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Application and Modification of a Wakasagi Hatching Box for Delta Smelt Propagation

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Application and Modification of a Wakasagi Hatching Box for Delta Smelt Propagation

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Abstract

Delta smelt (*Hypomesus transpacificus*) (DS) are an endangered species of fish in the Osmeridae family. They are endemic to the Sacramento/San Joaquin Delta (Delta). This thesis explores the application of an Egg Frame Box (EFB) created by the Lake Suwa Fishing Collective (LSFC) as a means to hatch DS eggs into the Delta in an effort to reduce hatchery characteristics by investigating the fluid dynamics inside and around the EFB through computational fluid dynamics simulations and found that the water decelerates significantly before entering the EFB and further decelerates upon entering the EFB, to a suitable water flow velocity for DS egg incubation. Studying the effects of water temperatures on the hatching and survival rate of DS eggs adhered to the EFB found that temperature plays a significant role in the fertilization and survival of DS eggs, as well as showing that DS eggs can hatch at the water flow velocity found during simulations. Another study focused on testing how alternative egg frame material for the DS eggs to adhere to affects their hatching and survival rate and found that DS eggs can adhere to and hatch from the substrates tested in a similar rate as the egg frame from the LSFC, demonstrating that locally bought, biodegradable items can be used to as a substitute for the egg frame substrate. Last, we studied the effects of different humidities on DS eggs to evaluate how transporting the eggs outside of water will affect their hatching rate and found that DS eggs are extremely sensitive to being removed from their aquatic environment and should remain submerged throughout their transport to the deployment site. Overall, this thesis provides valuable insight into DS eggs in an effort to restore the wild population from its endangered status in the Delta.

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Table of Contents

Chapter 1: General Introduction and Literature Review

1.1 The San Francisco Estuary

The San Francisco Estuary (SFE), combined with the Sacramento-San Joaquin Delta (Delta), is the largest estuary on the West Coast (Davoren & Ayres, 1984). Starting from the west coast at the Golden Gate Bridge and reaching the Delta, the SFE consists of four smaller bays, Central Bay, South Bay, North Bay, and Suisun Bay, spanning over 1600 square miles and includes a variety of habitats like marshlands, tidal channels, and mudflats. It is an important ecological system that supports a diverse range of fishes, wildlife, and plant species. It also serves as a passage for shipping vessels and as a source of water for many parts of California. Many threats, including habitat and water flow regime alteration, invasive species, and water diversions, have affected aquatic organisms in the SFE (Bennett & Burau, 2015; Moyle et al., 2016; Sommer et al., 2007).

Communities living around the estuary, as well as the state of California, heavily rely on the estuary for food, water, and recreation. Cities in Southern California rely on the SFE as a water source, as a portion of the water is diverted using the California Aqueduct to supply water to the region (DWR Public Affairs Office, 2002). Two rivers that play a key role in the physical and ecological characteristics of the estuary are the Sacramento River and the San Joaquin River. These rivers are the largest rivers in California, and a substantial portion of them drain into the Central Valley. However, the SFE faces many challenges and threats, including habitat loss, pollution, and climate change. These threats have contributed to the decline of many fish species that live in the estuary like the delta smelt (*Hypomesus transpacificus*), longfin smelt (*Spirinchus thaleichthys*), chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*Oncorhynchus*

mykiss), white sturgeon (*Acipenser transmontanus*), and green sturgeon (*Acipenser medirostris*) (Miller et al., 2012).

The SFE is continuously facing threats like climate change, sea level rises, drought, and invasive species (Poff et al., 1997). These problems have already caused severe damage to the ecosystem of the Delta and are expected to increase in the coming years. Climate change has a large effect on the fish population in the SFE. Rising temperatures, droughts, and random rainy seasons lead to alterations in the physical characteristics of the estuary like the salinity, water temperature, clarity, and habitat, which are critical for the development and survival of fishes. Climate change also affects the rise in sea level, further impacting the habitats and food web by allowing seawater to enter the inland water (Parker et al., 2011).

Invasive species are also a significant threat to native species of the SFE. Non-native species like the Asian clam (*Corbicula fluminea*) compete with native filter-feeding invertebrates for prey, the Chinese mitten crab (*Eriocheir sinensis*), which has a burrowing behavior that can damage riverbanks and levees, as well as the striped bass (*Morone saxatilis*), which prey on native fishes in the estuary.

There are many conservation efforts underway to restore the SFE. Habitat restoration has been a key focus of the conservation efforts for the estuary (Figure 1.1). One example of a habitat restoration project is the California EcoRestore program (EcoRestore). The multi-agency initiative, launched in 2015, focuses on restoring 30,000 acres of wetland and floodplains within the Delta, Suisun Marsh, and Yolo Bypass region by 2025. The project is led by the Department of Water Resources and currently involves 32 projects. A majority of the projects are focusing on actions supporting the long-term health of the Delta and its native fish and wildlife species. In 2006, the first project toward the 9,000-acre tidal wetland target broke ground and as of 2020,

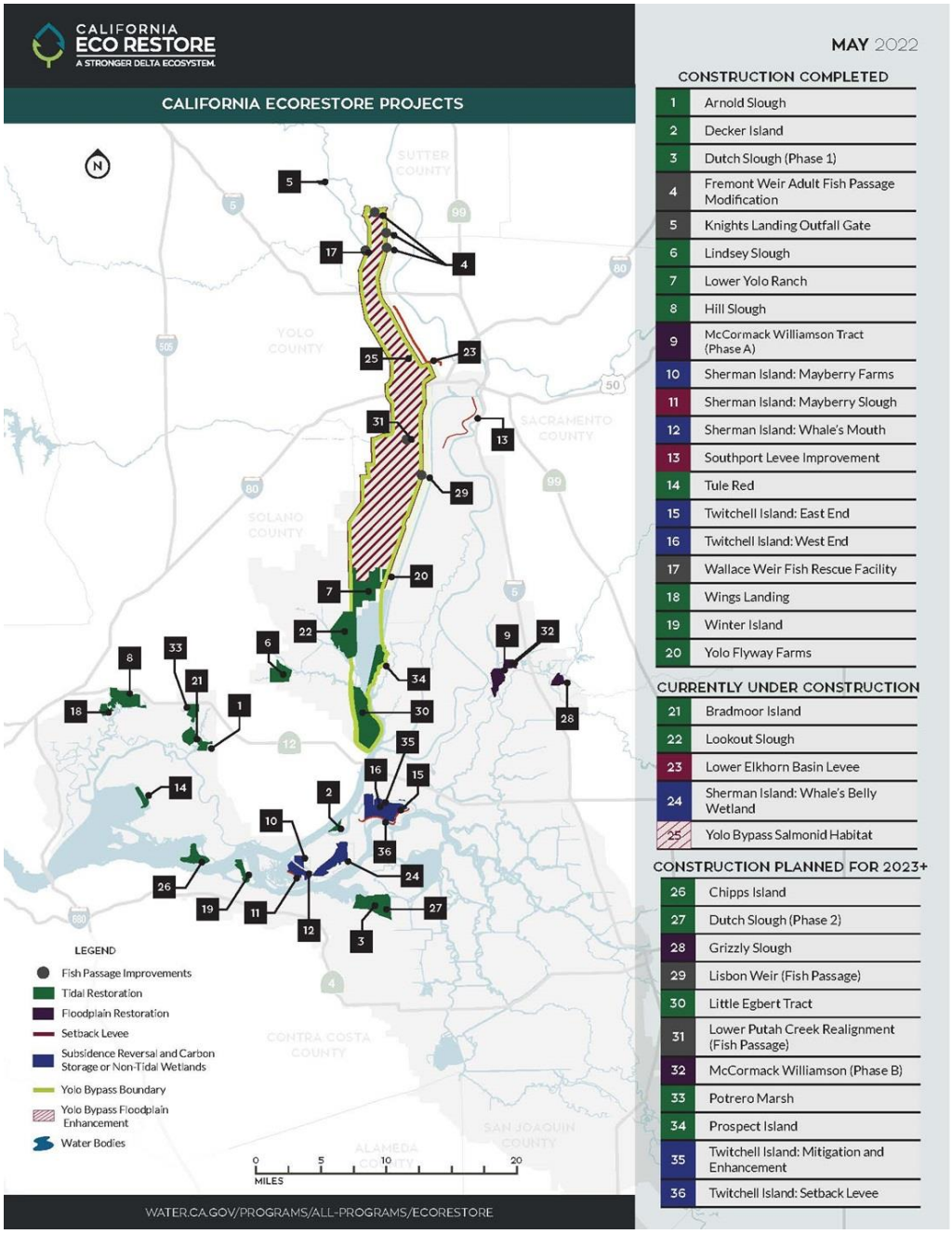


Figure 1.1: Map of California Ecosystem Projects (California Eco Restore)

EcoRestore has nearly doubled the restored and protected habitat lands in the Delta with all planned tidal wetland projects expected to be under construction or completed.

The SFE is a vital ecosystem that supports a wide range of fish species, wildlife, and plants as well as the communities nearest to the estuary as well as the rest of the state of California. However, with the number of challenges that the estuary faces including the loss of habitat, pollution, climate change, and water diversion, these problems have caused a major decline in many native fish species like the delta smelt. Though conservation efforts, like habitat restoration and fishery management have been successful in addressing threats, it is important to continue research, monitoring, and effective management of the estuary and the native fish species to ensure the long-term health of the San Francisco estuary and the diverse food web for future generations.

1.2 Delta Smelt Biology

Delta smelt (DS) are an endangered species endemic to the Delta (Moyle, 2002). They are a small (5-7 cm), slender smelt, as seen in Figure 1.2, residing in the Osmeridae family (Moyle et al., 2018). DS are known as an indicator species of the health of the Delta (Hasenbein et al., 2013). They are usually a semelparous fish, most living only 1 year, with a few able to survive to age 2, consisting of a single distinct wild population that are usually found in the oligohaline. DS eggs are small (1 mm), transparent appearance, and possess an adhesive stalk that enables them to adhere onto substrates such as vegetation, sand, or gravel, preventing them from getting swept away by the water current as seen in Figure 1.3 (Lindberg et al., 2020). Then, larvae grow into juveniles in the spring and migrate into a low salinity zone in

Figure 1.2: Adult delta smelt (Photo credit: Marzieh Aghbolaghi. FCCL.)

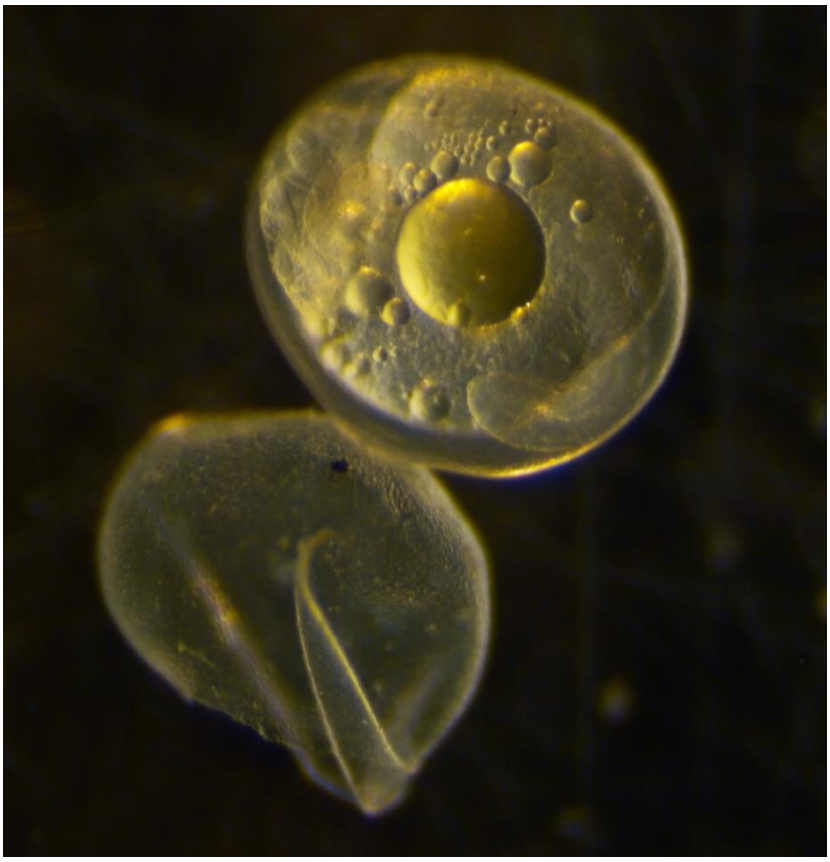


Figure 1.3: Delta smelt egg with adhesive stalk (Photo Credit: Bradd Baskerville-Bridges, FCCL, 2017)

the summer. In the fall, the juveniles will start to mature, before swimming upstream into the estuary in the winter where they spawn multiple times, most likely on sandy beaches and other substrate for the eggs to stick on, and then die (Sommer et al., 2011). The other remains in the fresh tidal waters of the northern delta for their entire life (Moyle et al., 2018). Recent research from Hobbs et al., (2019) has revealed the presence of three distinct life-history phenotypes in DS including freshwater resident, brackish-water resident, and semi-anadromous DS that should be considered into future conservation and management efforts aimed at preventing the extinction of DS in the wild.

Usually following migration are substantial risks from natural sources, such as predation, starvation, and extreme climate, as well as anthropogenic sources, including hunting, fishing, and barricades (Sommer et al., 2011). As they are an annual fish, DS migration and spawning are the most important periods of their life cycles in terms of survival of the species. Thus, the existence and steadiness of the species depends on the successful spawning and migration of adult DS. This migration takes place in a highly sought out area of water, as the route must travel through the Delta, which is found in the upper east region of the SFE, to arrive at their spawning grounds.

DS are a weak swimming fish, using a stroke and glide style of swimming (Swanson et al., 1998). The majority of DS found in 2011 were semi-anadromous, where larval DS are reared in low salinity zones and migrate back to freshwater regions for spawning. This is believed to begin with the "first flush" of turbid water from land runoff after the first major winter rainstorm (Bennett & Burau, 2015; Sommer et al., 2011). Several studies have investigated the migration and distribution of DS during spawning season. Sommer et al., (2011) reported that DS migrate upstream to regions in Cache Slough. Murphy & Hamilton, (2013) supported their findings when they found an increase in DS density in Montezuma and Cache Slough and a decrease in Suisun

Bay during spawning season. Bennett & Burau, (2015) found that DS exploit tidal action to migrate upstream to their spawning grounds in the northern region of the Delta. Kurobe et al., (2022) found that during spawning season, mature DS are found in freshwater locations where the salinity is below 1.0. However, there are still aspects of DS migration that are not understood. For example, it is unclear whether DS have specific spawning grounds that they migrate to or whether they spawn in locations where water quality parameters are suitable. Specific requirements like substrate type and depth are also still unknown.

Though a once common fish in the delta, the decline of their population can be attributed to many different factors, ecological and anthropological (Miller et al., 2012). Since DS migrate from the brackish waters of the Delta into the freshwater of the Sacramento River to lay their eggs, their population faces critical threats when the waterways that connect their migration routes to their spawning grounds are altered. Significant factors like water diversion in their migration paths, climate change, and several historical droughts have confined the population of DS into a fraction of the habitat that they once easily traversed for many years (Moyle et al., 2016).

As an annual species, DS can experience significant shifts in their population from minor changes in their habitats. Thus, ecological, and anthropological factors such as long-lasting droughts and diversions of water runways that prevent DS from returning to their spawning grounds, can also have extremely detrimental effects to their populations. DS are low on the food web and are a prey species for many fish such as Chinook salmon, steelhead trout, striped bass, sturgeon, and many more species of fishes (Moyle et al., 1992). Their population has been on a steep decline in the last couple of decades and since, their numbers have dwindled enormously.

The International Union for Conservation of Nature (IUCN) Red List of Threatened Species, or IUCN Red list, one of the most extensive inventories of global conservation statuses for biological species (IUCN 2022). They employ a precise set of criteria, which are relative to each species and all regions, to evaluate the risk of extinction of thousands of species and subspecies. There are nine groups that species are categorized into based on rate of decline, population size, and other criteria. Potential for future threats is also considered in the classification of the conservation status of the species. DS are listed by the IUCN Red list as critically endangered, which is when a species is in a particular and extremely critical state when a species has a population size reduction, a reduction across a geographic range, a population decline, a very small population size, or probability of extinction.

In the past, DS were abundant in the upper Delta. Populations started declining dramatically in the 1980s. A key threat leading to the extinction of the species is a reduction across the geographic range of their native habitat, due to environmental changes. A large, historic drought all over the state of California ranging from 2012 to 2016, followed by an extremely wet 2017 has caused a large decline of DS (Boxall, 2017). Another environmental change that is continuing to contribute to the decline of DS is the diversion of water sources in Northern and Central California. The diversions created to supply water to civilization are cutting off migration routes used by adult DS from their spawning grounds (Moyle et al., 2018).

Since 1959 the California Department of Fish and Game (DFG) has been conducting annually the Summer Townet Survey (STN), which was designed to quantify the abundance of age 0 striped bass as well as collect DS data to analyze abundance, distribution, and habitat use. The STN sampled thirty-two historic stations ranging from eastern San Pablo Bay to Rio Vista on the Sacramento River as well as Stockton on the San Joaquin River. In 2011, the STN added

eight additional stations in Cache Slough and the Sacramento Deep Water Ship Channel. The STN begins sampling in mid-June using a conical net of 1.5 m^2 mouth and 2.5 mm cod-end mesh, which is towed obliquely through the water column.

The Fall Midwater Trawl Survey (FMWT) is an annual trawl conducted since 1967, initiated in the SFE to determine the relative abundance and distribution of young striped bass, but it also sampled other pelagic species in the upper estuary as well as DS. The FMWT samples 116 separate locations from September to December to calculate an annual abundance index. These sampling locations vary from areas covered by the STN like the San Pablo Bay to Stockton on the San Joaquin River, Hood on the Sacramento River, and the Sacramento Deep Water Ship Channel. The midwater trawl has mouth dimensions of 12 ft wide by 12 ft high with smaller dimensions in the mouth when under tension during a tow. These mesh sizes vary from 0.5 inch at the cod-ends and gradually increase in nine different sections until the largest mesh of eight inches. The FMWT was consistently used to conduct sampling of mature, adult DS, from 1991 until 2002, when it was replaced by the Spring Kodiak Trawl.

The Spring Kodiak Trawl Survey (SKT) is a yearly survey that takes place from January to May with the purpose of determining the relative abundance and distribution of adult DS during the time they ripen and spawn (Sommer et al., 2011). Starting from January or February, the SKT samples thirty-nine separate locations from the Napa River upstream from the Suisun Bay and the Delta. A 10-minute surface sample is conducted at each location, by two boats towing a 7.6 m wide by 1.8 m high Kodiak trawl. The mesh sizes vary from 5.1 cm knotted stretched mesh at the mouth of the net, 1.3 cm in five panels, and 0.6 cm knotless stretched mesh at the cod end. The DS collected in the survey are counted, measured, and then categorized into groups in terms of six spawning condition categories.

The Fish Conservation and Culture Laboratory (FCCL), located in Byron, California, is a research and education facility that has been a part of the Biological and Agricultural engineering department of the University of California, Davis since 1996. The main goals of the laboratory are to maintain a refuge population of DS in captivity that is very genetically close to the wild population as a safeguard against extinction (Hung et al., 2019). The FCCL also houses and maintains a research population used for collaborative research with researchers from different agencies and institutions, providing fishes of all life stages. The FCCL is focused on developing and implementing effective strategies for improving the survival and hatching rate of cultured DS at their labs. They have also started housing and conducting research on wakasagi and longfin smelt. As of the last couple of years, the FCCL has added as well as renovated existing facilities to increase the capacity for culture and research purposes. They also provides tours and educational programs for the general public, school groups, and community organizations. The FCCL has a significant role in the conservation efforts to conserve and restore the native fish populations in the Delta.

1.3 Wakasagi

Wakasagi (*Hypomesus nipponensis*) *also* known as the Japanese smelt, are a Japanese commercial food fish, found widely in lakes and estuaries in Japan, such as Honshu, and Hokkaido, as well as in parts of North and South Korea, Russia, and the Delta. They are a small, thin fish, also in the Osmeridae family. They are usually around 10-20 cm long (Figure 1.3), and have a cylindrical, slender body and a pointy snout. They look very similar to DS. In fact, wakasagi and DS both share very similar biological characteristics (Swanson et al., 2000). Wakasagi have short life cycles, and they typically live for 1 or 2 years, and are able to spawn multiple times in one season (Sasaki et al., 2003).

Figure 1.3: Adult wakasagi. (Photo credit: Marzieh Aghbolaghi. FCCL.)

Wakasagi typically spawn in flowing water, with adults from coastal populations as well as freshwater lake populations often swimming into rivers to spawn. The eggs each come with an adhesive stalk so that the eggs can adhere to submerged vegetation and keep from getting carried out by the water current (Peterson et al., 2021). Lake spawning in landlocked lakes and reservoirs are likely to occur as well, although it has now to scientifically described yet. After hatching, the larval fish stay in the shallow water currents. When they then grow into juvenile fish and start to swim on their own accord into shallow waters to feed on plankton and other small organisms like Copepoda, Rotifera, Cladocera, and Ostracoda, just like DS (Peterson et al., 2021). Factors such as warmer water temperatures and higher levels of prey species can accelerate the growth of wakasagi. Typically, when wakasagi usually reach adulthood, they will migrate from the deep waters to shallower areas near shores to spawn their eggs onto vegetation.

Wakasagi were introduced to the Delta in 1959 by the California Department of Fish and Game for stocking in upper California reservoirs and streams to serve as prey trout and salmon (Davis et al., 2022). Being a non-native species that thrives in the Delta makes wakasagi an invasive species. An invasive species refers to a non-native species that has been introduced into an ecosystem with the ability to establish and spread rapidly, causing ecological, economic, or social harm to the surrounding ecosystem. It is a term usually used to describe non-native species that have negative effects on the ecosystem. Wakasagi and DS share many ecological similarities, including overlapping habitats and food resources and thus, will compete for these resources. Wakasagi and DS share many ecological similarities, including overlapping habitats and food resources and thus, will compete for these resources (Peterson et al., 2021).

The Lake Suwa Fishing Collective (LSFC) is an organization in Japan that manages and regulates fishing activities in Lake Suwa. This organization consists of local fishing businesses

as well as fishermen. They set rules and regulations for fishing in Lake Suwa and are responsible for promoting sustainable fishing practices and preserving the ecosystem. To supply the demand of wakasagi to Japanese fishermen, the LSFC created an egg holding device that houses fertilized wakasagi eggs. This device, known as an egg frame box (EFB), is designed to hold numerous egg frames that the wakasagi eggs adhere to. Given their similarities in life cycles, egg spawning, and adhesion qualities, it is reasonable to assume that the EFB deployed by the LSFC could potentially be adapted for introducing newly hatched larval DS into the Delta.

1.4 Artificial Incubation of Fish Eggs

The practice of using egg incubators for fish egg incubation has created a significant improvement in large scale aquaculture operations. These devices can be used for many different fishes in aquaculture as a method for fish reproduction. Fish egg incubation has an especially vital role for the aquaculture industry and the use of frames for fish egg incubation has been a significant part of aquaculture, mainly in large scale operations. With the ability to control and optimize environmental conditions such as water temperature, salinity, water quality, oxygen levels, etc., incubation methods have ensured higher hatching and survival rates for fish eggs. This has led to more sustainable and efficient techniques to produce fish.

The gentle flow of water provides oxygen exchange for the eggs and helps keep bacteria from proliferating on them(Watson & Chapman, 1996). Fish egg incubators can be classified into three major types based primarily on the density of the eggs to be hatched, their stickiness, and the sensitivity of the eggs: egg frames, trays, and conical incubators.

A tray-type incubator (Figure 1.4) consists of a container that has a screen through which a flow of water permeates to supply the eggs with oxygen and flush away waste products. They are often designed so that water penetrates the tray from below and flows out over the upper

Figure 1.4: MariSource 8-tray Vertical Incubator for Salmon (Photo Credit: MariSource)

edge. Since the eggs lay over a screen, tray-type incubators are ideal for fish eggs that can be injured by movement during incubation. The newly hatched larvae can drop through the screen holes minimizing handling and removal of the eggshells. can also be placed outside the spawning tank and then used as incubators.

Conical shaped tanks (Figure 1.5) are usually for fish eggs that are non-adhesive and require constant movement where water flows into the bottom or top of the container. In this type of incubator, the eggs are gently suspended, and the flow of water constantly tumbles the eggs in the lower portion of the cone. The flowing water ensures that well oxygenated water is constantly being replaced in the incubator and the tumbling of the eggs keeps them from collecting debris which can lead to fungal infections. These types of incubators can be set in series above a catching tank, where the hatched larvae pour out of the incubators as they hatch and flow into the tank.

Egg frames (Figure 1.6) are usually for adhesive eggs. By simulating a spawning, they serve as egg collectors and provide a place for egg attachment. Frames consist of bundles of fibrous material arranged in a variety of forms and made from a variety of varied materials like plastic shreds, air filters, moss, tree fibers, horse hairs, and other thin materials. Typically, egg frames are suspended in the water column or laid along the bottom or sides of the spawning container. Some egg frames have a grid structure or have a mesh on the bottom of them to allow for better oxygen exchange for the eggs. The size of the grid/mesh depends on the size of the fish eggs. As oxygen exchange and water flow are a crucial factor in multiple aspects of successful egg hatching, the placement of the frames is crucial to allow water to flow over the eggs. Regular maintenance of the frames is crucial for the health of the eggs, as accumulation of dead eggs or

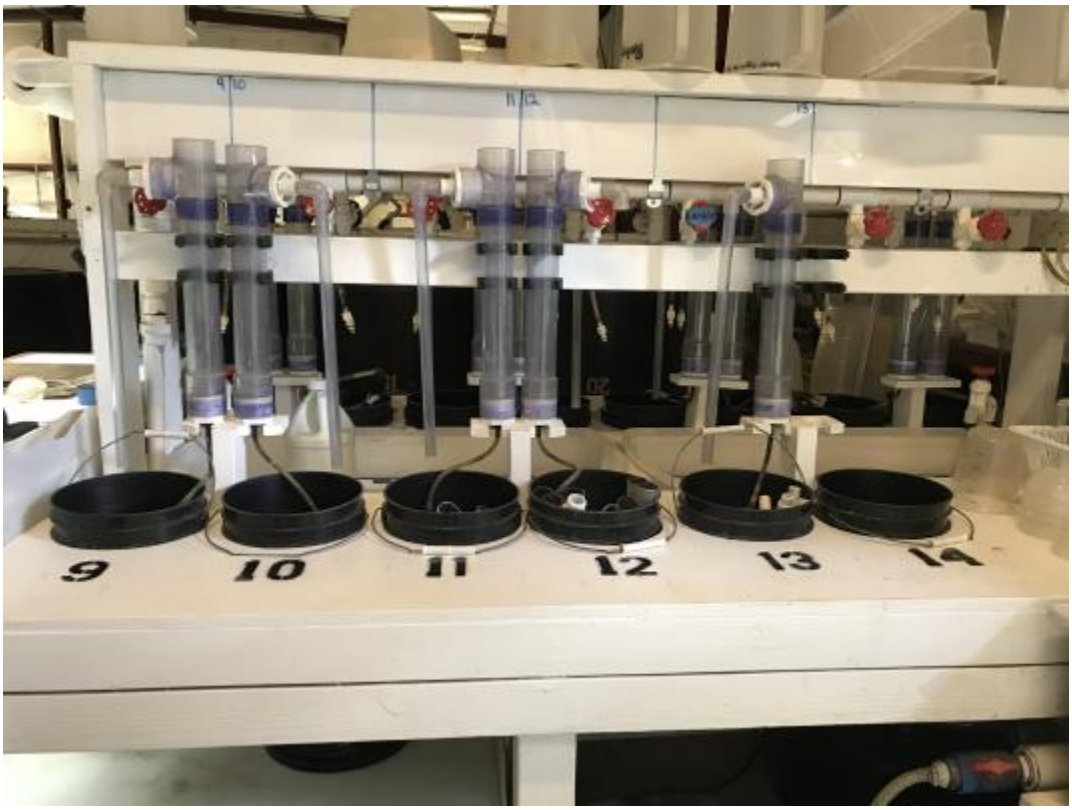


Figure 1.5: Conical shaped tanks at the FCCL (Photo Credit: FCCL)

Figure 1.6: Egg frame from the Lake Suwa Fishing Collective (Photo Credit: Lake Suwa Fishing Collective)

detritus can lead to fungus proliferation and can impact the health of the remaining eggs. Egg frames are designed to hold fish eggs in place while allowing water to flow over them. Traditional methods rely on using natural bodies of water to deploy the egg frames, while more advanced practices employ tanks specially designed as incubators with controlled environments.

