

Effect of Gonadotropin Releasing Hormone (Ovaplant-L) Dose on Induced Spawning of Female Striped Bass (*Morone saxatilis*)

by

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Abstract

To identify the lowest effective dose to achieve successful spawning in striped bass, three doses of salmonid gonadotropin releasing hormone analogue (Ovaplant-L; Syndel) were compared: 10, 30 and 70 $\mu\text{g kg}^{-1}$, each in triplicate. The experimental unit was a 1500 L tank stocked with 1 female and three male striped bass; there were nine tanks total. Broodstock overwintered in a pond were ‘cold-banked’ at 12°C starting 24 May followed by an increase to 18°C over 14 (Trial 1) and six (Trial 2) days. Oocyte maturation (Bayless hour) was slower than predicted, confounding the effect of Ovaplant dose. Ovaplant-L injection resulted in 33.3% (6 of 18) females spawning. Seven required a follow-up injection of human chorionic gonadotropin (350 IU kg^{-1} ; hCG). Remaining five females failed to oviposit fertile oocytes. Three of four females who received 70 $\mu\text{g kg}^{-1}$ and whose eggs were sufficiently matured (≤ 15 Bayless hours) successfully spawned, compared to the other treatment groups’ (3 of 5; 10 $\mu\text{g kg}^{-1}$) (0 of 5; 30 $\mu\text{g kg}^{-1}$) spawning success solely using Ovaplant-L. Effect of Ovaplant-L dose on successful spawning was not evident.

List of Abbreviations and Symbols Used

cGnRH – Chicken gonadotropin releasing hormone

DO – Dissolved oxygen

DPH – Days post-hatch

FOM – Final oocyte maturation

FSH – Follicle-stimulating hormone

GnRH – Gonadotropin releasing hormone

GnRHa – Gonadotropin releasing hormone analogue

GTH – Gonadotropin

hCG – Human chorionic gonadotropin

HPG - Hypothalamo-pituitary-gonadal

IM – Intramuscular

LH – Luteinizing hormone

mGnRH – Mammalian gonadotropin releasing hormone

mGnRHa – Mammalian gonadotropin releasing hormone analogue

MIS – Maturation-inducing steroid

NRFF – North River Fish Farms Ltd.

sbGnRH – Seabream gonadotropin releasing hormone

sGnRH – Salmonid gonadotropin releasing hormone

sGnRHa – Salmonid gonadotropin releasing hormone analogue

Chapter 1: Introduction

Land-based striped bass (*Morone saxatilis*) farming can potentially diversify finfish aquaculture to capitalize on the established demand for the fish, particularly in the United States. In Atlantic Canada, the sustainability of the Atlantic salmon aquaculture industry is being questioned due to rising seawater temperatures, the threat of sea lice (*Lepeoptherius salmonis*), and public controversy surrounding farming in sea cages (Chen et al. 2015; Shephard & Gargan 2020; Weitzman et al. 2023). In Nova Scotia, the sea-cage controversy led to a call for diversification to include more land-based operations (Doelle & Lahey 2014). Striped bass, a euryhaline native fish with a higher tolerance to warm water than salmonids, is a potential alternative which can be held year-round in constructed ponds and reared in freshwater land-based operations, avoiding public controversy. Exploring the potential for striped bass aquaculture aligns with the current call for investment in research and innovation in Nova Scotia to encourage small and medium operators (Davis Pier 2023).

In the United States, there is exceptional demand for seafood, resulting in a trade deficit of 15 billion USD/annum which continues to increase (Engle et al. 2023). The high popularity of striped bass as a food fish is evident in the recreational fishing harvest that is three times larger than commercial fishing quota; the latter strictly limited due to conservation concerns (7100 t in 2021 vs. <2000 t ; NOAA 2023). Despite this demand, no striped bass aquaculture production has been reported in either the U.S. or Canada (USDA 2024; Engle et al. 2023). An important limiting factor has been the difficulty in spawning captive female striped bass.

Successful completion of spawning in captivity has been achieved in the South-Eastern United States at the research level in academia with the use of human chorionic gonadotropin (hCG) and gonadotropin releasing hormone (GnRH), but this has not yet transferred to industry (Hodson &

Sullivan 1993; Andersen et al. 2021a). Volitional spawning success has been achieved, but only in academia (Andersen et al. 2021b). Only hybrid striped bass (male *Morone saxatilis* x female *Morone chrysops*) is commercially produced in the US, ranked #4 in the aquaculture industry in 2018 by sales, generating a modest 8,688 tonnes with a value of US\$35 million (USDA 2024).

In New Brunswick, spawning of Bay of Fundy striped bass was achieved, however production did not surpass the laboratory scale (Peterson et al. 1996). Within the Bay of Fundy, there were historically three spawning populations: Saint John River, Annapolis River and Shubenacadie River (LeBlanc et al. 2018). Currently, the dominant population in the Bay of Fundy utilizes the Shubenacadie/Stewiacke River estuary as the spawning and nursery habitat (LeBlanc et al. 2018). The Saint John River stock is extant but fragile. An additional population, the Gulf of Saint Lawrence stock spawns in the Miramichi River, and the population has increased tremendously this century to ~720,000 adults as of 2019 (LeBlanc et al. 2018; Chaput & Douglas 2022).

At Dalhousie University Agricultural Campus (Dal-AC), capture of wild eggs from the Stewiacke River enabled studies on larvae and juveniles (Cook et al. 2010; Duston et al. 2018). Broodstock derived from the wild eggs were used in the research described here, ensuring there was increased genetic variability. US researchers have been conducting striped bass breeding research on Chesapeake Bay stock (Hodson & Sullivan 1993; Andersen et al. 2021b), which are genetically discrete from the Bay of Fundy stock (LeBlanc et al. 2018).

At Dalhousie University, Agricultural Campus (Dal-AC) from 2000 to 2019, spawning attempts used Ovaplant-S, a silastic implant produced by Syndel (Nanaimo, BC V9T 6A7) containing 150 µg salmonid gonadotropin releasing hormone (sGnRH). Egg quality at Dal-AC was typically poor, associated with broodstock held in a single small holding tank (3.5m³), limiting their growth potential. Broodstock growth and general health improved greatly when reared in a cage in a

constructed pond at North River Fish Farms Ltd. (NRFF), 20 km North of Truro, NS. In 2022, using broodstock reared at NRFF and trucked to Dal-AC, and a new product Ovaplant-L: a liquid sGnRH analog (sGnRHa; Syndel), spawning success improved greatly. Each spawning trials used a ratio of three males and one female, following procedures from North Carolina. Among the 12 females tested in 2022, final oocyte maturation (FOM) was independent of Ovaplant-L dose (10 vs. 30 μg per kg body weight), and an additional hormone injection of hCG (350 international units (IU) per kg body weight) was needed (Duston et al. unpubl. data).

Broodstock were fed pellets with squid meal (Europa, Skretting; St. Andrews, N.B) since 2019, when Canadian Food Inspection Agency (CFIA) approved the inclusion of squid meal as an ingredient in feed. The presence of squid meal/oil was believed to improve the quality of produced oocytes in marine fish through a more nutritious yolk (Watanabe & Vassallo-Agius 2003). This occurs through a superior protein quality, and higher phospholipid and cholesterol content of squid meal compared to steam dried pellets, having 14.6 times greater amounts of astaxanthin, which promotes antioxidant capacity and 1.35 times more crude protein (Watanabe & Vassallo-Agius 2003). However, in September 2024, Dr. Steve Backman changed his recommendation on Europa diet, so broodstock diet was switched to “Vitalis” Skretting (Vitalis, Skretting; St. Andrews, N.B).

As spawning of captive striped bass broodstock mostly rely on hormone injections to stimulate FOM in the absence of suitable environmental cues, including a diet resulting in a more nutritious yolk aids in improving the chances of spawning success.

The primary objective of this thesis was to identify a single injection dose of Ovaplant-L that achieved successful FOM without a stressful follow-up injection of hCG. Chapter 2 reviews striped bass taxonomy and phylogeny, and the endocrine control of sexual maturation, building the rationale for the experimental work. Chapter 3 presents the materials and methods of the

experimental work. All activities were conducted at NRFF due to Dal-AC lab being closed for renovations from 2022 to 2024.

Chapter 2: Review of state of knowledge and rationale for experimental work

2.1 Striped bass life cycle

Striped bass is an anadromous (coastal) fish that spawns in estuaries along the Atlantic coast of North America. It can be found from the Bay of Fundy (45° 00' 00"N, 65° 47' 59"W) and Gulf of St. Lawrence (49° 36'N, 61° 24'W) in Atlantic Canada, to the Apalachicola River (30° 42' 31"N, 84° 51' 50"W) in Florida, a coastal range of 1500 km (LeBlanc et al. 2018; Wooley & Crateau 1983; Waldman et al. 1990). US stocks migrate north and east during spring and summer, and south and west during autumn and winter (Waldman et al. 1990). Their spring migration typically brings them to river mouths and estuaries to spawn (Waldman et al. 1990).

The broodstock for this study originated from wild eggs collected from the Stewiacke-Shubenacadie River system over several years and were incubated and reared to juvenile stage at Dal-AC and later transported to NRFF in spring as 1-year-olds (50-80g). The current broodstock (n = 93; avg. 3.40kg ca. July 2024) for the past three years have been held in a single cage in a constructed freshwater pond at NRFF (annual temperature range 0.5 - 25.0°C).

The average age at sexual maturity of Bay of Fundy male striped bass is about 3 years, and females about 6 years (Bradford et al., 2015). While farming striped bass in captivity has not been accomplished commercially, hybrid striped bass production using male striped bass and female white bass (*Morone chrysops*) occurs on 57 farms in the United States (USDA 2024).

Notable differences exist between captive striped bass and white bass spawning methods. Striped bass oviposit eggs, which are fertilized by males in a spawning tank (Hodson & Sullivan 1993). Hand-stripping of female striped bass was not attempted, following spawning procedures of North Carolinian researchers (Sullivan et al. 2003; Andersen et al. 2021c). In contrast, white bass eggs

are manually stripped from ripe females, and sperm is added directly for fertilization (Ohs et al. 2009). Additionally, white bass exhibit a multiple-clutch, group-synchronous spawning pattern (Berlinsky et al. 1995), while striped bass are single-clutch, group-synchronous spawners (Specker et al. 1987). Spawning attempts to culture striped bass have occurred through tank spawning since 1966 (Stevens 1966). However, hand-stripping of white bass females provides an advantage over striped bass as large quantities of striped bass oocytes cannot be accurately stripped without an accurate prediction of FOM (Harrell et al. 1990). This has resulted in the formation of hybrid striped bass industry since the 1990s (Andersen et al. 2021a)

Females (Bay of Fundy stock) with a fork length of 45 cm produce around 50,000 eggs, while those with a fork length of 91 cm can yield up to 2,100,000 eggs (Paramore 1998). In the Bay of Fundy, the mature males and females migrate upstream to spawn in the tidal freshwater of the Stewiacke River (Duston et al. 2018). In the river system, spawning occurs between 3 and 6 km upstream of the confluence of the Shubenacadie river (Rulifson & Tull 1999). Spawning populations typically spawn in several episodes between late-May and early-July, lasting 31 to 49 days each year, typically associated with a rise in temperature to 16°C following the frequent cold spells of a typical Nova Scotia spring (Duston et al. 2018). The increase in temperature is a critically important cue for spawning both in the wild and among captive fish (Hodson & Sullivan 1993). To induce spawning in striped bass broodstock, a thermal inducement phase is essential, raising water temperature to 18°C to simulate natural spawning conditions (Hodson & Sullivan 1993).

Once fertilized, eggs hatch in about 48 hours (Rulifson & Tull 1999). Striped bass larvae grow in brackish water where they transition to juveniles at ~ 40 days post hatch (DPH). The growth of larvae has a positive correlation based on female size, with larvae from large (>15kg) females of

Chesapeake Bay stock at 25 days post hatch being 22% larger than larvae from smaller (<4kg) females, however no similar studies have been conducted on Canadian striped bass (Monteleone & Houde 1990). Juveniles remain in brackish water until they reach one to two years old, before entering the ocean to participate in migrations (Rulifson & Dadswell 1995).

