Sidney Alexandre Poidevin

Improving commercial protocols of Russian sturgeon at larval & fingerling life stages

Measuring the effects of weaning on the survival of Russian sturgeon larvae using live feed & measuring the effects of increasing stocking densities on the growth and survival of Russian sturgeon fry

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Declaração de autoria

Declaro ser a autor/a deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Acknowledgments

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Abstract

The present study was performed to improve protocols for the commercial production of Russian sturgeon at the larval and fingerling life stages. Two trials were undertaken, the first trial ran for 35 days and focused on reducing mortality rates at the larval stage by implementing a co-feeding strategy using live artemia cultures. 3 different dietary regimes were tested, G1: Mixed Diet (Inert + Artemia), G2: Inert Diet (Control), & G3 Live Diet (Artemia). The dietary regimes containing live feeds underwent an 11-day weaning period before being replaced completely with commercial inert feeds. Mortality was significantly lower for G1 (Mixed Diet) when compared to the other treatment groups. The mortality rates of G2 were lower than the mortality rates produced by G3, but the differences were not significant. The study revealed a favorable effect of the mixed diet of G1, containing live and inert food on the survival of Russian sturgeon larvae. The production of live food is expensive and laborious, but the results of this trial ensure that it is not necessary for the entire larval stage. The second trial also ran for 35 days and focused on maximizing available space and water at the fingerling life stage by testing the effects of increasing stocking densities on survival and growth. 3 treatment groups were formed containing a different population density, ranging from low to high (Low: 800 ind., Medium: 1,200 ind., High: 1,600 ind.). The results of this trial revealed no significant differences in average body weight, FCR, SGR or weight gain. The biomass density in the high animal density treatment group reached a concentration of 18 kg.m^{-3}, with no negative effects on growth or survival. The results of this trial indicate that the threshold of biomass concentrations at Caviar de Neuvic can be increased without loss of performance.

Keywords: Russian sturgeon, weaning, diet, larviculture, stocking density, fingerling

Resumo

Os esturjões são uma ordem primitiva de peixes que existe há mais de 150 milhões de anos. No entanto, devido à sobrepesca e às pressões ambientais como a poluição da água e a construção de barragens, as populações mundiais de esturjões sofreram um rápido declínio no último século. Consequentemente, várias espécies de esturjão tornaram-se interessantes para o sector da aquacultura, inicialmente com a intenção de reconstituir as populações selvagens. Recentemente, registaram-se vários avanços na tecnologia de incubação, embriologia e reprodução neste grupo de animais. No entanto, devido à crescente procura mundial de caviar e ao aumento exponencial da população humana, o sector da aquacultura do esturjão começou a investir na produção direta de caviar, encerrando o ciclo de vida de várias espécies de esturjão em cativeiro. Atualmente, a cultura do esturjão e a produção de caviar encontram-se em vários países dos continentes da América do Norte, Ásia e Europa. Embora tenham sido feitos muitos avanços e a produção comercial tenha provado ser viável, existem ainda muitos obstáculos à produção que diminuem a rentabilidade de algumas espécies seleccionadas. No caso do esturjão russo, que é famoso pelo seu premiado caviar "Ossetra", o mais importante é a dependência de alimentação viva durante a fase larvar e os longos períodos de cultura necessários para produzir caviar.

No presente trabalho, realizado em colaboração com a empresa Caviar de Neuvic e o Grupo de investigação em Aquacultura do CCMAR, explorámos potenciais soluções para melhorar os protocolos de produção comercial do esturjão-russo através de dois ensaios realizados durante as fases larvar e de alevim. Os investigadores na área da aquacultura têm sido bem sucedidos no desmame de algumas espécies de esturjão para alimentos inertes comerciais, mantendo baixas taxas de mortalidade. Isto continua a ser problemático para o esturjão-russo, uma vez que as taxas de mortalidade são ainda bastante elevadas quando se utilizam apenas alimentos inertes no regime alimentar durante a fase larvar. O primeiro ensaio deste estudo teve como objetivo reduzir as taxas de mortalidade através do desenvolvimento de uma estratégia de desmame misturando alimentos inertes e vivos no início da alimentação exógena, com a intenção de minimizar a dependência de alimentos vivos nesta espécie. Este regime alimentar reduziu significativamente a quantidade de alimento vivo que é normalmente utilizado numa maternidade de esturjão-russo, com a expetativa de manter uma mortalidade mínima. Uma população de 33.000 larvas foi dividida em 3 grupos experimentais (G1, G2, & G3), cada um com 2 réplicas. O ensaio decorreu durante 35 dias, tendo cada grupo recebido um regime alimentar diferente, com os grupos experimentais G1 & G3 a ser alimentados com culturas de Artémia durante os primeiros 11 dias de alimentação exógena. Durante o período experimental, a alimentação do G1 consistiu numa dieta mista (alimentos vivos e inertes), enquanto o regime alimentar do G3 apenas continha alimento vivo. O G2 serviu de controlo, com as larvas a serem alimentadas apenas com alimento inerte durante todo o ensaio. No final da experiência, a mortalidade foi significativamente mais baixa nas lavras do grupo G1, quando comparada com os outros regimes de alimentação. As taxas de mortalidade registadas no grupo G2 foram inferiores às taxas de mortalidade no grupo G3, no entanto esta diferença não foi significativa. Todos os regimes alimentares registaram um pico de mortalidade diária no 16º dia pós-eclosão (dph), tendo o G3 apresentado o pico de mortalidade mais elevado, com 14,68% num só dia. O estudo revelou um efeito positivo da dieta mista do G1, contendo alimentos vivos e inertes, na sobrevivência das larvas de esturjão-russo. A produção de alimento vivo numa maternidade é dispendiosa e trabalhosa, mas os resultados aqui apresentados demonstram que não é necessária durante toda a fase larvar. A aplicação de um curto período de co-alimentação revelou taxas de mortalidade semelhantes às observadas quando se utiliza alimento vivo durante toda a fase larvar.