However, there is an unorthodox egg frame used in Japan by the Lake Suwa Fishing Collective (LSFC) for supplementing lakes with wakasagi. Due to its immense popularity as a recreational activity in Japan, every year, wakasagi are replenished using a variety of creative methods. Wakasagi support a thriving commercial freshwater fishery that is sustained through an aquaculture program, with annual hatchery releases exceeding ten billion eggs and newly hatched larvae into lakes and reservoirs (Davis et al., 2022).

A method that is particularly intriguing for DS population rehabilitation in the wild is the one devised by the LSFC, which uses an EFB containing 32 gg frames made out of palm tree fiber that can hold fertilized wakasagi eggs as a method to replenish the wild population, as seen in figure 1.6. The LSFC utilize the adhesive stalk of wakasagi eggs to secure them onto the mesh of the egg frame during incubation. The frames are kept at the LSFC facility for 7-8 days during incubation to maintain and care for the eggs. Maintenance includes removing fungus and dead eggs from the egg frames. Prior to hatching, the egg frames are transferred into the EFB, which is then covered by a polypropylene tarp. The boxes are transported by car and deployed into various lakes and rivers throughout Japan (LSFC, n.d., section 6). The frame should strategically be positioned at the mouth of the body of water with shallow, slow flowing water (LSFC, n.d., section 6). This allows the water to flow into the slits of the EFB and over the eggs attached to the egg frames. As the larval wakasagi hatch, the water current carries them into their natural environment, as if naturally incubated and hatched in the wild.

Chapter 2: Fluid Dynamics Modeling of Water Interaction Within a Wakasagi Egg Frame Box

2.1 Introduction

Delta smelt (DS), a species of fish that are endemic to the Sacramento-San Joaquin Delta (Delta), are facing an imminent threat of extinction. The factors contributing to their demise are numerous, including climate change and habitat loss (Sommer et al., 2007). In response, significant efforts have been set in motion to conserve and rehabilitate the species. One such initiative is the UC Davis Fish Conservation and Culture Laboratory (FCCL) in Byron, CA, which hosts a breeding program to provide researchers with healthy DS for studies. The FCCL also houses a refuge population of DS with genotypes similar to their wild counterparts (Lindberg et al., 2013). They are also collaborating with several different agencies to test methods to introduce DS from the laboratory in the Delta.

One of these approaches involves the use of an egg frame box (EFB, Figure 2.1a) to hatch larval DS directly into the waters. Originally developed by the Lake Suwa Fishing Collective (LSFC), an organization that manages and regulates the fishing activities of Lake Suwa, Japan, as a method to repopulate the lake with wakasagi (*Hypomesus nipponensis*), which are a popular recreation fish for many Japanese fishermen. The EFB consists of the box, the lid, and the egg frames that fit inside the box. All of the EFB is made out of palm tree (*Cycas revoluta*) and the inside of the egg frames is made with palm tree fibers so that eggs can adhere to the frame more efficiently and water can flow through the frames inside the EFB. The EFB consists of two components: the wooden frame box with the lid and egg frames

(a)

(b)

(c)

Figure 2.1: Wakasagi (a) EFB with (b) slots to secure egg frames and (c) egg frame purchased from the Lake Suwa Fishing Collective. (Photo credit: Will Mulvaney, FCCL).

held together using small nails. The EFB is a rectangular enclosure constructed with sturdy wooden slats. The base of the box is solid, providing a secure foundation, while the sides are made up of vertically aligned slats with uniform gaps between them, allowing for water circulation. The top of the box features a set of parallel wooden slats affixed to a frame, which are used to hold or support the egg frames substrates for the eggs to adhere to during incubation. These slats are spaced evenly to ensure each egg receives equal exposure to the water's oxygen while also permitting light penetration. Around the slats on the top frame, there is a thicker border frame, reinforcing the structure and providing rigidity. This frame is essential for maintaining the shape of the box and preventing warping or damage when immersed in water. There are wooden slots inside the EFB that hold the thirty-two egg frames vertically inside the box, as depicted in Figure 2.1a. These frames were made with palm tree (*Cycas revoluta*) bark fibers (Figure 2.1b) through the middle that fish eggs with adhesive stalks can stick to. This screen is a crucial element of the box's internal structure, providing a substrate for the adhesive sacs of sticky eggs to attach. When in the field, the EFB is anchored in place near the shore of a lake with weights on top of it to keep it under the water surface.

As DS and wakasagi have eggs that are similar sizes (>1 mm) and the two species are very similar, it would be plausible to use the EFB in the Delta for DS species rehabilitation in their natural habitat (Benjamin et al., 2018; Fisch et al., 2014; Xie & Li, 1987). These similar qualities could make it possible to use the EFB as a method to introduce newly hatched larval DS into the Delta. One way to evaluate the device is by using Computational Fluid Dynamics (CFD) simulations, which can be a very efficient and cost-saving tool in studying the effectiveness of an EFB in rehabilitating the DS population in the Delta. Through simulations of the Delta conditions and analysis of the hydrodynamics inside the EFB under those conditions, researchers

can identify potential challenges and optimize its efficacy for incubating and hatching DS eggs. Additionally, simulations can help to identify the best deployment strategies for the EFB, such as where to release the frames into the Delta and when would be the right time to deploy the EFB. With the aid of simulations, researchers can increase the likelihood of success for the EFB in rehabilitating the DS population and protecting the species from extinction.

CFD is a field in applied mathematics and engineering involving numerical simulations and analysis of fluid flows and related applications using computers to solve fluid flow problems. It is able to simulate and analyze the behavior of fluids in a wide range of applications, like aerodynamics, hydrodynamics, chemical reactions, heat transfer, and many more. CFD has been used as an application for many experiments regarding fluid dynamics. Shen & Diplas, (2008) conducted experiments using CFD to model two and three dimensional CFD models of complex ecological stream flows. They also conducted experiments to validate their simulations. Rajabzadeh et al., (2015) conducted a study focusing on the flow dynamics, development, and wastewater treatment in a subsurface vertical flow wetland mesocosm. They employed CFD as a tool to analyze and simulate the various processes occurring in this wetland system. Kolden et al., (2016) also conducted simulations to model the whitewater park hydraulics and fish habitat in Colorado. Dong et al., (2009) created models for the float collar of a gravity fish cage to simulate the motion effects and tension on the lines from different waves of various heights, curls, and widths. They also validated the simulations with physical model experiments, concluding that their computational method can correctly predict the relationship between waves and the movement of the float collar and the tension of the mooring ropes. Liu et al., (2019) studied the structural strength and failure of a floating collar in a single-point mooring fish cage and were able to quantify.

The basic principles of CFD starts with discretizing the model domain into a grid of cells, also known as the mesh. Governing equations are then used to iteratively solve the behavior of the fluid flow dynamics simulations. When CFD simulations are used correctly, these simulations can be used for many applications to conduct simulations that would more cost and time saving compared to conducting an actual experiment.

Since the EFB was made for water conditions for Wakasagi in Japan, what is not known is what will happen inside the EFB when placed inside the Delta. Thus, to study the fluid interactions within the EFB when it is applied in the field here, a CFD simulation was created to model the effects using water flow velocities found in the Delta. The objective for this chapter is to use CFD simulations to analyze the water interactions within the EFB using water flow velocities found in the Delta.

2.1.1 Governing Equations

One of the main concepts of CFD is the governing equations. For the simulations conducted for this thesis, the most fundamental equation used is the Navier-Stokes equations (NS equations) which govern the motion of fluids. The NS equations are nonlinear equations used to mathematically describe the conservation of mass, momentum, and energy for a fluid in a continuum. These equations can be used in many applications, including hydrodynamics, biomedical engineering, and aerodynamics to predict and understand fluid flow. In vector form, the equations are:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho u) = 0$$

$$\rho\left(\frac{\partial u}{\partial t} + u \cdot \nabla u\right) = -\nabla p + \mu \nabla^2 u + f$$

The first equation is the continuity equation, which states that the rate of change of fluid density (ρ, kg/m^3) with respect to time (t, s) plus the divergence of the mass flux (ρu) is equal to zero. The second equation, the momentum equation, represents the conservation of momentum in a fluid flow. It describes how the velocity field (u) evolves over time due to various forces, including pressure gradients ($-\nabla p$), viscous forces ($\mu \nabla^2 u$), and external forces (f). In both equations, u is the velocity vector (m/s), p is the pressure (Pa), μ is the dynamic viscosity (Pa·s), ∇ represents the gradient operator, and ∇^2 is the Laplacian operator. The NS equations are particularly challenging to solve analytically. These equations are fundamental in fluid dynamics and are used to simulate fluid flows in various engineering and physical contexts.

2.1.2 Discretization

In order to solve the governing equations numerically, they must first be initially discretized into simpler, algebraic equations that can be solved through computational methods. This is usually achieved using Finite Difference (FDM), Finite Elements (FEM), or Finite Volume Methods (FVM). These methods involve converting the NS equations into algebraic equations by dividing the computational domain into a finite number of discrete cells/elements. The equations are then solved by approximating the solution variables at the center of the cells/elements. Each method has its own advantages and disadvantages, so the choice of the numerical method depends on the specific problem being investigated.

The FDM is used to approximate the derivatives in the NS equation using a set of discrete points or nodes. These solution variables at each node are approximated by the values of the neighboring nodes. This method is simple to implement but usually does not produce the most accurate results, especially for complex geometries and non-uniform shapes. As there are not

complex geometries or non-uniform shapes modeled in this simulation, for the topics covered in this thesis, the FDM method is used.

The FEM approximates the solution variables using finite elements. Each element is defined using a set of nodes and shape functions, describing the variation of the solution within that element. The solution variables are approximated as linear combinations of those shape functions within each element. This method is more flexible than the FDM and can solve more complex geometries but is more computationally intensive.

The FVM solves the governing equations by dividing the computational domain into a set of finite control volumes. The solution variables used are averaged for each control volume, while the fluxes found at the boundaries of each control volume are calculated using interpolation or numerical integration. This method is more useful for simulating flows with shocks or discontinuities because it preserves the mass, energy, and momentum of the fluid.

2.1.3 Turbulence Modeling

Turbulence modeling is the construction and use of a mathematical model in predicting the effects of turbulence in a simulation. Turbulence in CFD is described as a chaotic and random behavior of fluid dynamics. The propensity of an incompressible flow to become turbulent can be measured by the Reynolds number:

$$Re = \frac{\rho UL}{\mu}$$

where U is the velocity of the incoming water (m/s), ρ is the density of the water (kg/m^3), L is the characteristic length (m), and μ is the dynamic viscosity of the water. These fluid movements are influenced by the shape of the object that the fluid is flowing over. A high Re (>2300)

indicates that a flow is turbulent, while a lower Re (<2300) would indicate the laminar nature of a flow.

The fluid motion under these conditions on COMSOL Multiphysics (COMSOL Inc., Stockholm, Sweden) were expressed using the Reynolds-Averaged Navier-Stokes' equation (RANS equation), which is the most commonly used model in industrial flow applications:

$$\rho(u \cdot \nabla)u = \nabla \cdot [-pI + K] + F + \rho g$$

$$\rho \nabla \cdot u = 0$$

where K is the stress tensor for a Newtonian fluid, encompassing both viscous and turbulent stresses:

$$K = (\mu + \mu_T)(\nabla u + (\nabla u)^T).$$

Here, K is the total stress tensor, μ is the dynamic viscosity of the fluid, representing the fluid's resistance to shear or viscous stress, μ_T is the eddy viscosity (turbulent viscosity), which is a property of the flow and not the fluid, representing the increased effective viscosity due to turbulent eddies, ∇u is the velocity gradient tensor, and $(\nabla u)^T$ is the transpose of the velocity gradient tensor, accounting for the fact that the stress tensor is symmetric. The transport equations for the turbulent kinetic energy (k) and its rate of dissipation (ϵ), which are specific to the k-ε model:

$$\rho(u \cdot \nabla)k = \nabla \cdot \left[\left(\mu + \frac{\mu_T}{\sigma_k}\right)\nabla k\right] + P_k - \rho\epsilon$$

$$\rho(u \cdot \nabla)\epsilon = \nabla \cdot \left[\left(\mu + \frac{\mu_T}{\sigma_\epsilon}\right)\nabla \epsilon\right] + C_{\epsilon 1}\frac{\epsilon}{k}P_k - C_{\epsilon 2}\frac{\epsilon^2}{k}P_k$$

Where, μ_T, the turbulent viscosity, is computed as

$$\mu_T = \rho C_\mu \frac{k^2}{\epsilon}$$

And the turbulent kinetic energy production, P_k, is given by:

$$P_k = \mu_T [\nabla u : (\nabla u + (\nabla u)^T$$

The k-ε model equations are used to close the RANS equations by providing expressions for the turbulent viscosity and the production of turbulent kinetic energy. These equations, together with the momentum and continuity equations, allow for the calculation of the time-averaged flow fields in turbulent flow conditions. The k-ε model is widely used in CFD due to its robustness and reasonable accuracy for a wide range of turbulent flows. The continuity equation of the equation in the form, with the assumption that the fluid is incompressible and Newtonian in which case the Navier-Stokes equations as seen in section 2.1.1. Turbulence modeling is one of the most important aspects of CFD simulations because turbulence plays an important role in many fluid flow problems, including blood flow in the cardiovascular system. Some other examples of turbulence modeling used in CFD are the Large Eddy Simulation (LES) models and the Direct Numerical Simulation (DNS) models. For the topics covered in this thesis, RANS simulations are used to create these simulations. These models are required because the NS equations are unable to predict the behavior of turbulence.

RANS models are simulated by averaging the NS equations over time and space, while separating the mean flow properties, like the velocity and pressure from the turbulent fluctuations. RANS equations require closure models to account for the effects of turbulence in the average flow properties, which are based on statistical assumptions and experimental data. This can introduce some uncertainties into a simulation model which can cause some errors in the simulation results. Because of this, it is important that the user chooses the model depending

on the specific application and the level of accuracy that is required when creating a simulation. It is also important that the simulation can be validated using experimental data and that it can be calibrated to match the experimental results.

2.1.4 Boundary and Initial Conditions

In CFD, boundary conditions are used to specify the behavior of the fluid flow variables at the boundaries of the computational domain. Boundary conditions are an essential aspect of turbulence modeling as they define the behavior of the flow at the boundaries of the computational domain. They show how different elements with different physical properties can interact with each other. These boundary conditions are necessary for solving governing equations for fluid dynamics like the NS equation and obtaining a numerical solution accurately so that it represents the physical system that is being simulated. There are three main classifications of boundary conditions: Dirichlet, Neumann, and mixed boundary conditions.

Dirichlet boundary conditions, also known as fixed-value boundary conditions, specify the values of the solution variables at the boundary of the computational domain. They are used to specify the exact value of the solution variables at the boundary. Examples of Dirichlet boundary conditions used in CFD are the no-slip condition, which is used to model fluid flow near a solid boundary. In the no-slip boundary condition, the fluid velocity at the boundary is set to 0 m/s. Other common Dirichlet boundary conditions are the inlet and outlet conditions which are used to specify the fluid velocity, pressure, and other flow variables at the inlet/outlet of the computational domain.

The Neumann Boundary Conditions, also known as the free-value boundary condition, are used to specify the value of the normal derivative of the solution variables at the boundary of the computational domain. They are used to specify the rate of change of the solution variables at

the boundary, like the rate of heat transfer at the boundary. An example of the Neumann boundary conditions in CFD are the wall heat flux condition, which is used to model the transfer of heat from a fluid to a solid wall with a known heat flux. Another example is the wall shear stress condition which is used to model the effects of friction from fluid flow on a solid wall, with a known shear stress. Complex structures may be simplified to reduce computational effort, assuming that the simplifications do not affect the flow field significantly.

The Mixed Boundary Conditions are a combination of the Dirichlet and Neumann boundary conditions. They are used to specify the values of both the values of the solution variables and their normal derivatives at the boundary of the computational domain.

Just like there are different types of discretization that the user can choose based on the specific problem that is being simulated, the user should take into consideration the type of problem that they are simulating when considering the type of boundary conditions to use in CFD simulations, since incorrect boundary conditions can lead to erroneous results and inaccurate simulations.

2.1.5 CFD Software

CFD software are computer programs that are used to perform numerical simulations for fluid dynamics phenomena. A CFD software usually include pre-processing, solving, and post-processing tools that allow the user to generate and analyze simulations.

The pre-processing tools used in CFD software are used to create the geometry of the model and to set up initial and boundary conditions for the simulations. This process starts with defining the geometry of the flow domain like the shape of the object and volume of the fluid, as well as specifying the physical properties of the fluid, for example, the density, temperature, and viscosity of the fluid. This process is also where the discretization of the model, in the form of

generating a mesh, where the fluid domain is divided into discrete cells for the numerical solution of the governing equations. The user should decide the size of the mesh to be simulated based on where deformations or stresses occur. Those mesh areas should be more refined than other regions where deformation and stress is less likely to occur. Too coarse of a mesh can result in inaccuracies in the obtained results. Having smaller mesh element sizes in places with large deformations, stresses, and instabilities will allow for greater accuracy, without expending a large amount of computing time. Thus, it is especially important to assign the correct mesh size for each aspect of the model domain.

The solving aspect of CFD uses numerical algorithms to find solutions for the governing equations. This part of the software is especially important because it is when the user must select the correct numerical algorithms based on the type of simulation that is being conducted.

The post-processing tools in CFD software are used to analyze the results of a simulation. This allows the user to picture the results of the simulation in various aspects, such as plots, charts, and animations. They can also be used to extract quantitative information from the simulation, like the flow rates, pressure distribution, and the temperature profile.

There are many different commercial and open-source CFD software available to choose from, each with their own features as well as pros and cons. Popular commercial CFD software include COMSOL Multiphysics, ANSYS Fluent, and STAR-CCM+. Some open-source CFD software are SU2, Code_Saturne, and OpenFOAM. It is important for the user to consider factors such as user-friendliness of the software UI, the availability of the support and training needed, and the accuracy of the numerical algorithms of each software. For this study, COMSOL Multiphysics was used. COMSOL Multiphysics is a popular software program used in a variety of fields including fluid dynamics. It is well-known for its ability to manage Multiphysics

problems to solve phenomena that involve multiple fields of physics. It also has a very user-friendly UI, making it easier to create complex simulations compared to coding.

2.2 Methods

Simulations were conducted using a CFD software, COMSOL Multiphysics version 5.6 using a Lenovo ThinkPad P52s laptop (Lenovo Group Limited, Quarry Bay, Hong Kong). The EFB was purchased from the Lake Suwa Fishing Collective (LSFC). Measurements of the EFB and egg frames were conducted at the FCCL.

2.2.1 Model Construction

The EFB (Figure 2.1a) used by the LSFC is a 1 m length × 0.44 m height × 0.2 m width rectangular structure, composed of wooden blocks and planks made of palm fiber. Inside the EFB is where the egg frames are stored. As many as 32 egg frames (38 cm length × 19 cm height × 1.3 cm width, Figure 2.1b) can be held inside the EFB, separated by smaller blocks. The screen of the egg frame is made of palm bark fibers as a substrate for the eggs to adhere to. This also allows water to pass through the pores within the screen to create more aeration for the eggs and this important characteristic should be considered when creating the model for simulation. The design of the EFB is made to facilitate the passage of water through between the gaps in the wooden planks to aerate the eggs by creating a smooth flow over the eggs as seen in the diagrams in Figure 2.2.

To model the flow of water through the pores in the egg frames, the middle of the egg frames was simulated as a permeable material. The EFB manual created by the LSFC also included specific parameters for deployment of the EFB (LSFC, n.d., section 6). These parameters require that the water at the deployment site have no suspended particles, exhibit low

turbidity, and should be positioned within flows that are under 60 cm/s while being securely anchored to prevent it from drifting out from the water current. Additionally, the EFB should be appropriately weighted to ensure that all eggs on the frames within the EFB remain submerged beneath the water. These parameters were considered when creating the CFD model for simulating the water flow velocity inside the EFB.

2.2.2 Model Development

The measurements of the EFB and the egg frames were gathered and entered COMSOL's geometry which can be seen in Figure 2.3 and 2.4. In the simulation, the EFB was positioned adjacent to the bank of a flowing body of water. For the material of the EFB and the egg frames, the physical properties of American Red Oak (*Quercus rubra*) were assigned. For the mesh of the egg frames, the physical properties of nylon were assigned. All materials and the parameters can be seen on Table 2.1. Additional physical properties considered was the environment that the EFB model was simulated in. For this, the properties of water were assigned for the entire arena as a boundary, while the physical properties of brick were assigned to the floor of the arena.

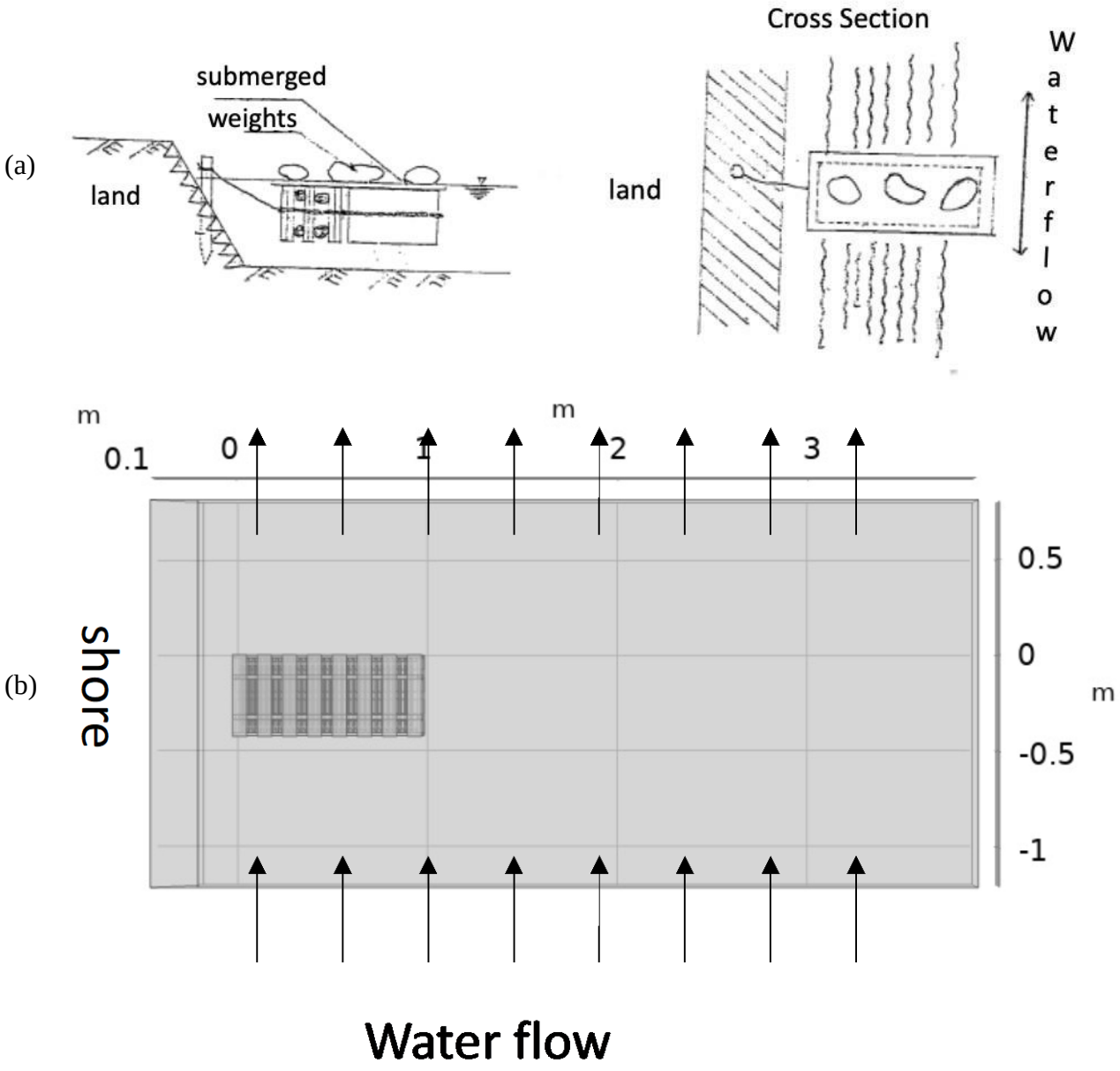


Figure 2.2: (a) The diagram used to construct models for CFD simulations (LSFC, n.d., section 6). (b) Horizontal view of simulation replicating the diagrams.

