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Brood Stock Establishment through Hormonal Induction of Gamete Expression and Cryopreservation of Spermatozoa in Bloaters (Coregonus hoyi)

Alexander John Presello
University of Windsor

Brood Stock Establishment through Hormonal Induction of Gamete Expression and Cryopreservation of Spermatozoa in Bloaters (*Coregonus hoyi*)

By

ALEXANDER JOHN PRESELLO

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by

Alexander Presello

APPROVED BY:

_____

O. Love

Department of Biological Sciences

_____

D. Heath

Great Lakes Institute for Environmental Research

_____

T. Pitcher, Co-Advisor

Department of Biological Sciences

_____

C. Wilson, Co-Advisor

Ontario Ministry of Natural Resources and Forestry; Aquatic Biodiversity and Conservation Unit

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DECLARATION OF CO-AUTHORSHIP

I hereby declare that this thesis incorporates material that is the result of joint research, as follows: both of my data chapters were co-authored with my supervisor, Dr. Trevor Pitcher. In each case, my co-author provided valuable feedback, helped with the project design and statistical analysis, and provided editorial input during the writing of each manuscript; however, in both cases, the primary contributions have all been by the author. Both Chapter Two and Chapter Three have been prepared as manuscripts, with Chapter Two being planned to submit for publication to Aquaculture.

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ABSTRACT

Animals raised in captivity typically exhibit reproductive dysfunction of varying degrees ranging from diminished to absent reproduction. This observation holds true for the bloater (*Coregonus hoyi*); a deepwater cisco extirpated from Lake Ontario. Efforts to rear bloaters in captivity have met with complications including the absence of egg expression in females and asynchronous gamete expression between sexes. As such, I examined whether the injection of an exogenous hormone (LHRHa) could induce gamete expression in both males and females. Also, I began the development of a sperm cryopreservation protocol for bloaters to overcome the observed asynchronous gamete expression. The exogenous hormone injections were effective at inducing the gamete expression of females, more so than males. Additionally, although the cryopreservation freezing rates examined were successful for closely-related species, they were not appropriate for bloater spermatozoa. However, all four extenders examined are suitable for bloater spermatozoa as none of them were biologically toxic.

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Chapter 1: General Introduction

1.1 Reproductive Biological Strategies in Captive Breeding

One of the greatest challenges associated with the captive breeding of vulnerable species is the establishment of a self-sustaining and reproductively viable population (Snyder et al. 1996). Reproductive viability, and thus population establishment, is often hindered by one or more aspects of reproductive dysfunction (an abnormality or impairment associated with the reproductive system of an organism) in captive animals (Snyder et al. 1996; Balmford et al. 1996). Reproductive dysfunction, in its many forms, may be attributed to factors such as inadequate environmental conditions, diet, behavioral incompatibility and inbreeding depression (Danielle and Murray 1986; Setchell et al. 1987; Milliam et al. 1988; Yamamoto et al. 1989; Merola, 1994). However, the incorporation of reproductive biological strategies into captive breeding has met with success in a number of vulnerable species. In mammals, for example, conservation of the giant panda (*Ailuropoda melanoleuca*) was greatly improved once a greater understanding of its chemical communication system was applied to its reproduction program (Swaisgood et al. 2004; Wei et al. 2015). Similarly, the Mauritius kestrel (*Falco punctatus*) population was restored from a group of only four individuals in part due to the use of artificial egg incubation – an assistive reproduction method (Jones et al. 1994). The use of assistive reproductive methods is not only limited to birds – Browne et al. (2006) incorporated exogenous hormone application into the conservation program of the Wyoming toad (*Bufo baxteri*) because its females experienced difficulty ovulating. The incorporation of reproduction biology in captive populations also includes a large number of vulnerable fish species such as the Atlantic salmon (*Salmo salar*) (Crim and Glebe

1984; Taranger et al. 1992) and lake sturgeon *(Acipenser fulvescens)* (Ciereszko et al. 1996). The two commonalities that bind all of these case studies together are (1) they are vulnerable due to low numbers in one or more populations and (2) their captive breeding efforts were improved through the understanding and application of reproduction biology.

1.2 Study Species: The Bloater

The bloater (*Coregonus hoyi*), a member of the family Salmonidae, is a deepwater cisco with silver-iridescent coloration and ranges from 20-30cm in length when fully grown (Scott and Crossman 1973). Parker (1989) noted that bloaters achieve sexual maturity at approximately three years of age and the exact spawning season of bloaters remains inconclusive with observed spawning events ranging from November through March in the Apostle islands region of Lake Superior (Becker, 1983). Unfortunately, not much is known regarding bloater reproduction or spawning behavior, likely because spawning occurs between November and March near the bottom of the lakes (Bunnell et al, 2014). Historically, bloaters, among other coregonids, were economically important to commercial fisheries of the Great Lakes, however, annual commercial yield in the Great Lakes decreased from an average of 12 million kilograms between 1910 and 1955 to 0.35 million kg by 1992. (Stockwell et al, 2009). Many species of deepwater cisco, including the kiyi (*Coregonus kiyi*), shortjaw (*Coregonus zenithicus*) and blackfin (*Coregonis Nigripinnis*) in addition to the bloater, experienced drastic reduction in their populations across the Great Lakes during the mid-20th century (Zimmerman and Kreuger, 2009).

More specifically, it is estimated that bloaters were extirpated from Lake Ontario in the 1950s for a variety of reasons (Clemens and Crawford, 2009). These hypothesized reasons include: fishing pressure, predator-prey ratios (Christie, 1973), increased food competition by invasive alewives (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*), sea lamprey (*Petromyzon marinus*) predation and diminished water quality (Smith, 1972; Christie, 1973, 1974). The last documented catch of a bloater in Lake Ontario was in 1983 (Baldwin, 1999) although there have been approximately 10 catches of deepwater ciscoes since 2002 (Fave & Turgeon, 2008). Reintroduction of bloaters to Lake Ontario has been suggested as an important step in optimizing the health of the Great Lakes as an ecosystem (Baldwin, 1999). The bloater is important on a number of ecological levels. There is evidence supporting bloaters as important trophic integrators (Eshenroder and Burham-Curtis, 1999) because they feed on various types of plankton as they vertically traverse the water column throughout their life cycle (Eshenroder et al., 1998). Adult bloaters have been known to feed on both benthic and pelagic macroinvertebrates as a results of this migration. (Zimmerman and Kreuger, 2009). Bloaters have additional ecological value as forage fish, particularly to lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*), thus their restoration could serve to reconnect energetic pathways at multiple levels within the lake's food web (Favé & Turgeon, 2008). As such, efforts to establish a self-sustaining broodstock of hatchery-reared bloaters in order to restore them to Lake Ontario are currently underway.

1.3 Bloater Culture and Restoration Efforts

As part of the Great Lakes Fish and Wildlife Restoration Act Regional Project entitled Development of Propagation Strategies to Support Reintroduction of Deepwater Coregonids in Lake Ontario, on an annual basis since 2011, fertilized eggs (acquired by mixing the sperm and eggs) collected from a population of wild bloaters native to Lake Michigan have been transported to White Lake Fish Culture Station (FCS) located in Sharbot Lake, Ontario. White Lake FCS then incubates the eggs and rears the resultant newly-hatched bloaters to adulthood. However, the wild collection is not viable long-term because the collections are very dangerous due to hazardous winter conditions on Lake Michigan in addition to their substantial financial cost. As such, the development of a self-sustaining broodstock has become a more favorable option than these wild collections. However, efforts to rear these wild-origin bloaters at White Lake FCS have been experiencing two main problems which provide the two central foci of my thesis: (1) the proportion of females that produce free flowing eggs on an annual basis are, currently, not enough to be able to establish a self-sustaining broodstock and (2) males and females are exhibiting asynchronous gamete expression (i.e. males and females become reproductively active at non-overlapping times of the year). Both of these events may be classified as reproductive dysfunctions which are common in hatchery-reared fish (Billard 1989; Berlinsky et al. 1997; Vermeirsen et al. 1998, 2000; Mylonas et al. 2010). Nonetheless, bloaters have been stocked annually for several years now and additional research is currently being conducted which examines their population dynamics, using acoustic telemetry, following their stocking.

1.4 Gamete Development in Fish

The reproductive cycle in fish may be divided into two main stages for both males and females. The first stage includes the proliferation, differentiation and growth of gametes (i.e. spermatogenesis and vitellogenesis) while the second stage includes the final maturation of gametes for release (spermiation and oocyte maturation) (Mylonas et al. 2010). In hatchery-reared males, the most commonly observed reproductive dysfunction is reduced sperm volume and quality while, in females, failure or unpredictable occurrence of oocyte maturation (OM) are the most common (Billard 1989; Berlinsky et al. 1997; Vermeirsen et al. 1998, 2000; Mylonas et al. 2010).

The first stage of gamete development in males, spermatogenesis, constitutes the proliferation of spermatogonia, spermatocyte I multiplication (through multiple meiotic divisions), the production of spermatocytes II through subsequent meiotic division and the differentiation of spermatocytes II into spermatids (Mylonas et al. 2010). The production of flagellated spermatozoa is what marks the end of spermatogenesis (reviewed in Billard 1989; Vizziano et al. 2008). The second stage, spermiation, commences with the release of spermatozoa into the sperm ducts. At this point, usually during the spawning season, sperm is ejaculated spontaneously or it can be manually expressed by gentle abdominal massage (Mylonas et al. 2010).

Conversely, in females the deposition of yolk precursors within the oocyte during oogenesis (also called vitellogenesis) is the first stage of gamete development. During vitellogenesis, important growth of the oocyte takes place as a result of the uptake of yolk precursor proteins; mainly low-density lipoproteins and vitellogenin (a key glycoprotein

in egg development) (Babin et al. 2007). Typically, it is the enzymatic cleavage of vitellogenin which produces the essential yolk proteins that, when adequately accumulated, trigger a hormonal stimulation which signals the end of vitellogenesis (Mylonas et al. 2010). At this stage, the oocytes undergo the process of oocyte maturation (OM). During this final stage of the development, the oocytes undergo several observable changes such as the coalescence of lipid and yolk droplets, clarification of the cytoplasm and a significant increase in volume due to water uptake by the oocyte (Cerdà et al. 2007). As mentioned previously, once spermiation and OM are complete the gametes are, under normal circumstances, ready to leave the body fully functional.

1.5 Hormonal Regulation of Fish Reproduction; the Hypothalamic-Pituitary-Gonadal Axis

Gamete development in fish, as it is with many other vertebrates, is largely regulated by the endocrine system and, more specifically, by the hypothalamic-pituitary-gonadal (HPG) axis. The HPG axis consists of a series of endocrine structures responsible for a hormonal cascade (See Figure 1.1). It is the coordinated release of a series of hormones which elicits the propagation of gamete development and reproduction in fish.

In fish, the cascade begins with the stimulation of sensory neurons by environmental cues such as photoperiod, water temperature and turbidity. This initial stimulation is interpreted by the hypothalamus which controls the production and release of hypophysiotropic hormones. The main hypophysiotropic hormone involved in

reproduction in fish is gonadotropic hormone releasing hormone (GnRH). GnRH targets the anterior pituitary (adenohypophysis) to initiate the release of pituitary hormones such as gonadotropic hormone (GtH) – the primary pituitary hormone involved in reproduction. Unlike mammals, GtH exists in two identifiable forms; GtH I and GtH II (Kawauchi et al. 1989; Swanson et al. 1991). These two forms, GtH I and GtH II appear to be analogous to the mammalian gonadotropic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), respectively (Redding and Patino, 1993). While both GtH I and GtH II are involved in gametogenesis, they have different physiological functions. GtH I is involved in the early stages of gametogenesis and in steroidogenesis whereas GtH II plays a larger role in the final stages of gametogenesis, which were discussed previously (Arcand-Hoy and Benson, 1998). As such, it is the role of the gonadotropic hormones to act on the gonads and elicit the production of steroidal sex hormones, namely, the androgens, estrogens and progestins associated with reproduction. The sex steroids, in turn, assist in regulating gametogenesis, reproduction and sexual phenotype in addition to a number of other behavioral traits (Arcand-Hoy and Benson, 1998). The specific effects of these hormones also differ depending on the sex of the fish.

In males, levels of GtH I are typically high throughout spermatogenesis and drops off towards the end of the reproductive cycle, while GtH II remains low during spermatogenesis and becomes elevated during the spawning period (Nagahama, 1994). Additionally, the gonadotropins stimulate the production of spermatogonia and the androgens necessary for both gametogenesis the development of secondary sexual characteristics for the species (Arcand-Hoy and Benson, 1998). Synthesis of the androgens takes place in the Leydig cells of the testes and, dependent on the stage of

reproduction, may include testosterone, 11-ketotestosterone and androstenedione (Redding and Patino, 1993). Finally, a decrease in plasma levels of androgens coupled with a rapid increase in progestins is common during spawning as these hormonal shifts, elicited by elevated GtH II, are required for spermiation (Arcand-Hoy and Benson, 1998).

A similar hormonal pattern is often observed in females. Plasma levels of GtH I are elevated during early oocyte development (i.e. vitellogenesis) as it binds to receptors located on both the granulosa and thecal layers of the developing follicle. Subsequently, the thecal cells produce testosterone which is then enzymatically altered into estradiol in the granulosa layer prior to release into the plasma (Redding and Patino, 1993; Cyr and Eales, 1996). It is then estradiol binding to estrogen receptors which initiates a series of events ending in the production of vitellogenin. However, as the development of the oocyte progresses, GtH I plasma levels drop off and GtH II levels begin to rise and bind to granulosa cells on the follicles to stimulate the release of progestins (Redding and Patino, 1993). Again, as with males, it is this increase in plasma progestin levels which govern the final steps of the maturation of oocytes and finally, initiate ovulation (Arcand-Hoy and Benson, 1998).

The HPG axis controls itself through a number of feedback systems, for example, testosterone generated in the testes may have either a stimulatory or inhibitory effect on the release of GtH from the pituitary depending on factors such as the stage of reproduction and the concentration of the hormone (Arcand-Hoy and Benson, 1998). Alterations to the factors that control the HPG axis could potentially contribute to various forms of reproductive dysfunction in fish.

1.6 Use of Exogenous Hormones to Overcome Reproductive Dysfunction

Animals that are raised in captivity often experience one or more forms of reproductive dysfunction that can be attributed to some incongruence between their wild and captive environment (Caughley, 1994; Balmford et al. 1996; Snyder et al. 1996). In recent years, the use of exogenous hormones has been touted as a method of overcoming reproductive dysfunction in captive populations. As mentioned previously, the endocrine system plays a crucial role in the reproductive cycle of most vertebrates. The relevant hormonal cascades are initiated by environmental cues, which are often lacking in captive populations in some respect, which elicit the release of hypophysiotropic hormones such as gonadotropic hormone releasing hormone (GnRH). These hormones perpetuate the reproductive endocrine cascade, thus, the most commonly utilized exogenous hormones for the purposes of overcoming reproductive dysfunction are analogs of gonadotropic hormone releasing hormone (GnRHa) and luteinizing hormone releasing hormone (LHRHa). For example, the use of LHRHa injections on both male and female Wyoming toads became part of an extensive captive breeding program as the toads initially failed to produce mature gametes, however, after the injections the toads (both sexes) experienced greater percentages of gamete expression (Browne et al. 2006). In addition to numerous other amphibian species, exogenous hormone use has also been utilised effectively in several species of fish (Browne et al. 2006; Malison et al. 1998).

One possible reason the number of species of fish currently being reared in captivity is increasing is the development of the aquaculture industry and supportive breeding programs (Duarte et al. 2007; Caughley, 1994). In a captive setting, environmental manipulation of factors such as photoperiod, water temperature and

turbidity can assist in controlling the reproduction of fish (Mylonas et al. 2010). The costs and feasibility of these manipulations (e.g. water depth) for large scale production of fish biomass are not practical, thus, the use of exogenous hormones to induce spawning in hatchery-reared fish is one economic and effective alternative (Mylonas et al. 2010). Some of the most common reproductive dysfunctions observed in captive fish include reduced sperm volume and quality in males and the failure or unpredictable onset of ovulation for females (Billard 1989; Berlinsky et al. 1997; Vermeirsen et al. 1998). Taranger et al (1992) utilized injections of LHRHa in female Atlantic salmon (*Salmo salar*) to induce and synchronize ovulation before its natural onset. In this case, the purpose of the use of the exogenous hormone was to overcome asynchronous spawning – not its complete absence. However, Mylonas et al (1992) administered injections of GnRHa in brown trout (*Salmo trutta*) in order to induce significantly greater levels of ovulation as compared to control fish. Although injection of exogenous hormone appears to be more popular in recent literature, the use of dissolvable pellets is an alternative method of administration. For example, Crim et al (1988) compared the effects of LHRHa single time injections and cholesterol pellets on plasma levels of gonadotropic hormones. Additionally, induction of spawning is not limited to females. Wojtczak et al (2005) used GnRHa injections to increase sperm production in European whitefish (*Coregonus lavaretus*). For a more extensive review of the dosages, and administration methods of exogenous hormone use in salmonids, the reader is referred to Table 1.1. At present, the use of exogenous hormone remains a viable alternative to environmental manipulation for the synchronisation and induction of spawning in captive fish populations.

1.7 Cryopreservation of Spermatozoa to Resolve Asynchronous Spawning between Sexes

Cryopreservation, as defined by Piironen (1993) refers to the storing of cells or tissues at -196°C, the temperature of liquid nitrogen. At this temperature, no known thermally driven chemical reaction can occur leaving only background radiation to limit the storage time to between 200 and 32,000 years (Ashwood-Smith, 1980). Cryopreservation of gametes is generally used for: synchronizing gamete availability in both males and females, sperm economy, simplifying transportation of gametes and for storage of genetic material in conservation programs (Cabrita et al. 2010). Although cryopreservation of eggs (oocytes) is possible, it is a much more complex process than cryopreserving sperm due to the large size and the two membranes of the egg possessing different permeabilities to water (Piironen, 1993). Consequently, spermatozoa, which do not possess the same difficulties associated with eggs, have been frozen and thawed successfully. To be able to cryopreserve spermatozoa, a complex protocol, consisting of appropriate extender (i.e. cryoprotectant and diluent) composition and freezing rate, must be established for the species of interest (Piironen, 1993). Although the terminology used in the literature has changed over time, hereafter, **cryoprotectant** will refer to those chemical substances that protect the interior of the cell and **diluent** will refer to those chemical substances which protect the exterior of the cell and render the sperm quiescent during the process of cryopreservation. Additionally, the mixture of a cryoprotectant and a diluent will be referred to as an **extender** or **extender solution**.

Cryopreservation has been utilized in many different fields, including both reproductive biology and conservation biology, for many years (Holt and Pickard, 1999; O'Connell et al. 2002). As such, protocols have been developed for many different cell

and tissue types. For example, a review by Barbas and Mascarenhas (2009) outlines the general status of cryopreservation for a number of domestic mammals including horses, bulls, pigs, rams and sheep. In addition to mammals, other species of amphibians and even plants possess cryopreservation protocols for their gametes for the purposes of genetic resource banking in addition to the ones outlined above (reviewed in Engelmann 2004; Kouba and Vance, 2009). However, one of the most common uses of cryopreservation, aside from an assistive reproductive technology for humans, is its use in the culture of fish.

For many years, cryopreservation of the milt (sperm) of fish has had a powerful impact on their culture and the banking of genetic resources. The uses of cryopreservation, as it applies to the culture of fish, according to Cabrita et al (2010) are: (1) that sperm can be stored and thawed when eggs become available, (2) the entire volume of sperm produce by a male can be cryopreserved (sperm economy), (3) gametes can be transported between different culture facilities and (4) the establishment of banks of genetic material. Although the culture of fish encompasses a great many species, most cryopreservation protocols have been developed for fish of the family Salmonidae (Cabrita et al. 2010; Lahnsteiner, 2000; Ciereszko et al. 2014). For an extensive (but not exhaustive) list of the cryopreservation protocols (i.e. cryprotectants, diluents, freezing rates etc.) of salmonids, refer to Table 3.1. In addition to salmonids, cryopreservation protocols have also been developed for other fish such as the redside dace (*Clinostomus elongatus*), sturgeon (*Acipenser spp.*) and channel catfish (*Ictalurus punctatus*) (Ciereszko et al. 1996; Glogowski et al. 2002; Christensen and Tiersch, 2005; Butts et al. 2013). Cryopreservation has been used in the culture of fish for many years and, to this

day, remains a useful method of banking genetic resources and synchronizing gamete availability between sexes.

1.8 Overview of Thesis

The objectives of my master's research were to: (1) evaluate the efficacy of intraperitoneal LHRHa injections in overcoming the observed reproductive dysfunction in hatchery-reared bloaters (Chapter 2); and (2) to begin the development of a cryopreservation protocol for the preservation of spermatozoa of hatchery-reared bloaters to overcome the asynchronous gamete expression observed between the sexes.

In my first data chapter (Chapter 2), I examined whether or not LHRHa induced spawning in hatchery-reared bloaters by examining the rates of expression of free flowing gametes following one of four, randomly assigned, treatments (control, sham, low or high injections of LHRHa) at one, three, four and five weeks post-treatment via pit-tag identification. This experiment took place at White Lake FCS in Sharbot Lake, Ontario and was replicated at two time points: (1) December 14 to January 20 (hereafter, December – January) and (2) January 18 to February 24 (hereafter, January – February). Additionally, I examined the differences observed in the average egg diameter (for both excised and free flowing eggs) of bloaters from all treatments (control, sham, low and high) as well as wild bloater eggs secured from specimens from Lake Michigan. This research will provide novel information regarding the culture of bloaters and potential methods for establishing a self-sustaining broodstock.

In my second data chapter (Chapter 3), I examined the toxic effects of four potential extender solutions on the sperm metrics (motility, velocity, and linearity metrics that are important for fertilization success) of hatchery-reared bloater spermatozoa. Furthermore, I also examined whether any of the four extenders, in conjunction with two different freezing rates, were capable of preserving sperm motility through the cryopreservation process. This research will provide essential and novel information regarding both the culture of bloaters as well as the characteristics of bloater spermatozoa.

References

Arcand-Hoy, L. D. & Benson, W. H. 1998. Fish reproduction: an ecologically relevant indicator of endocrine disruption. *Environ. Toxicol. Chem.*. **17**: 49-57.

Ashwood-Smith, M. J. 1980. Low temperature preservation of cells, tissues and organs. In: Ashwood-Smith, M. J., & Farrant J. (Eds.): Low temperature preservation in medicine and biology. Pitman Medical Ltd., Turnbridge Wells, Kent. pp.19-44.

Babin, P. J., Carnevali, O., Lubzens, E. & Schneider, W. J. 2007. Molecular aspects of oocyte vitellogenesis in fish. In: Babin, P. J., Cerdá, J., Lubzens, E. (Eds.), The Fish Oocyte: From Basic Studies to Biotechnological Applications. Springer, The Netherlands, pp. 39–76.

Baldwin, B. 1999. Native prey fish re-introduction into Lake Ontario: Bloater (*Coregonus hoyi*). Discussion Paper prepared for the Great Lakes Fishery Commission, Lake Ontario Committee. Available via www.glfc.org/lakecom/loc/lochome.php. Cited June 2016.

Balmford, A., Mace, G. M. & Leader-Williams, N. 1996. Designing the ark: setting priorities for captive breeding. *Conserv. Biol.*. **10**: 719-727.

Barbas, J. P. & Mascarenhas, R. D. 2009. Cryopreservation of domestic animal sperm cells. *Cell Tissue Bank*. **10**: 49-62.

Berlinsky, D. L., William, K., Hodson, R. G. & Sullivan, C.V. 1997. Hormone induced spawning of summer flounder *Paralichthys dentatus*. *J. World Aquat. Soc*. **28**: 79– 86.

Billard, R., 1989. Endocrinology and fish culture. *Fish Physiol. Biochem*. **7**: 49–58.

Browne, R. K., Seratt, J., Vance, C. & Kouba, A. 2006. Hormonal priming, induction of ovulation and in-vitro fertilization of the endangered Wyoming toad (*Bufo*

baxteri). *Reprod. Biol. Endocrin*. **4**: 34.

Bunnell, D. B., C. P. Madenjian, M. W. Rogers, J. D. Holuszko, & L. J. Begnoche. 2012. Exploring mechanisms underlying sex-specific differences in mortality of Lake Michigan Bloaters. *T. Am. Fish. Soc*. **141**:204–214.

Butts, I. A. E., Mokdad, A., Trippel, E. A. & Pitcher, T. E. 2013. Development of a sperm cryopreservation protocol for redside dace: implications for genome resource banking. *T. Am. Fish. Soc*. **142**: 671-680.

Cabrita, E., Sarasquete, C., Martinez-Páramo, S., Robles, V., Beirão, J., Pérez-Cerezales, S. & Herráez, M. P. 2010. Cryopreservation of fish sperm: applications and perspectives. *J. Appl. Icthyol*. **26**: 623-635.

Caughley, G. 1994. Directions in conservation biology. *J. Anim. Ecol*. **63**: 215-244.

Cerdá, J., Fabra, M., Raldúa, D., 2007. Physiological and molecular basis of fish oocyte hydration. In: Babin, P.J., Cerdá, J., Lubzens, E. (Eds.), The Fish Oocyte: From Basic Studies to Biotechnological Applications. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 349–396.

Christensen, J. M & Tiersch, T. R. 2005. Cryopreservation of channel catfish sperm: effects of cryoprotectant exposure time, cooling rate, thawing conditions, and male-to-male variation. *Theriogenology*. **63**: 2103-2112.

Christie, W. J. 1974. Changes in fish species composition of the Great Lakes. *J Fish Res Board Can*. **31**: 827–854.

Christie, W. J. 1973. A review of the changes in the fish species composition of Lake Ontario. *Great Lakes Fish Comm Tech Rep*. pp. 23-65.

Ciereszko, A., Dietrich, G. J., Nynca, J., Dobosz, S. & Zalewski, T. 2014.

Cryopreservation of rainbow trout semen using a glucose-methanol extender. *Aquaculture*. **420-421**: 275-281.

Ciereszko, A., Toth, G. P., Christ, S. A. & Dabrowski, K. 1996. Effect of cryopreservation and theophylline on motility characteristics of lake sturgeon (*Acipenser fulvescens*) spermatozoa. *Theriogenology*. **45**: 665-672.

Clemens, B. J. & Crawford, S. S. 2009. The ecology of body size and depth use by bloater (*Coregonus hoyi* Gill) in the Laurentian Great Lakes: patterns and hypotheses. *Rev. Fish. Sci*. **17**: 174-186.

Crim, L. W. & Glebe, B. D. 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. *Aquaculture*. **43**: 47-56.

Crim, L. W., Sherwood, N. M. and Wilson, C. E. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (LHRHa) administration by either single time injection of cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. *Aquaculture*. **74**: 87-95.

Cyr, D. G., Eales, J. G. 1996. Interrelationships between thyroidal and reproductive endocrine systems in fish. *Rev Fish Biol.* **6**:165–200.

Danielle, A. & Murray, N. D.. 1986. Effects of inbreeding in the Budgerigar (*Melopsittacus undulatus*) (Aves: *Psittacidae*). *Zoo Biol*. **5**:233-238.

Duarte, M., Marbá, N. & Holmer, M. 2007. Rapid domestication of marine species. *Science*. **16**: 382–383.

Engelmann, F. 2004. Plant cryopreservation: progress and prospects. *In Vitro Cell. Dev. Biol. –Plant*. **40**: 427-433.

Eshenroder, R. L. & Burnham-Curtis, M. K. 1999. Species succession and sustainability

of the Great Lakes fish community. Taylor, W. W. and Ferreri, C. P., (Eds.). Great Lakes Fishery Policy and Management: A Binational Perspective. East Lansing, (MI): Michigan State University Press. pp. 141–180.

Eshenroder, R. L., Argyle, R. L., and TeWinkel, L. M. 1998. Evidence for Buoyancy Regulation as a Speciation Mechanism in Great Lakes Ciscoes. *Arch. Hydrobiol*. 50: 207–217.

Favé, M. & Turgeon, J. 2008. Patterns of genetic diversity in Great Lakes bloaters (*Coregonus hoyi*) with a view to future reintroduction in Lake Ontario. *Conserv Genet.* **9**: 281-293.

Glogowski, J., Kolman, R., Szczepkowski, M., Horváth, A., Urbányi, B., Sieczyński, P., Rzemieniecki, A., Domagala, J., Demianowicz, W., Kowalski, R. & Ciereszko, A. 2002. Fertilization rate of Siberian sturgeon (*Acipenser baerii*, Brandt) milt cryopreserved with methanol. *Aquaculture*. **211**: 367-373.

Holt, W. V. & Pickard, A. R. 1999. Role of reproductive technologies and genetic resource banks in animal conservation. *Rev. Reprod*. **4**: 143-150.

Jones, C. G., Heck, W., Lewis, R. E., Mungroo, Y. Slade, G. & Cade, T. 1994. The restoration of the Mauritius kestrel Falco punctatus population. *IBIS*. **137**: 173-180.

Kawauchi, H., Suzuki, K., Itoh, H., Swanson, P., Naito, N., Nagahama, Y., Nozaki, M., Nakai, Y. & Itoh, S. 1989. The duality of teleost gonadotropins. *Fish Physiol. Biochem.* **7**:29–38.

Kouba, A. J. & Vance, C. K. 2009. Applied reproductive technologies and genetic resource banking for amphibian conservation. *Reprod. Fert. Develop*. **21**: 719-

737.

Lahnsteiner, F. 2000. Semen cryopreservation in the Salmonidae and in Northern pike. *Aquac. Res.*. **31**: 245-258.

Malison, J. A., Procarione, L. S., Kayes, T. B., Hansen, J. F. & Held, J. A. 1998. Induction of out-of-season spawning in walleye (*Stizostedion vitreum*). *Aquaculture*. **163**: 151-161.

Merola, M. 1994. A reassessment of homozygosity and the case for inbreeding depression in the cheetah. *Conserv. Biol.*. **8**: 961- 971.

Milliam, J. R., T. E. Roudybush, and C. R. Grau. 1988 Influence of environmental manipulation and nest-box availability on reproductive success of captive Cockatiels (*Nymphicus bollandicus*). *Zoo Biol.* **7**: 25-34.

Mylonas, C. C., Fostier, A. & Zanuy, S. 2010. Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endocr*. **165**: 516-534.

Mylonas, C. C., Hinshaw, J. M. & Sullivan, C. V. 1992. GnRHa-induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. *Aquaculture*. **106**: 379-392.

Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. *Int. J. Dev. Biol*. **38**: 217-229.

O'Connell, M. O., McLure, N. & Lewis, S. E. M. 2002. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. *Hum. Reprod*. **17**: 704-709.

Piironen, J. 1993. Cryopreservation of sperm from brown trout (*Salmo trutta* m. lacustris L.) and Arctic char (*Salvelinus alpinus* L.). *Aquaculture*. **116**: 275-285.

Redding, M. J. & Patino R. 1993. Reproductive physiology. In Evans, D. H. (Ed.). The Physiology of Fishes. Marine Science Series. CRC, Boca Raton, FL, USA, pp 503–534.

Setchell, K. D. R., Gosselin, S. J., Welsh, M. B., Johnston, J. O., Balistreri, W.F., Kramer, L. W., Dresser, B. L. & Tarr, M. J. 1987. Dietary estrogens a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology*. **93**: 225-233.

Smith, S. H. 1972. Factors of Ecologic Succession in Oligotrophic Fish Communities of the Laurentian Great Lakes. *J. Fish. Res. Board Can*. **29**: 717-730.

Snyder, N. F., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D & Miller, B. 1996. Limitations of captive breeding in endangered species recovery. *Conserv. Bio*. **10**: 338-348.

Stockwell, J.D., Ebener, M.P., Black, J.A., Gorman, O.T., Hrabik, T.R., Kinnunen, R.E., Mattes, W.P., Oyadomari, J.K., Schram, S.T., Schreiner, D.R., Seider, M.J., Sitar, S.P., Yule, D.L. 2009. A synthesis of cisco recovery in Lake Superior: implications for native fish rehabilitation in the Laurentian Great Lakes. *N. Am. J. Fish. Manag*. **29**: 626–652.

Swaisgood RR, Lindburg DG, White AM, Zhang H, Zhou X. 2004. Chemical communication in giant pandas: experimentation and application. Lindburg D. G. & Baragona K. (Eds.). Giant pandas: biology and conservation. University of California Press, Berkeley. pp. 106-120

Swanson, P., Suzuki, K., Kawauchi, H. & Dickhoff, W. W. 1991. Isolation and characterization of two coho salmon gonadotropins, GTH I and GTH II. *Biol*

Reprod. **44**: 29–38.

Taranger, G. L., Stefansson, S. O. & Hansen, T. 1992. Advancement and synchronization of ovulation in Atlantic salmon (*Salmo salar* L.) following injections of LHRH analogue. A*quaculture*. **102**: 169-175.

Vermeirssen, E.L.M., Scott, A.P., Mylonas, C.C., Zohar, Y., 1998. Gonadotrophin-releasing hormone agonist stimulates milt fluidity and plasma concentrations of 17,20b-dihydroxylated and 5b-reduced, 3a-hydroxylated C21 steroids in male plaice (*Pleuronectes platessa*). *Gen. Comp. Endocrinol.* **112**: 163– 177.

Vizziano, D., Fostier, A., Loir, M., Le Gac, F., 2008. Testis development, its hormonal regulation and spermiation induction in teleost fish. In: Alavi, S.M.H., Cosson, J., Coward, K., Rafiee, G. (Eds.), Fish Spermatology. Alpha Science International, Oxford, UK, pp. 103–139.

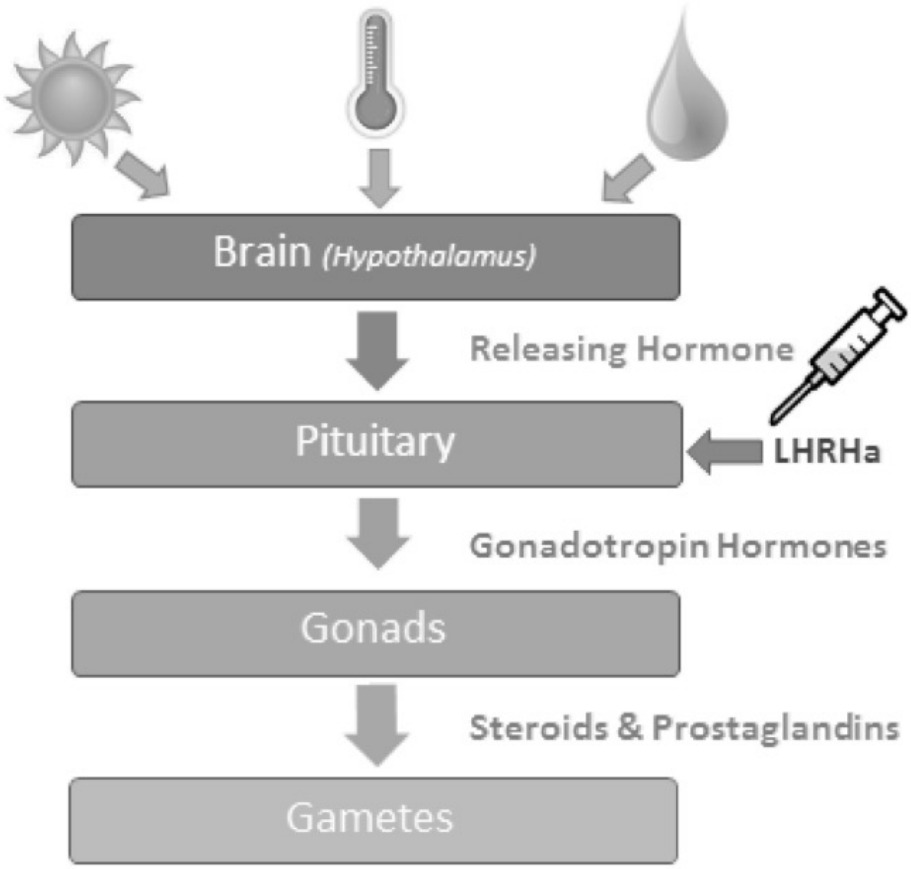
Wei, F., Swaisgood, R., Hu, Y., Nie, Y., Yan, L., Zhang, Z., Qi, D. & Zhu, L. 2015. Progress in the ecology and conservation of giant pandas. *Conserv. Biol*. **29**: 1497-1507.

Wojtczak, M., Kuźmiński, H., Dobosz, S., Mikołajczyk, T., Dietrich, G., Kowalski, R., Kotłowksa, M., Enright, W. J. & Ciereszko, A. 2005. Milt characteristics in European whitefish (*Coregonus lavaretus*) in relation to season and hormonal stimulation with a gonadotropin-releasing hormone analogue. *Advanc. Limnol*. **60**: 171-185.

Yamamoto, J. T., Shields, K. M., Milliam, J. R., Roudybush, T. E. & Grau, C. R. 1989. Reproductive activity of force-paired Cockatiels (*Nymphicus bollandicus*). *Auk*. **106**: 86-93.

Zimmerman, M. S. & Kreuger, C. C. 2009. An ecosystem perspective on re-establishing native deepwater fishes of the Laurentian Great Lakes. *N. Am. J, Fish. Manag*. **29**: 1352-1371.

Figure 1.1 In the hypothalamic-pituitary-gonadal axis, environmental cues such a photoperiod, water temperature and depth initiate a hormonal cascade in fishes that ends in gamete production and development. Luteinizing Hormone Releasing Hormone Analogue (LHRHa) can be used to induce gamete production in the absence of these cues. Adapted from Mylonas et al (2010).



Chapter 2: Induction of Free Flowing Gametes by Injection of Luteinizing Hormone Releasing Hormone Analog in Hatchery-Reared Bloaters

2.1 Introduction

Many fish species around the world are bred in captivity for a variety of reasons. Some are bred for food, such as in aquaculture, while others are bred for conservation purposes. A tremendous challenge often faced in captive breeding efforts is the establishment of a self-sustaining population (Snyder et al. 1996). Often, this difficulty can be attributed to some form reproductive dysfunction brought on by inadequate environmental conditions, diet, behavioral incompatibility or inbreeding depression (Danielle and Murray 1986; Setchell et al. 1987; Milliam et al. 1988; Yamamoto et al. 1989; Merola, 1994). However, the incorporation of reproductive biological strategies, such as the use of exogenous hormones, has improved the captive breeding efforts of many species including amphibians (Browne et al. 2006) and fishes (Crim et al. 1988; Mylonas, 1992; Wojtczak et al. 2005).

It has been well documented that the reproduction of hatchery-reared fish can be controlled through the manipulation of various environmental aspects such as water temperature and photoperiod (King and Pankhurst, 2007; Malison et al, 1998). Species for which there is comprehensive information regarding necessary ecological and biological aspects of their reproduction have been shown to benefit from these environmental manipulations (King and Pankhurst, 2007; Lafferty et al. 1999). However, there are still some species among those in aquaculture today for which either the

relevant information surrounding their reproduction is unknown or the appropriate environmental manipulations are neither practically nor economically feasible (i.e., migration, depth or turbidity) (Mylonas et al, 2010). Alternatively, another common method of controlling fish reproduction that does not solely rely on environmental cues exists; the use of exogenous hormones.

The use of exogenous hormones to manipulate and control fish reproduction has been utilized in aquaculture for many years and is founded on the understanding of reproductive endocrinology of fish (Noori et al. 2010; Crim et al. 1983). The reproductive cycle of fish can be separated into two distinct phases. The first phase is referred to as spermatogenesis and vitellogenesis in males and females, respectively. These initial phases are primarily associated with the proliferation, growth and differentiation of gametes while the second stage, spermiation and final oocyte maturation (FOM) constitute their maturation and preparation for release (Mylonas and Zohar, 2001). In general, to initiate the reproductive endocrine cascade there must first be adequate environmental stimuli (e.g. appropriate water temperature, depth, and turbidity cues) present to elicit the release of gonadotropin releasing hormone (GnRH) from the hypothalamus (see Figure 1.1). GnRH then stimulates the release of gonadotropic hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. Endocrine control of reproduction depends primarily on FSH during the first stage of reproduction while LH is the primary gonadotropic hormone of the second phase. By administering an exogenous hormone, the reproductive processes can be initiated without the presence of necessary environmental stimuli which, as mentioned previously, can be costly or impractical to create in a hatchery setting.

Often, the need to control the reproductive processes of cultured fish arises from the observation of some reproductive dysfunction of the broodstock. Mylonas and Zohar (2001) identified the unpredictable occurrence or absence of final oocyte maturation as the most commonly observed reproductive dysfunction in cultured fish. While females appear to be more commonly affected by a dysfunction, males, too, can experience dysfunction in the form of reduced milt volume and increased milt viscosity (Vermeirssen et al., 1998). Analogs of either gonadotropic hormone releasing hormone (GnRHa) or luteinizing hormone releasing hormone (LHRHa) are the most common exogenous hormones used in aquaculture when attempting to alleviate this reproductive dysfunction (Zohar and Mylonas, 2001).

GnRHa and LHRHa are both classified as gonadotropic releasing hormones as their physiological function is to stimulate the release of gonadotropic hormones from the anterior pituitary. Their efficacy on relieving reproductive dysfunction in fish appears to vary between species and depends on a number of factors such as vector of administration, dosage, timing and duration of administration, and environmental influences (Crim and Glebe, 1984; Billard et al. 1984). Typically, the exogenous hormone is administered through one of two main methods: via injection through a needle and syringe or the implantation of a slow-dissolving pellet. The appropriate dosage of a hormone can vary greatly between species but there is generally an accepted range of approximately 10-100ug/kg which is known to be effective for injections (See Table 2.1). Pellets provide a slower and longer lasting release of hormone compared to injections, however, it is much more difficult to determine what exact dosage a fish may be receiving at a given point in time (Crim et al, 1988; Garcia 1989). As different species

may have different spawning seasons, a hormonal administration time point that is effective for one species may prove ineffective for another. Other studies suggest that environmental factors, such as water temperature, can play an important role in determining the efficacy of the administered hormone in the induction of reproductive processes in hatchery-reared fish (King and Pankhurst, 2007). Often, there is no single optimal potential strategy to overcome reproductive dysfunction in cultured fish, especially for those species whose ecological and biological aspects of reproduction are not well-known (Crim et al. 1983,1984; King and Pankhurst, 2007). Such is the case with the bloater (*Coregonus hoyi*); an important aquatic species originally native to many of the Great Lakes.

The bloater (*Coregonus hoyi*) was extirpated from Lake Ontario in the early 1950's (Clemens and Crawford, 2009). Multiple factors such as fishing pressure, predator-prey ratios (Christie, 1973), increased food competition with alewives (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) as well as predation by sea lamprey (Petromyzon marinus) and reduced water quality (Smith 1972; Christie, 1973). In the wild, bloaters spawn via external fertilization at depths ranging from approximately 40 to 100 meters (Becker, 1983) with an average egg diameter of approximately 1.95 millimetres (Dryer and Beil, 1986). Additionally, adult bloaters have been found in water temperatures of between 4 and 11°C (TeWinkle and Fleischer, 1999). Given these natural environmental conditions, it is possible to understand the inherent difficulty of raising these fish in captivity for conservation purposes.

The main goal of the *Great Lakes Fish and Wildlife Restoration Act* Regional Project entitled *Development of Propagation Strategies to Support Reintroduction of Deepwater Coregonids in Lake Ontario (2014)*, is "to establish a self-sustaining population of one or more species of deepwater ciscoes in Lake Ontario within 25 years". To achieve this, the project also details two objectives: (1) to secure reliable sources of gametes from a self-sustaining broodstock by 2012 and (2) increase current culture capacity and stock 500,000 individuals by 2015. However, efforts from 2013 and 2014 yielded only 22,000 and 70,000 bloaters, respectively. Despite this, captive breeding efforts are still in progress at WLFCS.

Conservation efforts via a partnership between the United States Fish and Wildlife Service, Ontario Ministry of Natural Resources and Forestry (OMNRF), the Great Lakes Fishery Commission and the United States Geological Survey have been ongoing since 2010. As part of the initiative by the partnership, WLFCS has been attempting to produce a self-sustaining broodstock of bloaters for their annual reintroduction into Lake Ontario but have been experiencing difficulties including reproductive issues. For example, the hatchery-reared bloaters exhibit reproductive dysfunction in the forms of the absence of females expressing free-flowing eggs and males producing diminishing amounts of milt. Due to the complete lack of egg production in bloaters from the 2014-2015 season at WLFCS (compared to the consistent, albeit reduced milt expression in males), we elected to focus primarily on the quality of the eggs, rather than the sperm, that were produced this season.

In this study, the efficacy of the use of an exogenous hormone (LHRHa) to overcome the observed reproductive dysfunctions (i.e. asynchrony between sexes and absence of free-flowing eggs in females) exhibited by hatchery-reared bloaters was examined. Finally, because the production of eggs by the bloaters had been absent until this year, the egg diameter of both free-flowing eggs and eggs that have yet to be released were also examined and compared to the diameter of wild bloater eggs from Lake Michigan.

2.2 Materials and Methods

Broodstock

The sexually mature individuals from this study were derived from wild-caught bloaters from Lake Michigan in January 2012. Males and females from this broodstock population (n = 1,322 in total) were haphazardly assigned to one of four experimental treatments; control, sham control, low dosage and high dosage (see below for details). Following their respective treatments, each fish was checked in subsequent weeks to determine whether or not they were expressing free flowing gametes. This experimental design was replicated, once between December 14 and January 20 (hereafter December – January) and with another group of fish between January 18 and February 24 (hereafter January – February).

LHRHa Injection Protocol

Treatment injections took place over two days (half the individuals on day one and half the individuals on the second day) for both time points (December – January and

January – February). Each individual was anesthetized using tricaine methane-sulfonate (MS-222). Once each fish was under anesthesia it was checked immediately for expression of free-flowing gametes (eggs or milt) by gentle abdominal massage. Any fish which was found to be expressing free-flowing gametes was placed in a recovery tank to be excluded from the study because the efficacy of exogenous hormone at inducing free-flowing gamete expression could not be examined in these individuals. Individuals first had their sex guessed (bloaters are sexually monomorphic) and an approximately equal number of fish of each sex were randomly assigned to a treatment group (see details below), however, the sex ratio of the 2012 bloater cohort was approximately 2:1 in favor of females. Once it was determined a fish was not expressing free-flowing gametes, it was measured for mass (to the nearest gram), length (to the nearest mm) and girth just anterior to the dorsal fin (i.e. the widest part of the fish, to the nearest mm; see Appendix for details) in addition to receiving a pit-tag which was injected lateral to the dorsal fin. Following these morphometric measurements, each fish (n = 692 (241 males, 451 females) in December – January, n = 640 (241 males, 399 females) in January – February) was randomly assigned to one of four treatments: control (C, n = 175 (52 males, 123 females) in December – January, n = 160 (65 males, 95 females) in January – February), sham (S, n = 172 (65 males, 107 females) in December – January, n = 160 (64 males, 96 females) in January – February) control, low (L, n = 172 (58 males, 114 females) in December – January, n = 160 (55 males, 105 females) in January – February) dose, and high (H, n = 173 (66 males, 107 females) in December – January, n = 160 (57 males, 103 females) in January – February) dose. Each control fish was returned to a recovery tank following its morphometric measurements (see Figure 2.1). Fish in the low

dose group received an intraperitoneal priming injection (50%) of LHRHa (Syndel Laboratories Ltd., Vancouver, B.C.) in 0.9% saline, just below the pelvic fin, equivalent to 20ug/kg while high dose individuals received a similar injection but at a concentration of 40ug/kg. Sham control individuals received an intraperitoneal injection of 0.9% saline of the same volume that a fish of identical mass would receive had it received an LHRHa injection (i.e. low or high dose treatments). Following treatment for both the December – January and January – February groups, half of the individuals (first day of injections) were put into one recovery tank and half (second day of injections) into another tank – both containing individuals from all treatment groups to eliminate tank effects. This protocol was repeated for all fish from each of the treatments seven days following the initial handling and injections, this time to administer the resolving injection (50%) of LHRHa for the low and high fish, a second saline injection for sham fish and only anesthesia administration and handling for control fish. This brought the total amount of LHRHa injected to 40ug/kg for those individuals receiving a low dose and 80ug/kg for those receiving a high dose which is consistent with other species in the literature (see Table 2.1). Additionally, at this time point, fish were first checked by abdominal massage for the production of free-flowing gametes. All individuals were also checked again for free-flowing gametes at three, four and five weeks following their priming injections, which is consistent with other research regarding exogenous hormone injection in fish (Crim et al. 1988; Wojtczak et al. 2005) and is within the speculated spawning season of wild bloaters (Becker, 1983). Also, the average water temperatures during the December – January and January – February injections were 4 and 1°C, respectively. For all treatment and checks following the initial treatment, fish were identified by pit-tag

number and recorded for either expressing free-flowing gametes or not on the date of the check.

Gonadosomatic Index (GSI) Measurement

Weighing of hatchery-reared bloater gonads for GSI measurement took place during the third week after the priming injection. Prior to handing, all individuals were euthanized using MS-222. Subsequently, all individuals were weighed to the nearest gram using an electric scale and their girth was measured using a flexible tape measure, both, a second time in addition to the morphometric measurements taken during the injection protocol. Each male (3 high, expressing and 8 control, 9 sham, 12 low and 6 high non-expressing) and female (n = 47; 7 control, 7 sham, 5 low and 6 high, expressing females and 7 control, 5 sham, 4 low and 6 high, non-expressing) were dissected using a sagittal incision along the ventral midline so that the gonads could be extracted. All gonads were placed into medium plastic weigh boats and weighed on an electronic scale (to the nearest milligram) for the calculation of GSI (mass of individual divided by the mass of the gonads).

Egg Diameter Measurement

Photography of hatchery-reared bloater eggs for diameter measurement took place during the third week after the priming injection for both the December – January and January – February groups (i.e. same time as GSI measurement, see above). Additionally, unfertilized, wild bloater eggs from Lake Michigan were collected by staff from the United States Fish and Wildlife Service (USFWS) and delivered to WLFCS on January

28th approximately eight hours later. Prior to handing, all females (n = 26; 9 low and 7 high, expressing and 5 control and 5 sham non-expressing females for December – January, n = 47; 7 control, 7 sham, 5 low and 6 high, free flowing females and 7 control, 5 sham, 4 low and 6 high, excised females for January – February with the addition of n = 10 free flowing and 10 excised hatchery females each for both December – January and January – February groups), except the wild females (n = 24) which were processed on Lake Michigan, were anesthetized using MS-222. "Hatchery" females refer to individuals of the same 2012 cohort that did not participate in the LHRHa Injection Protocol (thus they were only anesthetized once) and "wild" female eggs were from Lake Michigan (see above). The females (n = 26; 9 low and 7 high, expressing and 5 control and 5 sham non-expressing females for December – January, n = 47; 7 control, 7 sham, 5 low and 6 high expressing females and 7 control, 5 sham, 4 low and 6 high, non-expressing females for January – February) had their morphometric measurements taken as in the injection protocol (see above). If a female was expressing free-flowing gametes, they were collected by gentle abdominal massage into medium plastic weigh boats. Once the free-flowing eggs were extracted or the female was not yet expressing them, each female was dissected using a sagittal incision along the ventral midline. Following extraction, subsets of eggs from each female were carefully placed on filter paper cut to fit inside small petri dishes. Once the eggs had been carefully separated on the filter paper using steel forceps, so that they were not in contact with one another, they were then photographed using a Celestron Handheld Digital Microscope Pro with each photograph including approximately 20 eggs (n = 352 subsets, mean = 22 +/- 0.25 eggs per subset (range: 10-37)). Following this imaging, the Celestron Portable Capture Pro software was used to

measure the diameters of each individual egg (mm).