O segundo objetivo desta tese foi o de melhorar os protocolos comerciais desta espécie na fase de alevim, testando os efeitos do aumento da densidade de cultivo na sobrevivência e no crescimento. Existe ainda pouca investigação sobre os efeitos desta variável, no entanto o aumento da densidade de cultivo permite aos Aquacultores maximizar o espaço e a água disponíveis, reduzindo os custos de produção. No início da experiência, a população de alevins de esturjão-russo foi dividida aleatoriamente em 3 grupos, que foram transferidos para 8 tanques de 3,7 m^3, para um período experimental de 35 dias. Cada grupo de tratamento estava a uma densidade de cultivo diferente, variando de baixa a alta (Baixa: 800 ind., Média: 1.200 ind., Alta: 1.600 ind.). Os tanques estavam inseridos num sistema semi-aberto, sendo todos os parâmetros de qualidade da água monitorizados diariamente. O parâmetro de maior preocupação durante este ensaio foi o oxigénio dissolvido (OD), uma vez que o consumo aumentou exponencialmente com o aumento das concentrações de biomassa. Os pesos médios dos alevins foram registados semanalmente (n=50), tendo sido calculadas as taxas de crescimento específico (SGR), de conversão alimentar (FCR) e o ganho de peso para cada tratamento. Os resultados deste ensaio não revelaram diferenças significativas no peso corporal médio, FCR, SGR ou ganho de peso. No final do ensaio, a densidade de biomassa no grupo de alta densidade atingiu um valor de 18 $kg.m^{-3}$, sem efeitos negativos no crescimento ou na sobrevivência. Segundo os protocolos anteriores utilizados na empresa Caviar de Neuvic, as concentrações de biomassa nunca deveriam ultrapassar os 12 $kg.m^{-3}$ para garantir um modelo de crescimento adequado. No entanto, os resultados deste ensaio indicam que o limite da concentração de biomassa pode ser aumentado sem perda de desempenho. Assumindo que os parâmetros de cultivo (DO, pH, temperatura, nitrito, nitrato e amónio) são adequados, a produção de alevins com concentrações de biomassa de 16 $kg.m^{-3}$ pode produzir com sucesso um crescimento e uma sobrevivência adequados. O diâmetro da ração é também um fator importante, uma vez que as rações com um diâmetro demasiado grande podem causar reduções na dispersão, induzindo uma distribuição desigual da ração entre os indivíduos. O aumento da variabilidade de tamanho entre indivíduos pode ter efeitos negativos nas taxas de crescimento médio de uma população. Tendo em conta estes fatores, o aumento do limite de biomassa em 6 $kg.m^{-3}$ para cada tanque pode aumentar os lucros da produção, através da maximização da água e do espaço disponíveis, e deverá compensar os custos acrescidos decorrentes do aumento do consumo de oxigénio. Continua a ser possível aumentar este limiar, uma vez que nunca foram registadas reduções do crescimento durante este ensaio.

Keywords: Russian sturgeon, weaning, diet, larviculture, stocking density, juvenile

1. State of the Art

Sturgeons from the order *Acipenseriformes* are one of the most ancient living vertebrates that still exist today. Researchers have discovered fossil records that date back to over 150 million years (Tzankova, 2007). Currently, there are 26 known species of sturgeon, all of which can be found in the temperate waters of the northern hemisphere, specifically in the continents of North America, Asia, and Europe (Birstein, 1993). However, due to a multitude of obstacles mainly caused by human intervention, global populations of all sturgeon species have experienced a rapid decline in the last century. Environmental pressures such as water pollution, the construction of dams, and irrigation systems have resulted in the loss of spawning grounds and have impeded the migration patterns of wild sturgeon (Gisbert & Williot, 2002). Most notably, however, the overall decline of sturgeon can be accredited to overfishing, due to the high demand for caviar, which remains as one of the most expensive fishery products today (Tzankova, 2007).