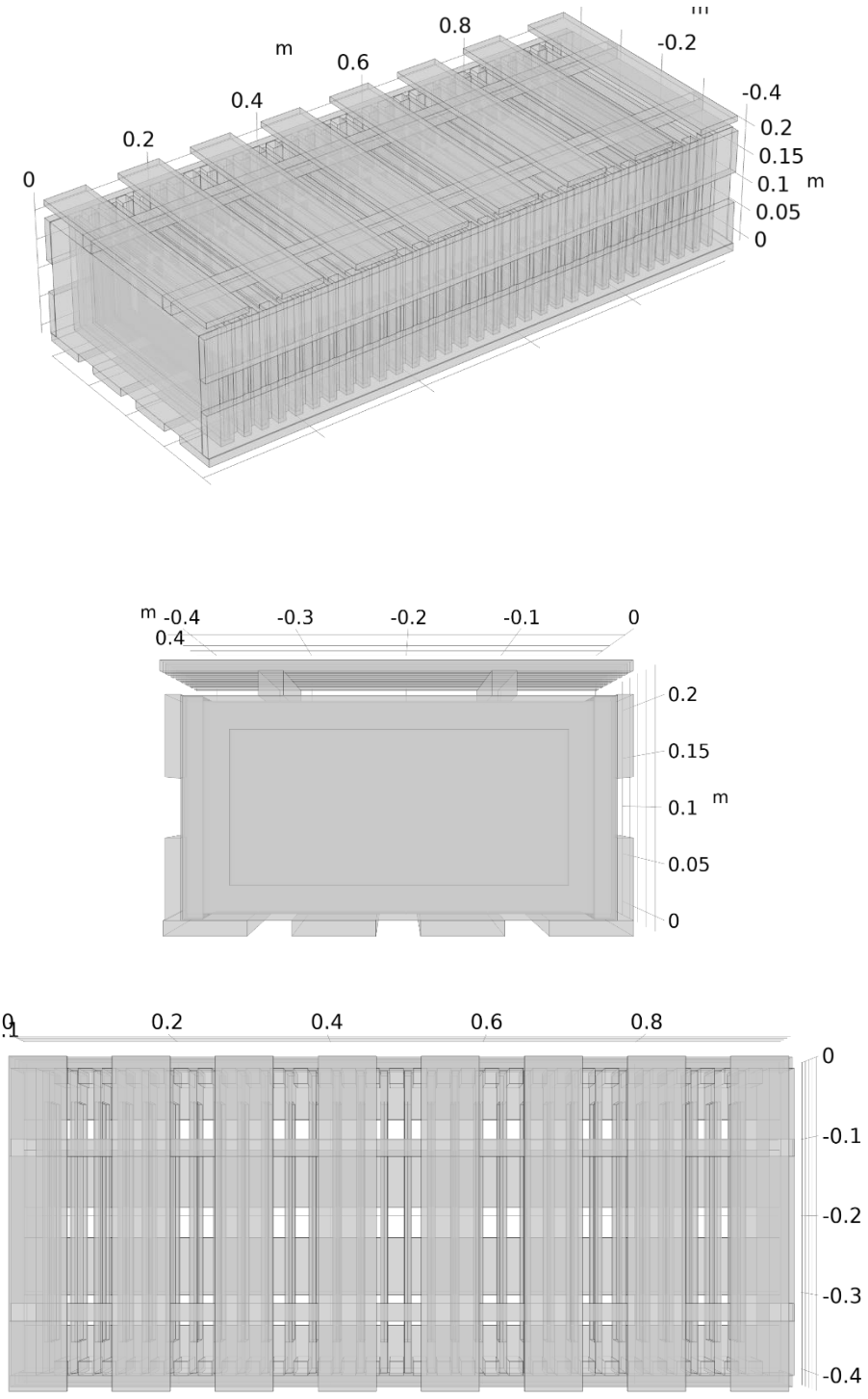


Figure 2.3: Life sized model for the EFB created on COMSOL Multiphysics.

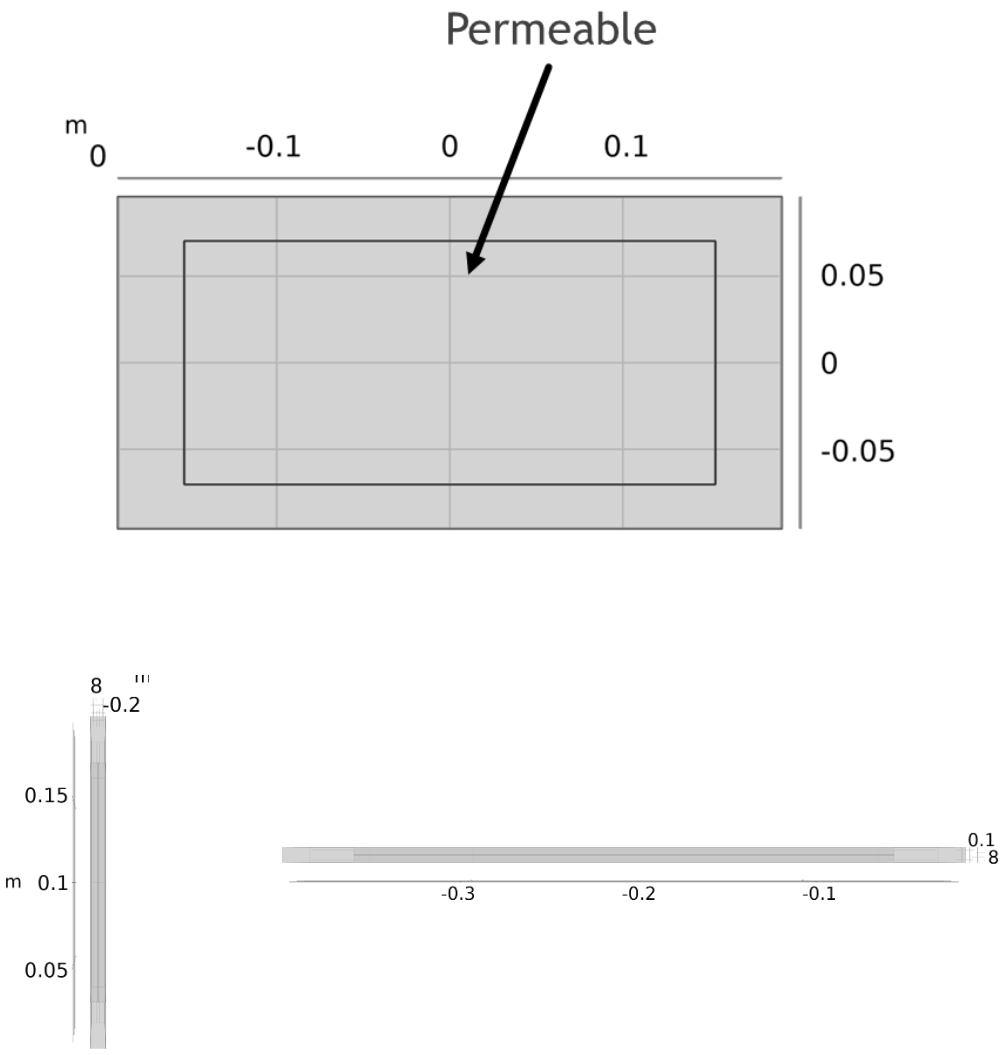


Figure 2.4: Life sized model for the egg frame created on COMSOL Multiphysics.

Table 2.1: Table of materials and properties used in simulation.

Material	Property	Variable	Value	Unit
American Red Oak	Density	ρ	630	kg/m^3
	Young's modulus	E	12.4e9	Pa
	Poisson's ratio	ν	0.3	
Concrete	Density	ρ	2300	kg/m^3
	Young's modulus	E	25e9	Pa
	Poisson's ratio	ν	0.2	
Nylon	Density	ρ	1150	kg/m^3
	Young's modulus	E	2e9	Pa
	Poisson's ratio	ν	0.4	

The EFB is deployed near the bank of a shallow, flowing body of water, held in place by weights, and anchored in place by a stake ((LSFC, n.d., section 6, Figure 2.2a). Accordingly, the simulation also replicated the stationary placement of the EFB in its location. Flow rates of 20 and 60 cm/s, which represent the lower and upper limit of the recorded flow velocity range recorded at the river where the wakasagi egg frames are deployed, respectively, were used to run the simulations (LSFC, n.d., section 6).

For the simulations in this project, it is assumed that at the inlet, a uniform inlet velocity profile based on water velocity given from the LSFC egg hatching manual. The water velocity used for the inlet velocity parameter was assumed from the data gathered from the water station near Cache Slough, where fertile female DS have been found. The water is assumed to be an incompressible, isothermal, steady state flow to help simplify simulation computation time. The model is also simplified by modelling the arena based off of the figures found in the LSFC egg hatching manual. The no-slip condition is applied at the walls of the EFB, which specifies the velocity of the fluid at the boundary will have zero velocity relative to the boundary, meaning the fluid velocity at the wall is zero.

2.2.2 Simulation Parameters

The steady, incompressible flow was created by a restricted momentum source going through the front portion of the EFB was assumed. Other assumptions include the wall boundary conditions assigned at the bottom, inner, and outer walls of the EFB, while symmetric boundary conditions were assigned to the water surface. The inlet boundary conditions were set as the long side facing the EFB, facing the side of the EFB that the water would be entering from, while the outlet boundary conditions were set as facing the opposite side facing the side of the EFB where the water is exiting as seen in Figure 2.2.

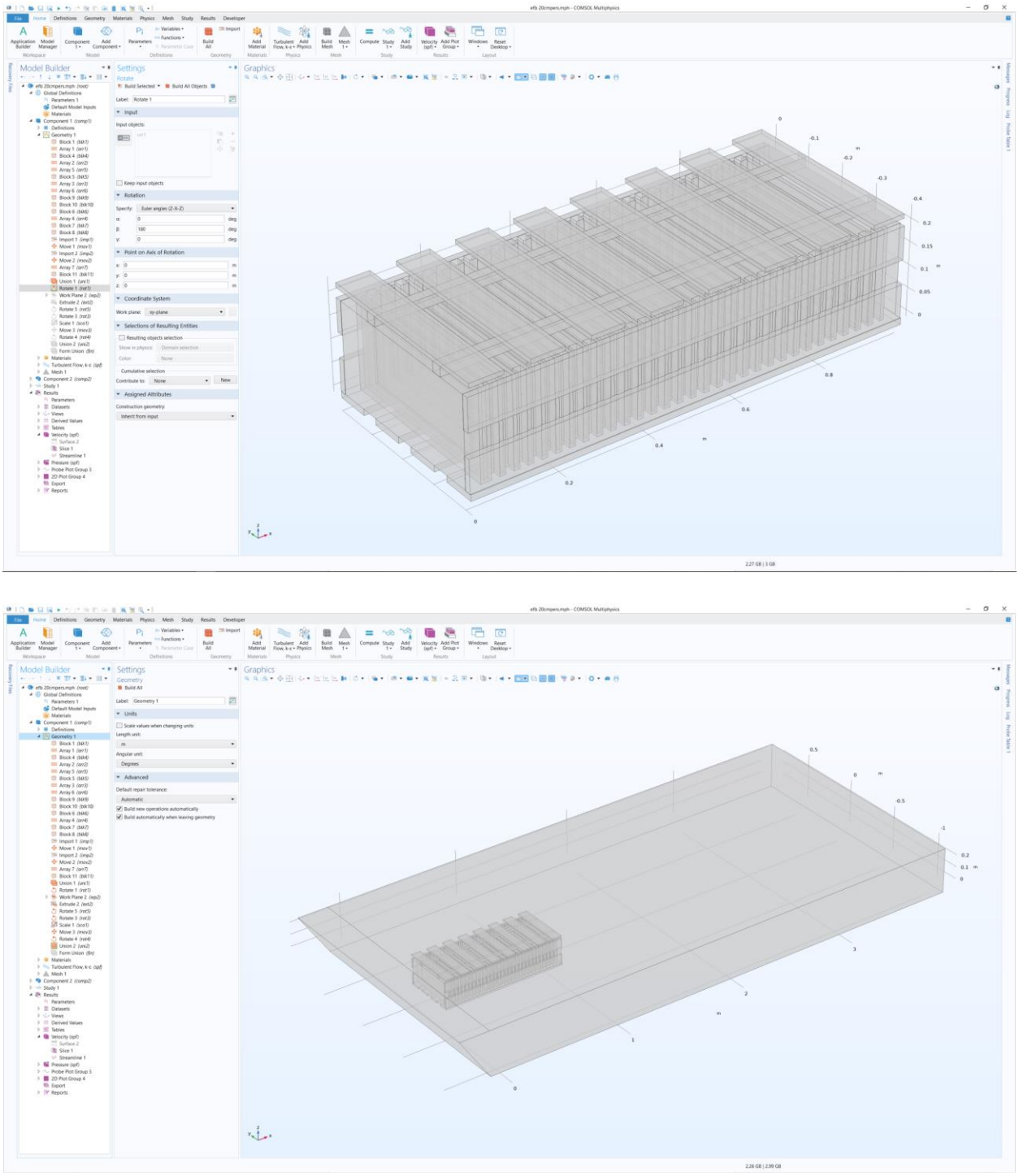


Figure 2.5: Created geometries of the EFB with egg frames placed inside (top) and the arena used for the simulation (Bottom).

(a)

(b)

(c)

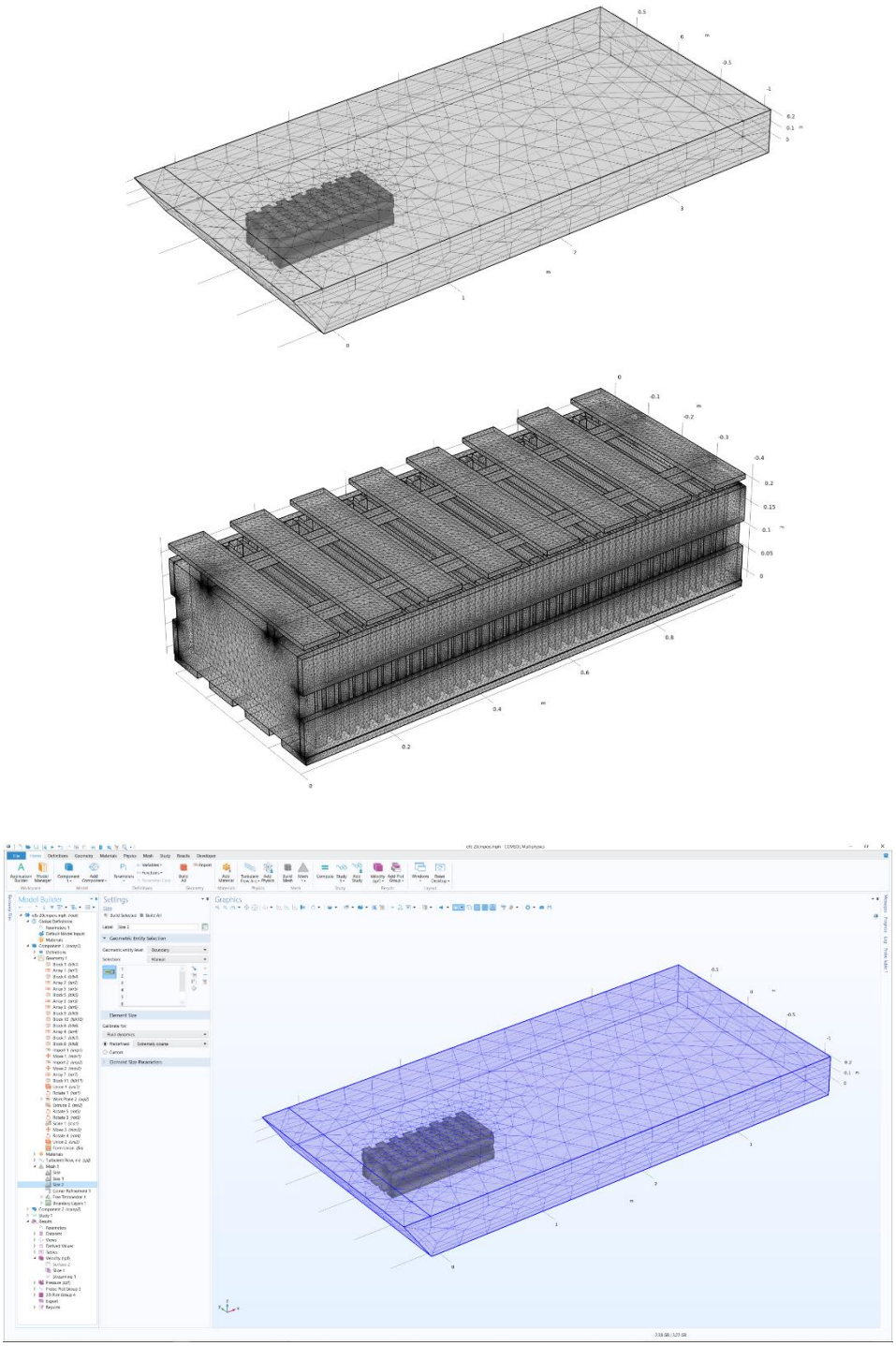


Figure 2.6: Mesh diagram of the (a) model for the simulations and the (b) egg frame box with (c) properties of EFB and the arena.

After creating the geometry and assigning the relevant physical properties to the model as seen in Figure 2.5, the next step in designing the model is to construct the mesh. The mesh of the computational volume is composed of tetrahedral elements that were automatically generated under user-specified settings on COMSOL Multiphysics by either assigning the mesh manually or utilizing the automatic mesh assignment feature using the software. For this simulation, the mesh assigned to create this simulation was finer for the EFB and its immediate surroundings, while a coarser mesh was assigned for the remaining parts of the modeled environment, considering the significance of simulating that specific mesh section would be (Figure 2.6). For the EFB, the maximum element size was 4.54 cm, and the minimum element size was 0.86 cm, with a maximum element growth rate of 1.13, a curvature factor of 0.5 and a resolution of 0.8 for the narrow regions. For the modeled environment, the maximum element size was 28.3 cm, and the minimum element size was 6 cm, with a maximum element growth rate of 1.4, a curvature factor of 1 and a resolution of 0.3 for the narrow regions. The final constructed mesh consists of 1,804,125 domain elements, 346,454 boundary elements, and 41,277 edge elements.

The inlet chosen corresponds to the side of the simulation through which water should enter though the front of the box and exit from the back of the box, directing towards the outlet positioned at the opposite side of the inlet in the simulation. Once the inlet and outlet have been defined, the simulation can be used to model conditions from various water flow velocities. The water velocity deducted from the simulations are given in three spatial axes representing how the velocity is distributed in each direction: X-direction (u), Y-direction (v), and Z-direction (w). To consolidate the three-dimensional velocity vectors into a single magnitude, several locations in the EFB simulation are randomly selected, and the 3D velocity magnitude is calculated using the formula:

$$Velocity = \sqrt{u^2 + v^2 + w^2}$$

Using this information, the magnitude of the flow velocity inside the EFB can be found in Table 2.2.

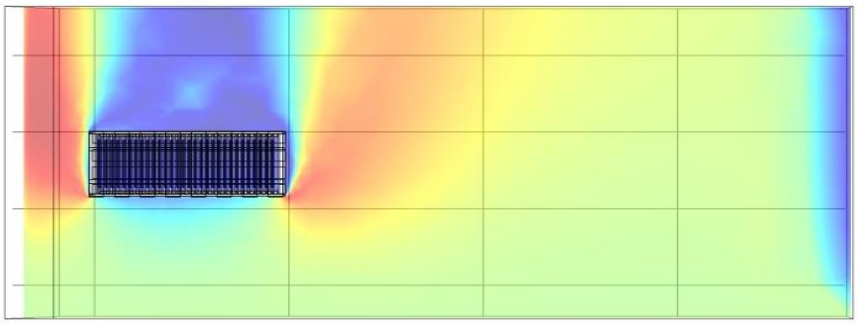
2.3 Results

After the simulation has been conducted, a clear pattern emerges in how the water behaves as it approaches and interacts with the EFB. As illustrated in Figures 2.7 and 2.8, the water enters the model through the inlet and then flows at a uniform stream towards the EFB. The water enters the EFB through the aligned slats in the front and interacts with the interior structure of the EFB and the egg frames. The water flows between the mesh of the egg frames in the EFB because it is porous. Afterwards, the water exits the EFB through the aligned slats in the back of the EFB and flows into the outlet at the end of the model.

It can be further seen that when the water enters at the given inlet velocity, the water's velocity experiences significant deceleration. In the case of the simulation with 60 cm/s inlet velocity (Figure 2.7), the water depreciated as low as 10 cm/s. In the case of the 20 cm/s inlet velocity simulation (Figure 2.8), the water also slowed down greatly from its initial speed to ab out 5 cm/s. The color legend on the right of the figures represents the velocity of the water in the simulation. It is observed that the water undergoes substantial deceleration as it approaches the EFB and continues to decelerate upon entering the EFB.

Using the particle tracing function of COMSOL Multiphysics, the movement of water can be traced as black lines in the simulation. These lines illustrate that the particles pass through the gaps within the egg frames (Figures 2.7c and 2.8c), which mimics the porous ability of the egg frames to have water be able to go between the fibers of the frames.

(a)



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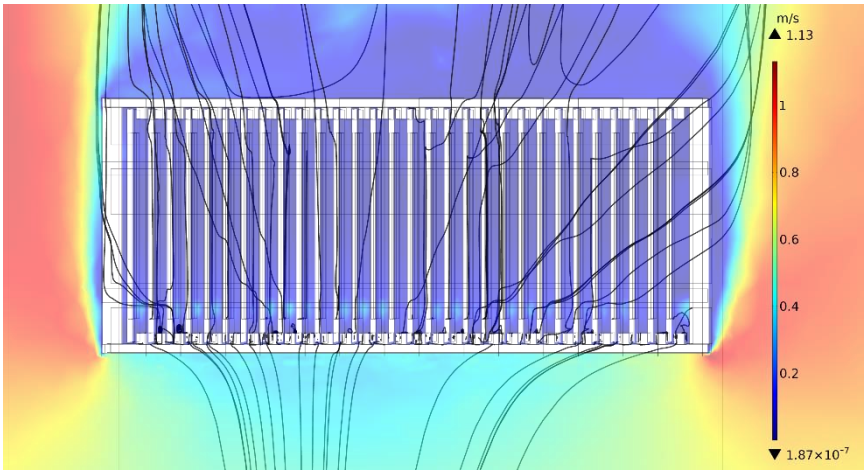

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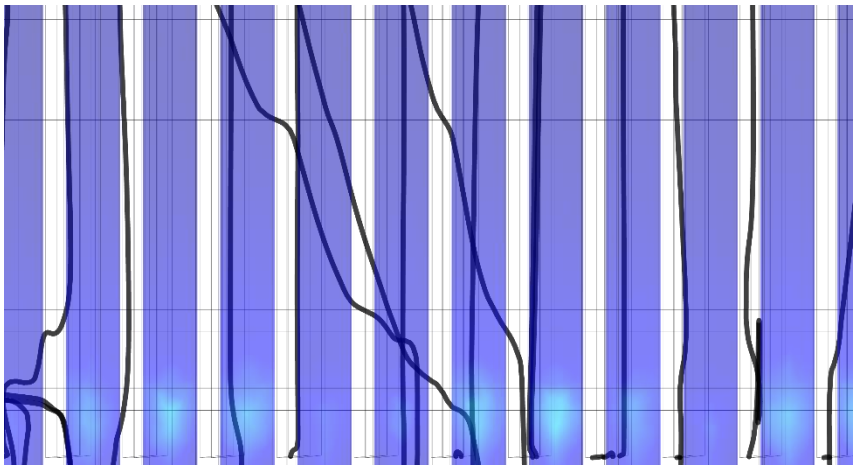
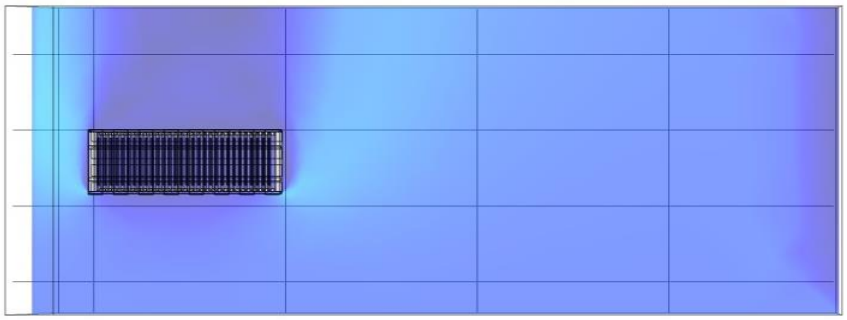


Figure 2.7: (a) Full horizontal velocity profile of 60cm/s simulation. (b) Focused view on EFB and (c) close up of particle tracing between frames.

(a)



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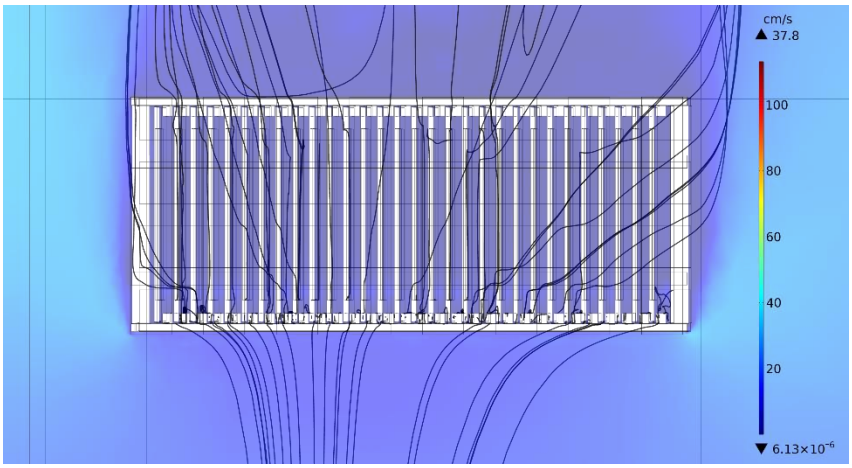

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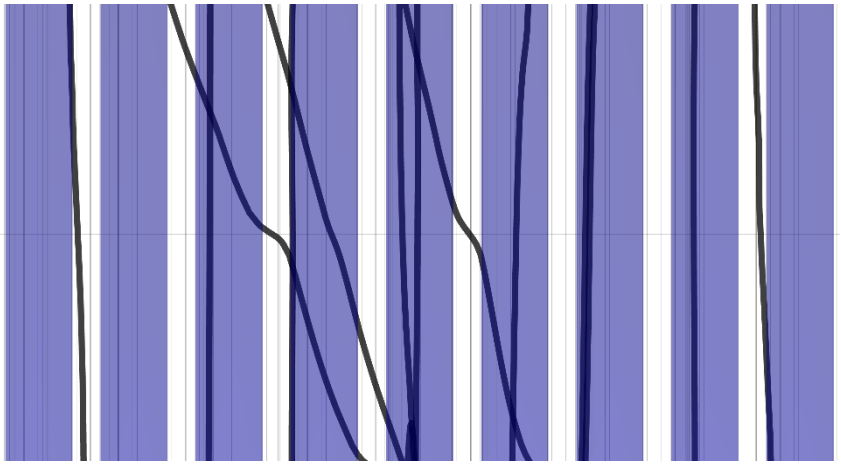


Figure 2.8: (a) Full horizontal velocity profile of 20 cm/s simulation. (b) Focused view on EFB and (c) close up of particle tracing between frames.