2.3 Results

LHRHa Injections

Cumulatively, LHRHa treatments of the December – January bloaters had a significant effect as to whether females (chi-squared test: $X^2 = 19.355$, $P < 0.0005$, Figure 2.2), but not males (chi-squared test: $X^2 = 4.76$, $P = 0.19$, Figure 2.2), were expressing free-flowing gametes. Similarly, treatment of the January – February bloaters had a significant effect as to whether females (chi-squared test: $X^2 = 23.07$, $P < 0.0001$, Figure 2.3), but not males (chi-squared test: $X^2 = 0.94$, $P = 0.82$ Figure 2.3), were expressing free-flowing gametes.

Additionally, after examining the free flowing expression percentages at each check, treatment of the December – January bloaters had a significant effect as to whether females at weeks one (chi-squared test: $X^2 = 10.57$, $P < 0.05$, Figure 2.4), three (chi-squared test: $X^2 = 16.33$, $P < 0.001$, Figure 2.4) and four (chi-squared test: $X^2 = 8.003$, $P <0.05$, Figure 2.4) but not five (chi-squared test: $X^2 = 2.69$, $P = 0.44$, Figure 2.4) post-priming injection were expressing free flowing gametes. However, treatment had no significant effect as to whether male December – January bloaters at weeks one (chi-squared test: $X^2 = 1.87$, $P = 0.60$, Figure 2.4), three (chi-squared test: $X^2 = 0.82$, $P = 0.84$, Figure 2.4), four (chi-squared test: $X^2 = 4.59$, $P = 0.20$, Figure 2.4) or five (chi-squared test: $X^2 = 4.27$, $P = 0.23$, Figure 2.4) post-priming injection were expressing free flowing gametes. Treatment of January – February bloaters had a significant effect as to whether

females at week three (chi-squared test: $X^2 = 41.25$, $P < 0.0001$. Figure 2.5), but not weeks one (chi-squared test: $X^2 = 4.33$, $P = 0.23$, Figure 2.5), four (chi-squared test: $X^2 = 2.75$, $P = 0.43$, Figure 2.5) and five (chi-squared test: $X^2 = 3.03$, $P = 0.39$, Figure 2.5), post-priming injection were expressing free flowing gametes. Treatment of January – February male bloaters did not have significant effects at weeks one (chi-squared test: $X^2 = 1.31$, $P = 0.73$, Figure 2.5) or three (chi-squared test: $X^2 = 1.97$, $P = 0.58$, Figure 2.5) post-priming injection were expressing free flowing gametes and no new males expressed free flowing gametes in weeks four and five.

Gonadosomatic Index (GSI)

The GSI of non-expressing (i.e. not producing free flowing milt) male bloaters was not significantly different among the treatment groups (One-way ANOVA: $F_{3,31} = 0.96$, $P = 0.42$, Figure 2.6). Similarly, the GSI of both expressing (One-way ANOVA: $F_{3,21} = 2.20$, $P = 0.12$, Figure 2.7) and non-expressing (One-way ANOVA: $F_{3,18} = 0.61$, $P = 0.62$) female bloaters was not significantly different among the treatment groups.

Egg Diameter

December – January bloaters of the control, sham and hatchery groups, whose eggs were excised, showed no significant difference among mean egg diameters (One-way ANOVA: $F_{2,17} = 0.45$, $P = 0.65$, Figure 2.8). However, mean egg diameter was significantly different among the free flowing eggs of the low, high and wild groups

(One-way ANOVA: $F_{2,37} = 16.90$, $P < 0.0001$, Figure 2.8) with wild eggs being significantly larger than both low and high dose eggs. Similarly, January – February bloaters of the control, sham, low, high and hatchery groups, whose eggs were excised, showed no significant difference among mean egg diameters (One-way ANOVA: $F_{4,27} = 0.77$, $P = 0.56$, Figure 2.9). Finally, mean egg diameters were significantly different among the free flowing eggs of the control, sham, low, high, hatchery and wild groups (One-way ANOVA: $F_{5,53} = 6.74$, $P < 0.0001$, Figure 2.9) with wild, low and control eggs being significantly larger than hatchery high and sham eggs.

2.4 Discussion

The usefulness of luteinizing hormone releasing hormone analog (LHRHa) for inducing the expression of free-flowing gametes in hatchery-reared bloaters appears to be effective, especially for females. However, there does not appear to be meaningful differences between the bloaters' GSI for either sex amongst the treatment groups. Additionally, there are observed differences between the egg diameter of the bloaters from the treatment groups, hatchery group (baseline) and wild group.

LHRHa Injection of Males

Males of the December – January group of bloaters did not appear to benefit from the LHRHa injections. Results indicated that control males (31%), followed by sham (22%), low (17%) and high (15%) experienced decreased occurrences of free flowing gamete expression, respectively, in the five weeks post-treatment. Alternatively, males of

the January – February group experienced little (if any) benefit from LHRHa injections (7% low and 9% high) as compared to control (5%) and sham (6%) individuals. This reduction in free flowing gamete expression is contradictory to what is typically observed following exogenous hormone injection of other hatchery-reared fish such as European whitefish (Wojtczak et al. 2005). Wojtczak et al injected 33 whitefish with GnRHa (25-32μg/kg) at the beginning of the spawning season (November) and all of the fish produced free flowing sperm – most of which occurred during the first 16 days after injection. However, this system differed from others on two key aspects: (1) the exact spawning season of the male hatchery-reared bloaters had not been previously identified and (2) based on the first aspect, the hormonal treatment was applied towards the end (December – January) and after (January – February) the observed spawning season for males whereas other studies regarding exogenous hormone injections of fish (see above), with well documented spawning seasons, were applied prior to or at the beginning of the spawning season. Additionally, it is possible that the males (December – January) experienced reduced levels of plasma gonadotropins due to increased plasm androgen levels via a negative feedback loop within the HPG axis (Soma et al. 1996). That is, while the males were producing free flowing milt (i.e. during their natural spawning season), their plasma androgen levels may have already been elevated, thus, by injecting LHRHa, a gonadotropin which eventually triggers the release of androgens from the testes, the abnormally high androgen levels reduced natural gonadotropin release from the pituitary, lowering plasma androgen levels and, consequently, stopped free flowing milt expression in some males (Nagahama, 1994). Finally, most of the males (January –

February) may also have spent their milt reserves earlier in the season, preventing the LHRHa injections from inducing further milt expression.

LHRHa Injection of Females

Contrary to the male bloaters, females experienced significantly greater percentages of free flowing egg expression from the LHRHa injections. Females from the December – January group that received low (19%) and high (20%) doses of LHRHa experienced greater levels of free flowing gamete expression as compared to sham (10%) and control (3%) groups over the five weeks post-treatment. This discrepancy between the gamete expression of the control and hormonal groups may be due to the fact that the observed spawning season for the majority of female bloaters had not yet begun at the time of the first injection. In the January – February females, low (49%) and high (46%) dose females experienced significantly greater than expected levels of free flowing gamete expression as compared to sham (24%) and control (30%) individuals. These levels of gamete expression, as compared to the December – January group, may be explained by the fact that at the time of the injection, the observed female spawning season had begun thus a higher proportion of control and sham females were expressing eggs. This increased level of free flowing gamete expression is what is typically observed in hatchery-reared fish that receive exogenous hormone injections such as brown trout (Noori et al. 2010) and Atlantic salmon (Crim and Glebe 1984). Noori et al achieved cumulative percent ovulations ranging from approximately 50% in control to 100% (in under 30 days post injection) in individuals who received a 100μg/kg injection. Similarly,

Crim and Glebe (1984) found that 94% of female Atlantic salmon had expressed free flowing gametes within 9 days of the LHRHa pellet implantation (27μg/kg) when it was conducted 4 weeks prior to the natural spawning season. This system differs from some others in that the exact spawning season of the female hatchery-reared bloaters had not been previously identified as compared to other species with well-known spawning season (see above). Additionally, expression of free flowing eggs from hatchery-reared bloaters at the levels observed in this research, have not been previously observed.

Gonadosomatic Index

There were no significant differences observed among the GSI's of bloaters of either sex among treatments in this experiment. This observation is consistent with studies that examine both exogenous hormone application and GSI measurement. For example, Trudeau et al (1995) observed that after implanting testosterone or estradiol (both 100μg/g) pellets into female goldfish (*Carassius auratus*), there was no significant difference in GSI at any point measured during its spawning season. Additionally, there appears to be a poor correlation between GSI and the different stages of reproductive development (i.e. expressing free flowing gametes or not), depending on the species of fish (DeVlaming et al. 1982). Not accurately knowing the spawning season of hatchery-reared bloaters prior to this experiment made GSI measurement for male bloaters difficult. In this experiment, male bloaters had stopped producing free flowing milt by the time the GSI measurement took place (February 8, 2016) except for a small number (n = 3) that began expressing milt between January 26 and February 8, which made the GSI

measurement of expressing males (other than the 3 above from, all from the high group) from the other treatment groups impossible. However, in the future, it would be beneficial to measure the reproductive hormone levels (e.g. estradiol, testosterone, vitellogenin etc.) in conjunction with the GSI and gamete expression (i.e. free flowing or not) status of the bloaters to better characterize and determine the physiological effects of LHRHa on the bloater reproductive cycle.

Egg Diameter

Significant differences were observed among the egg diameter of hatchery-reared and wild bloaters. For the December – January females, there were no observed significant differences among the egg diameter of excised eggs for the control, sham and hatchery bloaters. However, in regards to free flowing eggs, the wild female egg diameter was significantly greater than both the low and high individuals. Additionally, for the January – February females there were, again, no significant differences among the excised egg diameter for the control, sham, low, high and hatchery groups. Free flowing egg diameter of wild bloaters was significantly greater than sham, high and hatchery bloaters, but not the control and low groups. Additionally, body length (size) is one of the most common and strong predictors of egg size in teleosts (see Heath et al 1999 and the studies therein). The average length of the free flowing control, sham, low and high groups were 260 ± 17mm, 269 ± 10mm, 248 ± 5mm and 249 ± 4mm, respectively, for the December – January group and 262 ± 4mm, 242 ± 5mm, 249 ± 4mm and 258 ± 3mm, respectively for the January – February group. These groups were considerably larger

than the wild specimens (average length: 210 ± 3mm) yet they all had smaller average egg diameters than the wild group. It is expected that this difference is due to the incongruence between the environmental cues of the hatchery and those of the Lake Michigan bloater habitat.

This research was novel in that, to my knowledge, there has not been a study which compared the egg diameter of all three: hatchery, exogenous hormone injected and wild fish although, reproductive dysfunction has been observed to negatively impact egg diameter in hatchery-reared fish (reviewed in Mylonas & Zohar, 2001 and Mylonas et al. 2010). At the time of egg measurement (week 3 post-treatment) in the December – January group, there were no females from either the control or sham groups or other hatchery fish which had yet produced free flowing eggs so no measurements could be made at that time.

Overall, it was observed that, dependent on their timing, the use of intraperitoneal LHRHa injections can effectively induce free flowing gamete expression in hatchery-reared bloaters with minimal (< 1%) mortality. The times of injection for this study were more effective for females than males, however, modification of the timings, now that the spawning seasons of males and females are better understood, may increase the benefits observed for males. Additionally, up until this year, very few females had ever expressed free flowing eggs thus it was important to observe gamete quality (in the form of egg diameter) of females more so than males.

Future studies and practices pertaining to the use of exogenous hormone on hatchery-reared bloaters to induce the expression of free-flowing gametes could benefit

from injecting males in early November and females in mid-January (i.e. at the beginning of their respective observed spawning seasons) (Crim and Glebe 1984; Billard et al. 1984). Additionally, manipulation of photoperiod (Hansen et al. 2001) has been shown to drastically affect numerous factors of reproduction in species such as Atlantic cod (*Gadus morhua*). In their study, Hansen et al (2001) observed that only cod reared under natural (i.e. in the wild) lighting conditions spawned during the normal season and had significantly higher egg diameter and fecundity than cod which underwent photoperiod manipulation (e.g. continuous light etc.). A similar trend was observed by Taranger et al (1998) with Atlantic salmon exposed to natural and manipulated lighting – the proportion of sexually mature individuals was significantly reduced when lighting was unnatural. Another prominent factor which affects the reproduction of fish in captivity is their diet (reviewed in Izquierdo et al. 2001). For example, Duray et al (1994) observed that when dietary lipid level was increased for rabbitfish (*Siganus guttatus*) there were significant increases in both fecundity and hatching percentage. To conclude, intraperitoneal injections of LHRHa are an effective tool that can be utilized to create a reproductively viable and self-sustaining captive broodstock, however, there are a number of factors such as timing of exogenous hormone application, photoperiod manipulation (to natural levels) and even dietary manipulation, among many others which may continue to improve the reproduction of hatchery-reared bloaters.

References

Arabaci, M., Diler, I. & Sari, M. 2004. Induction and synchronization of ovulation in rainbow trout, *Oncorhynchus mykiss*, by administration of emulsified buserelin (GnRHa) and its effect on egg quality. *Aquaculture*. **237**: 475-484.

Becker, G. C. 1983. Fishes of Wisconsin. Madison (WI): University of Wisconsin Press. pp. 356-360.

Billard, R., Reinaud, P., Hollebecq, M. G. & Breton, B. 1984. Advancement and synchronization of spawning in *Salmo gairdneri* and *S. trutta* following administration of LRH-A combined or not with pimozide. *Aquaculture*. **43**: 57-66.

Browne, R. K., Seratt, J., Vance, C. & Kouba, A. 2006. Hormonal priming, induction of ovulation and in-vitro fertilization of the endangered Wyoming toad (*Bufo baxteri*). *Reprod. Biol. Endocrin*. **4**: 34.

Christie, W. J. 1974. Changes in fish species composition of the Great Lakes. *J Fish Res Board Can*. **31**: 827–854.

Christie, W. J. 1973. A review of the changes in the fish species composition of Lake Ontario. *Great Lakes Fish Comm Tech Rep*. pp. 23-65.

Clemens, B. J. & Crawford, S. S. 2009. The ecology of body size and depth use by bloater (*Coregonus hoyi* Gill) in the Laurentian Great Lakes: patterns and hypotheses. *Rev. Fish. Sci*. **17**: 174-186.

Crim, L. W. & Glebe, B. D. 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. *Aquaculture*. **43**: 47-56.

Crim, L. W., Evans, D. M. & Vickery, B. H. 1983. Manipulation of the seasonal

reproductive cycle of the landlocked Atlantic salmon (*Salmo salar*) by LHRH analogues administered at various stages of gonadal development. *Can. J. Fish. Aquat. Sci.* **40**: 61-67.

Crim, L. W., Sherwood, N. M. and Wilson, C. E. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (LHRHa) administration by either single time injection of cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. *Aquaculture*. **74**: 87-95.

Danielle, A. & Murray, N. D. 1986. Effects of inbreeding in the Budgerigar (*Melopsittacus undulatus*) (Aves: *Psittacidae*). *Zoo Biol*. **5**:233-238.

DeVlaming, V. Grossman, G. & Chapman, F. 1982: On the use of the gonosomatic index. Comp. Biochem. *Physiol. A Physiol*. **73**: 31–39.

Dryer, W. R. and Beil, J. 1986. Growth changes of bloater (*Coregonus hoyi*) of Apostle Islands region of Lake Superior. *T. Am. Fish. Soc*. **97**: 146-158.

Duray, M., Kohno, H. & Pascual, F., 1994. The effect of lipid enriched broodstock diets on spawning and on egg and larval quality of hatchery-bred rabbitfish (*Siganus guttatus*). *Philipp. Sci*. **31**: 42–57.

Garcia, L. M. 1991. Spermiation response of mature rabbitfish, *Siganus guttatus* Bloch, to luteinizing hormone releasing hormone analogue (LHRHa) injection. *Aquaculture*. **97**: 291-299.

Garcia, L. M. 1989. Dose-dependent spawning response of mature female sea bass, *Lates calcarifer* (Bloch), to pelleted luteinizing hormone-releasing hormone analogue (LHRHa). *Aquaculture*. **77**: 85-96.

Hansen, T., Karlsen, Ø., Taranger, G. L., Hemre, G., Holm, J. C. & Kjesbu, O. S. 2001. Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture*. **203**: 51-67.

Heath, D. D., Fox, C. W. & Heath, J. W. 1999. Maternal effects on offspring size: variation through early development of chinook salmon. *Evolution*. **53**:1605-1611.

Izquierdo, M. S., Fernández-Palacios, H. & Tacon, A. G. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture.* **197**: 25-42.

King, H. R. & Pankhurst, N. W. 2007. Additive effects of advanced temperature and photoperiod regimes and LHRHa injection on ovulation in Atlantic salmon (*Salmo salar*). *Aquaculture*. **273**: 729-738.

Lafferty, K. D., Swift, C. C. & Ambrose, R. F. 1999. Extirpation and recolonization in a metapopulation of an endangered fish, the tidewater goby. *Conserv. Biol.*. **13**: 1447-1453.

Lubzens, E., Young, G., Bobe, J. & Cerda, J. 2010. Oogenesis in teleosts: how fish eggs are formed. *Gen. Comp. Endocr*. **165**: 367-389.

Malison, J. A., Procarione, L. S., Kayes, T. B., Hansen, J. F. & Held, J. A. 1998. Induction of out-of-season spawning in walleye (*Stizostedion vitreum*). *Aquaculture*. **163**: 151-161.

Merola, M. 1994. A reassessment of homozygosity and the case for inbreeding depression in the cheetah. *Conserv. Biol*. **8**: 961- 971.

Milliam, J. R., T. E. Roudybush, and C. R. Grau. 1988 Influence of environmental manipulation and nest-box availability on reproductive success of captive

Cockatiels (*Nymphicus bollandicus*). *Zoo Biol.* **7**: 25-34.

Mylonas, C. C. & Zohar, Y. 2001. Use of GnRHa-delivered systems for the control of reproduction of fish. *Rev. Fish Biol. Fisher*. **10**: 463-491.

Mylonas, C. C., Fostier, A. & Zanuy, S. 2010. Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endocr*. **165**: 516-534.

Mylonas, C. C., Hinshaw, J. M. & Sullivan, C. V. 1992. GnRHa-induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. *Aquaculture*. **106**: 379-392.

Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. *Int. J. Dev. Biol*. **38**: 217-229.

Noori, A., Amiri, B. M., Mirvaghefi, A. & Baker, D. W. 2010. LHRHa-induced ovulation of the endangered Caspian brown trout (*Salmo trutta caspius*) and its effect on egg quality and two sex steroids: testosterone and 17α-hydroxyprogesterone. *Aquac. Res*. **41**: 871-877.

Olito, C., Loopstra, D. & Hansen, P. 2001. Acceleration of sexual maturation of Chinook salmon using luteinizing hormone-releasing hormone analog. *N. Am. J. Aquacult.*. **63**: 208-214.

Setchell, K. D. R., Gosselin, S. J., Welsh, M. B., Johnston, J. O., Balistreri, W.F., Kramer, L. W., Dresser, B. L. & Tarr, M. J. 1987. Dietary estrogens a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology*. **93**: 225-233.

Slater, C. H., Schreck, C. B. & Amend, D. F. 1995. GnRHa injection accelerates final maturation and ovulation/spermiation of sockeye salmon (*Oncorhynchus nerka*)

in both fresh and salt water. *Aquaculture*. **130**: 279-285.

Snyder, N. F., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D & Miller, B. 1996. Limitations of captive breeding in endangered species recovery. *Conserv. Biol.* **10**: 338-348.

Soma, K. K., Francis, R. C., Wingfield, J. C. & Fernald, R. D. 1996. Androgen regulation of hypothalamic neurons containing gonadotropin-releasing hormone in cichlid fish: integration with social cues. *Hormones and Behavior*. **30**: 216-226.

Taranger, G. L., Haux, C., Stefansson, S. O., Björnsson, B. J., Walther, B. T. & Hansen, T. 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradial-17β profiles in Atlantic salmon, *Salmo salar*. *Aquaculture*. **162**: 85-98.

Taranger, G. L., Stefansson, S. O. & Hansen, T. 1992. Advancement and synchronization of ovulation in Atlantic salmon (*Salmo salar* L.) following injections of LHRH analogue. A*quaculture*. **102**: 169-175.

TeWinkel, L. M., & Fleischer, G. W. 1999. Vertical migration and nighttime distribution of adult bloaters (*Coregonus hoyi*) in Lake Michigan. *T. Am. Fish. Soc*. **128**: 459-474.

Trudeau, V. L., Peter, R. E. & Sloley, B. D. 1991. Testosterone and estradiol potentiate the serum gonadotropin response to gonadotropin releasing hormone in goldfish. *Biol. Reprod.* **44**: 951-960.

Tyler, C. R. & Sumpter, J. P. Oocyte growth and development in teleosts. *Rev. Fish Biol. Fisher*. **6**: 287-318.

Vazirzadeh, A., Hajimoradloo, A., Esmaeili, H. R. & Akhlaghi, M. 2008. Emulsified versus saline administration of GnRHa on induction of ovulation in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. **280**: 267-269.

Vermeirssen, E.L.M., Scott, A.P., Mylonas, C.C., Zohar, Y., 1998. Gonadotrophin-releasing hormone agonist stimulates milt fluidity and plasma concentrations of 17,20b-dihydroxylated and 5b-reduced, 3a-hydroxylated C21 steroids in male plaice (*Pleuronectes platessa*). *Gen. Comp. Endocrinol.* **112**: 163– 177.

Wojtczak, M., Kuźmiński, H., Dobosz, S., Mikołajczyk, T., Dietrich, G., Kowalski, R., Kotłowksa, M., Enright, W. J. & Ciereszko, A. 2005. Milt characteristics in European whitefish (*Coregonus lavaretus*) in relation to season and hormonal stimulation with a gonadotropin-releasing hormone analogue. *Advanc. Limnol*. **60**: 171-185.

Yamamoto, J. T., K. M. Shields, J. R. Milliam, T. E. Roudybush, and C. R. Grau. 1989. Reproductive activity of force-paired Cockatiels (*Nymphicus bollandicus*). *Auk*. **106**: 86-93.

Zohar, Y. & Mylonas, C. C. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*. **197**: 99-136.

Table 2.1 Summary (non-exhaustive) of exogenous hormones, dosages and administration methods utilized for the induction gamete expression in salmonids. The exogenous hormones, gonadotropic hormone releasing hormone analog (GnRHa) and luteinizing hormone releasing hormone analog (LHRHa), are typically administered as surgically implanted pellet or an intraperitoneal injection.

Species	Exogenous Hormone	Maximum Dosage	Adminitration Method	Reference
Atlantic Salmon (*Salmo salar*)	LHRHa	125 µg	Pellet	Crim and Evans, 1983
	LHRHa	125 µg	Pellet	Crim and Glebe, 1984
	LHRHa	100µg/kg	IP Injection	Taranger et al. 1992
	LHRHa	10µg/kg	IP Injection	King and Pankhurst. 2007
Brown Trout (*Salmo trutta*)	LHRHa	100µg/kg	IP Injection	Noori et al. 2010
	GnRHa	20µg/kg	IP Injection	Mylonas et al. 1992
	LHRHa	20µg/kg	IP Injection	Billard et al. 1984
Rainbow Trout (*Oncorhynchus mykiss*)	LHRHa	38µg/kg	Pellet	Crim et al. 1988
	GnRHa	50µg/kg	IP Injection	Arabaci et al. 2004
	GnRHa	25µg/kg	IP Injection	Vazirzadeh et al. 2008
Chinook Salmon (*Oncorhychus tshawytscha*)	LHRHa	25µg/kg	IP Injection	Olito et al. 2001
European Whitefish (*Coregonus lavaretus*)	GnRHa	25µg/kg	IP Injection	Wojtczak et al. 2005
Sockeye Salmon (*Oncorhynchus nerka*)	GnRHa	10µg/kg	IP Injection	Slater et al. 1995

Figure Captions

Figure 2.1 Graphical representation of the experimental design for the bloater (*Coregonus* hoyi) Luteinizing Hormone Releasing Hormone Analogue (LHRHa) treatment protocol. All samples were anesthetized and pit-tagged for identification purposes. There were four treatment groups: a control (no injection), a sham control (0.9% saline intraperitoneal (IP) injection), low dose (0.9% saline + 40μg/kg (LHRHa) IP injection) and high dose (0.9% saline + 80μg/kg LHRHa IP injection). Following treatment, samples were returned to a holding tanks until the presence or absence of free-flowing gametes was assessed.

Figure 2.2 Percentage of Bloaters (*Coregonus hoyi*) that expressed free-flowing gametes between December 21 and January 20 (December – January sample) at White Lake Fish Culture Station in Sharbot Lake, Ontario. Males are indicated by dark gray bars and females by striped open bars for each of the treatment groups (control, sham, low and high).

Figure 2.3 Percentage of Bloaters (*Coregonus hoyi*) that expressed free-flowing gametes between January 25 and February 24 (January – February sample) at White Lake Fish Culture Station in Sharbot Lake, Ontario. Males are indicated by dark gray bars and females by striped open bars for each of the treatment groups (control, sham, low and high).

Figure 2.4 Percentage of Bloaters (*Coregonus hoyi*) that expressed free-flowing gametes at each of four time points on December 21/22 (1), January 4/5 (3), January 11/12 (4), and January 20 (5) (December sample) at White Lake Fish Culture Station in Sharbot

Lake, Ontario. Males are indicated by dark gray bars and females by striped open bars for each of the treatment groups (control (C), sham (S), low (L), and high (H)). In the case of an absent bar, no new individuals of that treatment group were found spawning at the given time point.

Figure 2.5 Percentage of Bloaters (*Coregonus hoyi*) that expressed free-flowing gametes at each of four time points on January 25/26 (1), February 8/9 (3), February 17/18 (4), and February 24 (5) (January sample) at White Lake Fish Culture Station in Sharbot Lake, Ontario. Males are indicated by dark gray bars and females by striped open bars for each of the treatment groups (control (C), sham (S), low (L), and high (H)). In the case of an absent bar, no new individuals of that treatment group were found spawning at the given time point.

Figure 2.6 Mean (± standard error) gonadosomatic index (GSI) of male Bloaters (*Coregonus hoyi*) at White Lake Fish Culture Station in Sharbot Lake, Ontario. GSI is the ratio of the mass of the gonads of an individual to the total mass of the individual. Individuals that expressed free-flowing gametes between January 25 and February 24 (January – February sample) are indicated by dark gray bars and individuals that did not (gonads were surgically excised) are indicated by striped open bars. In the case of an absent bar, no data could be collected for that group. Treatment means without a letter in common (with the same numbered subscript) for the same expression status were significantly different ($P < 0.05$).

Figure 2.7 Mean (± standard error) gonadosomatic index (GSI) of female Bloaters (*Coregonus hoyi*) at White Lake Fish Culture Station in Sharbot Lake, Ontario. GSI is the

ratio of the mass of the gonads of an individual to the total mass of the individual. Individuals that expressed free-flowing gametes between January 25 and February 24 (January – February sample) are indicated by dark gray bars and individuals that did not (gonads were surgically excised) are indicated by striped open bars. In the case of an absent bar, no data could be collected for that group. Treatment means without a letter in common (with the same numbered subscript) for the same expression status were significantly different ($P < 0.05$) and subscripts indicate individual one-way ANOVAs.

Figure 2.8 Mean (± standard error) egg diameter (mm) of Bloaters (*Coregonus hoyi*) from both White Lake Fish Culture Station (WLFCS) in Sharbot Lake, Ontario and Lake Michigan (wild). Egg diameter that was measured from free-flowing eggs are indicated by dark gray bars while eggs that were surgically excised are indicated by striped open bars for each treatment group (control, sham, low, high, hatchery and wild) for the December – January sample. Hatchery fish were from WLFCS and served as a baseline measure. Wild bloater eggs were from females in Lake Michigan. In the case of an absent bar, no data could be collected for that group. Treatment means without a letter in common (with the same numbered subscript) for the same egg status were significantly different ($P < 0.05$) and subscripts indicate individual one-way ANOVAs.

Figure 2.9 Mean (± standard error) egg diameter (mm) of Bloaters (*Coregonus hoyi*) from both White Lake Fish Culture Station (WLFCS) in Sharbot Lake, Ontario and Lake Michigan (wild). Egg diameter that was measured from free-flowing eggs are indicated by dark gray bars while eggs that were surgically excised are indicated by striped open

bars for each treatment group (control, sham, low, high, hatchery and wild) for the January – February sample. Hatchery fish were from WLFCS and served as a baseline measure. Wild bloater eggs were from females in Lake Michigan. In the case of an absent bar, no data could be collected for that group. Treatment means without a letter in common (with the same numbered subscript) for the same egg status were significantly different ($P < 0.05$) and subscripts indicate individual one-way ANOVAs.

Figure 2.1

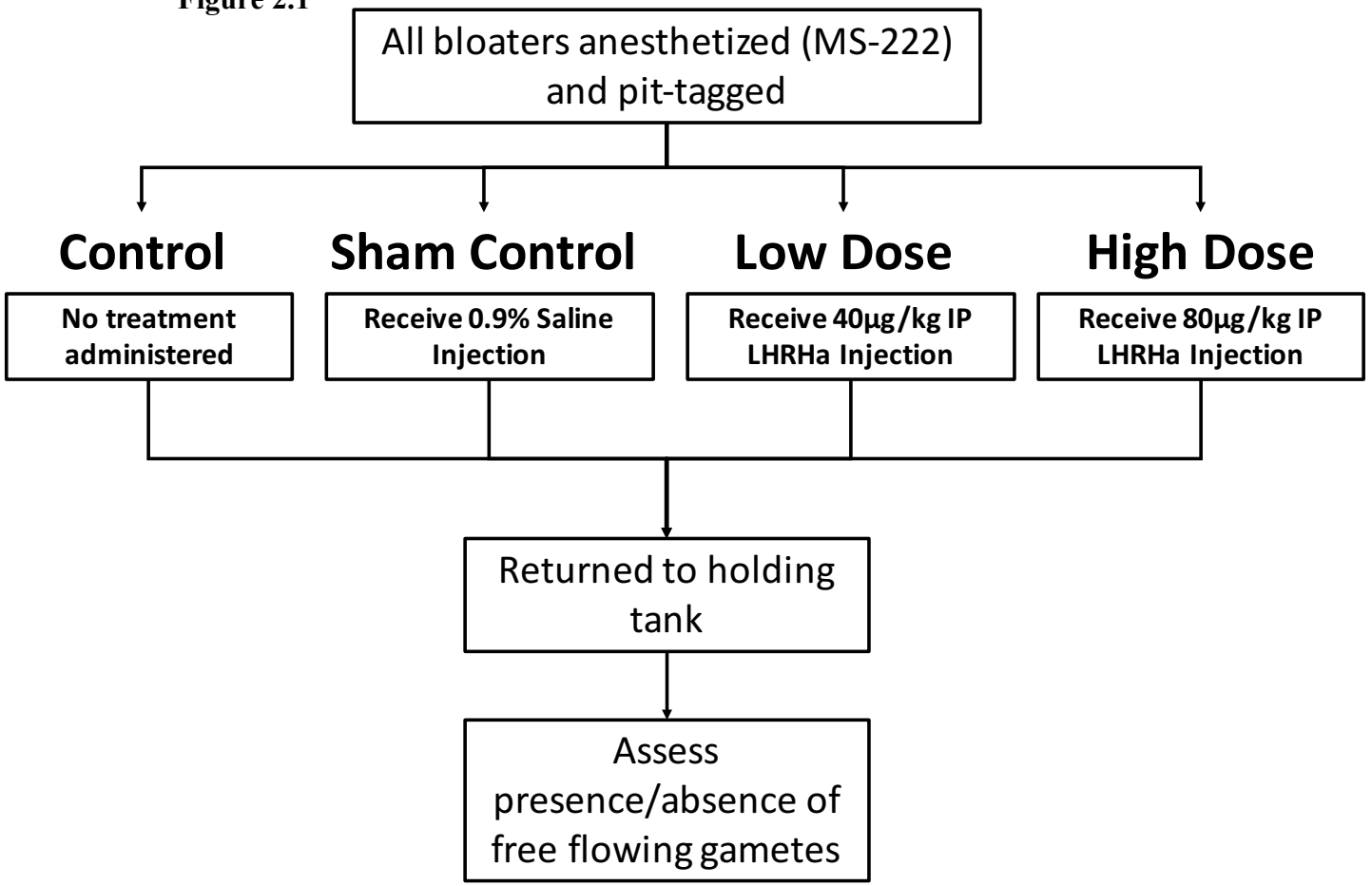

All bloaters anesthetized (MS-222) and pit-tagged
Control
Sham Control
Low Dose
High Dose
No treatment administered
Receive 0.9% Saline Injection
Receive 40µg/kg IP LHRHa Injection
Receive 80µg/kg IP LHRHa Injection
Returned to holding tank
Assess presence/absence of free flowing gametes

Figure 2.2

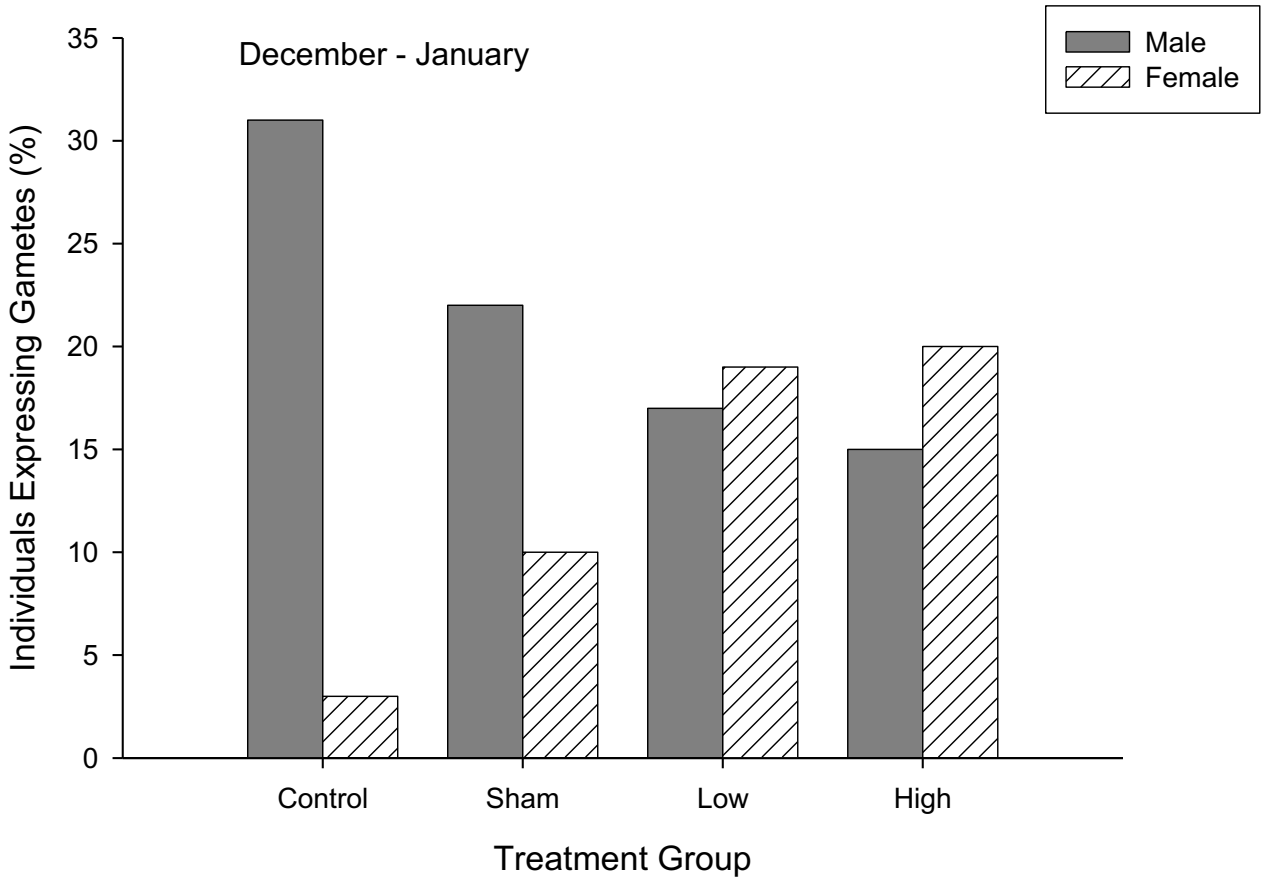

December - January
Male
Female
Individuals Expressing Gametes (%)
35
30
25
20
15
10
5
0
Control
Sham
Low
High
Treatment Group

Figure 2.3

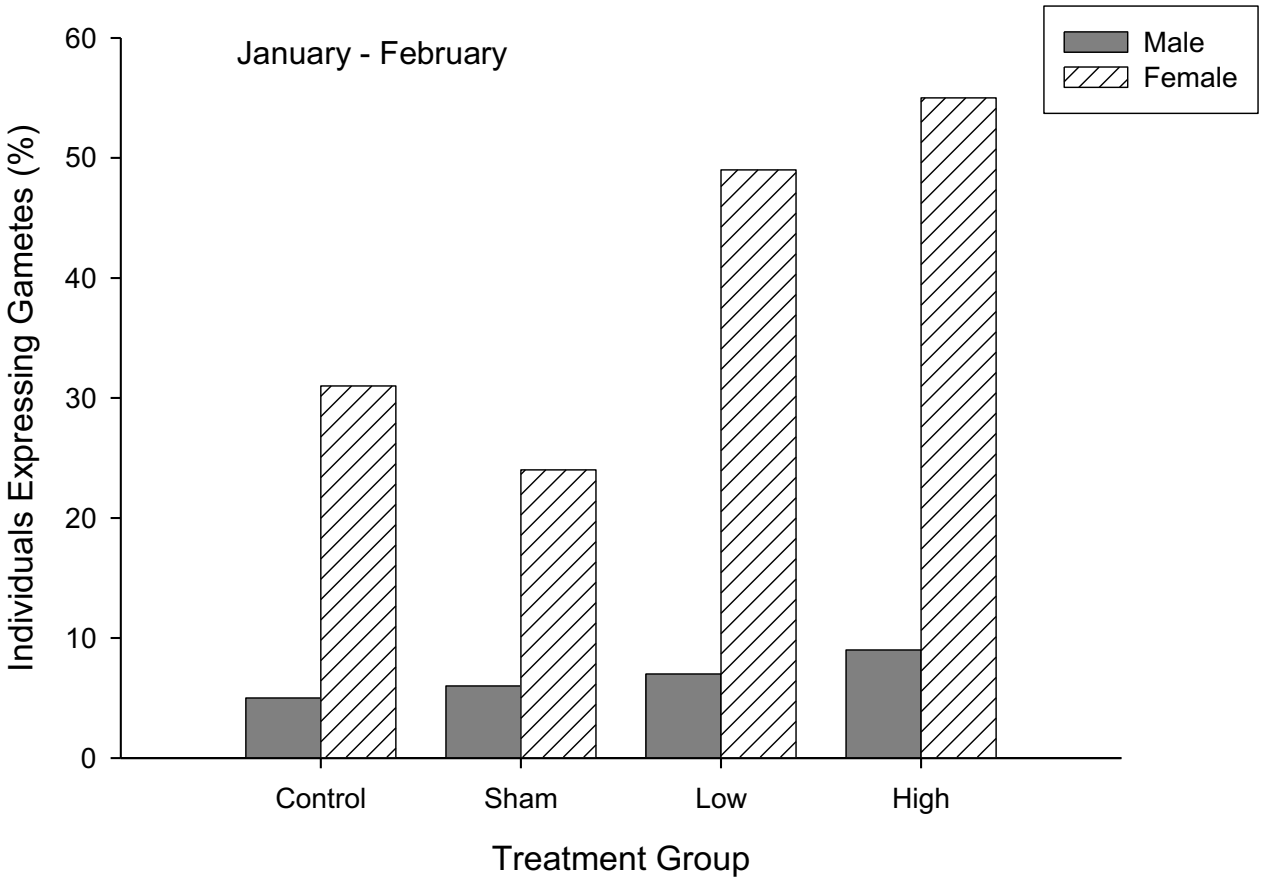

January - February
Male
Female
Individuals Expressing Gametes (%)
60
50
40
30
20
10
0
Control
Sham
Low
High
Treatment Group

Figure 2.4

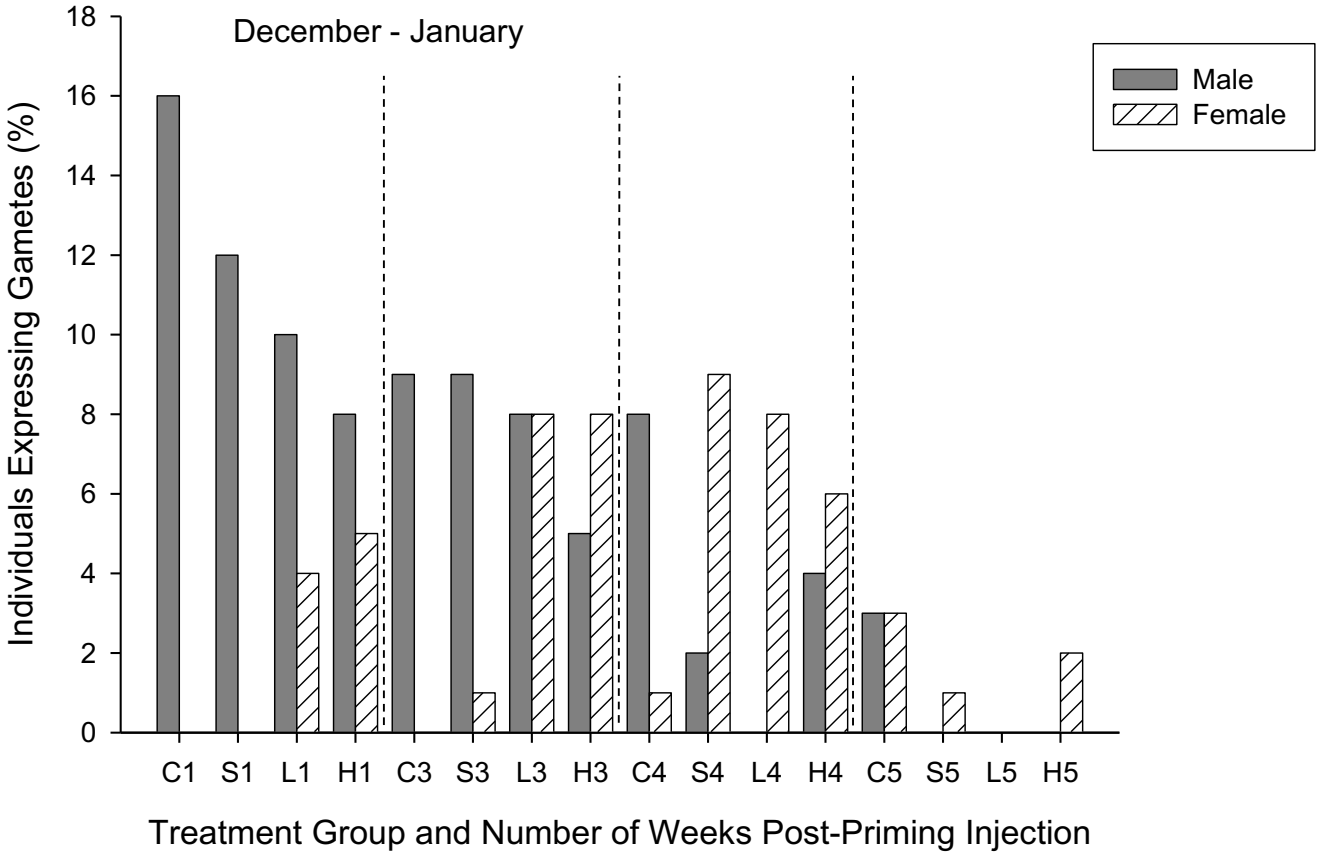

December - January
Male
Female
Individuals Expressing Gametes (%)
0
2
4
6
8
10
12
14
16
18
C1
S1
L1
H1
C3
S3
L3
H3
C4
S4
L4
H4
C5
S5
L5
H5
Treatment Group and Number of Weeks Post-Priming Injection

Figure 2.5

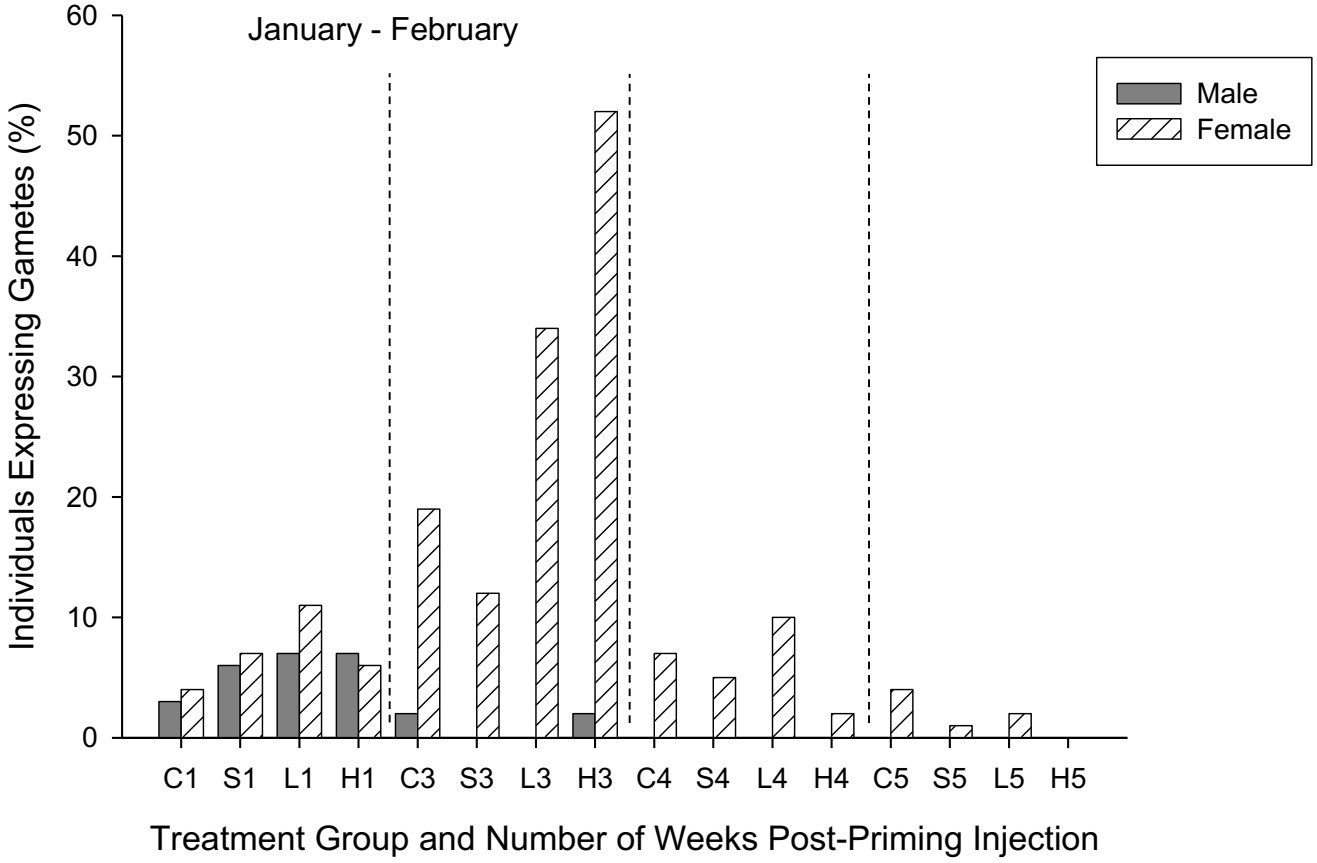

January - February
Male
Female
Individuals Expressing Gametes (%)
60
50
40
30
20
10
0
C1 S1 L1 H1 C3 S3 L3 H3 C4 S4 L4 H4 C5 S5 L5 H5
Treatment Group and Number of Weeks Post-Priming Injection

Figure 2.6

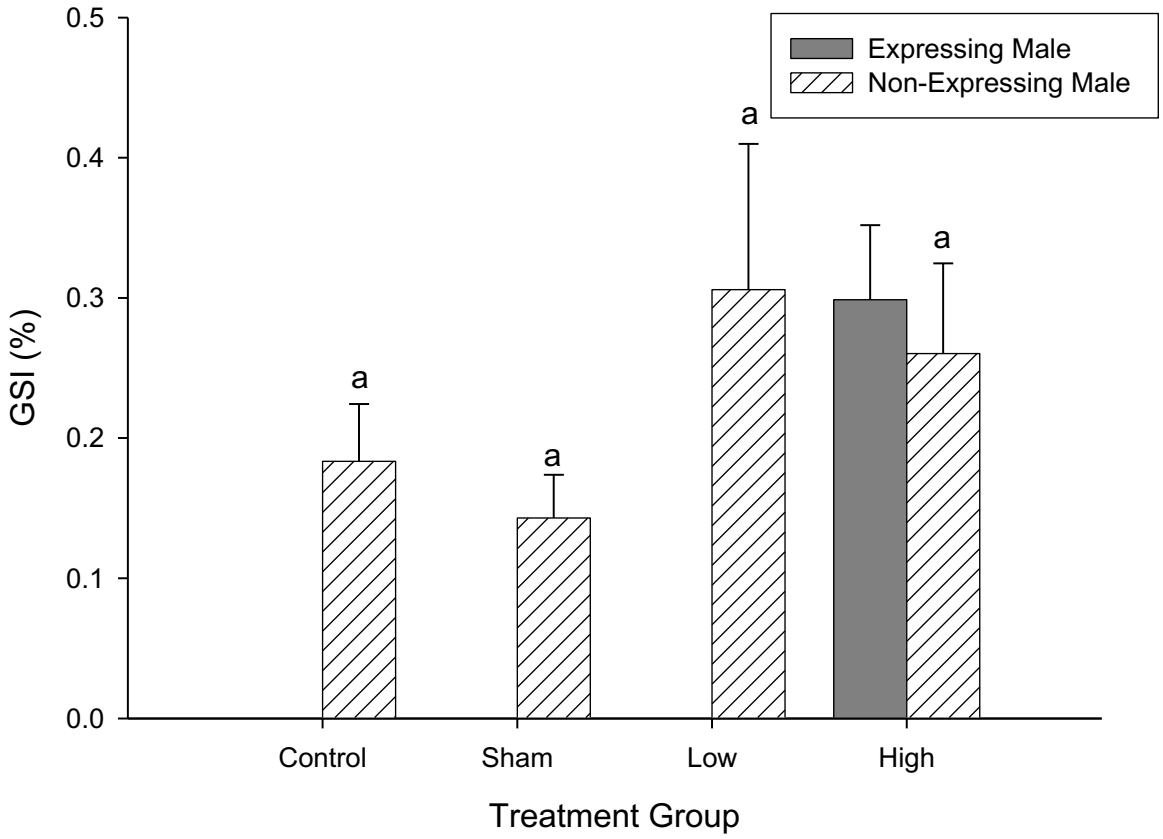

Expressing Male
Non-Expressing Male
GSI (%)
0.5
0.4
0.3
0.2
0.1
0.0
a
a
a
a
Control
Sham
Low
High
Treatment Group

