winter is quite predictable and stable. Burbot are an interesting candidate to study the influence of latitude and other environmental variables on life history and



reproductive traits for a number of reasons. They are the only mid-winter spawning freshwater fish in Canada, belonging to a diverse but otherwise entirely marine-dwelling family of fishes, Gadidae (Cohen et al. 1990). Burbot have an enormous geographic range, spanning 40° of latitude to the Arctic coast in North America (Scott and Crossman 1973, Cohen et al. 1990, Elmer et al. 2008, Stapanian et al. 2010), and occurring in a wide variety of freshwater ecosystem types and sizes (Scott and Crossman 1973, McPhail and Paragamian 2000) making a comparison of the life history and reproductive traits possible across wide environmental gradients for this species.

The primary objective of this study is to explore patterns and test predictions for selected life history and reproductive traits, using Burbot populations in boreal lakes as a model, with respect to environmental conditions across a wide spatial scale. A virtue of using Burbot from boreal lakes in Canada is that they are largely unexploited populations (Stapanian et al. 2010). Exploitation can confound the patterns that display the interactions of life history traits with the environment making the drivers of change difficult to discern (Olsen et al. 2004). It is hypothesized that variation in life history traits among populations will be primarily related to climate, ecosystem size, and ecosystem productivity. When resources are low, selection pressure favours fewer, larger young (Stearns 1976). Egg size and gonad size are predicted to increase with latitude, to produce larger offspring as a means to compensate for the shorter growing season in northern systems.

It is predicted that Burbot growth will decline as average annual temperatures decline from south to north across their range; this pattern has been observed in other fish species (e.g., Braaten and Guy 2002). Due to the environmental stability of their spawning period throughout their range, lake productivity should have more influence on reproductive traits than latitude or lake size, with more productive environments allowing for greater growth and gonadal development and demanding less energy storage. It is predicted that growth rate and GSI will be positively related to waterbody productivity, and that HSI will be negatively related to productivity. Lake size can influence food-chain length (Post et al. 2000), and community composition (Tessier and Woodruff 2002) due to increased heterogeneity of habitats (Rahel 1984). However, the relative size of lakes is also inversely related to productivity (Vadeboncoeur et al. 2008), therefore, it is predicted that traits compared to lake size will have inverse trends to those compared to productivity.

These predictions were tested by conducting analyses separately for each sex. In doing so, a secondary objective was met – to determine if the sex-related differences observed in the Windy Lake Burbot population (Chapter 2) are consistent across a wider range of Burbot populations. Burbot exhibit very little phenotypic variation across their range (Van Houdt 2003, Van Houdt et al. 2005) relative to co-occurring species such as Lake Trout (Krueger and Ihssen 1995, Blackie et al. 2003, Zimmerman et al. 2006, 2007). It is predicted that the sex-based differences observed in Windy Lake will also be evident in other Burbot populations.

## Methods

### *Site description and field sampling*

Burbot were collected from lakes across a spatial gradient spanning 17 degrees in latitude from Lake Manitou, in Ontario, to Drygeese Lake in the NWT (Table 4, Figure 23).

There is limited directed harvest of Burbot over this range but they are part of the bycatch of commercial and recreational fishers. Sampling was conducted from 2006 to 2012, during early January to early March. For most populations, Burbot were sampled with overnight sets of baited longlines deployed beneath the ice using a jigger board. For some populations, Burbot were captured by angling or sampled from the gill net by-catch of commercial fishermen. Burbot were killed with a sharp blow to the back of the head, packed on ice, and transported to the laboratory for processing. For some populations, Burbot were frozen whole and processed at a later date.

### *Laboratory analysis*

Frozen Burbot were thawed at room temperature prior to processing, but whole ovaries were always removed from females in a frozen state to ensure that eggs remained intact prior to freeze-drying (see below). All Burbot were processed to obtain the following information: total length ( $\pm 1.0$  mm), total body mass ( $\pm 1.0$  g wet), whole gonad mass ( $\pm 0.1$  g wet), somatic mass (total mass minus gonads;  $\pm 1.0$  g wet), liver mass ( $\pm 0.1$  g wet), sex, and state of maturity. Maturity was defined as: *immature* - gonad immature and not developing towards the next spawning period; *green* - gonad developing towards next spawning event but gametes not yet free-flowing; *ripe* - mature gametes free-flowing; *spent* - gonads recently spawned-out; or *unknown* - unable to determine sexual maturity.

Time of spawning for a given population was defined as the period between when the first ripe or spent female was captured, and when only spent fish of both sexes were captured. Gut contents were removed from each fish before determination of body mass. For green females, a subsample (~ 30 g) from the mid-section of the ovaries was removed, placed in a small plastic bag and frozen at -20 °C. Sagittal otoliths were removed, rubbed clean, placed in plastic vials and allowed to air-dry. Ovary subsamples were freeze-dried for seven days and mean egg size was determined by counting and weighing subsamples of the freeze-dried ova. Otoliths were prepared following the crack-and-burn technique (Edwards et al. 2011) and ages were determined by counting annuli on the burned surface under a dissecting scope using reflected light. The outer edge of the otolith was always counted as a complete annulus because fish were collected near the time of spawning.

Environmental variables included indices of climate, ecosystem size, and productivity. The correlation between latitude and accumulated growing degree days is strong and negative (Johnston and Leggett 2002), making latitude a good analog to thermal and photoperiod gradients, and therefore growing season. Waterbody productivity was chosen as more productive lakes generally having more zooplankton which is a key component of the diet of Burbot larvae (Ryder and Pesendorfer 1992, McPhail 2007). Water clarity is highly and inversely correlated with lake productivity (Wetzel 1983) and Secchi depth is a good index of relative productivity (e.g., Vander Zanden et al. 1999, Post et al. 2000, Johnston and Leggett 2002). Surface area and mean depth were used as indices of lake size.