2.2 Endocrine control of sexual maturation

The hypothalamo-pituitary-gonadal (HPG) axis is a regulatory system that controls sexual maturation (Figure 2.1). The axis regulates gametogenesis, the secretion of two gonadotropins (GTH) from the pituitary gland: follicle stimulating hormone (FSH) and luteinizing hormone (LH), and sex steroids (Steven et al. 2000). The hypothalamus is a region in the brain that produces gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland (Steven et al. 2000).

There are three types of GnRH ligands in fish; each is a 10 amino acid chain, and all regulate a separate facet of maturation (Kochman 2012). GnRH-1 (seabream-type GnRH or sbGnRH-I) is located in the preoptic-hypothalamic area (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Ser-Pro-Gly-NH₂; Kochman 2012; Zohar et al. 2010). It is responsible for stimulating the release of LH and is the most physiologically important form of GnRH toward the completion of FOM, ovulation, and spawning in red seabream (*Pagrus major*) and other perciform species such as striped bass (Okuzawa et al. 2003; Chow et al. 1998). GnRH-2 (chicken-type GnRH-2 or cGnRH-II) is synthesized in the midbrain and is likely the earliest evolved GnRH (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH₂; Kochman 2012). It promotes melatonin release, sexual behaviour, and stimulates the production of GTHs along with prolactin release *in vitro* (Millar 2005; Zohar et al. 2010). GnRH-3 (salmon-type GnRH-3 or sGnRH-III; pGlu-His-Trp-Ser-Tyr-Gly-Trp-Leu-Pro-Gly-NH₂) is found in olfactory bulbs and the rostral forebrain, potentially acting as a

neuromodulator in addition to contributing to FSH synthesis (Kochman 2012; Zohar et al. 2010). All three forms of GnRH were identified in the pituitary gland of pubertal female striped bass, at a ratio of 100:10:1 of sbGnRH:cGnRH-II:sGnRH (Holland et al. 2001).

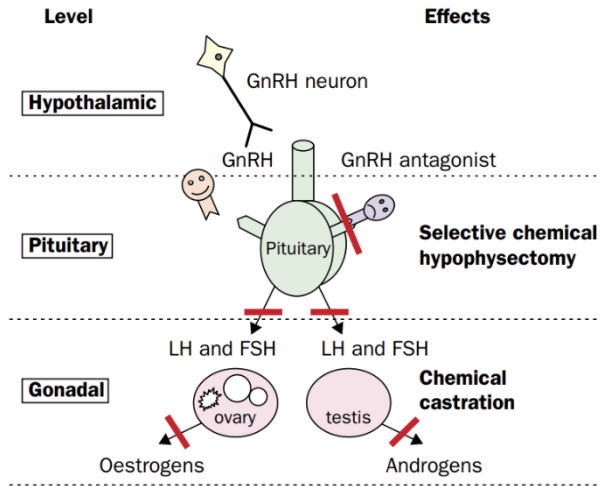


Figure 2.1. The interaction of gonadotropin releasing hormone (GnRH) neuron on the brain-pituitary-gonadal axis and hormonal therapy intervention sites for secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), acting on the gonads for induction of oocyte and sperm maturation. (From: Huirne & Lambalk, 2001).

GnRH binds to GnRH receptors on gonadotrope cells located on the surface of the anterior pituitary gland to promote the synthesis and secretion of two GTHs: FSH and LH (Flanagan & Manilall 2017; Figure 2.1). FSH acts on the gonads, stimulating spermatogenesis in males, and the development of ovarian follicles in females. The ovarian follicles secrete estradiol-17 β (E2) which stimulates vitellogenin production by the liver (Lubzens et al. 2017). To maintain homeostasis, dopamine is released by the hypothalamus, acting on the anterior pituitary gland to inhibit the secretion of FSH and LH and reduce the release of GnRH from the hypothalamus (Peter et al. 1993). Some spawning products include a dopamine antagonist, inhibiting dopamine and

increasing binding of GnRH to pituitary receptors (Figure 2.1; Ovaprim; Syndel). Ovaplant-L does not contain dopamine antagonists (Syndel). Ovaprim, another Syndel product, does contain dopamine antagonists, and is also used for induced spawning of finfish broodstock however Ovaprim's concentration of sGnRHa is not stated by the manufacturer. Ovaplant-L, by contrast, contains a known concentration of sGnRHa (100 µg/mL).

GnRH analogues (GnRHa) are synthetic GnRH, substituting at positions 6 and 10 of the decapeptide with a dextrorotatory (Glycine to D-Arginine (DArg)) amino acid and ethylamide group (Glycine to N-ethylamide(NEt)) respectively (Mylonas & Zohar 2007). Ovaplant-L is a sGnRHa, its sequence is pGlu-His-Trp-Ser-Tyr-DArg-Trp-Leu-Pro-NEt (Kochman 2012; Mylonas & Zohar 2007). These amino acid substitutions result in a GnRHa which has a greater resistance to enzymatic degradation (Goren et al. 1990). GnRHa remains longer in the fish than native GnRH, stimulating a release of LH from the pituitary gland for up to eight weeks, compared to the 23-minute half-life of native GnRH in gilthead seabream (*Sparus aurata L*; Mylonas et al. 1995; Zohar et al. 1990). The decay dynamics of all GnRHa are similar amongst fish species due to the structural similarity of GnRH (Lethimonier et al. 2004). While the use GnRHa can stimulate the release of FSH and LH to control ovulation in striped bass, primary successes have been twofold: spawned using researcher-synthesized GnRH pellets which are not approved by regulatory bodies for commercial aquaculture, and successes were limited to laboratory settings using Chesapeake Bay striped bass (Andersen et al. 2021c; Hodson & Sullivan 1993; Mizrahi & Levavi-Sivan 2023). Thus, successful spawning using pure striped bass on a commercial farm, with the Bay of Fundy population have yet to be successful.

2.2.1 Oocyte maturation

In the intricate process of oocyte maturation, the outer layer of the follicular envelope called thecal cells play a pivotal role by fostering the synthesis of cholesterol and its conversion to testosterone (Nagahama et al. 1995). Following this, FSH acts on the follicles, aromatizing testosterone and thereby instigating estradiol-17 β (E2) transportation to the liver. This estrogen surge, in turn, promotes synthesis of yolk protein from vitellogenin within oocytes: a vital stage referred to as vitellogenesis, typically occurring in striped bass from October to April among Chesapeake Bay stock (Tao et al. 1993).

LH released from the pituitary gland further enhances the synthesis of maturation inducing steroids (MIS) within the layers of follicular cells. MIS act as a trigger for final oocyte maturation and are released in response to environmental cues (Lubzens et al. 2010). In fish, MIS can be the progestin 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β DHP, or DHP) or 17 α ,20 β ,21-trihydroxy-4-pregnen-3-one (20 β -S; Thomas et al. 2007). In striped bass, both DHP and 20 β -S are associated with as MIS for FOM (King et al. 1994). Germinal vesicle breakdown (GVBD) signifies the resumption of meiosis; ovulation leads to expulsion of eggs into the water column, known as ovipositioning, concluding FOM, making the eggs available for fertilization (Tokumoto et al. 2006). Striped bass females in captivity face challenges in achieving FOM as their GTH levels remain low, resulting in the chemical and physical breakdown of the egg, otherwise known as oocyte atresia (Mylonas et al. 1998). GnRH delivery systems of subdermal implants (30 – 50 $\mu\text{g kg}^{-1}$), biodegradable injected microspheres (40 $\mu\text{g kg}^{-1}$) and free GnRH α dissolved in saline (20 $\mu\text{g kg}^{-1}$) caused induction of FOM in five of five, five of five, and one of five females respectively after first administration of hormone (Mylonas et al. 1998). A second injection of GnRH α four days after resulted in two additional females' oviposition (Mylonas et al. 1998). Use of GnRH α caused oviposition within 3 and 10 days, with 46% fertilization (avg. fecundity 174,000 eggs/kg;

Mylonas et al. 1998). The primary objective of my Master's thesis was to determine the minimum effective dose of Ovaplant-L to successfully induce spawning of striped bass females.

As hormonal induction is required for striped bass spawning, an appropriate spawning aid of GnRH is required. Syndel, a company that specializes in aquaculture pharmaceuticals, has supplied Dal-AC with its spawning hormone Ovaplant-S, a silastic implant delivery system that administers 150 µg GnRH for its striped bass broodstock program since 2000. In 2021, Syndel stopped the sale of Ovaplant-S to release its new product: Ovaplant-L. Ovaplant-L a liquid product, allows for adjusted the dose in terms of micrograms of hormone per kilogram body weight. Use of Ovaplant-L currently requires a Veterinary Drug Experimental Studies Certificate from Health Canada

2.3 Teleost reproductive dysfunction

Breeding fish, accustomed to seasonal changes in temperature, salinity, and depth due to migration, face a dearth of these cues in captivity, leading to reproductive inhibition (De Silva et al. 2008). Captive striped bass broodstock are typically unable to complete FOM without injection of either hCG or GnRH_a (Stevens 1966; Hodson & Sullivan 1993). The repercussions of reproductive inhibition extend to a stunting of gonadal growth, and in female striped bass this inhibition manifests in the progression of vitellogenesis, but inhibition of FOM leading to follicular atresia (Sullivan et al. 2003). Stress elevation and the absence of crucial exogenous cues are identified as additional contributors to reproductive dysfunction (Hodson & Sullivan 1993). Volitional spawning using 5th generation domesticated striped bass without the use of hormonal injection have had some recent success in the United States (Andersen et al. 2021a). However, in the absence of domesticated broodstock that are capable of successful volitional spawning, hormonal injection remains the most promising way to spawn captive striped bass broodstock.

2.4 Hormonally induced spawning

Successful induction of ovulation and spermiation in fish was first achieved through the injection of hCG into goldfish (*Carassius auratus*; Sneed & Clemens 1959). This was followed by successful induction of spawning in female striped bass broodstock captured just prior to spawning (Stevens 1966). Injections of hCG was first employed as a spawning hormone with female broodstock due to its high degree of structural homology to LH (Stevens 1966). First used in guinea pigs through injection of human placenta extract, hCG has been popularized as a sexual maturation hormone since 1912 (Cole 2010). Deriving from urine from pregnant women or through recombinant DNA, hCG typically is used to induce ovulation in cattle, and used in humans to induce follicular maturation and ovulation (Cole 2010). This human substitution directly stimulates the gonads, promoting ovulation but not further maturation of the oocytes (Ludwig et al. 2002). Intramuscular (IM) injections of hCG does not stimulate FOM, requiring mature oocytes to be effective (Goetz 1983). Wild female broodstock, fresh captured, received an injection of hCG at a dose of $280 \text{ IU}^{-1} \text{ kg}^{-1}$ (Stevens 1966). However, the use of hCG alone has been associated with the production of low-fertility eggs and success relied heavily on the oil globule coalescence of oocytes, a benchmark noted as being at ≤ 15 “Bayless” hours of development, requiring biopsies to identify egg development (Figure 2.2; Stevens 1966; Bayless 1972; Hodson & Sullivan 1993). Bayless hours are defined as an estimation of the number of hours a striped bass female will take to oviposition using hCG, keeping the fish near 19°C (Bayless 1972). Bayless hours are not a length of time indicator, rather a baseline for ovulation prediction (Bayless 1972).

Females at 13 – 14 Bayless were shown – after injection of hCG – to oviposite a mean of 569,000 oocytes vs. females ≤ 11 Bayless hour ovipositing a mean of 818,000 oocytes (Hodson & Sullivan

1993). Differing Bayless hours may exhibit varying levels of sensitivity to hormonal stimuli, which can affect the success of spawning (Hodson & Sullivan 1993).

In addition, hCG elicits an immune response in female striped bass due to the large molecular size of hCG and the heterologous nature of GtH preparations, resulting in a higher likelihood in a failure to spawn in the following seasons using hCG on the same fish (Mylonas & Zohar 2007).