Figure 2.7

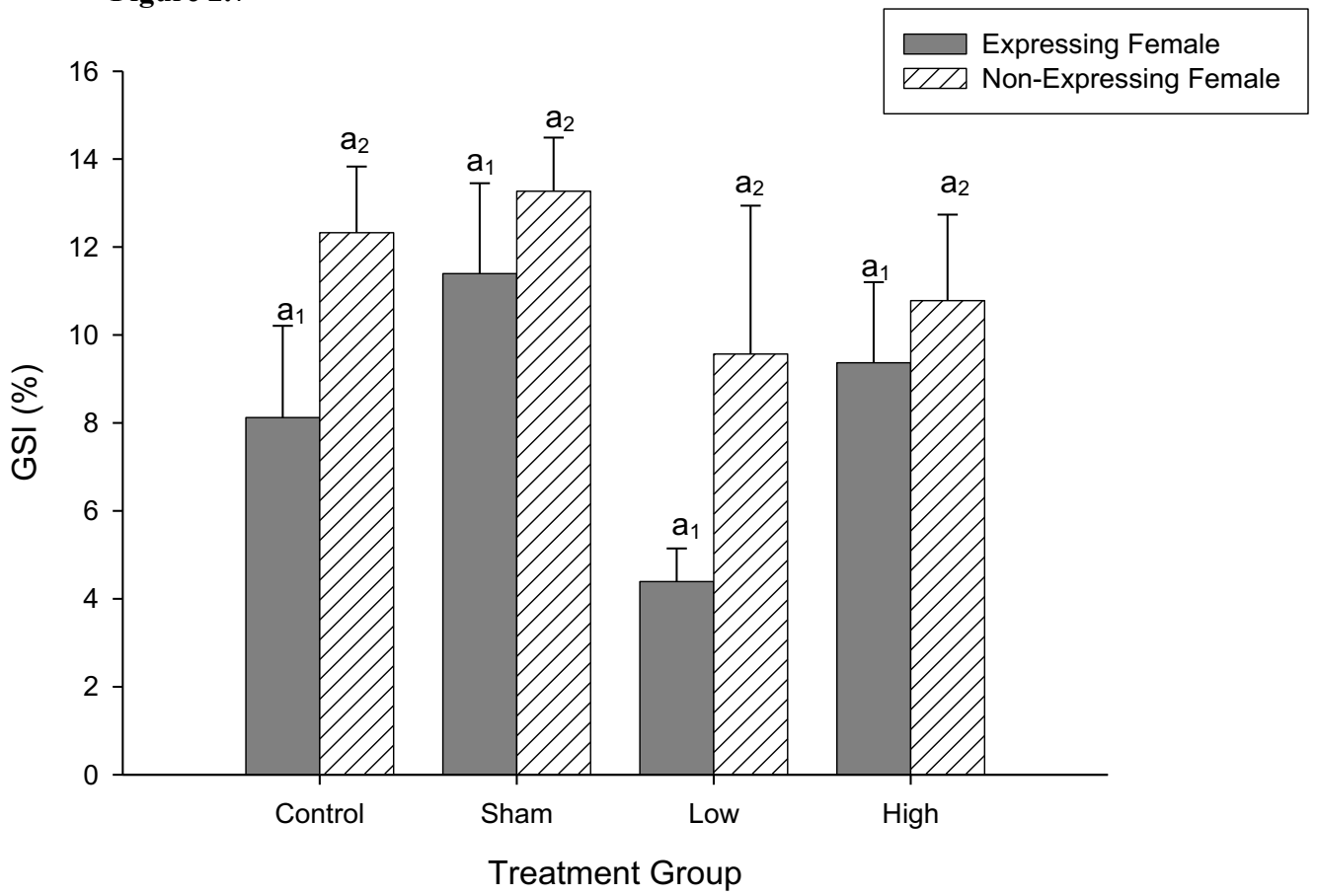

Expressing Female
Non-Expressing Female
GSI (%)
16
14
12
10
8
6
4
2
0
a1
a2
a1
a2
a1
a2
a1
a2
Control
Sham
Low
High
Treatment Group

Figure 2.8

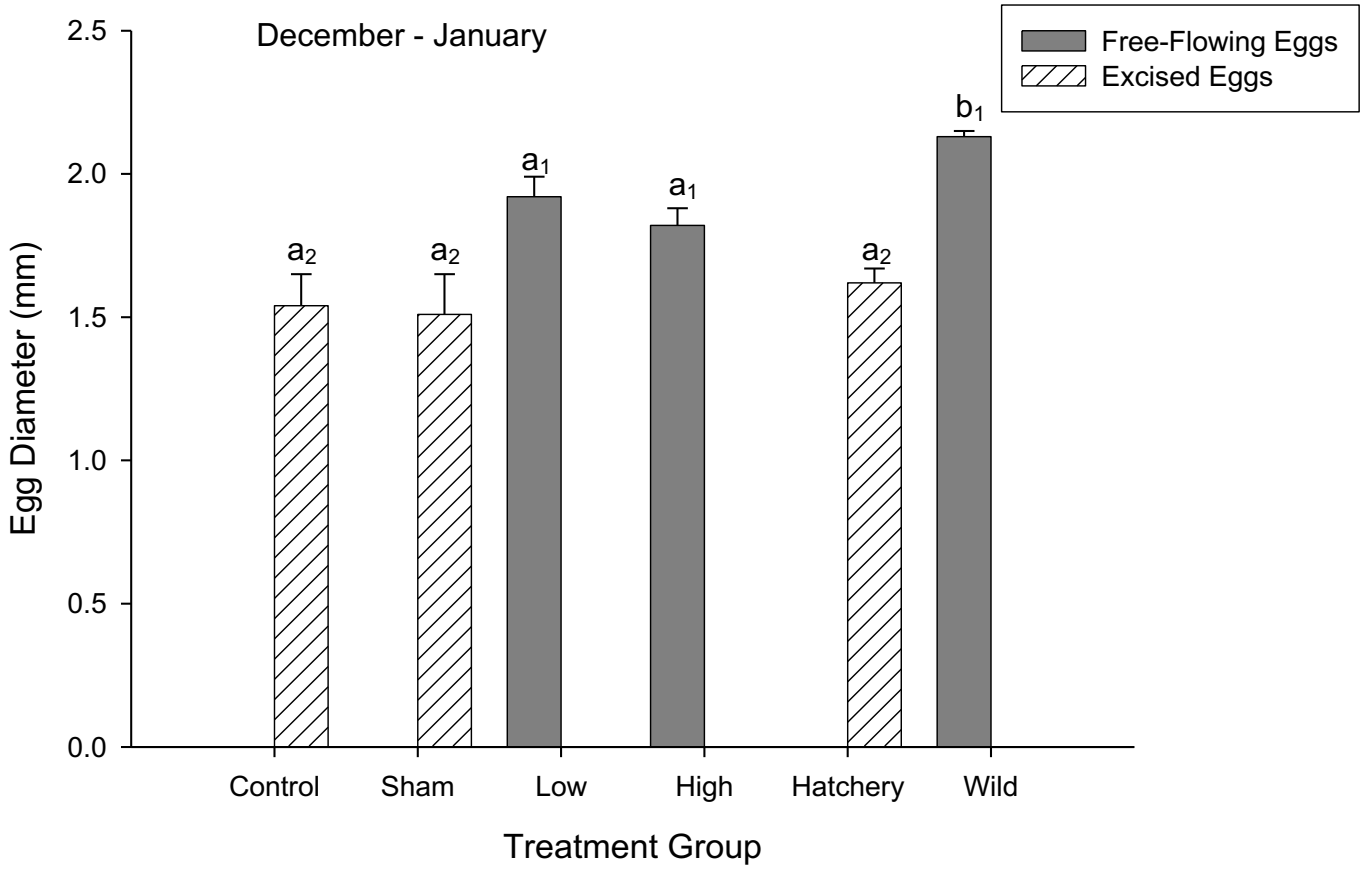
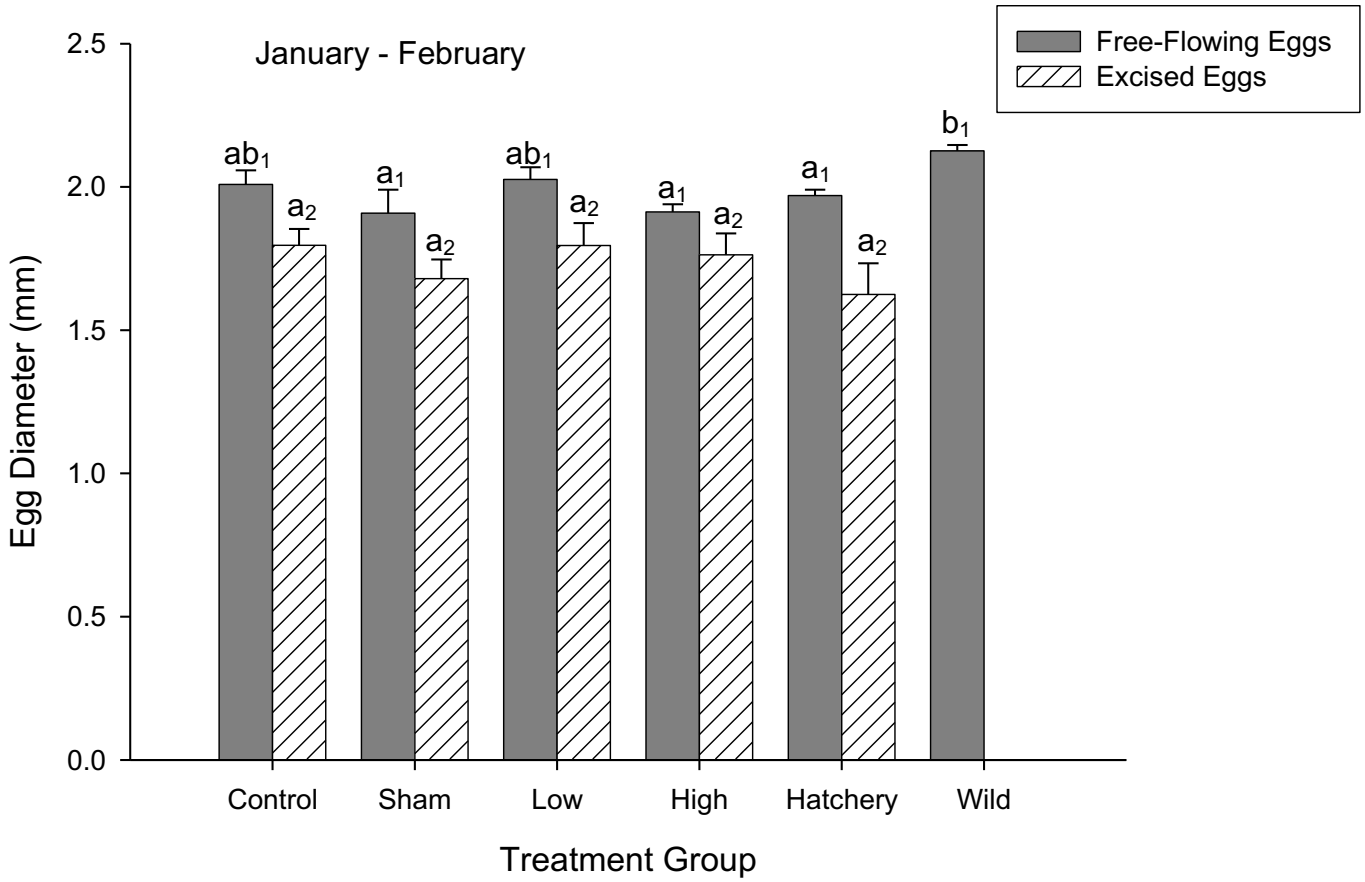

December - January
Free-Flowing Eggs
Excised Eggs
Egg Diameter (mm)
2.5
2.0
1.5
1.0
0.5
0.0
a2
a2
a1
a1
a2
b1
Control
Sham
Low
High
Hatchery
Wild
Treatment Group

Figure 2.9


January - February
Free-Flowing Eggs
Excised Eggs
Egg Diameter (mm)
2.5
2.0
1.5
1.0
0.5
0.0
ab1
a2
a1
a2
ab1
a2
a1
a2
a1
a2
b1
Control
Sham
Low
High
Hatchery
Wild
Treatment Group

Chapter 3: Development of a Cryopreservation Protocol for the Spermatozoa of Hatchery-Reared Bloaters

3.1 Introduction

Cryopreservation; the storage of cells or tissues at very low temperatures (-196 degrees Celsius), is often performed on spermatozoa. In recent years, cryopreservation of the spermatozoa of fish has become increasingly common as efforts to conserve vulnerable aquatic species and efforts to enhance the production of commercial aquaculture continue to develop (Ciereszko et al. 2014; Nynca et al. 2012). The cryopreservation of spermatozoa allows for a wide array of benefits including: synchronization of gamete availability, simplification of cultured fish management, transportation, and storage of genetic material for selection or conservation programs (reviewed in Cabrita et al., 2010). However, the development of cryopreservation protocols is highly individualistic to each species and tremendous differences are observed between the protocols of different fish taxa, in particular marine and freshwater species (Holt, 2000). Regardless of the specific differences between protocols, they all must address the same essential aspects in order to be effective.

A cryopreservation protocol hinges upon a multitude of factors with the most critical arguably being (i) the composition of the extender (consisting of both cryoprotectants and diluents) used during the freezing process and (ii) the rate of freezing. Cryoprotectants may be defined as those substances which protect the internal environment of the cell during the freezing process while diluents protect the external

components of the cell (Lahnsteiner, 2000). The solution formed by combination of cryoprotectants and diluents is commonly referred to as an extender. In addition to the composition of the extender, the rate at which the extender-spermatozoa solution is frozen is also important. Dependent on the species of fish and its habitat (freshwater or marine), freezing rates can either consist of a single step at one rate or they can consist of multiple steps each with its own rate (Conget et al. 1996; Suquet et al. 2000). The culmination of the optimization of each of these complex aspects is a method of cryopreserving the spermatozoa of the desired aquatic species while also maintaining the quality of the spermatozoa.

Sperm quality is a broad term often used to describe how well sperm are able to fertilize eggs. It is becoming increasingly common that computer-assisted sperm analysis (CASA) is used to determine the quality of the sperm of fish for both aquaculture and conservation purposes (reviewed in Kime et al. 2001). In light of this, it has been important to identify which sperm metrics provided by CASA (typically, the total and motile number of sperm in the recording, average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), straightness (STR; calculated by dividing VAP by VCL) and linearity (LIN; calculated by dividing VSL by VAP)) are indicative of successful fertilization. Typically, the percentage of motile sperm (hereafter sperm motility) has been shown to be the strongest correlate of successful fertilization and, thus, sperm quality (Lahnsteiner et al. 1998; Rurangwa et al, 2004; Kime et al. 2001). However, the individual metrics related to sperm velocity (VAP, VSL and VCL), or an average of the three, are also commonly used as indicators of fertilization success in fish (Rurangwa et al. 2001; Gage et al. 2004; Fitzpatrick et al. 2009). Because the primary

goal of all cryopreservation protocols is to optimize the post-thaw quality of the spermatozoa, analysis of these measurements is crucial in determining the efficacy of the protocol. Measurements are taken from the fresh sperm sample and again once the sperm has been cryopreserved and subsequently thawed (post-thaw) (Ciereskzo et al. 1996; Butts et al. 2010). Post-thaw motility is typically presented as a percentage of the fresh sperm motility while velocity is directly reported (um/s) (Butts et al. 2013). Arguably, the family for which the most is known, regarding the cryopreservation of spermatozoa, are the salmonids (Ciereszko et al. 2014; Lahnsteiner 2000; Conget et al. 1996).

Due to their profound importance and prevalence in aquaculture, a large proportion of cryopreservation protocols developed are for salmonids, while others often address more vulnerable aquatic species such as the Redside Dace (Butts et al. 2013) and many species of sturgeon (*Acipenser* spp.) (reviewed in Billard et al. 2004). While extender composition tends to vary between individual species, it has been observed that dimethylsulfoxide (DMSO) and methanol are commonly effective cryoprotectants for salmonids (Ciereszko et al. 2013, 2014; Lahnsteiner, 1996; see Table 3.1). Choice of diluent tends to vary more with some salmonids benefitting most from simple glucose while others are more elaborate (Ciereszko et al. 2013, 2014; Lahnsteiner, 2000). Often, when a protocol must be developed for a species for which one has not yet been found, the protocols of related species can prove to be a source of valuable insight (Ciereszko et al. 1996; Lahnsteiner, 2000). This is the case regarding the current study involving the bloater (*Coregonus hoyi*).

Currently, no cryopreservation protocol exists for bloaters. Although no protocol exists for bloaters, specifically, there is knowledge of sperm cryopreservation involving

whitefish (Coregonid spp.) from which the protocol of the current study is derived (see Piironen and Hyvärinen, 1983; Lahnsteiner, 2000; Ciereszko et al. 2013). Bloaters are a species which were native to Lake Ontario up until the 1950's at which time they became extirpated. A broodstock of bloaters, reared from the gametes of wild fish from Lake Michigan, is currently being cultured at White Lake Fish Culture Station (FCS) in Sharbot Lake, Ontario as part of an initiative by the Ontario Ministry of Natural Resources and Forestry (OMNRF) to reintroduce them to Lake Ontario. However, it has been observed that the broodstock experiences reproductive dysfunctions, one of which is the lack of suitable gamete production (see Chapter 2) and the asynchronous spawning season of the males and females, an observation not uncommon in cultured species (Reviewed in Zohar and Mylonas, 2001). In the past, males often finished expressing free-flowing milt before the females began expressing free-flowing eggs making broodstock management difficult. The development of a cryopreservation protocol for bloaters would allow spermatozoa to be stored until females began expressing eggs at which point fertilization could occur, circumventing the problematic reproductive asynchrony. As such, the purpose of this study was to try to determine which extender and freezing rate, of those most successful in related species (see Table 3.1), would be most effective at cryopreserving the spermatozoa of bloaters in addition to measuring their sperm quality.

3.2 Materials and Methods

Broodstock and Milt Collection

The sexually mature males from this study were derived from offspring produced by wild-caught bloater gametes from Lake Michigan in January 2012. Males were haphazardly selected from a tank of approximately 800 fish containing both males and females. Fish were anesthetized using MS-222 and subsequently checked for expression of free flowing milt. Slight pressure was applied to the abdomen of each male in the study, and milt was collected with a micropipette as it was released from the vent. Care was taken to ensure that milt was not contaminated with blood, urine, or feces. Milt samples were then micropipetted into 1,000-µL microcentrifuge tubes and were held on an electronic cooling block. Temperature of the block was set between 1 and 4°C (similar to that of the tank water, which ranged from 1 and 4°C).

Toxicity of Extenders

Sperm samples for this experiment were taken from individual male bloaters that were expressing free flowing milt and subsequently pooled to ensure adequate volume. The toxicity of two diluents and two cryoprotectants that have been used most successfully for other coregonids and salmonids (Ciereszko et al. 2014 & Lahnsteiner, 2000) were tested in full-factorial design (see Figure 3.1). Two diluents were evaluated: Glucose and the basic buffered physiological saline solution (BBPSS); NaCl (600mg), KCl (315mg), $CaCl_2 \cdot 2H_2O$ (15mg), $MgSO_4 \cdot 7H_2O$ (20mg), HEPES (470mg), sucrose

(500mg), bovine serum albumin (1.5g) and hen egg yolk (7ml) (Lahnsteiner, 2000). Additionally, two cryoprotectants were evaluated: methanol (9% v/v) and a mixture of dimethyl sulfoxide (5% v/v) and glycerol (1% v/v) (Ciereszko et al. 2014; Lahnsteiner, 2000). Diluents and cryoprotectants were mixed in 250ml glass Erlenmeyer flasks and were kept between 1 and 4◦C. Once homogeneous, these extender solutions (20μL) were micropipetted into 1,000-μL microcentrifuge tubes. Immediately following this, pooled milt (4μL) was micropipetted into the tubes containing the extender solution and subsequently mixed by flicking the tubes for 10–15 s. Sperm were allowed to incubate within each extender for 5 minutes at between 1 and 4◦C. Mean sperm activity for each of the eight experimental replicates pools of milt was used for statistical analyses; fresh sperm served as the control.

Effects of Extender Composition on Post-Thaw Sperm Quality

Milt was used from 6 individual males and 4 pools of males (composed of 2 males each due to insufficient milt volume per individual male). This allowed for 10 replicates to be conducted. Diluents and cryoprotectants were mixed in 250ml glass Erlenmeyer flasks and were kept between 1 and 4◦C. Once homogeneous, these extender solutions (20μL) were micropipetted into 1,000-μL microcentrifuge tubes. Immediately following this, pooled milt (4μL) was micropipetted into the tubes containing the extender solution. Using a full factorial design, milt samples were diluted with each possible combination of diluent (glucose and BBPSS) and cryoprotectant (DMSO + glycerol and methanol) at a sperm-to-extender ratio of 1:5, resulting in a total of 4

different extender combinations. Diluted milt samples were drawn up into 2,500µL cryogenic straws (Minitube Canada, Ingersoll, Ontario) by using a Microclassic micropipettor (Minitube Canada), and the straws were sealed with a microsonic sealer. An equilibration time of approximately 3 minutes was allotted, as this was the time required for two researchers to fill eight straws (one for each treatment combination) and load them into the cryogenic freezer (IceCube 14S; SyLab). SyLab software was used to freeze samples at a rate of either 35◦ C/min or 45◦ C/min from 1◦ C to −180◦ C. Once samples reached −180◦ C, they were plunged directly into a liquid nitrogen Dewar and were kept frozen for one hour. To evaluate post-thaw sperm quality traits, straws were transferred from the liquid nitrogen into a temperature-controlled water bath and were thawed for 8 seconds at 35◦C. Straw tips were cut off and the contents were released into small disposable weigh boats. Post-thaw activity of each sample was recorded within 30 seconds after thawing. Fresh sperm served as the control.

Effects of Freezing Rate on Post-Thaw Sperm Quality

Milt used in this part of the experiment was the same as what was used in effects of extender composition on post-thaw sperm quality (see above). Sperm from each male was diluted (see methods above) into the four extender combinations: Methanol + Glucose (C_C); Methanol + BBPSS (C_L); DMSO + Glycerol + BBPSS (L_L) and DMSO + Glycerol + Glucose (L_C) at a sperm-to-extender ratio of 1:5. Each sperm-extender combination was frozen at a rate of either 35◦ C/min or 45◦ C/min from 1◦ C to −180◦ C. Once samples reached −180◦ C, they were plunged directly into a liquid nitrogen Dewar

and were kept frozen for 1 h. The thawing of samples and the evaluation of post-thaw sperm activity were conducted according to the methods described in the Effects of extender composition on post-thaw sperm quality section (see above).

Male-to-Male Variation in Fresh Sperm Quality

Fresh milt samples from bloaters (n = 10; 8 individuals and 2 pools of 2 males each) were used in this part of the experiment. These 10 samples were from some of the same individuals as those used in the Toxicity of Extender experiment (n = 7) and from the Effects of Extender Composition on Post-Thaw Sperm Quality experiment (n = 3) above. Differences among sperm quality were recorded immediately following activation with 15µL of tank water and analyzed at 5 seconds post-activation (data for 10 and 15 seconds post-activation were qualitative similar, data not shown).

Sperm Quality Assessment

Milt (<0.2µL) was micropipetted into a chamber of a 2X-CEL glass slide (Hamilton Thorne Biosciences, Beverly, Massachusetts) and was covered with a coverslip (22 × 22 mm). Sperm were then activated with 15µL of tank water. Sperm were video-recorded by using a black-and-white video camera (charge-coupled device [CCD], Model XC-ST50; Sony, Japan) module at 50-Hz vertical frequency; the camera was mounted on an external phase contrast microscope (Model CX41; Olympus, Melville, New York) with a 10× negative-phase magnification objective (Butts et al. 2012). Once

recorded, sperm traits were analyzed by using the HTM-CEROS version 12 sperm analysis system (Hamilton Thorne Biosciences) set at the following parameters: number of frames = 60; minimum contrast = 11; minimum cell size = 3 pixels; and photometer = 55–65. To remove the potential effect of drift, sperm cells that had an average path velocity less than 20μm/s and a straight-line velocity less than 10μm/s were considered to be static and were excluded from the analyses (Rudolfsen et al. 2008). Percentage of motile sperm (hereafter sperm motility), average path velocity, straight line velocity, curvilinear velocity, straightness and linearity were assessed at 5, 10 and 15 seconds post-activation. Each video recording was manually checked for quality control. Sperm tracks were removed from analyses if the software incorrectly combined the crossing tracks of multiple sperm, if the software split the track of a single sperm, or if a sperm swam out of the field of view before it could be adequately assessed.

3.3 Results & Discussion

Toxicity of Extenders

Sperm motility was not found to be significantly different among the different extenders at 5 (One-way ANOVA: $F_{4,24} = 2.11$, $P = 0.11$, Figure 3.2), 10 (One-way ANOVA: $F_{4,24} = 1.76$, $P = 0.17$, Figure 3.2) or 15 (One-way ANOVA: $F_{4,24} = 2.28$, $P = 0.09$, Figure 3.2) seconds post-activation. Average path velocity was significantly different among extenders at 10 (One-way ANOVA: $F_{4,24} = 3.08$, $P = 0.04$, Figure 3.3a) and 15 (One-way ANOVA: $F_{4,24} = 6.50$, $P < 0.005$, Figure 3.3a) seconds post-activation but not at 5 seconds post-activation (One-way ANOVA: $F_{4,24} = 2.21$, $P = 0.10$, Figure

3.3a). Straight line velocity was significantly different among extenders at 15 (One-way ANOVA: $F_{4,24} = 4.50$, $P = 0.007$, Figure 3.3b) seconds post-activation but not at either 5 (One-way ANOVA: $F_{4,24} = 2.46$, $P = 0.07$, Figure 3.3b) or 10 (One-way ANOVA: $F_{4,24} = 2.53$, $P = 0.07$, Figure 3.3b) seconds post-activation. Curvilinear velocity was significantly different among extenders at both 5 (One-way ANOVA: $F_{4,24} = 3.27$, $P = 0.03$, Figure 3.3c) and 10 (One-way ANOVA: $F_{4,24} = 4.11$, $P = 0.01$, Figure 3.3c) seconds post-activation but not at 15 (One-way ANOVA: $F_{4,24} = 2.58$, $P = 0.63$, Figure 3.3c) seconds post-activation. Straightness was not significantly different among the extenders at 5 (One-way ANOVA: $F_{4,24} = 1.65$, $P = 0.19$, Figure 3.4a), 10 (One-way ANOVA: $F_{4,24} = 0.42$, $P = 0.79$ Figure 3.4a) or 15 (One-way ANOVA: $F_{4,24} = 1.29$, $P = 0.30$, Figure 3.4a) seconds post-activation. Similarly, linearity was also not significantly different among the extenders at 5 (One-way ANOVA: $F_{4,24} = 1.58$, $P = 0.21$, Figure 3.4b), 10 (One-way ANOVA: $F_{4,24} = 0.02$, $P = 1.0$, Figure 3.4b) or 15 (One-way ANOVA: $F_{4,24} = 0.14$, $P = 0.97$, Figure 3.4b) seconds post-activation.

Overall, it appears that all of the extenders examined are adequate for the cryopreservation of bloater spermatozoa, however, extender LC appears to be less suitable than the rest. These results are consistent with numerous findings in that a full factorial design of cryoprotectants and diluents is essential in determining which extenders examined are viable for cryopreservation of the spermatozoa for the species of interest (Suquet et al. 2000; Babiak et al. 2001; Butts et al. 2010, 2011). Previous studies have also indicated that extenders composed of methanol + glucose (CC) and DMSO + BBPSS (LL) have proven the least toxic for the spermatozoa of species such as rainbow trout (Lahnsteiner, 2000) and European whitefish (*Coregonus lavaretus*) (Ciereszko et al.

2013). It is beneficial to the development of a bloater spermatozoa cryopreservation protocol to know that the extenders examined in this experiment can be used without being biologically toxic to the sperm.

Effects of Extender Composition and Freezing Rate on Post-Thaw Sperm Quality

Following cryopreservation, each sperm sample was reduced from an average fresh motility of 54.74 ± 22.84% to 0.0 ± 0.0 % (see Table 3.1) motility for all extenders and freezing rates (see Table 3.2). Fresh sperm motility was recorded prior to cryopreservation and, at that time, all samples were motile following activation thus eliminating the possibility that the samples had been of poor quality (i.e. low motility), contaminated or prematurely activated with water.

Although the cryopreservation process decreased sperm quality to negligible levels for all frozen–thawed treatment combinations (See Table 3.2), the results can be used to gain insight into the development of a successful cryopreservation protocol for bloaters. However, this study revealed that the optimal cryopreservation protocols of other salmonids and coregonids cannot be effectively used for bloaters.

As mentioned above, the development of a sperm cryopreservation protocol for even a single species is both multi-faceted and complex. For example, Piironen (1987) and Hyvärinen (1983) achieved an unspecified post-thaw motility in the sperm of whitefish (*Coregonus muksun*) capable of fertilization rates of 85% using 20% glycerol as the cryoprotectant. When Ciereszko et al. (2008) attempted to use the same extender on European whitefish they did not observe any post-thaw motility (0%), however, methanol was found to be a better cryoprotectant for that particular species. This drastic

interspecific difference in optimal extender composition further reinforces the idea that the characterization of the sperm of the species of interest is essential to producing an effective cryopreservation protocol (Ciereszko et al. 2008; Butts et al. 2011). Furthermore, because the extenders were not completely toxic to the sperm, it appears that the freezing rate is a significant factor of the protocol which reduced the sperm motility to negligible levels during cryopreservation. If the freezing rate is too slow, then too much water leaves the cell and dehydration causes cell death; if the freezing rate is too fast, not enough water leaves the cell and large intracellular ice crystals form, causing the cell to rupture (Yang et al. 2007). For example, Conget et al (1996) observed that freezing rates of both -1 and -10°C/min reduced post-thaw rainbow trout sperm motility to 0% although these freezing rates have been successful in other species such as the Redside dace (*Clinostomus elongatus*) allowing for post-thaw motilities of between 10 and 45% (Butts et al. 2013). However, it is beyond the scope of this study to say, definitively, that it was the freezing rate alone which rendered the spermatozoa immotile following cryopreservation.

Male-to-Male Variation in Fresh Sperm Quality

Amongst male bloaters, fresh sperm quality was compared on the basis of motility, straight line velocity and straightness. The mean percent motility amongst males was 65.42% ± 6.70 (Figure 3.5a), the mean straight line velocity was 52.67μm/s ± 4.47 (Figure 3.5b) and the mean straightness was 71.20 ± 2.24 (Figure3.5c).

There was expected variation amongst the fresh sperm quality of male bloaters. The mean fresh sperm motility amongst the males examined was 65.42% (± 6.70, Figure 3.4a), the mean straight line velocity was 52.67um/s (± 4.67, Figure 3.4b) and the mean straightness of the sperm was 71.2 (± 2.24, Figure 3.4c). For measurements of additional sperm metrics of the 10 samples in this experiment, the reader is referred to Table A.9.

Analysis of the fresh sperm quality of the spermatozoa is an important step that should be completed during the development of a cryopreservation protocol, especially for species which the sperm quality is unknown (Ciereszko et al. 2008; Butts et al. 2011). For example, Ciereszko et al (2008) characterized the fresh sperm characteristics of European whitefish by measures of sperm motility and straight line velocity, amongst others, prior to cryopreservation. Additionally, Butts et al (2011) characterized the fresh sperm of Atlantic cod (*Gadus morhua*) by these same measures (sperm motility, straight line velocity and straightness), amongst others, prior to cryopreservation. Without these baseline measures, it would be impossible to determine the specific effects of the cryopreservation process on the quality (as determined by the metrics mentioned above) of the spermatozoa. The characterization of fresh bloater sperm has never been performed prior to this experiment. These measurements were essential as a baseline measure in determining the effects of the cryopreservation process on the various sperm metrics which were recorded and can serve as a reference point for future studies which may further examine the characteristics of bloater spermatozoa.

The need for a viable cryopreservation protocol is still important and a viable means of overcoming the asynchronous spawning times between sexes in the hatchery-reared bloaters. The information gained from this experiment will be used to narrow the

range of potentially beneficial cryopreservation protocols for this species and, ultimately, assist in the production of a reproductively viable and self-sustaining broodstock. In the future, should the establishment of a bloater cryopreservation protocol occur, careful monitoring of the degradation of genetic information (i.e. DNA) during the freezing process should be conducted.

Additionally, this research is novel in that, at the present time, there are no studies which utilize a controlled-rate freezer for the cryopreservation of coregonid sperm. Most studies on coregonids either place pellets of spermatozoa on dry-ice (which is equivalent to a freezing rate of approximately 35°C/min) or place straws on a styrofoam float at varying heights above liquid nitrogen (which are equivalent to freezing rates of approximately ≥45-50°C/min) (Lahnsteiner et al. 1996; Butts, unpublished data). These rates were unsuccessful in preserving motility of cryopreserved sperm in bloaters even though they may have been effective in other coregonids. Similar to the extender composition, freezing rates can also vary substantially between species (Piironen and Hyvärinen, 1983; Babiak et al. 1999).

In the future, it would be beneficial to test additional extender components that have proven non-toxic to the spermatozoa of other freshwater fish which include: glycerol, dimethylacetamide, DMSO and 1,2-propanediol (Piironen and Hyvärinen, 1983; Lahnsteiner, 2000; Butts et al. 2011). These components have produced less than optimal results in the cryopreservation protocols of many of the Salmonidae shown in Figure 3.1 (which is why they were excluded from the extenders examined in this experiment), however, as the effects of extender on spermatozoa are highly species-specific, it is

possible that these extenders may prove less (or more) toxic to the spermatozoa of bloaters, in particular.

In addition to testing a broader range of extenders, another method of cryopreservation; termed vitrification, could also be examined. Vitrification is an alternative method of cryopreservation characterized by plunging a suspension of spermatozoa directly into liquid nitrogen, however, the use of a modified extender is typically still used. This method has proven successful in species such as the Atlantic salmon (Figueroa et al. 2015; Merino et al. 2011) and channel catfish (*Ictalurus punctatus*) (Cuevas-Uribe et al. 2011) achieving post-thaw sperm motilities of approximately 50%. Use of vitrification may serve as an alternative method of overcoming the asynchronous spawning observed in the hatchery-reared bloaters.

It is also necessary that additional freezing rates, such as the rapid one utilized in vitrification, be examined for their efficacy regarding the cryopreservation of bloater spermatozoa. Optimal freezing rates of Salmonidae, such as those reported in Table 3.1, tend to be rapid (≥-35°C/min) when compared to other species such as redside dace (Butts et al. 2013) or sturgeon (Billard et al. 2004) with optimal freezing rates of between- 0.5 and -18.5°C/min. Thus, it may simply be that bloater sperm requires an alternative freezing rate to prevent the drastic loss of motility following cryopreservation.

This study utilized a control rate freezer, which is atypical compared to the studies in Table 3.1 which all utilized a Styrofoam float at varying heights above a pool of liquid nitrogen. This method does not allow for the freezing rate to be easily measured, however, the heights of the float are easily replicable and the freezing rates are assumed

to be identical at identical heights. Although this method seems less accurate than using a control rate freezer, it may be worth investigating as it has proven effective in the cryopreservation of the spermatozoa of many Salmonidae (see Table 3.1). Overall, there are many possible future directions which may contribute to the development of the cryopreservation protocol for bloater spermatozoa and this study contributes vital information towards that development.

References

Babiak, I., Fraser, L., Dobosz, S., Goryczko, K., Kuzminski, H. & Strzezek, J. 1999. Computer-controlled freezing of rainbow trout *Oncorhynchus mykiss* (Walbaum) spermatozoa for routine programmes. *Aquac. Res.*. **30**: 707-710.

Babiak, I., J. Glogowski, K. Goryczko, S. Dobosz, H. Kuzminski, J. Strzezek, and W. Demianowicz. 2001. Effect of extender composition and equilibration time on fertilization ability and enzymatic activity of rainbow trout cryopreserved spermatozoa. *Theriogenology*. **56**:177–192.

Billard, R., Cosson, J., Noveiri, S. B. & Pourkazemi, M. 2004. Cryopreservation and short-term storage of sturgeon sperm, a review. *Aquaculture*. **236:** 1-9.

Butts, I. A. E., Litvak, M. K., Kaspar, V. & Trippel, E. A. 2010. Cryopreservation of Atlantic cod *Gadus morhua* L. spermatozoa: effects of extender composition and freezing rate on sperm motility, velocity and morphology. *Cryobiology*. **61**: 174-181.

Butts, I. A. E., Mokdad, A., Trippel, E. A. & Pitcher, T. E. 2013. Development of a sperm cryopreservation protocol for redside dace: implications for genome resource banking. *T. Am. Fish. Soc*. **142**: 671-680.

Butts, I. A. E., N. Feindel, S. Neil, E. Kovacs, B. Urbanyi, and E. A. Trippel. 2011. Cryopreservation of Atlantic Cod (*Gadus morhua*) sperm in large-volume straws: applications for commercial production and gene banking. *Aquac. Res.*. **42**:1714–1722.

Butts, I. A. E., O. P. Love, M. Farwell, and T. E. Pitcher. 2012. Primary and secondary sexual characters in alternative reproductive tactics of Chinook Salmon:

associations with androgens and the maturation-inducing steroid. *Gen. Comp. Endocr.* **175**:449–456.

Cabrita, E., Sarasquete, C., Martinez-Páramo, S., Robles, V., Beirão, J., Pérez-Cerezales, S. & Herráez, M. P. 2010. Cryopreservation of fish sperm: applications and perspectives. *J. Appl. Icthyol*. **26**: 623-635.

Christensen, J. M & Tiersch, T. R. 2005. Cryopreservation of channel catfish sperm: effects of cryoprotectant exposure time, cooling rate, thawing conditions, and male-to-male variation. *Theriogenology*. **63**: 2103-2112.

Ciereszko, A., Dietrich, G. J., Nynca, J., Dobosz, S. & Zalewski, T. 2014. Cryopreservation of rainbow trout semen using a glucose-methanol extender. *Aquaculture*. **420-421**: 275-281.

Ciereszko, A., Dietrich, G. J., Nynca, J., Liszewska, E., Karol, H. & Dobosz, S. 2013. The use of concentrated extenders to improve the efficacy of cryopreservation in whitefish spermatozoa. *Aquaculture*. **408-409**: 30-33.

Ciereszko, A., Dietrich, G. J., Wojtczak, M., Sobocki, M., Hliwa, P., Kuźmiński, H., Dobosz, S., Słowińska, M. & Nynca, J. 2008. Characterization and cryopreservation of whitefish (*Coregonus lavaretus* L.) semen from Lake Łebsko, Poland. *Fund. Appl. Limnol*. **173**: 59-65.

Ciereszko, A., Toth, G. P., Christ, S. A. & Dabrowski, K. 1996. Effect of cryopreservation and theophylline on motility characteristics of lake sturgeon (*Acipenser fulvescens*) spermatozoa. *Theriogenology*. **45**: 665-672.

Conget, P., Fernández, M. Herrera, G. & Minguell, J. J. 1996. Cryopreservation of rainbow trout (Oncorhynchus mykiss) spermatozoa using programmable freezing.

Aquaculture. 143: 319-329.

Cuevas-Uribe, R., Leibo, S. P., Daly, J. & Tiersch, T. R. 2011. Production of channel catfish with sperm cryopreserved by rapid non-equilibrium cooling. *Cryobiology*. **63**: 186-197.

Figueroa, E., Merino, O., Risopatrón, J., Isachenko, V., Sánchez, R., Effer, B., Isachenko, E., Farias, J. G. and Valdebenito, I. 2015. Effect of eminal plasma on Atlantic salmon(*Salmo salar*) sperm vitrification. *Theriogenology*. **83**: 238-245.

Fitzpatrick, J. L., Montgomerie, R., Desjardins, J. K., Stiver, K. A., Kolm, N. & Balshine, S. 2009. Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *PNAS*. **106**: 1128-1132.

Gage, M. J., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B. & Parker, G. A. 2004. Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. *Curr. Biol.* **14:** 44-47.

Holt, W. V. 2000. Fundamental aspects of sperm cryobiology: importance of species and individual differences. *Theriogenology*. **53**: 47-58.

Kime, D. E., Van Look, K. J., McAllister, B. G., Huyskens, G., Rurangwa, E. & Ollevier, F. 2001. Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. *Comp. Biochem. Phys. C.* **130**: 425-433.

Lahnsteiner, F. 2000. Semen cryopreservation in the Salmonidae and in Northern pike. *Aquac. Res*. **31**: 245-258.

Lahnsteiner, F., Berger, B., Weismann, T. & Patzner, R. 1998. Determination of semen quality of the rainbow trout, *Oncorhychus mykiss*, by sperm motility, seminal

plasma parameters, and spermatozoal metabolism. *Aquaculture*. **163**: 163-181.

Lahnsteiner, F., Berger, B., Weismann, T. & Patzner, R. 1996. The influence of various cryoprotectants on semen quality of the rainbow trout (*Oncorhychus mykiss*) before and after cryopreservation. *J. Appl. Ichthyol*. **12**: 99-106.

Merino, O., Sánchez, R., Risopatrón, J., Isachenko, E., Katkov, I. I., Figueroa, E., Valdebenito, I., Mallmann, P. & Isachenko, V. 2012. Cryoprotectant-free cryopreservation of fish (*Oncorhyncus mykiss*) spermatozoa: first report. *First International Journal of Andrology*. **44**: 390-395.

Nynca, J., Dietrich, G. J., Fopp-Bayat, D., Dietrich, M. A., Słowińska, M., Liszewska, E. Karol, H., Martyniak, A. & Ciereszko, A. 2012. Quality parameters of fresh and cryopreserved whitefish (*Coregonus lavaretus* L.) semen. *J. Appl. Ichtyol*. **28**: 934-940.

Piironen, J. 1987. Factors affecting fertilization rate with cryopreserved sperm of whitefish (*Coregonus muskun* Pallas). A*quaculture*. **66**: 347-357.

Piironen, J. and Hyvärinen, H. 1983. Cryopreservation of spermatozoa of whitefish *Coregonus muksun* Pallas. *J. Fish. Biol*. **22**: 159-163.

Rideout, R. M., E. A. Trippel, and M. K. Litvak. 2004. The development of Haddock and Atlantic Cod sperm cryopreservation techniques and the effect of sperm age on cryopreservation success. *Journal of Fish Biology*. **65**:299– 311.

Rudolfsen, G., L. Figenschou, I. Folstad, and O. Kleven. 2008. Sperm velocity influence paternity in the Atlantic Cod (*Gadus morhua* L.). *Aquac. Res.*. **39**: 212–216.

Rurangwa, E., Kime, D. E., Ollevier, F. & Nash, J. P. 2004. The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*. **234**: 1-28.

Rurangwa, E., Volckaert, F.A.M., Huyskens, G., Kime, D.E. & Ollevier, F. 2001. A concerted strategy for the quality control of refrigerated and cryopreserved semen using computer-assisted sperm analysis (CASA), viable staining and standardised fertilisation: application to preservation of sperm of African catfish (*Clarias gariepinus*). *Theriogenology* **55**: 751-769.

Suquet, M., Dreanno, C., Fauvel, C., Cosson, J. & Billard, R. 2000. Cryopreservation of sperm in marine fish. *Aquac. Res*. **31**: 231-243.

Tiersch, T. R., Figiel Jr., C. R., Wayman, W. R., Williamson, J. H., Carmichael, G. J., & Gorman, O. T. 1998. Cryopreservation of sperm of the endangered razorback sucker. *T. Am. Fish. Soc*. **127**: 95–104.

Yang, H., C. Carmichael, Z. M. Varga, and T. R. Tiersch. 2007. Development of a simplified and standardized protocol with potential for high-throughput for sperm cryopreservation in Zebrafish *Danio rerio*. *Theriogenology*. **68**:128– 136.

Table 3.1 Summary of cryopreservation protocol components including cryoprotectant, diluent, freezing rate and post-thaw sperm motility in salmonids. Common successful cryoprotectants include dimethyl sulfoxide (DMSO), methanol and glycerol at varying concentrations. Common successful diluents include glucose and a basic buffered physiological saline solution composed of sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), Hepes salt, hen egg yolk, bovine serum albumin (BSA) and sucrose. The most common successful freezing rate is a rapid one of approximately -45°C/min. Successful post-thaw sperm motilities range from approximately 10-50% depending on the species and protocol.

Species	Cryoprotectant	Diluent	Freezing Rate (°C/min)	Post-thaw Motility (%)	Reference
Atlantic Salmon (*Salmo salar*)	DMSO	Glucose	~ -45	~10	Dziewulska et al. 2011
European Whitefish (*Coregonus lavaretus*)	Methanol	Glucose	~ -45	27.5	Ciereszko et al. 2013
	Methanol	Glucose	~ -45	23	Ciereszko et al. 2008
	Methanol	Glucose	~ -45	35	Nynca et al. 2012
Rainbow Trout (*Oncorhynchus mykiss*)	DMSO + Glycerol	NaCl, KCl, $CaCl_2$, $MgSO_4$, Hepes, Hen Egg Yolk	~ -45	19.1	Lahnsteiner et al. 1996
	Methanol	Glucose	~ -45	49.9	Ciereszko et al. 2014
	Methanol	NaCl, KCl, $CaCl_2$, $MgSO_4$, Hepes, Hen Egg Yolk, BSA, Sucrose	~ -45	26.6	Lahnsteiner, 2000
Arctic Char (*Salvelinus alpinus*)	Methanol	Glucose	~ -45	10 - 25	Mansour et al. 2006

Table 3.2 Mean sperm motility (%) ± standard error (SE) of male bloaters (*Coregonus hoyi)* before (fresh) and after (post-thaw) the cryopreservation process. Results were recorded for fresh sperm as well as with each of the 4 extenders: methanol with glucose (CC), methanol with basic buffered physiological saline solution (CL), dimethyl sulfoxide + glycerol with basic buffered physiological saline solution (LL) and dimethyl sulfoxide + glycerol with glucose (LC) at each of the examined freezing rates.

			Motility (%)
Treatment	**n**	**Freezing Rate (-℃/min)**	**Mean ± SE**
			Fresh
Fresh	3	-	54.74 ± 22.84
			Post-thaw
CC	10	35	0.00 ± 0.00
		45	0.00 ± 0.00
CL	10	35	0.00 ± 0.00
		45	0.00 ± 0.00
LL	10	35	0.00 ± 0.00
		45	0.00 ± 0.00
LC	10	35	0.00 ± 0.00
		45	0.00 ± 0.00

Figure Captions

Figure 3.1 Graphical representation of the full-factorial experimental design of the cryopreservation protocol. The leftmost column indicates the cryoprotectants (methanol and dimethylsulfoxide (DMSO) + glycerol) examined. The center column indicates the diluents (glucose and basic buffered physiological saline solution (BBPSS)) examined. Finally, the rightmost column indicates the freezing rates (-35 and -45°C/min) examined in this experiment. Overall, a total of 8 different treatment combinations were examined, as displayed by the number of boxes in the rightmost column.

Figure 3.2 Mean percent motility (± standard error) of hatchery-reared bloater (*Coregonus hoyi*) spermatozoa at 5, 10 and 15 seconds post-activation after 5 minutes of incubation in extender solution or, in the case of fresh sperm, 5 minutes after collection. Results were recorded for fresh sperm as well as the 4 extenders: methanol with glucose (CC), methanol with basic buffered physiological saline solution (CL), dimethyl sulfoxide + glycerol with basic buffered physiological saline solution (LL) and dimethyl sulfoxide + glycerol with glucose (LC). Treatment means without a letter in common (with the same numbered subscript) for the same post-activation time were significantly different ($P < 0.05$) and subscripts indicate individual one-way ANOVAs.

Figure 3.3 Mean average path velocity (VAP) in μm/s (A), straight line velocity (VSL) in μm/s (B) and curvilinear velocity (VCL) in μm/s (C) (± standard error) of hatchery-reared bloater (*Coregonus hoyi*) spermatozoa at 5, 10 and 15 seconds post-activation after 5 minutes of incubation in extender solution or, in the case of fresh sperm, 5 minutes after collection. Results were recorded for fresh sperm as well as the 4 extenders: methanol

with glucose (CC), methanol with basic buffered physiological saline solution (CL), dimethyl sulfoxide + glycerol with basic buffered physiological saline solution (LL) and dimethyl sulfoxide + glycerol with glucose (LC). Treatment means without a letter in common (with the same numbered subscript) for the same post-activation time were significantly different ($P < 0.05$) and subscripts indicate individual one-way ANOVAs.

Figure 3.4 Mean straightness (STR; A) and percent linearity (LIN; B) (± standard error) of hatchery-reared bloater (*Coregonus hoyi*) spermatozoa at 5, 10 and 15 seconds post-activation after 5 minutes of incubation in extender solution or, in the case of fresh sperm, 5 minutes after collection. Results were recorded for fresh sperm as well as the 4 extenders: methanol with glucose (CC), methanol with basic buffered physiological saline solution (CL), dimethyl sulfoxide + glycerol with basic buffered physiological saline solution (LL) and dimethyl sulfoxide + glycerol with glucose (LC). Treatment means without a letter in common (with the same numbered subscript) for the same post-activation time were significantly different ($P < 0.05$) and subscripts indicate individual one-way ANOVAs.

Figure 3.5 Mean percent motility (A), straight line velocity (VSL) in μm/s (B), and straightness (STR) (C) at 5 seconds post-activation for each of the 10 males or pools hatchery-reared bloaters (*Coregonus hoyi*) recorded. In each graph, the first bar represents the average value for the indicated metric among the 10 males or pools. Samples 8 and 10 are pools of sperm from 2 to 3 males and were required since the volume of sperm from only a single male was inadequate for the cryopreservation process. All other samples are individual males.