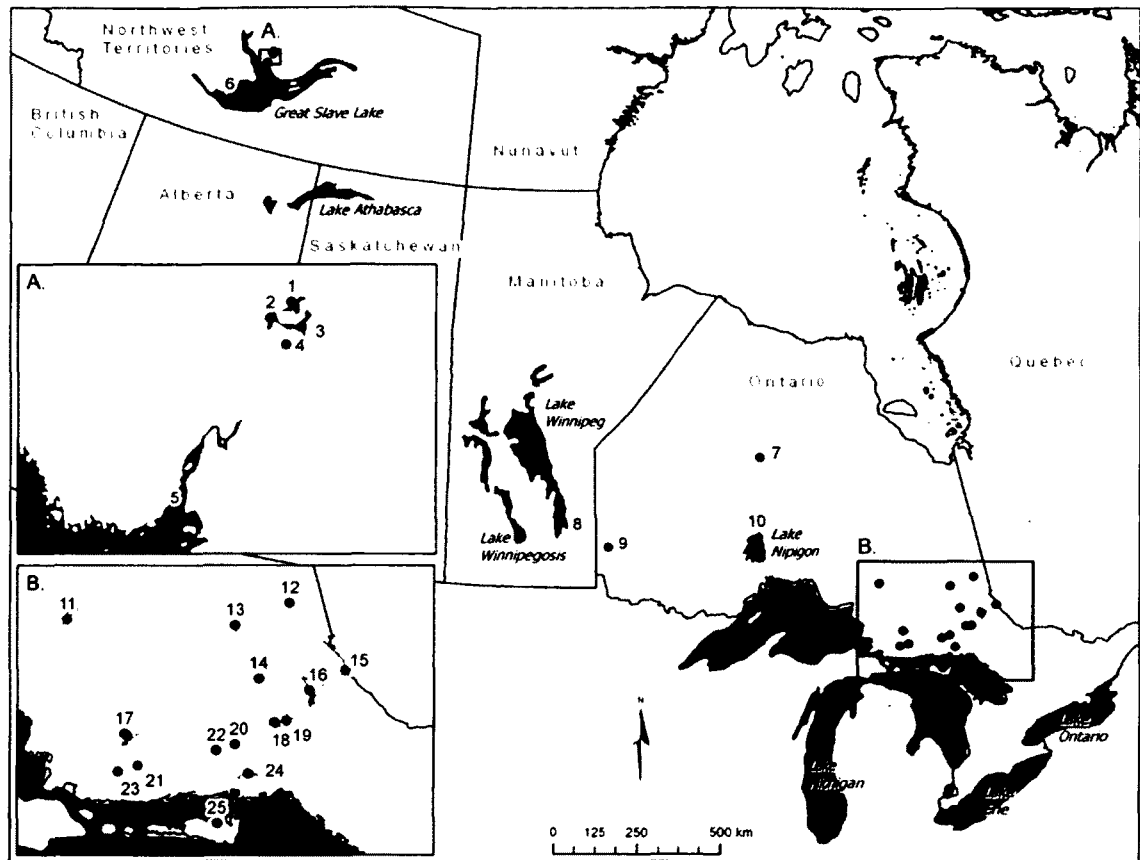
### *Data analysis*

Gonadosomatic index (GSI) was estimated as the whole gonad mass of pre-spawn Burbot divided by somatic mass, and hepatosomatic index (HSI) was estimated as liver mass divided by somatic mass. For the assessment of the proportion of the adult fish that were spawning, only adult females collected during the spawning period (January and February) were considered. The maturity of females was assigned as outlined above. Females with gonads < 2.0 g were deemed reproductively immature and excluded from the analysis of the proportion of spawning fish. Variables expressed as proportions were arcsine-square-root transformed, and other variables were  $\log_e$ -transformed, as required, to normalize residuals or to linearize relationships. All statistical analyses were performed with SAS<sup>®</sup> version 9.2 (SAS 2009) procedures. Growth was examined by fitting length at age data to a von Bertalanffy model (Ricker 1975, Lester et al. 2004) using non-linear least-squares (NLIN procedure). Predicted mean sizes at age seven and ten were derived from these models and used as the estimates of growth rate. These ages were chosen to ensure that only fish of reproductive age were assessed, as Burbot in slow-growing northern populations reach sexual maturity by age seven (Chen 1969). To facilitate comparisons within and among populations all data were adjusted to a common representative body size of 500 g where somatic mass was used as a covariate and 435 mm when total length was used as a covariate. In each population, adjusted means (least-square means) were estimated for each sex using an ANCOVA model (GLM procedure) with sex as the class variable and body size as the covariate. To test whether sex-based differences observed in the intensively-sampled Windy Lake population (Chapter 2) were consistent across Burbot populations, paired-comparisons t-tests for each life history trait

(growth rate, GSI, HSI, etc.) were conducted using the adjusted population means. Data were pooled for subsequent analyses where no significant differences between the sexes were found. To determine how these life history traits varied with respect to environmental conditions each dependent variable was regressed against the environmental variables using a multiple regression model with a stepwise selection procedure (REG procedure).