Female striped bass broodstock are injected with 350 IU per kilogram body weight hCG to induce ovulation (Hodson & Sullivan 1993). Male striped bass broodstock are capable of spawning in captivity as they do not have a limiting dysfunction, however they are injected with 75 IU kg⁻¹ hCG to assist with additional sperm production (Hodson & Sullivan 1993; Andersen et al. 2021c). The requirement for mature oocytes requires biopsy of female broodstock to assess egg development prior to injection. As GnRHa stimulates the natural secretion of FSH and LH, it facilitates a more natural completion of FOM in fish like Nile tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*; Mizrahi & Levavi-Sivan 2023).

To prepare for hormone injection, gonadal maturation of female striped bass was slowed through a process called “cold-banking”, where the water is held at ~10°C to slow FOM, thus extending the spawning season (Hodson et al. 1999). This process was critical for females as to halt oocyte maturation but was less important for males (Hodson et al. 1999). Striped bass broodstock are biopsied to confirm oocyte maturation and eligibility, with acceptable oocytes typically measuring ~600 - 900 µm in diameter with some oil globule coalescence at ~15 h Bayless (Figure 2.2; Sullivan et al. 2003).

Biopsies required manual handling of the broodstock, anaesthetizing, and oocyte extraction by inserting a plastic catheter (0.3mm internal diameter) into one of the oviducts and applying light

mouth suction. Oocyte stage was assessed by observation under a dissecting microscope (Hodson & Sullivan 1993). Staged oocytes are observed for size and germinal vesicle migration, a precursor to GVBD, taking place <15 Bayless hours prior to ovulation (Bayless 1972; Kerby 1986).

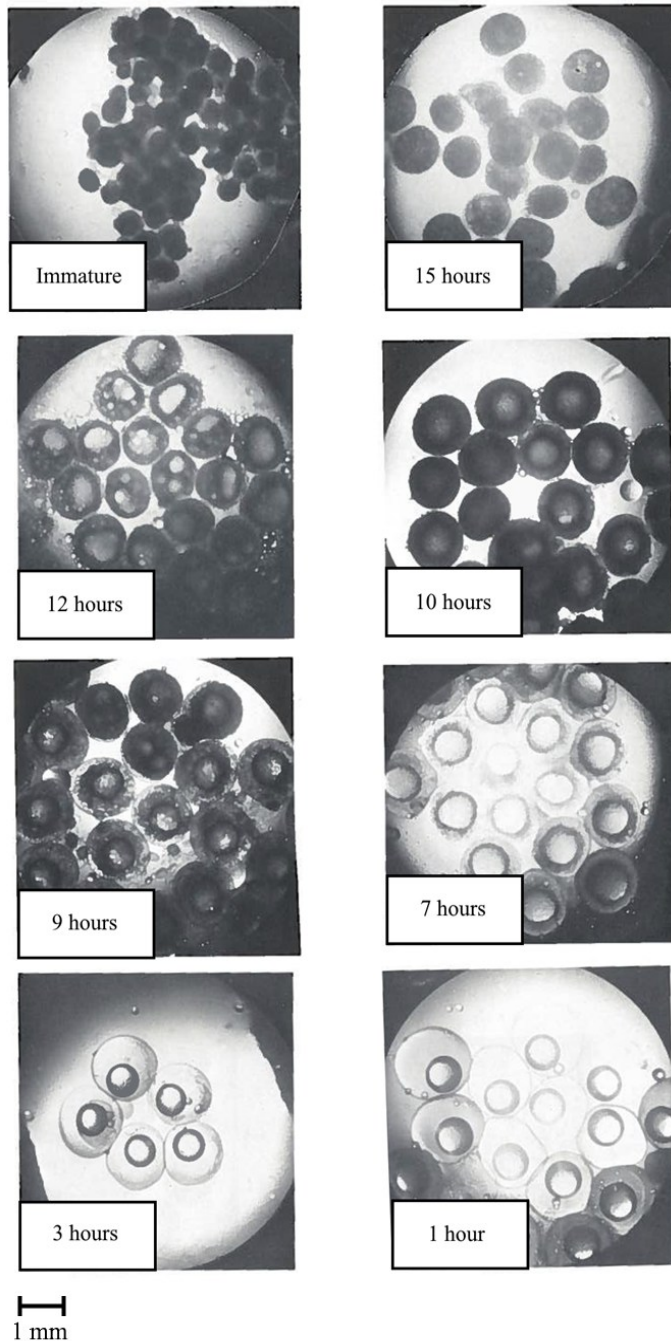


Figure 2.2. Striped bass egg development “Bayless hours” used for ovulation prediction viewed at 20X magnification. Immature oocytes are small, often less than 600 μm in size and yellow-ish in color. 15 hour oocytes are opaque, with oil globule droplets present; oocytes are typically 1 mm in diameter. 12 hour oocytes remain opaque, but with partial polarization of oil globule. 10 hour oocytes have finished polarization. 9 hour oocytes, opaque color begins to dissipate, showing transparency. 7 hour oocytes have clearer transparency around oil globule, and chorion remains rigid. In 3 hour oocytes, yolk is transparent. At 1 Bayless hour, chorion loses some rigidity and oocytes begin to hydrate slightly, increasing in size to 1.2 mm or larger (From: Bayless 1972).

Better success at spawning female striped bass was achieved by the injection of constructed implants containing mammalian gonadotropin releasing hormone [D-Ala⁶-Pro⁹-NEt]-LHRH (mGnRH) at a dose range of 48.8 to 62.5 µg per kg of body weight, resulting in a mean (SD) of 140,810 ± 18,983 eggs per female (n = 10), with a fertilization success of 67.4% (Hodson & Sullivan 1993). Two outlier females produced fewer than 20,000 eggs with a 0 and 6% fertilization success (Hodson & Sullivan 1993). The mean (SD) body weights of the 12 females was 3.3 ± 0.1 kg (Hodson & Sullivan 1993). Each pellet weighed 30mg, containing approximately 100 µg mGnRH, and was a matrix of cholesterol and cellulose (Sherwood et al. 1988). Two types of pellets were used: 80% and 95% cholesterol, and water temperature was kept at 18°C (Hodson & Sullivan 1993). There was no significant difference between mGnRH release of 80% and 95% cholesterol pellets until 48 h, when only 95% pellets would release >100 ng/h mGnRH, and was projected to release 7-10 ng/h mGnRH after 600 h (Sherwood et al. 1988). Andersen et al. (2021c) synthesized implants containing 150 µg mGnRH following the same protocol of Hodson and Sullivan (1993) with a 95% cholesterol pellet (1993); their effective dose range was 30 to 37 µg kg⁻¹. These implants were injected into the fish into the posterior base of the second dorsal fin, resulting in 18 of 23 females spawning successfully, producing a mean of 839,303 oocytes (Andersen et al. 2021c). Of those 18 females, nine successfully produced fry at a mean of 124,359 fry per female (Andersen et al. 2021c).

At Dal-AC, attempts to induce spawning of striped used silastic implants from Syndel as they were the only commercially available product approved for use by Health Canada for experimental purposes. Each implant contained 150 µg GnRH (sGnRH_a; Ovaplant-S; Syndel). Thermal induction at Dal-AC followed SOPs from North Carolina elevating the water temperature from 10

to 18°C over about 7 days to mimic spawning conditions in the wild, and examination of the eggs through a second biopsy followed at the end of thermal inducement phase (Sullivan et al. 2003).

Immature eggs are identified by the absence of an oil globule, and are typically less than 600 µm (Harrell et al. 1990; Figure 2.2). By 15 Bayless hours, oil globule formation is visible and oocytes are shown to clump together; size increases to ~ 1 mm (Figure 2.2). Bayless hours 14 through 11, central coalescence of oil globule formation occurs, while oocyte size remains relatively unchanged (Figure 2.2). At 10 Bayless hours, polarization is complete, yet nucleus remains unclear (Figure 2.2). Bayless hours 9 and 8 show clearing of nucleus and less adhesion to other oocytes (Figure 2.2). By 7 Bayless hours oocytes are transparent, however chorion is rigid (Figure 2.2). From 6 to 2 Bayless hours, differences are subtle, observing only minor differences in oocyte clarity and transparency (Figure 2.2). At 1 Bayless hour, chorion loses rigidity and slight hydration occurs, inflating size to 1.2 mm or larger (Figure 2.2). Once the oil globule is formed, injection of GnRH is a viable option. Injection of female broodstock ranging from 10 to 15 Bayless hours before ovulation with mGnRH_a resulted in 41 of 55 fifth generation female broodstock to spawn (Andersen et al. 2021c).

Health Canada issued an Experimental Studies Certificate to J. Duston, enabling the testing of Syndel's new product "Ovaplant-Liquid", which has not yet been approved for use in aquaculture. Syndel recommends a dose of 10 µg kg⁻¹ based on their experience with salmonid broodstock. Syndel suggested a higher dose would be worth testing on striped bass females (A. McCool, Syndel, pers. comm. to J. Duston February 2022). In 2022, using broodstock trucked in 20 km from NRFF to Dal-AC, both 10 and 30 µg kg⁻¹ Ovaplant-L doses were equally effective, resulting in 12 of 12 females successfully spawning but 10 of 12 required a follow-up IM injection of 350 IU/kg hCG 48-72h after the Ovaplant (Duston et al. unpubl. data). Egg quality was good, and 40,000 juveniles

were produced. In the published literature, there is no data on the response of striped bass to Ovaplant-L, however spawning attempts were made in 2023 to spawn white bass females (*Morone chrysops*) in the United States. The use of 75 $\mu\text{g kg}^{-1}$ Ovaplant-L on white bass females resulted in an 80% spawning rate (n=155; Johnson 2023).

NRFF's hatchery was used for this study because the Dal-AC wet lab was closed in October 2022 for essential renovations and remained closed through 2024. The use of NRFF allowed for sufficient space for installation of nine spawning tanks, to allow for testing of three doses each in triplicate. Hence, for this MSc project the principal objective was to test Ovaplant-L on female striped bass in concentrations of 10, 30, and 70 $\mu\text{g kg}^{-1}$. The objective is to identify a reliable protocol to produce good quality striped bass eggs without both resorting to a follow-up injection of hCG due to immune response to hCG, and to reduce extra handling to reduce additional stress.

2.5 Rationale for the experimental design

The experimental portion of this study was conducted over the summers of 2023 and 2024. This time period coincided with the spawning season of wild striped bass in Nova Scotia and was selected to ensure that the gonadal development of most broodstock would be nearing maturity.

After consulting with Dr. T. Astatkie, a statistician at Dal-AC, he recommended two trials, each three replicates of three experimental doses of Ovaplant-L: 10, 50, and 75 $\mu\text{g kg}^{-1}$. A randomized block design (RBD) would have been implemented, and females of similar body size used within each block. Oocyte observation would have been performed to measure the scale of oil globule coalescence (Bayless hours), as well as body size would be introduced as covariates to provide $df = 8$. 2023's trials would have been analyzed using an ANCOVA of RBD using $x_1 =$ oil globule coalescence and $x_2 =$ fish length.

Based on results of 2023, a revised dose would have been developed, and similar layout would be proposed: two trials observing three replicates of three experimental doses of Ovaplant-L. As Ovaplant-L was unobtainable during 2023, a revised dose response of 10, 30, and 70 $\mu\text{g kg}^{-1}$ was implemented: following previous Dal-AC spawning attempts at 10 and 30 $\mu\text{g kg}^{-1}$, and a higher dose at 70 $\mu\text{g kg}^{-1}$ following successful spawning attempts with white bass (Johnson 2023).

A randomized block was implemented using females of similar size within each block. Oocyte observation would have been performed to measure the scale of oil globule coalescence, as well as fish length as covariates to provide $df = 8$. Spawning success of 2024's trials would have been analyzed using an ANCOVA of RBD using $x_1 = \text{oil globule coalescence}$ and $x_2 = \text{fish length}$.

Egg stage of development was chosen as a covariate because it influences how the oocytes respond to hormonal induction (Hodson & Sullivan 1993). By including the egg stage of development as a covariate, differences are accounted for to ensure the analysis accurately reflects the relationship between Ovaplant-L dose and spawning response of female striped bass broodstock.

Syndel could not supply Ovaplant-L in 2023, despite promising imminent deliveries to line with spawning attempts. As the procurement of Ovaplant-L was unsuccessful during 2023, hormonal induction solely used Chorulon (hCG) and thus were not included in the results of this document.