Figure 3.1

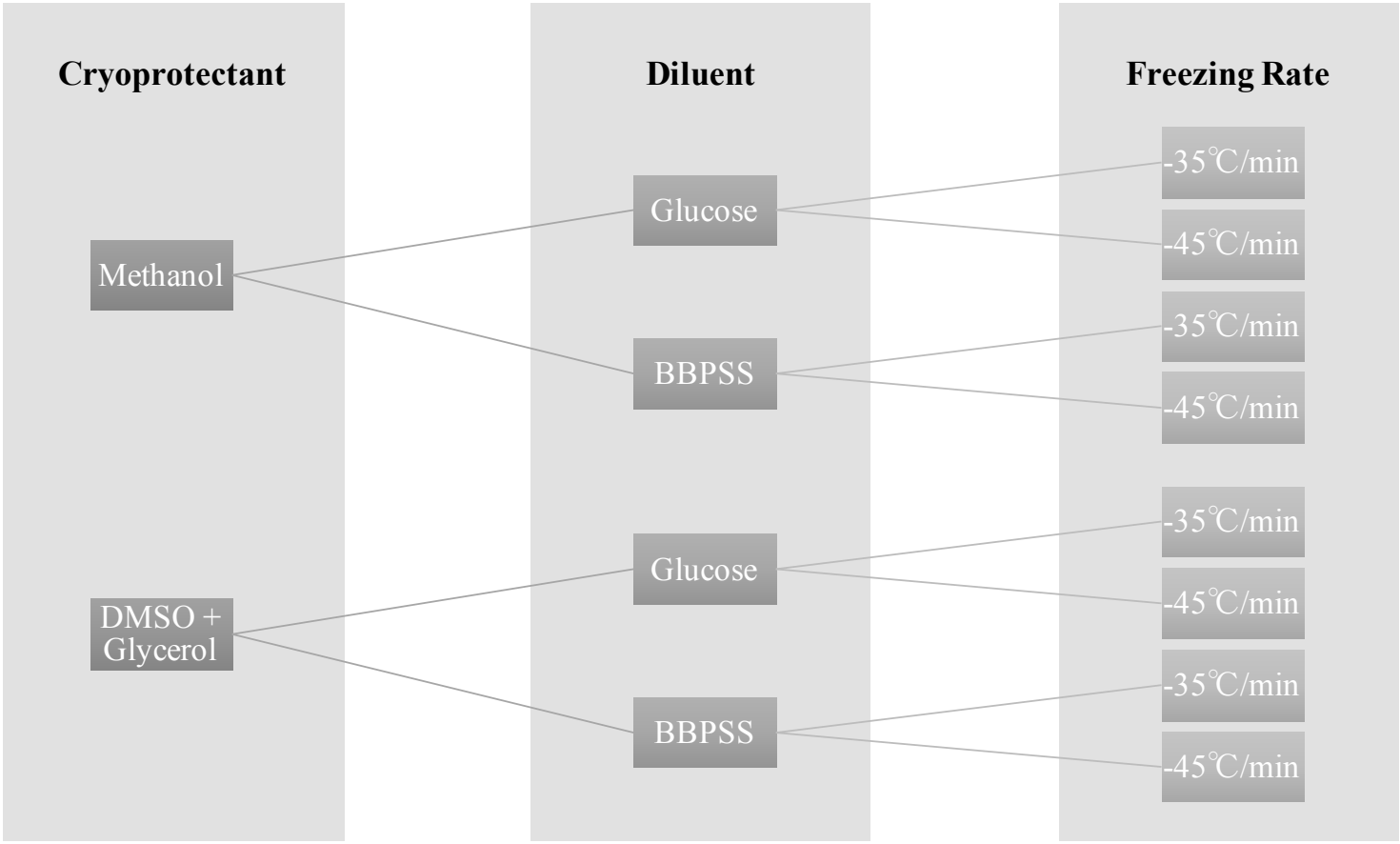

Cryoprotectant
Diluent
Freezing Rate
Methanol
DMSO + Glycerol
Glucose
BBPSS
Glucose
BBPSS
-35°C/min
-45°C/min
-35°C/min
-45°C/min
-35°C/min
-45°C/min
-35°C/min
-45°C/min

Figure 3.2

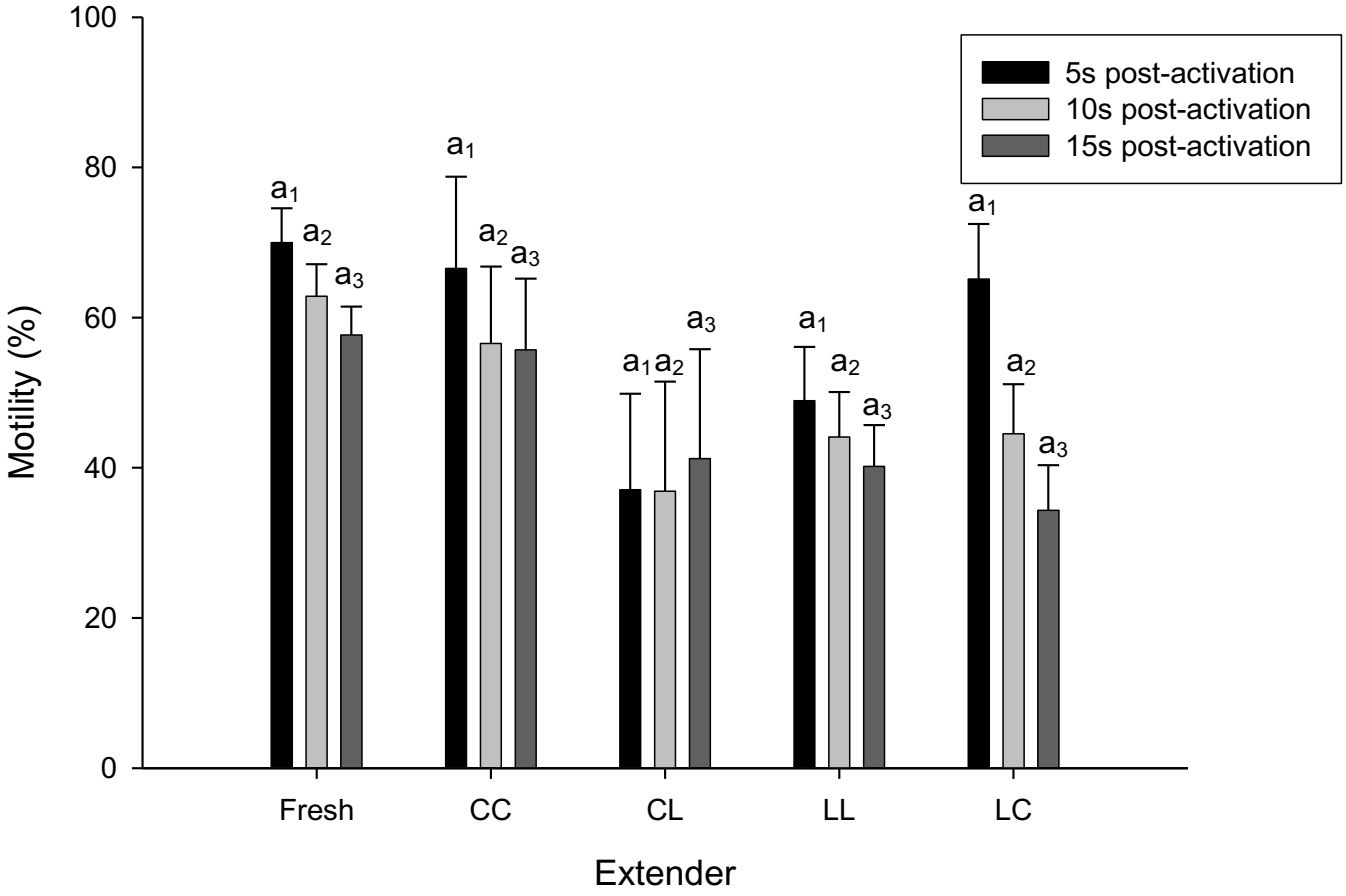

100
80
60
40
20
0
Motility (%)
5s post-activation
10s post-activation
15s post-activation
a1
a2
a3
a1
a2
a3
a1
a2
a3
a1
a2
a3
a1
a2
a3
Fresh
CC
CL
LL
LC
Extender

Figure 3.3

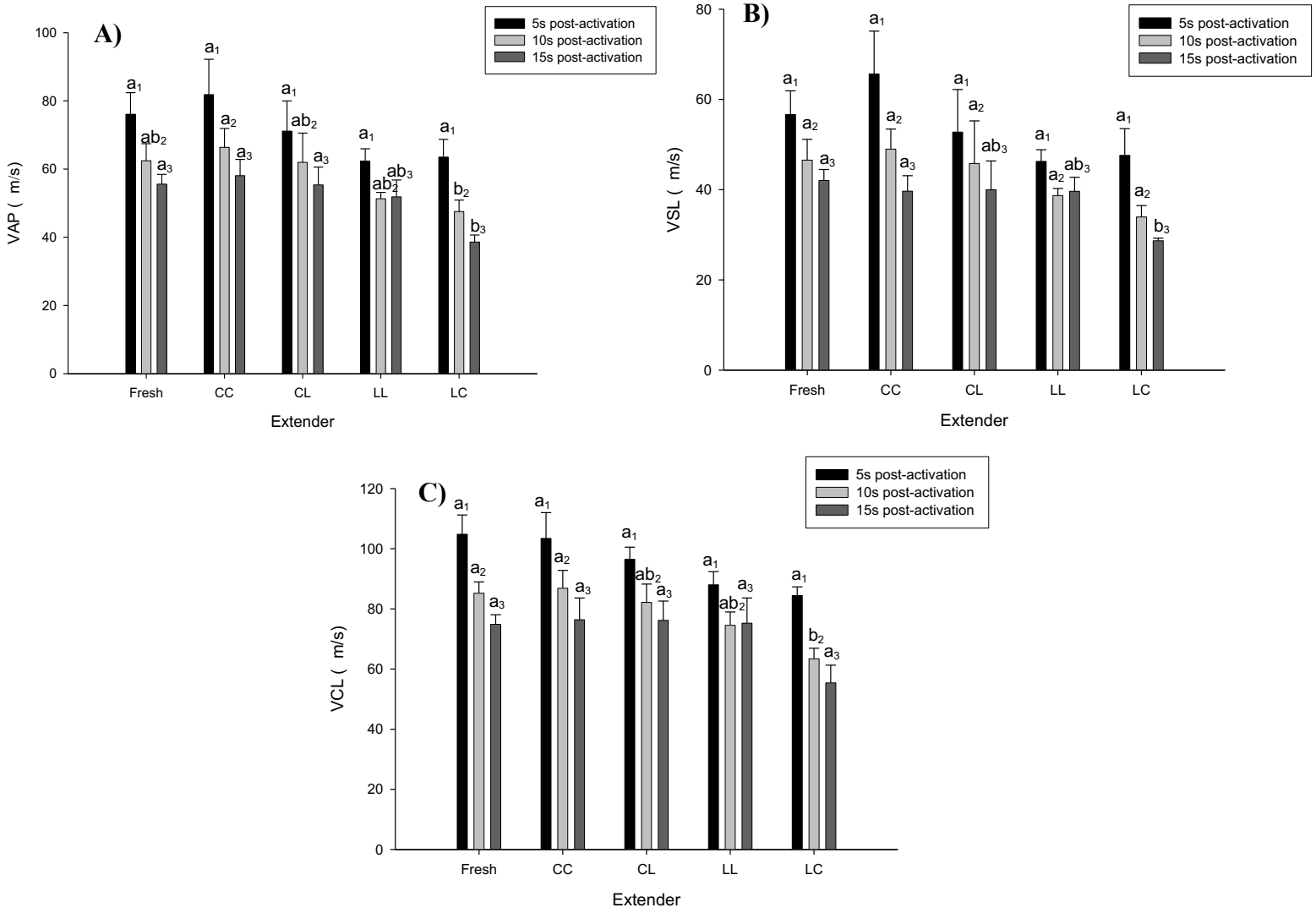

A)
VAP (m/s)
5s post-activation
10s post-activation
15s post-activation
Fresh
CC
CL
LL
LC
Extender
B)
VSL (m/s)
C)
VCL (m/s)

Figure 3.4

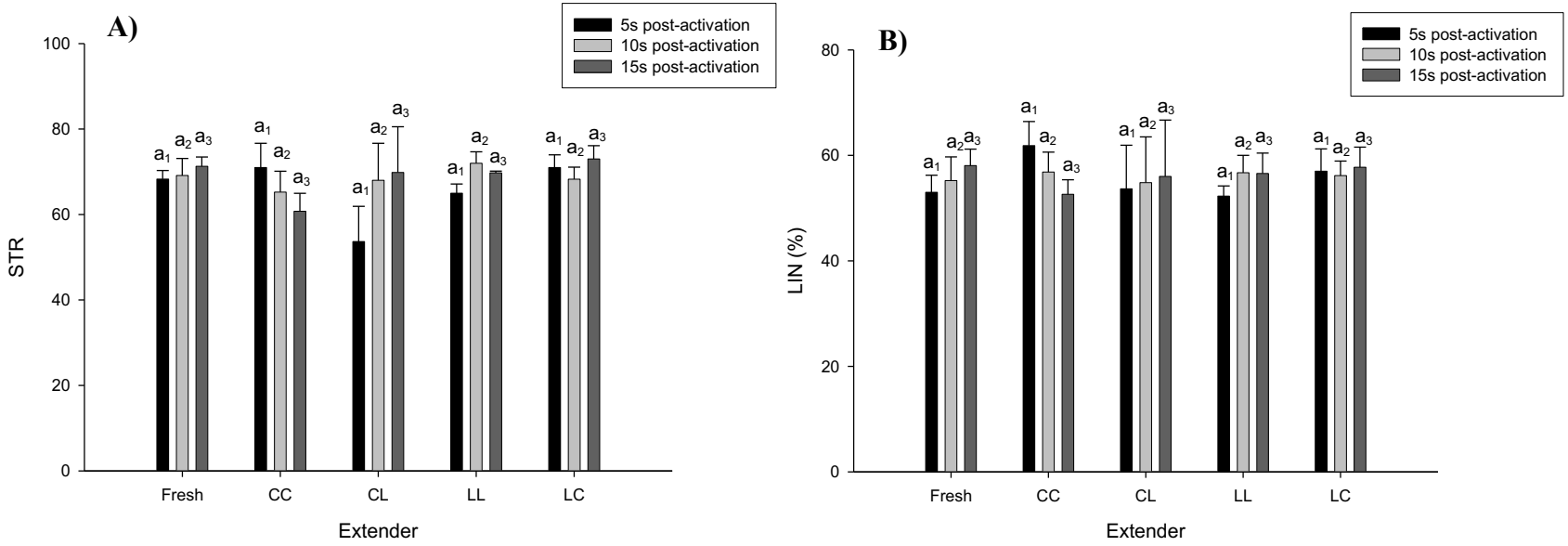
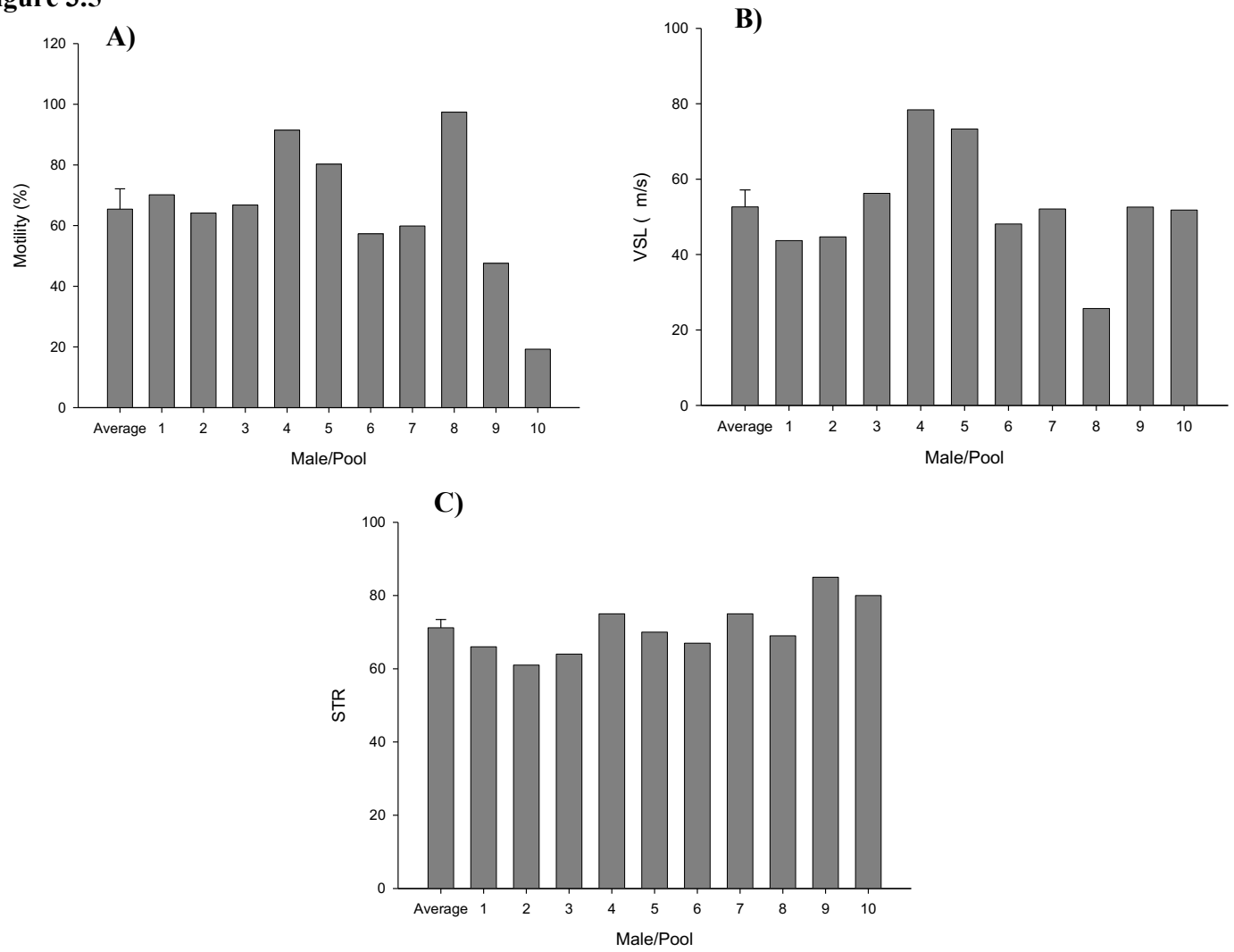

A)
5s post-activation
10s post-activation
15s post-activation
STR
100
80
60
40
20
0
Fresh
CC
CL
LL
LC
Extender
B)
5s post-activation
10s post-activation
15s post-activation
LIN (%)
80
60
40
20
0
Fresh
CC
CL
LL
LC
Extender

Figure 3.5


A)
Motility (%)
120
100
80
60
40
20
0
Average 1 2 3 4 5 6 7 8 9 10
Male/Pool
B)
VSL (m/s)
100
80
60
40
20
0
Average 1 2 3 4 5 6 7 8 9 10
Male/Pool
C)
STR
100
80
60
40
20
0
Average 1 2 3 4 5 6 7 8 9 10
Male/Pool

Chapter 4: General Discussion

4.1 Summary

The integration of reproductive biological strategies into captive breeding programs has been beneficial for many species of varying taxa (Taranger et al. 1992; Swaisgood et al. 2004; Browne et al. 2006). The bloater (*Coregonus hoyi*) is an example of an aquatic species which was locally extirpated from Lake Ontario, and is currently undergoing captive breeding efforts (Clemens and Crawford, 2009). These efforts are being impaired by the reproductive dysfunction experienced by the hatchery-reared bloaters in the form of both asynchronous and absent gamete expression (the latter especially in females). The objective of this thesis was to assess the efficacy of and apply reproductive biological techniques in the form of exogenous hormone administration and cryopreservation of spermatozoa to overcome reproductive dysfunction in hatchery-reared bloaters. In the first data chapter (Chapter 2) the efficacy of exogenous luteinizing hormone releasing hormone analog (LHRHa) injections for inducing free-flowing gamete expression in hatchery-reared bloaters was investigated. The LHRHa injections were effective at inducing the expression of free flowing gametes at both doses (40ug/kg and 80ug/kg), however, females exhibited greater percentages of gamete expression compared to males. In the second data chapter (Chapter 3), the development of a cryopreservation protocol for the sperm of hatchery-reared bloaters was initiated. Results indicated that while none of the examined extenders was critically toxic to the spermatozoa, neither of the freezing rates, which have been successful in other coregonids, were capable of preserving any post-thaw sperm motility (Lahnsteiner, 2000;

Ciereszko et al. 2013). Together these results indicate that the captive breeding of hatchery-reared bloaters can benefit from the incorporation of reproductive biological strategies and that, as more is discovered regarding the reproductive biology of the bloater, certain strategies will become more effective than at present. The following will examine the key findings from each chapter and also provide directions for future research in this area.

4.2 Chapter 2

Exogenous hormone injections have been used to improve the captive breeding efforts of many species including amphibians and fishes (Mylonas et al. 1992; Taranger et al. 1992; Browne et al. 2006). For salmonids, such as the bloater, dosages of exogenous gonadotropic hormones have varied widely from approximately 10 – 100ug/kg (see Table 2.1). The captive breeding of hatchery-reared bloaters is an essential aspect of current efforts to reintroduce the bloater into Lake Ontario, however, it has been hindered by reproductive dysfunction in a majority of its sexually mature population. As such, the aim of this chapter was to determine the efficacy of injections of LHRHa at inducing the expression of free-flowing gametes in hatchery-reared bloaters.

While numerous studies have examined the efficacy of exogenous hormone use to induce free-flowing gamete expression in fishes (e.g. Crim et al. 1983; Billard et al. 1984; Noori et al. 2010), the results from this study are the first to formally examine the efficacy of LHRHa injections in overcoming asynchronous and absent gamete expression in hatchery-reared bloaters. Although it is unknown which factor(s) related to the captive

rearing environment are impacting the reproductive cycle of the bloaters, the use of exogenous gonadotropic hormones (as outlined in Chapter 2) appear adequate in overcoming the apparent reproductive dysfunction with very low levels (< 1%) of mortality. A previous study by King and Pankhurst (2007) suggested that, through the manipulation of photoperiod and water temperature, the efficacy of LHRHa injections to advance ovulation could be improved for Atlantic salmon (*Salmo salar*). Over the course of the study, timing of ovulation could be advanced by several months when an advanced (constant) lighting photoperiod regime and an advanced (chilled) temperature regime were used in conjunction with LHRHa injections (ovulation completed in March) as compared to using natural lighting and temperature regimes (ovulation completed in June) (King and Pankhurst, 2007). The results of this study illustrate the influence of environmental cues on the expression of gametes in fish (see Figure 2.2). This provides further evidence that the manipulation of environmental cues, if possible in a hatchery setting, may help control the reproduction of hatchery-reared fishes.

Based on the results discussed in Chapter 2, several recommendations could be made to improve the captive breeding efforts of bloaters. First, as exogenous LHRHa injections become a part of the captive breeding efforts of bloaters, the injections should be administered earlier in the spawning season (e.g. November) because LHRHa injections (administered in December) appeared to inhibit sperm expression through a hormonal negative feed-back loop which reduced the proportion of spawning males (see Chapter 2; Nagahama, 1994). Also, based on observations during the experiment, males appeared to be well into their spawning season by the time of the first injection (December – January sample) while females had not yet begun. However, at the time of

the first injection for the January – February sample, the majority of males had concluded expressing sperm while females were only beginning to express free-flowing eggs. Females, at both injection times, were successfully induced to produce free-flowing eggs (i.e. before and at the beginning of their spawning season), thus, it may be possible that injecting males before or at the beginning of their spawning season may increase the number of free-flowing males. Overall, an earlier injection would appear to induce both males and females at the same time, overcoming both the asynchronous and absent expression of free-flowing gametes. Second, it may be of benefit to examine the effectiveness of exogenous gonadotropic hormones in the form of an implanted pellet. Several studies have utilized the pellets to induce the expression of free-flowing gametes in the past (Crim and Glebe, 1984; Crim et al. 1988; Garcia 1989). Crim et al (1984) conducted a study which compared the plasma gonadotropin levels in rainbow trout (*Oncorhynchus mykiss*) following an LHRHa injection or pellet implantation. Results indicated that pellet implants maintained elevated levels of gonadotropins (necessary for spawning) for several weeks, depending on the dosage, as compared to the injections which lasted no longer than 48 hours. Unlike exogenous hormone injections, implanted pellets are designed for continuous and long-term hormone delivery, however, they are likely more stressful on the fish (Crim et al. 1983). Finally, although it may not be the most economical solution, manipulation of photoperiod or diet have also been shown to improve captive breeding efforts in fishes (Bromage et al. 1992). Manipulation of photoperiod (Hansen et al. 2001) has been shown to affect many factors of reproduction in species such as Atlantic cod (*Gadus morhua*) (Hansen et al. 2001) and Atlantic salmon (Taranger et al. 1998). In their studies, Hansen et al (2001) observed that only cod reared

under natural lighting conditions spawned during the normal season and had significantly higher egg diameter and fecundity than cod which underwent photoperiod manipulation (e.g. continuous light). Similarly, Taranger et al (1998) observed that captive Atlantic salmon exposed to natural lighting had a greater proportion of individuals become sexually mature as compared to those exposed to continuous lighting. Additionally, it may be beneficial to examine the effects of diet on fish reproduction (reviewed in Izquierdo et al. 2001) regarding bloaters. A change in diet may help to improve proportion of reproductive individuals and quality of gametes. For example, Duray et al (1994) observed that when dietary lipid level was increased for rabbitfish (*Siganus guttatus*) there were significant increases in both fecundity of females and hatching percentage of fertilized eggs. Future studies will be able to examine the different modalities of exogenous hormones use (i.e. injection vs. pellet), the effects of the administration time of the exogenous hormone (i.e. November vs. December and January) and also the efficacy of manipulating environmental factors for improving the captive breeding efforts of hatchery-reared bloaters.

4.3 Chapter 3

The cryopreservation of spermatozoa of fishes to improve captive breeding efforts and overcome reproductive dysfunction has been utilized for many years (reviewed in Cabrita et al. 2010). Although there is no cryopreservation protocol for bloaters, specifically, protocols do exist for other salmonids – including some coregonids (see Table 3.1). Captive-breeding efforts at White Lake Fish Culture Station have been

hindered by asynchronous expression of gametes by male and female bloaters, preventing the optimal fertilization of eggs. Given the urgency of the reproductive dysfunction dilemma, a timely attempt to develop a cryopreservation protocol for bloaters was to test the two most successful cryoprotectants, diluents and freezing rates as observed in other coregonids (Lahnsteiner 2000; Ciereszko et al. 2013). Consequently, the aim of this chapter was to develop a cryopreservation protocol for hatchery-reared bloaters which would overcome the observed asynchronous gamete expression through the successful cryopreservation of bloater spermatozoa.

In the future, it would be beneficial to test additional extender components such as: dimethylacetamide, DMSO and 1,2-propanediol (Piironen and Hyvärinen, 1983; Lahnsteiner, 2000; Butts et al. 2011) which have proven to not be critically toxic to freshwater fish spermatozoa. Since the effect of extenders on the spermatozoa of a species is highly individualistic, it is possible that these extenders may prove less (or more) toxic to the spermatozoa of bloaters, specifically, despite the fact that they produced lower post-thaw sperm metrics than those in Table 3.1. Additionally, it is also necessary that a broader range of freezing rates be examined for their efficacy regarding the cryopreservation of bloater spermatozoa. Typical freezing rates of Salmonidae, such as those reported in Table 3.1, are considered rapid (≥-35°C/min) when compared to what is optimal for other species such as sturgeon (*Acipenser* spp.) (Billard et al. 2004) or redside dace (Butts et al. 2013) with freezing rates of between- 0.5 and -18.5°C/min. Based on the results of the current study, it appears likely that bloater sperm requires an alternative freezing rate to prevent the complete loss of motility following cryopreservation.

In addition to testing a broader range of protocol components, an alternative method of cryopreservation; termed vitrification, could be examined. Vitrification is characterized by plunging a suspension of spermatozoa, withiin a modified extender, directly into liquid nitrogen (an extremely rapid freezing rate). This method has proven successful in species such as the Atlantic salmon (Figueroa et al. 2015; Merino et al. 2011) and channel catfish (*Ictalurus punctatus*) (Cuevas-Uribe et al. 2011) achieving post-thaw sperm motilities of approximately 50%. Alternatively, the cryopreservation of the eggs of female bloaters, as opposed to the spermatozoa of males, could be investigated. The cryopreservation of the eggs of fish has not received nearly as much attention as the cryopreservation of spermatozoa (reviewed in Chao and Liao, 2001). Fish ova are relatively large, contain a large amount of yolk and are encapsulated with a thick chorion (compared to spermatozoa) which render conventional cryoprotectants much less effective in the cryopreservation process. However, despite this inherent difficulty, Tsai et al (2009) managed to achieve a late-stage ovarian follicle post-thaw viability of 50.7% for zebrafish (*Danio rerio*). It is possible that such a protocol could be investigated and developed for the eggs of bloaters, however, since the hatchery-reared female bloaters spawn after the males (as observed in this study), the eggs would have to be frozen for an entire year resulting in the loss of a single seasons' worth of fish production.

Overall, this thesis provides an examination of the some of the factors associated with a successful cryopreservation protocol for hatchery-reared bloaters such as extender composition and freezing rate. Future studies should examine additional permutations of the protocol components (as discussed above) to further optimize the cryopreservation protocol. Additionally, it may be worth investigating alternative methods of

cryopreservation such as vitrification or even egg cryopreservation. Each of the methods and factors discussed has the potential to overcome the observed spawning asynchrony between sexes in hatchery-reared bloaters which would make their broodstock establishment considerably less difficult.

4.4 Conclusions

This thesis demonstrated that the incorporation of reproductive biological strategies into captive-breeding programs can improve the efficacy of such programs. Additionally, it reinforces the importance a thorough understanding of the reproductive biology (reproductive season for both sexes, for example) for the species of interest, especially in a captive environment such as a hatchery.

The use of exogenous hormones and spermatozoa cryopreservation were used in compliment of one another, in this research, to overcome the originally observed reproductive dysfunction in the hatchery-reared bloaters. However, I believe it is the knowledge regarding the use of LHRHa which will be of more use to bloater rearing efforts in the future, more so than cryopreservation. The use of exogenous LHRHa injections, if applied to both males and females at a time point before their natural spawning season (i.e. early to mid-November) may induce the gamete expression of both sexes. This could effectively eliminate the need to cryopreserve spermatozoa since fertilization would be possible at that time so long as both sexes are expressing the adequate amounts of gametes to achieve desired brood stock numbers.

Cumulatively, the results of this thesis provide a greater understanding of the culture of hatchery-reared bloaters in addition to revealing effective methods of overcoming reproductive dysfunction in this species for the purposes of creating a self-sustaining and reproductively viable broodstock. Continued research on the applications of reproductive biological strategies in captive-breeding programs is important for many species that have become or will become vulnerable in the future. As such, the results of this thesis have important applications regarding the improvement of captive breeding programs as well as the conservation of genetic material for species conservation efforts through cryopreservation.

In conclusion, this thesis has provided vital information that can make the establishment of a self-sustaining, reproductively viable broodstock of bloaters much simpler in addition to contributing novel information regarding the circumvention of reproductive dysfunction in captive fishes. Consequently, the establishment of such a broodstock will eventually allow for the carefully planned reintroduction of bloaters to Lake Ontario which, in turn, can contribute to improving the health of more economically valuable fish, such as lake trout (*Salvelinus namaycush*), and possibly the Lake Ontario ecosystem in general by restoring one of its ecologically valuable native species.

References

Billard, R., Cosson, J., Noveiri, S. B. & Pourkazemi, M. 2004. Cryopreservation and short-term storage of sturgeon sperm, a review. *Aquaculture*. **236:** 1-9.

Billard, R., Reinaud, P., Hollebecq, M. G. & Breton, B. 1984. Advancement and synchronization of spawning in *Salmo gairdneri* and *S. trutta* following administration of LRH-A combined or not with pimozide. *Aquaculture*. **43**: 57-66.

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J. R. C., Duston, J. & Barker, G. 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **100**: 141–166.

Browne, R. K., Seratt, J., Vance, C. & Kouba, A. 2006. Hormonal priming, induction of ovulation and in-vitro fertilization of the endangered Wyoming toad (*Bufo baxteri*). *Reprod. Biol. Endocrin*. **4**: 34.

Butts, I. A. E., Litvak, M. K., Kaspar, V. & Trippel, E. A. 2010. Cryopreservation of Atlantic cod *Gadus morhua* L. spermatozoa: effects of extender composition and freezing rate on sperm motility, velocity and morphology. *Cryobiology*. **61**: 174-181.

Butts, I. A. E., Mokdad, A., Trippel, E. A. & Pitcher, T. E. 2013. Development of a sperm cryopreservation protocol for redside dace: implications for genome resource banking. *T. Am. Fish. Soc*. **142**: 671-680.

Butts, I. A. E., Feindel, N., Neil, S., Kovacs, E., Urbanyi, B. & Trippel, E. A. 2011. Cryopreservation of Atlantic Cod (*Gadus morhua*) sperm in large-volume straws:

applications for commercial production and gene banking. *Aquac. Res.*. **42**:1714–1722.

Cabrita, E., Sarasquete, C., Martinez-Páramo, S., Robles, V., Beirão, J., Pérez-Cerezales, S. & Herráez, M. P. 2010. Cryopreservation of fish sperm: applications and perspectives. *J. Appl. Icthyol*. **26**: 623-635.

Chao, N. & Liao, I. C. 2001. Cryopreservation of finfish and shellfish gametes and embryos. *Aquaculture*. **197**: 161-189.

Ciereszko, A., Dietrich, G. J., Nynca, J., Liszewska, E., Karol, H. & Dobosz, S. 2013. The use of concentrated extenders to improve the efficacy of cryopreservation in whitefish spermatozoa. *Aquaculture*. **408-409**: 30-33.

Ciereszko, A., Dietrich, G. J., Wojtczak, M., Sobocki, M., Hliwa, P., Kuźmiński, H., Dobosz, S., Słowińska, M. & Nynca, J. 2008. Characterization and cryopreservation of whitefish (*Coregonus lavaretus* L.) semen from Lake Łebsko, Poland. *Fund. Appl. Limnol*. **173**: 59-65.

Clemens, B. J. & Crawford, S. S. 2009. The ecology of body size and depth use by bloater (*Coregonus hoyi* Gill) in the Laurentian Great Lakes: patterns and hypotheses. *Rev. Fish. Sci*. **17**: 174-186.

Crim, L. W. & Glebe, B. D. 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. *Aquaculture*. **43**: 47-56.

Crim, L. W., Evans, D. M. & Vickery, B. H. 1983. Manipulation of the seasonal reproductive cycle of the landlocked Atlantic salmon (*Salmo salar*) by LHRH analogues administered at various stages of gonadal development. *Can. J. Fish. Aquat. Sci.* **40**: 61-67.

Crim, L. W., Sherwood, N. M. and Wilson, C. E. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (LHRHa) administration by either single time injection of cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. *Aquaculture*. **74**: 87-95.

Garcia, L. M. B. 1989. Dose-dependent spawning response of mature female sea bass, *Lates calcarifer* (Bloch), to pelleted luteinizing hormone-releasing hormone analogue (LHRHa). *Aquaculture*. **77**: 85-96.

Hansen, T., Karlsen, Ø., Taranger, G. L., Hemre, G., Holm, J. C. & Kjesbu, O. S. 2001. Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture*. **203**: 51-67.

Holt, W. V. 2000. Fundamental aspects of sperm cryobiology: importance of species and individual differences. *Theriogenology*. **53**: 47-58.

Izquierdo, M. S., Fernández-Palacios, H. & Tacon, A. G. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture.* **197**: 25-42.

King, H. R. & Pankhurst, N. W. 2007. Additive effects of advanced temperature and photoperiod regimes and LHRHa injection on ovulation in Atlantic salmon (*Salmo salar*). *Aquaculture*. **273**: 729-738.

Lahnsteiner, F. 2000. Semen cryopreservation in the Salmonidae and in Northern pike. *Aquac. Res*. **31**: 245-258.

Lahnsteiner, F., Berger, B., Weismann, T. & Patzner, R. 1996. The influence of various cryoprotectants on semen quality of the rainbow trout (*Oncorhychus mykiss*) before and after cryopreservation. *J. Appl. Ichthyol*. **12**: 99-106.

Mylonas, C. C., Hinshaw, J. M. & Sullivan, C. V. 1992. GnRHa-induced ovulation of

brown trout (*Salmo trutta*) and its effects on egg quality. *Aquaculture*. **106**: 379-392.

Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. *Int. J. Dev. Biol*. **38**: 217-229.

Noori, A., Amiri, B. M., Mirvaghefi, A. & Baker, D. W. 2010. LHRHa-induced ovulation of the endangered Caspian brown trout (*Salmo trutta caspius*) and its effect on egg quality and two sex steroids: testosterone and 17α-hydroxyprogesterone. *Aquac. Res.*. **41**: 871-877.

Piironen, J. and Hyvärinen, H. 1983. Cryopreservation of spermatozoa of whitefish *Coregonus muksun* Pallas. *J. Fish. Biol*. **22**: 159-163.

Swaisgood RR, Lindburg DG, White AM, Zhang H, Zhou X. 2004. Chemical communication in giant pandas: experimentation and application. Lindburg D. G. & Baragona K. (Eds.). Giant pandas: biology and conservation. University of California Press, Berkeley. pp. 106-120

Taranger, G. L., Haux, C., Stefansson, S. O., Björnsson, B. J., Walther, B. T. & Hansen, T. 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradial-17β profiles in Atlantic salmon, *Salmo salar*. *Aquaculture*. **162**: 85-98.

Taranger, G. L., Stefansson, S. O. & Hansen, T. 1992. Advancement and synchronization of ovulation in Atlantic salmon (*Salmo salar* L.) following injections of LHRH analogue. A*quaculture*. **102**: 169-175.

Tsai, S., Rawson, D. M. & Zhang, T. 2009. Development of cryopreservation protocols for early stage zebrafish (*Danio rerio*) ovarian follicles suing controlled slow

cooling. *Theriogenology*. **71**: 1226-1233.

Yang, H., C. Carmichael, Z. M. Varga, and T. R. Tiersch. 2007. Development of a simplified and standardized protocol with potential for high-throughput for sperm cryopreservation in Zebrafish *Danio rerio*. *Theriogenology*. **68**:128– 136.

APPENDICES

APPENDIX 1: SUMMARY AND RAW DATA OF MORPHOMETRIC MEASUREMENTS AND GAMETE EXPRESSION CHECKS

Table Captions

Table A.1 Summary statistics for the comparison of the mean mass (g) between sexes, expression status (expressing; free flowing gametes present and non-expressing; free flowing gametes absent) and treatment group for hatchery-reared bloaters (*Coregonus hoyi*). Sample size (n), standard error (SE) and the range of data are also included for both the December – January sample bloaters (A) and the January – February sample (B).

Table A.2 Summary statistics for the comparison of the mean total length (mm) between sexes, expression status (expressing; free flowing gametes present and non-expressing; free flowing gametes absent) and treatment group for hatchery-reared bloaters (*Coregonus hoyi*). Sample size (n), standard error (SE) and the range of data are also included for both the December – January sample bloaters (A) and the January – February sample (B).

Table A.3 Summary statistics for the comparison of the mean girth (mm) between sexes, expression status (expressing; free flowing gametes present and non-expressing; free flowing gametes absent) and treatment group for hatchery-reared bloaters (*Coregonus hoyi*). Sample size (n), standard error (SE) and the range of data are also included for both the December – January sample bloaters (A) and the January – February sample (B).

Table A.4 Summary statistics for the comparison of the mean gonadosomatic index (GSI) (%) between sexes, expression status (expressing; free flowing gametes present and

non-expressing; free flowing gametes absent) and treatment group for hatchery-reared bloaters (*Coregonus hoyi*). Sample size (n), standard error (SE) and the range of data are also included for both the December – January sample bloaters (A) and the January – February sample (B).

Table A.5 Raw data of morphometric measurements, sex estimate for male (M) or female (F), treatment group, original tank (from tank) and gamete expression check results (nothing, hard eggs, free flowing eggs (FF eggs) or free flowing milt (milt)) for the December – January sample of hatchery-reared bloaters (*Coregonus hoyi*).

Table A.6 Raw data of morphometric measurements, sex estimate for male (M) or female (F), treatment group, original tank (from tank) and gamete expression check results (nothing, hard eggs, free flowing eggs (FF eggs) or free flowing milt (milt)) for the January – February sample of hatchery-reared bloaters (*Coregonus hoyi*).

Table A.1

A)

	Treatment	n	Mass (g) Mean ± SE	Range
Expressing Male	Control	16	116 ± 13	64-239
	Sham	14	125 ± 12	56-189
	Low	10	136 ± 15	74-211
	High	10	120 ± 12	77-199
Non-Expressing Male	Control	36	133 ± 9	62-273
	Sham	51	145 ± 6	77-301
	Low	48	125 ± 7	50-287
	High	55	129 ± 7	61-277
Expressing Female	Control	4	201 ± 59	97-351
	Sham	11	231 ± 34	55-473
	Low	22	174 ± 12	87-346
	High	21	159 ± 9	83-236
Non-Expressing Female	Control	119	161 ± 7	56-490
	Sham	96	177 ± 7	65-429
	Low	92	165 ± 7	57-380
	High	86	159 ± 7	69-359

B)

	Treatment	n	Mass (g) Mean ± SE	Range
Expressing Male	Control	3	94 ± 10	74-108
	Sham	4	142 ± 33	75-207
	Low	4	159 ± 16	111-178
	High	5	104 ± 19	56-167
Non-Expressing Male	Control	62	123 ± 6	54-247
	Sham	59	132 ± 6	53-231
	Low	51	125 ± 7	53-263
	High	52	120 ± 7	56-232
Expressing Female	Control	29	180 ± 11	92-331
	Sham	23	139 ± 12	62-287
	Low	51	165 ± 10	53-401
	High	57	182 ± 9	59-391
Non-Expressing Female	Control	66	150 ± 8	55-409
	Sham	73	160 ± 8	57-368
	Low	54	142 ± 7	50-267
	High	46	146 ± 9	56-289

Table A.2

A)

	Treatment	n	Length (mm) Mean ± SE	Range
Expressing Male	Control	16	222 ± 8	143-285
	Sham	14	233 ± 6	188-265
	Low	10	236 ± 7	210-273
	High	10	234 ± 7	209-275
Non-Expressing Male	Control	36	238 ± 4	195-286
	Sham	51	242 ± 3	206-300
	Low	48	229 ± 5	87-290
	High	55	233 ± 4	111-285
Expressing Female	Control	4	260 ± 17	233-307
	Sham	11	269 ± 10	195-310
	Low	22	248 ± 5	199-305
	High	21	249 ± 4	209-286
Non-Expressing Female	Control	119	245 ± 3	110-320
	Sham	96	254 ± 3	193-330
	Low	92	250 ± 3	190-323
	High	86	244 ± 3	160-315

B)

	Treatment	n	Length (mm) Mean ± SE	Range
Expressing Male	Control	3	223 ± 9	210-240
	Sham	4	239 ± 13	210-270
	Low	4	251 ± 9	225-265
	High	5	227 ± 11	200-260
Non-Expressing Male	Control	62	235 ± 3	190-290
	Sham	59	238 ± 3	185-280
	Low	51	236 ± 4	190-295
	High	52	236 ± 5	190-415
Expressing Female	Control	29	262 ± 4	220-320
	Sham	23	242 ± 5	195-290
	Low	51	249 ± 4	195-330
	High	57	258 ± 3	195-305
Non-Expressing Female	Control	66	244 ± 3	195-345
	Sham	73	247 ± 3	190-315
	Low	54	243 ± 3	185-295
	High	46	244 ± 4	190-310

Table A.3

A) **Girth (mm)**

	Treatment	n	Mean ± SE	Range
Expressing Male	Control	16	109 ± 4	89-140
	Sham	14	112 ± 4	85-135
	Low	10	115 ± 5	90-140
	High	10	110 ± 4	94-133
Non-Expressing Male	Control	36	114 ± 3	88-158
	Sham	51	119 ± 2	90-164
	Low	48	112 ± 2	75-160
	High	55	112 ± 2	85-155
Expressing Female	Control	4	132 ± 15	100-165
	Sham	11	141 ± 9	86-200
	Low	22	127 ± 3	100-165
	High	21	124 ± 2	105-140
Non-Expressing Female	Control	119	122 ± 2	90-195
	Sham	96	128 ± 2	85-185
	Low	92	124 ± 2	85-180
	High	86	123 ± 2	88-200

B) **Girth (mm)**

	Treatment	n	Mean ± SE	Range
Expressing Male	Control	3	108 ± 7	95-115
	Sham	4	128 ± 12	105-150
	Low	4	136 ± 6	120-145
	High	5	115 ± 7	95-135
Non-Expressing Male	Control	62	125 ± 3	100-245
	Sham	59	125 ± 2	90-165
	Low	51	123 ± 2	90-160
	High	52	122 ± 2	95-170
Expressing Female	Control	29	143 ± 3	115-180
	Sham	23	130 ± 4	100-180
	Low	51	138 ± 3	95-190
	High	57	143 ± 3	100-200
Non-Expressing Female	Control	66	135 ± 3	95-210
	Sham	73	138 ± 3	95-230
	Low	54	130 ± 3	100-170
	High	46	131 ± 3	95-175

Table A.4

A)

	Treatment	n	GSI (%) Mean ± SE	Range
Expressing Male	Control	9	0.76 ± 0.15	0.14-1.76
	Sham	6	0.70 ± 0.17	0.28-1.34
	Low	8	0.63 ± 0.12	0.29-1.21
	High	7	0.77 ± 0.29	0.13-2.45
Non-Expressing Male	Control	0	-	-
	Sham	0	-	-
	Low	0	-	-
	High	0	-	-
Expressing Female	Control	0	-	-
	Sham	0	-	-
	Low	9	10.85 ± 1.79	3.37-16.64
	High	7	8.82 ± 1.92	1.98-15.84
Non-Expressing Female	Control	5	11.27 ± 1.22	6.80-14.10
	Sham	5	13.17 ± 1.67	9.66-18.51
	Low	0	-	-
	High	0	-	-

B)

	Treatment	n	GSI (%) Mean ± SE	Range
Expressing Male	Control	0	-	-
	Sham	0	-	-
	Low	0	-	-
	High	3	0.30 ± 0.05	0.21-0.40
Non-Expressing Male	Control	8	0.18 ± 0.04	0.06-0.37
	Sham	9	0.14 ± 0.03	0.05-0.28
	Low	12	0.31 ± 0.1	0.04-1.29
	High	6	0.26 ± 0.06	0.07-0.47
Expressing Female	Control	7	8.12 ± 2.08	2.59-15.32
	Sham	7	11.40 ± 2.06	4.52-20.24
	Low	5	4.39 ± 0.75	2.50-6.21
	High	6	8.12 ± 2.25	2.59-15.32
Non-Expressing Female	Control	7	12.32 ± 1.5	7.34-19.40
	Sham	5	13.27 ± 1.22	9.12-16.36
	Low	4	9.57 ± 3.37	2.49-18.12
	High	6	10.78 ± 1.96	4.76-18.86

Table A.5

							Gamete Expression Check Date and Result			
Pit-tag ID	**Sex Estimate**	**Treatment**	**Mass (g)**	**Length (mm)**	**Girth (mm)**	**From Tank:**	**Dec 21-22**	**Jan 4-5**	**Jan 11-12**	**Jan 20**
000866	F	High	201	260	133	10	Nothing	Hard Eggs	Hard Eggs	Nothing
003454	F	Low	175	250	130	3	Nothing	FF Eggs		
003570	M	Sham	199	250	140	3	Nothing	Nothing	Nothing	Nothing
006824	M	Low	140	235	120	3	Nothing	Nothing	Nothing	Nothing
007839	M	Control	270	275	155	4	Nothing	Nothing	Nothing	Nothing
017566	M	Low	62	196	93	6	Nothing	Nothing	Nothing	Nothing
021259	F	High	210	275	133	4	Nothing	FF Eggs		
021420	F	Low	84	210	100	10	Nothing	Nothing	Nothing	Nothing
021693	F	Control	212	284	135	3	Nothing	Hard Eggs		
021804	F	Control	150	245	120	3	Nothing	Nothing	Nothing	Nothing
021827	F	Low	182	250	133	4	Nothing	FF Eggs		
022163	F	Control	100	225	115	10	Nothing	Nothing	Nothing	Nothing
022973	F	High	118	233	111	3	Nothing	Nothing	Nothing	Nothing
023220	M	Sham	141	244	118	3	Nothing	Nothing	Nothing	Nothing
023256	F	Control	147	238	125	3	Nothing	Hard Eggs	Hard Eggs	Nothing
023519	M	Sham	301	280	164	4	Nothing	Nothing	Nothing	Nothing
023584	F	Sham	132	251	114	3	Nothing	Hard Eggs	Hard Eggs	Nothing
023596	F	Low	295	281	160	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
023718	F	High	114	229	114	3	Nothing	Nothing	Nothing	Nothing
023986	M	Control	66	196	89	4	Nothing	Milt		
024026	F	Sham	76	205	94	6	Nothing	Nothing	Nothing	Nothing

024328	F	Sham	137	240	120	4	Nothing	Nothing	Nothing	Nothing
024570	F	Control	168	244	128	3	Nothing	Hard Eggs	Hard Eggs	Nothing
025405	F	Low	89	217	105	10	Nothing	Nothing	Nothing	Nothing
025630	F	Control	97	235	100	10	Nothing	Hard Eggs	Hard Eggs	FF Eggs
029228	M	High	88	213	101	6	Nothing	Nothing	Nothing	Nothing
033692	F	High	198	260	131	6	Nothing	Nothing	Hard Eggs	Hard Eggs
034579	M	Sham	178	254	129	6	Nothing	Milt		
034594	M	Sham	196	220	111	4	Nothing	Nothing	Nothing	Nothing
034596	F	Control	217	269	145	3	Nothing	Hard Eggs	Nothing	Nothing
034847	F	High	104	220	108	4	Nothing	Nothing	Nothing	Nothing
034909	F	Control	158	259	120	6	Nothing	Nothing	Nothing	Nothing
034937	F	Sham	112	225	111	6	Nothing	Nothing	Nothing	Nothing
035117	M	Low	118	235	111	3	Nothing	Nothing	Nothing	Nothing
035258	M	High	167	255	125	10	Nothing	Nothing	Nothing	Nothing
035286	F	Sham	103	231	104	3	Nothing	Nothing	Nothing	Nothing
035375	M	Control	118	230	110	10	Nothing	Nothing	Nothing	Milt
035512	M	Low	103	228	102	6	Nothing	Milt		
035548	M	Control	87	205	97	6	Nothing	Nothing	Nothing	Nothing
035569	M	Control	152	260	120	3	Nothing	Nothing	Nothing	Nothing
035634	M	Low	126	249	111	10	Nothing	Nothing	Nothing	Nothing
035850	F	Low	207	269	138	3	Nothing	Nothing	Nothing	Nothing
035882	F	High	212	275	114	4	Hard Eggs	Hard Eggs	Hard Eggs	Nothing
036030	M	Control	136	248	115	10	Nothing	Nothing	Nothing	Nothing
036149	M	Low	97	225	97	3	Nothing	Nothing	Nothing	Nothing
036305	M	Low	127	230	111	4	Nothing	Nothing	Nothing	Nothing
036334	F	High	192	260	131	6	Nothing	Nothing	Nothing	Nothing
036907	M	High	173	255	130	10	Nothing	Nothing	Nothing	Nothing