**Table 4. Location and physical characteristics of lakes where Burbot were sampled across Canada. Numbers in parentheses correspond to lake numbers on Figure 21.**

Lake	Location	Latitude ° N	Longitude ° W	Surface Area (ha)	Max. Depth (m)	Secchi (m)
Drygeese (1)	NWT	62.73	114.17	547	35	8.2
Baptiste (2)	NWT	62.70	114.22	365	32	5.5
Chitty (3)	NWT	62.70	114.12	305	20	5.0
Alexie (4)	NWT	62.67	114.15	420	32	7.5
Great Slave - YK Bay (5)	NWT	62.40	114.33	2856800	614	5.0
Great Slave - Slave Point (6)	NWT	61.13	115.83	2856800	614	5.0
Attawapiskat (7)	Ontario	52.30	87.90	28100	6	1.5
Winnipeg - South Basin (8)	Manitoba	50.80	96.75	2438700	28	0.8
Big Sand (9)	Ontario	50.11	94.63	7965	62	4.3
Nipigon (10)	Ontario	49.83	88.50	448060	137	6.5
Missinaibi (11)	Ontario	48.36	83.69	7707	94	4.2
Round (12)	Ontario	48.02	80.04	1213	36	1.5
Midlothian (13)	Ontario	47.91	81.00	367	32	4.6
Stull (14)	Ontario	47.26	80.82	257	34	5.0
Temiskaming (15)	Ontario	47.45	79.57	29484	214	0.9
Temagami (16)	Ontario	47.01	80.08	20972	110	10.7
Peshu (17)	Ontario	46.97	83.14	389	51	6.1
Wanapitei (18)	Ontario	46.75	80.75	13257	142	5.8
Kukagami (19)	Ontario	46.73	80.55	1865	55	8.0
Windy (20)	Ontario	46.60	81.44	1111	66	5.1
Endikai (21)	Ontario	46.59	83.03	592	48	5.8
Fox (22)	Ontario	46.58	81.75	471	42	4.4
Wakomata (23)	Ontario	46.57	83.36	2469	73	8.4
Panache (24)	Ontario	46.25	81.33	8034	55	6.9
Manitou (25)	Ontario	45.78	81.98	10461	49	8.4



**Figure 23.** Locations of lakes where Burbot were sampled across Canada. Numbers correspond to lakes identified in Table 4.

## Results

### *Sex-based differences*

There were no significant sex-based differences found in reproductive traits of Burbot among the 25 populations. Gonad size, expressed as GSI, ranged from 3.9% to 27.5% of somatic mass. In some populations the mean GSI of females surpassed that of males, and vice versa in others (Figure 24), but overall there was no significant difference between sexes for GSI across populations (paired-comparisons *t*-test,  $t = 0.37$ ,  $n = 19$ ,  $P = 0.71$ ). Similarly, liver size (expressed as HSI) was highly variable among populations (Figure

25), but there was no significant difference between sexes for HSI for the populations analysed (paired-comparisons  $t$ -test,  $t = 0.36$ ,  $n = 22$ ,  $P = 0.72$ ).

The von Bertalanffy model fit the growth trajectory well for both sexes of Burbot for most populations. Across populations, there was no significant difference between sexes for length at age seven (paired-comparisons  $t$ -test,  $t = 1.41$ ,  $n = 21$ ,  $P = 0.17$ ) or age 10 (paired-comparisons  $t$ -test,  $t = 0.68$ ,  $n = 21$ ,  $P = 0.50$ ). For most populations of Burbot spawning took place during February regardless of location.

#### *Environmental gradient based differences*

No statistically significant trends were observed between GSI and any of the environmental variables (latitude, secchi, lake area, or lake depth), with a only a very weak, positive but non-significant relationship observed between GSI and log-transformed latitude (regression analysis,  $F_{1,19} = 1.00$ ,  $P = 0.33$ ,  $r^2 = 0.05$ ). The relative liver size (HSI) of Burbot had a weak positive relationship with latitude (regression analysis,  $F_{1,22} = 4.76$ ,  $P = 0.04$ ,  $r^2 = 0.18$ ).

Of the traits investigated, egg size showed the strongest relationship with a metric of lake size. Egg dry mass increased with log lake surface area (regression analysis,  $F_{1,11} = 26.26$ ,  $P = 0.0003$ ), with 70% of the variation explained (Figure 26), as well as log lake depth ( $F_{1,11} = 6.07$ ,  $P = 0.03$ ;  $r^2 = 0.82$ ). The proportion of females that were in reproductive condition (i.e. green or ripe) was highest in productive lakes ( $r^2 = 0.15$ ) (Figure 27), but this relationship was not significant (regression analysis,  $F_{1,22} = 3.00$ ,  $P$



= 0.10). The same general pattern seen for egg size and percent spawners was observed for Burbot growth. There was a slight positive trend between Burbot growth (size at age seven) and productivity ( $r^2 = 0.15$ ), and a negative trend for size at age ten and log latitude ( $r^2 = 0.13$ ). However, neither of these relationships were statistically significant (regression analysis,  $F_{1,19} = 3.39$ ,  $P = 0.08$  and  $F_{1,19} = 2.81$ ,  $P = 0.11$ , respectively).

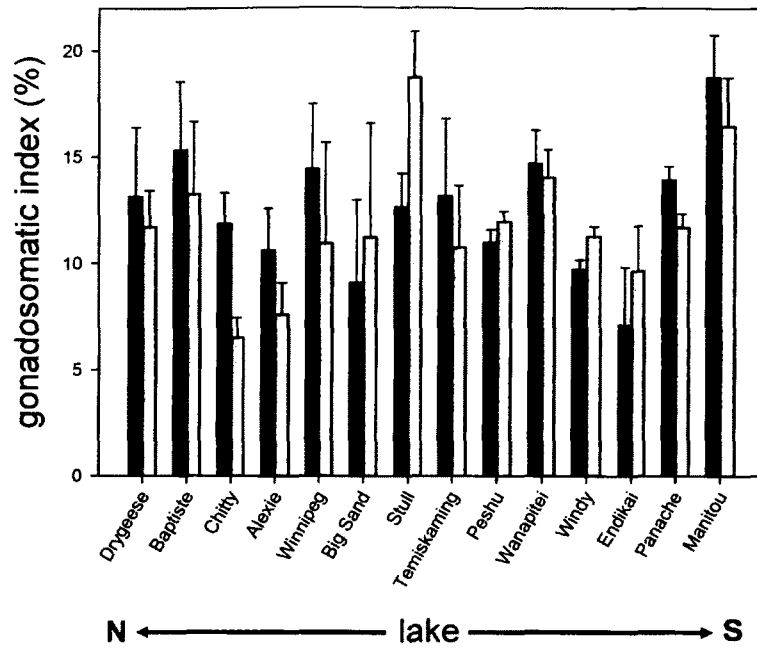


Figure 24. Gonadosomatic index of Burbot from lake dwelling populations across Canada. Bars represent body size-adjusted means ( $\pm$  SE), with black bars for female Burbot and grey bars for male Burbot. Only populations where data for the trait examined were available are presented.