Spawning attempts in 2024, preceded by cold-banking, began on 24 May 2024 with first biopsy of eligible fish, and a randomized block design was implemented for the study. Three blocks were used to study three experimental doses: 10, 30, and 70 $\mu\text{g kg}^{-1}$. Assignment of broodstock was set at random, and attempts were made to standardize conditions within blocks. Spawning success was low, thus, did not meet conditions for statistical analyses requiring all nine females to successfully spawn.

Grubb's test was performed to quantify differences of fertility between eggs spawned with Ovaplant-L, vs. Ovaplant-L with a follow-up injection of hCG.

2.6 Project objectives

The project objective was to quantify the spawning response in relation to doses of 10, 30, and 70 $\mu\text{g kg}^{-1}$ sGnRH α (Ovaplant-L). Time until spawning was measured, as was percent fertilization and egg diameter.

Based on the literature review, the null hypothesis (H_0) for this study was that there is no significant relationship between Ovaplant-L dose and spawning response of female striped bass broodstock. The alternate hypothesis (H_A) was that a significant relationship exists between Ovaplant-L dose range of 10 to 70 $\mu\text{g kg}^{-1}$ and spawning response of female striped bass broodstock. To adequately measure the project objective, it was required to determine if NRFF has proper infrastructure for spawning and early rearing of striped bass eggs and larvae through to juvenile, and to identify weaknesses.

Chapter 3: Methods

3.1. North River Fish Farms Ltd. infrastructure

The study was conducted at NRFF, a commercial finfish producer in North River, Nova Scotia (45° 29' 53"N, 63° 12' 31"W). The farm is divided between a hatchery site and a pond site about 1.5km apart; each requires its own license from the provincial government. There are three constructed freshwater ponds: the upper (82 x 92 x 3.9 m depth), middle (138 x 63 x 4.3 m depth), and lower (106 x 91 x 4.8 m depth; Toning 2019). Water source is mostly from the ground and flows by gravity between the three ponds, hence they share the same chemistry (annual range 0.5 – 25.0°C; 0 to >200L/min flow; pH 6.5 – 7.1; hardness 12 – 18mg/L; alkalinity 7 – 14 mg/L; Toning 2019; unpubl. data 2023). Occasional aeration sometimes occurs through use of industrial air pumps from July to September. In summer the DO is highly stratified with depth, showing a difference of 5.0 mg L⁻¹ oxygen saturation between 0 and 4 m depth (Figure 3.2). Heavy rainfall on 26 July, 28 August and 01 September 2023 had a large effect on DO stratification, increasing oxygen saturation after rainfall events at 2 m depth from 3.1 to 4.3 mg/L between 26 July and 4 August (Figure 3.2), and increasing oxygen saturation at 4 m depth to 5.4 mg L⁻¹ on 1 September from 0 mg L⁻¹ on 25 August (Figure 3.2). Temperature stratification was also present during the summer months, showing an average 2.9°C difference between surface temperature and 4 m depth between 26 July and 1 September 2023 (Figure 3.3). During cooling in September, temperature and oxygen stratification disappeared (Figures 3.2; 3.3). Four pond cages (6.1 x 12.2 x 3.7 m. deep) are connected together in a rectangular pattern: three hold larger (> 500g) striped bass year-round and is where broodstock are reared, with one empty pond cage (Figure 3.1).



Figure 3.1. Aerial photo of North River Fish Farms Ltd.'s Middle Pond, with its four visible rearing cages (6.1 x 12.2 x 3.7 m. deep). Altitude of 203 m. Source: Google Earth, 7 June, 2025.

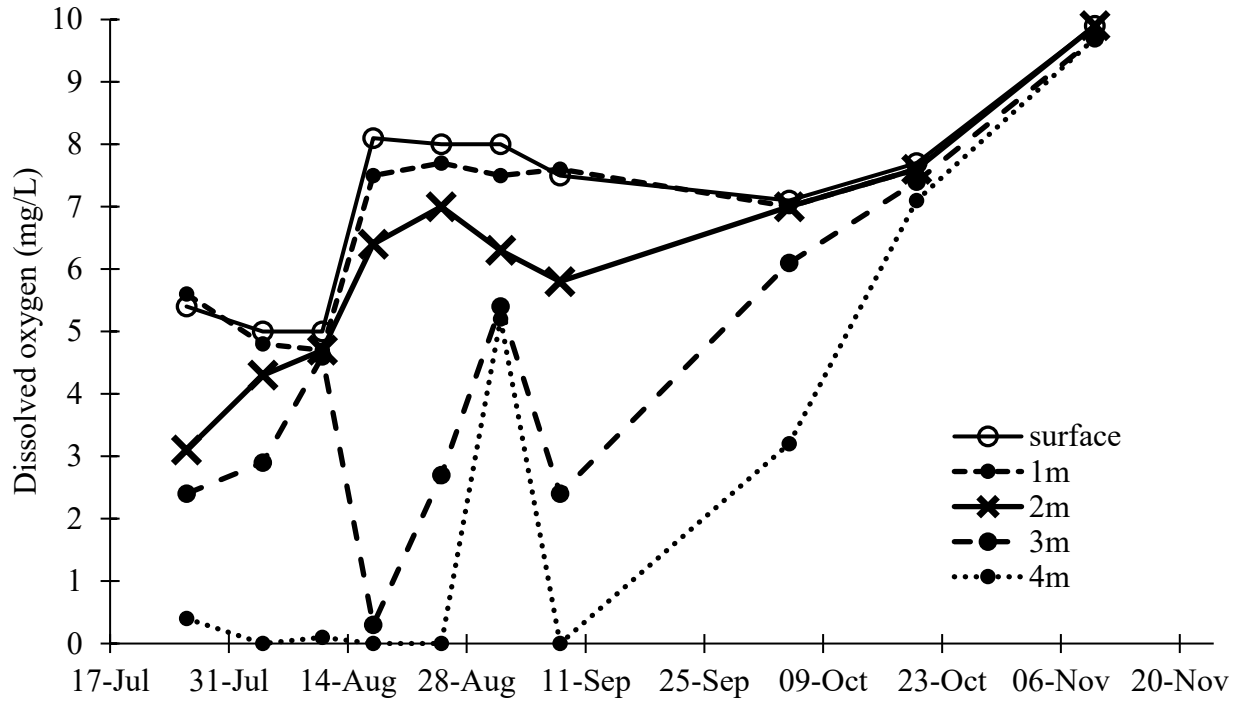


Figure 3.2. Striped bass broodstock rearing cage dissolved oxygen (mg/L) with depth (observed) from 26 July to 10 November 2023.

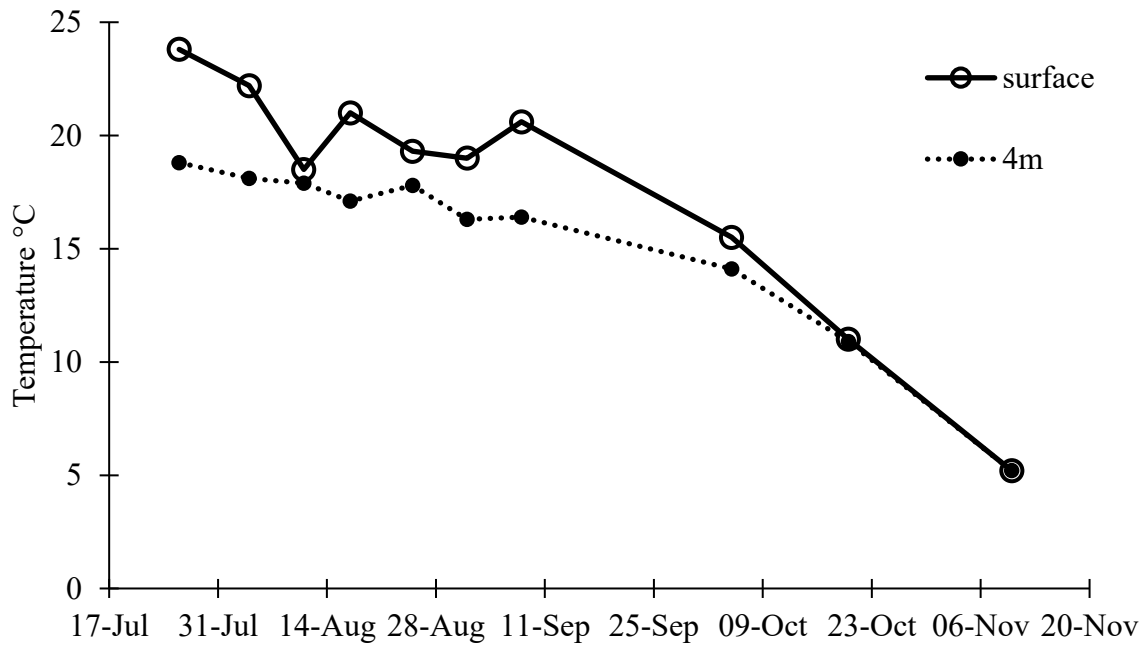


Figure 3.3. Striped bass broodstock rearing cage surface temperature (°C) and four-meter depth temperature observed from 26 July to 10 November 2023.

The hatchery building is 10 x 30m, with two levels. Freshwater is supplied from three sources: North River, natural Artesian springs, and groundwater. Seawater was stored in two 15,000L fibreglass storage tanks and gravity-fed to the hatchery. Seawater was trucked in from Cape John on the Northumberland Strait about 30km from NRFF.

The ground level had seven green “Swede” fibreglass tanks (3x3x0.9m depth). In mid-May broodstock were transported via tractor from the Middle Pond 1.4 km to the hatchery, where they were dip netted into two Swede tanks, and separated by sex. To slow oocyte maturation ‘cold-banking’ broodstock tanks were supplied with spring water at a mean of 12.5°C (range 9.3 to 16.1°C). An additional Swede tank was used as a mixing tank to create ~3 ppt brackish water (Hodson et al. 1999).

Freshwater intake from North River fed into a sump tank (1.22 x 1.83 x 0.86m depth) where temperature was controlled through the titanium heat exchanger and propane powered heater (Lochinvar Model 285). Heated water from the heater sump was circulated to a concrete sump (2.5 x 1.5 x 1m depth). A 0.5 hp pump circulated water from the concrete sump to the heater sump to equalize temperatures. The sump included a moving bed biofilter (Kaldnes beads, ca. 1.5 x 1.5 x 1m depth), and a 2 hp pump which supplied water to the spawning tanks. Nine green fibreglass spawning tanks (ca. 1.5m diameter, 1500L) sat on ground level: tanks five through nine sat in the three Swede tanks on the left, and spawning tanks one through four sat on the concrete floor of the hatchery. A regenerative blower (Sweetwater; 0.5hp) supplied compressed air throughout the hatchery.

Compressed air to each spawning tank was provided by a ~15 cm silica diffuser. Overflow water was recirculated to the concrete sump through connection to the main water line in tanks one

through four, and through Cornell drains in tanks five through nine via the egg collector through a 1.27 cm pipe.

For eggs and larvae, brackish water (3ppt; ca. 16-18°C) was held in a green swede mixing tank. Warm freshwater was obtained from the concrete sump or cold freshwater from spring water, gravity fed from an artesian spring. A saltwater line was plumbed to the mixing tank, where it could be controlled manually. A Danner Pondmaster magnetic drive pump (Model 12B) supplied brackish water to the egg incubators, and a 2 hp pump supplied brackish water upstairs to the nursery tanks. Egg incubators were in an adjoining room to the spawning tanks. Six hemispherical upwelling egg incubators sat on a metal frame supplied with brackish water.

Upstairs, the nursery room held 36 light blue tanks (each 1.5m diameter 30cm deep; ca, 275L working volume). Nursery tanks proved ideal from larvae 1 dph to juveniles reaching 15g. In another room upstairs, a laboratory was set up primarily for artemia production.

3.2 Broodstock rearing and handling

Broodstock were derived from wild eggs captured in the Stewiacke River using a plankton net (1.5 m; 1000 µm mesh) and reared at Dal-AC, before being transported to NRFF as 1 year olds. Broodstock ages ranged from an estimated four to eight years old.