038009	F	Sham	210	270	135	6	Nothing	Hard Eggs	Hard Eggs	Nothing
039466	F	Control	265	280	155	6	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
040025	M	Sham	184	261	135	4	Nothing	Milt		
041370	F	Control	144	250	120	3	Nothing	Hard Eggs	Nothing	Hard Eggs
041733	F	High	158	251	126	6	Nothing	Nothing	Nothing	Nothing
042741	F	Control	154	241	125	4	Nothing	Nothing	Nothing	Nothing
043125	F	High	76	215	96	6	Nothing	Nothing	Nothing	Nothing
043417	F	Sham	194	273	134	10	Nothing	Nothing	Nothing	Nothing
043471	M	Low	131	241	113	3	Nothing	Nothing	Nothing	Nothing
043638	F	Control	106	230	105	4	Nothing	Nothing	Nothing	Nothing
043988	F	Sham	254	245	158	3	Nothing	Nothing	Nothing	Nothing
044832	F	Sham	201	255	135	6	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
044844	F	Sham	155	250	127	6	Nothing	Nothing	Hard Eggs	Nothing
044854	M	Low	180	250	130	4	Nothing	Nothing	Nothing	Nothing
045195	F	Low	241	291	140	4	Nothing	Nothing	Nothing	Nothing
045989	F	Low	60	190	85	10	Nothing	Nothing	Nothing	Nothing
046267	F	Control	200	265	137	4	Nothing	Nothing	Nothing	Nothing
046604	M	Control	137	241	115	10	Nothing	Nothing	Nothing	Nothing
046682	F	High	122	286	120	3	Nothing	Nothing	Nothing	FF Eggs
046694	M	Low	87	87	119	10	Nothing	Nothing	Nothing	Nothing
046700	F	Control	163	240	132	6	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
046703	M	High	112	237	102	3	Nothing	Nothing	Nothing	Nothing
046854	F	Control	80	210	96	3	Nothing	Nothing	Nothing	Nothing
047757	M	Low	128	240	113	6	Nothing	Nothing	Nothing	Nothing
047823	M	Control	157	245	123	10	Nothing	Nothing	Nothing	Nothing
047950	M	Sham	106	230	104	6	Nothing	Nothing	Nothing	Nothing
048012	F	High	192	270	130	4	Nothing	Nothing	Nothing	Nothing

048139	F	High	153	254	125	6	Nothing	Nothing	Nothing	Nothing
054094	M	High	77	209	94	10	Nothing	Milt		
054096	F	Control	139	240	115	3	Nothing	Hard Eggs	Nothing	Nothing
054212	F	Sham	169	248	126	4	Nothing	Hard Eggs	Nothing	Nothing
054228	M	Sham	88	215	95	10	Nothing	Nothing	Nothing	Nothing
054534	F	Control	193	271	130	3	Nothing	Hard Eggs		
054633	F	Control	248	270	150	3	Nothing	Nothing	Nothing	
054723	F	Low	123	222	120	10	Nothing	Hard Eggs	Nothing	Nothing
054768	M	Control	115	235	109	6	Nothing	Nothing	Nothing	Nothing
054846	M	High	79	210	95	10	Nothing	Nothing	Nothing	Nothing
054923	F	High	132	245	115	10	Nothing	FF Eggs		
055025	F	Sham	96	225	100	6	Nothing	Nothing	Nothing	Nothing
055306	M	Low	146	250	120	4	Nothing	Nothing	Nothing	Nothing
055321	F	High	231	275	143	10	Nothing	Hard Eggs	Nothing	Hard Eggs
055467	M	Sham	166	260	125	6	Nothing	Nothing	Nothing	Nothing
055478	F	Low	57	190	89	6	Nothing	Nothing	Nothing	Nothing
055483	M	Sham	134	248	111	4	Nothing	Nothing	Nothing	Nothing
057250	M	Sham	145	241	121	4	Nothing	Milt		
057422	F	Low	177	258	130	6	Nothing	Nothing	Nothing	Nothing
058736	M	High	97	225	100	10	Nothing	Nothing	Nothing	Nothing
058930	F	High	161	254	125	6	Nothing	Hard Eggs	FF Eggs	
060822	F	Sham	93	215	100	6	Nothing	Nothing	Nothing	Hard Eggs
064700	F	High	189	245	130	3	Nothing	Hard Eggs	FF Eggs	
064741	M	Sham	102	270	103	3	Nothing	Nothing	Nothing	Nothing
064940	F	High	117	231	111	3	Nothing	FF Eggs		
065696	F	Control	128	241	115	10	Nothing	Nothing	Nothing	Nothing
066030	F	Control	69	210	90	6	Nothing	Hard Eggs	Nothing	Nothing

066222	F	High	238	273	143	6	Hard Eggs	Hard Eggs	Nothing	Nothing
066347	F	High	123	239	115	10	Nothing	Hard Eggs	Nothing	Nothing
066702	M	Low	145	250	120	4	Nothing	Nothing	Nothing	Nothing
066755	F	Sham	169	247	124	10	Hard Eggs	Hard Eggs		
066844	M	Sham	160	260	125	3	Nothing	Nothing	Nothing	Nothing
067579	F	Control	201	265	136	4	Nothing	Nothing	Nothing	Nothing
067785	M	Sham	206	206	136	10	Nothing	Nothing	Nothing	Nothing
068495	M	High	99	210	100	6	Nothing	Nothing	Nothing	Nothing
068661	M	High	92	215	105	6	Nothing	Nothing	Nothing	Nothing
070201	M	High	126	249	105	6	Nothing	Nothing	Nothing	Nothing
070314	M	High	61	201	86	10	Nothing	Nothing	Nothing	Nothing
071359	F	Control	154	244	125	4	Nothing	Nothing	Nothing	Nothing
071888	M	Sham	200	275	134	10	Nothing	Nothing	Nothing	Nothing
072536	M	Control	93	218	100	3	Nothing	Nothing	Nothing	Nothing
072619	M	Control	89	218	100	6	Nothing	Nothing	Nothing	Nothing
072880	F	Sham	141	245	119	3	Nothing	Nothing	Nothing	Nothing
073555	M	Control	91	217	98	4	Nothing	Milt		
073616	M	Sham	157	245	125	6	Nothing	Milt		
073619	F	High	303	285	150	4	Nothing	Hard Eggs	Hard Eggs	Nothing
073777	M	Low	125	240	111	10	Nothing	Nothing	Nothing	Nothing
073921	M	Low	65	193	98	3	Nothing	Nothing	Nothing	Nothing
074016	M	Low	140	243	125	6	Nothing	Nothing	Nothing	Nothing
074191	F	High	69	185	95	4	Nothing	Nothing	Nothing	Nothing
074356	F	High	121	230	111	3	Nothing	Nothing	Nothing	Nothing
074371	M	Low	65	195	90	4	Nothing	Nothing	Nothing	Nothing
074594	F	High	127	231	113	3	Nothing	Hard Eggs	Nothing	Nothing
074595	F	Low	120	235	110	10	Nothing	Hard Eggs	Hard Eggs	Hard Eggs

074650	M	Control	186	266	130	4	Nothing	Nothing	Nothing	Nothing
074659	F	Sham	226	274	140	6	Nothing	Nothing	Nothing	Nothing
074716	F	Low	179	250	130	4	Nothing	Nothing	Nothing	Nothing
074808	F	Low	169	259	124	10	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
074902	F	High	173	260	130	3	Hard Eggs	Hard Eggs	FF Eggs	
075113	F	Low	132	273	132	10	Hard Eggs	Hard Eggs	Nothing	Nothing
075196	F	Control	197	281	133	6	Nothing	Nothing	Nothing	Nothing
075502	F	Control	86	210	108	10	Nothing	Hard Eggs	Hard Eggs	Nothing
076077	F	Low	285	290	155	6	Nothing	Nothing	Nothing	Nothing
076460	M	Sham	110	230	108	10	Nothing	Nothing	Nothing	Nothing
077939	M	High	150	255	118	10	Nothing	Milt		
079002	F	Low	145	239	123	10	Nothing	FF Eggs		
079929	F	High	169	254	123	6	Nothing	Hard Eggs	FF Eggs	
080011	M	Sham	136	237	119	6	Nothing	Nothing	Nothing	Nothing
080446	M	High	110	235	105	6	Nothing	Nothing	Nothing	Nothing
080602	M	Control	62	208	88	6	Nothing	Nothing	Nothing	Nothing
080774	M	High	148	245	118	3	Nothing	Nothing	Nothing	Nothing
080815	F	High	118	228	114	6	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
081143	M	Low	287	290	160	4	Nothing	Nothing	Nothing	Nothing
081245	F	Control	110	220	111	6	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
081309	M	Control	273	286	158	4	Nothing	Nothing	Nothing	Nothing
081316	M	High	127	235	120	6	Nothing	Nothing	Nothing	Nothing
081501	F	Sham	95	235	109	10	Nothing	Nothing	Nothing	Nothing
081508	F	Low	108	235	109	3	Nothing	FF Eggs		
082556	M	High	166	260	125	4	Nothing	Nothing	Nothing	Nothing
082678	M	Sham	112	223	110	3	Nothing	Nothing	Nothing	Nothing
083206	F	Low	132	230	114	4	Nothing	Nothing	Nothing	Nothing

083959	F	High	85	220	97	6	Nothing	Hard Eggs	Nothing	Nothing
085728	F	High	140	244	115	10	Nothing	Nothing	Nothing	Nothing
097953	F	Control	199	270	135	3	Nothing	Nothing	Nothing	Nothing
098258	F	Sham	193	271	132	3	Nothing	Nothing	Nothing	Nothing
099291	F	Low	225	262	135	4	Nothing	Nothing	Nothing	Nothing
099638	F	Low	180	265	125	6	Nothing	Hard Eggs	Hard Eggs	Nothing
100413	F	Sham	91	215	111	10	Nothing	Nothing	Nothing	Nothing
100423	F	High	82	213	97	6	Nothing	Nothing	Nothing	Nothing
100961	F	Control	209	265	135	4	Nothing	Nothing	Nothing	Nothing
101222	F	High	113	230	110	10	Nothing	Nothing	Nothing	Nothing
101791	F	Low	142	236	119	3	Nothing	Hard Eggs	Nothing	Nothing
101877	M	Sham	113	223	113	6	Nothing	Nothing	Nothing	Nothing
102034	M	Low	128	240	112	4	Nothing	Nothing	Nothing	Nothing
102669	F	High	164	240	125	3	Nothing	Hard Eggs	Nothing	Nothing
102783	F	Sham	174	270	125	6	Hard Eggs	Hard Eggs	Hard Eggs	Nothing
102985	F	Low	249	280	145	4	Nothing	Nothing	Nothing	Nothing
103674	M	Sham	77	215	90	4	Nothing	Nothing	Nothing	Nothing
107502	F	Low	136	235	112	6	Nothing	Hard Eggs	Nothing	Nothing
108485	F	Sham	137	245	119	10	Nothing	Hard Eggs	Hard Eggs	Nothing
108721	F	Control	275	300	141	4	Nothing	Nothing	Nothing	Nothing
108928	F	High	152	250	127	10	Nothing	Hard Eggs	Nothing	Nothing
109053	M	Control	159	250	119	3	Nothing	Nothing	Nothing	Nothing
109784	F	Control	119	233	116	4	Nothing	Nothing	Hard Eggs	FF Eggs
109878	M	Control	221	285	136	4	Nothing	Nothing	Milt	
109959	F	Sham	110	230	105	10	Nothing	Nothing	Nothing	Nothing
110676	M	Sham	116	230	111	6	Nothing	Nothing	Nothing	Nothing
111165	F	Sham	135	250	113	10	Nothing	Hard Eggs	Nothing	Nothing

111278	F	Low	362	323	164	6	Nothing	Hard Eggs	Nothing	Nothing
111639	F	Sham	289	305	150	4	Nothing	Hard Eggs	FF Eggs	
111773	M	High	69	195	90	6	Nothing	Nothing	Nothing	Nothing
112324	F	High	270	280	155	6	Nothing	Hard Eggs	Hard Eggs	Nothing
117568	F	Low	66	200	90	10	Nothing	Hard Eggs	Nothing	Nothing
117800	M	Sham	75	205	90	3	Nothing	Milt		
118024	F	Sham	176	260	175	6	Nothing	Nothing	Hard Eggs	Nothing
118186	M	Low	75	205	95	6	Nothing	Nothing	Nothing	Nothing
118379	F	High	92	216	100	6	Nothing	Nothing	Nothing	Nothing
118392	M	Control	203	269	135	3	Nothing	Nothing	Nothing	Nothing
118398	F	Sham	138	244	115	4	Nothing	Nothing	Nothing	Nothing
118630	F	Low	63	195	95	6	Nothing	Hard Eggs	Nothing	Nothing
118804	M	High	220	282	134	4	Nothing	Nothing	Nothing	Nothing
119308	F	Control	164	249	125	4	Nothing	Nothing	Nothing	Nothing
119759	F	High	115	225	110	4	Nothing	Nothing	Nothing	Nothing
119827	M	Sham	105	228	105	6	Nothing	Nothing	Nothing	Nothing
121581	F	Control	121	225	115	6	Nothing	Nothing	Nothing	Nothing
121620	M	Low	128	225	115	3	Milt	Milt		
121999	F	Sham	174	250	130	3	Nothing	Hard Eggs	Hard Eggs	Nothing
123119	M	Low	93	215	105	4	Nothing	Nothing	Nothing	Nothing
123811	M	Control	65	195	90	6	Nothing	Nothing	Nothing	Nothing
123992	F	Control	156	254	120	6	Nothing	Nothing	Nothing	Nothing
125961	F	High	197	256	140	3	Hard Eggs	Nothing	Nothing	Nothing
126088	F	Control	351	307	165	10	Nothing	Nothing	Hard Eggs	FF Eggs
126097	F	Low	133	235	115	3	Nothing	Hard Eggs	Hard Eggs	Nothing
126244	F	High	199	265	132	4	Nothing	FF Eggs		
126432	M	Control	123	241	115	6	Nothing	Nothing	Nothing	Nothing

126516	F	Control	145	245	125	6	Nothing	Nothing	Nothing	Nothing
126563	M	High	128	247	115	10	Nothing	Nothing	Nothing	Nothing
126739	F	Sham	223	278	140	3	Nothing	Nothing	Nothing	Nothing
127088	M	Low	107	238	103	4	Nothing	Nothing	Nothing	Nothing
127170	F	High	108	235	114	10	Nothing	Nothing	Nothing	Nothing
127234	F	Low	155	245	122	3	Nothing	Nothing	Nothing	Nothing
128022	F	High	216	274	136	4	Nothing	Nothing	Nothing	Nothing
131425	F	Control	106	225	111	4	Nothing	Hard Eggs	Nothing	Nothing
132077	F	High	149	244	119	3	Nothing	Hard Eggs	Nothing	Nothing
134020	F	Control	118	235	115	10	Hard Eggs	Hard Eggs	Nothing	Hard Eggs
139799	F	Control	56	285	147	3	Nothing	Nothing	Hard Eggs	Nothing
139974	M	Low	167	246	125	4	Nothing	Milt		
140188	F	Sham	473	310	200	4	Nothing	Hard Eggs	Nothing	FF Eggs
141327	F	Control	80	212	94	6	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
141397	F	High	179	250	136	4	Nothing	Hard Eggs	Hard Eggs	FF Eggs
141452	F	High	114	235	105	10	Nothing	Nothing	Nothing	Nothing
142022	F	High	276	285	150	6	Nothing	Nothing	Nothing	Nothing
142668	F	Sham	149	248	120	3	Nothing	Nothing	FF Eggs	
142975	M	High	87	210	100	3	Nothing	Nothing	Nothing	Nothing
143867	F	Low	203	265	135	3	Nothing	Hard Eggs	Nothing	Nothing
144348	F	Control	490	310	195	3	Nothing	Nothing	Hard Eggs	Hard Eggs
144407	M	Sham	204	268	133	10	Nothing	Nothing	Nothing	Nothing
147748	M	High	111	111	116	10	Nothing	Nothing	Nothing	Nothing
147869	M	Sham	96	215	100	3	Nothing	Nothing	Nothing	Nothing
147977	F	Control	228	280	145	10	Nothing	Nothing	Nothing	Nothing
148137	F	High	97	216	102	6	Nothing	Hard Eggs	Hard Eggs	Nothing
148217	F	Low	109	226	105	6	Nothing	Nothing	Nothing	Nothing

148573	F	Sham	175	260	133	6	Nothing	Nothing	Nothing	Nothing
148839	M	Low	108	230	111	4	Nothing	Nothing	Nothing	Nothing
149558	F	Sham	223	275	135	10	Nothing	Nothing	Nothing	Nothing
149671	F	Low	185	256	133	6	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
150091	F	Sham	239	282	140	4	Hard Eggs	Hard Eggs	Nothing	Nothing
150490	F	Sham	185	265	130	10	Nothing	Nothing	Nothing	Nothing
150498	M	Sham	119	236	112	10	Nothing	Nothing	Nothing	Nothing
150525	F	Sham	193	265	135	10	Hard Eggs	Nothing	Nothing	Nothing
150739	M	Control	115	233	111	10	Nothing	Nothing	Nothing	Nothing
150817	F	Low	165	256	125	3	Nothing	Nothing	Nothing	Nothing
152166	M	High	133	240	120	3	Nothing	Nothing	Nothing	Nothing
152638	F	Control	239	265	148	3	Hard Eggs	Nothing	Nothing	Nothing
153564	F	Control	118	239	111	6	Nothing	Hard Eggs	Nothing	Nothing
153671	F	Low	179	258	138	4	Nothing	Nothing	FF Eggs	FF Eggs
160368	F	Low	100	227	100	6	Nothing	Nothing	Nothing	Nothing
160552	F	Low	219	265	140	4	Hard Eggs	Hard Eggs	Nothing	Nothing
161034	F	High	174	259	126	4	Hard Eggs	Nothing	Nothing	Nothing
165108	M	High	166	249	127	6	Nothing	Nothing	Nothing	Nothing
169120	F	Sham	238	280	138	10	Hard Eggs	Nothing	Nothing	Nothing
169142	F	Sham	257	270	150	4	Nothing	Nothing	Nothing	Nothing
169144	F	Sham	65	195	85	4	Hard Eggs	Nothing	Nothing	Nothing
169586	M	High	204	174	138	3	Nothing	Nothing	Nothing	Nothing
170280	M	Sham	130	225	120	3	Nothing	Nothing	Nothing	Nothing
170639	F	Sham	84	220	105	6				
289786	F	Control	80	110	100	4	Nothing	Nothing	Nothing	Nothing
294727	M	Low	64	205	88	6	Nothing	Nothing	Nothing	Nothing
299322	F	Low	175	250	123	4	Nothing	Hard Eggs	FF Eggs	FF Eggs

299329	M	High	92	220	101	4	Milt			
299503	F	Control	88	225	95	6	Nothing	Nothing	Nothing	Nothing
299516	M	High	79	213	93	6	Nothing	Nothing	Nothing	Nothing
299571	F	High	116	225	110	10	Nothing	Nothing	Nothing	Nothing
299603	F	Control	181	246	135	4	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
299691	F	Sham	271	285	155	4	Hard Eggs	FF Eggs		
299704	M	Low	71	205	90	10	Nothing			
299809	F	Sham	153	250	125	3	Nothing	Nothing	Nothing	Nothing
300766	M	Sham	235	300	140	10	Nothing	Nothing	Nothing	Nothing
302959	F	High	164	255	124	6	FF Eggs			
302983	F	Low	142	245	118	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
303044	M	High	79	210	94	10	Nothing	Milt		
303052	F	Sham	73	206	93	10	Nothing	Nothing	Nothing	Nothing
303072	F	Control	151	250	120	6	Hard Eggs	Nothing	Nothing	Nothing
305377	F	Sham	106	223	110	10	Hard Eggs	Hard Eggs	Nothing	Nothing
317342	F	Low	114	240	105	10	Nothing	Nothing	Nothing	Nothing
326476	M	Sham	126	230	120	10	Nothing			
328873	F	High	144	241	120	4	Nothing	Nothing	Nothing	Nothing
364203	M	High	86	205	100	4	Nothing	Nothing	Nothing	Nothing
366857	M	High	164	249	124	3	Nothing	Nothing	Nothing	Nothing
366861	F	Low	193	260	135	3	Hard Eggs	Nothing	FF Eggs	FF Eggs
366926	F	High	140	234	120	4	Nothing	Nothing	Nothing	Nothing
366984	F	Sham	158	251	125	3	Nothing	Nothing	Nothing	Nothing
366986	M	High	262	273	150	4	Nothing	Nothing	Nothing	Nothing
367051	F	Sham	118	227	111	4	Nothing	Nothing	Nothing	Nothing
367120	F	Control	131	253	115	6	Hard Eggs	Nothing	Hard Eggs	Hard Eggs
367123	M	Control	122	235	111	6	Nothing	Nothing	Nothing	Nothing

367171	M	Low	154	250	120	6	Nothing	Nothing	Nothing	Nothing
367179	F	Control	107	231	107	4	Hard Eggs	Nothing	Hard Eggs	Hard Eggs
367381	F	Control	82	218	95	10	Nothing	Nothing	Nothing	Nothing
367425	F	Low	159	269	123	6	Nothing	Nothing	Nothing	Nothing
367455	M	High	168	264	125	3	Nothing	Nothing	Nothing	Nothing
367456	M	High	98	220	100	6	Nothing	Nothing	Nothing	Nothing
367583	F	Low	180	258	132	4	Hard Eggs	Nothing	Nothing	Nothing
367743	F	High	196	260	135	3	Hard Eggs	Hard Eggs	Nothing	Nothing
367781	F	High	278	290	155	10	Nothing	Nothing	Nothing	Nothing
367862	F	High	206	260	140	4	Hard Eggs	Nothing	Nothing	Nothing
367868	F	High	102	216	105	3	Hard Eggs	Hard Eggs	FF Eggs	
367907	F	Low	131	236	118	3	Hard Eggs	FF Eggs		
368075	F	Control	128	235	115	6	Hard Eggs	Hard Eggs	Nothing	Nothing
368241	M	Control	156	250	121	4	Nothing	Nothing	Nothing	Nothing
368310	F	Low	233	268	150	3	Nothing	Nothing	Nothing	Nothing
368314	M	Sham	151	235	122	4	Nothing	Nothing	Nothing	Nothing
368366	F	High	227	270	144	10	Nothing	Nothing	Hard Eggs	Hard Eggs
368407	F	High	99	218	115	6	Nothing	Nothing	Nothing	Nothing
368478	F	Low	235	277	145	3	Nothing	Nothing	Nothing	Nothing
368479	F	Control	89	220	100	4	Hard Eggs	Nothing		
368635	M	Low	148	237	123	10	Nothing	Nothing	Nothing	Nothing
368650	M	Control	84	220	109	10	Nothing	Nothing	Nothing	Nothing
368702	F	Control	140	251	116	10	Nothing	Nothing	Hard Eggs	Hard Eggs
368714	F	Low	99	230	101	6	Hard Eggs	Hard Eggs	Nothing	Nothing
368717	F	Control	79	200	94	6	Hard Eggs	Nothing	Nothing	Nothing
368748	M	Control	150	251	116	4	Nothing	Nothing	Nothing	Nothing
368853	F	Low	164	260	125	4	Nothing	Nothing	FF Eggs	

368939	F	High	95	210	100	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
368979	F	Low	202	282	130	3	Nothing	FF Eggs		
369046	M	Control	86	215	95	6	Nothing	Milt		
369114	F	Low	157	259	120	6	Hard Eggs	Hard Eggs	Nothing	Nothing
369153	F	Control	267	285	157	10	Hard Eggs	Nothing		
369173	M	Sham	123	243	111	10	Nothing	Nothing	Nothing	Nothing
369200	F	Control	115	230	110	6	Nothing	Nothing	Nothing	Nothing
369211	F	Control	124	240	111	3	Hard Eggs	Nothing	Nothing	Nothing
369225	F	High	126	240	111	4	Hard Eggs	Nothing	Nothing	Nothing
369253	F	Control	193	260	120	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
369259	M	Sham	241	270	145	4	Nothing	Nothing	Nothing	Nothing
369272	M	Low	123	239	110	3	Nothing	Nothing	Nothing	Nothing
369292	F	High	197	255	139	3	Nothing	FF Eggs		
369302	M	Sham	157	255	120	10	Nothing	Nothing	Nothing	Nothing
369367	F	Low	124	247	115	6	Nothing	Nothing	Nothing	Nothing
369425	F	Sham	106	229	104	10	Nothing			
369488	M	High	105	219	110	6	Milt			
369491	F	Control	145	235	120	4	Nothing	Nothing	Nothing	Nothing
369498	F	Sham	263	245	140	4	Hard Eggs	Hard Eggs	Nothing	Nothing
369524	F	High	185	270	128	6	Nothing	Nothing	Nothing	Nothing
369585	F	Sham	185	270	130	4	Hard Eggs	Hard Eggs	Nothing	Nothing
369739	F	Low	133	247	110	4	Nothing	Nothing	Nothing	Nothing
369751	F	Sham	232	280	145	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
369863	M	Sham	194	265	138	4	Nothing	Nothing	Nothing	Nothing
369890	F	Control	61	195	90	3	Hard Eggs	Hard Eggs	Nothing	Nothing
369901	M	High	66	207	85	10	Nothing	Nothing	Nothing	Nothing
369946	F	Low	380	310	180	3	Hard Eggs	Nothing	Nothing	Nothing

369962	F	High	184	268	127	10	Nothing	Nothing	Nothing	Nothing
369987	F	Control	109	230	106	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
370043	M	Low	91	220	99	10	Milt			
370055	F	High	116	232	115	6	Hard Eggs	Hard Eggs	Nothing	Nothing
370095	M	Sham	118	239	108	6	Nothing	Nothing	Nothing	Nothing
370157	F	Control	173	265	125	4	Nothing	Nothing	Nothing	Nothing
370263	F	Control	196	265	138	10	Nothing	Nothing	Nothing	Nothing
370314	F	High	142	245	115	4	Hard Eggs	Hard Eggs	Nothing	Nothing
370315	M	High	91	220	105	3	Nothing	Nothing		
370357	M	Low	77	215	92	6	Nothing	Nothing	Nothing	Nothing
370448	M	Sham	137	242	125	6	Milt			
370716	F	Sham	170	256	125	3	Nothing	Nothing	Nothing	Nothing
370722	F	Low	98	220	105	3	FF Eggs			
370742	F	Sham	190	261	131	3	Nothing	Nothing	Hard Eggs	Hard Eggs
370760	F	Control	145	250	118	10	Nothing	Nothing	Nothing	Nothing
370795	F	Low	131	235	118	4	Nothing	Nothing	Nothing	Nothing
370873	F	Sham	172	247	129	4	Hard Eggs	Nothing	Nothing	Nothing
370918	M	Control	89	210	98	6	Nothing	Nothing	Nothing	Nothing
370978	M	Low	196	265	137	10	Nothing	Nothing	Nothing	Nothing
371093	F	High	73	200	91	3	Hard Eggs	Nothing	Nothing	Nothing
371102	F	Sham	152	250	124	3	Hard Eggs	Nothing	Nothing	Nothing
371120	M	High	199	275	133	10	Nothing	Nothing	Milt	
371124	F	Low	132	240	120	6	Nothing	Nothing	Nothing	Nothing
371237	F	Sham	55	195	86	3	Hard Eggs	Nothing	FF Eggs	
371274	M	Sham	132	238	114	10	Milt			
371286	F	Control	125	225	115	4	Nothing			
371315	M	High	133	245	112	10	Nothing	Nothing	Nothing	Nothing

371446	M	High	277	285	155	4	Nothing	Nothing	Nothing	Nothing
371481	M	Low	92	219	99	4	Nothing	Nothing	Nothing	Nothing
371505	F	Low	114	227	106	10	Nothing	Nothing	Nothing	Nothing
371528	F	High	165	250	129	6	Nothing	Nothing	Nothing	Nothing
371572	F	Sham	171	252	134	3	Hard Eggs	Hard Eggs	FF Eggs	FF Eggs
371576	F	Sham	126	237	118	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
371763	F	Control	162	260	119	3	Nothing	Nothing	Hard Eggs	Hard Eggs
371765	M	High	83	213	105	10	Nothing	Nothing	Nothing	Nothing
371945	F	Sham	184	266	130	10	Hard Eggs			
372010	F	Low	350	310	170	3	Nothing	Nothing	Nothing	Nothing
372051	M	Low	74	210	90	10	Milt			
372141	F	Control	126	235	115	10	Nothing	Nothing	Nothing	Nothing
372185	M	Sham	56	188	85	6	Milt	Milt		
372187	M	Sham	188	260	130	4	Nothing	Nothing	Nothing	Nothing
372198	F	Control	213	265	150	4	Nothing	Hard Eggs	Nothing	Nothing
372235	F	High	95	215	105	4	FF Eggs			
372325	M	Control	139	240	120	6	Milt			
372363	F	Low	96	224	105	6	Hard Eggs	Nothing	Nothing	Nothing
372381	M	Low	190	260	140	4	Nothing	Nothing	Nothing	Nothing
372425	F	Low	86	226	94	6	Nothing	Nothing	Nothing	Nothing
372439	F	Low	143	240	120	4	Nothing	Nothing	Nothing	Nothing
372447	F	Sham	241	280	145	3	Nothing	Nothing	Nothing	Nothing
372463	M	Sham	154	251	125	6	Nothing	Nothing	Nothing	Nothing
372483	F	Sham	204	270	140	4	Hard Eggs	Hard Eggs	Nothing	Nothing
372498	F	Sham	113	220	111	6	Nothing	Nothing	Nothing	Nothing
372506	M	Sham	120	246	111	10	Milt	Nothing	Nothing	Nothing
372536	M	Control	100	225	105	4	Milt			

372576	M	Sham	114	235	110	3	Nothing	Nothing	Nothing	Nothing
372597	F	Control	205	276	138	10	Nothing	Nothing	Nothing	Nothing
372599	M	Control	149	249	123	6	Nothing	Milt		
372704	F	Control	123	229	112	6	Nothing	Nothing	Nothing	Nothing
372774	F	Control	131	245	114	4	Nothing	Nothing	Nothing	Nothing
372918	F	Low	161	264	123	10	Nothing	Nothing	Nothing	Nothing
373071	M	High	118	234	109	3	Milt			
373272	F	Low	238	285	145	4	Hard Eggs	Nothing	Nothing	Nothing
373340	F	Low	149	246	123	10	Nothing	Nothing	Nothing	Nothing
373341	M	Low	109	235	105	4	Nothing	Nothing	Nothing	Nothing
373363	F	Low	191	265	138	3	Hard Eggs	Hard Eggs	Nothing	Nothing
373412	F	Control	70	110	98	10	Nothing	Nothing	Nothing	Nothing
373471	F	High	136	235	118	10	Hard Eggs	Nothing	Nothing	Nothing
373521	M	Low	111	234	101	10	Nothing	Nothing	Nothing	Nothing
453923	M	High	135	246	111	6	Milt			
460134	M	Sham	102	221	106	6	Nothing	Nothing	Nothing	Nothing
499585	F	Control	136	249	115	3	Hard Eggs	Nothing	Nothing	Nothing
532581	F	Low	236	275	143	4	Nothing	Hard Eggs	FF Eggs	FF Eggs
565264	F	Control	82	212	90	3	Nothing	Nothing	Nothing	Nothing
571557	M	Low	125	231	115	10	Nothing	Nothing	Nothing	Nothing
573218	M	High	93	220	95	3	Nothing			
574739	M	Sham	107	227	103	10	Nothing	Nothing	Nothing	Nothing
575470	F	Sham	156	274	116	4	Nothing	Hard Eggs	Nothing	Nothing
575955	F	Sham	191	259	135	6	Hard Eggs	Nothing	Nothing	Nothing
581179	M	Control	145	259	115	10	Nothing	Nothing	Nothing	Nothing
588122	F	High	96	230	99	4	Nothing	Nothing	Nothing	Nothing
595117	F	High	93	221	99	10	Nothing	Nothing	Nothing	Nothing

597279	M	High	125	238	111	4	Nothing	Nothing	Nothing	Nothing
599717	M	Sham	202	260	135	6	Nothing	Nothing	Nothing	Nothing
609804	M	High	186	250	130	4	Nothing	Nothing	Nothing	Nothing
612266	F	Sham	251	265	160	4	Hard Eggs	Hard Eggs	Nothing	Nothing
612705	F	Low	120	225	114	10	Hard Eggs	Hard Eggs	Nothing	Nothing
622064	F	Sham	124	210	115	4	Nothing	Nothing	Nothing	Nothing
622311	M	Sham	105	230	105	6	Nothing	Nothing	Milt	
626799	F	Low	131	247	113	6	Hard Eggs	FF Eggs		
627383	F	Sham	261	278	150	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
631950	F	Low	108	227	114	10	Hard Eggs	Hard Eggs	Nothing	Nothing
632584	F	Low	324	295	160	10	Nothing	Nothing	Nothing	Nothing
636523	F	Sham	134	250	119	6	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
641621	F	Low	227	271	145	6	Nothing	Nothing	Hard Eggs	Hard Eggs
641829	F	Control	214	214	140	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
642848	M	Sham	191	265	131	4	Nothing	Nothing	Nothing	Nothing
644099	F	Control	220	276	145	4	Hard Eggs	Hard Eggs	Nothing	Nothing
645340	M	High	98	225	110	10	Nothing	Nothing	Nothing	Nothing
646768	M	Sham	76	210	93	10	Milt			
648739	F	Low	203	258	140	6	Nothing	Nothing	Nothing	Nothing
648869	F	High	155	257	130	3	Hard Eggs	FF Eggs		
650897	F	High	320	295	160	4	Hard Eggs	Nothing	Nothing	Nothing
654813	F	High	307	300	165	6	Hard Eggs	Nothing	Hard Eggs	Hard Eggs
662434	F	Control	102	225	106	3	Nothing	Nothing	Hard Eggs	Hard Eggs
670593	F	High	125	239	110	3	Hard Eggs	Hard Eggs	Nothing	Nothing
671193	M	High	122	229	116	10	Nothing	Nothing	Nothing	Nothing
677370	F	Control	174	245	130	4	Nothing	Nothing	Nothing	Nothing
679452	F	Sham	69	193	90	10	Nothing	Nothing	Nothing	Nothing

680681	F	Low	126	235	115	6	Nothing	Nothing	Nothing	Nothing
690208	F	High	236	278	140	4	Hard Eggs	Hard Eggs	FF Eggs	
694418	F	High	232	280	150	4	Nothing	Hard Eggs	Nothing	Nothing
709845	M	High	187	270	128	4	Nothing	Nothing	Nothing	Nothing
726039	F	Control	131	250	115	10	Hard Eggs	Nothing	Nothing	Nothing
729791	M	Control	86	234	94	6	Nothing	Nothing	Nothing	Nothing
730450	M	Low	268	275	148	4	Nothing	Nothing	Nothing	Nothing
736042	M	High	143	240	120	3	Nothing	Nothing	Nothing	Nothing
755765	F	Control	401	231	99	10	Nothing	Nothing	Nothing	Nothing
782499	M	Low	125	240	110	3	Nothing	Nothing	Nothing	Nothing
782549	F	Control	97	226	101	3	Nothing	Nothing	Nothing	Nothing
785590	F	Low	187	271	125	10	Hard Eggs	Hard Eggs	Nothing	Nothing
791210	F	Control	80	210	96	3	Nothing	Nothing	Nothing	Nothing
791356	M	Sham	186	267	130	4	Nothing	Nothing	Nothing	Nothing
792389	F	High	175	250	123	3	FF Eggs			
792879	M	Sham	101	215	111	3	Nothing	Nothing	Nothing	Nothing
798686	F	Control	155	250	125	4	Hard Eggs	Nothing	Nothing	Nothing
810951	F	Low	143	245	121	3	Hard Eggs	Nothing	Nothing	Nothing
812161	M	High	172	265	120	10	Nothing	Nothing	Nothing	Nothing
812898	F	Sham	132	253	115	10	Nothing	Nothing	Nothing	Nothing
815148	M	High	99	220	104	4	Nothing	Nothing	Nothing	Nothing
818298	F	High	225	283	136	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
820466	F	Low	121	235	105	4	Nothing	Nothing	Nothing	Nothing
912702	F	Sham	249	265	158	10	Hard Eggs	Nothing	Hard Eggs	Hard Eggs
916784	F	Low	119	239	109	4	Nothing	Nothing	Nothing	Nothing
917488	F	Control	245	280	145	6	Hard Eggs	Nothing	Nothing	Nothing
932099	M	Control	96	233	99	10	Milt			

946345	M	Control	95	230	98	10	Nothing	Nothing	Nothing	Nothing
952084	F	Sham	222	280	142	6	Nothing	Nothing	FF Eggs	
975492	M	Control	143	143	115	10	Milt			
976748	M	Control	99	230	105	10	Nothing	Nothing	Nothing	Nothing
977995	F	Sham	250	280	150	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
978437	M	Low	50	199	75	3	Nothing	Nothing	Nothing	Nothing
978581	F	Control	93	224	99	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
981079	F	Control	122	235	111	4	Hard Eggs	Nothing	Nothing	Nothing
982362	F	High	93	210	105	4	Nothing	Nothing	Nothing	Nothing
982365	M	High	186	261	129	6	Nothing	Nothing	Nothing	Nothing
982423	F	Control	152	260	121	3	Hard Eggs	Nothing	Nothing	Nothing
982432	F	Control	138	240	120	6	Hard Eggs	Hard Eggs	Nothing	Nothing
982445	F	Sham	78	215	90	6	Nothing	Nothing	Nothing	Nothing
982490	M	High	130	240	112	10	Nothing	Nothing	Nothing	Nothing
982516	F	Control	238	265	146	4	Nothing	Nothing	FF Eggs	
982568	M	Control	104	225	111	3	Nothing	Nothing	Nothing	Nothing
982586	M	Control	173	255	124	3	Nothing	Nothing	Nothing	Nothing
982608	F	High	165	253	125	3	FF Eggs			
982653	M	Low	107	125	109	10	Nothing	Nothing	Nothing	Nothing
982654	F	Control	98	220	112	3	Hard Eggs	Nothing	Nothing	Nothing
982700	M	High	94	225	99	10	Nothing	Nothing	Nothing	Nothing
982746	F	Control	65	195	90	10	Hard Eggs	Hard Eggs	Nothing	Nothing
982819	F	High	122	240	111	6	Nothing	Nothing	Nothing	Nothing
982921	F	High	108	220	107	4	Nothing	Nothing	Nothing	Nothing
982980	F	Sham	187	270	133	10	Nothing	Nothing	Nothing	Nothing
982996	F	Low	140	240	130	4	Nothing	Nothing	Nothing	Nothing
983004	F	High	83	210	109	3	Nothing	Nothing	Nothing	Nothing

983042	F	Low	133	250	115	6	Nothing	Nothing	FF Eggs	
983072	M	High	77	215	90	10	Nothing	Nothing	Nothing	Nothing
983115	M	Low	180	255	135	10	Nothing	Milt		
983157	M	Control	140	243	117	10	Nothing	Nothing	Nothing	Nothing
983187	M	High	116	227	110	4	Nothing	Nothing	Nothing	Nothing
983197	F	Sham	138	250	115	10	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
983240	F	Low	271	295	146	6	Nothing	Hard Eggs	Nothing	Nothing
983331	F	Control	235	275	130	4	Nothing	Nothing	Nothing	Nothing
983377	M	High	205	270	140	6	Nothing	Nothing	Nothing	Nothing
983416	F	Sham	84	215	94	6	Nothing	Nothing	Nothing	Nothing
983509	M	High	83	216	100	6	Nothing	Nothing	Nothing	Nothing
983659	M	High	134	255	113	10	Nothing	Nothing	Nothing	Nothing
983677	F	Control	72	200	100	4	Nothing	Nothing	Nothing	Nothing
983746	M	Sham	189	265	131	6	Milt			
983791	F	Control	159	243	125	10	Nothing	Nothing	Nothing	Nothing
983837	F	Sham	245	284	143	10	Nothing	Nothing	Nothing	Nothing
983846	F	Low	202	202	134	10	Hard Eggs	Hard Eggs	FF Eggs	FF Eggs
983859	F	Sham	280	284	155	3	Nothing	Nothing	FF Eggs	FF Eggs
983903	F	Low	249	280	145	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
983933	F	Low	251	280	148	4	FF Eggs			
984150	M	Control	105	220	105	3	Nothing			
984235	M	Low	181	255	130	6	Nothing	Nothing	Nothing	Nothing
984252	M	Low	171	279	129	4	Nothing	Nothing	Nothing	Nothing
984299	F	Sham	169	268	130	10	Nothing	Hard Eggs	Nothing	Nothing
984333	F	Control	240	278	145	6	Nothing	Nothing	Nothing	Nothing
984345	F	Control	390	320	175	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
984357	F	Low	67	198	85	6	Hard Eggs	Hard Eggs	Nothing	Nothing

984391	F	Control	184	250	130	3	Hard Eggs	Nothing	Nothing	Nothing
984514	F	Control	124	235	106	3	Hard Eggs	Hard Eggs	Nothing	Nothing
984533	F	Control	79	210	100	3	Nothing	Hard Eggs	Nothing	Nothing
984724	F	Low	116	230	115	3	Nothing	FF Eggs		
984952	F	Low	199	199	133	3	FF Eggs			
985069	F	High	331	315	165	3	Hard Eggs	Nothing	Nothing	Nothing
985160	M	Low	155	240	120	10	Nothing	Nothing	Nothing	Nothing
985190	F	Sham	280	275	163	3	Hard Eggs			
985335	F	High	83	209	111	4	Hard Eggs	FF Eggs		
985510	M	Sham	116	230	106	4	Milt			
985717	F	Sham	84	220	95	4	Nothing	Nothing	Nothing	Nothing
985733	M	Low	169	250	124	3	Milt			
985945	F	Sham	167	269	125	3	Nothing	Nothing	FF Eggs	
986034	F	Low	109	220	110	6	Nothing	Nothing	Nothing	Nothing
986080	F	High	143	235	127	6	Nothing	Hard Eggs	Nothing	Nothing
986084	F	Sham	159	250	125	4	Hard Eggs	Nothing	Nothing	Nothing
986088	M	Control	67	200	98	3	Milt			
986133	F	Low	135	238	120	4	Nothing	Nothing	Nothing	Nothing
986165	M	Control	64	205	97	6	Milt			
986215	M	High	192	265	130	6	Nothing	Nothing	Nothing	Nothing
986439	M	High	87	220	100	3	Nothing	Nothing	Nothing	Nothing
986469	M	Low	151	247	123	4	Milt			
986598	F	Control	105	230	105	3	Nothing	Nothing	Nothing	Nothing
986612	M	High	129	230	120	3	Nothing	Nothing	Milt	
986755	M	Low	211	273	140	10	Nothing	Milt		
986783	M	High	117	240	114	6	Milt			
986895	M	Control	87	210	100	6	Nothing	Nothing	Nothing	Nothing

986949	F	Low	135	242	115	3	Nothing	Hard Eggs	Nothing	Nothing
986958	M	Low	89	218	95	4	Nothing	Nothing	Nothing	Nothing
986967	F	Low	292	298	150	4	Hard Eggs	Nothing	Nothing	Nothing
987125	F	High	70	204	88	10	Nothing	Hard Eggs	Nothing	Nothing
987128	F	Low	230	286	135	3	Nothing	Nothing	Hard Eggs	Hard Eggs
987149	F	Sham	245	272	150	3	Nothing	Nothing	Nothing	Nothing
987177	F	Sham	181	260	130	6	Nothing	Nothing	Nothing	Nothing
987184	F	Sham	191	270	132	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
987210	M	High	104	232	103	3	Nothing	Nothing	Nothing	Nothing
987227	F	High	109	225	110	4	Nothing	Nothing	Nothing	Nothing
987240	F	Control	293	280	158	10	Hard Eggs	Nothing	Nothing	Nothing
987253	F	Low	110	235	115	10	Nothing	Nothing	Nothing	Nothing
987340	M	High	119	235	117	4	Nothing	Nothing	Nothing	Nothing
987387	F	Control	167	259	124	4	Nothing	Nothing	Nothing	Nothing
987392	F	Sham	304	300	165	3	Nothing	Nothing	Nothing	Nothing
987456	F	Low	165	264	122	4	Hard Eggs	Nothing	Nothing	Nothing
987498	F	High	175	160	130	4	Nothing	Nothing	Nothing	Nothing
987537	F	Low	215	260	140	3	Hard Eggs	Nothing	Hard Eggs	Hard Eggs
987596	M	Control	223	265	145	4	Nothing	Nothing	Nothing	Nothing
987605	M	Low	152	266	128	6	Nothing	Nothing	Nothing	Nothing
987634	F	High	228	270	141	3	Nothing	Nothing	Nothing	Nothing
987707	F	High	84	215	91	6	Nothing	Nothing	Nothing	Nothing
987713	F	Sham	95	214	105	4	Nothing	Hard Eggs	Nothing	Nothing
987758	M	Control	130	245	111	6	Nothing	Nothing	Nothing	Nothing
987779	F	Control	355	315	160	3	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
987789	F	Sham	106	225	105	10	Nothing	Nothing	Nothing	Nothing
987893	M	Sham	127	255	111	10	Nothing	Nothing	Nothing	Nothing

988041	M	Sham	98	230	100	4	Nothing	Nothing	Nothing	Nothing
988084	M	Sham	78	210	94	6	Milt			
988095	F	Control	99	220	107	6	Hard Eggs	Nothing	Nothing	Nothing
988099	F	Sham	133	265	114	6	Nothing	Nothing	Nothing	Nothing
988109	M	Low	98	220	105	10	Nothing	Nothing	Nothing	Nothing
988136	M	Sham	158	249	120	3	Nothing	Nothing	Nothing	Nothing
988246	F	Low	161	255	130	10	Hard Eggs	Hard Eggs	Nothing	Nothing
988269	F	High	104	220	111	10	Hard Eggs	Nothing	Nothing	Nothing
988301	F	Low	87	217	100	3	Hard Eggs	Hard Eggs	FF Eggs	
988374	M	Low	120	240	118	6	Nothing	Nothing	Nothing	Nothing
988526	F	Sham	427	330	185	3	Hard Eggs	Hard Eggs	Nothing	Nothing
988537	F	Control	205	260	140	4	Hard Eggs	Hard Eggs	Nothing	Nothing
988550	F	High	207	271	136	3	Hard Eggs	Hard Eggs	Nothing	Nothing
988601	F	Low	257	285	145	3	Nothing	Nothing	Nothing	Nothing
988678	F	Low	239	275	140	10	Hard Eggs	Hard Eggs	Nothing	Nothing
988711	F	Control	228	275	142	6	Hard Eggs	Nothing	Nothing	Nothing
988718	F	Low	192	265	130	4	FF Eggs			
988734	F	Control	177	248	126	4	Hard Eggs	Nothing	Nothing	Nothing
988912	F	Control	105	232	103	4	Nothing	Nothing	Nothing	Nothing
988919	F	High	79	216	92	6	Nothing	Nothing	Nothing	Nothing
989009	F	Sham	259	285	145	3	Hard Eggs	Hard Eggs	Nothing	Nothing
989032	F	Low	119	235	109	4	Nothing	Nothing	Nothing	Nothing
989044	F	Control	165	249	124	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
989055	M	Sham	145	245	120	4	Nothing	Nothing	Nothing	Nothing
989056	F	Control	406	315	175	4	Hard Eggs			
989085	F	Control	99	222	101	6	Hard Eggs			
989098	F	Control	101	240	106	10	Nothing	Nothing	Nothing	Nothing

989123	F	Sham	129	240	115	6	Hard Eggs	Hard Eggs	Nothing	Nothing
989131	M	Low	83	210	96	6	Milt			
989178	F	Sham	177	259	128	10	Hard Eggs	Nothing	Hard Eggs	Hard Eggs
989196	F	Control	89	213	101	4	Nothing	Nothing	Nothing	Nothing
989201	F	High	192	260	133	4	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
989285	F	Control	107	235	111	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
989330	F	Control	280	285	155	4	Nothing	Nothing	Nothing	Nothing
989341	M	Sham	149	230	120	6	Nothing	Nothing	Nothing	Nothing
989382	F	Low	154	252	120	3	Nothing	Nothing	Nothing	Nothing
989415	F	Sham	429	320	185	4	Hard Eggs	Nothing	Nothing	Nothing
989555	F	Low	182	275	125	10	Hard Eggs	Hard Eggs	Nothing	Nothing
989637	F	Sham	244	281	140	4	Hard Eggs	Hard Eggs	Nothing	Nothing
989690	F	Sham	325	289	163	4	Hard Eggs			
989725	M	Control	75	204	91	10	Nothing	Nothing	Nothing	Nothing
989792	NA	Low	112	228	120	6	Nothing	Nothing	Nothing	Nothing
989822	F	High	184	250	135	4	Hard Eggs	Hard Eggs	Nothing	Nothing
989892	F	Sham	197	271	134	6	Nothing	Nothing	Hard Eggs	Hard Eggs
989916	F	High	359	298	170	4	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
990007	M	High	86	229	94	3	Nothing	Nothing	Nothing	Nothing
990034	F	Sham	177	273	125	10	hard	Hard Eggs	Nothing	Nothing
990204	F	Sham	196	260	133	3	Hard Eggs	Hard Eggs	Nothing	Nothing
990347	F	Low	92	215	105	6	Nothing	Nothing	Nothing	Nothing
990352	M	Sham	125	235	115	4	Nothing	Nothing	Nothing	Nothing
990375	M	Sham	81	220	113	10	Nothing	Nothing	Nothing	Nothing
990388	F	High	79	210	95	10	Nothing	Nothing	Nothing	Nothing
990640	F	Control	105	225	105	3	Nothing	Hard Eggs	Nothing	Nothing
990651	F	High	85	200	98	4	Hard Eggs	Nothing	Nothing	Nothing