Based on the simulations of the EFB placed in a stream with velocities of 20 and 60 cm/s, the relationship found between the incoming flow and the flow in the box is that the water velocity in the box was significantly decreased. In the case of the simulation with an inlet water velocity speed of 60 cm/s, when the water approaches the EFB at about 40 cm away, the average water flow velocity decelerated to about 34.88 cm/s, about 58% reduction from the inlet flow velocity. As the water enters the EFB, the water velocity further decelerated to about 1.16 cm/s, which was about 2% of the inlet velocity. The water stayed at a relative constant speed while moving inside the EFB, retaining an average velocity of 1.37 cm/s, also about 2% of the selected inlet velocity.

Around the EFB is an entirely different scenario. As presented in Figure 2.9 showing the different sections of velocity profiles of the simulation, the water flowing directly on the left and right side of the EFB accelerated significantly when compared to the selected inlet velocity of 60 cm/s. On the left side of the EFB, the average velocity of the water was 94.51 cm/s, while the average velocity of the water on the right side of the EFB was 93.98 cm/s, about 158% increase when compared to the inlet velocity. In a real-life scenario, this may become a problem as the acceleration of the water may dislodge the anchors holding the EFB in place. Measurements and coordinates of where they were taken can be found in Table 2.2 below.

2.4 Discussion

The optimum flow velocity for DS eggs adhered to egg frames is not fully understood. When consulting the LSFC regarding a suitable flow rate, they replied that 133.5 L/s is the preferred flow rate used for wakasagi egg incubation, which is converted to 0.1335 m^3/s. Based on the cross-sectional area of the inlet, the water flow velocity can vary. When testing different substrates for DS eggs (Chapter 4), it was found that 10 cm/s was a suitable water flow velocity

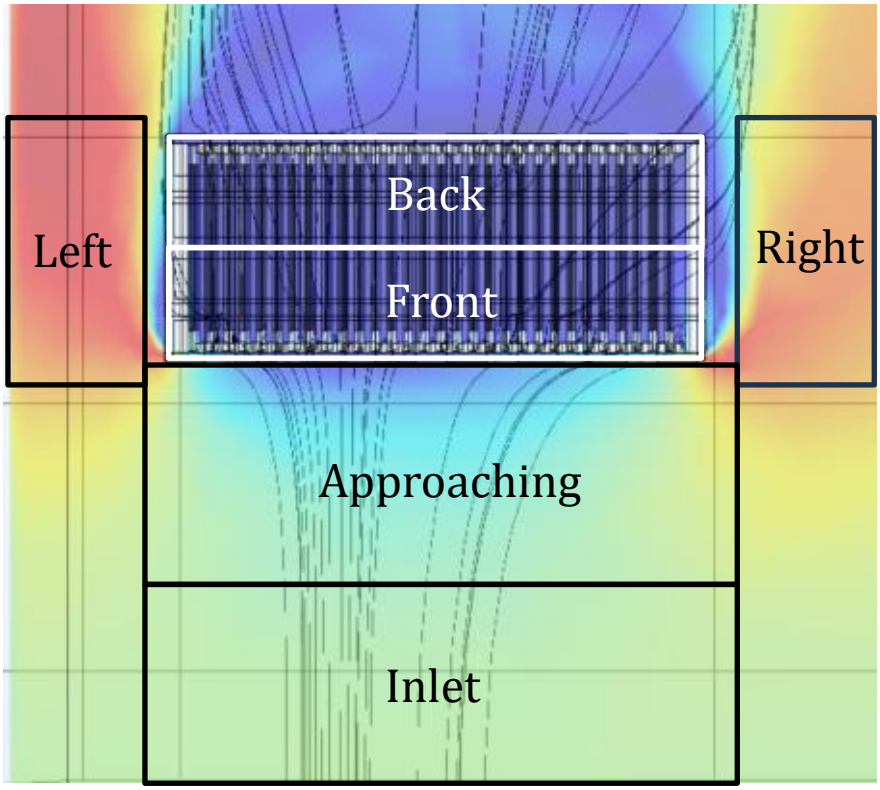


Figure 2.9: Different sections in the 60 cm/s simulation for the EFB.

Table 2.2: Coordinates and water velocity in different sections of the 60 cm/s simulation.

Area	**X**	**Y**	**cm/s**	**Area**	**X**	**Y**	**cm/s**	**Area**	**X**	**Y**	**cm/s**
Inlet	0.69	-0.97	58.80	*Right*	1.08	-0.36	96.88	*Back*	0.81	-0.30	1.71
	0.49	-1.00	58.37		1.10	-0.36	94.98		0.22	-0.09	1.30
	0.24	-1.01	58.78		1.07	-0.39	96.53		0.24	-0.09	1.27
	0.77	-1.02	59.44		1.19	-0.29	91.82		0.63	-0.09	1.13
	0.16	-0.95	58.79		1.06	-0.37	95.45		0.48	-0.08	1.47
	0.48	-1.05	58.73		1.15	-0.27	89.12		0.48	-0.13	1.59
	0.52	-1.11	59.27		1.12	-0.31	93.06		0.60	-0.09	1.15
Area	**X**	**Y**	**cm/s**	**Area**	**X**	**Y**	**cm/s**	**Area**	**X**	**Y**	**cm/s**
Approaching	0.55	-0.51	34.64	*Left*	-0.08	-0.36	98.28	*Front*	0.49	-0.31	1.28
	0.29	-0.48	26.60		-0.03	-0.37	84.57		0.33	-0.30	1.19
	0.69	-0.49	37.30		-0.06	-0.36	92.14		0.33	-0.30	1.13
	0.28	-0.49	31.46		-0.07	-0.32	96.41		0.49	-0.33	0.98
	0.35	-0.53	37.58		-0.09	-0.31	98.47		0.60	-0.32	1.10
	0.09	-0.45	35.18		-0.06	-0.37	97.94		0.86	-0.30	1.11
	0.74	-0.50	41.46		-0.07	-0.30	93.78		0.81	-0.30	1.30

for hatching the eggs. Lindberg et al., (2020) found that DS prefer pebble and sand substrates at higher water velocities (8.8 cm/s, 15.4 cm/s, and 14.6 cm/s) for spawning under certain laboratory conditions. With the information from the simulations of the flow of the Delta, the most suitable location to deploy the EFB is still not fully known. A suitable location for deployment should be in an area suitable for DS eggs to incubate and hatch while, not damaging or dislodging the EFB from its location. As spawning behavior for wild DS is still a mystery, their spawning grounds have still not been discovered. In an article by Kurobe et al., (2022), they cited several papers regarding the spawning habitats of fertile female DS.

The Spring Kodiak Trail (SKT) is an annual survey conducted from January to May that aims to assess the relative abundance and distribution of spawning DS within the Delta. The SKT data reveals that Cache Slough is the primary location where all the captured, ripe female DS were located. Furthermore, Kurobe et al., (2022) provided a significant insight that the majority of ripe female DS in Cache Slough were observed in the month of February.

Future studies that can be conducted to better understand using the wakasagi EFB. One focus can be to study how the hydrologic forces from the water current can affect the tension on the anchor and mooring lines that keep the EFB in place. The tensions in the mooring line and the EFB can be predicted using simulation outcome. This information will be useful when deciding the type of anchoring and mooring equipment needed, and the site where the EFB is to be deployed. Another idea that could be studied is modeling different EFB and egg frames in various shapes and sizes that could improve various aspects of incubating DS eggs on the EFB. This could include redesigning the EFB to facilitate improved water flow within the EFB in an effort to improve egg incubation or adjusting its size to accommodate a smaller number of eggs.

There are some limitations to this study as well. One limitation of our simulations is the inability to validate them through real-life experiments due to the high cost associated with small velocity flow meters. Another limitation of our simulation was the extended duration required to complete each simulation due to the computational demands, often taking several days to run a single simulation.

2.5 Conclusion

Using the wakasagi EFB created by the LSFC to supplement the delta with larval DS seems like a plausible idea, given the similarities of wakasagi eggs with DS eggs like egg size and adhesive properties. The results of the simulation demonstrated that the water flowing towards the EFB at the specified inlet velocity undergoes significant deceleration as it approaches the EFB, ultimately to a flow velocity suitable for hatching DS eggs. Further research to deduce the area of the EFB with the most consistent flow velocity indicated that the front portion of the device has the most uniform water flow velocity.

Chapter 3: Temperature Effects on Delta Smelt Egg Hatching Using a Wakasagi Egg Frame Box

3.1 Introduction

Delta smelt (DS) are a critically endangered species of fish found only in the Sacramento-San Joaquin Delta (Delta). Diversion of water resources as well as historic drought conditions are some of the reasons for the decline of the population of the species (Moyle et al., 2018). DS play a crucial role in the Delta ecosystem for several compelling reasons. First, they serve as an indicator species, giving insight into the overall health of Delta. As an endemic species that is extremely sensitive, monitoring the status and health of the DS population and the trends provides valuable knowledge about the overall ecological condition of the region (Moyle et al., 2016). The preservation of their wild population helps maintain biodiversity and ecological balance within the Delta. Given the ongoing threat of invasive species introduction in the Delta, safeguarding as many native species like the DS becomes increasingly important (Poff et al., 1997).

The Fish Conservation and Culture Laboratory (FCCL) located in Byron, CA, is a facility affiliated with the University of California, Davis, specializing in the research, conservation, and cultivation of DS along with the threatened longfin smelt (*Spirinchus thaleichthys*). The lab is collaborating with multiple agencies on implementing methods to prevent extinction of the wild population of DS (Ellison et al., 2023; Lindberg et al., 2013). Reduction of hatchery effects is a large aspect of conservation programs that would incorporate cultured populations into conservation and management (Hindar et al., 1991). This involves modifying propagation methods or minimizing exposure to hatchery conditions in order to enhance the success of reintroducing cultured populations into their natural habitats (Kostow, 2009). One example of

this is a method involving the use of the egg frame box (EFB) from the LSFC in Japan, to hatch larval DS into the Delta using the same method that they employ for replenishing Lake Suwa with fertilized wakasagi (*Hypomesus nipponensis*) eggs that hatch and grow in the water for recreational purposes. Wakasagi and DS have potential to naturally hybridize in the SFE, and their eggs are very similar in terms of size and adhesiveness as previously mentioned in Chapter 2. Therefore, this approach for introducing larval DS with minimal domestic traits into the Delta appears plausible (Benjamin et al., 2018; Fisch et al., 2014; Xie Yuhao & Li Bo, 1987).

Early in the wakasagi spawning season, mature wakasagi are caught in Lake Suwa, Japan using trimmer nets when they travel upstream to their spawning grounds (Fujikawa & Katayama, 2014). The lake is divided into sections, and the fish are gathered in the center where they are caught using nets. Then, ripe males and females are immediately separated for egg spawning. It is important to emphasize the prevention of any moisture from contacting the eggs as they are water activated (Mizuno et al., 2010).

When spawning wakasagi, first, the eggs from a female are expressed into a dry container. Then, the milt from the male wakasagi is applied onto the eggs and the container is agitated using a waterfowl feather, which are hydrophobic due to its waxy coating (LSFC, n.d., section 6). The fertilized eggs are then poured onto the frames and spread evenly using the feather. Afterwards, the frames are then placed into a bowl filled with water to activate the eggs and milt. This addition also causes the adhesive stalks of the eggs to adhere to the fibers in the middle of the egg frame.

Since it would be important to determine whether the EFB would be suitable for DS in the Delta as it was for wakasagi in Lake Suwa, simulations were conducted to model the water flow velocity inside the compartments of the EFB to determine water flow rate that the eggs

attached to the egg frames would have to face (as described in Chapter 2). Results from the simulations demonstrated that as the water approaches the EFB, it experiences a substantial deceleration and further decelerates inside the EFB. The objectives in this chapter aims to compare the fluid interactions within the EFB observed in lab conditions to the predictions made by the simulations. We also evaluate the fertilization and survival rate of DS eggs within the EFB based on water temperatures found in the natural conditions found in the Delta and compare them to the rate observed under normal hatchery operation conditions.

3.2 Methods

3.2.1 Wakasagi Egg Frame Box

The wakasagi EFB was acquired from the LSFC, an organization that oversees and regulates fishing activities of Lake Suwa, as a method to replenish lakes with wakasagi, a popular recreation fish amongst Japanese fishermen (Japan Travel, n.d.). The EFB consists of two parts: the EFB itself and the egg frames that are held inside the compartments of the EFB. The characteristics of the box can be found in Section 2.2.1.

3.2.2 Egg Fertilization and Attachment to the Egg Frames

Spawning procedures for DS were adapted from methods employed by the FCCL with some slight modifications (Lindberg et al., 2013). Ripe DS were carefully selected and sorted by gender (Ellison et al., 2023). To ensure the safety and well-being of the fish, the DS were anesthetized using a diluted, non-lethal dose of buffered tricaine methane sulfonate (MS222, $NH_2C_6H_4COOC_2H_5 \cdot CH_3SO_3H$, Western Chemical, Inc.; 0.1 mg/L), and then gently dried off using paper towels. It is crucial to maintain a dry vent area in DS to prevent early activation of

the eggs. The spawning process involved first strip-spawning the eggs from the female, followed by application of the milt from the male to fertilize the eggs on the egg frame. The eggs were then evenly distributed across the frame using a hydrophobic duck (Welsh Harlequin, *Anas platyrhynchos domesticus*) feather as seen in Figure 3.1. Once the eggs were submerged in water, fertilization began and the eggs naturally adhered to the substrate (Tsai et al., 2021). The egg frames were then placed into the EFB for incubation.

3.2.3 Egg Incubation Trials

Five trials were conducted to evaluate the fertilization and survival rates of DS eggs incubated in the wakasagi EFB. A total of 23954 eggs spawned from multiple females were adhered on the frames, ranging from 686 to 3516 for each frame, with an average of 1597 eggs per frame. In each trial, only three egg frames containing eggs were placed in the EFB along with empty egg frames and placed into a 15 × 2.5 × 3 m, 1900-L rectangular tank on site at the FCCL in one of the facilities as seen in Figure 3.2. During the first, second-, and third-days post-fertilization (DPF), the eggs were soaked in a diluted Pond Rid-Ich solution (55 mL Pond Rid-Ich to 3.73 L system water, Kordon LLC., 0.014:1) for a minute each day. This process minimizes the potential for bacterial and fungal accumulation on the dead eggs, which could negatively impact the hatching success of the viable eggs. Formalin, an aqueous solution of formaldehyde (CH_2O), is an active ingredient in Pond Rid-ich, commonly used to kill bacteria and fungus. A constant water flow velocity of 30 cm/s was chosen and maintained because this flow velocity was achievable and within the range typically encountered when the EFB deployed in Lake Suwa in Japan (LSFC, n.d., section 6). Temperatures of 15.5, 16.3, 17.6, 20.2, and 20.7°C were selected for the trials because they represented natural conditions found in the Delta, with each temperature tested once.

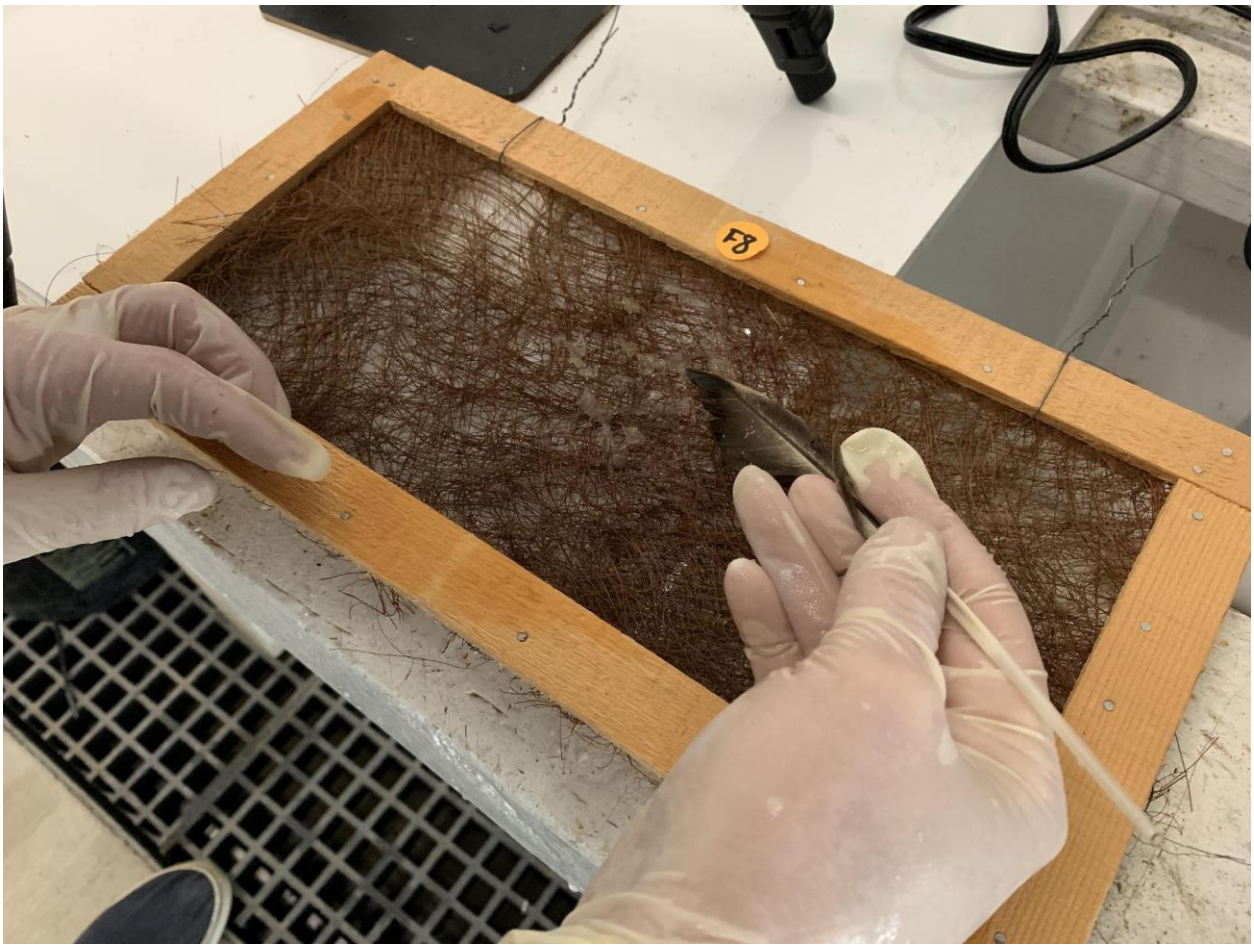
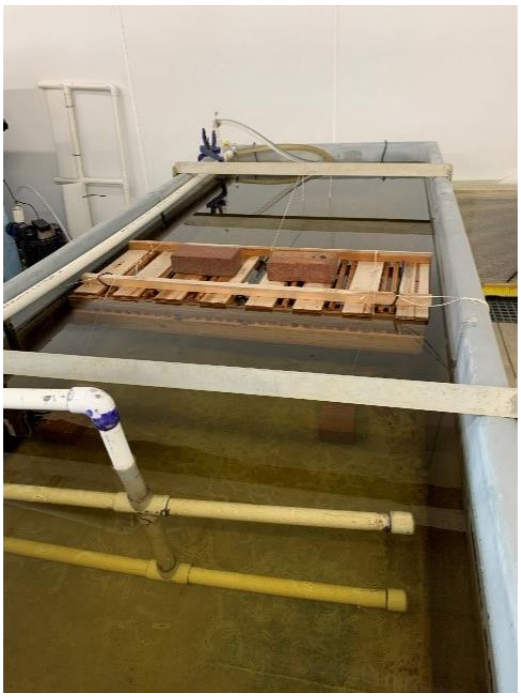


Figure 3.1: Hydrophobic duck feather used to spread delta smelt eggs evenly across egg frames.

(a)



(b)

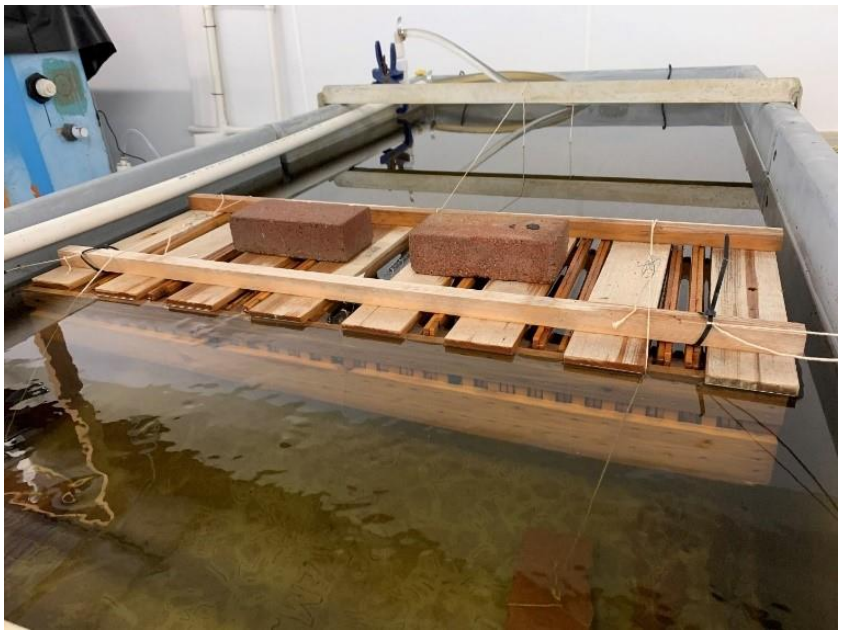


Figure 3.2: (a) The wakasagi EFB inside the 1900-L rectangular trough. The inlet can be seen at the bottom of the picture. (b) A close up of the EFB inside the trough.

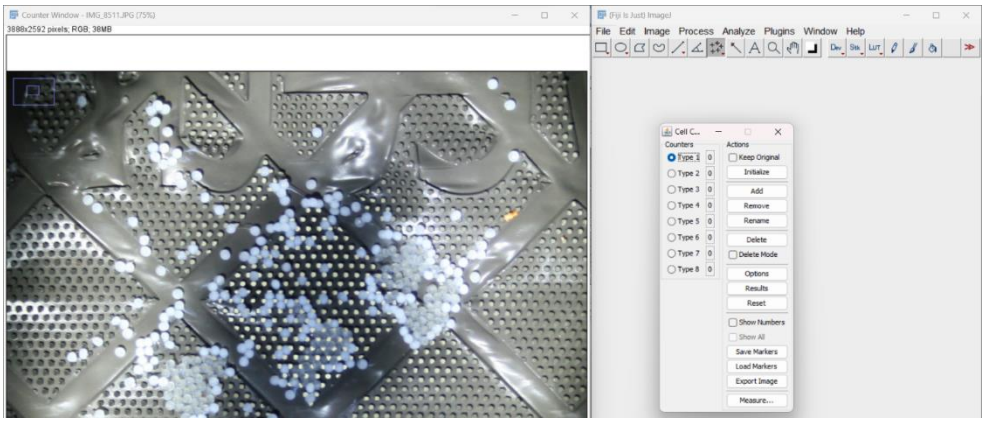
3.2.4 Data Collection

The eggs on the egg frames were photographed using a camera (Canon EOS Rebel XTi, Canon Technologies Corp., Japan) on 3 and 10 DPF to find the fertilization and survival rates of the eggs. After 10 DPF, any live egg that has not hatched would be labeled as dead eggs. Live and dead eggs were counted using an image processing extension called Fiji on ImageJ, a public domain image analysis software developed by the National Institutes of Health (NIH), to determine the success of fertilization and incubation on each of the frames. The photographs of the eggs adhered to the frames were first imported into ImageJ with the Fiji extension for cell counting, and then each individual egg was manually selected and marked as either live or dead (Schindelin et al., 2012; Schneider et al., 2012; Tsai et al., 2021). The process of selecting and counting live and dead eggs are shown in Figure 3.3. A live egg would be attached to the substrate and look clear with visible eyespots on the embryo, and an egg would be considered a dead egg when it was detached from the substrate, was opaque, covered in fungus, or had no distinguishable eyespots (Tsai et al., 2021). The software was then used to automatically count the number of live and dead eggs, based on the markings manually made on the images. This method was chosen because it allowed for a quick and accurate counting of a large quantity of eggs, which would have been difficult to achieve manually.

3.2.5 Statistical Analyses

All statistical analyses in this study were performed using RStudio (PBC, Boston, MA). The comparison of fertilization and hatching parameters among treatment means was conducted using Tukey's HSD (honestly significant difference) test. Prior to conducting the ANOVA, the normality of the residuals was assessed using the Shapiro-Wilk test and the Bartlett test for homogeneity of variances. Given the violations of the assumptions of normality and

(a)



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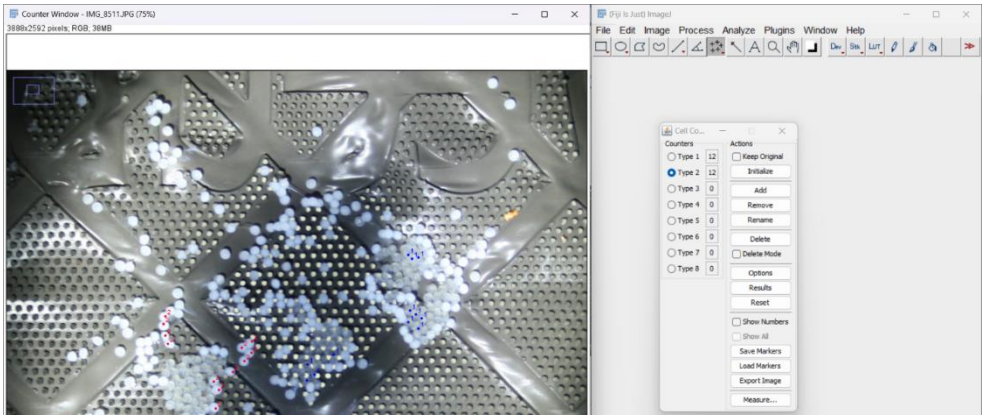


(c)

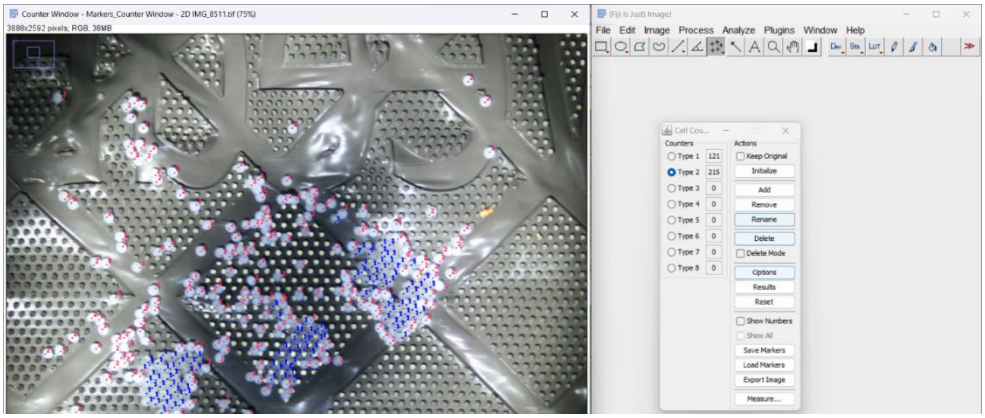


Figure 3.3: Process of counting 3 DPF live and dead DS eggs on an egg frame using Fiji extension on ImageJ starting from (a) initializing the image then (b) starting the counting to (c) tallying the final count. Red symbols signify a dead egg and blue symbols signify a live egg.

homogeneity of variances, a non-parametric Kruskal-Wallis test followed by a post-Hoc Dunn's test for significance between substrates is employed. This method was well-suited for the study as it provides a more comprehensive understanding of the temperature variations. Each temperature was evaluated once, and three frames were used simultaneously as replicates.