Broodstock June 2023 (n = 98; avg. 2.49kg), and July 2024 (n = 93, 44 males, 49 females; avg. 3.40kg) were held year-round at NRFF in a single cage in the middle pond. Broodstock were fed Skretting Europa diet (12mm) pellets with squid meal on the recommendation of Dr. S. Backman following CFIA approval in 2019 of squid meal in finfish diet (Europa, Skretting; St. Andrews, N.B.) once per day from April to November when water temperatures were above 10°C at an

estimated 1% total body weight (3.1 kg). During winter, the broodstock were dormant under the ice.

On 12 May 2024, broodstock were moved from the pond to hatchery and cold-banked. In the pond, they were crowded to the net's edge and were dip-netted one fish at a time to avoid injury from spines.

They were anaesthetised in a 250L insulated tank (Xactics) next to the cage, part-filled with pond water made brackish (10ppt) by adding marine salt mix (Coralife; Aquamerik, PQ), and Tricaine methanesulfonate (MS-222; Syncaïne, Syndel; 0.15g/L, plus NaHCO₃ 0.3g/L). At loss of reflex, the fish was identified using a Passive Integrated Transponder (PIT) tag reader, and sex was determined by applying hand pressure to the abdomen. PIT-tags were implanted into the dorsal musculature post-spawning, never before. Mature males in mid-May all released seminal fluid, and maturing females were identified by an enlarged soft abdomen and the morphology of the urogenital vent. The sex of immature fish could not be determined. Once sex was determined, fish were carried one at a time via 1m rubber sling to one of two Xactics tanks (1.5 x 1 x 1m) and separated by sex. Each Xactics tank was $\frac{3}{4}$ filled with brackish water (10 ppt) at 10°C supplied with oxygen via a ceramic diffuser. Each holding tank was transported 1.4 km by tractor fork-lift to the hatchery. On arrival to the hatchery, broodstock were dip-netted one at a time into two Swede tanks used for cold-banking, supplied by flow-through, and separated by sex. The temperature during the cold bank period averaged 12.5°C (range 9.3 – 16.1°C).

3.2.1 Spawning tanks

The nine spawning tanks were installed to allow three replicates of three Ovaplant-L dose responses. Water fed into each spawning tank drained into an egg collector (ca. 100 L black polyethylene bin) and was then recirculated to the concrete sump (Figure 3.4). A 2 inch bulkhead,

connected to a 1.27cm inch hose was added to allow recirculation of water from egg collectors. Freshwater temperature was controlled via the heater, and increased prior to spawning from 12 to 18°C over 11 days for trial 1, and 8 days for trial 2, with a target flow of 5L/min per tank to stimulate FOM.

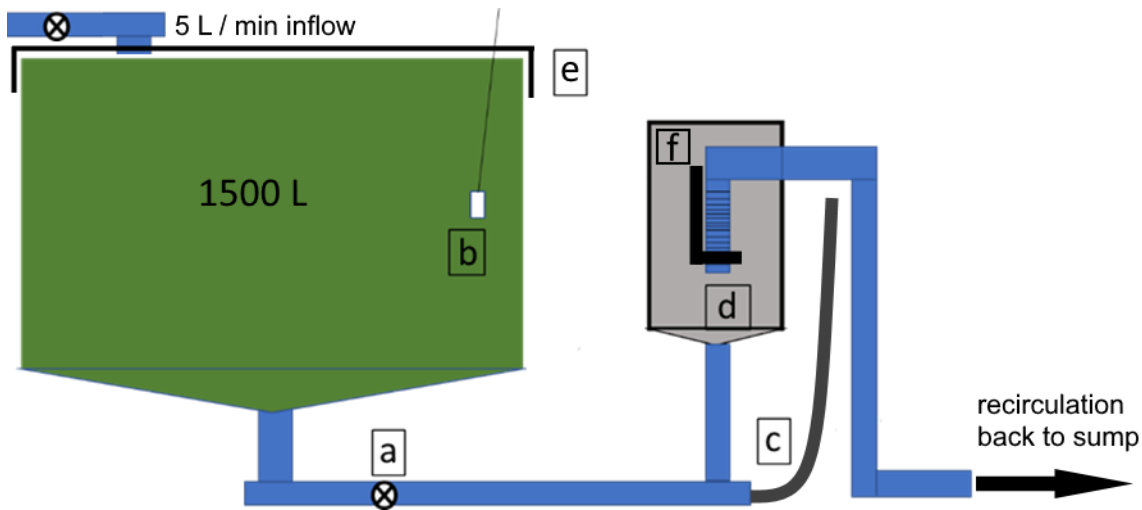


Figure 3.4. 1500 L capacity spawning tank supplied with 5 L min⁻¹ inflow (left) and 100 L capacity egg collector (right). (a) Egg collector valve, (b) airstone, (c) egg collector hose, (d) 2 in well-screen pipe, (e) 1 cm diameter black mesh net, (f) bubble ring for aeration.

The light intensity at the water surface was low (0 to 60 lux) provided by incidental ambient daylength (latitude 45°N) through a sliding door. A net (1 cm mesh) was tightly secured on top of each tank to prevent escape.

3.2.2 Hatchery

Six hemi-spherical “Kriesel” upwelling egg incubators were installed in the incubator room (Hughes et al. 1974; 90 L; Aquabiotech, Coaticook, QC). Water flow was adjusted with fine-adjustment valves to allow for rotational upwelling and suspension of eggs within the water column. A 500 µM Nitex screen was put around standpipe and secured with black electrical tape.

The incubators were supplied with brackish water from the swede mixing tank via a small pump (Danner Pondmaster Model 12B). Water was kept between 16 and 18°C at a salinity between 2.8 and 5.1 ppt mixing river water and salt water, using immersion heaters. Maintaining eggs in suspension was difficult, requiring frequent adjustment of water inflow from the bottom and side jets. Egg buoyancy changes post-fertilization confounded the issue, and water flow required frequent adjustment (range 0.3 to 1 L min⁻¹), and the light intensity was low (range 0 to 45 lux).

3.2.3 Larvae rearing tanks

Nine light blue fibreglass tanks (1.2m diameter; ca. 275L) were set up each with a centre screen (7.62 cm PVC well screen; ~ 25 cm height; 0.5 mm slits spaced 7mm apart). A slow rotation of the water column (ca. 1mm per sec) was generated by a water inflow manifold just above the water surface (1/2 inch PVC with about 8 drilled holes, each 1.5mm). Brackish water (18-20°C; ca. 3ppt) was pumped up from the mixing tank on the ground floor. An upwelling water current was generated by a bubble ring (flexible air diffuser) secured at the base of the centre screen. Debris was siphoned out of the tanks at least once daily. Husbandry was conducted with the aid of red headlights, and light intensity during the larval stage was very low (< 5 lux).

3.3 Spawning

3.3.1 Biopsy and oocyte development

Broodstock were anesthetized with MS-222 (0.15g/L, plus NaHCO₃ 0.3g/L) in a 250L insulated bin (Xactics) filled to 30 cm water depth (80L at 10ppt salinity). Recovery from anesthesia took place in a shallow 500L Xactics tank (30 cm water depth; 243L volume) with a salinity of 10ppt and a large airstone (15 cm) supplied with compressed air. Twelve females were selected for biopsy; thirty males were examined to verify they were producing milt. The fish were dip-netted one at a time, and at loss of reflex, PIT tag number, fork length and body weight recorded. Not all

fish had a PIT tag. An oocyte sample was taken by biopsy by inserting into one of the oviducts a flexible plastic insemination straw (~ 13 cm length; OD 3 mm; ID ~ 1.5 mm) connected to a flexible polyethylene tube (~ 90cm length; OD 3 mm; ID 2 mm). Light mouth suction was applied to the catheter to extract oocytes. The sample was immersed in Cortland's saline (4.179g NaCl, 0.186g KCl, 0.083g CaCl₂, 0.060g MgSO₄, 0.210g NaHCO₃, 0.180g NaH₂PO₄, 0.450g Glucose, 290mOsmol, 7.5 pH in 500 mL water). Oocyte development was assessed by pipetting oocytes onto a depression microscope slide, then developed with an inverted microscope (EVOS XL Core Imaging System). Two depression slides were developed per fish, and three oocytes were measured per depression slide. Oocyte development was based on oocyte diameter and coalescence of oil globule following "Bayless hours" (Bayless 1972). Egg diameter was determined by comparing images of the oocytes against a 1.5 mm calibration circle with the inverted microscope. Females were selected for hormonal induction if oocytes were at 15 Bayless or had advanced further. Oocytes that have been staged for 15 hours or fewer show ooplasm clearing and reach a size greater than 600 μm .

3.3.2 Hormone injection and post-injection

Post-biopsy, selected females and males were moved to thermal induction tanks and separated by sex. Four spawning tanks were used; females were split among two (n = 6 per tank), and males were split among the other two (n = 15 per tank). Controlling water temperature was challenging due to make-up water for the recirculation system coming from a small shallow river, the temperature of which fluctuated greatly depending on weather. Water temperature of the spawning tanks in trial one started at 16.6°C due to unseasonably hot weather conditions. Temperature drop to 13.9°C on 26 May occurred due to cold weather conditions affecting makeup water and sump water temperature (Figure. 3.5).

Water was elevated from 13.9 to 18.4°C from 26 May to 6 June 2024 (Figure 3.5). Water remained heated during the duration of the spawning period of female broodstock (Figure 3.5).

Water temperature of the spawning tanks in trial two was elevated from 15.1 to 18.4°C from 14 June to 20 June (Figure 3.6). Water remained heated during the duration of the spawning period of female broodstock (Figure 3.6).

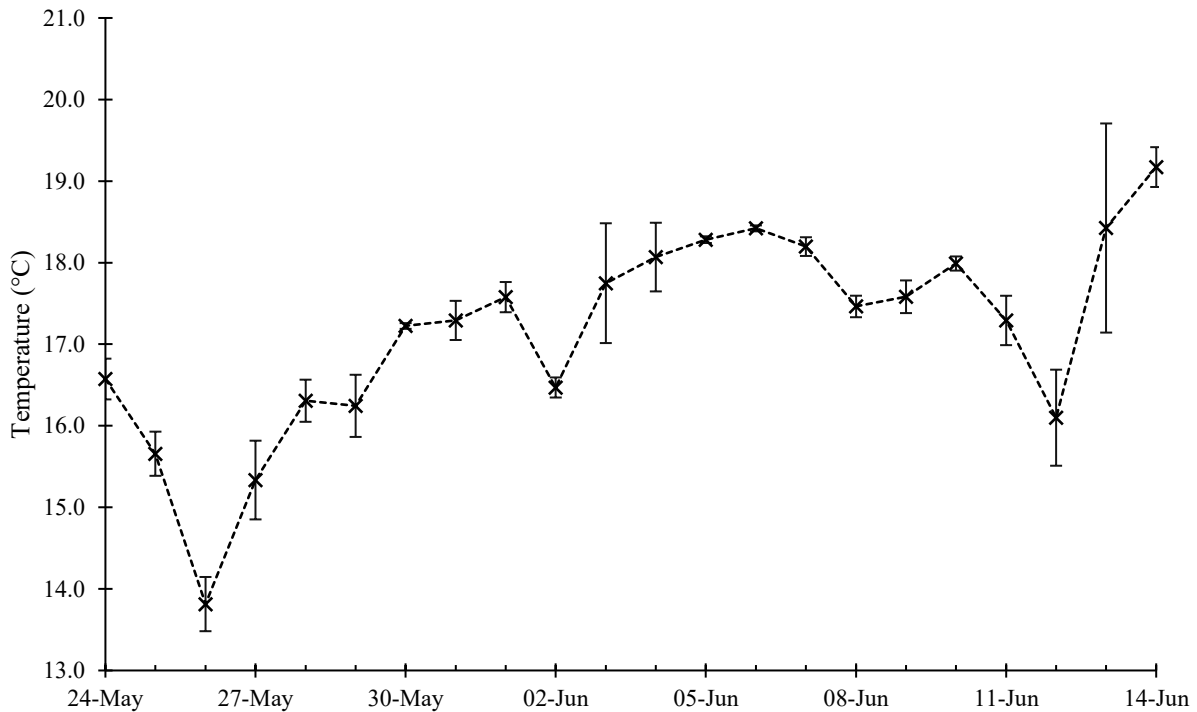


Figure 3.5. Average and standard deviation of daily mean (SD) temperature (°C) of broodstock holding tanks during thermal induction and spawning phase during trial one (24 May to 14 June, 2024), measured hourly. Ovaplant-L injection of females, and hCG injection of males occurred on 6 June. Temperature was recorded hourly using a CTD-Diver (Van Essen Instruments).