990692	M	Low	92	220	100	3	Nothing	Nothing	Nothing	Nothing
990711	F	Control	299	283	160	3	Nothing	Nothing	Nothing	Nothing
990884	F	High	165	250	135	4	Hard Eggs	Hard Eggs	Nothing	Nothing
990909	F	High	173	255	145	3	Hard Eggs	Nothing	Hard Eggs	Hard Eggs
990928	F	High	149	235	122	6	Nothing	Nothing	Nothing	Nothing
990935	F	Sham	313	285	164	3	Nothing	Nothing	FF Eggs	
990980	F	Low	207	263	147	3	Nothing	Hard Eggs	Nothing	Nothing
991002	F	Sham	226	273	145	6	Hard Eggs	Nothing	Nothing	Nothing
991047	F	Low	75	205	100	6	Nothing	Nothing	Nothing	Nothing
991084	F	Low	171	250	130	6	Nothing	Nothing	Nothing	Nothing
991150	M	Control	85	205	102	6	Milt			
991156	F	Low	86	222	94	10	Nothing	Nothing	Nothing	Nothing
991157	M	Sham	113	235	118	10	Nothing	Nothing	Nothing	Nothing
991164	F	High	175	24	135	3	FF Eggs			
991198	F	Low	168	245	128	4	Nothing	Nothing	Nothing	Nothing
991229	F	Sham	131	235	119	6	Hard Eggs			
991351	F	Sham	228	260	145	4	Nothing	Nothing	Nothing	Nothing
991391	M	Sham	133	225	120	4	Nothing	Nothing	Nothing	Nothing
991448	F	Control	172	255	130	6	Hard Eggs	Nothing	Nothing	Nothing
991468	F	Sham	246	278	145	4	Nothing	Nothing	Nothing	Nothing
991516	F	High	188	260	135	6	hard	Nothing	Nothing	Nothing
991542	M	Low	145	235	120	6	Nothing	Nothing	Nothing	Nothing
991548	F	Low	346	305	165	6	freeflowing			
991585	F	High	197	260	131	4	Nothing	Nothing	Nothing	Nothing
991615	F	Low	145	245	120	3	Hard Eggs	Hard Eggs	Nothing	Nothing
991617	F	Low	171	268	121	10	Hard Eggs	Nothing	Nothing	Nothing
991676	F	Sham	147	245	123	4	Hard Eggs	Hard Eggs	FF Eggs	

991682	F	Low	128	225	115	10	Nothing	Nothing	Nothing	Nothing
991743	M	Control	239	275	140	3	Milt			
991755	M	Sham	146	240	125	3	Nothing	Nothing	Nothing	Nothing
991850	F	Low	98	210	105	3	Hard Eggs	Hard Eggs	Nothing	Nothing
991894	F	Control	106	225	108	3	Hard Eggs	Nothing	Nothing	Nothing
991929	M	Control	119	230	110	6	Nothing	Nothing	Milt	
991952	F	Low	179	253	136	6	Nothing	FF Eggs		
992014	F	High	156	242	20	3	Nothing	Nothing	Nothing	Nothing
992085	F	Control	217	270	134	10	Nothing	Nothing	Nothing	Nothing
992093	F	Control	151	250	120	6	Hard Eggs	Hard Eggs	Nothing	Nothing
992102	F	High	144	239	120	3	Hard Eggs	FF Eggs		
992258	F	High	162	259	121	10	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
992324	M	Control	154	245	121	6	Nothing	Nothing	Nothing	Nothing
992344	M	Control	78	205	100	10	Nothing	Nothing	Milt	
992370	F	Low	128	239	122	10	Nothing	Hard Eggs	Nothing	Nothing
992384	F	Sham	300	300	150	3	Nothing	Nothing	Nothing	Nothing
992412	F	Control	160	262	122	10	Hard Eggs			
992483	M	Low	100	225	106	10	Nothing	Nothing	Nothing	Nothing
992494	M	Control	162	249	127	3	Nothing	Nothing	Nothing	Nothing
992541	F	Sham	78	213	93	6	Nothing	Nothing	Nothing	Nothing
992883	F	Control	112	224	110	4	Nothing	Nothing	Nothing	Nothing
993229	F	Control	223	275	140	4	Nothing	Nothing	Nothing	Nothing
994587	M	Sham	127	240	115	6	Nothing	Nothing	Nothing	Nothing

Figure A.6

							Gamete Expression Check Date and Result			
Pit-Tag ID	**Sex Estimate**	**Treatment**	**Mass (g)**	**Length (mm)**	**Girth (mm)**	**From Tank:**	**Jan 25-26**	**Feb 8-9**	**Feb 17-18**	**Feb 24**
001199	F	Low	121	240	125	8	Nothing	Nothing	Nothing	Nothing
002199	F	Control	176	250	150	11	Nothing	Nothing	Nothing	Nothing
009561	F	Low	112	230	120	8	Nothing	Nothing	Nothing	Nothing
009647	F	Sham	106	220	125	8	Nothing	Nothing	Nothing	Nothing
010449	F	Low	144	255	130	11	Hard Eggs	FF Eggs		
010512	F	High	103	225	120	8	Nothing	Nothing	Nothing	Nothing
011798	F	Sham	127	240	125	10	Nothing	Nothing	Nothing	Nothing
016009	F	Sham	57	190	95	8	Nothing	Nothing	Nothing	Nothing
020573	M	Control	54	190	100	8	Nothing	Nothing	Nothing	Nothing
020726	M	Low	53	195	90	11	Nothing	Nothing	Nothing	Nothing
021123	M	Sham	83	220	105	8	Nothing	Nothing	Nothing	Nothing
021187	F	Control	101	220	120	8	Nothing	Nothing	Nothing	Nothing
021269	F	Sham	133	230	125	8	Nothing	Nothing	Nothing	Nothing
021568	M	Control	247	275	165	11	Nothing	Nothing	Nothing	Nothing
021573	F	Control	163	255	135	11	Nothing	FF Eggs		
022246	F	Low	188	265	135	8	Nothing	FF Eggs		
023096	F	Low	150	240	145	10	Nothing	FF Eggs		
023101	M	High	68	205	105	11	Nothing	Nothing	Nothing	Nothing
023116	M	Sham	131	240	130	8	Nothing	Nothing	Nothing	Nothing
024507	M	Control	120	240	125	8	Nothing	Nothing	Nothing	Nothing
025551	F	Control	188	270	140	11	Nothing	FF Eggs		

029100	F	Sham	147	255	130	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
029400	M	Control	70	205	105	11	Nothing	Nothing	Nothing	Nothing
029515	M	Control	94	220	110	8	Nothing	Nothing	Nothing	Nothing
030168	F	Low	143	255	130	10	Nothing	Nothing	Nothing	Nothing
033542	M	Sham	144	250	130	11	Nothing	Nothing	Nothing	Nothing
033604	F	High	138	240	130	8	Nothing	Nothing	Nothing	Nothing
034493	F	Sham	94	225	115	8	Nothing	Nothing	Nothing	Nothing
034844	M	Control	82	210	110	11	Nothing	Nothing	Nothing	Nothing
035146	F	Sham	169	255	140	11	Nothing	Nothing	Nothing	Nothing
035502	M	High	98	220	120	11	Nothing	Nothing	Nothing	Nothing
035731	F	High	147	255	130	11	Hard Eggs	FF Eggs		
035895	F	Sham	227	280	155	11	Nothing	Nothing	Nothing	Nothing
036116	F	Low	124	230	130	8	Nothing	FF Eggs		
036155	M	High	177	265	140	8	Nothing	Nothing	Nothing	Nothing
036239	F	Control	133	235	130	8	Nothing	Nothing	Nothing	Nothing
039707	M	Control	80	220	105	8	Nothing	Nothing	Nothing	Nothing
041985	F	Low	345	310	180	8	Nothing	FF Eggs		
042245	F	Control	159	255	135	8	Nothing	Nothing	Nothing	Nothing
043127	F	High	271	300	170	7	Nothing	FF Eggs		
043567	M	Sham	56	190	95	8	Nothing	Nothing	Nothing	Nothing
043642	F	Control	196	275	145	8	Nothing	Nothing	Nothing	Nothing
044004	M	Low	197	265	150	11	Nothing	Nothing		
045256	F	Sham	83	225	110	8	Nothing	Hard Eggs		
045323	F	Low	112	225	125	11	Nothing	Nothing	Nothing	Nothing
045428	F	Control	128	235	130	8	Nothing	FF Eggs		
045439	M	Control	87	205	115	11	Nothing	Nothing	Nothing	Nothing
045687	F	Sham	157	250	140	11	Nothing	FF Eggs		

046019	M	Low	116	225	125	11	Nothing	Nothing	Nothing	Nothing
046062	F	Low	111	240	120	8	Nothing	FF Eggs		
047363	M	High	124	240	125	8	Milt			
047547	M	Sham	207	270	150	11	Milt			
047578	M	Sham	91	220	110	8	Nothing	Nothing	Nothing	Nothing
047804	M	Low	134	245	130	7	Nothing	Nothing	Nothing	Nothing
047825	F	Control	78	205	110	11	Nothing	Nothing	Nothing	Nothing
047949	M	High	83	210	110	8	Milt			
048168	M	High	170	260	140	10	Nothing	Nothing	Nothing	Nothing
054166	F	Control	150	260	135	11	Nothing	FF Eggs		
054296	M	Sham	83	215	110	8	Nothing	Nothing	Nothing	Nothing
054751	M	High	60	200	100	8	Nothing	Nothing	Nothing	Nothing
054843	M	High	110	240	120	11	Nothing	Nothing	Nothing	Nothing
054844	F	Control	111	220	125	11	Nothing	Nothing	Nothing	Nothing
054948	F	Sham	72	205	105	11	Nothing	Nothing	Nothing	Nothing
055022	M	High	262	280	170	10	Nothing	Nothing	Nothing	Nothing
055045	F	High	272	295	170	8	Nothing	FF Eggs		
055210	F	Sham	128	235	130	8	Nothing	Nothing	Nothing	Nothing
055214	F	Control	139	245	135	10	Nothing	Nothing	Nothing	Nothing
055261	F	Low	170	260	145	11	Nothing	FF Eggs		
055460	M	Sham	192	265	150	11	Nothing	Nothing	Nothing	Nothing
055545	M	Sham	88	225	110	11	Nothing	Nothing	Nothing	Nothing
055673	M	High	68	200	105	11	Nothing	Nothing	Nothing	Nothing
057189	F	Sham	104	230	120	8	Nothing	Nothing	Nothing	Nothing
059041	M	Low	101	220	115	8	Nothing	Nothing	Nothing	Nothing
059316	F	Control	143	230	140	8	FF Eggs			
059635	F	High	141	250	130	8	Nothing	Nothing	Nothing	Nothing

060044	F	Control	85	220	110	11	Nothing	Nothing	Nothing	Nothing
060196	M	Low	100	225	120	8	Nothing	Nothing	Nothing	Nothing
060479	F	High	250	285	160	11	Nothing	Nothing	Nothing	Nothing
065470	F	High	62	210	100	7	Nothing	Nothing	Nothing	Nothing
065567	F	High	155	255	130	8	FF Eggs			
067355	F	High	209	285	150	11	Nothing	Nothing	Nothing	Nothing
067412	F	Low	130	235	130	11	Hard Eggs	FF Eggs		
067834	F	Low	153	255	130	8	Nothing	Nothing	Nothing	Nothing
069058	F	Sham	196	270	150	8	Nothing	Nothing	Nothing	FF Eggs
069152	F	Sham	144	235	140	8	Nothing	Nothing	Nothing	Nothing
069563	F	Control	89	230	110	11	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
069749	F	Sham	271	290	165	8	Nothing	Nothing	Nothing	Nothing
070075	F	High	225	275	160	8	Nothing	Nothing	Nothing	Nothing
070469	F	Low	139	235	135	8	Nothing	FF Eggs		
072510	F	High	270	280	175	10	Nothing	Nothing	Nothing	Nothing
073134	M	Sham	67	215	110	11	Nothing	Nothing	Nothing	Nothing
073419	F	Low	53	200	100	11	Nothing	Nothing	Nothing	FF Eggs
073801	F	Control	99	220	120	8	Nothing	Nothing	Nothing	Nothing
073906	F	High	207	265	160	11	Nothing	FF Eggs		
074467	M	High	99	230	115	11	Nothing	Nothing	Nothing	Nothing
074567	M	Sham	160	205	95	11	Nothing	Nothing	Nothing	Nothing
074720	M	Control	134	245	130	11	Nothing	Nothing	Nothing	Nothing
074983	F	Control	276	290	180	10	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
075063	M	Control	103	230	115	11	Nothing	Nothing	Nothing	Nothing
075075	M	High	119	225	125	11	Nothing	Nothing	Nothing	Nothing
075081	F	Control	241	275	165	8	Nothing	Nothing	Nothing	Nothing
075295	M	Sham	94	225	110	10	Milt			

075350	M	Control	155	240	135	11	Nothing	Nothing	Nothing	Nothing
075397	F	Sham	231	260	160	11	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
075517	F	Low	148	240	130	11	Nothing	Nothing	Nothing	Nothing
075609	F	High	61	190	100	8	Nothing	Nothing	Nothing	Nothing
076017	F	Sham	86	220	110	11	FF Eggs			
076622	F	Sham	78	215	110	10	Nothing	FF Eggs		
076840	F	High	315	300	180	8	FF Eggs			
077024	F	Low	217	275	150	11	Nothing	Nothing	Nothing	Nothing
077490	M	Control	108	235	120	8	Nothing	Nothing	Nothing	Nothing
078065	F	Low	255	290	165	11	Nothing	FF Eggs		
079045	M	Control		220	110	11	Nothing	Nothing	Nothing	Nothing
079179	M	Low	90	215	110	11	Nothing	Nothing	Nothing	Nothing
079223	F	Control	130	230	135	11	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
079240	F	High	187	270	145	11	Hard Eggs	FF Eggs		
079638	F	Sham	75	210	110	11	Nothing	Nothing	Nothing	Nothing
079852	F	Control	89	210	120	7	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
080033	M	Low	140	235	130	11	Nothing	Nothing	Nothing	Nothing
080272	F	Low	175	240	150	8	Nothing	Nothing	Nothing	Nothing
080432	M	Control	69	210	100	11	Nothing	Nothing	Nothing	Nothing
080465	M	High	140	260	125	11	Nothing	Nothing	Nothing	Nothing
080495	M	Low	124	240	120	8	Nothing	Nothing	Nothing	Nothing
080503	M	High	197	270	150	11	Nothing	Nothing	Nothing	Nothing
080659	F	Low	112	230	120	8	Nothing	Nothing	Nothing	Nothing
080988	F	High	283	290	170	8	Nothing	Hard Eggs		
081399	F	Sham	136	245	130	8	Nothing	Nothing	Nothing	Nothing
081738	M	Sham	114	235	120	8	Nothing	Nothing	Nothing	Nothing
081892	F	High	221	290	150	8	Hard Eggs	FF Eggs		

081907	F	High	173	265	140	10	Hard Eggs	FF Eggs		
082405	F	Low	210	275	155	11	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
082963	F	Low	90	215	115	11	Nothing	Nothing	Nothing	Nothing
085335	F	High	115	230	120	11	Nothing	Nothing	Nothing	Nothing
092521	F	Low	105	235	115	10	FF Eggs			
092607	F	High	140	250	130	11	Nothing	Nothing	Nothing	Nothing
097785	F	Sham	129	240	130	10	Nothing	Nothing	Nothing	Nothing
098087	M	Low	92	215	110	11	Nothing	Nothing	Nothing	Nothing
098262	F	High	213	270	155	11	Nothing	FF Eggs		
098271	M	High	77	210	105	8	Nothing	Nothing	Nothing	Nothing
098740	F	Low	162	250	140	8	Nothing	Nothing	Nothing	Nothing
098909	F	High	120	245	125	8	Nothing	Nothing	Nothing	Nothing
099256	M	Low	82	210	105	11	Nothing	Nothing	Nothing	Nothing
099319	F	Sham	89	210	115	11	Nothing	Nothing	Nothing	Nothing
099592	F	Control	131	260	125	8	Nothing	Nothing	Nothing	Nothing
099909	F	Low	213	255	155	11	FF Eggs			
100014	M	Control	187	255	140	8	Nothing	Nothing	Nothing	Nothing
100032	M	Control	115	240	120	11	Nothing	Nothing	Nothing	Nothing
100055	F	Low	194	265	150	11	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
100303	F	Low	74	205	110	8	Nothing	Nothing	Nothing	Nothing
100350	F	High	132	235	130	11	FF Eggs			
101153	F	Low	107	220	120	10	FF Eggs			
101264	F	Low	147	245	140	11	Nothing	Nothing	Nothing	Nothing
101469	F	Low	187	260	165	11	Nothing	Nothing	Nothing	Nothing
101594	F	Control	179	250	145	11	Nothing	FF Eggs		
102071	F	Sham	235	275	165	8	Nothing	Nothing	Nothing	Nothing
103170	F	Control	135	235	130	10	Nothing	Nothing	Nothing	Nothing

103287	F	Low	141	245	130	11	Nothing	Nothing	Nothing	Nothing
103306	F	High	166	265	125	11	Nothing	Nothing	Nothing	Nothing
103542	F	Low	180	265	145	8	FF Eggs			
107691	F	Control	93	230	110	8	Nothing	Nothing	Nothing	Nothing
108210	M	Low	213	270	155	11	Nothing	Nothing	Nothing	Nothing
108803	F	Low	156	265	135	11	Nothing	FF Eggs		
108805	F	High	138	250	130	8	Nothing	Nothing	Nothing	Nothing
109454	F	Low	95	215	115	11	Nothing	Nothing	Nothing	Nothing
109513	F	Sham	125	235	125	8	Nothing	Nothing	Nothing	Nothing
109712	F	High	291	290	175	10	Hard Eggs	FF Eggs		
110120	F	Control	201	275	150	10	Nothing	Nothing	Nothing	Nothing
111130	M	Low	98	225	115	11	Nothing	Nothing	Nothing	Nothing
111504	F	High	93	220	115	8	Nothing	Nothing	Nothing	Nothing
112342	M	Control	83	215	100	11	Nothing	Nothing	Nothing	Nothing
112454	M	Control	100	230	120	11	Nothing	Nothing	Nothing	Nothing
115121	F	Control	300	300	170	8	Nothing	Nothing	Nothing	Nothing
116865	F	Low	83	215	105	11	Nothing	Nothing	Nothing	Nothing
117352	M	Control	121	240	125	10	Nothing	Nothing	Nothing	Nothing
117915	F	Control	148	250	135	8	Nothing	FF Eggs		
118187	M	Sham	96	215	120	8	Nothing	Nothing	Nothing	Nothing
118240	F	High	229	275	160	8	Nothing	FF Eggs		
118356	F	High	217	275	150	11	Nothing	FF Eggs		
118678	M	Sham	220	275	150	7	Nothing	Nothing	Nothing	Nothing
119288	F	High	311	305	175	11	Nothing	FF Eggs		
119314	F	Sham	173	250	140	11	Nothing	Nothing	Nothing	Nothing
122201	F	High	124	235	130	10	Nothing	FF Eggs		
123631	M	Control	74	210	95	11	Milt			

125232	F	Sham	95	230	115	8	FF Eggs			
125314	F	Control	260	280	165	10	Nothing	Nothing	Nothing	Nothing
125350	F	Sham	215	260	155	8	Nothing	Nothing	Nothing	Nothing
125498	F	Low	53	195	95	11	Nothing	Nothing	FF Eggs	
125624	M	Low	121	225	125	8	Nothing	Nothing	Nothing	Nothing
125808	M	High	84	210	115	11	Nothing	Nothing	Nothing	Nothing
125970	F	Low	208	275	150	11	FF Eggs			
126347	M	Control	95	220	110	8	Nothing	Nothing	Nothing	Nothing
126656	F	Low	50	225	100	11	Nothing	Nothing	Nothing	Nothing
126674	F	Low	158	255	135	8	FF Eggs			
126977	F	Sham	332	290	180	11	Nothing	Nothing	Nothing	Nothing
126998	F	Sham	153	240	140	8	Nothing	Nothing	Nothing	Nothing
127414	F	High	122	230	125	11	Nothing	FF Eggs		
127644	M	Control	104	230	115	8	Nothing	Nothing	Nothing	Nothing
141235	F	Low	111	240	120	8	Nothing	FF Eggs		
141961	M	Control	187	255	145	11	Nothing	Nothing	Nothing	Nothing
142082	F	Control	116	230	125	8	Nothing	Nothing	Nothing	Nothing
142171	F	High	108	225	120	8	Nothing	Nothing	Nothing	Nothing
142270	M	Sham	95	215	105	11	Nothing	Nothing	Nothing	Nothing
142523	F	Low	170	270	140	10	Nothing	Nothing	Nothing	Nothing
142543	F	Low	148	245	140	8	Nothing	FF Eggs		
142593	F	Low	401	330	190	11	Nothing	FF Eggs		
142800	F	Control	119	225	130	8	Nothing	Nothing	Nothing	Nothing
143027	M	High	101	230	120	8	Nothing	Nothing	Nothing	Nothing
143038	M	Low	90	220	110	10	Nothing	Nothing	Nothing	Nothing
143362	M	Low	56	200	90	11	Nothing	Nothing	Nothing	Nothing
143371	F	Low	123	215	150	11	Nothing	FF Eggs		

143912	F	Control	169	265	140	8	Nothing	Nothing	Nothing	Nothing
143937	F	Control	145	245	130	11	Nothing	FF Eggs		
144086	F	Control	227	275	160	10	Nothing	FF Eggs		
144108	F	Control	112	230	120	8	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
148016	M	High	175	255	145	11	Nothing	Nothing	Nothing	Nothing
148202	M	Control	205	275	150	8	Nothing	Nothing	Nothing	Nothing
148306	F	Sham	174	265	140	11	Nothing	Nothing	Nothing	Nothing
148400	F	Low	84	225	105	8	Nothing	Nothing	Nothing	Nothing
148744	F	Control	117	250	140	11	Nothing	Nothing	Nothing	Nothing
149090	F	High	93	215	120	11	Nothing	Nothing	Nothing	Nothing
149320	M	Sham	93	220	105	11	Nothing	Nothing	Nothing	Nothing
149348	F	Control	316	300	180	11	Hard Eggs	Hard Eggs	FF Eggs	
150364	M	Sham	114	225	125	8	Nothing	Nothing	Nothing	Nothing
150700	F	Sham	197	275	150	10	Nothing	Nothing	Nothing	Nothing
150775	F	High	101	225	120	8	Nothing	Nothing	Nothing	Nothing
150814	F	Sham	243	280	165	11	Nothing	FF Eggs		
164231	F	Sham	62	195	100	8	FF Eggs			
168718	F	High	210	275	150	11	Nothing	FF Eggs		
186006	M	Low	92	215	115	7	Nothing	Nothing	Nothing	Nothing
186201	F	Low	142	235	135	7	Nothing	Nothing	Nothing	Nothing
186998	F	Sham	267	285	170	7	Nothing	Nothing	Nothing	Nothing
187108	F	High	236	285	160	7	Nothing	FF Eggs		
187195	M	Control	115	230	120	7	Nothing	Nothing	Nothing	Nothing
299340	F	Low	108	225	120	10	Nothing	Nothing	Nothing	Nothing
299343	F	Low	243	265	165	11	Nothing	FF Eggs		
299400	M	Sham	204	275	140	11	Nothing	Nothing	Nothing	Nothing
299437	M	High	201	265	140	11	Nothing	Nothing	Nothing	Nothing

299533	F	Low	90	230	110	11	Nothing	Nothing	Nothing	Nothing
299542	F	Low	95	215	115	11	Nothing	Nothing	Nothing	Nothing
299564	M	Low	61	205	95	10	Nothing	Nothing	Nothing	Nothing
299566	F	Sham	102	235	115	10	Nothing	Nothing	Nothing	Nothing
299616	M	High	221	280	150	11	Nothing	Nothing	Nothing	Nothing
299635	F	High	135	255	125	7	Nothing	FF Eggs		
299696	M	Low	111	225	120	11	Milt			
299800	F	Sham	210	275	150	8	Nothing	FF Eggs		
300740	M	Control	119	240	120	10	Nothing	Nothing	Nothing	Nothing
301111	M	Sham	194	260	150	11	Nothing	Nothing	Nothing	Nothing
301801	F	Low	192	230	105	11	Nothing	Nothing	Nothing	Nothing
302449	F	High	391	305	200	8	Nothing	FF Eggs		
302452	F	Sham	287	290	180	8	Nothing	FF Eggs		
303009	M	Control	69	205	105	11	Nothing	Nothing	Nothing	Nothing
303084	M	Sham	113	235	120	8	Nothing	Nothing	Nothing	Nothing
309027	M	Control	108	235	120	11	Nothing	Nothing	Nothing	Nothing
311675	F	High	81	210	110	7	Nothing	Nothing	Nothing	Nothing
313999	M	Control	127	240	125	8	Nothing	Nothing	Nothing	Nothing
316555	M	Control	101	225	120	7	Nothing	Nothing	Nothing	Nothing
316593	F	Sham	60	200	95	11	Nothing	Nothing	Nothing	Nothing
329967	F	Control	268	290	165	11	Nothing	Nothing	Nothing	Nothing
334254	F	Control	161	245	145	7	Nothing	Nothing	Nothing	Nothing
345820	F	High	152	235	145	7	Nothing	Nothing	Nothing	Nothing
346227	M	High	142	245	130	7	Nothing	Nothing	Nothing	Nothing
346304	M	Low	106	235	120	7	Nothing	Nothing	Nothing	Nothing
346895	F	Low	135	245	130	7	Nothing	Nothing	Nothing	Nothing
350687	M	High	151	250	135	7	Nothing	Nothing	Nothing	Nothing

351420	F	Sham	204	260	155	7	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
351870	M	High	152	245	135	11	Nothing	Nothing	Nothing	Nothing
352886	M	Low	56	190	95	7	Nothing	Nothing	Nothing	Nothing
353514	M	Low	153	245	135	7	Nothing	Nothing	Nothing	Nothing
353787	F	Sham	81	215	115	7	Nothing	Nothing	Nothing	Nothing
353866	F	Control	162	260	135	7	Nothing	Nothing	Nothing	Nothing
353955	M	Control	204	270	155	7	Nothing	Nothing	Nothing	Nothing
354306	F	Control	195	260	150	7	Nothing	Hard Eggs	Hard eggs	Hard eggs
354349	M	Control	91	225	110	7	Nothing	Nothing	Nothing	Nothing
354562	F	Sham	61	200	95	7	Nothing	Nothing	Nothing	Nothing
354626	M	High	112	220	120	7	Nothing	Nothing	Nothing	Nothing
361181	M	Control	81	215	110	7	Nothing	Nothing	Nothing	Nothing
363914	F	Sham	236	270	160	7	Nothing	Nothing	Nothing	Nothing
363947	M	Control	178	265	145	7	Nothing	Nothing	Nothing	Nothing
365251	F	Low	220	275	160	7	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
365846	F	Low	197	265	150	7	Hard Eggs	FF Eggs		
366627	M	High	158	250	140	7	Nothing	Nothing	Nothing	Nothing
366756	M	Low	74	215	105	11	Nothing	Nothing	Nothing	Nothing
366821	F	Low	128	235	130	8	Nothing	FF Eggs		
366836	F	Control	124	230	130	11	Nothing	FF Eggs		
366894	F	Sham	108	230	230	11	Nothing	Nothing	Nothing	Nothing
366924	M	Low	109	220	125	8	Nothing	Nothing	Nothing	Nothing
367019	M	High	125	235	125	8	Nothing	Nothing	Nothing	Nothing
367061	F	Sham	167	265	140	10	Nothing	FF Eggs		
367257	F	Control	112	220	125	11	Nothing	Nothing	Nothing	Nothing
367300	F	Control	163	255	140	11	Hard Eggs	FF Eggs		
367438	M	Control	182	255	150	8	Nothing	Nothing	Nothing	Nothing

367500	M	High	122	225	120	8	Nothing	Nothing	Nothing	Nothing
367532	F	Low	145	250	130	10	FF Eggs			
367544	M	Control	235	290	150	11	Nothing	Nothing	Nothing	Nothing
367635	M	Control	91	220	110	8	Nothing	Nothing	Nothing	Nothing
367663	F	Control	108	225	125	8	Nothing	Nothing	Nothing	Nothing
367691	F	Sham	240	280	160	8	Nothing	Nothing	Nothing	Nothing
367714	M	Control	92	225	110	11	Nothing	Nothing	Nothing	Nothing
367765	F	Sham	324	305	180	7	Nothing	Nothing	Nothing	Nothing
367769	F	High	67	205	100	11	Nothing	Nothing	Nothing	Nothing
367889	M	Control	127	240	125	8	Nothing	Nothing	Nothing	Nothing
367956	F	Control	107	220	120	8	Nothing	Nothing	Nothing	Nothing
368003	M	Sham	108	235	110	11	Nothing	Nothing	Nothing	Nothing
368022	F	High	286	290	170	8	Nothing	FF Eggs		
368091	F	Control	125	240	125	8	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
368358	M	Sham	72	195	105	11	Nothing	Nothing	Nothing	Nothing
368689	F	Control	180	265	145	11	Nothing	Nothing	Nothing	Nothing
368692	F	High	140	250	130	8	Hard Eggs	FF Eggs		
368759	M	Low	136	250	125	11	Nothing	Nothing	Nothing	Nothing
368802	M	Sham	130	245	120	11	Nothing	Nothing	Nothing	Nothing
368915	F	High	219	270	160	11	Hard Eggs	FF Eggs		
368955	M	Low	135	250	125	11	Nothing	Nothing	Nothing	Nothing
368999	M	Control	111	225	120	11	Nothing	Nothing	Nothing	Nothing
369161	F	Control	169	215	145	10	Nothing	Nothing	Nothing	Nothing
369172	M	High	75	205	110	11	Nothing	Nothing	Nothing	Nothing
369174	F	High	130	240	130	11	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
369274	M	High	86	220	110	7	Nothing	Nothing	Nothing	Nothing
369289	M	Sham	230	270	155	11	Nothing	Nothing	Nothing	Nothing

369343	F	High	149	240	130	11	Nothing	FF Eggs		
369372	M	Sham	106	225	125	11	Nothing	Nothing	Nothing	Nothing
369486	F	Control	124	240	120	11	Nothing	Nothing	Nothing	Nothing
369519	F	Low	136	260	130	8	Nothing	Nothing	Nothing	Nothing
369538	M	Low	141	240	135	8	Nothing	Nothing	Nothing	Nothing
369561	F	High	204	265	150	11	FF Eggs			
369666	M	Control	127	235	125	8	Nothing	Nothing	Nothing	Nothing
369707	M	Low	110	225	120	8	Nothing	Nothing	Nothing	Nothing
369775	M	Sham	119	235	125	8	Nothing	Nothing	Nothing	Nothing
369798	F	Low	237	290	160	8	Nothing	Nothing	Nothing	Nothing
369869	F	Control	151	260	135	8	Nothing	Nothing	Hard eggs	FF Eggs
370015	F	Sham	141	250	130	8	Nothing	Nothing	Nothing	Nothing
370045	F	Sham	118	245	120	10	Nothing	Nothing	Nothing	Nothing
370124	F	Control	331	320	180	11	Hard Eggs	FF Eggs		
370136	F	Low	105	230	120	11	Nothing	Nothing	FF Eggs	
370251	M	Low	102	225	115	7	Nothing	Nothing	Nothing	Nothing
370287	F	Low	70	295	105	8	Nothing	Nothing	Nothing	Nothing
370409	M	High	83	210	105	10	Nothing	Nothing	Nothing	Nothing
370442	M	Control	71	210	105	11	Nothing	Nothing	Nothing	Nothing
370598	F	Control	195	265	155	11	Nothing	Nothing	FF Eggs	
370699	F	High	242	275	160	11	Hard Eggs	FF Eggs		
370706	F	High	106	235	115	11	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
370875	F	Low	242	280	165	8	Nothing	FF Eggs		
370903	F	Control	179	265	145	8	Nothing	FF Eggs		
371023	F	Low	267	290	170	8	Nothing	Nothing	Nothing	Nothing
371115	F	High	106	225	125	10	Nothing	FF Eggs		
371167	M	Sham	220	270	155	7	Nothing	Nothing	Nothing	Nothing

371225	M	High	110	230	120	11	Nothing	Nothing	Nothing	Nothing
371382	F	Low	100	225	120	8	Nothing	FF Eggs		
371442	F	Sham	72	195	105	11	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
371524	M	Low	174	265	140	8	Nothing	Nothing	Nothing	Nothing
371629	M	Sham	88	230	110	8	Nothing	Nothing	Nothing	Nothing
371714	F	Sham	116	230	125	7	Nothing	Nothing	Nothing	Nothing
371735	F	Sham	101	230	120	8	Nothing	FF Eggs		
371748	F	Control	68	205	100	8	Nothing	Nothing	Nothing	Nothing
371777	F	Sham	151	250	135	10	Nothing	Nothing	Nothing	Nothing
371938	M	Low	88	220	110	11	Nothing	Nothing	Nothing	Nothing
371974	F	Low	233	275	170	8	Nothing	Hard Eggs	FF Eggs	
372089	F	Control	168	250	130	11	Nothing	Nothing	Nothing	Nothing
372099	F	Control	101	230	120	11	Nothing	Nothing	FF Eggs	
372114	F	Low	63	185	105	8	Nothing	Nothing		
372160	M	Low	160	260	135	8	Nothing	Nothing		
372184	F	Sham	96	220	120	11	Nothing	Nothing		
372193	M	Sham	89	215	110	8	Nothing	Nothing		
372242	F	High	135	235	135	8	Nothing	Nothing		
372274	F	Low	196	255	155	11	Nothing	FF Eggs		
372398	F	High	157	255	140	8	Nothing	Nothing	Nothing	Nothing
372412	M	High	174	250	140	11	Nothing	Nothing	Nothing	Nothing
372480	F	High	56	190	95	8	Nothing	Nothing	Nothing	Nothing
372504	M	Sham	95	225	115	8	Nothing	Nothing	Nothing	Nothing
372543	F	High	201	255	150	11	Nothing	Nothing	Nothing	Nothing
372618	F	High	289	310	170	8	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
372725	F	Control	127	235	125	8	Nothing	Nothing	Nothing	Nothing
372770	F	Sham	145	255	135	11	Nothing	Nothing	FF Eggs	

372779	F	Control	205	280	145	11	Nothing	FF Eggs		
372834	F	Control	212	280	155	8	Nothing	Hard Eggs	Hard eggs	FF Eggs
372836	M	Low	123	230	130	8	Nothing	Nothing	Nothing	Nothing
372858	M	Low	71	210	110	8	Nothing	Nothing	Nothing	Nothing
372915	M	Sham	66	205	100	10	Nothing	Nothing	Nothing	Nothing
372923	F	Sham	107	230	120	8	Nothing	FF Eggs		
372976	F	Control	75	210	105	10	Nothing	Nothing	Nothing	Nothing
373048	M	Control	88	220	110	7	Nothing	Nothing	Nothing	Nothing
373057	F	High	195	260	155	11	Nothing	Nothing	Nothing	Nothing
373129	M	Control	107	245	120	10	Nothing	Nothing	Nothing	Nothing
373193	F	Low	266	270	170	8	Nothing	Nothing	Nothing	Nothing
373795	F	Control	216	275	150	7	Hard Eggs	Nothing	Nothing	Nothing
375883	F	Sham	74	200	105	7	Nothing	FF Eggs		
375955	F	Control	272	300	165	7	FF Eggs			
377570	M	Low	154	255	140	7	Nothing	Nothing	Nothing	Nothing
379866	M	Sham	75	210	105	7	Milt			
381787	M	Sham	125	240	125	11	Nothing	Nothing	Nothing	Nothing
382492	F	Sham	151	245	135	7	Nothing	Nothing	Hard eggs	
383163	F	High	217	265	155	7	Nothing	FF Eggs		
390934	M	Sham	145	240	130	11	Nothing	Nothing	Nothing	Nothing
392544	F	High	121	245	125	7	Hard Eggs	FF Eggs		
392640	M	Sham	217	270	165	7	Nothing	Nothing	Nothing	Nothing
434059	F	Control	228	275	160	8	Nothing	Nothing	Nothing	Nothing
454695	F	Control	242	290	155	11	Nothing	FF Eggs		
459933	M	Low	128	240	125	7	Nothing	Nothing	Nothing	Nothing
472998	F	Sham	196	270	140	11	Nothing	Nothing	Nothing	Nothing
521715	F	High	120	230	125	11	Nothing	FF Eggs		

574939	M	Sham	133	240	130	8	Nothing	Nothing	Nothing	Nothing
575541	M	Sham	97	235	115	8	Nothing	Nothing	Nothing	Nothing
576860	M	Low	131	250	130	8	Nothing	Nothing	Nothing	Nothing
577973	F	High	183	265	145	8	Nothing	Nothing	Nothing	Nothing
580943	M	High	114	230	130	8	Nothing	Nothing	Nothing	Nothing
581213	M	Sham	177	255	145	11	Nothing	Nothing	Nothing	Nothing
588802	F	Low	83	215	110	8	Nothing	Nothing	Nothing	Nothing
599361	F	Low	174	270	135	7	Nothing	Nothing	Nothing	Nothing
601067	F	Sham	280	300	170	11	Nothing	Nothing	Nothing	Nothing
604759	F	Low	233	285	160	8	FF Eggs			
611292	M	Control	133	245	120	11	Nothing	Nothing	Nothing	Nothing
612802	F	Sham	152	250	135	11	FF Eggs			
619045	F	Sham	107	230	120	8	FF Eggs			
620667	F	High	129	245	125	8	Nothing	Nothing	Nothing	Nothing
629940	F	Sham	101	230	120	11	Nothing	Nothing	Nothing	Nothing
633335	M	Control	96	235	110	11	Nothing	Nothing	Nothing	Nothing
642849	M	Low	172	255	145	8	Milt			
656266	M	High	180	255	145	8	Nothing	Nothing	Nothing	Nothing
656962	F	Control	131	240	130	8	Nothing	Nothing	Nothing	Nothing
657718	F	Control	88	220	110	8	Nothing	Nothing	Nothing	Nothing
662134	F	High	130	245	130	11	Hard Eggs	FF Eggs		
665113	F	Sham	177	265	140	8	Nothing	Nothing	Nothing	Nothing
677540	F	Sham	91	215	105	8	Nothing	Nothing	Nothing	Nothing
714514	F	Sham	187	270	150	8	Hard Eggs	Nothing	Nothing	Nothing
714585	M	Sham	130	240	130	8	Nothing	Nothing	Nothing	Nothing
722746	M	Control	158	250	140	8	Nothing	Nothing	Nothing	Nothing
722748	F	High	215	265	155	11	Nothing	FF Eggs		

727752	F	Control	68	205	100	8	Nothing	Nothing	Nothing	Nothing
751698	F	Low	135	240	130	8	FF Eggs			
753815	M	Low	178	260	145	11	Milt			
755292	M	High	64	205	100	11	Nothing	Nothing	Nothing	Nothing
757706	F	High	298	280	180	8	FF Eggs			
765717	M	Control	108	240	115	10	Milt			
765965	M	High	105	225	120	11	Nothing	Nothing	Nothing	Nothing
768396	F	Control	177	255	150	8	Nothing	FF Eggs		
771636	F	Low	212	275	155	8	Nothing	FF Eggs		
772317	F	Low	123	230	130	11	Nothing	Nothing	Nothing	Nothing
779390	M	High	103	225	120	8	Nothing	Nothing	Nothing	Nothing
780422	F	High	161	255	140	11	Nothing	FF Eggs		
783531	F	Control	182	240	155	11	Nothing	Nothing	Nothing	Nothing
796473	M	Sham	205	275	145	11	Nothing	Nothing	Nothing	Nothing
798599	F	Control	132	245	125	11	Nothing	Nothing	Nothing	Nothing
805140	F	Control	151	240	135	8	Nothing	Nothing	Nothing	Nothing
811531	F	Low	67	200	105	8	FF Eggs			
815716	F	High	107	220	120	11	Nothing	FF Eggs		
819036	M	High	169	255	140	8	Nothing	Nothing	Nothing	Nothing
824238	F	High	111	230	120	11	Hard Eggs	FF Eggs		
878371	F	Low	193	265	150	11	Nothing	FF Eggs		
887005	M	High	118	240	125	8	Nothing	Nothing	Nothing	Nothing
904621	M	Control	132	245	120	11	Nothing	Nothing	Nothing	Nothing
914911	F	Low	163	250	130	11	Nothing	Nothing	Nothing	Nothing
916492	F	Low	187	265	145	8	Nothing	FF Eggs		
930201	M	High	193	265	145	11	Nothing	Nothing	Nothing	Nothing
930672	F	High	116	240	120	8	Nothing	FF Eggs		

945957	M	Sham	77	215	110	11	Nothing	Nothing	Nothing	Nothing
952004	F	Sham	139	235	130	10	Nothing	Nothing	Nothing	Nothing
955492	M	Control	140	250	130	8	Nothing	Nothing	Nothing	Nothing
955829	M	High	90	225	110	8	Nothing	Milt		
959156	M	High	82	225	95	11	Nothing	Nothing	Nothing	Nothing
961442	F	Sham	259	290	165	11	Nothing	Nothing	Nothing	Nothing
967123	M	Sham	53	185	90	11	Nothing	Nothing	Nothing	Nothing
968096	M	High	76	210	110	8	Nothing	Nothing	Nothing	Nothing
976593	F	High	167	250	140	8	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
978497	F	Low	125	235	110	10	Nothing	Nothing	FF Eggs	
978656	M	Low	173	265	135	11	Milt			
978687	F	Sham	82	225	110	11	Hard Eggs	FF Eggs		
979387	M	Sham	211	270	150	11	Nothing	Nothing	Nothing	Nothing
979656	M	Control	168	260	140	8	Nothing	Nothing	Nothing	Nothing
980093	M	Control	131	240	130	8	Nothing	Nothing	Nothing	Nothing
980224	F	Control	205	265	140	11	Nothing	Nothing	Nothing	Nothing
980658	F	Low	157	260	140	8	Nothing	Nothing	Nothing	Nothing
982391	M	Low	161	265	140	8	Nothing	Nothing	Nothing	Nothing
982441	M	High	167	260	135	8	Milt			
982518	M	High	123	235	125	7	Nothing	Nothing	Nothing	Nothing
982522	F	Sham	94	225	115	8	Nothing	Hard Eggs	Hard eggs	Hard eggs
982542	F	Low	180	255	145	11	Nothing	Nothing	Nothing	Nothing
982551	F	Sham	140	250	130	8	FF Eggs			
982615	M	Low	112	240	120	8	Nothing	Nothing	Nothing	Nothing
982736	M	Sham	143	250	130	8	Nothing	Nothing	Nothing	Nothing
982760	M	Control	136	245	245	8	Nothing	Nothing	Nothing	Nothing
982826	F	Control	113	230	125	10	Nothing	Nothing	Nothing	Nothing

982906	F	High	205	275	155	10	Nothing	Nothing	Nothing	Nothing
982946	M	Control	198	265	150	11	Nothing	Nothing	Nothing	Nothing
982948	F	Sham	151	245	140	11	Nothing	Nothing	FF Eggs	
982975	M	Sham	103	230	115	8	Nothing	Nothing	Nothing	Nothing
983006	F	Low	109	230	120	8	Nothing	Nothing	Nothing	Nothing
983069	M	Sham	190	250	145	8	Milt			
983106	F	Sham	164	265	140	8	Nothing	Nothing	Nothing	Nothing
983118	M	High	103	220	120	8	Nothing	Nothing	Nothing	Nothing
983119	F	Sham	176	255	140	11	Nothing	Nothing	Nothing	Nothing
983162	M	Sham	132	250	125	11	Nothing	Nothing	Nothing	Nothing
983183	F	Control	161	260	145	8	Nothing	Nothing	Nothing	Nothing
983185	F	Sham	77	210	105	11	Hard Eggs	Hard Eggs	FF Eggs	
983268	F	Low	110	230	120	8	Nothing	FF Eggs		
983360	M	Sham	213	275	155	7	Nothing	Nothing	Nothing	Nothing
983373	M	Sham	78	210	110	11	Nothing	Nothing	Nothing	Nothing
983391	M	High	56	200	95	11	Milt			
983401	F	Sham	120	240	130	8	Nothing	Nothing	Nothing	Nothing
983409	F	High	136	240	130	11	Hard Eggs	FF Eggs		
983465	M	Sham	157	255	130	8	Nothing	Nothing	Nothing	Nothing
983466	M	Sham	110	230	120	11	Nothing	Nothing	Nothing	Nothing
983596	F	Control	196	265	150	8	Nothing	Nothing	Nothing	Nothing
983603	F	Sham	368	310	190	8	Hard Eggs	Hard Eggs	Hard Eggs	Hard Eggs
983815	M	Sham	231	280	155	11	Nothing	Nothing	Nothing	Nothing
983866	F	Control	122	245	120	11	Nothing	FF Eggs		
983908	F	Control	147	245	135	8	Nothing	Nothing	Nothing	Nothing
983945	F	Sham	274	280	170	8	Nothing	Nothing	Nothing	Nothing
984000	F	Control	105	240	115	10	Nothing	Nothing	Nothing	Nothing

984046	F	Low	127	240	130	11	Nothing	FF Eggs		
984071	F	Sham	148	245	135	10	Nothing	Nothing	Nothing	Nothing
984226	M	Sham	67	195	105	11	Nothing	Nothing	Nothing	Nothing
984305	F	Low	142	240	135	11	Nothing	Nothing	Nothing	Nothing
984308	M	Low	263	295	160	11	Nothing	Nothing	Nothing	Nothing
984336	F	Sham	129	240	130	8	Nothing	Nothing	FF Eggs	
984435	F	High	121	235	130	8	Nothing	FF Eggs		
984494	F	High	105	225	120	11	Hard Eggs	FF Eggs		
984680	F	Sham	91	225	115	8	Nothing	Nothing	Hard Eggs	Hard Eggs
984747	M	Low	154	245	130	8	Nothing	Nothing	Nothing	Nothing
984792	F	Control	145	255	135	8	Nothing	Nothing	Nothing	FF Eggs
984817	F	Sham	152	240	140	11	Nothing	Nothing	Nothing	Nothing
984828	M	Low	130	255	130	8	Nothing	Nothing	Nothing	Nothing
984876	M	Control	156	250	140	8	Nothing	Nothing	Nothing	Nothing
984885	F	Low	141	240	130	11	Nothing	Nothing	Nothing	Nothing
985001	M	High	84	415	100	11	Nothing	Nothing	Nothing	Nothing
985004	F	Low	148	255	120	11	Nothing	Nothing	Nothing	Nothing
985181	M	High	56	190	95	8	Nothing	Nothing	Nothing	Nothing
985312	M	Control	76	210	105	11	Nothing	Nothing	Nothing	Nothing
985322	F	Control	188	275	135	11	FF Eggs			
985423	M	Sham	114	230	125	11	Nothing	Nothing	Nothing	Nothing
985434	M	Low	137	245	120	11	Nothing	Nothing	Nothing	Nothing
985507	F	High	135	255	125	8	Nothing	FF Eggs		
985567	M	Sham	137	250	130	8	Nothing	Nothing	Nothing	Nothing
985653	F	High	138	235	135	11	Nothing	Nothing	Nothing	Nothing
985664	F	High	312	300	180	8	Nothing	FF Eggs		
985675	F	High	112	235	120	11	Nothing	Nothing	Nothing	Nothing

985697	F	Sham	96	220	115	7	Nothing	Nothing	Nothing	Nothing
985708	F	Low	114	220	130	11	Nothing	Nothing	Nothing	Nothing
985712	F	Sham	201	250	155	11	Nothing	Nothing	Nothing	Nothing
985718	F	Low	67	200	105	8	Nothing	Nothing	Nothing	Nothing
985737	M	Low	233	290	150	8	Nothing	Nothing	Nothing	Nothing
985912	M	Low	56	195	95	11	Nothing	Nothing	Nothing	Nothing
985970	F	Sham	122	225	130	11	Hard Eggs	Nothing	Nothing	Nothing
986039	F	Low	94	225	110	8	Nothing	FF Eggs		
986106	F	High	186	265	130	8	Nothing	FF Eggs		
986230	M	High	73	205	105	8	Nothing	Nothing	Nothing	Nothing
986386	F	Low	120	245	125	8	Nothing	Hard Eggs	FF Eggs	
986409	F	Low	167	255	140	11	FF Eggs			
986513	F	Low	102	215	125	11	Nothing	FF Eggs		
986523	F	High	177	260	145	10	Hard Eggs	FF Eggs		
986599	F	Sham	172	250	145	8	Nothing	FF Eggs		
986604	F	Low	245	245	165	11	Nothing	FF Eggs		
986675	F	Sham	177	265	140	11	FF Eggs			
986759	M	Control	184	275	140	8	Nothing	Nothing	Nothing	Nothing
986973	M	High	257	280	160	10	Nothing	Nothing	Nothing	Nothing
987053	F	High	181	260	145	8	Nothing	FF Eggs		
987164	M	Low	220	275	150	8	Nothing	Nothing	Nothing	Nothing
987214	F	Control	211	275	150	11	Hard Eggs	FF Eggs		
987412	F	High	59	195	100	8	Nothing	FF Eggs		
987506	F	Low	127	235	130	8	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
987660	F	Low	216	270	160	8	Nothing	Nothing	Nothing	Nothing
987716	M	Sham	125	225	125	11	Nothing	Nothing	Nothing	Nothing
987728	M	Control	101	220	115	7	Nothing	Milt		

987784	M	Sham	179	255	145	10	Nothing	Nothing	Nothing	Nothing
987839	F	High	86	220	110	8	Nothing	Nothing	Nothing	Nothing
987885	F	Sham	140	245	130	11	Nothing	Nothing	Nothing	Nothing
988005	F	High	131	240	125	8	Nothing	FF Eggs		
988026	F	High	223	275	150	8	Nothing	Nothing	Nothing	Nothing
988077	F	Sham	158	255	135	8	Nothing	Nothing	Nothing	Nothing
988277	F	Control	69	210	210	11	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
988302	M	High	57	215	95	11	Nothing	Nothing	Nothing	Nothing
988316	F	Low	188	220	110	11	Nothing	FF Eggs		
988324	F	Control	92	220	115	7	Nothing	Nothing	FF Eggs	
988340	M	Sham	149	245	135	11	Nothing	Nothing	Nothing	Nothing
988350	M	High	77	210	105	8	Nothing	Nothing	Nothing	Nothing
988359	M	Control	72	205	105	8	Nothing	Nothing	Nothing	Nothing
988467	F	High	237	285	165	8	Nothing	FF Eggs		
988475	F	Control	104	230	120	11	Nothing	Nothing	Nothing	Nothing
988566	F	Control	155	255	130	11	Nothing	Nothing	Nothing	Nothing
988649	F	Control	174	255	145	8	Nothing	Nothing	Nothing	Nothing
988684	F	High	124	240	130	8	Nothing	FF Eggs		
988864	F	Control	55	195	95	11	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
988892	F	High	161	260	140	8	Nothing	FF Eggs		
988914	M	Control	112	235	125	8	Nothing	Nothing	Nothing	Nothing
989004	F	High	139	240	135	11	Nothing	FF Eggs		
989111	F	High	124	235	125	11	Nothing	Nothing	Nothing	Nothing
989150	F	High	137	245	130	11	Nothing	Nothing	FF Eggs	
989231	F	High	109	235	110	11	Nothing	FF Eggs		
989233	F	Low	123	230	125	8	Nothing	Nothing	Nothing	Nothing
989295	M	Sham	111	230	125	11	Nothing	Nothing	Nothing	Nothing

989324	F	Sham	227	280	160	8	Nothing	Nothing	Nothing	Nothing
989385	F	Low	107	220	120	8	Nothing	Nothing	Nothing	Nothing
989461	F	High	118	230	120	8	Nothing	Nothing	Nothing	Nothing
989495	F	Control	149	255	130	8	Nothing	Nothing	FF Eggs	
989504	F	High	180	260	145	10	Hard Eggs	FF Eggs		
989591	F	Sham	141	250	130	8	Nothing	Nothing	Nothing	Nothing
989839	M	Sham	150	255	130	8	Nothing	Nothing	Nothing	Nothing
989910	F	Sham	191	250	150	11	Nothing	Nothing	Nothing	Nothing
989919	F	Low	132	240	135	7	Nothing	FF Eggs		
989936	M	Low	221	275	155	8	Nothing	Nothing	Nothing	Nothing
989939	F	High	170	250	140	11	Nothing	FF Eggs		
989953	M	High	75	220	100	8	Nothing	Nothing	Nothing	Nothing
990103	F	Low	156	245	140	11	Nothing	Nothing	Nothing	Nothing
990165	F	High	154	255	130	11	Nothing	Nothing	Nothing	Nothing
990181	M	High	71	210	105	8	Nothing	Nothing	Nothing	Nothing
990238	F	High	173	265	135	8	Nothing	Nothing	Nothing	Nothing
990314	M	Sham	180	270	140	11	Nothing	Nothing	Nothing	Nothing
990483	M	Control	99	230	115	8	Nothing	Nothing	Nothing	Nothing
990508	M	Low	110	240	115	11	Nothing	Nothing	Nothing	Nothing
990539	M	Low	62	195	100	10	Nothing	Nothing	Nothing	Nothing
990545	M	Low	88	220	110	11	Nothing	Nothing	Nothing	Nothing
990559	M	Control	166	260	140	11	Nothing	Nothing	Nothing	Nothing
990747	M	Control	65	195	100	11	Nothing	Nothing	Nothing	Nothing
990767	M	Control	77	210	105	8	Nothing	Nothing	Nothing	Nothing
990878	M	High	96	225	115	8	Nothing	Nothing	Nothing	Nothing
990912	F	Control	113	215	130	11	Nothing	Nothing	Nothing	Nothing
991083	M	Low	97	225	115	8	Nothing	Nothing	Nothing	Nothing

991204	F	Control	233	275	160	8	Nothing	Nothing	Nothing	Nothing
991252	F	High	75	215	110	11	FF Eggs			
991296	M	High	71	205	105	10	Nothing	Nothing	Nothing	Nothing
991314	F	Sham	339	315	180	10	Nothing	Nothing	Nothing	Nothing
991339	F	High	74	200	100	11	Nothing	Nothing	Nothing	Nothing
991534	F	Low	205	270	155	8	Nothing	FF Eggs		
991579	F	Low	115	240	120	11	FF Eggs			
991629	F	Control	179	260	145	11	FF Eggs			
991681	F	High	122	235	125	11	Nothing	Nothing	Nothing	Nothing
991744	F	High	165	260	135	11	Nothing	Nothing	Nothing	Nothing
991847	F	Sham	170	250	145	11	Nothing	Nothing	Nothing	Nothing
991900	M	Sham	119	240	120	8	Nothing	Nothing	Nothing	Nothing
991995	F	High	138	245	130	8	Nothing	Hard Eggs	Hard Eggs	Hard Eggs
991997	F	Low	95	230	100	11	Nothing	Nothing	Nothing	Nothing
992061	F	Sham	93	225	115	8	Hard Eggs	Nothing	Nothing	Nothing
992171	F	Control	153	250	140	8	Nothing	Nothing	Nothing	Nothing
992182	F	Low	222	285	150	10	Hard Eggs	Nothing	Nothing	Nothing
992232	F	Control	213	270	150	11	Nothing	Nothing	Nothing	Nothing
992256	M	Control	160	245	140	8	Nothing	Nothing	Nothing	Nothing
992310	F	Control	409	345	195	8	Nothing	Nothing	Nothing	Nothing
992421	F	Control	85	215	110	11	Nothing	Nothing	Nothing	Nothing
992465	F	Low	277	285	170	8	Nothing	Nothing	FF Eggs	
992485	M	Sham	152	255	140	8	Nothing	Nothing	Nothing	Nothing
992533	F	Sham	147	255	135	8	Nothing	Nothing	Nothing	Nothing
993445	M	Low	240	290	160	10	Nothing	Nothing	Nothing	Nothing

APPENDIX 2: RAW DATA OF MANUALLY MEASURED EGG DIAMETERS

Table Captions

Table A.7 Raw data of egg diameter (mm) of female hatchery-reared bloaters (*Coregonus hoyi*) for the December – January sample. Treatment group, presence of free flowing eggs, ID and subset of eggs which were either surgically excised (in the case of non-free flowing individuals) or collected by gentle abdominal massage (in the case of free flowing individuals). Each diameter measure represents a single egg.