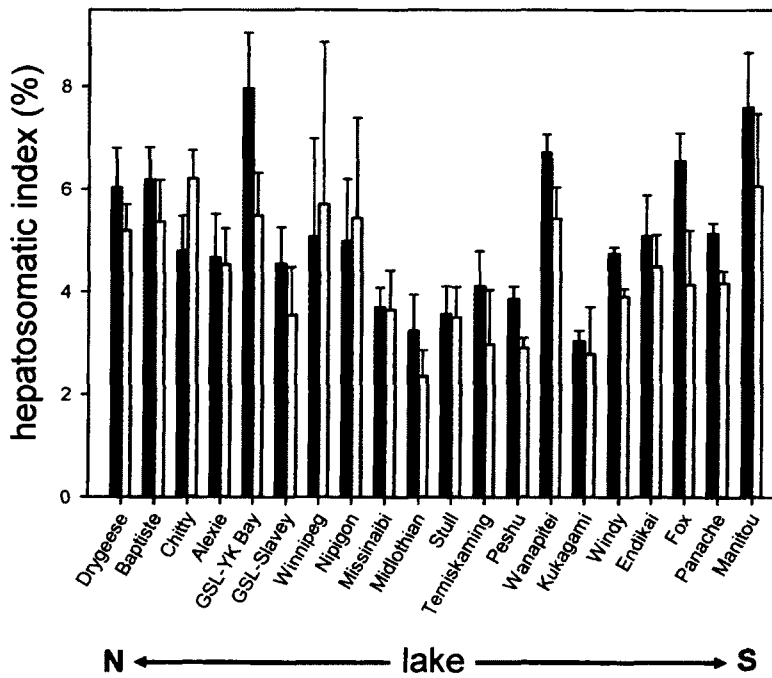


Figure 25 Hepatosomatic index of Burbot from lake dwelling populations across Canada. Bars represent body size-adjusted means ( $\pm$  SE), with black bars for female Burbot and grey bars for male Burbot. Only populations where data for the trait examined were available are presented.

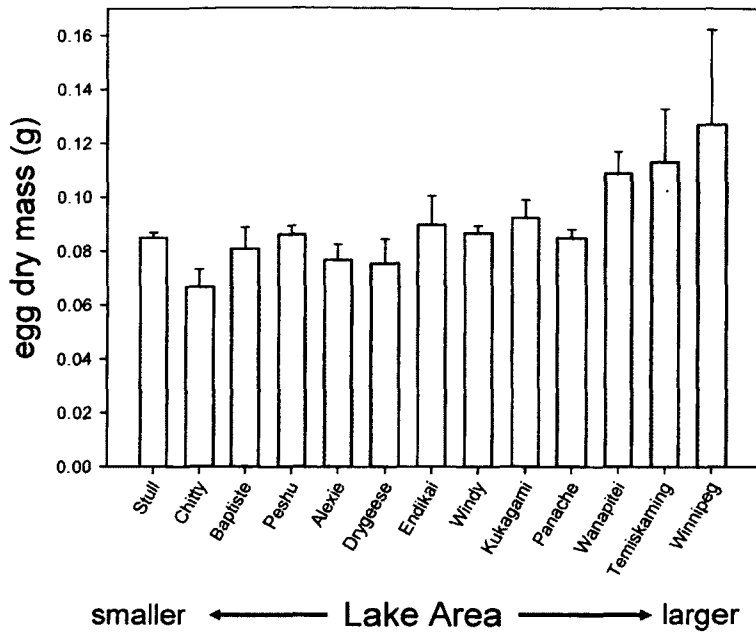


Figure 26. Egg dry mass of Burbot from lake dwelling populations across Canada contrasted against ecosystem size. Bars represent body size-adjusted means ( $\pm$  SE). Only populations where data for the trait examined were available are presented.

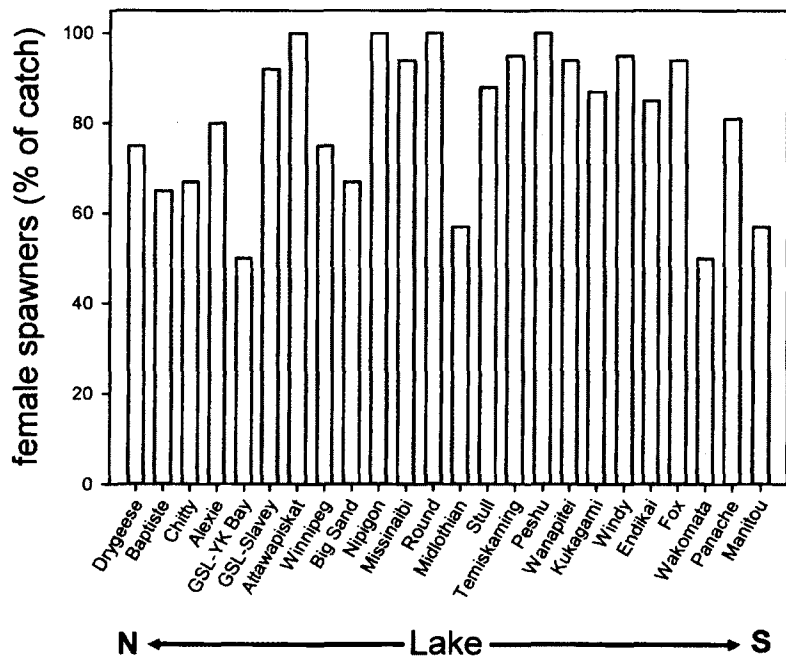


Figure 27. The proportion of captured female Burbot that were in reproductive condition during the spawning period from lake dwelling populations across Canada. Reproductively immature fish were excluded. Only populations where data for the trait examined were available are presented.