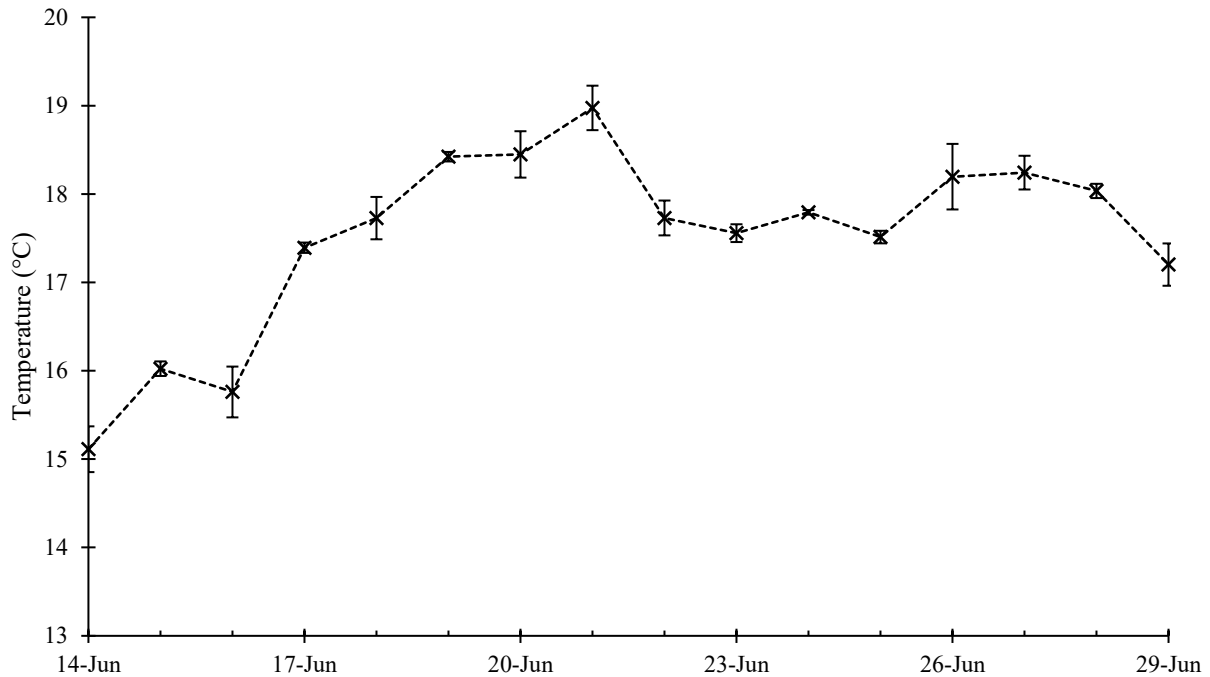


Figure 3.6. Average and standard deviation of daily mean (SD) temperature (°C) of broodstock holding tanks during thermal inducement and spawning phase during trial two (14 June to 29 June, 2024), measured hourly. Ovaplant-L injection of females and hCG injection of males occurred on 20 June. Temperature was recorded hourly using a CTD-Diver (Van Essen Instruments).

On the day when water temperature reached 18°C, an anesthetic tank and recovery tank were prepared following earlier parameters, and the body weight and fork length of each female were recorded using a large analytical balance (Mettler Toledo). Female was assigned by experimental dose and injected with either 10, 30, or 70 $\mu\text{g}^{-1} \text{kg}^{-1}$ Ovaplant-L at the posterior base of the second dorsal fin (sGnRHa; Syndel). Males received a muscular injection of Chorulon (hCG) behind the second dorsal fin (75IU/kg). Post-recovery, fish were transferred to spawning tanks at a 3♂:1♀ sex ratio.

Post-injection, the fish remained in their respective spawning tank for up to 4 days, and checked about every 2 hours for eggs. DO was checked daily and maintained at >80% saturation. Water temperature in the sump was recorded hourly by a CTD-Diver (Van Essen Instruments). During

spawning, large numbers of eggs quickly accumulated in the egg collector. Eggs were carefully siphoned out via a ¾ inch PVC hose at the base of the egg collector into a 500µm sieve (made from 8 inch PVC sewer pipe) in a 10L plastic bucket with a side drain. Flow was controlled by adjusting the height of the collecting bucket. The eggs were gently transferred from the sieve in the bucket to the incubator using a plastic kitchen sifter (15.24 cm diameter; 1.5mm mesh). Oocyte fertilization rate was quantified around 2 to 4h post-spawning by mounting 10 to 30 eggs on a custom built plexiglass slide with a 3 mm wide groove. Using a standard dissecting microscope, the blastocyst was hidden beneath the oil globule. Using a blunt dissecting needle, the blastocyst was revealed through partial rotation of the egg. Each female's eggs were assessed, ranging from 10 to 126 per female.

After the induced spawning attempt was complete, either with or without oviposition, each fish was anaesthetised their fork length and body weight was recorded. Females who did not oviposit within 69 to 95 h were biopsied and oocytes examined, and received an injection of Chorulon behind the second dorsal fin (350IU/kg). Males were checked for milt production. They were netted one at a time into a 1.5 x 1 x 1m Xactics filled with brackish water (10ppt), oxygen supplied ceramic diffuser, transported via tractor back to their holding cage in the middle pond. Dip-netting fish one at a time ensured handling mortalities were very rare (n = 1). Fish resumed feeding within a few days.

Chapter 4: Results

Spawning attempts during 2023 were conducted without use of Ovaplant-L and were unsuccessful. Biopsy performed on 2 June 2023 confirmed all oocytes had not advanced to 15 Bayless hours, indicating immaturity. The heater was not operational, and thus insufficient heating made it impossible to perform thermal induction. Spawning with hCG was attempted on 16 and 19 June however females failed to oviposit oocytes that had finished FOM. Volitional spawning was attempted on 27 June to stimulate oviposition of females, but females failed to oviposit. Thus, no results are presented from 2023.

From the 2024 spawning trials, 13 of 18 females between two trials spawned. Statistical analysis was not permitted due to the requirement of all nine females yielding data in both trials. Therefore, the results of the spawning trials are limited to descriptive statistics.

Differences in percent fertilization was quantified, first using Grubbs' test to determine if there were outliers in the data. One female (female #6, Trial 2; Table 4.2) whose percent fertilization was the lowest (8.0%, $n = 24$) was an outlier ($p = 0.038$, $Z = 2.40$)

A two sample t-test was conducted to quantify differences in percent fertilization using Ovaplant-L (79%, range 50 to 89%) and Ovaplant-L then hCG (66%, range 46 to 85%), showing no significant difference between the two ($p = 0.25$).

4.1 Trial 1 (May 24 to June 09, 2024)

After 10 days in the cold-bank tank at 10.2 to 16.7°C, twelve females were biopsied on 24 May 2024 and were selected as potential spawners. For the thermal induction phase, females were divided between two 1500L tanks, $n=6$ and $n=6$ per tank, thermal induction commenced, elevating

temperature from 16.6 to 18.4°C from 24 May to 6 June. 30 males were biopsied and examined for milt production and were divided between two tanks for the thermal induction phase.

On June 3, biopsies revealed the first three females' oocytes failed to advance since the previous examination on 24 May. Average body weights of the three females were 3.8 kg (range 3.2 to 4.3 kg) with an average fork length of 63.2 cm (range 58.7 to 67.1 cm). Oocyte diameter ranged from 915 to 1247 μm and Bayless hours from 11 to 15 (Table 4.1). The body weights of the females ranged from 3.1 to 5.9 kg; fork length ranged from 60.5 to 77 cm (Table 4.1). Believing not enough time had passed, biopsied fish were returned to the pond cage and remaining nine females remained in thermal induction phase and were biopsied again on 6 June and used for trial one.

On 6 June after the thermal induction period had reached 18°C, the females were biopsied again. Their average body weight decreased by 3.1% since May 24th, from 4248 g (range 3184 to 5964 g) to 4118 g (range 3096 to 5810 g; Table 4.1). Oocyte diameter and Bayless hour ranged from 836 to 1184 μm and 12 to 15 Bayless respectively (Table 4.1). On June 6, all nine females were injected with Ovaplant-L, despite the oocytes of five fish failing to advance from the Bayless 15h stage during the warming stage. Four females spawned successfully after Ovaplant injection, with all four females' oocytes advancing beyond 15 Bayless hours. Four of the five females who failed to spawn with Ovaplant-L had oocytes that did not advance past 15 Bayless hours (Table 4.1). On June 9, remaining five females were injected with Chorulon. Four of five females that received 350 IU kg^{-1} spawned successfully between 5 and 28 h post-injection (Table 4.1). All three females that received 70 $\mu\text{g kg}^{-1}$ Ovaplant-L spawned successfully between 33.5 and 42.5 h post-injection (Table 4.1).

Female #1 was observed to have advanced from 15 to 1h Bayless post-Ovaplant injection, with an oocyte diameter of 1009 (range 899 to 1112, $n = 7$) before thermal inducement, before spawning

successfully after Chorulon injection (Table 4.1). Female #2 oocytes shrank during thermal inducement from 1074 (range 935 to 1288, n = 8) to 973 μm (range 895 to 1009, n = 10), however advanced from 15h to between 12 and 14h, spawning successfully following 10 $\mu\text{g kg}^{-1}$ Ovaplant-L injection. Female #3's oocytes before (915 μm ; range 783 to 1048, n = 6) and after thermal inducement (836 μm ; range 785 to 865, n = 3) were the smallest, did not advance during thermal inducement, and did not advance post-Ovaplant injection; failed to spawn post-Chorulon injection (Table 4.1).

Female #4 advanced from 15 to 10h Bayless post-injection of Ovaplant-L, and despite shrunken oocytes from 1005 (range 909 to 1080, n = 8) to 848 μm (range 697 to 956, n = 4), spawned after Chorulon injection. Female #5 Bayless was judged to have advanced to from 15 to 7 after Ovaplant injection, with oocyte diameter decreasing from 1056 (range 952 to 1213, n = 5) to 943 μm (range 851 to 1023, n = 6), spawning successfully after hCG injection (Table 4.1). Female #6 did not advance Bayless during thermal inducement, and had slightly shrunken oocytes before (916 μm ; range 815 to 975, n = 4) and after (898 μm ; range 848 to 994, n = 4) thermal inducement, but advanced between 10 and 11 h post-Ovaplant, and spawned successfully after injection of Chorulon (Table 4.1).

Female #7 successfully spawned using Ovaplant-L despite both shrinking of oocytes before (1247 μm ; range 1072 to 1499, n = 10) and after (1012 μm ; range 960 to 1029, n = 7) thermal inducement, and Bayless = 15h at time of injection (Table 4.1). However, Bayless was inconsistent through thermal inducement phase, exhibiting 11h oocytes on first biopsy on 24 May. Female #8 had large oocytes and successful spawning resulting from Ovaplant injection; egg diameter increased from 1018 (range 899 to 1107, n = 8) to 1028 μm (range 954 to 1072, n = 6), and Bayless was judged to have advanced from 15 to 14. Female #9 was the first female to spawn, and her oocytes advanced

after the thermal inducement period from 1067 (range 922 to 1137, n = 8) to 1184 μm (range 1158 to 1242, n = 13; Table 4.1).

Of the females that successfully spawned following Ovaplant-L injection, oocyte diameter and percent fertilization ranged from 2.8 to 3.4 mm and 50.0 to 89% respectively with sample sizes of 22 (Female #2), 80 (Female #7), 44 (Female #8), and 126 (Female #9; Table 4.1). Of the females that spawned successfully with Chorulon, oocyte diameter and percent fertilization ranged from 2.9 and 4.0 mm and 61.3 and 89% respectively, with sample sizes of 80 (Female #1), 92 (Female #5), and 50 (Female #6).