3.3 Results

The fertilization rate of the DS eggs was determined after the 10-day incubation period on the egg frames as seen in Table 3.1. Of the eggs evaluated in all the 5 trials, an average of 48% of the eggs were successfully fertilized, ranging from 14 to 88% successful fertilization rate. At lower temperatures, a consistent fertilization rate was observed, but as the water temperature increased, the fertilization rate decreased significantly but there was an increase in the fertilization rate at 20.7°C. Similarly, survival rates were higher at lower temperatures compared to higher ones. This shows that fertilization rate of DS eggs were significantly influenced by the temperature ($p = 0.028$, Figure 3.4).

The average survival rate of the DS eggs tested had an average of 50%, ranging between 22 and 100% survival rate. Similarly, the egg survival rate after 10 days was also analyzed and is shown to be significantly affected by the temperature ($p = 0.031$, Figure 3.4). Table 3.1 below has the fertilization and hatching results from the completed trials. Further, a post-hoc Tukey HSD test was conducted to further investigate which groups differ from one another. For the fertilization rate, it seems like the difference between Temp 20.7°C and 20.2°C is statistically significant with $p = 0.027$. As for the survival rate, there is a significant difference between the survival rates when the water temperature was 17.6°C and 16.3°C. These significant differences are indicated in Figure 3.4 using letters to denote statistical significance when comparing multiple groups.

Table 3.1: Egg fertilization and hatching results from five completed trials.

Start Date	Temp (°C)	Frame #	Live 3 DPF	Unviable 3DPF	Live 10DPF	Unviable 10DPF	Fertilization rate (%)	Survival rate (%)
3/13/20	15.5	1	826	530	360	739	61	44
3/13/20	15.5	2	281	859	73	1155	25	26
3/13/20	15.5	3	605	81	471	117	88	78
3/31/20	16.3	4	400	332	380	202	55	95
3/31/20	16.3	5	739	706	739	546	51	100
3/31/20	16.3	6	686	349	522	279	66	76
4/14/20	17.6	7	1026	1089	291	339	49	28
4/14/20	17.6	8	716	1176	296	258	38	41
4/14/20	17.6	9	288	676	79	135	30	27
4/28/20	20.2	10	728	2373	163	308	23	22
4/28/20	20.2	11	507	3009	325	461	14	64
4/28/20	20.2	12	346	1418	96	257	20	28
5/12/20	20.7	13	467	323	316	239	59	68
5/12/20	20.7	14	1826	455	519	552	80	28
5/12/20	20.7	15	711	426	209	450	63	29

(a) (b)

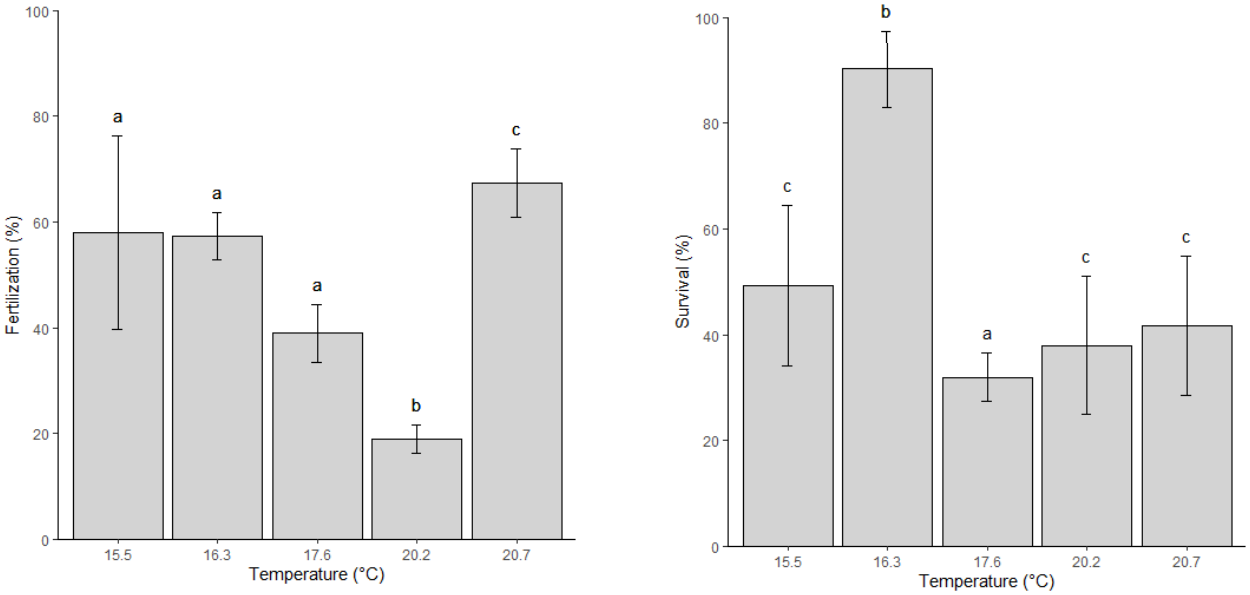


Figure 3.4: (a) Fertilization rate (%) and (b) survival rate (%) after 10 days of DS eggs on egg frames across 5 different temperatures (mean ± SE). Temperatures with the same letter, (e.g., a, b, or c) indicate that they are not significantly different from each other.

3.4 Discussion

From the results obtained, it is evident that the fertilization rate as well as survival rate of DS eggs were significantly affected by the temperature of the water it is contained in. Specifically, the 16.3°C incubation trial had the most successful survival rate as well as above average fertilization rate between the temperatures tested. The FCCL observed better hatching results at 15°C compared to temperatures of 10°C and 20°C during DS egg incubation (Baskerville-Bridges et al., 2005). This preference for 15°C could potentially indicate captive traits in DS. The FCCL maintains a water temperature of 16°C during incubation, and this temperature preference for hatching at around 16°C may have been unintentionally selected in DS bred at the FCCL. Though the 20.7°C trial yielded the highest fertilization rate, it was from one group tested that had a much higher rate when compared to the other two groups that were tested. This could be the reason for that anomaly when compared to the FCCL observations, though more studies need to be conducted to come to a more concrete conclusion.

When the eggs were adhered onto the frames during incubation, fungus grew on some of the dead eggs as seen in Figure 3.5. During FCCL daily operations, *Saprolegnia* fungus would grow when there were dead eggs and would spread rapidly. The LSFC also encountered this fungus problem and would carefully remove the fungus from the eggs daily using prongs (LSFC, n.d., section 6). The fungus would proliferate on the frames around the dead eggs. The same problem has been seen for eggs of many different freshwater and marine fish species as there is a strong inverse relationship between the time required for a

Figure 3.5: Egg frame with dead fertilized DS eggs affected with fungal infection.

fertilized fish egg to develop and the surrounding water temperature (Dannevig, 1895; Pauly & Pullin, 1988). At the FCCL, using the diluted Pond Rid-Ich treatment (55 mL Pond Rid-Ich to 3.73 L system water, Kordon LLC., 0.014:1) for 3 days as referenced in Section 3.2.3 has been effective in reducing the growth of the fungus, but not completely, so daily maintenance was still required to pick the fungus off the frames.

Examining how water temperature influences egg fertilization and survival could contribute to conservation strategies for DS for determining the precise time frame to deploy the EFB into the Delta based on temperature of the water. Though each temperature was successful in hatching and survival of DS eggs, the optimum water temperature for egg incubation is not yet fully understood. The experiment concluded that fertilization (80%) and survival rates (76%) were slightly lower than under normal laboratory conditions for DS at the FCCL, so additional trials should be conducted to identify water temperatures that yield similar fertilization and hatching rates comparable to rates under normal laboratory conditions (97%) (Y. J. Tsai et al., 2022).

While modeling the simulation with an inlet velocity of 60 cm/s, the water inside the EFB decelerated to an average of 10cm/s. When comparing the results of the simulations discussed in chapter 2 with the results obtained from these trials, it became apparent that the water velocity experienced inside the EFB is suitable for successfully hatching DS eggs. This observed water velocity was corroborated when testing with DS eggs on the EFB, which resulted in the successful hatching of the eggs.

3.5 Conclusion

Given the similarities found in wakasagi and DS eggs, using the wakasagi EFB seems like a plausible idea to supplement the Delta with larval DS. This study found that temperature

had a significant effect on the fertilization and survival of DS eggs. These findings will contribute to conservation efforts that aim to protect and restore the wild population of the DS. As DS are an endangered species that is endemic only to the Delta, it is important to keep them from becoming extinct as they would be a valuable tool for monitoring and studying changes in the environment. Overall, this study investigates the optimum temperature for DS incubation inside the EFB, but also shows that it can be used to hatch DS eggs. Further research on incubation temperature when planning strategies for DS on the EFB conservation should be considered.

Chapter 4: Testing of Alternative Egg Frame Materials

4.1 Introduction

Delta smelt (DS) are an imperiled fish species found only in the Sacramento-San Joaquin Delta (Delta). They are annual, multi-spawning fish from the Osmeridae family that are split into three groups based on major life-history phenotypes of DS: freshwater resident, brackish-water resident, and semi-anadromous (Bush & Hobbs, 2017; Hobbs et al., 2019). Their eggs are laid onto substrate material like gravel, sand, or vegetation (Kurobe et al., 2022). Each egg possesses an adhesive stalk to facilitate its attachment to the substrate and keep it from getting carried into the water current (Lindberg et al., 2020). The relative abundance of each group varies yearly, with the semi-anadromous phenotype being the most common and the brackish-water resident being the least common.

Wakasagi are a Japanese fish species that are found in lakes and rivers throughout Japan. They are also an introduced species found in the Delta and phenotypically as well as genetically, have very similar characteristics to DS (Swanson et al., 2000). Wakasagi, similar to DS, typically live for one year (Singh et al., 2015). They are both similar in size and use an adhesive stock to adhere the egg to substrate as described in Chapter 3. In Japan, wakasagi are a popular target for recreational fishing in the winter (Japan Travel, n.d.). Chartered boats carry anglers to fishing spots where they can catch wakasagi.

One method used to resupply the population of wakasagi into these lakes, is an EFB that fertilized wakasagi eggs adhere onto. Several of these frames are contained together in an EFB. The EFB is placed into the mouth of a shallow, flowing body of water. Over the course of several days, the fertilized eggs develop and hatch into the water. The flowing water gently aerates the eggs, while adhesive stalks of the eggs keep them adhered onto the frame, so they are

not swept away into the current. An EFB was obtained from the Lake Suwa Fishing Collective (LSFC) as this approach is hypothesized to be feasible because of the many similarities that eggs of the two species share. The EFB and egg frame are constructed from palm fiber, while the mesh of the egg frame is made from palm tree bark fiber.

The FCCL, which houses the refuge population of DS that are genetically similar to wild DS, purchased an EFB along with 32 egg frames, to evaluate their application for DS eggs. Previous studies (Chapter 3) have concluded that DS eggs can adhere, incubate, and hatch from the frames of the EFB created by the LSFC. The LSFC, the manufacturer of the EFB, is planning to discontinue production due to the loss of knowledge and expertise necessary for its manufacture.

Thus, the objective for this study is to find a new material to substitute for the egg frames that we used in the EFB. The material must be both biodegradable and thin enough to fit into the slots of the EFB, providing a suitable surface for egg adhesion while allowing water to flow between the EFB slots. For this experiment, DS egg fertilization and hatching rates were evaluated on two different substrates, burlap, and garden fabric (Figure 4.1).

4.2 Methods

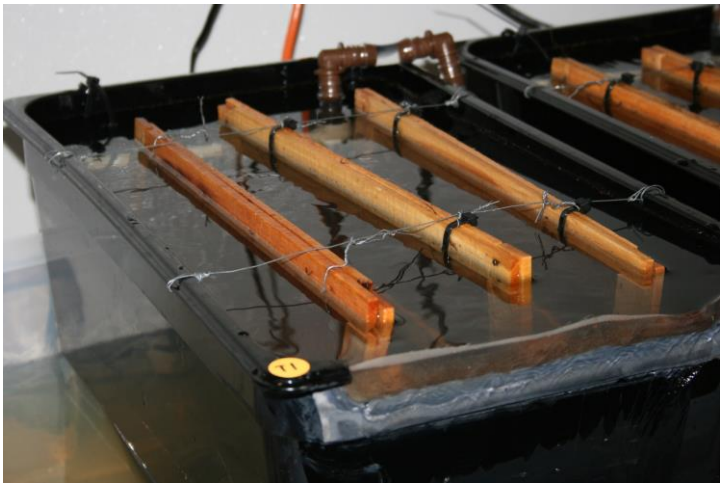
Frames and materials for substrate were created using plywood bought from Home Depot, and the substrates (garden fabric and burlap) were secured to the frames using yarn. These materials were selected because they are biodegradable and readily available in most locations. The prototype frame made from plywood was created using the exact measurements of one of the egg frames purchased from the LSFC (38 cm length × 19 cm height× 1.3 cm width). After assembly, the frames were placed in tanks with flowing water for a day to allow for the leaching of any excess chemicals from the exterior of the materials. The spawning procedure

Figure 4.1: Different egg frame materials (right to left: palm tree fiber, burlap, and garden fabric).

followed the methods outlined in Chapter 3, with the exception that the methods being that the eggs were spawned onto different substrates used as the frame mesh.

Two trials were conducted, each frame type was replicated three times. Fertilized eggs from three different female DS were randomly distributed onto the frames. Each tray contained three different types of egg frames. The trays were all placed in a larger water bath to control the temperature of the system. Each tray had a spray bar to recirculate the water flow from the front of the trays to the back where the water flows through a catch screen (Figure 4.2). The flow velocity for the two trials were both 10 cm/s and a temperature of 12°C, which was based on the LSFC egg management book (LSFC, n.d., section 6). Similar to Chapter 3, fungus would accumulate on dead eggs, so they had to be carefully removed daily. The eggs were photographed daily from Day 0 until Day 10 using a camera (Canon EOS Rebel XTi, Canon Technologies Corp., Japan). The camera was mounted onto a tripod to ensure consistent positioning and focus. The trials concluded after 10 days post fertilization, and it was determined that the unhatched eggs were dead after the 10-day incubation. After completion of the trials, data collection was conducted using the same methodology outlined in Chapter 3.2.4. Live and dead egg counts were recorded on Days 1, 5, and 10. The fertilization and survival rates of the DS eggs adhered to the different substrates were determined after 3 and 10 DPF days of incubation, respectively, on the experimental egg frames.

(a)



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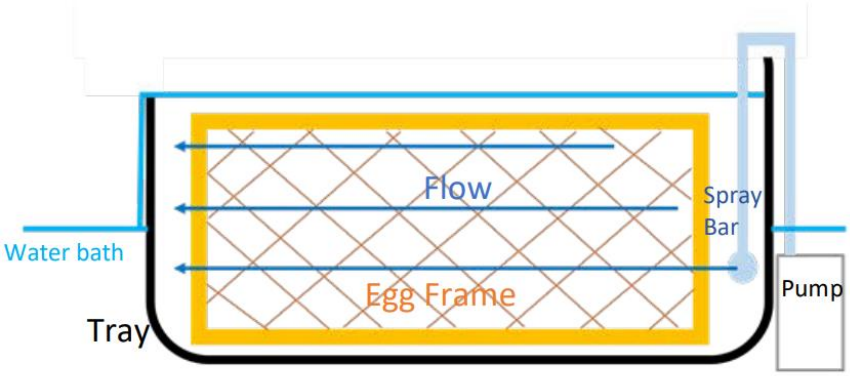

Figure 4.2: (a) The layout of the system used for the trials and (b) diagram of the tray system.

4.2.1 Statistical Analyses

All statistical analysis in this study was performed using RStudio (PBC, Boston, MA). In the analysis of the experimental data, the significant difference in the survival and hatching rates between the different treatments and placements between the means between attached and detached eggs were analyzed using a one-way ANOVA followed by Tukey's HSD test used to compare the means of different groups. Prior to conducting the ANOVA, the normality of the residuals was assessed using the Shapiro-Wilk test and the Bartlett test for homogeneity of variances. Given the violations of the assumptions of normality and homogeneity of variances, a non-parametric Kruskal-Wallis test followed by a post-Hoc Dunn's test for significance between substrates is employed.

4.3 Results

The experiment was initially performed using 48 groups of DS eggs that were divided into 3 based on frame substrate. Photographed used to as illustrated in Figures 4.3, 4.4, and 4.5. Table 4.1 shows the fertilization and survival rates of the eggs on day 1, 5, 10 and 15. The Shapiro-Wilk test indicated that the residuals were not normally distributed ($p > 0.05$), while the Bartlett test confirmed the assumption of homogeneity of variances ($p > 0.05$). In this case, a non-parametric Kruskal-Wallis test was used to assess the effect of different substrates on fertilization rate and did not reveal a statistically significant difference between the treatment groups (Kruskal-Wallis chi-squared = 3.41, df = 2, $p = 0.18$) meaning that the DS eggs showed no significant difference in fertilization between the substrates tested for the frames. The average fertilization rate, determined after 5 DPF, was 35%.

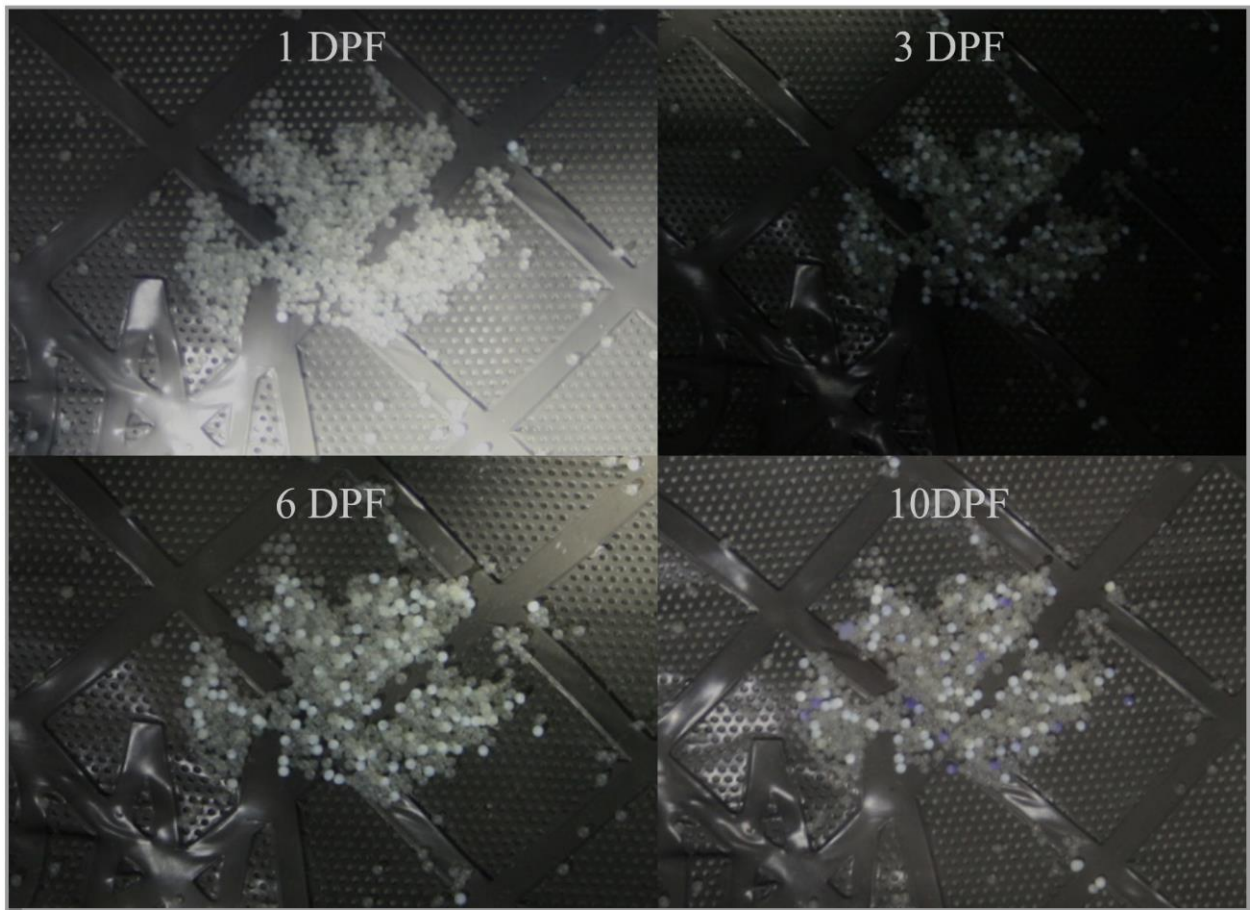

Figure 4.3: Delta smelt eggs from group 4C (garden fabric) on different DPFs taken on a Canon EOS Rebel XTi. Live eggs stayed attached to the substrate and looked clear. Dead eggs were detached from the substrate, opaque, or covered in fungus.

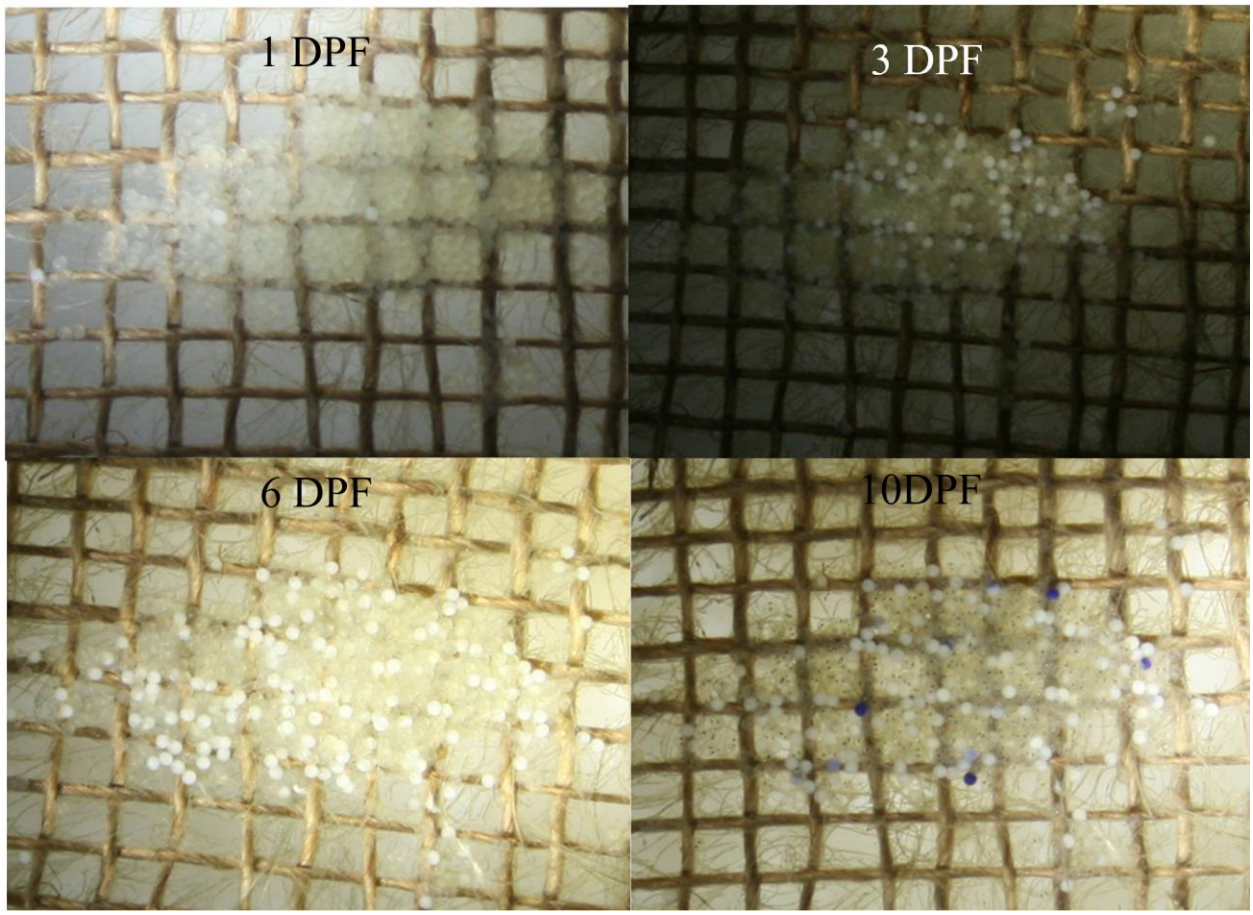

Figure 4.4: Delta smelt eggs from group 3I (burlap) on different DPFs taken on a Canon EOS Rebel XTi. Live eggs stayed attached to the substrate and looked clear. Dead eggs were detached from the substrate, opaque, or covered in fungus.