## **Discussion**

Burbot showed little variation in life history traits across the environmental gradients examined. Of the traits examined, only egg size, and relative liver size (HSI) exhibited any significant trends relative to environmental gradients, with differences in lake size and latitude explaining most of the variation observed. The strongest predictive power ( $r^2$ ) was observed between egg size and lake depth (82%) and egg size and lake area (70%). None of the other life history traits of Burbot populations varied with respect to latitude, productivity, or lake-size. Further, no sex-based differences in the traits assessed were found to be significant across populations.

Reproduction is costly in terms of energy (Wong and Jennions 2003, Rideout and Tomkiewicz 2011). Lipids stored in body tissues are often used to supply energy demands relating to reproduction of iteroparous fishes (Casselman and Schulte-Hostedde 2004, Jonsson and Jonsson 2005, Johnston et al. 2012) and are particularly important to lean fish, such as members of the codfishes (Marshall et al. 1999, Skjæraasen et al. 2006a) including Burbot (Chapter 2 of this thesis). Liver size of Burbot was observed to increase with latitude in this study. Perhaps larger livers enable more lipid storage to assist in gonad development in northern environments. However, the gonad size and overall growth of Burbot remained consistent across all gradients examined. These results may indicate that Burbot in northern climates are able to compensate for the stresses of reproduction and growth by relying more heavily on stored lipid-based energy reserves in their livers than their southern counterparts, possibly trading-off stored energy for reproductive effort. Striking a balance between gonad production and energy required for adult survival is a critical life history requirement (Cole 1954, Stearns 1976). Skipping

spawning years may also provide a mechanism that enables Burbot in more demanding environments to replenish the energy required for reproduction, and perhaps growth.

It has been suspected that Burbot in some populations skip spawning seasons (Chen 1969, McPhail and Paragamian 2000), and the data presented here supports this hypothesis, with some adult females found to be in non-spawning condition during the spawning period in most populations. However, no trend was noted for percent of adult females in spawning condition and the environmental gradients tested. In Atlantic Cod, it is thought that low energy reserves at the onset of vitellogenesis may make individuals in the population skip spawning for that year (Burton et al. 1997, Skjæraasen et al. 2006a). Skipping spawning enables fish to retain a portion of their stored energy to endure adverse conditions and maintain productive potential (Rideout and Tomkiewicz 2011).

Contrary to what was predicted; egg size did not increase with latitude, but rather increased with lake size. The trends seen in lake size are often opposite to what would be expected in productivity, as lake size is often inversely correlated with water clarity (Fee et al. 1996, Vadeboncoeur et al. 2008). The diet of Burbot larvae is almost exclusively zooplankton (Ryder and Pesendorfer 1992, McPhail 2007). Presumably, if larger lakes are less productive, there should be less food available to emerging Burbot than in smaller systems. Selection pressure would be expected to favour fewer, larger young in resource poor conditions (Stearns 1976), and this may offer an explanation for the significant relationship between egg size and lake size observed in this study.

From an investigation of several populations of Burbot throughout Quebec, Magnin and Fradette (1977) commented that size-at-age is likely latitude dependent with more northerly populations of Burbot growing more slowly. The results of this study do not support their hypothesis. No trends were detected for Burbot size at age with any environmental gradients tested. Similarly, no trend for age at maturity and latitude was discovered for American Shad (Leggett and Carscadden 1978), and no correlation was found with size-at-age in an assessment of 21 minnow species against a latitudinal gradient (Gotelli and Pyron 1991). How size-at-age and growth change along latitudinal gradients can vary among species. For example, in a study comparing life history attributes of river-dwelling fish along a latitudinal gradient, the growth rate increased with increasing latitude for Emerald Shiners (*Notropis atherinoides*), but decreased for Sauger (*Sander canadensis*) (Braaten and Guy 2002). Burbot are speculated to grow more in the winter than summer (Nelson and Paetz 1992), and perhaps this offsets temperature related latitudinal effects. Other factors, not investigated in this study, can influence the size at age of Burbot such as inter-specific competition for resources in a given lake (Stapanian et al. 2011). The role that selection pressure from inter-specific competition plays on Burbot life history traits is an avenue for future research.

Despite differences in the duration of ice-cover from south to north, spawning time for most populations sampled was usually confined to the first three weeks of February. It is therefore possible that the circannual reproductive cycle for Burbot is more endogenously driven than it is for other fishes. Even for fish where day-length is thought to largely drive their circannual reproductive clock, intrinsic drivers govern their cycle to some degree