Table 4.1. Trial 1. Fork length (FL), body weight, oocyte diameter (OD), Bayless hour maturation stage and spawning times among female striped bass (n=9) following a temperature increase from May 26 to June 6 of 12 to 18°C then Ovaplant-L injection (10, 30, and 70 µg kg⁻¹). Females that failed to spawn by 9 June were injected with Chorulon (hCG; 350 IU/kg). One female did not spawn (DNS) after injection of Chorulon. Asterisk indicates missing data. Egg diameter and fertilization rate was assessed about 4h post spawning.

#	Ovaplant µg/kg	FL (cm)	Body weight (g)		OD (µM)		Bayless (hr)			Hours to spawn post-injection		Egg quality	
			24-May	06-Jun	24-May	06-Jun	24-May	06-Jun	09-Jun	Ovaplant L 06-Jun	hCG 09-Jun	Diameter (mm)	Fert. %
1	10	64.5	3627	3511	1009	*	15	15	1	--	5	3.2	61.3
2	10	63.5	3710	3636	1074	973	15	12 - 14	--	42	--	*	81.6
3	10	62.2	3286	3149	915	836	15	15	15, 1 at 10	--	DNS	DNS	DNS
4	30	70.5	5183	5071	1005	848	15	14	10	--	27	*	*
5	30	77	5964	5810	1056	943	15	15	7	--	5	4	84.8
6	30	61.1	3184	3096	916	898	15	15	10 - 11	--	28	2.9	72
7	70	71	4580	4469	1247	1012	11	15	--	42.5	--	3.4	89
8	70	60.5	3865	3573	1018	1028	15	14	--	40.5	--	3.2	50
9	70	69.8	4836	4749	1067	1184	15	12	--	33.5	--	2.8	84.1

4.2 Trial 2 (June 12 to June 24, 2024)

Twelve females were biopsied on 12 June 2024, after 30 days in the cold-bank (mean 11.9°C, range 9.3 – 16.7°C). Three females' oocytes were immature and were not chosen for Trial 2. Among the remaining nine females, their body weights ranged from 3.4 to 5.7 kg (Table 4.2). Oocyte diameter ranged from 906 to 1082 μm and the development stage was 15 Bayless hours for eight fish, with one female (#4) with oocytes that were borderline between immature and 15h, having 824 μm oocytes (range 758 to 891 μm , n = 6; Table 4.2). Post-biopsy, the females were divided between two tanks for the thermal induction phase. Males (n=33) were checked and biopsied for milt production and were divided between two tanks for the thermal induction phase. The temperature was then raised from 14.0 to 18.4°C between 13 June to 20 June following cooling of water from 16.2 to 14.0°C from 12 June to 13 June (Figure. 3.5).

On 20 June after the thermal induction temperature had reached 18°C, the females were biopsied again. Average body weight of the females decreased in eight days by 0.4% from an average of 4358 g (range 3392 to 5744 g) to 4342 g (range 3434 to 5686 g; Table 4.2). Oocytes ranged from 12 to 15 Bayless, with one female (#4) showing immature oocytes (Table 4.2). Oocyte development between June 12 and 20 advanced in only two fish: female #1 advanced from 15 to 13-14 Bayless, with an oocyte diameter of 906 μm (range 817 to 962, n = 7) and female #3 oocytes advanced to 12-13 Bayless, with an oocyte diameter of 1082 μm (range 1029 to 1124, n = 8; Table 4.2). Female #2 failed to progress in Bayless hours despite having large oocytes (1071 μm ; range 977 to 1156, n = 8; Table 4.2). Female #4 advanced from immature to 15 Bayless hours but died post-injection (Table 4.2). Female #5 had small (987 μm ; range 960 to 1008, n = 8) oocytes and failed to progress in Bayless hours. Female #6 was small in size and had larger oocytes and failed to advance in Bayless hours (1026 μm ; range 979 to 1063, n = 6; Table 4.2). Females #7 through

#9 also failed to advance in Bayless hour despite oocyte diameters of female #7 (914 μm ; range 844 to 986, $n = 7$), female #8 (1006 μm ; range 939 to 1027, $n = 8$) female fish #9 (1007 μm ; range 958 to 1065, $n = 6$) being larger than female #1, who successfully spawned (Table 4.2).

On June 20, the nine females were injected with Ovaplant-L. The random assignment of dose resulted in both females that showed some oocyte advancement receiving the lowest dose, 10 ug kg^{-1} Ovaplant-L. These two females, #1 and #3 were the only two to spawn successfully, between 34 and 40 h post-injection (Table 4.2). Of the two females that spawned with Ovaplant-L, their mean oocyte diameter was 3.1 mm and percent fertilization ranged from 80.0 to 89.0% (Table 4.2), with sample sizes of 10 (Female #1) and 38 (Female #3).

The remaining seven females failed to spawn with Ovaplant-L. Female #4 was found dead on June 21 without spawning. Ovaries were examined; no abnormalities were evident during necropsy and the cause of death was unclear. On June 24, the remaining six females were injected with hCG (350 IU kg^{-1}). Two of six females spawned successfully between 33 and 56 h post-injection (Table 4.2). Female #9's oocytes were judged to have advanced from 15 h to 8 – 9 h Bayless post-Ovaplant-L injection and spawned first (Table 4.2). The second female to spawn had larger oocytes (1026 μm) and advanced her oocyte maturation post-injection of Ovaplant-L from 15 h to 12 – 14 h Bayless (female #6; Table 4.2). The remaining four females failed to spawn, with female #2, 7, and 8's oocytes remaining immature. Female #5's oocytes advanced to 12 – 14 h Bayless post-injection of Ovaplant-L, and – on June 28 – was administered a second injection of Chorulon at 350 IU kg^{-1} . Spawning occurred the next day, 119 h after the first Chorulon injection (Table 4.2). Of the two females that spawned following hCG injection, oocyte diameter and percent fertilization ranged from 1.3 to 2.5 mm and 8.0 to 46.0% respectively (Table 4.2), with sample sizes of 24 (Female #6) and 24 (Female #9).

Table 4.2. Trial 2. Body weight, oocyte diameter (OD), Bayless hour maturation stage and spawning times among female striped bass (n=9) following a temperature increase between June 12 to June 20 from 12 to 18°C then Ovaplant-L injection (10, 30, and 70 µg kg⁻¹). Chorulon (hCG; 350 IU/kg) was injected on 24 June for fish that did not spawn following Ovaplant-L. Female #5 received additional injection of hCG on 28 June. Three females did not spawn (DNS) after injection of Chorulon, and one female died after injection of Ovaplant-L. Asterisk indicates missing data. Time to spawn post-injection of Ovaplant-L, and Chorulon was measured, as well as egg diameter (mm) and fertilization percent.

#	Ovaplant ug/kg	Body weight (g)		OD (µM)	Bayless (hr)			Hours to spawn post-injection		Egg quality	
		12-Jun	20-Jun	12-Jun	12-Jun	20-Jun	24-Jun	Ovaplant L 20-Jun	hCG 24-Jun	Diameter (mm)	Fert. %
1	10	3392	3434	906	15	13 - 14	--	38 to 40	--	3.1	80.0
2	10	4715	4735	1071	15	15	imm	DNS	DNS	--	--
3	10	4510	4479	1082	15	12 - 13	--	34	--	3.1	89.0
4	30	5744	5686	824	imm/15	15		Female died post-injection of Ovaplant			
5	30	4700	4696	987	15	15	12 - 14	--	119	*	*
6	30	3955	3937	1026	15	15	12 - 14	--	56	1.3	8.0
7	70	4462	4446	914	15	imm/15	7 to imm	DNS	DNS	--	--
8	70	3680	3647	1006	15	15	imm	DNS	DNS	--	--
9	70	4064	4019	1007	15	15	8 - 9	--	33	2.5	46.0

Chapter 5: Discussion

This study aimed to quantify spawning success in response to three doses of Ovaplant-L: 10, 30, and 70 $\mu\text{g kg}^{-1}$. While spawning success was inconsistent, with insufficient data to quantify Ovaplant-L's effect on spawning success, the results suggested Bayless hour may be more closely related to spawning success than a specific dose of Ovaplant-L. These findings build upon previous academic work conducted by Andersen et al. (2021c), who successfully spawned domestic striped bass broodstock using mGnRHa and hCG.

There was insufficient data to quantify the effect of Ovaplant-L on spawning success, however trends have been observed through successful spawning females' oocytes being staged at ≤ 15 Bayless hours. However, spawning success in this study post-injection Ovaplant, when administration to females whose oocytes < 15 Bayless hour was 83% (5 of 6; Tables 4.1 and 4.2). These findings indicate that Bayless hour may have a greater influence on spawning success than Ovaplant-L dose and should be the consideration when choosing broodstock for hormonal induction in striped bass hatcheries.

Several potential explanations can account for these results. Variability in gonadal development of broodstock, indicated by differences in Bayless hour after thermal induction may have played a crucial role. Similar spawning trials suggest that successful spawning is attributed to oocyte development stage, and inconsistent spawning response in this study may indicate that hormonal induction as a standalone is insufficient without proper oocyte maturation at < 15 Bayless hours (Andersen et al. 2021c; Hodson & Sullivan 1993).

Additionally, external environmental conditions like water temperature fluctuations, extended periods of cold-banking and handling stress may have negatively impacted spawning success of

this study. Broodstock stress is known to negatively affect hormonal responses in striped bass (Hodson & Sullivan 1993; Zohar et al. 2010). One of the stress indicators is haemorrhages on the surface of the caudal fin, and at the base of the other fins, known as red-tail syndrome (Harrell et al. 1990). These stressors can disrupt the striped bass' reproductive neuroendocrine system, and thus prohibit their ability to complete FOM (Hodson et al. 1990; Andersen et al. 2021c). Presence of red-tail syndrome was observed on striped bass broodstock during this study, and thus, the extended cold-banking period between Trial 1 and Trial 2, compounded with water temperature fluctuations and handling stress may have contributed to the inability of advancing in Bayless hours during trial 2.

Lastly, nutrition of pre-spawning broodstock may have contributed to results. Broodstock diet used in this study was Europa Skretting grower feed, which Skretting advised in September 2024 was found to be not formulated for reproductive conditioning. Use of grower feed also may have influenced oocyte quality, further complicating females' ability to finish FOM.

These factors highlight the difficulty of induced spawning in female striped bass, suggesting that success cannot be attributed solely to Ovaplant-L dose. The following discussion will explore these themes in greater detail, to examine the underlying relationships between Bayless hour, environmental factors, and broodstock nutrition to better understand conditions necessary for greater spawning success.

5.1. Effect of Ovaplant-L dose on spawning success

Spawning trials of 2024 using Ovaplant-L successfully spawned 6 of 18 females in a freshwater RAS at NRFF. In Trial 1, all three females that were administered $70 \mu\text{g kg}^{-1}$ successfully spawned without a follow-up injection of hCG (Table 4.1). Three females' oocytes advanced to less than 15

Bayless hours post-thermal induction to a range of 11 to 14 Bayless (Table 4.1). One female (Female #1) was shown to have advanced to 1 Bayless hour on 9 June, who may have spawned if given more time prior to hCG injection.

Contrasting with Trial 2, all females who were administered $70 \mu\text{g kg}^{-1}$ failed to spawn until injected with hCG, and only one successfully spawned after hCG (Table 4.2). The two females who successfully spawned solely using Ovaplant were administered $10 \mu\text{g kg}^{-1}$, but whose oocytes advanced during thermal induction phase (Table 4.2). It seems that if thermal induction results in oocytes advancing beyond 15 Bayless hours, there is a high probability that Ovaplant-L will result in spawning with good quality eggs independently of Ovaplant-L dose.

These trials suggest Ovaplant-L has promise for inducing spawning of Bay of Fundy striped bass. This is supported by previous studies, as use of mGnRH α has been used to successfully spawn striped bass since the 1990s (Andersen et al. 2021c; Hodson & Sullivan 1993). As GnRH decay dynamics are similar amongst fishes due to the structural similarities among GnRH types, there should be no difference between the use of mGnRH α and sGnRH α (Lethimonier et al. 2004).