Table A.8 Raw data of egg diameter (mm) of female hatchery-reared bloaters (*Coregonus hoyi*) for the January – February sample as well as the wild bloater eggs collected in mid-January (wild). Treatment group, presence of free flowing eggs, ID and subset of eggs which were either surgically excised (in the case of non-free flowing individuals) or collected by gentle abdominal massage (in the case of free flowing individuals). Each diameter measure represents a single egg.

Table A.7

Treatment	Free flowing Eggs?	ID	Subset #	Egg Diameter (mm)
Hatchery	No	121715-F1	1	1.8 1.6 1.8 1.5 1.8 1.7 1.8 1.7 1.7 1.7 1.6 1.8 1.7 2.0 1.7 1.7 1.8 1.7 1.7 1.7 1.9 1.5 1.7 1.6 2.1 1.5 1.7
			2	1.9 1.7 1.8 1.6 1.7 1.7 1.9 2.0 1.6 1.9 1.8 1.7 2.0 1.7 1.7 2.1 2.1 1.7 1.8 1.6 2.0 1.7 1.6 1.7 1.9 1.7 1.7 1.6 1.9 1.7
			3	1.8 1.7 1.7 1.8 1.8 1.7 1.9 1.7 1.8 1.7 1.8 1.7 1.8 1.7 1.7 1.8 1.8 1.8 1.8 1.8 1.8
Hatchery	No	121715-F2	1	1.6 1.2 1.5 1.6 1.7 1.7 1.7 1.5 1.6 1.6 1.7 1.7 1.5 1.5 1.5 1.9 1.8
			2	2.1 1.8 1.9 1.7 1.7 1.9 1.5 1.8 1.7 1.7 1.2 1.7 1.7 1.8 1.4 1.6
			3	1.7 1.5 1.6 1.6 1.5 1.8 1.7 1.8 1.6 1.8 1.7 1.7
Hatchery	No	121715-F3	1	1.6 1.5 1.6 1.5 1.6 1.1 1.7 1.6 1.5 1.5 1.5 1.6 1.5 1.7 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.5 1.6 1.6 1.7 1.6 1.4 1.6 1.7 1.7 1.8 1.5 1.6 1.5 1.5
			2	1.6 1.6 1.5 1.5 1.7 1.8 1.5 1.5 1.6 1.6 1.5 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.7 1.6 1.6 1.7 1.6 1.6
			3	1.5 1.6 1.5 1.7 1.5 1.5 1.5 1.5 1.6 1.6 1.7 1.8 1.5 1.6 1.7 1.7 1.5 1.5 1.6 1.6 1.5 1.6 1.6 1.5 1.5 1.6
Hatchery	No	121715-F4	1	1.3 1.3 1.4 1.0 1.2 1.4 1.4 1.3 1.3 1.2 1.3 1.2 1.3 1.1 1.4 1.3 1.6 1.3 1.3 1.2 1.1 1.3
			2	1.3 0.7 0.8 1.1 1.5 1.2 1.2 1.1 1.2 1.0 1.4 1.1 1.4 1.2 1.3 1.4 1.2 1.3 1.0 1.1 1.3
			3	1.1 1.3 1.3 1.3 1.3 1.2 1.3 1.1 1.3 1.3 1.3 1.2 1.3 1.3 1.3
Hatchery	No	121715-F5	1	1.4 1.6 1.4 1.5 1.4 0.8 0.7 1.5 1.4 1.4 1.4 1.5 1.5 1.5 1.4 1.5 1.6 1.0 1.5 1.5 1.6 1.5 1.5 1.5
			2	1.4 1.5 1.5 1.5 1.5 1.4 1.4 0.8 1.5 1.5 1.6 1.4 1.5 1.3 1.4 1.5 1.6 1.6 1.7 1.4 0.6 1.5 1.4 1.5 1.1 1.8 0.6
			3	1.6 1.6 1.4 1.6 1.6 1.5 1.4 1.6 1.5 1.5 1.5 1.6 1.6 1.5 1.6 1.8 1.4 1.8 1.5 1.4 1.4
Hatchery	No	121715-F6	1	1.6 1.5 1.7 1.8 1.7 1.6 1.7 1.8 1.6 1.6 1.6 1.7 1.6 1.8 1.6 1.6 1.7 1.6 1.6
			2	1.6 1.6 1.7 1.8 1.7 1.7 1.8 1.6 1.6 1.8 1.6 1.7 1.7
			3	1.8 1.6 1.7 1.8 1.7 1.6 1.8 1.7 1.7 1.9 1.7 1.7 1.6 1.8 1.8 2.0 2.0 1.7
Hatchery	No	121715-F7	1	1.7 1.9 1.7 1.7 1.8 1.8 1.8 1.7 1.6 1.8 1.7 1.8 1.6 1.6 1.7 1.7 2.1
			2	1.7 1.8 1.6 1.7 1.7 1.6 1.8 1.6 1.7 1.6 1.6 1.8 1.6 1.7 1.6 1.8 1.8 1.7 1.8 1.7 1.7 1.6 1.6
			3	1.8 1.6 1.7 1.7 1.8 1.8 1.9 1.7 1.8 1.8 1.7 1.6
Hatchery	No	121715-F8	1	1.8 1.9 1.8 1.9 1.7 1.8 1.7 1.7 1.8 1.7 1.7 1.9 1.8 1.9 1.8 1.9 1.7 1.7 1.8 1.8 1.9 1.8 1.6 1.7 2.1
			2	1.6 1.7 1.8 1.8 1.9 1.8 1.8 1.7 2.3 1.6 1.7 1.8 2.0 1.7 1.8 1.6 1.8 1.8 2.0 1.8 1.5 1.9
			3	1.8 1.8 1.9 1.8 1.8 1.7 1.8 1.8 1.9 1.9 1.8 1.9 1.8 1.7 1.8 1.7 1.6 1.9 1.9 1.8 1.6 1.6 1.6
Hatchery	No	121715-F9	1	1.6 1.8 1.7 1.7 1.6 1.9 1.9 1.8 1.6 1.5 1.9 1.8 1.7
			2	1.9 1.8 1.9 1.7 1.6 1.8 1.7 1.5 1.8 1.9 1.8 1.6 1.6 1.7 1.8 1.8 1.7 1.7 1.6 2.4
			3	1.7 1.8 1.7 1.2 1.3 1.8 2.0 1.9 2.1 1.9 1.7 1.6 1.5 1.9 1.7 1.7 0.9 0.9 0.6 0.6
Hatchery	No	121715-F10	1	1.7 1.5 1.5 1.7 1.6 1.6 1.7 1.7 1.7 1.7 1.6 1.6 1.6
			2	1.6 1.8 1.6 1.6 1.5 1.7 1.5 1.6 1.6 1.7 1.7 1.3 1.7 1.6 1.4 1.7
			3	1.6 1.7 1.5 1.5 1.6 1.5 1.5 1.5 1.7 1.6 1.6 1.6 1.6 1.7 1.6 1.7 1.5 1.6 2.0 1.5 1.8
High	Yes	F2	1	2.1 1.9 1.8 1.8 2.0 2.0 2.1 1.9 2.0 2.0 2.3 2.1 2.4 2.0 1.8 2.4 1.8 2.1 2.0 2.1
			2	2.0 2.1 2.0 2.0 1.8 1.9 2.1 1.9 1.9 2.0 2.0 1.6 1.8 1.9 1.8 1.8 1.9 2.0 1.9 1.8
			3	2.5 2.0 1.8 1.9 2.0 2.0 2.1 2.1 1.8 2.1 2.4 1.9 1.9 2.4 1.8 1.9 2.3 1.8 1.9 2.1
High	Yes	F5	1	1.8 1.6 2.0 1.8 1.8 1.8 2.0 1.6 1.8 1.8 1.8 1.8 1.9 1.9 1.9 2.0 2.0 1.8 1.9 2.0
			2	1.8 2.0 1.9 1.9 1.8 1.9 1.6 1.6 1.9 1.6 1.9 1.8 1.9 1.6 1.8 1.9 1.6 1.9 2.0 2.0
			3	1.8 1.9 1.9 1.9 1.6 1.8 2.0 1.6 1.6 1.8 1.8 1.6 1.8 1.9 1.9 1.8 1.6 1.8 1.9 1.9
Low	Yes	F7	1	1.4 1.5 1.4 1.4 1.5 1.4 1.4 1.5 1.5 1.5 1.4 1.3 1.5 1.5 1.4 1.4 1.4 1.3 1.3 1.5
			2	1.3 1.4 1.3 1.3 1.5 1.4 1.5 1.4 1.6 1.4 1.4 1.5 1.3 1.3 1.4 1.4 1.5 1.4 1.4 1.5
			3	1.4 1.3 1.4 1.5 1.4 1.4 1.5 1.5 1.4 1.5 1.4 1.5 1.4 1.5 1.6 1.3 1.4 1.5 1.4 1.3
High	Yes	F8	1	1.9 1.9 2.0 2.0 2.1 2.0 1.9 2.0 2.0 2.1 2.0 1.9 1.9 2.4 2.0 2.4 1.8 1.9 2.0 2.4
			2	2.0 2.0 1.9 2.0 1.9 2.0 1.9 1.9 1.9 2.1 1.9 1.8 1.9 2.0 1.9 2.0 2.0 1.9 2.0 2.0
			3	2.0 2.3 2.3 2.1 2.1 2.0 2.1 2.1 2.1 1.9 1.9 2.0 1.9 1.9
High	Yes	F9	1	1.8 1.6 1.6 1.6 1.8 1.6 1.9 1.9 1.8 1.6 1.6 1.8 1.8 1.6 1.8 1.8 1.9 1.8 1.8 1.6
			2	1.6 1.6 1.6 1.6 1.9 1.6 1.5 1.8 1.6 1.8 1.6 1.5 1.6 1.6 1.6 1.8 1.8 1.5 1.8 1.8
			3	1.8 1.5 1.9 2.1 1.6 1.8 1.8 1.5 1.8 1.8 1.8 1.6 1.5
Low	Yes	F10	1	1.9 1.9 1.9 1.9 2.0 1.8 1.8 1.9 1.9 2.0 1.9 1.8 1.9 2.1 2.0 1.9 1.9 1.8 1.9 2.0
			2	1.9 1.8 1.9 2.0 1.9 1.9 2.0 1.8 1.9 1.9 2.0 1.8 1.8 1.9 1.9 1.8 1.9 1.6 2.1 1.8

			3	1.8 1.8 2.6 1.8 1.8 2.4 1.9 1.8 1.9 1.9 1.9 1.9 1.8 1.8 1.8 1.9 1.9 1.6 1.9 1.8
			1	1.6 1.9 2.0 2.0 1.8 2.0 1.9 1.9 1.9 2.0 1.8 1.8 1.6 2.0 2.1
High	Yes	F12	2	1.8 1.8 1.8 2.0 2.1 2.0 1.8 1.8 1.8 1.8 1.6 2.1 1.8 1.8 2.0 1.8
			3	
			1	1.6 1.4 1.6 1.6 1.5 1.5 1.5 1.6 1.6 1.5 1.6 1.8 1.5 0.6 1.5 1.4 0.8
High	Yes	F14	2	1.8 1.6 1.6 1.5 1.8 1.6 1.8 1.6 0.9 1.6 1.8 1.5 1.6 1.5 1.8 1.8 1.6 1.5 1.8 1.6
			3	1.8 1.6 1.8 2.0 1.5 1.6 1.6 1.5 1.6 1.9 1.6 1.8 1.9 1.8 1.8 1.5 1.6 1.6 1.4 1.6
			1	1.8 1.8 1.9 1.8 1.9 1.9 1.8 1.9 1.8 1.8 1.9 1.8 1.9 2.0 1.9 1.8 1.9 1.8 2.0 1.9
High	Yes	F16	2	1.9 1.9 1.8 2.0 1.6 1.9 1.8 1.9 1.8 1.9 1.8 2.0 1.9 2.0 1.9 2.0 1.9 1.9 1.9 1.9
			3	1.8 1.8 1.8 1.8 1.9 1.8 1.8 1.8 1.8 1.9 1.9 1.9 1.8 2.0 1.8 1.9 1.9 1.8 1.9 2.0
			1	2.1 2.0 1.9 1.9 1.9 2.0 1.9 1.8 2.0 2.0 1.9 1.9 2.0 1.8 1.9 1.9 2.0 1.9 2.0 2.0
Low	Yes	F17	2	2.0 2.0 1.9 2.0 1.9 2.0 2.0 2.1 1.9 2.1 1.9 1.8 1.9 1.9 1.9 2.0 1.9 1.8 1.9 2.0
			3	2.0 2.0 1.9 1.8 1.9 2.0 1.9 1.8 1.9 1.9 2.0 1.8 1.8 1.9 1.9 2.0 2.0 1.9 1.8 1.9
			1	1.8 1.8 1.8 1.8 1.8 1.9 1.9 1.8 1.8 1.7 1.8 1.7 1.8 1.8 1.8 1.9 1.7 1.8 1.7 1.9 1.8 1.8
Control	No	F1	2	1.8 1.7 1.7 1.8 1.7 1.7 1.8 1.8 1.9 1.7 1.7 1.7 1.9 1.7 1.8 1.7 1.8 1.7 1.9 1.8
			3	1.8 1.8 1.8 1.8 1.7 1.8 1.7 1.7 1.9 1.9 1.7 1.8 1.8 1.7 1.9 1.8 1.7 1.9 1.9 1.7 1.8 1.8
			1	1.7 1.8 1.6 1.7 1.5 1.6 1.8 1.7 1.6 1.6 1.7 1.6 1.7 2.0 1.7 1.6 1.6 1.5 1.6 1.9 1.6 1.7 1.7 1.6 1.8 1.6 1.8 1.2 1.6 1.7 1.7 1.6 1.2
Control	No	F2	2	1.5 1.5 1.8 1.7 1.7 1.6 1.7 1.7 1.5 1.2 1.7 1.4 1.6 1.6 1.7 1.7 1.7 1.7 1.5 1.8 1.7 1.7 1.5 1.7 1.6 1.8 1.7 1.7 1.7 1.8 1.8 1.6 1.7 1.5 1.8
			3	1.7 1.8 1.8 1.8 2.0 1.8 1.7 1.7 1.7 1.6 1.8 1.8 1.8 1.8 1.8 1.8 1.7 1.9 1.7 1.6 1.5 1.8 1.5 1.7 1.8 1.6 1.6 1.8 1.5 1.5 1.8 1.7 1.7 1.8 1.8 1.6 1.6
			1	1.1 1.1 1.1 1.0 1.0 1.0 1.1 1.1 1.1 1.1 1.2 1.0 1.0 1.0 0.9 1.1 1.0 1.1 1.0 1.0 1.1 1.1 1.0 0.8 0.9 0.7 0.8 1.0 1.7 1.0 1.0 1.0 1.1
Sham	No	F3	2	1.0 1.0 1.0 1.0 1.1 1.1 1.1 1.0 1.1 1.6 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.0 1.0 1.0 1.0 1.0 1.0 0.9 0.9 0.8 1.1 1.1 1.1 1.1 0.9
			3	1.1 1.0 1.1 1.1 1.1 1.0 0.8 0.6 0.8 0.9 1.0 1.0 1.0 1.0 1.1 1.0 1.0 1.1 1.0 1.0 1.0 1.1 0.9 1.0 1.1 1.1 1.1 1.1 1.1 1.0 0.9
			1	2.1 2.1 1.8 1.6 1.8 1.8 1.9 1.8 1.8 1.8 1.7 1.7 1.7 1.8 1.7 1.9 1.7 1.7 1.7
Sham	No	F4	2	2.0 1.9 1.8 1.8 1.7 1.8 1.7 1.9 1.9 1.6 1.7 1.8 1.9 1.8 2.0 1.8 1.7 1.7
			3	2.1 2.1 1.9 1.7 1.7 1.8 1.9 1.8 1.8 1.7 1.8 1.7 1.7 1.7 1.8 1.8 1.7 1.7 1.8 1.7 1.8
			1	1.8 1.7 1.7 1.8 1.8 1.7 1.8 1.9 1.8 1.7 1.6 1.9 1.4 1.8 1.7 1.7 1.8 1.7 1.7 1.8 1.8
Sham	No	F5	2	2.0 2.0 1.7 1.8 1.7 1.7 1.7 1.8 1.8 1.7 1.7 1.8 1.8 1.8 1.8 1.8 2.0 2.0 1.7 1.7 1.8
			3	1.7 1.8 1.8 1.7 1.7 1.6 1.7 1.8 2.0 1.7 1.8 1.7 1.8 1.4 1.7 1.8 1.8 1.8 1.7 1.8 1.8 1.7
			1	1.6 1.7 1.8 1.6 1.7 1.6 1.7 1.7 1.6 1.6 1.6 1.6 1.3 1.6 1.7 1.7 1.6 1.5 1.7 1.4 1.4 1.3 1.6 1.6 1.6 1.6 1.6 1.5 1.6 1.6
Control	No	F6	2	1.7 1.6 1.7 1.7 1.7 1.6 1.7 1.8 1.6 1.6 1.4 1.6 1.6 1.6 1.6 1.6 1.7 1.7 1.5 1.6 1.7 1.7 1.6 1.6 1.5 1.7 1.7 1.7 1.5 1.6
			3	1.7 1.8 1.6 1.7 1.6 1.9 1.9 1.6 1.7 1.6 1.8 1.7 1.7 1.6 1.7 1.8 1.4 1.7 1.6 1.7 1.7 1.6 1.7
			1	1.7 1.8 1.6 1.6 1.7 1.7 1.7 1.6 1.7 1.7 1.6 1.7 1.7 1.7 1.7 1.7 1.7 0.7 1.6 1.7 1.7 1.7 1.6 1.6 1.8 1.6 1.6 1.7 1.7
Sham	No	F7	2	1.7 1.7 1.6 1.7 1.8 1.7 1.7 1.7 1.6 1.9 1.6 1.7 1.7 1.7 1.7 1.8 1.6 1.7 1.6 1.8 1.6 1.7 1.7 1.6 1.9 1.7 1.7 1.6 1.6
			3	1.8 1.7 1.8 1.6 1.7 1.7 1.8 1.7 1.6 1.8 1.5 1.6 1.6 1.5 1.8 1.6 1.8 1.7 1.7 1.6 1.6 1.7 1.7 1.7 1.8 1.7 1.5 1.7 1.7 1.8
			1	
Sham	No	F8	2	1.3 1.4 1.4 1.5 1.4 1.5 1.6 1.3 1.6 1.5 1.0 1.2 1.0 1.3 1.2 1.3 1.3 1.3 1.6 1.3 1.3 1.3 1.4 1.4 1.8 1.2 1.3 1.3 1.5 1.3
			3	1.5 1.5 1.7 1.4 1.4 1.4 1.3 1.3 1.5 1.5 1.4 1.5 1.3 1.3 1.3 1.5 1.4 1.3 1.2 1.4 1.3 1.4 1.4 1.5 1.4 1.3 1.3 1.2 1.3 1.3 1.5 1.4 1.3
			1	1.2 1.1 1.1 1.2 1.2 1.1 1.1 1.1 1.2 1.3 1.2 1.1 0.9 1.2 1.2 1.2 1.2 1.2 1.2 1.1 1.1 1.2 1.2 1.2 1.2 1.2 1.1 1.2 1.2 1.2 1.3 1.0 0.9
Control	No	F9	2	1.1 1.2 1.2 1.1 1.2 1.1 1.3 1.2 1.2 1.1 1.1 1.2 1.2 1.1 1.1 1.1 1.3 1.1 1.3 1.2 1.1 1.1 1.2 1.2 1.2 1.2 1.1 1.2 1.1
			3	1.2 1.2 1.2 1.2 1.2 1.3 1.2 1.2 1.1 1.2 1.2 1.2 0.8 1.2 1.0 1.1 1.2 1.0 1.2 1.1 1.0 1.1 1.1 1.1 1.1 1.1 1.2 1.2 1.2 1.2 1.2 1.2 0.9
			1	1.4 1.6 1.5 1.6 1.6 1.5 1.5 1.5 2.1 1.6 1.5 1.4 1.5 1.5 1.6 1.5 1.4 1.6 1.5 1.5 1.6 1.6 1.3 1.5 1.5 1.6 1.5
Control	No	F11	2	1.5 1.3 1.6 1.8 1.5 1.6 1.4 1.2 1.5 1.7 1.6 1.6 1.4 1.5 1.5 1.7 1.5 1.5 1.6 1.4 1.3 1.5 1.5 1.5 1.5 1.6
			3	1.6 1.5 1.6 1.6 1.5 1.6 1.6 1.5 1.4 1.7 1.5 1.3 1.7 1.6 1.4 1.4 1.4 1.5 1.4 1.4 1.5 1.6 1.4 1.6 1.4 1.6 1.5 1.7 1.6 1.7 1.5
			1	1.2 1.3 2.1 1.8 1.8 2.0 1.7 2.0 2.0 2.1 1.6 2.2 2.0 1.8 1.7 2.0 2.1 1.8 1.8 2.0
Low	Yes	F1	2	2.0 1.8 1.7 1.8 2.0 2.2 2.2 2.0 2.0 1.8 1.7 2.0 2.2 1.8 2.0 2.0 2.1 2.0 1.7 2.0
			3	2.0 2.1 2.0 1.8 2.0 1.7 1.8 2.0 2.1 2.0 2.2 1.7 2.1 2.2 1.7 1.8 2.1 2.1 2.2 2.1
			1	2.0 2.1 2.0 1.8 2.3 1.8 1.8 2.0 2.1 1.8 2.2 1.8 2.1 2.0 1.8 2.0 1.8 2.2 2.0 2.0
Low	Yes	F4	2	2.0 2.0 2.1 2.0 2.2 1.8 2.1 2.2 2.2 2.0 1.8 1.8 1.8 2.3 2.4 2.0
			3	1.8 1.8 2.1 1.7 2.2 2.1 2.2 2.6 2.2 2.1 2.2 2.0 2.0 2.2 2.2 2.0 1.8 2.0 2.0 2.1

			1	2.0	2.0	2.2	2.1	2.1	2.1	2.0	1.7	2.0	2.1	2.1	2.0	1.8	2.1	2.1	2.0	2.0	2.1	2.2	2.1
Low	Yes	F15	2	2.0	2.0	2.1	2.1	2.1	2.1	2.2	1.8	2.0	2.1	2.1	2.1	2.0	2.2	2.0	2.3	2.0	2.1	2.2	2.2
			3	2.1	2.1	2.0	2.1	2.1	2.2	2.1	2.2	2.1	2.0	2.1	2.1	2.1	2.0	2.2	1.8	2.0	2.1	2.2	2.1
			1	2.1	2.2	2.0	2.1	2.2	2.0	2.2	1.8	2.3	1.8	2.0	2.3	1.8	2.1	2.2	2.2	2.1	2.2	2.1	2.2
Low	Yes	F16	2	2.2	2.2	2.1	2.1	1.8	2.2	2.2	2.2	2.0	2.2	2.1	2.2	2.1	2.3	2.2	2.1	1.8	2.0	2.2	
			3	2.2	2.0	2.0	2.1	2.2	2.1	2.1	2.1	2.1	2.2	2.0	2.0	2.1	2.2	2.3	1.8	2.1	2.0	2.1	2.1
			1	2.0	2.1	2.1	1.8	2.0	2.0	2.1	1.8	2.0	2.0	2.0	2.1	2.0	2.1	2.3	2.0	2.0	2.3	2.0	1.8
Low	Yes	F17	2	2.1	2.0	2.2	2.0	2.1	2.2	1.8	2.2	2.2	2.0	1.8	2.1	2.2	2.2	2.1	2.1	2.0	1.8	2.0	2.2
			3	2.2	2.0	2.1	2.1	2.1	2.1	2.2	2.1	2.2	2.1	2.1	2.2	2.2	2.2	2.1	2.1	2.2	2.3	2.1	2.3
			1	1.8	2.1	2.2	1.8	2.2	2.2	2.4	1.8	1.8	1.7	2.0	1.7	2.0	2.1	1.7	1.8	2.0	2.1	1.7	1.7
Low	Yes	F6	2	1.7	1.8	2.0	2.0	2.3	2.1	2.1	1.7	2.6	1.7	2.1	1.8	2.0	2.0	1.8	2.0	2.1	2.0		
			3	1.8	2.0	2.0	1.7	2.0	1.8	1.8	1.7	2.0	2.2	1.7	2.2	1.7	2.0	2.2	2.1	1.7	2.0	1.8	2.0

Table A.8

Treatment	Free flowing Eggs?	ID	Subset #	Egg Diameter (mm)
			1	1.7 1.6 1.8 1.7 1.7 1.7 1.7 1.8 1.8 1.7 1.7 1.7 1.7 1.7 1.8 1.7 1.7 1.7 1.8 1.7 1.8 1.7 1.7 1.8 1.7 1.8
Hatchery	No	F1	2	1.7 1.9 1.7 1.8 1.7 1.8 1.7 1.7 1.8 1.9 1.8 1.7 1.7 1.7 1.7 1.9 1.6 1.8 1.5 1.8 1.8 2.0 1.8 1.7 1.8 1.7 1.5
			3	1.7 1.6 1.6 1.6 1.7 1.7 1.7 1.8 1.6 1.6 1.7 1.7 1.6 1.7 1.7 1.8 1.6 1.7 1.6 1.7 1.5 1.7 1.7 1.6 1.7 1.6
			1	1.7 1.7 1.7 1.8 1.6 1.8 1.7 1.7 1.9 1.7 1.7 1.7 1.5 1.7 1.7 1.6 1.8 1.7 1.8 1.8 1.8 1.8 1.7 1.7
Hatchery	No	F2	2	1.6 1.7 1.6 1.7 1.7 1.7 1.7 1.8 1.6 1.6 1.6 1.6 1.5 1.6 1.6 1.6 1.6 1.8 1.6 1.7 1.7 1.5 1.6 1.5 1.7 1.6 1.3 1.4 1.8
			3	1.7 1.7 1.6 1.7 1.7 1.6 1.6 1.8 1.7 1.7 1.7 1.7 1.7 1.6 1.6 1.6 1.7 1.6 1.6 1.6 1.7 1.5 1.7 1.6 1.7 1.6 1.6
			1	0.8 0.7 0.7 0.8 1.0 0.7 0.6 0.6 0.6 0.6 0.6 0.5 0.8 0.8 0.8 0.6 0.8 0.7 0.7 0.8 0.6 0.7 0.8 0.7
Hatchery	No	F3	2	0.7 0.8 0.8 0.7 0.6 0.5 0.8 0.6 0.7 0.6 0.7 0.9 0.6 0.8 0.7 0.6 0.6 0.6 0.7 0.9 0.8 0.7 0.6 0.6
			3	0.6 0.6 0.7 0.7 0.8 0.6 0.8 0.7 0.9 0.8 0.7 0.8 0.8 0.7 0.7 0.8 1.0 0.5 0.8 0.8 0.6 0.6 0.7
			1	2.0 2.0 1.9 1.8 1.8 1.9 1.8 1.8 1.9 1.8 1.9 1.8 1.9 1.9 1.9 1.8 1.9 1.9 1.9 1.7 1.8 1.8 1.7 1.8 1.8 1.8 1.8 1.7 1.8
Hatchery	No	F4	2	2.1 2.0 2.1 1.8 1.8 1.9 1.7 1.7 2.0 1.8 1.8 1.9 1.8 1.6 1.8 1.8 1.7 1.8 1.8 1.8 1.8 1.8 1.9 1.9
			3	2.1 2.1 2.2 2.1 2.0 1.9 1.9 2.0 1.7 1.8 1.9 1.8 1.8 1.9 1.9 1.8 1.7 1.8 1.8 1.8 1.7 1.8 1.8 1.8 1.9
			1	2.0 2.0 2.0 1.8 1.8 1.9 1.7 2.0 1.7 1.9 1.6 1.7 1.8 1.9 1.8 1.8 1.9 1.9 1.9 1.8
Hatchery	No	F5	2	2.1 2.1 2.0 1.9 1.8 2.0 1.9 2.0 2.0 1.7 1.9 1.8 1.9 2.0 1.9 1.8 1.9 2.0 1.9 1.8 1.9 2.0 1.8 1.9 1.9 2.0 1.9
			3	2.0 1.7 1.7 1.7 1.7 1.7 1.7 1.8 1.7 1.7 1.6 1.9 1.6 1.8 2.0 1.9 1.8 1.8 1.6 1.8 1.8 1.7 2.0 1.9 1.9 1.7 1.9 1.8 2.0 1.7
			1	2.1 1.7 1.8 1.8 1.8 1.8 1.9 1.9 1.8 1.8 1.7 1.8 1.8 1.8 1.8 1.7 1.9 1.6 1.8 1.8 1.9 1.9 1.8 1.8 1.8 1.7 1.9 1.8 1.7
Hatchery	No	F6	2	1.9 1.9 1.9 1.8 1.8 2.0 2.0 2.0 2.0 2.0 1.8 1.9 1.8 1.7 1.8 1.9 1.8 1.7 1.7 1.9 1.8 1.9 1.8 1.8 1.8 1.9 1.8
			3	2.0 2.1 2.0 2.0 1.9 1.9 2.0 1.8 1.8 1.9 1.8 1.8 1.9 1.8 2.0 1.7 1.8 1.9 1.8 1.7 1.9 1.8 1.9 1.9 1.9 1.9 1.9 1.9 1.8
			1	2.1 2.1 2.1 2.0 1.9 1.9 1.4 1.8 1.4 1.4 1.5 1.9 1.9 1.9 1.3 1.8 1.5 1.8 2.0 1.8 1.3 1.9
Hatchery	No	F7	2	2.3 2.0 2.0 1.9 1.9 2.0 1.9 1.7 1.3 1.9 1.7 1.9 1.8 1.7 1.4 1.9 1.8 1.8 1.9 1.1 1.9 1.5
			3	2.1 2.1 1.7 1.8 1.3 1.9 1.8 1.9 1.8 1.8 1.9 1.3 1.9 1.9 1.6 1.9 1.8 1.7 1.8 1.8 1.7 1.9 1.8 1.8
			1	1.6 1.6 1.7 1.5 1.6 1.8 1.6 1.5 1.6 1.4 1.6 1.6 1.7 1.7 1.6 1.5 1.8 1.6 1.5 1.5 1.6 1.7 1.5 1.6 1.6 1.6 1.4
Hatchery	No	F8	2	1.5 1.5 1.8 1.9 1.7 1.8 1.8 1.5 1.9 1.6 1.4 1.6 1.4 1.8 1.8 1.8 1.5 1.9 1.9 1.3 1.4 1.6 1.6 1.6 1.5 1.6 1.5
			3	1.6 1.4 1.5 1.6 1.7 1.5 1.6 1.6 1.6 1.5 1.8 1.6 1.7 1.5 1.9 1.5 1.5 1.6 1.6 1.5 1.7 1.6 1.5 1.6 1.6 1.7 1.5 1.4 1.7 1.5 1.5 1.5 1.7
			1	1.7 1.8 1.8 1.7 1.8 1.6 1.7 1.8 1.7 1.7 1.8 1.5 1.8 1.7 1.8 1.8 1.7 1.8 1.8 1.8 1.7 1.7 1.6 1.9
Hatchery	No	F9	2	1.7 1.7 1.7 1.7 1.8 1.8 1.9 1.6 1.9 1.8 1.6 1.8 1.8 1.7 1.6 1.7 1.8 1.8 1.7 1.8 1.6 1.6 1.8 1.8 1.7 1.7 1.8 1.6
			3	1.7 1.8 1.8 1.6 1.8 1.7 1.8 1.8 1.8 1.9 1.7 1.9 1.8 1.8 1.9 1.8 1.9 1.6 1.6 1.7 1.5 1.9 1.7 2.0
			1	2.2 0.9 1.4 1.7 1.7 1.3 1.5 1.4 1.5 1.5 1.5 1.4 1.5 1.3 1.4 1.3 1.2 1.6 1.6 1.5 1.3 1.4 1.4 1.5 1.8 1.5 1.8 1.5 1.8
Hatchery	No	F10	2	1.5 1.5 1.6 1.9 1.4 1.5 1.5 1.5 1.5 1.4 1.3 1.5 1.4 1.4 1.4 1.3 1.5 1.9 1.3 1.5 1.3 1.4 1.5 1.6 1.5 1.1 1.6
			3	1.9 1.8 1.1 1.5 1.3 1.6 0.4 1.6 1.4 1.4 1.5 1.4 1.8 1.5 1.4 1.5 1.6 1.5 1.5 1.4 1.5 1.4 1.4 1.4 1.5 1.9 1.5 1.4
			1	1.9 1.7 2.2 2.0 2.0 2.0 1.9 1.9 1.9 2.0 2.1 2.0 2.0 2.2 1.9 1.9 1.9 1.9 2.0 2.2
Hatchery	Yes	F1	2	2.0 2.0 2.0 2.3 2.1 2.0 2.2 2.0 2.2 2.1 2.2 2.2 2.1 2.1 2.0 1.9 2.0 2.0
			3	2.1 2.2 2.1 2.0 2.2 2.1 2.1 2.0 2.0 2.2 2.1 2.0 1.9 2.0 1.9 1.9 2.0 1.9 2.0 2.1
			1	1.9 2.1 1.9 2.0 1.9 2.0 2.1 1.9 1.9 2.0 2.1 2.1 1.9 1.9 2.0 2.1 2.1 1.9 2.0 1.9 2.0 1.9 2.0 1.9 2.0
Hatchery	Yes	F2	2	2.0 2.0 2.1 1.9 1.9 2.0 2.1 1.9 2.0 2.1 1.9 2.0 2.1 2.2 2.1 1.9 1.5 1.9
			3	2.0 2.0 2.1 2.1 2.1 1.9 1.8 2.0 2.0 2.0 1.9 2.0 1.9 2.0 1.9 2.0 2.1 2.2
			1	2.0 1.9 1.9 2.0 1.9 1.9 2.0 2.0 1.9 2.1 1.9 1.9 1.7 2.0 2.0 2.0 1.8 2.0 2.1 1.8 2.0 1.8 2.0 2.0
Hatchery	Yes	F3	2	2.0 2.1 2.1 2.2 1.9 2.0 2.1 2.2 1.9 1.9 2.1 1.8 1.9 2.0 2.1 1.9 1.9 2.0 1.8 1.9 1.9 2.1
			3	2.5 2.0 1.8 1.8 1.9 2.0 1.8 2.2 1.9 1.9 1.9 2.0 2.1 2.0 2.0 1.8 1.9 2.0 2.0 2.0 1.9 2.0 2.1
			1	2.0 1.8 1.9 2.0 1.8 1.9 2.0 2.0 1.9 1.7 2.0 1.9 1.9 1.8 2.0 2.0 1.9 2.1 2.1 2.0 1.9 1.8 1.9 1.9 1.9
Hatchery	Yes	F4	2	1.9 2.2 2.0 1.5 2.0 1.9 2.0 2.0 1.9 2.2 1.9 2.0 1.9 2.0 1.9 1.7 2.1 1.9 2.0 2.0 1.9 1.9 2.0
			3	2.0 1.9 1.9 2.1 2.3 1.8 1.9 1.9 1.8 1.8 2.0 2.0 2.1 2.0 1.9 1.7 1.9 1.8 2.0 2.0 2.1 2.0 2.1 1.9 2.0 1.9 2.0

			1	2.1 1.7 1.8 2.1 2.0 2.1 1.8 2.0 2.1 2.1 2.0 1.9 1.2 2.0 2.0 1.9 1.9 2.0 1.9 1.9 1.9
Hatchery	Yes	F5	2	2.0 1.9 1.9 1.9 1.8 1.8 1.9 1.9 1.9 1.9 1.9 1.9 1.9 2.0 2.0 1.8 2.0 1.9 1.8 1.9 1.8 1.8 1.9 1.9 1.9 1.8
			3	2.1 2.1 2.1 1.9 2.0 1.9 1.9 2.0 1.9 1.9 1.8 1.9 2.0 1.8 1.8 1.9 1.8 1.7 2.0
			1	2.1 2.0 2.0 2.0 2.1 2.0 2.0 2.1 2.0 1.9 1.9 2.0 1.9 1.9 1.8 1.9 1.9 1.9 2.0 2.1 2.1 2.1 1.9
Hatchery	Yes	F6	2	2.0 1.9 1.9 1.9 2.0 2.0 1.9 1.9 1.7 2.0 2.1 2.1 2.1 1.8 2.0 2.0 1.9 2.0 1.9 1.8 1.8 1.9 1.6 2.0 1.9 2.0
			3	1.9 1.9 2.0 1.9 2.0 1.9 2.0 1.9 2.1 1.9 2.0 2.0 1.8 1.9 1.9 2.0 2.0 1.8 2.0 1.9 1.9 2.0 2.2 2.0 1.9
			1	2.2 2.1 2.2 2.1 2.1 2.1 2.1 2.2 2.0 2.2 2.0 2.1 2.0 2.1 2.0 1.9 2.2 2.2 2.1 2.1 2.2 2.0
Hatchery	Yes	F7	2	2.2 1.9 2.1 2.1 2.1 2.0 2.0 2.1 2.2 2.0 2.1 2.0 2.1 2.1 2.0 2.1 2.4 2.1 2.0 2.2 2.2 2.2
			3	2.2 2.2 2.1 2.0 2.2 2.2 2.1 2.1 2.0 1.9 2.0 2.0 1.9 2.0 2.1 2.0 2.3 2.1 1.9 2.0 2.2 2.3 1.9 2.2 2.1
			1	2.6 1.9 2.0 1.8 2.1 2.1 1.8 2.0 1.9 1.9 1.9 2.0 1.8 1.9 1.8 1.9 1.9 1.8 1.9 1.9 2.0 1.9 1.9 1.9 2.0 1.9 1.9 1.8
Hatchery	Yes	F8	2	1.9 1.8 1.9 2.1 1.7 1.9 1.9 2.1 1.9 1.9 1.9 1.9 1.8 1.9 1.9 1.8 1.8 2.1 1.9 1.9 2.0 2.0 2.0 1.8 1.9 1.9 2.0 1.9
			3	1.9 1.9 1.9 1.9 1.8 2.0 1.8 2.0 1.8 2.0 1.7 1.9 1.9 1.9 1.8 1.8 2.0 2.0 2.0 2.0 1.7 2.0 1.8 2.1 2.0
			1	
Hatchery	Yes	F9	2	2.0 2.1 2.0 2.1 2.1 2.1 2.0 1.9 2.0 2.0 1.9 2.1 2.1 1.9 1.8 2.0 2.2 2.0 1.8 2.1 2.0
			3	2.0 2.0 2.0 2.2 2.1 2.1 2.0 2.0 2.0 2.1 1.9 2.0 2.0 1.9 2.1 2.1 2.0 2.1 1.9 2.0
			1	2.0 2.1 0.9 1.7 1.7 2.0 2.0 1.8 1.9 2.0 1.9 1.7 1.9 2.0 1.9 1.9 1.9 2.0 1.7 1.9 2.0 2.0 1.9 1.8 1.1 2.0 1.9 1.9
Hatchery	Yes	F10	2	1.9 1.8 1.8 2.0 1.8 1.8 2.0 2.0 1.9 1.9 1.9 1.9 1.8 1.7 1.8 1.8 2.2 2.0 1.9 1.9 1.9 1.8 1.8 1.9 1.8
			3	2.0 1.7 1.9 1.9 2.0 2.0 2.1 1.9 1.9 1.8 1.9 1.7 1.8 1.9 1.9 1.9 1.8 2.0 1.9 1.8 1.8 2.0 1.8 1.9 2.1 2.1 1.8 2.0 1.9 1.8
			1	1.7 1.7 1.7 1.6 1.6 1.7 1.2 1.6 1.8 1.9 1.8 1.8 1.4 1.5 1.5 1.7 1.5 1.8 1.6 1.5 1.6 1.5 1.5 1.4 1.7 1.7 1.6 1.6 1.7 1.5
Control	No	F1	2	1.7 1.6 1.6 1.6 1.7 1.7 1.5 1.7 1.6 1.4 1.7 1.5 1.4 1.6 1.7 1.7 1.5 2.0 1.8 1.5 1.5 1.6 1.6 1.6 1.6 1.5 1.8 1.6 1.7 1.5
			3	1.6 1.8 1.6 2.0 1.7 1.6 1.6 1.7 1.8 1.4 1.6 1.5 1.6 1.5 1.7 1.7 1.4 1.5 1.5 1.9 1.7 1.6 1.4 1.7 1.6 1.5 1.8
			1	2.0 2.1 2.0 1.4 1.4 1.8 1.8 1.3 1.6 1.6 1.7 1.5 1.5 1.4 1.2 1.6 1.7 1.7 1.3 1.6 1.8 1.7 1.4 1.3 1.8 1.9 1.8
Sham	Yes	F3	2	2.0 2.1 2.1 1.5 1.5 2.0 1.5 1.5 1.3 2.0 1.6 1.4 1.8 1.4 2.0 1.9 1.4 1.9 1.3 1.9 1.9 1.4 1.3 1.3 1.7 1.5 1.4
			3	1.6 1.3 1.4 1.4 1.8 1.5 1.3 1.5 1.6 1.4 1.9 1.6 1.4 1.1 1.5 1.4 1.6 1.3 1.9 1.1 1.5
			1	1.5 1.3 1.8 1.4 1.5 1.7 1.5 1.5 1.4 1.6 1.7 1.4 1.6 1.6 1.2 1.5 1.8 1.7 1.4 1.5 1.4 1.7 1.5 1.6 1.4 1.9
High	No	F4	2	1.6 1.6 1.5 1.7 1.6 1.9 1.5 1.6 1.5 1.5 1.4 1.4 1.8 1.6 1.5 1.5 1.4 1.6 1.5 1.3 1.5 1.5 1.6 1.4 1.4 1.4 1.4 1.5 1.7 1.4 1.2 1.4 1.3 1.5 1.7
			3	1.6 1.6 1.4 1.7 1.7 1.4 1.5 1.4 1.5 1.7 1.7 1.7 1.8 1.6 1.5 1.6 1.2 1.4 1.3 1.7 1.6 1.5 1.5
			1	2.0 1.8 2.0 1.8 2.2 1.7 1.5 1.9 2.2 2.0 2.0 1.9 1.8 2.0 1.8 1.9 1.8 1.9 2.1 1.5 1.9 1.7
Control	No	F5	2	1.7 2.0 1.6 1.7 1.6 1.5 1.6 2.1 1.7 1.8 1.6 1.6 1.8 1.8 1.9 1.5 1.9 1.8 1.8 1.7 1.7 1.7 1.8 1.7 1.8 1.8
			3	1.7 1.6 1.6 1.6 1.8 1.9 1.8 0.7 1.8 1.9 1.8 1.6 1.8 1.9 1.7 1.6 1.8 1.6 1.7 1.8 1.5 1.6 1.9 1.7
			1	1.8 1.7 1.8 1.7 1.7 1.8 1.7 1.7 1.8 1.7 1.7 1.7 1.7 1.7 1.9 1.8 1.9 1.6 1.8 1.9 1.7 1.8 1.6 1.8 1.6 1.8 1.7 1.7 1.7 1.7 1.8 1.7
Sham	Yes	F9	2	1.8 1.8 1.7 1.8 1.8 2.3 1.9 1.8 1.8 1.6 1.7 1.8 1.8 1.8 1.7 1.7 1.9 1.7 1.9 1.9 1.9 1.9 2.1 1.8
			3	1.7 1.7 2.0 1.7 1.8 1.8 1.9 1.6 1.9 2.0 2.0 1.8 1.8 1.8 1.7 1.6 1.8 1.8 1.7 2.0 1.8 1.7
			1	1.7 1.8 1.7 1.6 1.6 1.7 1.7 1.7 1.7 1.7 1.7 1.7 1.7 1.7 1.7 1.7 1.6 1.6 1.7 1.7 1.6 1.6 1.8 1.7 1.8 1.6 1.8
High	No	F12	2	1.8 1.8 1.8 1.7 2.0 1.9 1.9 1.6 1.6 1.7 1.8 1.7 1.8 1.7 1.6 1.8 1.7 1.6 1.7 1.9 1.6 1.5 1.9 1.7 1.9 1.5 1.7 1.6 1.6 1.6
			3	1.9 1.7 1.6 1.8 1.8 1.6 1.7 1.9 1.6 1.7 1.6 1.7 1.7 1.7 1.7 1.6 1.6 1.8 1.9 1.7 1.8 1.6 1.3 1.6 1.8 1.5 1.8 1.7 1.6 1.6
			1	1.5 1.8 1.8 1.5 1.7 1.7 1.7 1.6 1.5 1.4 1.7 2.2 1.7 1.8 1.6 1.7 1.6 1.8 1.6 1.5 1.7 1.7 2.4 1.6 1.6 1.9
Sham	Yes	F14	2	
			3	1.7 1.7 2.0 2.0 1.8 2.0 1.6 1.4 1.7 1.6 1.8 1.7 2.1 1.3 1.8 1.9 1.8 1.7 1.8 1.8 1.7
			1	1.7 1.7 1.5 1.9 2.0 1.8 2.1 2.1 1.8 1.5 1.9 1.7 2.0 2.0 2.0 1.9 1.7 1.8 1.7 1.4 1.6
Control	No	F18	2	1.8 1.7 1.6 1.8 1.7 1.6 2.3 1.8 1.8 1.6 1.8 1.9 1.8 1.8 1.7 1.8 1.5 1.7 1.8 1.8 2.1 1.7 1.6 2.0
			3	1.6 1.8 2.0 1.9 1.5 1.8 1.7 1.7 1.9 1.7 1.5 1.6 1.9 1.8 2.0 1.6 1.9 1.6 1.6 1.8 1.7 1.7 1.6 1.7
			1	1.7 1.7 1.6 1.9 1.9 1.7 1.6 1.8 2.1 1.6 2.2 1.7 1.6 1.9 1.6 2.1 1.5 2.2 1.7 1.6 1.5 1.6 1.6
Low	No	F19	2	1.8 1.9 1.7 1.7 1.6 1.8 2.0 2.0 1.7 1.8 1.8 1.8 1.8 1.7 1.6 1.6 1.6 2.0 2.3 1.7
			3	1.7 1.2 1.8 1.7 1.7 2.2 1.7 1.6 2.0 1.7 1.8 1.8 1.9 1.6 1.8 1.8 1.8 1.7 1.7 1.8 1.7 1.8 1.6 1.6 1.7 1.7 1.8 2.0 1.6 1.6 1.8