(Randall et al. 1998). The strategies used by existing taxa may therefore not be entirely viewed as adaptations to their current environment, but may be retained ancestral traits (Coddington 1988), where genetic programming can override environmental cues (Lagomarsino and Conover 1993). Perhaps, as Cahn (1936) had long ago speculated, the life history traits of Burbot are holdovers from its marine ancestry, with the timing of reproduction corresponding to that of many marine cods. Although the dark, cold under-ice conditions appear extreme, the variability of these winter conditions is low and predictable and animals can adjust accordingly (Winemiller and Rose 1992). In a correlation analysis of life history variables North American marine cods (Vila-Gipert et al. 2002), including Burbot (Winemiller and Rose 1992), were categorized as having a periodic life history strategy; larger size at maturity, episodic or seasonal spawning, and large clutches of small eggs. A periodic life history strategy is more common in temperate and arctic freshwater fish species in comparison to tropical species (Vila-Gipert et al. 2002). In unpredictable environments, smaller broods should be selected as a trade-off for adult fitness, while minimizing the cost of failure should any single reproductive event be unsuccessful (Stearns 1976). Although egg size was larger in larger lakes, suggesting an adaptation to assist larval survival in resource deplete environments, there were no significant differences in GSI between the sexes or across the environmental gradients tested. These results indicate that regardless of the system or geographic location of the Burbot population investigated, the overall gonadal investment did not change. Burbot in the populations studied appeared to use the stability of the winter environment and a periodic spawning strategy to define their reproductive period. This life history strategy has allowed Burbot to successfully occupy an enormous geographic range under a

spectrum of environmental conditions, with very little variation in life history traits. In fact, Burbot today remain morphologically similar to the proto-Burbot *L. hulai* which first colonized freshwater systems over 5 million years ago (Van Houdt 2003, Van Houdt et al. 2005).



## CHAPTER 5 - General Discussion

This study investigated several aspects of life history theory and sexual selection using Burbot as a model organism. In doing so, many advances were made in the understanding of the life history and reproductive ecology of this poorly studied fish. For example, several facets of Burbot ecology, such as their circannual reproductive cycle, are now supported with field-based evidence. Furthermore, this work adds to the existing knowledge regarding gadoid fish mating systems in general.

Life history theory predicts that an organism's traits are developed as adaptations to their environment in order to maximize adult survival and reproductive output (Cole 1954, Stearns 1976). Overall, the results of this study indicate that Burbot show a remarkable consistency in life history traits across a wide range of environmental conditions. For example, although egg size was shown to increase with lake size, GSI remained consistent among populations. This indicates that, regardless of egg size, Burbot are putting the same overall effort into gonadal development. Burbot appear to do this by skipping spawning years, similar to other codfishes (Burton et al. 1997, Skjæraasen et al. 2006a). Evidence of reproductively mature but non-spawning Burbot was found throughout the populations sampled, with no difference in the percent of non-spawning females found, indicating that skipped spawning is a ubiquitous strategy. Withholding reproductive effort is a trade-off mechanism to maximize adult survival (Stearns 1976).

Every existing life history pattern should be assumed to have value, adapted to survive under prevailing environmental conditions (Cole 1954). Such consistency in the life

history of Burbot, coupled with the low level of phenotypic (Chen 1969) or genetic variation shown across their range (Elmer et al. 2008), lend support to the concept of life history stability. Stability in life history traits means that the species is so well-adapted to its environment, that it has achieved an evolutionary stable strategy (Maynard Smith and Price 1973).

When viewed collectively, the results of this study provide field-based support to the notion that Burbot employ a periodic strategy similar to other gadoids (Winemiller and Rose 1992, Vila-Gipert et al. 2002). Perhaps one of the most fundamental advancements is that Burbot, as the only freshwater member of the cod family (Cohen et al. 1990), appear to exhibit the same life history architecture as their marine ancestors and existing kin. Simply stated, Burbot may be regarded as a marine cod in a freshwater environment. This gadoid life history template has proven to be immensely successful allowing Burbot to have a common life history pattern not only among the 25 populations studied herein, but also to occupy among the greatest geographic (Stapanian et al. 2010) and depth (Sitar et al. 2008) distribution of any freshwater fish.

This study is the first to demonstrate that Burbot vocalize (Chapter 2). Also, this study was the first to document fish calls of any type under ice-cover. The fact that swim bladder mass (including drumming muscles) increased toward the spawning period, that the hearing sensitivity of adult Burbot was found to be concomitant with the sound energy of their calls, and that the calls were coincident with the onset of the spawning period, strongly suggests that Burbot calls serve a practical purpose to their reproductive

activities, and are not simply an unused vestige of their marine ancestry. Spawning vocalizations are yet another similarity that Burbot share with many marine gadoids (Hawkins and Rasmussen 1978), suggesting a common gadoid mating system where acoustic signalling is used in mate choice in the absence of other obvious dimorphic characters.

One possible deviation from the gadoid mating system is that there is no evidence of aggressive behaviour in Burbot. Other codfishes are known to have aggressive displays towards rivals (Brawn 1961a, Hawkins and Amorin 2000, Rowe and Hutchings 2006). Burbot on the other hand, seem to show a great affinity towards one another (Edsall et al. 1993), even during the spawning period (Cahn 1936, Fabricius 1954). The huge gonads of male Burbot, surpassing those of females in many populations, indicate that sperm competition is an important aspect of the mating system of Burbot. However, their enormous testes size also support the idea that Burbot are a non-aggressive species, as gonad size is inversely proportional to behavioural based intrasexual sperm competition (Taborsky 1998). If male Burbot fought with one another, they would not likely have evolved such large testes, as energy for agonistic behaviour would need to be traded-off with gonad production, as it is with other fishes (Blanchfield and Ridgway 1999). Rather, their large testes are likely the product of selective pressures from their apparent group spawning behaviour; this topic begs further investigation.

While this study provided many insights into Burbot ecology with respect to their mating system and how they fit into the life history spectrum, it also underscores how little is

known about much of our freshwater aquatic ecosystems. This study addressed many data gaps in our understanding of the basic biology of this species, such as the timing and duration of their spawning period. Through this study, many new questions have surfaced. The questions posed throughout the preceding thesis, in addition to those expanded upon below, can be the basis for future research.

It was shown that there were seasonal and sex-based differences in Burbot gas gland mass. Overall, males had larger gas glands, and male gas glands were heaviest in the winter – whereas female gas glands were largest in the spring (see Chapter 2). The physoclist gas gland allows Burbot to undertake effective buoyancy control for depth transitioning through gas exchange with blood (Alexander 1993), and proportionately larger gas glands should allow for more rapid gas exchange. The sex-based and seasonal differences noted here, imply that there may be habitat use differences between the sexes, perhaps related to different diet or thermal requirements.