Spawning success of Chesapeake Bay striped bass at 3♂:1♀ sex ratio injected with mGnRH α was 78% (18 of 23), however only 50% (9 of 18) successfully produced fry (Andersen et al. 2021c). While spawning attempts for this study followed procedures developed by North Carolina researchers, notable differences were found in used GnRH, broodstock, and hatchery facilities between similar spawning studies of striped bass.

Hodson & Sullivan (1993) reported on spawning induction in Chesapeake Bay striped bass using pellets containing $150 \mu\text{g mGnRH}\alpha$, with a dose range of 33 to $111 \mu\text{g kg}^{-1}$. All nine wild and three domestic females tested had oocytes measured at > 15 Bayless hour and thus required follow-up

injection of hCG regardless of initial mGnRHa dose (Hodson & Sullivan 1993). Timing for hCG injection varied from 10 to 75 hours post administration of mGnRHa; spawning was completed 8 to 25 hours post-injection of hCG (Hodson & Sullivan 1993). While spawning occurred in all individuals, the need for a follow-up injection of hCG, combined with wide variability in oocyte quality (15.0 to 80.2% hatching success) that was independent of mGnRHa dose suggests that the method was partially successful and comparable to the present study.

In Mylonas et al. (1998), the spawning induction protocol relied on hormonal induction of mGnRH with an extended observation period of up to 10 days. Using injections of 30 to 50 $\mu\text{g kg}^{-1}$ to induce spawning of striped bass in Israel, this longer window allowed females' oocytes sufficient time to complete FOM, denoted through onset of GVBD, corresponding to < 15 Bayless hour development (Mylonas et al. 1998). Despite this strategy inducing FOM and ovulation and oviposition in 13 of 15 females, the overall success was partial, with 46% fertilization (Mylonas et al. 1998).

In contrast, this current study used a more compressed timeline, which may have resulted in three of six females' oocytes remaining immature at the time of hCG injection during Trial 2 (Table 4.2). During the spawning trials of 2024, females who failed to spawn after 3 to 4 days were injected with hCG, thus it is uncertain as to whether these females would spawn if given 10 days prior to hCG injection. While Mylonas et al. (1998) managed their water temperature effectively over a longer induction period (17.2 – 20.1°C), experimental conditions within the current study may have imposed more rapid or variable changes given its more compressed timeline, reducing the opportunity for optimal oocyte maturation. Thus, while the protocol of Mylonas et al. (1998) achieved success with their spawning trials, their moderate fertilization rate suggests that their methodology could be improved.

This helps to explain the results of this study, which diverged in two ways compared to Mylonas et al. (1998). The current study induced spawning with Ovaplant-L, with a follow-up injection of hCG three (Trial 1) and four (Trial 2) days after. Mylonas et al. (1998) had an observation period of 10 days, using multiple delivery systems of inducing spawning with GnRH: microspheres, implants, and injections. Additionally, this study differed through its environmental conditions, with Mylonas et al. (1998) using larger (50m³) holding tanks compared to the current study's 8.1m³ spawning tanks, potentially resulting in additional broodstock stress. Lastly, Mylonas et al. (1998) used broodstock that may have been potentially domesticated, as they were produced by wild Chesapeake Bay broodstock, compared to the current study, which derived from wild eggs.

Employment of mGnRHa for induced spawning of 5th generation domestic female striped bass seems effective only at ≤ 15 Bayless hours (Andersen et al. 2021c). Optimal spawning was achieved when oocytes were advanced sufficiently, resulting in an initial spawning success of 78%, with about 50% of spawned eggs developing into fry, indicating the protocol was effective in triggering spawning with effective reproductive success (Andersen et al. 2021c).

A potential critical factor of Andersen et al. (2021c) is the use of 5th generation domestic striped bass broodstock, which may inherently differ in their physiological responses compared to the earlier generation broodstock used in the present study. These genetic differences, combined with control over environmental variables and precise timing of GnRHa induction relative to oocyte maturity may have contributed to the observed outcomes. These differences in achieving broodstock with greater generations of domestication may explain why this current study, despite following the procedures of Andersen et al. (2021c), was unable to achieve full spawning success.

5.2. Optimal Bayless hour to spawn

The effectiveness of GnRH induced spawning in striped bass broodstock depends greatly on the Bayless hour at time of administration, with oocytes beyond ≥ 15 Bayless hour typically advancing to an average of 11 Bayless hours 10 to 75 hours post-administration of mGnRH α (range 8 to 14 Bayless, $n = 12$; Hodson & Sullivan 1993). Use of hCG has shown to successfully spawn striped bass broodstock at even 15 Bayless hours; however, its employment does not trigger GTH production to further mature the oocytes (Hodson & Sullivan 1993; Andersen et al. 2021c). In the present study, one of 11 females who received injection of Ovaplant-L at 15 Bayless hours spawned successfully, however Bayless was inconsistent through thermal inducement phase, exhibiting 11h oocytes on first biopsy on 24 May (Table 4.1). Spawning trials on North Carolinian captive striped bass had success with hormonal induction when oocytes were at 9 – 15 Bayless hour, but exact details were not provided (Andersen et al. 2021c).

The fertilization rate of striped bass was also observed, as females with oocytes at 13 – 14 Bayless hour injected with hCG oviposited a mean of 569,000 oocytes compared to females at ≤ 11 Bayless hours ovipositing a mean of 818,000 oocytes (Hodson & Sullivan 1993). Fertilization in the present study of females who spawned with Ovaplant (79%, range 50 – 89 %) vs. Ovaplant then hCG (66%, range 46 – 85%) were contrasted (Table 4.1; Table 4.2). Female #6 in Trial 2 whose oocytes' percent fertilization was 8.0% was counted as an outlier as per Grubb's test. Additionally, that female's oocytes were 1.25 mm diameter, indicative of low quality and ruptured chorion. Fertility of eggs spawned with Ovaplant vs. Ovaplant then hCG were not significantly different ($p = 0.25$, $T = 1.27$, $DF = 6$).

5.3. Spawning success and nutrition in striped bass

Spawning success in striped bass broodstock is not solely attributed to hormonal induction protocols employed; it is also critically dependent on the nutritional status of broodstock. Andersen et al. (2020) showed that domestic, 5th generation broodstock were maintained on a commercial broodstock diet designed for striped and white bass broodstock for hybrid striped bass production (Bass Brood, Ziegler, Gardners, PA: 45% protein, 15% lipid).

A similar broodstock feed was also used in Hodson et al. (1999), which is based on the U.S. Fish & Wildlife Service open formula salmon diet, substituting squid meal for herring/menhaden meal, and up to 25% of fish oil is replaced with squid meal (Hodson et al. 1999; CAF-Striped Bass Broodstock Diet “B”, Ziegler, Gardners, PA). High-protein, balanced lipid diets support the energetic and biochemical demands of oocyte development, thus contributing to the spawning success found in their spawning trials, and increased levels of ω -3 and ω -6 fatty acids promote larval development and survival (Hodson et al. 1999).

While prior spawning protocol development highlighted in Hodson & Sullivan (1993) focused on hormonal induction, domestic broodstock were fed a 38% protein commercial trout diet, also sourced from Ziegler Bros (Gardners, PA; Hodson & Sullivan 1993).

In contrast, the current study used Europa Skretting: a 12 mm pellet feed supplemented with squid meal (Europa, Skretting; St. Andrews, N.B: 50% protein, 18% oil). After receiving notice from Dr. Backman from Skretting, he stated that Europa diet is a grower diet, not a brood diet (N. Mann, Skretting, pers. comm. to J. Duston 18 September 2024). Dr. Backman recommended use of Vitalis SA feed for broodstock spawning for future spawning efforts, also containing squid meal and greater quantities of Vitamin C and Vitamin E (Vitalis, Skretting; St. Andrews, N.B: 50% protein,

24% fat). The use of Europa diet may have led to compromised oocyte quality and inconsistent spawning outcomes for striped bass, following poor egg quality in lumpfish broodstock at Huntsman Marine Science Centre (*Cyclopterus lumpus*; P. Wiper, pers. comm. to J. Duston September 2024).

These findings underscore the importance of integrating nutritional strategies with hormonal induction protocols with striped bass spawning efforts.

5.4. Study limitations

A key limitation of this study was the inability to spawn using Ovaplant-L during summer 2023 due to Syndel's inability to supply the product, and low spawning success of Trial 2 in 2024, which prevented application of robust statistical analysis. While there were trends that could be observed, caution must be taken in drawing conclusions from the data. Individual variation to dose response could be contributed to inconsistent results, or exogenous factors such as handling stress, or water temperature fluctuations. Although every effort was made to maintain optimal spawning conditions throughout the study, effect of handling stress is shown to negatively affect spawning attempts of striped bass broodstock through disruption of broodstock's reproductive neuroendocrine system, prohibiting their ability to complete FOM (Andersen et al. 2021c). Future spawning efforts should aim to control these external variables more strictly.

An additional limitation can be found in the relatively small sample size and the resulting low replication in spawning trials. Statistical analysis was not permitted due to the model's requirement of all nine females yielding data between two spawning trials, thus this limited replication compounded by individual variability among broodstock made it difficult to generalize observed trends across larger populations.

Another limitation to this study can be found in the feed fed to broodstock prior to removal from net pens: spawning attempts post 2019 used Europa Skretting 12 mm after advice from Skretting. Given that Europa is for grower broodstock, this may present itself as a confounding variable for oocyte quality. Thus, a lower-fat, higher-protein broodstock feed may contribute to greater spawning success for future trials.

Lastly, a key limitation of this study was the use of prolonged cold banking to half oocyte maturation and acclimate broodstock to tank conditions. While it was necessary for logistical consistency, the differences in cold banking duration between Trial 1 (10 days) and Trial 2 (30 days), combined with the use of a smaller holding tank (8,100L) may have introduced additional stressors not present in previous studies that used larger systems (31,139L in Andersen et al. 2021c; 50,000L in Mylonas et al. 1998). These factors may have disrupted reproductive neuroendocrine function, impairing FOM and reducing spawning success (Andersen et al. 2021c). These effects can be confounding comparisons between trials, limiting the interpretation of efficiency of Ovaplant-L dose on spawning success.

5.5. Future research directions

To further refine spawning procedures of Bay of Fundy striped bass, several areas require additional investigation:

1. Optimization on Bayless hour effect on hormonal induction. Greater quantification on spawning success focusing on Bayless hour can be examined to determine which oocyte development stage is required for consistent spawning response for Ovaplant treatment.

2. Extend observation period. Similar research on Israeli striped bass observed spawning response of GnRH for up to 10 days (Mylonas et al. 1998). Female #1 of Trial 1 was shown at 1 Bayless hour on 9 June 2024; more time may have resulted in completion of FOM.
3. Assessment of exogenous conditions. Monitoring effect of water temperature and handling on broodstock stress and hormonal responsiveness can enhance consistency of spawning attempts in hatchery settings. The observation of broodstock stressors such as red-tail syndrome and comparing with spawning attempts to quantify effect and intensity of broodstock stress on spawning response.
4. Refinement of knowledge on conditions prior to spawning. Allowing broodstock more time in net-pen results in less stress and more feeding prior to spawning trials, and warming late-spring waters contribute to furthering FOM to result in lower Bayless hour after cold-bank period.

5.6. Conclusion

The spawning success of female Bay of Fundy striped bass broodstock is influenced by multiple interacting factors. Failed vitellogenesis while in captivity, attributed to outward stresses on broodstock result in failed FOM and inability to spawn without a reproductive aid. GnRH has been used for decades in academia to spawn striped bass broodstock with varying degrees of success, however, only recently have commercial products been tentatively approved by Health Canada for use on commercial broodfish. Ovaplant-L, approved for use on commercial striped bass, as a liquid analogue allows for adjusted doses to quantify spawning response. Ovaplant injections of 10, 30, and 70 μg^{-1} kg resulted in 83% successful spawning in females regardless of dose at < 15 Bayless hour. Thus, impact of Bayless hour rather than Ovaplant dose should be considered more carefully moving forward with similar studies. Additionally, importance of exogenous conditions such as

water temperature, handling stress, cold-bank timing and broodstock feed should not be underestimated as impacts of these conditions can be seen both in literature and in this study. Further research into the Bayless hour that will lead to successful spawning could be beneficial in furthering the state of knowledge of creating a land-based industry of striped bass farming.

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