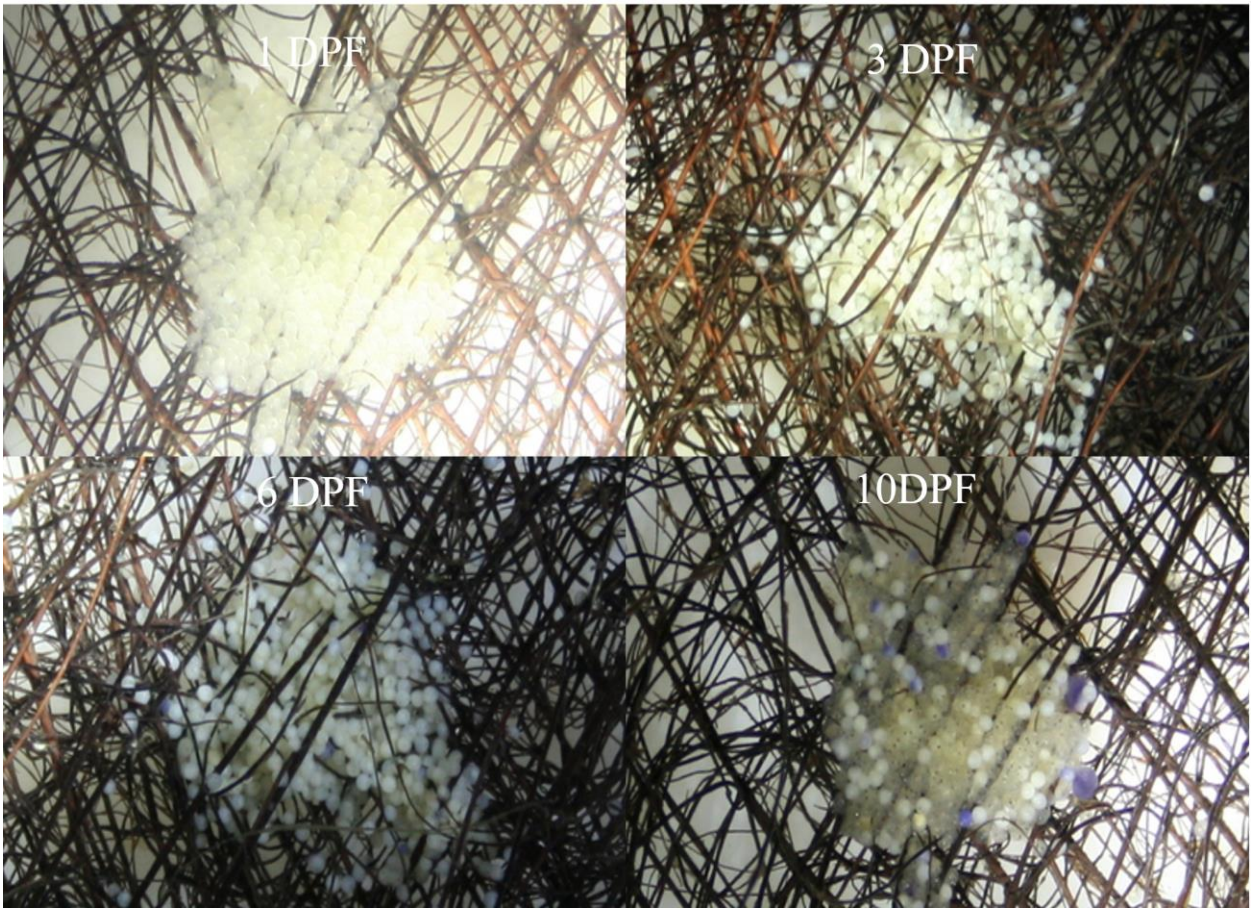

Figure 4.5: Delta smelt eggs from group 1F (palm fiber) on different DPFs taken on a Canon EOS Rebel XTi. Live eggs stayed attached to the substrate and looked clear. Dead eggs were detached from the substrate, opaque, or covered in fungus.

Table 4.1: Average and standard error of fertilization and survival rate of DS eggs on egg frames with different substrates found on day 1, 5, and 10 DPF.

Frame Type	Fertilization	D1 Survival	D5 Survival	D10 Survival
Control	0.54 ± 0.07	1	0.67 ± 0.37	0.48 ± 0.27
Burlap	0.53 ± 0.08	0.89 ± 0.08	0.27 ± 0.07	0.20 ± 0.07
Garden Fabric	0.69 ± 0.07	0.94 ± 0.06	0.38 ± 0.06	0.33 ± 0.06

The Shapiro-Wilk test indicated that the residuals were not normally distributed (W = 0. 0.85, p = 6.65E-6), while the Bartlett test confirmed the assumption of homogeneity of variances (K-squared = 0.55, df = 2, p = 0.76). In this case, a non-parametric Kruskal-Wallis test was used to assess the effect of different substrates on fertilization rate and did not reveal a statistically significant difference between the treatment groups (Kruskal-Wallis chi-squared = 2.51, df = 2, p = 0.29), meaning that the DS eggs showed no significant difference in survival between the substrates tested for the frames. The average survival rate, determined between 5 and 10 DPF, was about 83% of the eggs fertilized.

After live and dead DS eggs for each day have been tallied, the results are analyzed using the Kruskal-Wallis test, it was found that DS eggs had no significant difference in fertilization rate (p = 0.18) and survival rate (p = 0.29) between the treatments and were tolerant to the frame types used.

4.4 Discussion

This study aimed to investigate the effects of different treatments on fertilization and survival rate in DS eggs. The results from this experiment showed no significance in the fertilization or survival rate of DS eggs amongst the substrates used to make the egg frame. The study found no significant differences in fertilization and survival rate amongst the substrates tested. These findings are similar with the results of another project conducted at the FCCL, particularly the fertilization rate (Tsai et al., 2021). However, it is important to note that the Shapiro-Wilk test indicated a violation of the normality assumption and, while the Bartlett test confirmed that variances were homogeneous, the violation of normality suggests that the results should be interpreted with caution.

Despite the lack of statistical significance, the trends observed in this study demonstrate that DS eggs can survive on the wakasagi egg frame, burlap, and garden fabric. It also shows that many different materials may be used to substitute the palm fibers used for the mesh for the DS eggs to adhere to, although there are some substrates, acrylic for example, that DS eggs do not adhere to very well (Tsai et al., 2022). Future studies could benefit from a larger sample size to mitigate the impact of violations of statistical assumptions.

Another problem found during this experiment was the recurring occurrence of fungus that would appear on the frames to proliferate on the dead eggs. The fungus would proliferate on the eggs and could potentially impact the overall outcome of the study, so it is crucial to prioritize the removal of the fungus. Using the same methods of bathing the egg frames in a diluted Pond Rid-Ich solution in Chapter 3.4, the picking process was much easier on the burlap and garden fabric substrates than from the synthetic fibers substrate that is used by the LSFC, mainly because the fungus was easier to identify and pick through when using the prototypic frames compared to the original substrate, where there were fiber strands layered on top of each other, so it was harder to see the fungus and pick them out using the forceps.

4.5 Conclusion

DS are an endangered species that are only found in the Delta. Using an EFB can help replenish the Delta with DS that have fewer domestic traits than larvae born in laboratory/fishery conditions. The LSFC, the manufacturer of the EFB, is planning to discontinue, so various substrates are being tested to determine their effectiveness as a potential replacement for the egg frames made by the LFSC. The two materials that were tested were common items that can be easily found. The outcome indicates that DS eggs can successfully adhere, incubate, and hatch on a variety of substrates, including items that can be easily accessed. This study also

demonstrates that DS eggs are adapted to be able to adhere to different materials and suggests that creating an egg frame using alternative materials can be cost-effective and also easily reproducible if this method for wakasagi supplementation can be adapted for hatching DS.

This study contributes to our understanding of the effects of different substrates on fertilization and survival rate on DS by demonstrating that locally available materials like burlap and garden fabric could be just as effective for the egg frame, this shows that there are many possibilities for biodegradable, cost-effective and easily reproducible alternatives to replace the egg frames. Future studies could also expand on this project by exploring the use of other locally accessible materials for egg frames, as well as different shapes and sizes of both the egg frames and EFB. This research can lead to optimization of the EFB for hatching DS larvae into the Sacramento-San Joaquin Delta that are genetically similar to wild DS.

Chapter 5: Humidity Effects on the Delta Smelt Eggs

5.1 Introduction

Delta smelt (DS) are an endangered fish that reside only in the Sacramento-San Joaquin Delta (Delta). They are small, silvery fish that can reach a size of up to 5-7 cm in length (Moyle et al., 2016). As an indicator species of the delta, their health and status as a population are a proportional representation of the ecosystem that DS reside in (Hasenbein et al., 2013). Their lifespan usually only lasts for one year. They are multi-spawning fish from the Osmeridae family that are split into three groups based on major life-history phenotypes of DS: freshwater resident, brackish-water resident, and semi-anadromous (Bush & Hobbs, 2017; Hobbs et al., 2019). Each egg is about 1 mm in diameter and is equipped with an adhesive stalk, allowing them to cling onto substrates such as sand or vegetation to prevent them from being carried away by the water currents (Lindberg et al., 2020). Subsequently, larval DS hatch from the eggs and develop in the waters.

There are factors that can be attributed for their status as an endangered species, including water diversion and long droughts (Moyle et al., 2016). Given their annual life cycle, years of disruptions in the migration process between locations have led to a decline in the population of DS. Thus, efforts to protect and propagate this species has led to the idea of implementing an Egg Frame Box (EFB) created by the Lake Suwa Fishing Collective (LSFC, n.d., section 6). The EFB is designed to contain 32 egg frames that are used to hold wakasagi (*Hypomesus nipponensis*) eggs. DS and wakasagi eggs have very similar characteristics that make using the EFB for DS eggs a viable option, as mentioned in Chapters 2-4.

Another important consideration is to determine the coordination of transporting the eggs from the Fish Conservation and Culture Laboratory (FCCL) to the deployment site. The LSFC

transports their egg frames covered by a polypropylene tarp. As the feasible location for the deployment of the EFB should be somewhere in the Delta, the endemic habitat for the DS, the eggs should have to be transported for several hours to reach the destination. The fundamental question is the transportation method for the EFB containing DS eggs and whether it is feasible to transport the EFB without keeping it submerged, thereby exposing the eggs to ambient conditions during transportation. This should be studied to better understand how DS eggs fare in conditions with different temperatures and humidity than they would experience under water.

This chapter focuses on studying the impact of varying temperatures and humidity levels on the hatching and survival rate of DS eggs. Gaining insight into the response of DS eggs to these conditions during transportation could be crucial to refining methods to transport the EFB containing DS eggs. This could contribute to ensuring the successful reintroduction of this endangered species to its native habitat as well as future procedures for spawning and research on DS eggs.

5.2 Methods

5.2.1 Ambient Temperature and Humidity Inside the Egg Frame Box

To evaluate the effects of ambient conditions on DS eggs inside the EFB during transportation from the FCCL to the deployment site of the EFB, a series of trials were conducted on August 19, 2022, and January 21, 2023, to observe the effects during different seasons. One of the trials took place in the summer, characterized by significantly high temperatures and low air humidity, while the other trial was conducted during the winter when the temperature was low, and the humidity was high. Initially, the EFB was kept submerged in water inside a circular tank for a day. Afterward, it was removed from the water and positioned

on a dry table in the laboratory. Over the course of five hours, the humidity and temperature inside the box were recorded for five hours using seven Bel-Art H-B Instruments Thermometer-Hygrometers (Bel-Art Products, Inc., Wayne, NJ) are placed inside the EFB at various locations, as shown in Figure 5.1.

Humidity and temperature were recorded for 5 hours, and the data was used to determine the different humidity levels tested (Tables 5.1 - 5.4 and Figures 5.2 - 5.5). It should be noted that after 78 minutes, the box was moved from inside to outside to replicate conditions that would be seen if the box was moved from where it was submerged in the lab and loaded onto a truck outdoors to be moved to its destination. Another thing to be noted for the ambient conditions recorded on 8/19/2022 is that during the afternoon hours, the probe for recording ambient temperature had difficulty reading the humidity and gave no reading. However, it can be seen that the relative humidity inside the EFB on 8/19/2022 declined as low as 40%. This was used to form a baseline for the lowest humidity tested during the study. After the baseline of 40% relative humidity for the lowest humidity tested during the study was found, four humidity levels are chosen for the next experiment, 40%, 60%, 80%, and 100%.

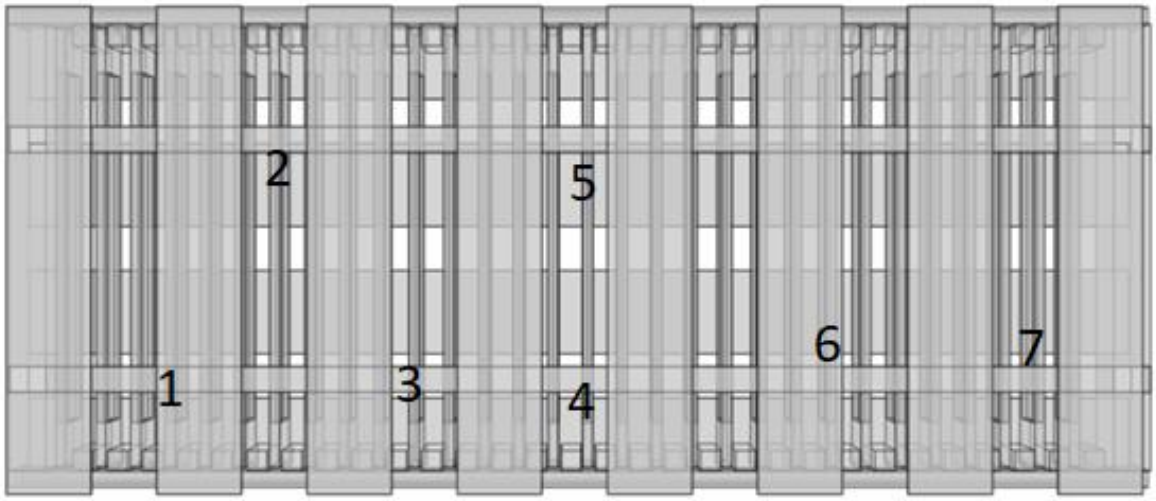

Figure 5.1: Placement of Bel-Art H-B Instruments Thermometer-Hygrometers inside the wakasagi EFB. Probes were all positioned on the bottom of the box.

Table 5.1: Relative Humidity in EFB on 1/21/2023

Time	P1	P2	P3	P4	P5	P6	P7	Ambient
8:40	66%	63%	54%	63%	58%	61%	61%	39%
8:41	67%	64%	54%	63%	58%	61%	61%	39%
8:42	67%	64%	55%	64%	59%	62%	61%	39%
8:43	69%	66%	58%	66%	61%	64%	64%	39%
8:45	71%	69%	61%	69%	63%	67%	68%	40%
8:50	73%	73%	69%	73%	68%	72%	73%	42%
8:55	75%	74%	73%	75%	71%	74%	74%	44%
9:00	76%	76%	75%	77%	73%	75%	76%	46%
9:10	79%	80%	79%	81%	78%	77%	78%	49%
9:40	86%	84%	85%	86%	83%	84%	86%	50%
9:58	88%	87%	87%	88%	87%	83%	83%	57%
10:40	87%	85%	86%	89%	88%	87%	88%	62%
11:40	90%	85%	88%	93%	92%	90%	90%	57%
12:40	91%	83%	85%	93%	91%	89%	89%	52%
13:40	92%	87%	89%	93%	92%	88%	89%	57%
13:58	89%	86%	87%	90%	90%	90%	90%	56%

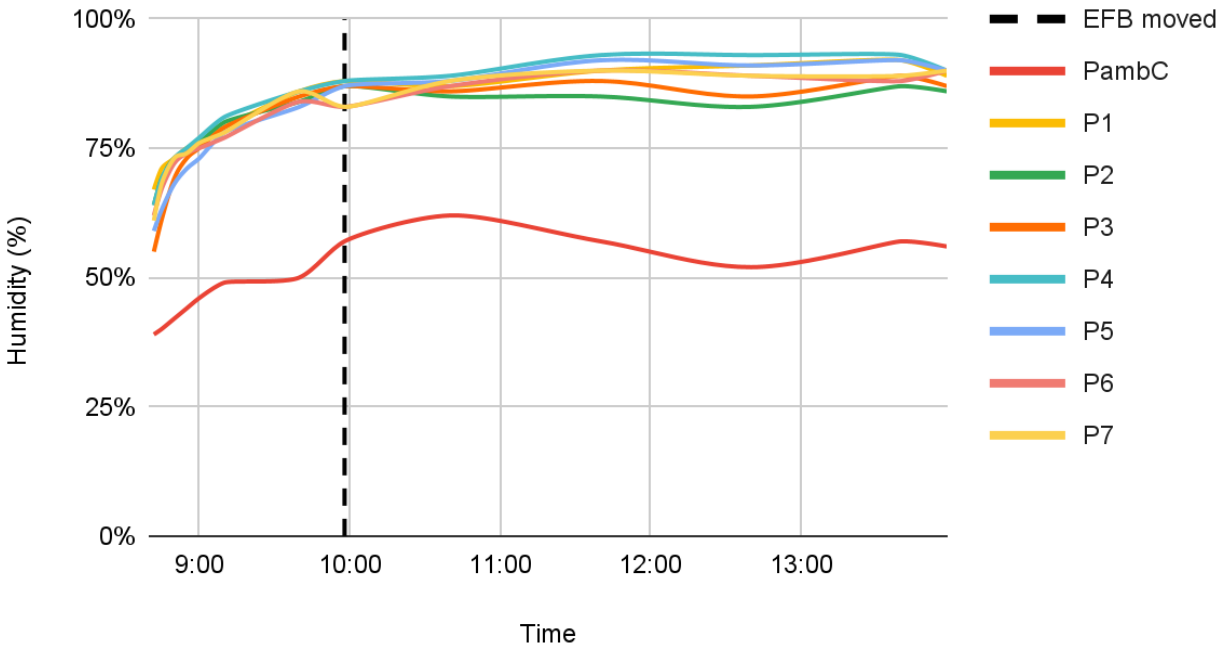

Figure 5.2: Relative Humidity in EFB on 1/21/2023

Table 5.2: Temperature in °C in EFB on 1/21/2023

Time	P1	P2	P3	P4	P5	P6	P7	Ambient
8:40	9.9	9.9	10.2	10.2	10.5	10	10.6	10.2
8:41	9.9	10	10.3	10.2	10.6	10.1	10.7	10.2
8:42	10	10	10.3	10.2	10.6	10.1	10.7	10.2
8:43	10.3	9.9	10.1	10	10.4	10	10.4	9.9
8:45	10.1	9.7	9.8	9.8	10.1	9.9	10.2	9.6
8:50	9.9	9.5	9.6	9.5	9.8	9.8	9.9	9.5
8:55	9.8	9.3	9.4	9.3	9.5	9.7	9.7	9.5
9:00	9.6	9.7	9.1	9	9.2	9.5	9.4	9.5
9:10	9	8.5	8.6	8.6	8.7	9.2	9	9.6
9:40	8.8	8.4	8.4	8.4	8.6	8.9	8.7	9.8
9:58	5.8	5.8	5.8	6	5.9	5.9	6	7.5
10:40	5.4	5.8	5.6	5.5	5.6	5.7	5.8	8.3
11:40	5.8	6.3	6.2	5.9	6	6.1	6.3	9.2
12:40	6.4	6.2	6.7	6.7	6.6	6.9	7	10
13:40	7.1	7	7.2	7.1	7.1	7	7.2	10.1
13:58	9.9	9.9	10.2	10.2	10.5	10	10.6	10.2

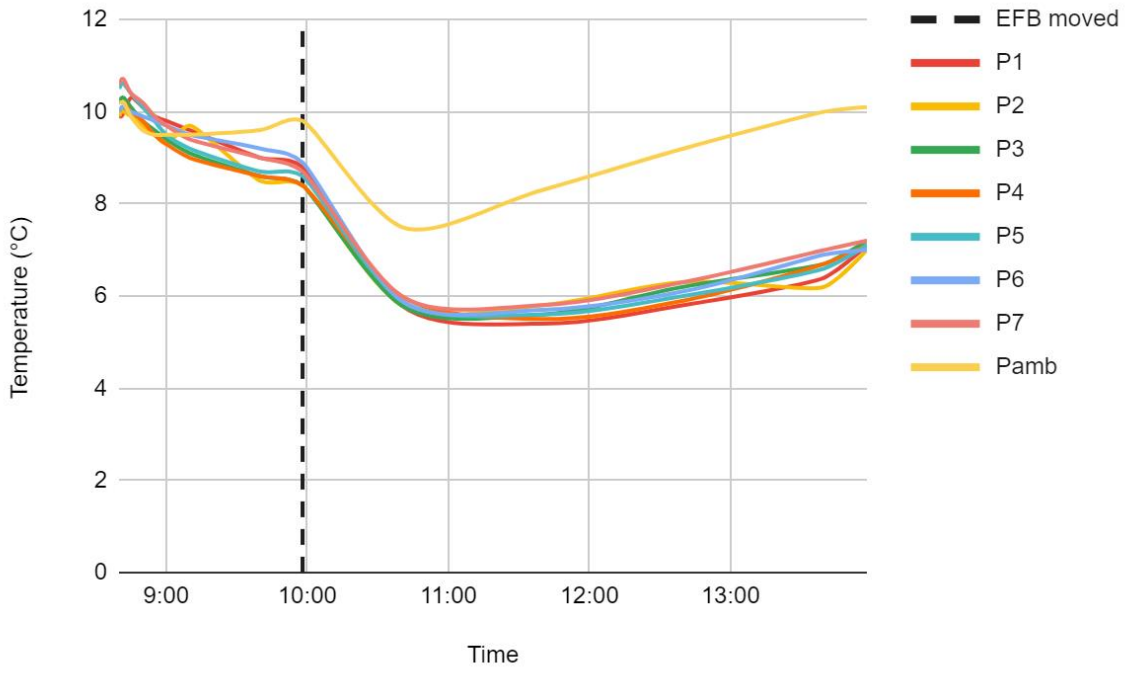

Figure 5.3: Temperature (°C) in EFB on 1/21/2023

Table 5.3: Relative Humidity in EFB on 8/19/2022

Time	P1	P2	P3	P4	P5	P6	P7	Ambient
8:40	67%	65%	64%	62%	66%	73%	73%	51%
8:41	68%	66%	66%	63%	67%	74%	74%	50%
8:42	69%	68%	65%	69%	70%	76%	75%	50%
8:43	70%	69%	71%	66%	70%	77%	76%	51%
8:45	73%	72%	74%	69%	73%	79%	78%	52%
8:50	73%	78%	81%	74%	79%	83%	82%	47%
8:55	81%	82%	83%	78%	83%	86%	84%	48%
9:00	84%	85%	87%	81%	85%	89%	87%	48%
9:10	88%	88%	89%	84%	88%	92%	89%	49%
9:40	91%	93%	91%	90%	93%	95%	93%	50%
9:58	93%	96%	90%	90%	92%	87%	80%	53%
10:40	77%	73%	63%	62%	60%	76%	63%	--
11:40	65%	62%	55%	52%	55%	64%	52%	--
12:40	63%	59%	47%	45%	46%	53%	45%	33%
13:40	51%	56%	46%	47%	47%	48%	40%	30%
13:58	53%	53%	43%	49%	45%	62%	45%	29%

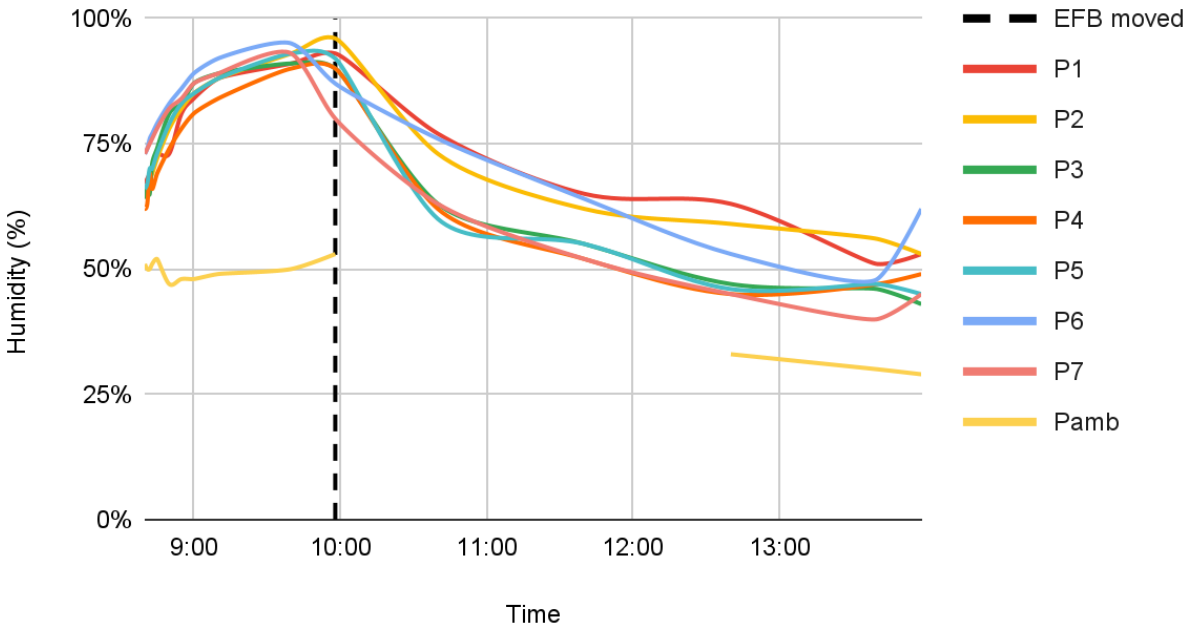

Figure 5.4: Relative Humidity in EFB on 8/19/2022

Table 5.4: Temperature in °C in EFB on 1/21/2023

Time	P1	P2	P3	P4	P5	P6	P7	Ambient
8:40	22.0	22.1	22.4	21.7	20.9	20.7	20.3	23.1
8:41	21.8	21.9	22.2	21.7	20.9	20.6	20.2	23.2
8:42	21.6	21.9	21.1	20.9	20.4	20.4	19.9	23.3
8:43	21.3	21.3	21.7	21.4	20.5	20.2	19.8	23.2
8:45	20.9	20.6	21.5	20.9	20.2	19.5	19.5	23.0
8:50	20.9	20.4	20.3	20.4	19.6	19.3	18.9	22.7
8:55	19.6	19.5	19.7	19.6	19.2	19.0	18.7	22.6
9:00	19.2	19.2	19.4	19.0	18.9	18.9	18.4	22.2
9:10	18.8	18.9	19.1	18.9	18.5	18.7	18.1	21.7
9:40	18.0	18.5	18.6	18.7	18.0	18.5	17.5	21.6
9:58	19.4	19.3	19.6	19.8	19.4	20.2	19.9	25.7
10:40	22.6	23.2	24.4	24.7	24.9	22.6	24.1	45.7
11:40	24.9	26.1	27.1	27.3	27.0	24.8	26.3	45.2
12:40	25.5	26.0	27.8	28.2	28.2	26.1	27.9	31.8
13:40	26.6	27.0	28.3	28.3	28.6	28.2	29.8	33.5
13:58	27.2	27.7	29.1	28.7	29.0	26.9	28.8	32.8

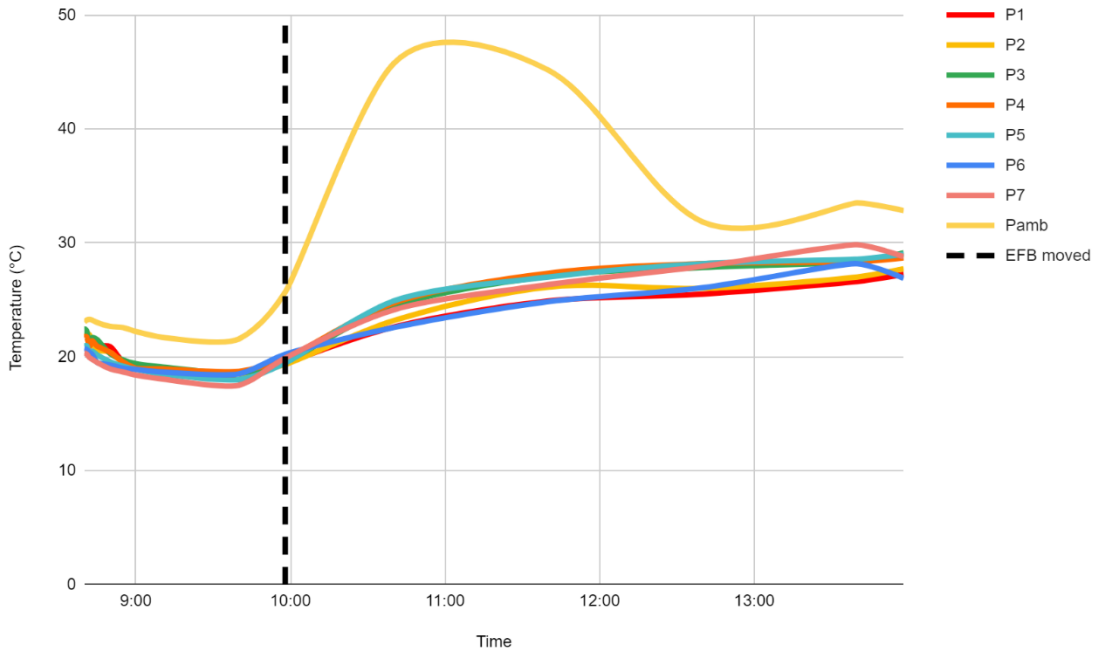

Figure 5.5: Temperature (°C) in EFB on 8/19/2022

5.2.2 Temperature and Humidity Trials on Delta Smelt Eggs

For this experiment, 5 ripe DSs were collected using the refuge population from the FCCL. Eggs were spawned and fertilized at the FCCL using the spawning procedure outlined in Chapter 3.2.2, with the exception that the methods being that the eggs were spawned into bowls. The eggs were pooled together and then transported under water in bowls to the Hung lab at Bainer Hall at UC Davis campus, where they were then incubated using a water bath at 16°C. For the initial 3 days, a diluted Pond Rid-Ich solution (55 mL Pond Rid-Ich to 3.73 L system water, Kordon LLC, Hayward, CA, 0.014:1) was used daily for 1 minute daily. On the third day of maintenance, a bentonite solution (100mL Bentonite to 1.5 L system water, Sigma-Aldrich ,0.067:1) was gently applied to the outer coating of the eggs. This procedure was done to remove their adhesive stalk to make the eggs easier to move. Before starting the trial, the live eggs are selected and placed into groups of 30.