Discovering that Burbot made calls was enlightening to our understanding of the reproductive ecology of Burbot. The fact that they do call is a platform upon which to expand upon our understanding of their mating systems. One obvious research direction (but one admittedly difficult to accomplish) would be to simultaneously video and sound record the calling of Burbot in order to link sound production with particular behaviours, and then compare these results with those for other gadoids. Also, the use of acoustic monitoring could be developed as a non-lethal and cost effective technique for

determining the presence and timing of spawning Burbot, and perhaps other difficult-to-sample codfishes such as Arctic Cod.

This study assessed life history traits across a wide range of whole-lake environmental conditions. However, during the winter when Burbot spawn, their environment across populations is much more stable and predictable, and this appears to have led to stability among populations in their reproductive traits (see Chapter 4). In order to test the influence of environmental stochasticity on the life history traits of this species, future research should expand sampling to populations at the extremes of water temperature or productivity and including the most southern and northern populations. Increasing the range of measured environmental gradients would offer the added benefit of establishing an ecological baseline in which to track or predict environmental change, particularly climate.

A caution to note is that only Burbot from lotic environments were used in this study. In addition to being lake dwelling, Burbot are known to have fluvial and adfluvial life history types (McPhail and Paragamian 2000, Richardson et al. 2001, Evans et al. 2002, Evans 2007, McPhail 2007), but fall into the same overall subspecies separation (Van Houdt 2003). There are however a few differences in these life history types that are worth noting. Due to their cold water preferences, fluvial Burbot are more commonly found in northerly latitudes where rivers are generally cooler than those in the southern parts of their range (McPhail and Paragamian 2000, McPhail 2007). These Burbot are tolerant of, and may even prefer, very turbid areas of rivers (Breeser et al. 1988), or the

confluence of clear streams running into large rivers (McPhail and Paragamian 2000). Also, fluvial Burbot populations often migrate to access spawning grounds; migrations can be quite extensive (Breeser et al. 1988), with the longest recorded movement by an individual Burbot being 450 km (McPhail and Paragamian 2000). A detailed comparison of the ecology of riverine, adfluvial, and lake dwelling Burbot should be conducted. It is possible that the sperm of riverine Burbot would be more motile than lake dwelling Burbot to ensure adequate fertilization where current may be present. More and stronger swimming sperm have evolved in some fishes as a means to contend with current (Shapiro and Giraldeau 1996, Levitan 2005). Comparing the motility of sperm between lake and river dwelling Burbot would be an interesting research question on the adaptation of sperm in varied environments.

Most Burbot populations throughout their Holarctic range are stable, however in many regions they are in decline or threatened (Paragamian et al. 2008, Dixon and Vokoun 2009). Burbot have been extirpated from the United Kingdom (Worthington et al. 2010) and parts of Western Europe where they are the focus of recent reintroduction efforts (Stapanian et al. 2010). The main causes for declining Burbot populations are pollution, and habitat alterations; notably hydroelectric dams that alter water temperatures, disrupt flow regimes, and block migrations to spawning grounds (Paragamian et al. 2002, Paragamian et al. 2008, Stapanian et al. 2010). Burbot in southern parts of their range are potentially the most sensitive, particularly when anthropogenic stressors are coupled with changing climates (Reist et al. 2006). Although secure, Arctic and sub-arctic Burbot, along with other northern fishes are not immune from impact (Reist et al. 2006, Stapanian

et al. 2010). In northern Eurasia, particularly Finland, Sweden, and parts of Russia, Burbot populations have been severely impacted by eutrophication, acidification (acid rain), pollution, water draw downs, and invasive species. The status of Burbot is largely unknown for Mongolia and northern China, however they do contribute to subsistence and commercial fisheries (Stapanian et al. 2010). In the Alaskan and Canadian north, Burbot are stable but there have been localized declines. In Alaska, Burbot were targeted (non-commercially) using winter set lines. This practice led to local declines in Burbot populations, especially those with road access. However, regulations prohibiting the use of setlines have since been established and many Alaskan populations have recovered (Stapanian et al. 2010). In the Yukon and NWT, high levels of chlorinated hydrocarbons and other atmospheric deposited pollutants have been detected in Burbot (Muir et al. 1990, Kidd et al. 1995, Kidd et al. 1998, Carrie et al. 2010). For a comprehensive review of the world wide status of Burbot see Stapanian et al. (2010).

Burbot are not actively managed as a fishery in most jurisdictions (McPhail and Paragamian 2000, Paragamian and Bennett 2008), or considered in other resource management decisions. For example, timing restrictions of industrial activities to avoid sensitive periods, such as spawning, are widely used to mitigate impacts to fishes; however, impacts on Burbot are rarely considered, despite the fact that they are the only fish to spawn in the winter, coinciding with the majority of the industrial activity and development in northern areas (Cott et al. 2008). As shown in Chapter 3 of this thesis, Burbot vocalize in association with their reproductive activities, and may be vulnerable to noise impacts. Winter-based natural resource exploration and development activities have

the potential to transmit noise under ice at levels that may disturb fish (Mann et al. 2009a, Cott et al. 2012). The extent to which this anthropogenic under-ice noise could disturb Burbot reproduction is not known and should be researched. The interactions of natural environmental variation with climate change and other anthropogenic impacts are complex. These influences would likely have a cumulative and negatively synergistic effect, making the separation of acute anthropogenic impacts from pervasive influences difficult (Reist et al. 2006). Research on the life history traits and reproductive ecologies of northern species like the Burbot is important because it can provide valuable insights and indicators of anthropogenic perturbations (Holmgren and Appleberg 2000, Schindler et al. 2000, Braaten and Guy 2002, Power et al. 2005, Reist et al. 2006). For example, information gained through an examination of life history over latitudinal gradients can help refine climate change predictions (Power et al. 2005).