Sham	No	F21	1	1.6 1.6 1.6 1.9 1.7 1.5 1.8 1.7 1.8 1.8 1.5 2.0 1.8 1.9 1.8 1.8 1.4 1.6 1.6 1.5 1.6 2.0 2.1 1.5 1.3 1.4
			2	1.4 1.4 1.6 1.5 1.4 1.8 1.6 1.5 1.9 1.7 2.2 1.4 1.7 1.8 2.2 1.7 1.7 1.7 1.6 1.4 1.8 1.4 1.7 1.8
			3	1.7 1.6 1.5 1.6 1.5 1.4 1.4 1.6 1.9 1.5 1.8 1.5 1.7 1.4 1.8 1.7 1.5 1.5 1.8 1.7 1.6 1.5 1.5 1.7 1.5 1.5 1.5 1.5 1.3 1.4 1.5 1.6 1.8
Control	Yes	F23	1	1.7 1.8 1.6 1.7 1.5 1.8 1.6 1.8 1.6 1.7 1.6 1.7 1.4 1.3 1.5 1.8 1.7 1.7 1.9 1.8 1.4 1.7 1.4 1.9
			2	1.7 1.7 1.8 1.6 1.7 1.4 1.6 1.7 1.8 2.0 1.8 1.5 1.4 1.8 1.7 1.6 1.7 1.6 1.7 1.9 1.7 1.6 1.7 1.9 1.5 1.9
			3	1.7 1.5 1.5 1.3 1.6 1.6 1.7 1.6 1.6 2.0 1.7 1.6 1.7 1.6 1.9 1.7 1.6 1.5 1.4 1.6 1.7 1.4 1.7 1.7 1.5 1.6 1.7 1.8 1.7 1.3 1.7
Sham	Yes	F31	1	1.5 1.3 1.4 1.4 1.3 1.5 1.4 1.5 1.6 1.4 1.3 1.2 1.3 1.4 1.3 1.3 1.3 1.8 1.4 1.3
			2	1.5 1.6 1.3 1.4 1.5 1.4 1.3 1.4 1.4 1.7 1.4 1.4 1.5 1.2 1.4 1.5 1.5 1.6 1.3 1.5 1.6 1.6 1.7 1.6 1.4
			3	1.6 1.7 1.4 1.2 0.8 1.7 1.6 1.3 1.6 1.6 1.3 1.5 1.6 1.5 1.4 1.6 1.3 1.8
Sham	Yes	F35	1	1.8 2.1 2.3 1.6 1.9 1.7 1.8 1.8 1.9 1.7 1.8 1.8 1.9 1.8 1.8 1.6 2.0 1.9 1.9 1.7 1.7 1.7 1.8 1.9 1.6 1.8
			2	2.0 1.8 1.8 1.7 1.9 1.7 1.7 1.6 1.8 1.7 1.9 2.0 1.7 2.1 1.8 1.8 2.1 1.9 1.6 1.9 1.9 1.8 1.9 1.8 1.9 1.7 1.7 2.0
			3	2.0 1.8 2.0 1.7 1.7 1.6 1.8 2.3 1.9 1.8 1.8 1.9 2.0 1.7 1.9 1.5 1.6 1.8 1.7 2.1 1.8 1.9 1.8 1.7 2.2 1.8 1.7 1.8 2.0
Control	Yes	F39	1	2.2 1.9 1.9 2.1 2.0 2.2 2.2 2.2 2.1 2.1 2.0 2.2 2.4 2.1 2.1
			2	2.0 2.2 2.0 2.1 2.0 2.2 2.2 2.0 2.2 2.0 2.1 1.9 2.1 2.1 2.1 2.0 2.0 1.9 2.1 2.1 2.3
			3	2.3 2.1 2.3 2.5 2.2 2.3 2.0 2.2 2.2 2.2 2.5 2.5 2.3 2.0 1.9 2.2
High	Yes	F40	1	1.9 2.0 2.0 1.8 2.0 1.9 1.9 2.1 1.9 2.0 2.1 1.6 1.9 1.9 2.1 1.7 1.8 1.8 2.1 2.0 1.9
			2	1.9 2.0 1.9 1.9 1.7 2.3 2.0 2.2 1.9 1.9 1.9 2.0 1.9 1.8 1.9 1.6 2.0 1.9 2.0 2.4
			3	2.1 2.1 1.8 1.9 2.0 2.2 1.9 1.9 2.2 1.7 1.6 2.0 1.7 2.0 2.0 1.9 2.2 1.8 1.9 1.6 2.0
High	Yes	F41	1	1.9 1.8 1.9 1.9 1.8 1.8 1.9 1.9 1.8 1.9 1.8 1.8 1.7 1.7 1.9 1.9 1.9 1.7 1.8 1.9 1.8 1.9
			2	1.8 1.6 1.7 1.7 1.9 1.9 1.6 1.9 1.7 1.6 2.0 2.1 1.7 1.8 1.9 1.8 1.9 1.9 1.8 1.9 2.0 1.8 1.8 1.8 1.9 1.7
			3	1.6 1.8 1.7 1.7 1.8 1.9 1.8 1.9 1.6 1.8 1.7 1.7 1.8 1.8 1.8 1.8 1.5 1.9 1.6 1.8 1.8 1.9 1.7 1.9 1.8
Low	No	F42	1	2.2 2.0 2.3 2.2 2.1 2.2 2.2 2.3 2.1 2.1 1.9 1.8 2.0 1.9 1.9 2.0 2.0 2.2 2.1
			2	2.1 2.0 2.0 1.9 1.9 1.9 2.0 2.1 2.0 2.0 1.9 2.1 2.3 2.1 1.8
			3	2.0 1.7 2.1 2.0 2.2 1.9 2.0 2.0 1.8 2.0 2.2 2.0 2.1 1.9 2.0 2.1 1.9 2.1
Sham	Yes	F43	1	2.1 2.2 2.2 2.2 2.2 2.0 2.1 2.3 2.3 2.2 2.1 2.5 2.5 2.5 2.5 2.1 2.3 2.1 2.8
			2	2.1 2.1 1.9 2.2 2.3 2.5 2.3 1.9 2.0 2.7 2.3 1.9 2.7 2.2 2.3 2.2 2.1 2.2 2.0 2.5 2.0 2.1 1.9 2.0 2.2
			3	1.9 2.0 2.0 2.1 2.1 2.0 3.0 2.3 2.0 1.9 2.3 2.2 2.0 2.1 1.8 2.1 2.3 2.4 2.1 2.3 2.3 2.2 1.9 2.2 2.1
Control	Yes	F44	1	1.5 1.5 1.7 1.8 1.8 1.6 2.0 1.8 1.8 1.8 1.8 1.8 1.7 1.7 1.8 1.7 1.7 1.8 1.7 1.8 1.8 1.7 1.6 1.8 1.8 1.8 1.8
			2	1.8 1.8 2.3 2.3 1.7 1.8 1.9 2.0 2.0 1.9 1.7 1.8 1.9 2.1 1.8 1.8 1.6 1.7
			3	1.8 1.9 1.5 1.7 1.8 1.7 1.8 1.8 1.7 1.8 1.5 1.6 1.5 1.8 1.6 1.9 1.6 1.8 1.8 1.9 1.5 1.9 1.7 1.7 1.7 1.6
High	Yes	F45	1	1.8 1.8 1.9 1.9 1.9 1.9 1.9 1.9 1.7 1.8 2.1 1.8 1.7 2.0 2.0 2.0 1.8 2.0 1.8 2.0 1.8
			2	2.1 2.0 2.1 1.8 1.8 2.0 1.9 2.1 1.8 2.0 2.2 2.1 2.0 2.2 2.1 2.3 1.8 2.4 1.6 2.2 1.8 1.8 1.9 2.0
			3	1.9 1.9 1.8 2.1 1.9 1.9 1.9 1.9 2.2 1.7 1.9 1.9 1.8 2.0 1.8 2.1 2.1
Control	Yes	F46	1	2.2 2.2 2.4 2.0 1.8 2.1 2.0 1.8 2.0 1.9 1.9 2.1 1.9 1.9 1.9 1.8 1.9
			2	2.2 2.2 1.9 1.9 1.8 2.1 1.9 1.9 2.0 1.9 2.1 1.8 2.0 2.0 1.9 2.1 1.8
			3	2.3 2.0 2.3 1.9 2.0 2.0 2.0 2.1 1.9 2.0 2.1 2.1 2.0 2.2 2.1 1.9 2.0
Low	Yes	F47	1	2.1 2.0 2.3 2.5 2.2 1.8 2.4 2.0 2.1 2.1 2.0 2.0 2.3 2.2 2.1 2.2 2.0 2.1
			2	2.1 1.9 2.1 1.9 2.0 2.3 2.0 2.1 2.0 2.1 2.0 2.3 2.2 1.9 2.6 1.9 2.0 2.1
Sham	Yes	F48	1	1.9 2.1 1.6 1.5 1.9 1.8 2.0 1.8 2.0 2.1 1.9 2.1 1.9 2.0 1.9 2.0 2.0 2.1 1.9
			2	1.9 2.1 1.9 2.1 2.0 1.9 1.9 1.9 1.7 2.0 1.8 2.2 1.9 2.4 1.9 2.1 2.1 1.8
			3	1.9 1.9 1.8 2.0 1.9 2.1 1.9 2.1 2.2 1.9 1.8 2.0 2.1 2.3 1.9 2.1 2.0 1.9 2.1

Low	Yes	F49	1	1.8	2.4	2.3	2.4	2.1	2.1	2.1	2.3	1.9	2.2	2.1	2.3	2.1	1.8	2.4	2.1	2.4	2.4	2.3	1.9	1.8
			2	2.0	1.7	1.9	2.0	2.0	1.9	2.1	1.5	2.2	2.1
Control	Yes	F50	1	1.9	1.9	1.9	1.8	1.8	1.9	1.8	1.8	1.8	2.0	1.7	1.6	1.9	1.8	1.8	1.9	1.7	2.0	2.1
			2	2.0	2.1	1.9	2.0	1.9	1.8	1.8	2.1	1.8	1.9	1.7	1.9	1.9	2.0	2.0	2.0	1.9	1.9	1.7	2.1	1.8	1.9
			3	1.8	1.9	1.8	1.8	1.9	2.0	2.1	1.6	2.1	1.8	1.9	1.8	1.9	1.8	1.9	1.9	1.9	2.0	1.9	2.0	2.0
High	Yes	F51	1	1.9	1.9	2.0	2.1	2.0	1.9	2.0	1.8	1.8	1.9	1.9	1.9	2.0	1.9	1.7	1.7	1.7	1.8	1.6	2.0	1.9
			2	2.0	2.1	1.8	2.0	1.9	1.9	1.9	2.0	2.1	2.0	2.1	2.1	2.2	1.9	2.0
			3	1.9	1.9	2.0	2.0	1.8	1.7	2.1	1.7	1.7	1.9	1.8	2.1	2.0	1.8	1.8	2.1
Sham	No	F2	1	2.0	1.9	1.8	2.2	1.7	1.9	1.9	2.0	2.0	1.9	1.8	1.9	1.7	1.9	2.1	1.8	1.6	1.8
			2	1.8	1.7	1.9	1.8	1.8	2.0	1.8	1.6	1.6	1.5	1.8	1.8	1.8	1.8	1.8	1.8	1.9	2.0	1.7	1.7
			3	1.7	1.7	1.8	1.7	1.8	1.7	1.7	1.7	1.6	1.8	1.7	1.7	1.7	1.6	1.9	1.8	1.7	1.5	1.7	1.7	1.7	1.8
High	Yes	F3	1	1.8	1.8	1.9	1.8	1.9	1.8	2.0	1.9	1.7	1.8	1.6	1.9	2.0	1.8	2.0	2.1	1.7	1.9	2.0	2.1	2.0
			2	2.0	1.8	1.9	1.7	1.9	1.8	1.8	1.9	2.0	1.8	1.6	2.0	1.9	1.9	2.0	1.8	1.8	1.8	1.9	2.1	2.0	1.8
			3	1.8	2.0	1.8	2.0	1.8	2.0	1.7	1.9	1.8	1.9	2.0	1.9	1.9	1.8	2.0	2.1	1.7	1.9	1.8	2.1	1.8	2.0	1.9
High	No	F4	1	1.6	2.3	1.6	1.6	1.7	1.7	1.6	1.7	1.7	1.7	1.6	1.7	1.6	1.7	1.5	1.8	1.4	1.7	1.6	1.8	2.1	2.0
			2	1.8	1.7	1.9	1.6	2.0	1.9	1.9	1.8	1.6	1.6	2.0	1.9	1.6	1.9	1.8	1.7	1.7	1.5	1.9	1.6
			3	1.8	1.8	1.8	1.6	1.7	1.6	2.3	2.0	1.6	1.5	1.7	1.5	1.7	2.3	1.8	1.8	1.8	1.6	1.5
Control	No	F9	1	1.8	1.8	1.9	2.0	1.8	2.0	1.8	2.0	1.9	1.8	1.9	2.0	1.9	1.8	1.7	1.9	1.5	1.9	1.9
			2	2.0	1.9	2.1	1.9	1.8	2.0	1.9	1.7	1.8	2.0	2.0	1.9	2.0	2.0	1.9	1.7	1.9	1.9	1.7	1.7
			3	2.3	1.6	1.9	2.1	1.8	1.9	2.1	2.1	2.0	1.8	2.0	2.0	1.9	1.9	1.8	1.7	1.7	1.9	2.0	1.9	2.0	2.0	1.8	2.1	2.1
Sham	No	F10	1	1.7	1.7	1.7	1.7	1.7	1.6	1.8	1.7	1.7	1.7	1.7	1.7	1.6	1.9	1.8	1.6	1.7	1.6	1.7	1.6	1.7	1.7	1.7	1.7
			2	1.6	1.7	1.7	1.8	1.8	1.6	1.6	1.7	1.7	1.6	1.7	1.6	1.7	1.7	1.8	1.7	1.7	1.8	1.7	1.5
			3	1.6	1.6	1.6	1.6	1.6	1.7	1.8	1.6	1.6	1.7	1.9	1.7	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.8	1.8	1.7
High	Yes	F12	1	2.0	2.1	1.9	2.0	1.9	1.9	1.9	2.0	2.1	2.0	2.0	1.9	1.9	2.0	1.9	1.9	2.1	2.1	2.0	2.1
			2	2.1	2.1	2.1	2.1	2.1	2.1	2.0	2.0	2.0	2.0	2.0	2.1	1.8	2.0	1.9	2.1	1.9	2.0	2.1	1.9
			3	2.0	2.0	1.9	2.2	2.2	2.0	2.0	2.0	2.1	1.9	2.0	2.1	2.1	2.2	2.1	1.9	2.0	2.0	1.9	2.0	1.9	2.0	1.7	2.0	2.0	2.1	1.8
Control	No	F13	1	1.8	1.8	2.0	1.9	1.7	1.6	1.5	1.9	1.9	1.8	1.9	1.8	1.8	1.7	1.8	1.8	1.8	1.9	1.6	1.8	1.9	1.8	1.3	1.7	1.8	1.6
			2	1.6	1.7	1.8	1.7	1.9	1.7	1.9	1.9	1.9	1.9	1.8	1.9	1.8	1.9	1.8	1.8	1.9	1.8	1.8	1.9	1.9	1.8	1.7	1.8	2.0	1.8	1.7
			3	2.5	2.0	1.8	1.8	1.8	1.8	1.9	1.6	1.7	1.8	1.8	1.9	1.6	1.8	1.8	1.9	1.8	1.7	1.8	1.9	2.1
High	No	F17	1	1.8	1.9	1.8	1.8	1.9	2.1	1.8	2.2	1.8	1.9	2.0	1.8	2.1	2.1	2.0	2.0	2.0	1.9	2.0
			2	2.0	2.1	2.0	2.3	1.9	1.9	2.1	1.7	2.1	1.9	2.0	2.1	2.1	2.0	1.9	2.1	2.1	1.9
			3	1.9	2.1	1.8	2.1	1.9	2.2	1.9	1.9	2.0	1.9	2.0	1.9	1.9	1.8	2.1	2.0
High	No	F19	1	2.1	2.1	2.1	1.9	2.0	2.0	1.9	2.0	1.9	2.0	2.0	1.9	2.0	1.9	2.1	2.0	2.0	2.2
			2	2.1	2.1	1.9	2.2	2.0	1.9	2.1	1.9	1.9	1.9	2.0	2.1	2.0	2.0	2.1	1.7	2.0	1.9	2.0
			3	1.9	2.0	2.0	1.9	2.2	2.0	1.8	2.0	1.8	2.0	1.8	1.9	2.1	1.9	1.9	1.9	2.3
Low	No	F20	1	1.7	1.7	1.8	1.7	1.5	1.8	1.7	1.7	1.6	1.8	1.7	1.7	1.7	1.7	1.8	1.8	1.8	1.7	1.7	1.7	1.6	1.8	1.6	1.8	1.6	1.8	1.8
			2	1.9	1.8	1.6	1.6	1.8	1.7	1.7	1.8	1.8	1.8	1.8	1.8	1.7	1.6	1.8	1.8	1.8	1.8	1.7	1.6	1.8	1.8	1.8
			3	1.8	1.7	1.7	1.8	1.8	1.8	1.7	1.8	1.7	1.8	1.9	1.6	1.8	1.8	1.8	1.7	1.7	1.8	1.6	1.8	1.6	1.9
Low	No	F24	1	1.7	2.0	1.7	1.4	1.5	1.9	1.6	1.5	1.8	1.6	1.7	1.5	1.6	1.7	1.8	1.8	1.7	1.8	1.8	1.7	1.6
			2	1.6	1.7	1.7	1.5	1.9	1.9	1.5	1.6	1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.8	1.5	1.8	1.7	1.6	1.6	1.5	1.6
			3	1.8	1.7	1.7	1.8	1.6	1.8	1.6	1.8	1.6	1.4	1.3	1.6	1.4	1.4	1.5	1.8	1.8	1.7	1.7	1.7
Control	Yes	F25	1	2.0	2.0	2.2	2.1	2.4	1.8	2.1	2.0	2.0	2.1	2.2	2.0	1.9	2.1	2.0	2.1	2.0	2.1	2.2	2.0	2.1	2.0
			2	2.1	2.1	2.1	2.2	2.1	2.1	2.1	2.2	2.1	1.9	2.0	2.0	2.0	2.1	2.0	2.2	2.1	2.1	2.1	1.9	2.1	2.1
			3	2.1	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.1	2.0	2.3	2.1	2.3	2.6	2.1	2.0	2.4	2.2	2.3	2.0

			1	1.9	1.9	2.0	1.9	2.1	2.0	1.9	1.9	2.0	2.0	1.9	2.2	2.0	2.1	2.0	2.1	1.9	2.0	2.1	2.0	2.0	2.2	2.0	2.1	1.9	1.9		
Sham	Yes	F27	2	1.8	1.9	2.1	2.1	2.1	2.1	2.1	1.9	1.8	1.9	2.0	2.2	2.1	2.1	1.9	1.9	2.0	2.1	1.9	2.0	1.7	2.0	2.0	2.1	2.1	2.0	2.0	2.0
			3	1.9	2.0	2.2	2.0	2.1	2.1	1.9	2.0	2.0	2.1	2.1	2.1	2.0	2.0	2.0	1.8	2.0	1.9	1.9	2.0	2.1	2.2	2.1	2.0	2.0	1.9	1.9	
			1	2.0	2.1	2.3	2.0	2.1	1.9	2.1	2.1	1.9	2.1	2.0	2.0	1.8	2.0	2.2	2.1	2.1	2.1	1.9	2.1								
Control	Yes	F29	2	2.2	2.2	2.0	2.0	2.2	2.1	2.1	2.2	2.2	2.2	2.1	2.2	2.1	2.2	2.3	2.2	2.1	2.0	2.1	2.1	2.1	2.0	2.0	2.1				
			3	2.1	2.2	2.1	2.1	2.2	2.2	2.0	2.0	2.0	2.2	2.1	2.0	2.2	2.2	2.2	2.2	2.2	2.1	2.1	2.1	2.2	2.1	2.1	2.1				
			1	2.1	1.9	2.8	2.1	2.0	2.0	2.1	2.1	2.0	2.0	2.1	2.0	2.1	2.0	2.0	1.9	2.0	2.2	2.0	1.9	2.0							
Low	Yes	F31	2	2.0	2.1	2.2	2.1	2.2	2.0	1.9	2.1	2.1	2.0	2.2	2.0	2.2	2.1	2.1	2.0	2.1	1.9	2.3									
			3	2.1	2.2	2.2	2.2	2.2	2.2	2.0	2.1	2.2	2.1	2.1	1.9	2.0	2.1	2.0	2.1	2.1	1.9	1.9	2.0								
			1	2.1	1.9	1.9	1.9	1.7	1.8	1.8	1.9	1.9	2.0	1.8	1.9	1.8	2.0	1.8	1.7	1.8	1.9	1.9	1.7	1.8	1.8	1.9					
Low	Yes	F32	2	1.9	2.0	1.8	1.9	2.0	1.8	1.9	1.9	2.0	2.0	1.9	1.8	1.8	2.0	1.9	2.0	2.0	1.9	1.8	1.9	1.8	1.8	1.8	2.0				
			3	2.0	1.8	1.8	1.8	1.8	1.9	1.9	1.8	1.8	1.7	1.8	1.8	1.8	1.8	1.8	2.2	1.9	1.5	1.9	2.0	1.7	1.8	1.9					
			1	2.2	2.1	1.7	2.0	1.8	1.6	2.1	1.7	2.2	1.9	2.1	2.2	2.1	2.1	2.1	2.2	2.4	2.4	2.7									
Control	No	F33	2	2.4	2.2	2.1	2.2	2.2	2.0	2.0	2.1	2.0	2.1	2.3	2.1	2.1	2.0	1.8	2.0	2.2	2.0	2.3	2.1								
			3	1.7	2.1	2.4	2.2	1.9	1.7	1.9	2.0	2.1	2.2	1.9	2.3	2.1	1.9	1.7	1.6	2.1	2.1	1.7	1.7	2.0	1.7	2.2	2.2				
			1	2.0	2.0	2.1	2.2	2.0	2.1	2.1	2.1	1.9	2.2	1.9	2.2	2.0	2.0	2.0	2.0	2.0	2.0	2.0									
Control	Yes	F34	2	1.9	2.1	2.1	1.9	2.1	2.1	2.0	2.1	2.1	1.9	2.2	2.1	2.1	2.2	2.0	2.2	2.0	2.3	2.2	1.9	2.0	1.9						
			3	2.1	2.0	2.0	2.1	2.0	2.1	2.3	2.0	2.1	2.0	2.0	2.0	2.0	2.1	2.2	2.1	2.1	1.9	2.1	2.2	2.1	2.0	1.9	2.2	2.0	2.1	2.0	
			1	2.1	2.1	1.9	2.0	2.0	2.0	2.0	1.9	2.1	2.1	2.1	2.0	2.0	2.5	2.0	2.5	2.1											
Low	Yes	F36	2	2.2	2.1	2.1	2.0	1.9	1.9	2.0	2.1	1.9	2.0	2.0	2.1	2.1	2.0	2.1	2.2	2.0	1.9										
			3	1.9	2.1	2.0	2.0	2.0	1.9	2.0	2.1	2.1	1.8	2.1	1.9	1.9	2.1	2.0	2.0	2.0	2.2	2.1	1.9	2.1	2.1						
			1	2.2	2.0	2.2	2.2	2.1	2.2	2.0	2.2	2.0	2.3	2.2	2.1	2.1	1.8	2.1	2.1	2.2	1.9	2.0									
Sham	Yes	F37	2	2.1	2.3	2.1	2.0	2.1	2.1	2.1	2.2	2.0	2.1	1.9	2.2	2.3	2.2	1.9	2.0												
			3	1.8	2.1	1.9	2.1	2.2	2.2	2.1	2.2	2.1	2.0	2.3	1.9	2.1	2.1	2.2	2.0	2.2											
			1	1.4	1.6	1.7	1.8	1.4	1.8	1.5	1.4	1.7	1.6	1.6	1.7	1.4	1.5	1.7	1.5	1.6	1.7	1.6	1.8	1.4	1.6	1.6	1.6				
High	No	F39	2	1.9	1.9	2.1	1.8	1.7	1.6	1.6	1.9	1.7	1.6	1.3	1.7	1.5	1.5	1.6	2.0	1.6	1.8	2.1	1.6	1.6							
			3	1.7	1.7	1.7	1.6	1.7	1.6	1.8	1.5	1.6	1.7	1.5	1.7	1.8	1.6	1.6	1.6	1.7	1.6	1.6	1.6	1.6	1.6	1.5	1.8	1.8			
			1	2.1	2.1	2.2	2.2	2.4	2.3	2.0	2.0	2.1	2.1	2.1	2.1	2.0	2.0	2.1	2.2	2.2	1.9	2.1	2.2	2.0	2.1	2.1					
Wild	Yes	W1	2	2.1	2.3	2.2	2.1	2.0	2.2	2.3	2.3	2.3	2.3	2.1	2.1	2.5	2.0	2.1	2.2	1.9	2.3	2.3	2.1	2.1	2.0						
			1	1.9	2.0	2.0	2.1	2.0	2.0	2.1	2.0	2.0	2.2	1.9	1.8	2.1	2.1														
Wild	Yes	W2	2	2.2	2.0	2.4	1.9	2.0	2.2	1.9	2.0	2.2	2.1	2.0	2.0	2.0	2.3	2.1	1.9												
			1	2.1	2.3	2.3	2.1	2.4	2.2	2.4	2.4	2.2	2.2	2.2	2.2	2.2	2.5	2.4	2.5	2.1	2.1										
Wild	Yes	W3	2	2.3	2.1	1.7	2.5	2.2	2.3	2.4	2.3	2.3	2.6	2.4	2.3	2.4	2.4	2.2	2.1	2.2	2.0	2.3	2.5								
			1	2.1	2.1	1.8	2.1	2.2	2.0	2.3	2.0	2.0	2.2	2.0	2.0	2.1	2.0	2.4													
Wild	Yes	W4	2	2.1	1.9	2.1	2.0	2.0	2.2	2.0	2.1	2.1	2.0	2.2	2.1	1.9	2.1	2.0	1.8	2.0	2.2										
			1	2.0	2.0	2.2	1.0	1.9	2.3	2.2	1.7	2.2	2.2	1.5	2.3	2.3	2.2	1.4	1.9	2.2	0.9	1.8	2.1	2.1	2.3						
Wild	Yes	W5	2	2.5	2.3	1.1	2.1	2.4	2.1	1.3	1.0	2.1	2.2	2.5	2.5	2.2	2.3	1.8	2.9	2.2	2.1	2.5									
			1	2.3	2.1	2.4	2.0	2.3	2.1	2.1	2.0	2.0	1.9	2.1	2.4	2.2	2.2	2.1	2.1	2.5	2.0	1.9	1.8								
Wild	Yes	W7	2	2.4	2.1	2.4	2.1	2.0	1.9	1.9	2.3	2.0	2.0	2.0	2.0	2.1	2.4	2.5	2.1	2.3	2.4	2.1	2.5	2.5							
			1	2.2	2.1	2.1	2.1	2.6	2.3	2.2	2.2	2.0	2.0	2.3	2.3	2.3	2.2	2.0	2.2	2.2	2.4										
Wild	Yes	W8	2	2.0	1.8	2.2	2.0	2.0	2.0	2.0	2.5	1.8	2.0	2.2	2.3	2.1	2.0	2.3	2.2	2.1	2.2	2.1	2.1	2.1	2.2	2.0					
			1	2.1	2.1	2.1	2.4	2.3	2.3	2.0	2.1	2.1	2.2	2.1	1.9	2.2	2.2	2.3	2.2	2.1											
Wild	Yes	W9	2	1.9	2.1	2.0	2.1	2.3	2.2	2.1	2.3	2.0	2.2	2.3	2.0	2.3	2.2	2.3													
			1	2.2	2.0	2.3	2.1	2.0	2.5	2.3	2.0	2.2	2.2	2.3	2.1	2.2	2.2	2.0	2.0	2.2	2.3	2.1	2.0	2.2							
Wild	Yes	W10	2	2.3	2.2	2.1	2.2	2.0	2.2	2.1	2.1	2.2	2.1	2.1	2.1	2.0	2.0	2.1	2.1	2.1											

Wild	Yes	W11	1	2.1 2.3 2.2 2.3 2.3 2.5 2.1 2.1 2.3 1.8 2.1 2.4 2.7 2.2 3.0 2.4 2.0 1.9 2.2 2.1 2.1
			2	2.0 2.2 2.1 2.4 2.1 2.1 2.0 2.0 1.9 1.9 2.1 2.4 2.1 2.1 2.3 2.1 2.1 2.2 2.1
Wild	Yes	W12	1	2.0 2.2 2.1 2.1 2.0 2.3 2.0 1.9 2.0 2.1 2.1
			2	2.3 2.1 2.1 2.2 2.2 2.0 2.1 2.2 2.1 2.1 2.0 1.9 1.9 1.9 2.2 2.1 2.4 2.0 2.1
Wild	Yes	W13	1	2.1 2.1 2.0 2.9 1.9 1.9 2.2 2.2 2.0 2.7 2.2 2.3 2.4 1.9 2.2 2.1 2.1 2.1 2.8 2.2 2.1 2.2
Wild	Yes	W14	1	1.9 2.0 1.9 1.7 2.0 1.8 1.8 2.0 1.9 1.7 1.6 1.8 1.8 1.9 1.7 1.7 1.8 1.9 1.9 1.8 1.8 2.1 2.0
			2	1.7 1.9 1.9 2.1 2.0 1.9 1.9 1.7 2.0 1.8 1.9 2.0 1.9 1.9 1.7 2.0 1.8 1.7 1.7 2.1 1.9 1.9 2.0 2.1 2.0 2.0 1.8
Wild	Yes	W15	1	2.7 2.1 2.2 1.9 2.3 2.1 2.3 2.2 2.2 2.2 1.9 2.6 2.3 2.2 2.3 2.2 2.1 2.3 2.0 2.2 2.0 2.4
			2	2.1 2.4 2.4 2.3 2.0 2.2 1.9 2.1 2.0 2.2 2.3 2.3 2.2 2.4 2.0 1.9
Wild	Yes	W16	1	2.4 2.0 2.0 2.1 1.9 2.1 2.1 1.9 1.9 2.0 1.9 1.9 2.1 1.9 1.9 2.0 2.2 2.3 1.9 2.0 2.0 2.0 2.5 1.9
			2	2.0 2.0 1.9 2.0 2.1 1.9 2.0 2.2 2.4 2.0 2.0 2.0 2.3 2.0 1.8 2.1 2.1 1.9 2.2 2.2
Wild	Yes	W17	1	2.3 2.4 2.2 2.2 2.3 2.1 2.4 2.5 2.2 2.4 2.1 2.2 2.3 2.2 2.4 2.2 2.5 2.5 2.4 2.1 2.5 2.3 2.0 2.2
			2	2.6 2.4 2.2 2.9 2.3 2.4 2.4 2.4 2.4 2.3 2.6 2.2 2.1 2.3 2.7 2.5 2.3 2.2 2.3 2.5 2.4 2.5
Wild	Yes	W18	1	2.1 2.2 2.1 2.2 2.1 2.1 1.9 1.9 2.0 2.2 2.1 2.1 2.0 2.1 1.9 2.0 2.1 2.1 2.1 2.1 2.1 2.0 2.3 2.3 2.1
			2	2.1 2.1 2.2 2.2 2.3 2.1 2.1 2.1 2.0 2.1 2.2 1.9 2.1 2.4 2.0 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.1 2.0 2.1 2.3 2.1 2.2 2.5 1.8
Wild	Yes	W19	1	2.2 2.2 2.1 2.2 2.4 2.3 2.1 2.4 2.2 2.4 2.2 2.1 2.0 2.0 2.1 2.5 2.2 2.3 2.0 2.1 2.2
			2	2.2 2.4 2.2 2.2 2.5 1.9 2.0 2.1 2.3 2.1 2.1 2.3 2.0 2.5 2.3 2.0 2.3 2.2 2.4 2.4 2.0 2.2 2.1 1.9 2.0 1.8 2.2
Wild	Yes	W20	1	2.3 2.1 2.2 2.2 2.2 2.3 2.3 2.3 2.3 2.3 2.4 2.3 2.4 2.4 2.1 2.5 2.3 2.1 2.2 2.3 2.4
			2	2.3 2.1 2.1 2.3 2.4 2.2 2.3 2.3 2.1 2.4 2.4 2.3 2.3 2.1 2.1 2.1 2.2 2.4 2.4
Wild	Yes	W21	1	1.9 2.2 2.1 2.1 2.2 1.7 2.0 2.1 2.1 1.8 1.8 2.0 1.9 2.1
			2	2.0 2.0 1.9 1.9 2.1 2.0 2.0 2.0 1.9 2.0 2.0 2.0 2.0 1.9 2.1 2.0 2.1 2.0
Wild	Yes	W22	1	2.0 2.2 2.0 2.1 2.1 2.1 2.0 2.3 1.8 2.0 2.1 2.0 2.1 2.0 2.0 2.1 1.9 2.4 2.1 1.8
			2	2.3 2.2 2.1 1.9 2.2 2.1 2.1 2.3 2.1 2.3 2.2 1.9 2.0 2.6 2.0 2.5 2.1
Wild	Yes	W23	1	1.9 2.2 1.9 1.9 1.9 2.0 2.0 2.0 2.1 2.1 2.0 2.1 2.4 2.2 2.0 1.9 2.0 1.9 1.9
			2	2.4 2.2 2.0 2.3 2.0 2.0 2.0 2.0 2.3 2.4 2.0 2.0 2.0 2.3 2.0 2.3 2.1 2.1 2.4 1.8 2.6
Wild	Yes	W24	1	2.1 2.4 1.8 2.4 2.1 1.9 2.0 1.8 1.5 2.0 1.9 1.9 2.0 2.1 1.9 2.2 1.8 1.9 2.0 1.8 1.7 1.8 2.2 1.9 2.1 1.8 1.9 1.9 2.1 2.1 2.0 1.9 1.8 1.8
			2	2.1 2.1 2.0 2.1 2.2 2.3 2.1 2.5 2.2 2.0 2.5 2.0 2.0 2.1 2.2 2.2 2.0 1.8 2.5 2.1 2.2 1.8
Wild	Yes	W25	1	2.3 2.1 2.0 2.5 2.1 2.3 2.5 2.1 2.1 2.6 2.2 2.3 1.9 2.2 2.1 2.0 2.0 2.1 2.4 2.1 2.2
			2	2.1 2.2 2.1 2.1 2.2 1.9 2.2 2.0 2.1 2.2 2.2 2.3 2.2 2.3 2.3 2.3 2.0 2.0 2.3
Wild	Yes	W26	1	2.1 2.0 2.0 2.1 2.1 2.1 2.0 2.2 2.1 2.1 2.1 2.2 2.1 2.1 2.0 2.2 2.2 2.0 1.9 2.1 2.0 1.9 2.0
			2	2.3 2.1 2.2 2.2 2.6 2.0 2.2 2.3 2.2 2.3 2.0 2.3 2.3 2.3 2.3 2.0 2.1 2.1 2.1 2.3 1.9

APPRENDIX 3: RAW DATA OF SPERM METRICS FROM COMPUTER ASSISTED SPERM ANALYSIS OUTPUT

Table A.9 Raw data from computer assisted sperm analysis output of hatchery-reared bloater (*Coregonus hoyi*) sperm. Sperm metrics for each sample include the total number of sperm (total #), the number of motile sperm (motile), progression (prog), average path velocity (VAP, um/s), straight line velocity (VSL, um/s), curvilinear velocity (um/s), lateral head displacement (ALH, um), beat cross frequency (Hz), straightness (STR), linearity (LIN, %) and longevity (Long, s).

Male ID	Post-Activation (s)	Total #	Motile	Prog	VAP (µm/s)	VSL (µm/s)	VCL (µm/s)	ALH	BCF (Hz)	STR	LIN (%)	Long (s)
1 A_1	5	102	78	35	81.1	59.4	121.6	5.3	29.1	67	50	
1 A_1	10	170	137	54	80.8	58.1	116.1	5.2	32.2	67	49	
1 A_1	15	214	166	72	74.6	54.7	110.1	5.7	37.0	69	51	72
1 A_2	5	42	34	15	86.3	62.2	115.6	3.9	31.0	69	55	
1 A_2	10	44	33	10	73.6	46.1	106.6	2.2	34.8	56	46	
1 A_2	15	67	48	16	63.6	42	100.1	3.5	42.2	63	45	49
1 A_3	5	143	106	51	70	51.4	107	4.7	40.4	68	50	
1 A_3	10	154	115	56	70.5	51.9	107.2	5.1	40.7	69	52	
1 A_3	15	159	103	31	70.2	46.8	107.8	5	30.0	61	44	47
1 B_2	5	144	69	36	71.8	56.3	100.4	3.5	36.2	72	56	
1 B_2	10	155	85	34	59.9	43.1	81	3.7	41.6	68	53	
1 B_2	15	158	96	51	58.7	44.9	77.9	2.8	40.5	74	60	53

1 B_3	5	185	140	65	82.5	62.1	108.7	3.4	32.0	71	58	
1 B_3	10	271	207	103	72.7	55.9	102.2	4.3	40.5	72	56	
1 B_3	15	254	203	86	67.2	48.6	94.9	3.5	36.7	69	54	48
1 C_1	5	143	79	32	56.6	42.6	88.9	4	42.7	68	49	
1 C_1	10	124	60	22	55.5	38.7	89.1	4.3	44.9	68	48	
1 C_1	15	118	56	19	40.9	27.8	67.5	4.2	47.5	66	47	50
1 C_2	5	177	123	52	63.3	47.9	103.5	4.8	33.0	68	47	
1 C_2	10	206	139	67	59	43.5	96.3	4.5	40.3	71	50	
1 C_2	15	195	108	44	47.4	33.5	80.6	5	45.3	66	46	54
1 D_3	5	115	52	14	47.6	32.1	77.0	3.1	46.9	67	50	
1 D_3	10	104	42	19	37.7	28.4	59.5	2.9	39.6	75	56	
1 D_3	15	102	33	14	39.5	28.6	66.9	2.4	41.2	71	51	43
1 $Fresh_3$	5	67	47	24	59.6	43.7	96.7	3.5	42.5	66	53	
1 $Fresh_3$	10	49	32	9	67.6	41.8	93.1	2.3	41.4	56	45	
1 $Fresh_3$	15	48	26	11	51.4	36.3	73.1	3.6	47.1	64	53	43
2 $Fresh_3$	5	198	127	38	65.5	44.7	108.7	7.1	30.6	61	38	
2 $Fresh_3$	10	137	76	17	45.3	28.2	84.4	4.5	49.9	56	36	
2 $Fresh_3$	15	148	83	29	50.2	34.7	82.8	4.5	44.3	64	43	44
2 D_2	5	215	118	38	49.5	33.9	78.6	5.1	44.6	65	45	
2 D_2	10	162	85	28	41.3	27.3	63.5	2.8	46.1	63	47	
2 D_2	15	172	92	27	41.8	25.4	71.7	3.7	48.8	58	41	40
2 D_3	5	276	181	63	58.5	38.7	91.8	4.7	42.6	64	44	
2 D_3	10	224	113	41	49.6	33.7	79	4.7	45.5	66	47	
2 D_3	15	215	112	32	52.1	34.5	85.9	5.6	44.4	63	44	45

3 B_1	5	142	27	13	53.8	34.2	92.6	4.1	32.6	59	38	
3 B_1	10	132	24	5	45.4	27.9	70.8	1.8	52.4	57	40	
3 B_1	15	138	36	12	45.3	27.2	77.8	5.6	40.8	56	37	29
3 C_1	5	236	154	49	65	43.3	100.9	4.8	32.4	61	42	
3 C_1	10	277	168	64	67	48.4	102.6	4.7	33.1	67	48	
3 C_1	15	295	157	67	56.1	40.4	84	4.4	34.9	69	51	48
4 $Fresh_1$	5	152	91	27	71.9	47.7	102.2	3.9	26.4	64	46	
4 $Fresh_1$	10	158	74	31	52.6	38.7	79.3	3.7	46.9	66	51	
4 $Fresh_1$	15	155	76	31	57.5	42.7	81.1	3.3	44.1	68	55	45
4 $Fresh_2$	5	114	84	36	85.8	64.8	129.9	6.3	38.1	64	46	
4 $Fresh_2$	10	106	69	25	63.2	42.6	96.9	4.8	32.0	62	46	
4 $Fresh_2$	15	98	58	30	50.2	39	82.5	4	43.2	74	56	40
4 A_1	5	227	31	10	44.8	31.5	79.9	3.1	43.4	64	46	
4 A_1	10	199	29	11	44	31.3	76.9	4.3	43.3	62	43	
4 A_1	15	196	30	10	40.4	28.4	63.5	3.9	38.2	63	46	34
4 C_2	5	176	44	18	44.2	33.7	69.3	3.5	46.5	68	50	
4 C_2	10	182	45	17	44.3	31.2	81.9	4.2	38.7	67	45	
4 C_2	15	153	35	15	58.5	43.3	93.6	4.4	40.0	67	48	28
5 Fresh	5	199	182	100	100.0	78.4	122.9	4.5	17.0	75	63	
5 Fresh	10	202	168	99	64.8	52.8	79.7	2.9	39.4	78	66	
5 Fresh	15	195	152	81	69.3	53	83.7	3.1	34.6	73	63	50
5 A_2	5	155	146	81	113.8	89.1	130.3	4.5	11.8	75	67	
5 A_2	10	149	127	53	79.4	54.2	93.7	3.4	24.9	66	58	
5 A_2	15	139	116	52	64.9	47.8	79.4	2.9	38.3	70	61	46

5 C_1	5	92	84	84	75.8	62.3	92.1	3.1	23.1	84	71	
5 C_1	10	99	79	41	45	35.6	51.8	2.2	49.9	80	68	
5 C_1	15	103	66	40	41.7	36.6	55.9	2.3	47.5	89	73	30
5 C_2	5	56	25	9	75.5	51.4	98.3	3.4	36.2	62	49	
5 C_2	10	93	41	21	64.9	52.3	78.7	2.4	45.6	76	66	
5 C_2	15	55	30	17	51.2	39.9	60	1.6	41.9	76	68	38
5 D_1	5	95	80	53	76.2	64.3	86.4	2.7	33.1	82	74	
5 D_1	10	99	60	35	59.8	49.8	67.6	2	43.1	81	73	
5 D_1	15	90	35	15	35.4	26.7	46.5	1.7	51.8	75	62	33
5 D_2	5	131	129	102	90.4	80.9	108.5	3.6	21.4	88	76	
5 D_2	10	123	105	33	60.3	40.6	74.2	2.7	22.8	65	54	
5 D_2	15	107	70	26	47.4	32.8	59.1	2.7	40.7	65	56	37
6 Fresh	5	76	61	23	97.9	73.3	121.9	5.1	15.9	70	57	
6 Fresh	10	71	51	29	87.6	68	101.7	3.7	25.9	73	64	
6 Fresh	15	60	37	18	62.9	47.6	74.6	3.6	31.1	72	63	40
6 A_1	5	118	114	81	106.7	92.1	123.4	6.5	17.1	86	75	
6 A_1	10	103	71	36	66	51.5	85.8	3.3	45.2	70	58	
6 A_1	15	86	49	24	66.9	51.1	80.1	2.2	19.5	74	65	41
6 A_2	5	81	52	36	103.2	88.4	115.2	3.7	11.8	84	74	
6 A_2	10	71	40	23	83.3	65.8	95.2	2.3	18.6	70	64	
6 A_2	15	54	23	10	63.5	43.6	69.9	2	14.1	66	62	47
6 C_1	5	151	71	34	70.5	51.9	92.4	3.7	21.9	69	55	
6 C_1	10	134	61	31	55.8	42.3	71.3	3	35.4	75	61	
6 C_1	15	131	53	36	50.7	44.9	63.7	3	28.7	86	72	38

6 C_2	5	100	72	24	56	38.9	83.7	4.8	24.8	65	44	
6 C_2	10	210	78	32	43.6	31.8	70.7	3.7	42.9	71	52	
6 C_2	15	180	68	27	40.1	29.8	65.7	4.2	30.2	71	49	36
6 D_2	5	66	54	24	64.1	45	81.0	2.7	35.8	71	58	
6 D_2	10	70	21	9	43.3	29.6	50.1	1.8	52.9	59	54	
6 D_2	15	70	19	11	34.2	29	44.5	1.8	44.4	82	66	33
7 Fresh	5	192	110	44	66.1	48.1	84.2	3.3	20.7	67	54	
7 Fresh	10	154	82	44	59	46.7	74.6	2.9	21.2	75	63	
7 Fresh	15	145	67	43	51.2	42.6	63.1	2.5	40.5	80	67	44
7 A_1	5	28	12	10	106.8	98.1	120.7	3.1	22.5	89	78	
7 A_1	10	30	9	6	77.1	66.8	105.2	3.4	26.7	79	63	
7 A_1	15	7	4	1	67.5	25.8	69.4	0.4	31.6	37	36	30
7 A_2	5	41	23	13	107.7	91.7	119.8	3.1	16.2	77	70	
7 A_2	10	49	30	19	76.1	61.4	80.6	1.6	39.0	77	73	
7 A_2	15	48	29	15	58.1	43.7	64.8	1.7	43.6	72	67	39
7 B_1	5	69	21	11	82.5	64.9	92.3	4.7	16.9	76	66	
7 B_1	10	67	18	10	74.2	60	84.1	1.7	42.0	77	70	
7 B_1	15	66	18	12	57.8	46	64.4	2.1	38.6	82	74	34
7 C_1	5	171	77	26	54.6	39.6	85.2	4.9	34.2	66	44	
7 C_1	10	82	36	17	53	39.4	67.9	3.9	46.5	69	57	
7 C_1	15	73	29	12	52	38	65.4	2.9	29.3	67	56	38
7 D_1	5	75	29	10	60.4	42.6	73.1	2.8	44.2	68	56	
7 D_1	10	65	12	8	36.1	27.5	56.2	1.8	54.1	75	64	
7 D_1	15	48	10	6	37.2	30.6	44.2	1.6	39.1	81	70	34

7 C_2	5	242	23	12	73.5	56.8	90.8	5.4	21.9	72	59	
7 C_2	10	264	51	24	40.3	33.6	54.6	3.4	26.2	85	69	
7 C_2	15	142	20	9	44.5	34.1	66.7	2.5	43.1	74	57	30
7 D_2	5	135	84	43	82.7	65.3	97.7	3	18.3	72	62	
7 D_2	10	114	47	27	52.3	40.3	62.2	2.2	32.6	75	65	
7 D_2	15	110	25	12	34.3	26.9	46.3	1.6	56.0	76	62	43
8 Fresh	5	157	94	48	64.7	52.1	83.1	3.3	29.7	75	60	
8 Fresh	10	171	94	58	55	47.7	74.8	2.8	40.9	82	64	
8 Fresh	15	140	75	32	50.3	39.2	65	2.5	45.5	75	62	53
8 A_1	5	86	59	33	54.5	46.2	69.7	2.9	28.6	81	66	
8 A_1	10	76	36	14	52.6	36.7	71.4	3.2	48.1	61	49	
8 A_1	15	68	36	10	41.4	27.6	58.7	2.3	50.3	62	48	50
8 A_2	5	31	31	24	88.4	77.9	99.9	3.7	39.2	87	77	
8 A_2	10	73	45	32	63.6	53.1	73.9	2.6	51.2	80	70	
8 A_2	15	72	42	23	64.1	51.4	73.1	2.8	43.2	76	68	48
8 C_1	5	135	55	24	67.7	49.1	82.2	3.2	19.3	70	57	
8 C_1	10	115	32	21	53.3	45.7	65.2	2.6	42.4	83	72	
8 C_1	15	102	32	18	35.1	29.2	50.7	1.6	53.3	81	64	40
8 C_2	5	170	50	23	57.1	43.1	71.1	2.9	43.1	73	61	
8 C_2	10	148	49	25	47	35.7	65.8	4.4	43.0	73	59	
8 C_2	15	134	34	23	47.7	42.1	58.1	3.1	44.9	84	72	43
8 D_1	5	160	99	46	62.9	52.5	84.6	2.7	19.6	72	60	
8 D_1	10	135	55	20	58.7	41.4	74.9	2.1	22.6	66	55	
8 D_1	15	106	22	11	36.3	30.1	46.8	2.2	46.6	81	67	44

8 D_2	5	107	66	27	58.1	39	76.1	3.3	19.7	65	51	
8 D_2	10	102	45	17	50.6	30.7	64.1	2.1	21.3	60	49	
8 D_2	15	96	18	5	31.2	22.1	41.5	1.9	51.3	71	57	46
Pool 1 Fresh	5	115	112	55	40.3	25.7	55.3	3.0	35.3	69	56	
Pool 1 Fresh	10	151	78	14	42.2	24.9	67.1	2.7	47.1	57	38	
Pool 1 Fresh	15	142	49	13	35.2	25.8	49.4	2.8	53.4	69	53	86
6 Fresh	5	82	39	28	58.3	52.6	70.3	2.9	47.1	85	73	
6 Fresh	10	33	20	18	71	66.5	74.6	1.2	42.3	93	88	
6 Fresh	15	24	13	5	59.1	44.3	63.5	0.9	36.4	73	67	41
Pool 5 Fresh	5	244	47	27	58.0	51.8	71.5	2.0	34.8	80	67	
Pool 5 Fresh	10	243	33	20	55.7	47.5	77.4	2.4	45.6	78	62	
Pool 5 Fresh	15	231	30	16	50.3	40.5	70.6	1.9	33.4	78	59	45

VITA AUCTORIS

NAME:	Alexander John Presello
PLACE OF BIRTH:	Windsor, ON
YEAR OF BIRTH:	1992
EDUCATION:	University of Windsor Windsor, Ontario 2010 – 2014 BHK (Hons) Human Kinetics
	University of Windsor Windsor, Ontario 2015 – 2016 M.Sc. Biological Sciences