Based on the relative humidity found in the EFB on 8/19/2022 and 1/21/2023, humidities of 40%, 60%, 80%, and 100%, along with a control, were selected for testing. Triplicates of each group were made, with the controls being under 100% humidity and 15°C for the duration of the study. On the fourth day after fertilization, the eggs were put on trials. The eggs were taken out of their bowls and placed on a dry screen (1 μm) to remove the moisture from the eggs (Figure 5.6). The eggs were then placed into a Caron Environmental Chamber 7000-25 (Caron, Marietta, OH), set at the corresponding humidity and 25°C for 5 hours. Humidity and temperature of each group are monitored using a Bel-Art H-B Instruments Thermometer-Hygrometer placed next to each screens. Another set of 3 groups were placed into a 25°C water bath for 5 hours and another set of 3 groups functioned as the control group. The same procedures were replicated for a 3-hour trial under the same four conditions. After the experiments, the eggs were returned to their

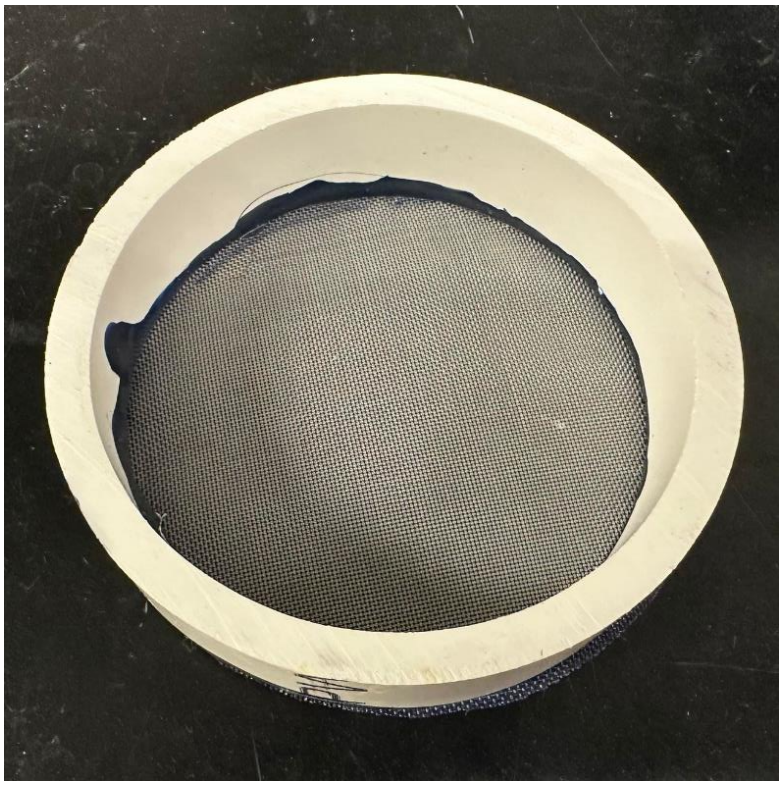

Figure 5.6: A round white PVC with a 1 μm mesh screen used to remove moisture from delta smelt eggs.

respective egg bowls and submerged for incubation once again. The eggs were then monitored daily over a span of seven days, during which, both live and dead eggs were counted. Any dead eggs identified were promptly removed. Hatching was also induced on the eggs by gently swirling the eggs in a circular pattern using a pipette.

5.2.4 Egg Hatching Determination

DS eggs generally hatch in 8-10 days at 15-17°C, although it is not unusual to have a few larvae hatch early (Baskerville-Bridges et al., 2005). This usually signifies that the major hatching event should take place within the next two days. Thus, every day after the first larval DS hatched until the conclusion of the trial, the eggs were checked daily and then counted.

After 10 Days Post Fertilization (DPF), the live eggs that remained unhatched were considered dead eggs. The hatched larvae were counted and then subsequently transferred to a separate tank for euthanasia. The experiment conducted for the 3-hour trials for the study followed the same procedure as the 5-hour trial.

5.2.5 Statistical Analyses

All statistical analyses in this study were performed using RStudio (PBC, Boston, MA). In the analysis of the experimental data, the significant difference in the hatching rates in the different humidities between the means and standard errors between attached and detached eggs were analyzed using Tukey’s HSD test. Prior to conducting the ANOVA, the normality of the residuals was assessed using the Shapiro-Wilk test and the Bartlett test for homogeneity of variances. Given the violations of the assumptions of normality and homogeneity of variances, a non-parametric Kruskal-Wallis test followed by a post-Hoc Dunn's test for significance between substrates is employed.

5.3 Results

The results show that when exposed to 40% and 60% humidity for 5 hours, none of the eggs were able to hatch. When exposed to 80% humidity, 43.62% and 8.89% of the eggs hatched after being exposed for 3 and 5 hours, respectively. All of the eggs hatched in the 100% humidity as well as the control. This shows that exposure to unsaturated air that is less than 100% humidity would be detrimental to their hatching success.

In the 3-hour trial (Table 5.5 and Figure 5.7), the many of the eggs subjected to 40% and 60% humidity did not survive the experiment with 4.40% and 10% of the eggs hatching, respectively, while the eggs subjected to the 80% humidity fared much better with a 43.62% hatching rate. The 100% humidity and the control both had 100% hatching rate. The Shapiro-Wilk test indicated that the residuals were not normally distributed ($p > 0.05$), while the Bartlett test confirmed the assumption of homogeneity of variances ($p > 0.05$). The Kruskal-Wallis test indicated a significant overall effect of humidity on hatching rate ($\chi^2 = 12.42$, $p = 0.01$).

Post-hoc Dunn's tests were conducted to further investigate these differences revealing that the hatching rate at 100% humidity was significantly higher compared to 40% ($p=0.004$) and 60% ($p=0.01$). However, no significant difference was observed between 40% and 60% humidity levels ($p=0.39$).Similarly, no significant differences were observed between 100% and 80% ($p = 0.07$), 40% and 80% ($p = 0.13$), and 60% and 80% ($p = 0.20$) humidity levels. When compared to the control group, the 40%, 60% , and 80% humidity levels showed significant differences ($p = 0.004$, $p = 0.01$, and $p = 0.07$ respectively), but 60% and 80% did not ($p = 0.20$). The 100% humidity showed no significant difference from the control ($p > 0.5$). These significant differences are indicated in Figure 5.7 using letters to denote statistical significance when

Table 5.5: Delta smelt eggs hatching rate for tested humidities for 3 hours.

Temp	Humidity	Live	Hatched	Hatching rate
25	40	24	0	13%
25	40	4	0	0%
25	40	30	0	0%
25	60	30	0	32%
25	60	30	6	0%
25	60	30	1	0%
25	80	30	6	16%
25	80	30	0	81%
25	80	19	2	23%
25	100	29	29	100%
25	100	30	30	100%
25	100	30	30	100%
control	control	30	30	100%
control	control	30	30	100%
control	control	30	30	100%

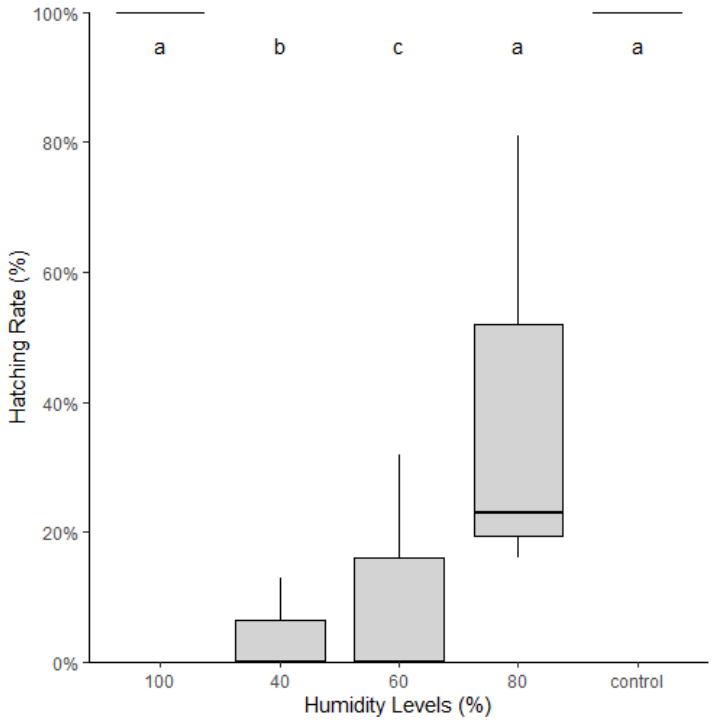

Figure 5.7: Box plot of delta smelt eggs hatching rate for tested humidities for 3 hours. Temperatures with the same letter, (e.g., a, b, or c) indicate that they are not significantly different from each other.

comparing multiple groups. Temperatures with the same letter, (e.g., a, b, or c) indicate that they are not significantly different from each other.

In the 5-hour trial (Table 5.6 and Figure 5.8), none of the eggs subjected to 40% and 60% humidity survived the experiment, while the eggs subjected to the 80% humidity fared much better with an 8.89% hatching rate. The 100% humidity and the control both had 100% hatching rate. The Shapiro-Wilk test indicated that the residuals were not normally distributed ($p > 0.05$), while the Bartlett test confirmed the assumption of homogeneity of variances ($p > 0.05$). The Kruskal-Wallis test also indicated a significant overall effect of humidity on hatching rate ($\chi^2 = 12.26$, $p = 0.02$).

The Dunn's test also indicated significant differences in hatching rates among various humidity levels. Specifically, the hatching rate at 100% humidity was significantly different from that at 40% ($p = 0.003$) and 60% ($p = 0.03$). When compared to the control group, the 40% humidity level showed a significant difference ($p = 0.003$), as well as 60% and 80% did ($p = 0.03$ and $p = 0.03$, respectively). However, the hatching rate at 40% was not significantly different from that at 60% ($p = 0.21$). Additionally, no significant differences were observed between 40% and 80% ($p = 0.18$) and 60% and 80% ($p = 0.46$) humidity levels. The 100% humidity showed no significant difference from the control ($p > 0.5$). These significant differences are indicated in Figure 5.8 using letters to denote statistical significance when comparing multiple groups. Temperatures with the same letter, (e.g., a, b, or c) indicate that they are not significantly different from each other.

Table 5.6: Delta smelt eggs hatching rate for tested humidities for 3 hours.

Temp	Humidity	Live	Hatched	Hatching rate
25	40	24	0	0%
25	40	4	0	0%
25	40	30	0	0%
25	60	30	0	0%
25	60	30	6	20%
25	60	30	1	3%
25	80	30	6	20%
25	80	30	0	0%
25	80	19	2	11%
25	100	29	29	100%
25	100	30	30	100%
25	100	30	30	100%
control	control	30	30	100%
control	control	30	30	100%
control	control	30	30	100%

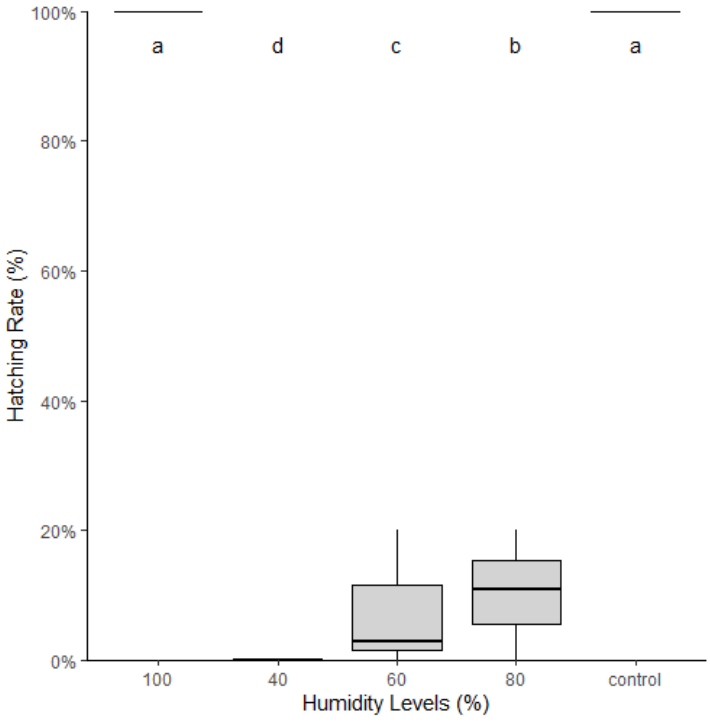

Figure 5.8: Box plot of delta smelt eggs hatching rate for tested humidities for 3 hours. Temperatures with the same letter, (e.g., a, b, or c) indicate that they are not significantly different from each other.

5.4 Discussion

The findings suggest that DS eggs are highly sensitive to being outside of the aquatic environment. One explanation for this could be that lower humidity levels would dry out the chorion, leaving the entire egg to dry out, leading to their failure to hatch. Comparatively, all eggs in the control group (100% humidity with no exposure to air) were able to hatch at the conclusion of the experiment. This reinforces the understanding that DS eggs are best adapted for fully aquatic conditions. Therefore, to ensure better hatching rates, it is important to maintain 100% humidity conditions or by keeping the eggs submerged during transportation to the deployment site. More research would be needed to understand the mechanism behind how DS eggs fare when unsubmerged.

One challenge encountered was that the humidity chamber used could not lower the temperature to the desired 16°C, as the lowest temperature allowed was 25°C. To address this limitation, a control group trial was conducted to investigate potential differences in egg hatching between 16°C and 25°C. The results indicated no significant difference, although it should be noted that this trial was relatively small in scale. This is noteworthy because during the summer, the temperature inside the EFB reached up to 29°C, as indicated in Table 5.4 and Figure 5.5.

These findings provide much needed insight on the future transportation methods for the EFB for DS conservation efforts. This was the only experiment conducted where fungus did not accumulate on the dead eggs during incubation. This may be attributed to the daily maintenance conducted on the eggs. Another factor that may have also eliminated the appearance of fungus was because the number of eggs used was much less, so they were easier to manage.

While there are fish species that come onto land to spawn and fertilize their eggs, like the California grunion (*Leuresthes tenuis*), DS lack the adaptations that these fishes have to survive

and incubate on land (K. L. M. Martin et al., 2004). For California grunion, their spawning is closely tied to the moon cycle and will usually spawn on beaches during the highest tide by swimming onto land to lay and bury their eggs on the beach (Martin et al., 2011). Once the female has landed on the beach, she will burrow herself up to her pectoral fins using her tail to lay her eggs. Numerous male grunions will follow the females and attempt to fertilize the eggs with their milt and then will retreat back into the water. Though the eggs are laid on land and not submerged by the water, they are protected from ambient temperatures and humidity by the damp sand.

Another adaptation that California grunions exhibit that DS do not seem to do is the ability to extend their incubation period and prolong their hatching process when conditions are not favorable. These eggs have cues that react when water comes into contact with the outer layer of their eggs that they follow to be able to hatch at the right time. This adaptation leads to a better chance of survival by waiting until the next set of high tides is high enough to carry the grunion larvae into the ocean. These adaptations are essential for the California grunion and the main reason that their eggs are able to survive being un-submerged. As DS eggs do not have any similar adaptations, their eggs should not be left un-submerged, but more research needs to be conducted on this topic.

5.5 Conclusion

DS are an endangered species of fish only found in the Delta. As an indicator species, their survival is especially important for the health of the delta. Multiple agencies and companies have been cooperating on ideas and efforts to bring the species back from the brink of extinction. The idea of adapting the wakasagi EFB to supplement the Delta with fertilized DS eggs that are ready to hatch has been discussed and from that, tests were conducted to examine the feasibility

of the contraption for DS eggs. The findings indicate that DS eggs are extremely sensitive to being removed from their aquatic environment. Therefore, to maintain high hatching rates, the eggs should remain submerged throughout their transport to the release site. From the results of this study, it shows that the EFB should be submerged during transportation to the deployment site when DS eggs are being held in it.

Chapter 6: Summary, Conclusion, and Recommendations

6.1 Summary

Delta smelt (DS) are an endangered species of smelt, endemic to the Sacramento-San Joaquin Delta (Delta). Though there are many reasons for their status as an endangered species, as a keystone species as well as an indicator species, it is paramount that their conservation for DS be successful and therefore should include different efforts to preserve the species. This study aims to contribute to the conservation of the endangered DS by exploring innovative methods, such as the wakasagi egg frame box (EFB) and evaluating its efficacy for DS eggs through a series of tests.

First, the computation fluid dynamics simulations conducted on COMSOL Multiphysics revealed that waters flowing into the box experiences significant deceleration (about 83% reduction) as it enters into the EFB. This along with the physical experiments using the EFB in a large flowing tank (see Chapter 3) demonstrated its viability for adaptability for accommodating DS eggs.

Previous studies have been conducted to determine the feasibility of the EFB in the previous chapters, including CFD simulations to determine the water flow velocity inside the EFB, testing to analyze the viability of the EFB on DS eggs, and testing different substrates to replace the palm fibers of the original EFB.

From simulations on COMSOL Multiphysics modeling the EFB using water flow velocities recorded in the Delta, it was calculated that water velocity decelerates rapidly as it travels in a unidirectional motion towards the openings of the EFB. The water velocity inside the EFB was found to be suitable for DS egg incubation and hatching from a separate study. Since the contraption was originally made for wakasagi eggs, the second study focused on the viability

of the egg frames for DS eggs. Another study focused on finding alternative materials as a substrate for DS eggs to adhere to in the case that the egg frames need to be reproduced, testing burlap and garden fabric. It was found that there are many easily accessible, yet suitable materials that DS eggs can adhere to.

The research presented demonstrates the potential of the wakasagi EFB as a feasible strategy for DS conservation. The research affirmed that DS eggs can successfully adhere, incubate, and hatch on a variety of different substrates at a hatching rate worse than in the incubators at the FCCL. These substrates were biodegradable and readily available at Home Depot, indicating their accessibility at common stores.

This study also revealed the sensitivity of DS eggs to changes in environmental changes, particularly when unsubmerged and experiencing different humidities. This underlines the imperative to maintain constant aquatic conditions during transportation to the deployment site to ensure higher egg hatching rates.

6.2 Conclusion

This research substantiates that the wakasagi EFB created by the LSFC can potentially serve as a suitable candidate to facilitate the hatching of DS larvae within the Delta. The research demonstrates that DS eggs can successfully adhere to the wakasagi egg frames under water flow velocities found when simulating conditions inside the EFB, incorporating real parameters from the Delta. Furthermore, when testing these conditions on fertilized DS eggs in a lab environment, the eggs were able to hatch using the water flow velocities observed from the CFD simulations. The incubation and maintenance procedures conducted by the LSFC could similarly be implemented at the FCCL.

Another study also showed that biodegradable materials for creating additional egg frames can be conveniently sourced from any home improvement store. Two materials, burlap, and garden fabric were tested against the control and showed no difference in DS egg survival and hatching rate.

One study helped determine how to transport the EFB from the FCCL to the deployment area. The study concluded that DS eggs should be kept submerged under water when being transported. The studies showed that when comparing fertilized DS eggs that have been left unsubmerged for various hours (3-5) to fertilized DS eggs kept fully submerged for the entire incubation duration, the unsubmerged eggs were heavily affected by being left unsubmerged. As DS are a fully aquatic fish, with no adaptation in themselves as well as their eggs, it should not be a surprise that the eggs should be kept submerged for the entire transportation process to the deployment to optimize egg hatching rate.

6.3 Recommendations

After determining the feasibility of the wakasagi EFB for use on DS eggs, the next step should be to determine where the EFB should be deployed. From the article written by Kurobe et al., (2022), using the Spring Kodiak Trail (SKT), a yearly survey taking place from January to May to determine the relative abundance and distribution of spawning DS in the Sacramento-San Joaquin Delta, it can be seen that Cache Slough is the area that all of the ripe female DS were found in. Another valuable piece of information from Kurobe et al., (2022), was that a majority of the ripe female DS found in Cache slough was in the month of February. Water quality data such as water depth and flow rate can then be extrapolated from two stations found in and around the slough. The first station, 11455385, is located upstream from the Cache slough, and the

second station, 11455420, is located downstream from it, as seen in Figure 6.1. Using the flow rate and water depth data from the stations, the flow velocity can be determined using the following equation:

$$Q = AV$$

where Q is the volumetric flow rate (m^3/s), V for the water velocity (m/s), and A is the cross-sectional area of flow (m^2). Using the water velocity for February 2020-2022, a figure can be created for each year, overlaying the water velocity from each station to determine the better location to deploy the EFB as diagramed in Figures 6.2, 6.3, and 6.4. From the graphs, it can be seen that the water velocity in the first station, 11455385, would have more adequate flows suited for hatching DS eggs, while the second station would not have the adequate flows. With this information, the most suitable location to deploy the EFB would be near the first station.

Next, to quantify the water velocity further, to find the best 4-day span to deploy the EFB, each flow velocity data was given a value depending on the respective velocity as shown the following table:

V>60cm/s → 1

V>50cm/s → 0.75

V>25cm/s → 0.4

V>10cm/s → 0.1

V<10cm/s → 0

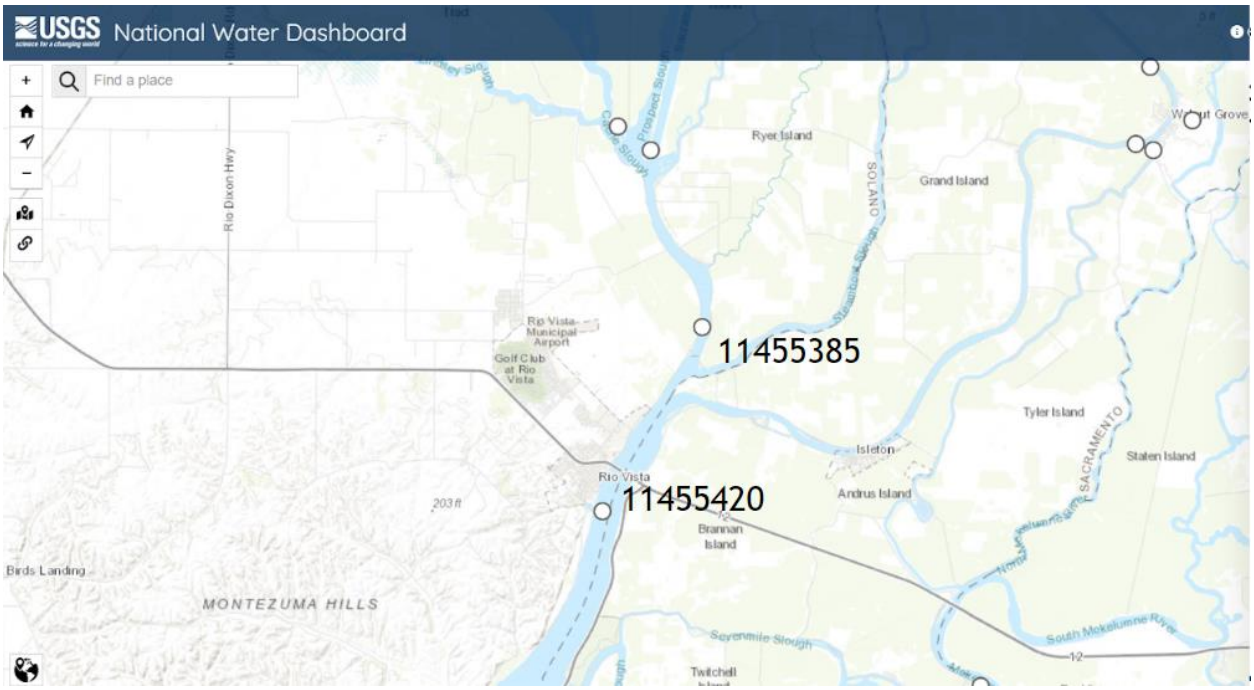

Figure 6.1: Water Stations 11455385 and 11455420 located around Cache Slough.