Burbot are a good indicator species for detecting environmental change in Arctic and sub-Arctic environments. Burbot are known to be sensitive to temperature fluctuations (Paragamian et al. 2002, Jackson et al. 2008) and like other cold water adapted fishes, may be particularly vulnerable to climate related impacts (Reist et al. 2006, Jackson et al. 2008), and may be good early indicators of change in cold-water systems (Stapanian et al. 2010). Further, because they have fatty livers and live in remote northern lakes having no point sources of pollution, Burbot are well suited for monitoring the long-range atmospheric transport of persistent organic pollutants (Muir et al. 1990).



The emphasis or lack of emphasis on a species can bias the collection and interpretation of ecological information (Caro and Stoner, 2003; Seddon et al., 2005; Link, 2007; Pysěk et al., 2008). Although they are often overlooked, it has been demonstrated that Burbot play an important role in structuring and regulating aquatic ecosystems (Stewart and Watkinson 2004, Carl and McGuinness 2006, Knudsen et al. 2010, Cott et al. 2011, Stapanian et al. 2011). Knowing more about a species ecology is essential for the effective management and conservation of both the species and its habitat (Birtwell et al. 2005, Sanetra and Meyer 2005, Van Houdt et al. 2005, Reist et al. 2006). However, it is important to recognize there can be major differences in the ecology of an animal depending on where it is found within its range. This is especially true for species such as Burbot that occur broadly, across climatic zones into the Arctic. Often, in the absence of specific information for northern populations (which is usually limiting), southern models are used. This is not always appropriate, and may result in misinformed and even detrimental management decisions (Reist et al. 2006). Gaining a better understanding of the life history and reproductive ecology of Burbot in the context of freshwater ecosystems as well as within a gadoid context will allow for more informed decision making and better management of this species, co-existing species, and other gadoid fishes.

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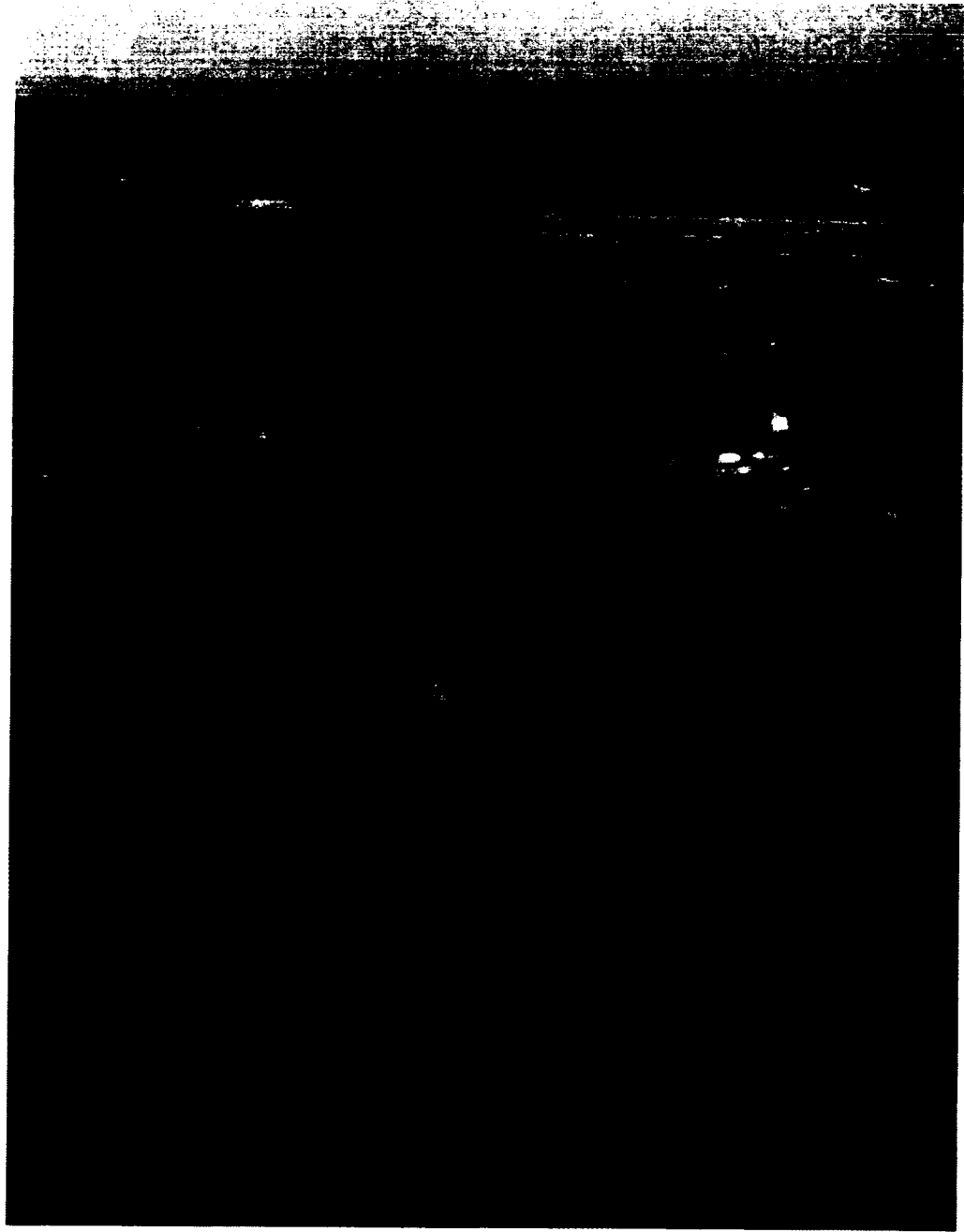
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