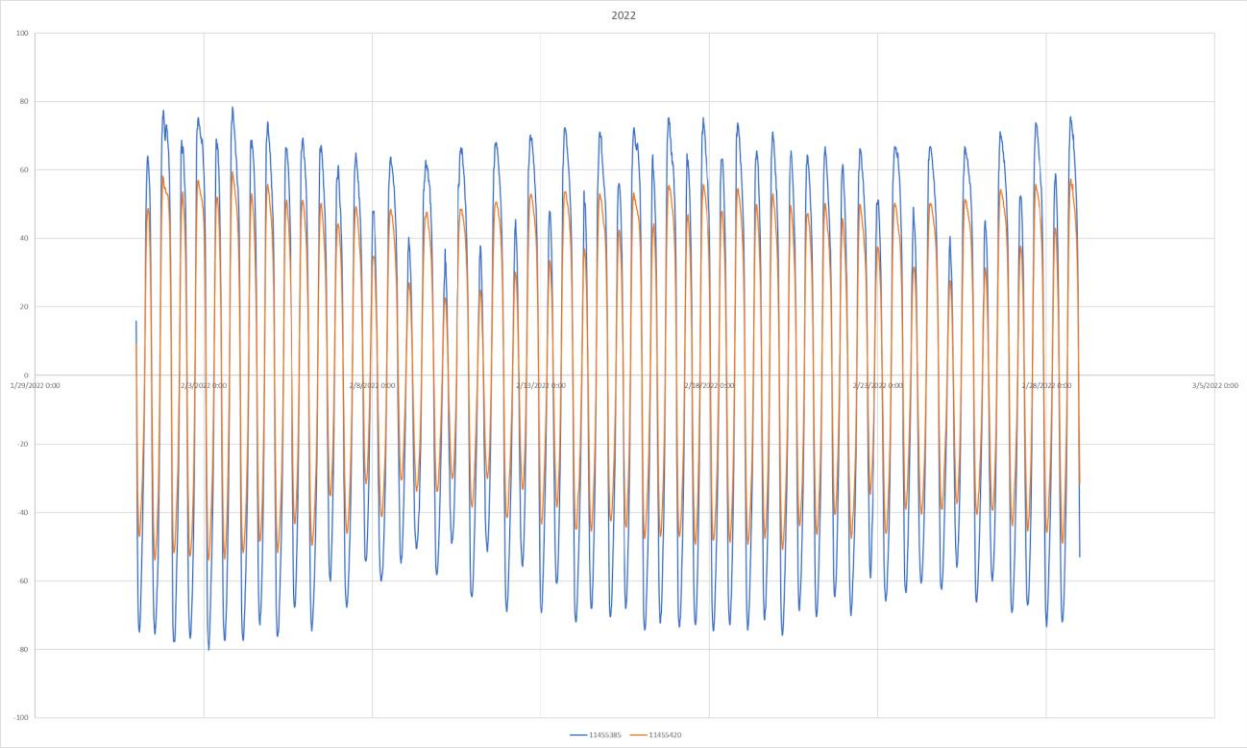

Figure 6.2: Mean flow velocity for discharge in cm/s for stations 11455385 (blue) and 11455420 (orange) for February 2020.

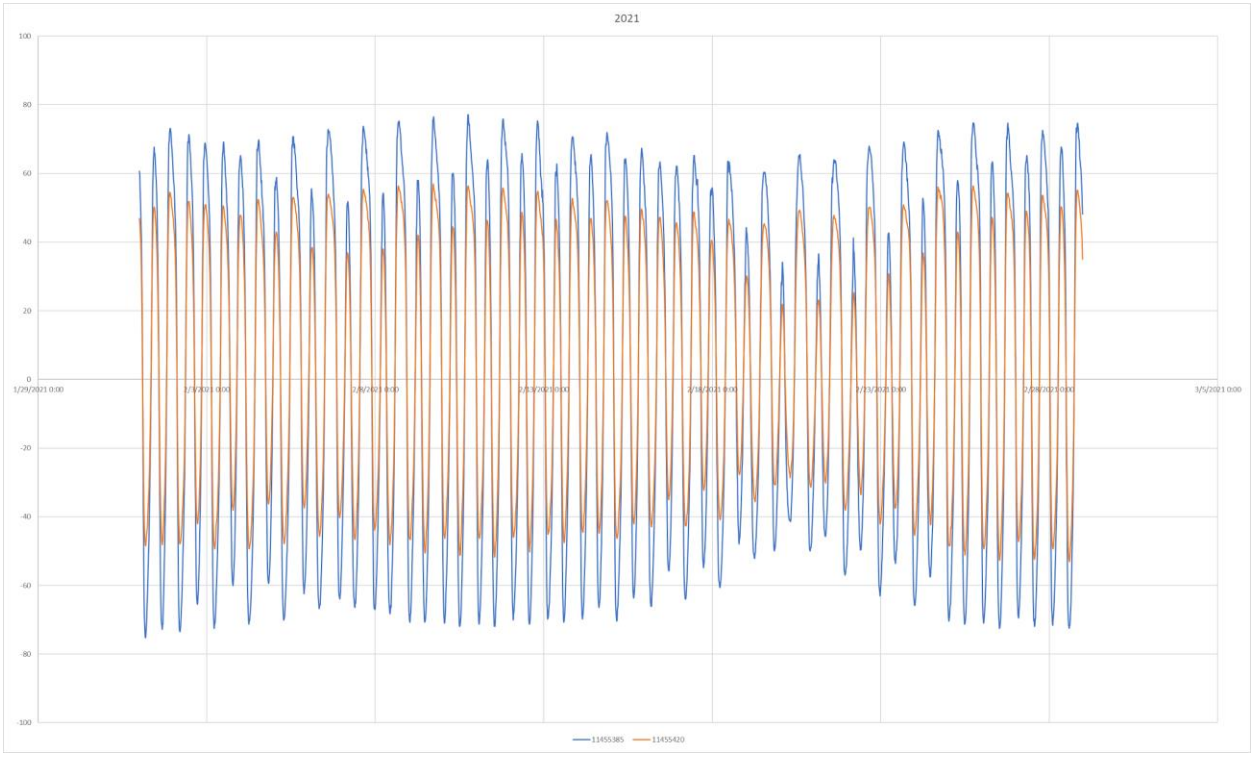

Figure 6.3: Mean flow velocity for discharge in cm/s for stations 11455385 (blue) and 11455420 (orange) for February 2021.

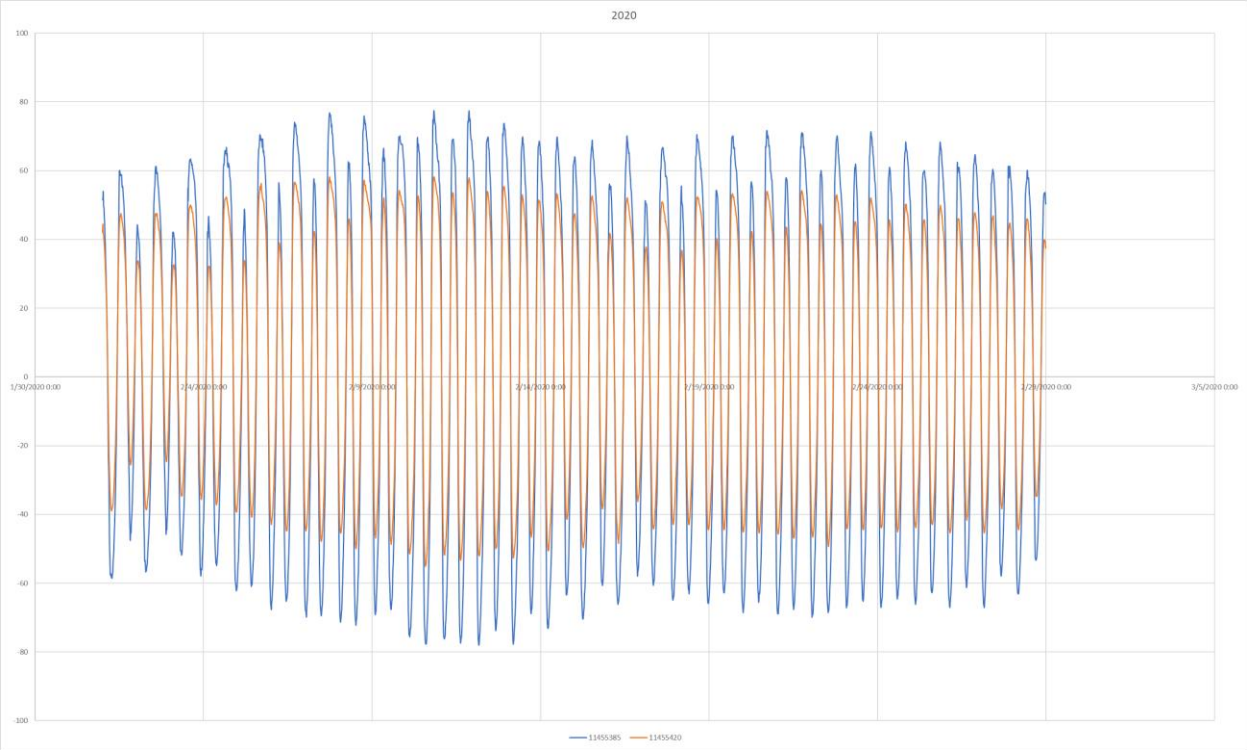

Figure 6.4: Mean flow velocity for discharge in cm/s for stations 11455385 (blue) and 11455420 (orange) for February 2022.

The values are inputted into a linear graph on excel (Figure 6.5) to determine which days would be the best to deploy the EFB. The best 4-day span to deploy the EFB should be able to be hypothesized using this information.

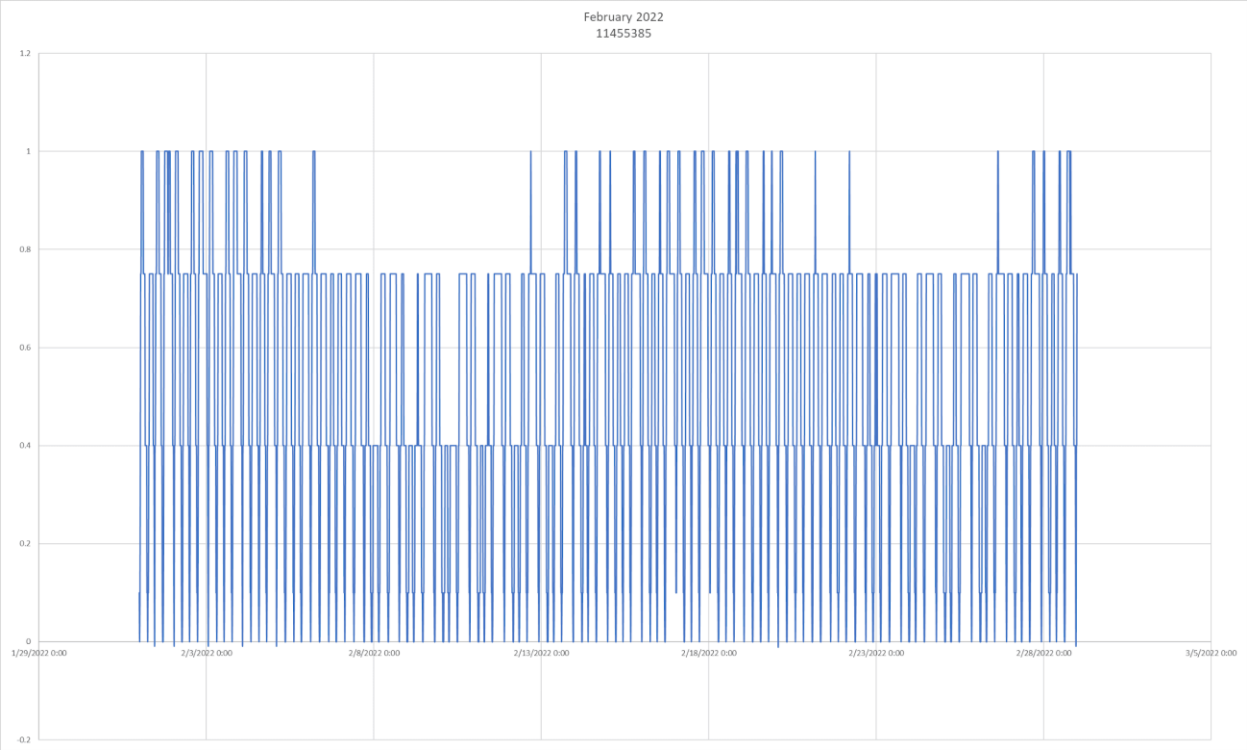

Figure 6.5: Linear graph to determine which days would be the best to deploy the egg frame box.

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Appendix

Appendix A : R Code for Analyses

Chapter 3 Code

```
#temp

#input data
TempData = read.csv("EggFrame_Trial.csv", header = T)
names(TempData)
TempData$Trial = as.factor(TempData$Trial)
TempData$Frame = as.factor(TempData$Frame)
TempData$Temp = as.factor(TempData$Temp)
summary(TempData)
str(TempData)
head(TempData)


#Summary
aggregate(Fert~Temp,TempData, FUN = function(x) c(Mean =
mean(x),min=min(x),max=max(x),N=length(x)))
aggregate(D3_Viable~Temp,TempData, FUN = function(x) c(Mean =
mean(x),min=min(x),max=max(x),N=length(x)))
aggregate(D10_Viable~Temp,TempData, FUN = function(x) c(Mean =
mean(x),min=min(x),max=max(x),N=length(x)))
aggregate(D03.Total~Temp,TempData, FUN = function(x) c(Mean =
mean(x),min=min(x),max=max(x),N=length(x)))
aggregate(D10.Total~Temp,TempData, FUN = function(x) c(Mean =
mean(x),min=min(x),max=max(x),N=length(x)))
aggregate(Survival~Temp,TempData, FUN = function(x) c(Mean =
mean(x),min=min(x),max=max(x),N=length(x)))


#Fert by temp
```

```
FertMod = aov(Fert~Temp,data=TempData)
Anova(FertMod)

# Survival By temp
FertMod2 = aov(Survival~Temp,data=TempData)
Anova(FertMod2)

# Tukey HSD test for Fert by Temp
tukey_results_Fert <- TukeyHSD(FertMod)

# View results
print(tukey_results_Fert)

# Tukey HSD test for Survival by Temp
tukey_results_Survival <- TukeyHSD(FertMod2)

# View results
print(tukey_results_Survival)

#Fert Plot
Fertplotdata = summarySE(TempData, measurevar="Fert", groupvars=c("Temp"),na.rm = T)

TempFertplot = ggplot(Fertplotdata,aes(x=Temp,y=Fert))+
  geom_bar(colour="black", fill="lightgrey", stat="identity")+
  geom_errorbar(aes(ymin=Fert-se, ymax=Fert+se), width=.1,position=position_dodge(.25)) +
  theme_classic()+ylab("Fertilization (%)")+xlab("Temperature (°C)") +
  coord_cartesian(ylim=c(0,110))+
  scale_y_continuous(expand = c(0,0),breaks=seq(0, 100, 20))+
  theme(plot.title = element_text(face="bold", hjust=-0.15))
TempFertplot

tiff("TempFertPlot.tiff",width=1200,height=1200,res=300, compression ="lzw")
grid.arrange(TempFertplot,ncol=1)
dev.off()

#Surb Plot
Survivalplotdata = summarySE(TempData, measurevar="Survival", groupvars=c("Temp"),na.rm
= T)

Survivalplot = ggplot(Survivalplotdata,aes(x=Temp,y=Survival))+
```

```
  geom_bar(colour="black", fill="lightgrey", stat="identity")+
  geom_errorbar(aes(ymin=Survival-se, ymax=Survival+se),
width=.1,position=position_dodge(.25)) +
  theme_classic()+ylab("Survival (%)")+xlab("Temperature (°C)") +
  coord_cartesian(ylim=c(0,100))+
  scale_y_continuous(expand = c(0,0),breaks=seq(0, 100, 20))+
  theme(plot.title = element_text(face="bold", hjust=-0.15))
Survivalplot
tiff("TempSurvivalPlot.tiff",width=1200,height=1200,res=300, compression ="lzw")
grid.arrange(Survivalplot,ncol=1)
dev.off()


install.packages("multcompView")
library(multcompView)


signif_letters_Fert <- data.frame(Temp = c("15.5", "16.3", "17.6", "20.2", "20.7"),
                                  Letter = c("d", "d", "d", "e", "f"))
signif_letters_Survival <- data.frame(Temp = c("15.5", "16.3", "17.6", "20.2", "20.7"),
                                      Letter = c("c", "b", "a", "c", "c"))

# Add the significance letters to your Fert plot
Survivalplot <- ggplot(Fertplotdata, aes(x=Temp, y=Survival)) +
  geom_bar(colour="black", fill="lightgrey", stat="identity") +
  geom_errorbar(aes(ymin=Survival-se, ymax=Survival+se), width=.1,
position=position_dodge(.25)) +
  geom_text(data=signif_letters_Survival, aes(label=Letter, y=100), vjust=-1) +  # Adjust y and
vjust as needed
  theme_classic() +
  ylab("Survival (%)") +
  xlab("Temperature (°C)") +
  coord_cartesian(ylim=c(0, 100)) +
  scale_y_continuous(expand = c(0, 0), breaks=seq(0, 100, 20)) +
  theme(plot.title = element_text(face="bold", hjust=-0.15))

# Add the significance letters to your Survival plot
Survivalplot <- ggplot(Survivalplotdata, aes(x=Temp, y=Survival)) +
  geom_bar(colour="black", fill="lightgrey", stat="identity") +
```

```
  geom_errorbar(aes(ymin=Survival-se, ymax=Survival+se), width=.1,
position=position_dodge(.25)) +
  geom_text(data=signif_letters_Survival, aes(label=Letter, y=100), vjust=-1) +  # Adjust y and
vjust as needed
  theme_classic() +
  ylab("Survival (%)") +
  xlab("Temperature (°C)") +
  coord_cartesian(ylim=c(0, 100)) +
  scale_y_continuous(expand = c(0, 0), breaks=seq(0, 100, 20)) +
  theme(plot.title = element_text(face="bold", hjust=-0.15))

#  significance letters to Fert plot

TempFert <- TempFert +
  geom_text(data=merge(Survivalplotdata, signif_letters_Survival, by="Temp"),
            aes(label=Letter, y=Survival+se), vjust=-1)
TempFert <- TempFert +
  theme(
    axis.title = element_text(size = 16),
    axis.text = element_text(size = 14),
    plot.title = element_text(size = 18)
  )

# Fert plot

Print(TempFertplot)



# significance letters to Survival plot
Survivalplot <- Survivalplot +
  geom_text(data=merge(Survivalplotdata, signif_letters_Survival, by="Temp"),
            aes(label=Letter, y=Survival+se), vjust=-1)
Survivalplot <- Survivalplot +
  theme(
    axis.title = element_text(size = 16),
    axis.text = element_text(size = 14),
    plot.title = element_text(size = 18)
  )
```

```
# Survival plot
print(Survivalplot)
```

Chapter 4 Code

```
setwd("C:/Users/brand/OneDrive/Desktop/egg frame")

#input data
substratetest = read.csv("chapter5stat.csv", header = T)
names(substratetest)

# ANOVA model

#Treatment Factor
substratetest$Treatment <- as.factor(substratetest$Treatment)
anova_model <- aov(Fertilization.rate ~ Treatment, data = substratetest)

# substrate fertilization test D1
tukey_test_fert <- TukeyHSD(anova_model)

#placement D10 test
substratetest$Placement <-as.factor(substratetest$Placement)
anova_model_D10placement <- aov(D10_Survival ~ Placement, data = substratetest)
tukey_test_D10placement <- TukeyHSD(anova_model_D10placement)


# Tukey test results
print(tukey_test_fert)

# substrate D15 survival test
anova_model_D15 <- aov(D15_Survival ~ Treatment, data = substratetest)

tukey_test_D15 <- TukeyHSD(anova_model_D15)

# substrate D10 survival test
anova_model_D10 <- aov(D10_Survival ~ Treatment, data = substratetest)
```

```
tukey_test_D10 <- TukeyHSD(anova_model_D10)

# Tukey Test results
print(tukey_test_D10)

# fertilization placement test day 1
substratetest$Placement <- as.factor(substratetest$Placement)

anova_model_placement <- aov(Fertilization.rate ~ Placement, data = substratetest)

tukey_test_placement <- TukeyHSD(anova_model_placement)
print(tukey_test_placement)

library(dplyr)

# Finding average and standard deviation
summary_table <- substratetest %>%
  group_by(Treatment) %>%
  summarise(
    Avg_D1_Viable = mean(D1_Viable, na.rm = TRUE),
    SE_D1_Viable = sd(D1_Viable, na.rm = TRUE) / sqrt(n()),
    Avg_D1_Unviable = mean(D1_Unviable, na.rm = TRUE),
    SE_D1_Unviable = sd(D1_Unviable, na.rm = TRUE) / sqrt(n()),
    Avg_D5_Viable = mean(D5_Viable, na.rm = TRUE),
    SE_D5_Viable = sd(D5_Viable, na.rm = TRUE) / sqrt(n()),
    Avg_D5_Unviable = mean(D5_Unviable, na.rm = TRUE),
    SE_D5_Unviable = sd(D5_Unviable, na.rm = TRUE) / sqrt(n()),
    Avg_D10_Viable = mean(D10_Viable, na.rm = TRUE),
    SE_D10_Viable = sd(D10_Viable, na.rm = TRUE) / sqrt(n()),
    Avg_D10_Unviable = mean(D10_Unviable, na.rm = TRUE),
    SE_D10_Unviable = sd(D10_Unviable, na.rm = TRUE) / sqrt(n()),
    Avg_D15_Viable = mean(D15_Viable, na.rm = TRUE),
    SE_D15_Viable = sd(D15_Viable, na.rm = TRUE) / sqrt(n()),
    Avg_D15_Unviable = mean(D15_Unviable, na.rm = TRUE),
    SE_D15_Unviable = sd(D15_Unviable, na.rm = TRUE) / sqrt(n())
  )

# Print summary table
print(summary_table)
```

```
# Check for normality
shapiro.test(residuals(anova_model))

#Check for variance
shapiro.test(residuals(anova_model_D10))
bartlett.test(Fertilization.rate ~ Treatment, data = substratetest)


hist(residuals(anova_model_D10))
qqnorm(residuals(anova_model_D10))
qqline(residuals(anova_model_D10))


kruskal.test(D10_Survival ~ Treatment, data = substratetest)
install.packages(dunn.test)
dunn.test(substratetest$D10_Survival, substratetest$Treatment, method = "bonferroni")

kruskal.test(Fertilization.rate ~ Treatment, data = substratetest)
library(dunn.test)
dunn.test(substratetest$Fertilization.rate, substratetest$Treatment, method = "bonferroni")

hist(residuals(anova_model))
qqnorm(residuals(anova_model))
qqline(residuals(anova_model))

anova_model_log <- aov(log_fertilization_rate ~ Treatment, data = substratetest)
summary(anova_model_log)

hist(residuals(anova_model_log))
qqnorm(residuals(anova_model_log))
qqline(residuals(anova_model_log))

kruskal.test(Fertilization.rate ~ Treatment, data = substratetest)
```

Chapter 5 Code

```
#Humidity3H

#input data
Humid3Data = read.csv("humidity3H.csv", header = T)
names(Humid3Data)

#Tukey Test

#convert humidity to factor variable
Hatch3Mod <- aov(Hatching.rate ~ Humidity, data = Humid3Data)
summary(Hatch3Mod)

# shapiro wilk test
shapiro.test(residuals(Hatch3Mod))

# Bartlett Test for Homogeneity of Variances
bartlett_test <- bartlett.test(Hatching.rate ~ Humidity, data = Humid3Data)
print(bartlett_test)

hist(residuals(Hatch3Mod))
qqnorm(residuals(Hatch3Mod))
qqline(residuals(Hatch3Mod))

#Kruskal-Wallis test
kruskal.test(Hatching.rate ~ Humidity, data = Humid3Data)


dunn_3h <- dunn.test(Humid3Data$Hatching.rate, g = Humid3Data$Humidity, method =
"bonferroni")
print(dunn_3h)

#tukey
TukeyTest <- TukeyHSD(Hatch3Mod)
print(TukeyTest)


# Create lettering for figure
text_data <- data.frame(
  x = c(1, 2, 3, 4, 5),
  y = c(0.95, 0.95, 0.95, 0.95, 0.95),
```

```
  label = c("a", "b", "c", "a", "a")
)

# Create the box and whiskers plot
ggplot(data = Humid3Data, aes(x = Humidity, y = Hatching.rate)) +
  geom_boxplot(colour="black", fill="lightgrey") +
  theme_classic() +
  ylab("Hatching Rate (%)") +
  xlab("Humidity Levels (%)") +
  coord_cartesian(ylim=c(0,1)) +
  scale_y_continuous(expand = c(0,0), breaks=seq(0, 1, 0.2), labels = scales::percent(seq(0, 1,
0.2))) +
  theme(plot.title = element_text(face="bold", hjust=-0.15)) +
  geom_text(data = text_data, aes(x = x, y = y, label = label))


#Humidity5H

#input data
Humid5Data = read.csv("humidity5H.csv", header = T)
names(Humid5Data)

#Tukey Test

#convert humidity to factor variable
Hatch5Mod <- aov(Hatching.rate ~ Humidity, data = Humid5Data)
summary(Hatch5Mod)

TukeyTest <- TukeyHSD(Hatch5Mod)
print(TukeyTest)

# shapiro wilk test
shapiro.test(residuals(Hatch5Mod))

# Bartlett Test for Homogeneity of Variances
bartlett_test <- bartlett.test(Hatching.rate ~ Humidity, data = Humid5Data)
print(bartlett_test)

hist(residuals(Hatch5Mod))
qqnorm(residuals(Hatch5Mod))
```

```
qqline(residuals(Hatch5Mod))

#Kruskal-Wallis test
kruskal.test(Hatching.rate ~ Humidity, data = Humid5Data)


dunn_5h <- dunn.test(Humid5Data$Hatching.rate, g = Humid5Data$Humidity, method =
"bonferroni")
print(dunn_5h)



#box and whiskers plot
ggplot(data = Humid5Data, aes(x = Humidity, y = Hatching.rate)) +
  geom_boxplot(colour="black", fill="lightgrey") +
  theme_classic() +
  ylab("Hatching Rate (%)") +
  xlab("Humidity Levels (%)") +
  coord_cartesian(ylim=c(0,1)) +
  scale_y_continuous(expand = c(0,0), breaks=seq(0, 1, 0.2), labels = scales::percent(seq(0, 1,
0.2))) +
  theme(plot.title = element_text(face="bold", hjust=-0.15)) +
  geom_text(aes(x = c(1, 2, 3, 4, 5), y = c(0.95, 0.95, 0.95, 0.95, 0.95), label = c("a", "b", "c", "d",
"a")))

# Create a data frame for the annotations
annotation_data <- data.frame(
  Humidity = c("100", "80", "60", "40", "control"),
  y = c(0.95, 0.95, 0.95, 0.95, 0.95),
  label = c("a", "b", "c", "d", "a")
)

# Create the plot
ggplot(data = Humid5Data, aes(x = Humidity, y = Hatching.rate)) +
  geom_boxplot(colour="black", fill="lightgrey") +
  theme_classic() +
  ylab("Hatching Rate (%)") +
  xlab("Humidity Levels (%)") +
  coord_cartesian(ylim=c(0,1)) +
```

```
  scale_y_continuous(expand = c(0,0), breaks=seq(0, 1, 0.2), labels = scales::percent(seq(0, 1,
0.2))) +
  theme(plot.title = element_text(face="bold", hjust=-0.15)) +
  geom_text(data = annotation_data, aes(x = Humidity, y = y, label = label))
```