The depletion of wild stocks has motivated an interest in aquaculture of this species, with the initial desired outcome being the restoration of the wild populations, while also providing a sustainable model for caviar production (Kim et al., 2019). This was typically done through the production of larvae and fry, which would then be released back into the wild. However, with the increase of human population (UN, 2019), and it's rising reliance on aquatic products generated through aquaculture, the commercial sturgeon sector began pursuing direct caviar production via completing life cycles in captive conditions, rather than fishing wild sturgeon carrying eggs. The first successful completion of a life cycle was achieved using the white sturgeon in the early 1980s by Dr. Serge Doroshov (Doroshov, 1985). This development led to the completion of life cycles of several other sturgeon species such as the Amur, Stellate, Siberian, Russian, and Beluga sturgeon, thus enabling the commercial expansion of sturgeon aquaculture worldwide.

There are six species currently farmed for their caviar, including white, Siberian, stellate, Russian, Adriatic and the beluga, which generates the highest valued caviar. The countries that are responsible for the world's supply of farmed caviar include France, USA, Italy, Spain, Germany, Austria, Russia, China, Iran, and Uruguay (Williot et al., 2005; Wei et al., 2004; Arnd et al., 2002;

Raymakers, 2002; Ivakhnenko, 2001; Williot et al., 2001), with France being the largest consumer of caviar in the E.U. and USA, China, and Russia, being the largest outside of the E.U. (EUMOFA, 2021). The country with the greatest caviar production worldwide is China, where they account for 60% of the global output (Bondarchuk, 2017).

While many advances in sturgeon aquaculture have been accomplished, such as improved hatchery techniques, reproduction, and embryology, there are still many issues within the protocols of sturgeon production (Doroshov, 1985). Firstly, while caviar is a very expensive delicacy, profit margins are still quite low due to the long culture period compared to other teleost species produced in aquaculture (Wei, et al., 2011). It takes roughly 6-8 years for white sturgeon to reach maturity, for example, with some other species taking even longer. Taking this into consideration, finding new ways to lower production costs, as well as increasing survival are crucial for the future of this sector. There is also the need for research towards adequate feed formulation. Most feeds that are used in sturgeon cultures are typically the same that are used for trout (Lu & Rasco, 2013). While some nutritional requirements are known, such as the need for fishmeal and fish oil, a specialized feed for sturgeon has yet to be globally developed (Bondarchuk, 2017). Many different dietary regimes have been tested at the larval stages, even using live feeds as a mean of stimulating increased growth and survival potential. However, very few have been successful in producing significant results or were simply not economically feasible to perform at a commercial level. The use of live feed during the larval stage is not ideal from a commercial standpoint, due to the operation costs of maintaining live feed cultures. It is also important to point out that future protocols need to be specific in regard to its respective species, since optimal rearing parameters differ between species, according to their physiological differences and ecological demands. For example, studies have shown that artificial diets can be successfully implemented at the larval stage during the onset of exogenous feeding, with species such as the White and Siberian sturgeon producing very low mortality rates. However, with the Russian sturgeon this is not the case (Gisbert & Williot, 1997), as mortality rates are significantly lower when using live feeds during the onset of exogenous feeding, when compared to artificial feeds. It appears that a significant proportion of the population refuses to consume inert feed, resulting in starvation and ultimately high mortality in many of these cultures, whereas with live feed most of the population will engage in feeding. The reasoning for this trend is still unknown, but it is theorized to be a result of heterogeneity or egg batch quality, due to varying results from different batches of eggs (Gisbert et al., 2018).

One of the most used live feeds in aquaculture is *Artemia naupli*i, and while they have a high protein content, nutritionally they are insufficient in key essential fatty acids, such as eicosapentaenoic acid (EPA) and docohexaenoic acid (DHA) which are needed for adequate growth and development. The various artificial feeds used in many sturgeon cultures typically contain these essential fatty acids (Hanaee et al., 2005; Morais et al., 2007). In many other fish species, the activity of digestive enzymes is a crucial indicator of development and survival (Zambonino & Cahu, 2001), and these essential fatty acids are key in enhancing the performance of these enzymes. In a recent study conducted by Kamaszewski et al., (2014), a trial was conducted comparing the effects of diets containing artemia enriched with DHA & EPA against non-enriched artemia. The results found that the enriched diet exhibited an improved development of the digestive system, specifically the digestive tract, which in turn promotes a better capability to catabolize nutrients. However, there were no significant differences in survival. Taking this into consideration, it is also important to note that previous studies have shown that an insufficient consumption of essential fatty acids in sturgeon species, even though not detrimental to larval survival, can lead to morphological deformities in later stages of life. Thus, it is still important to maintain adequate essential fatty acids concentrations when formulating a dietary regime for these species (Gisbert & Williot, 2002).

The constant use of live feed through larval stages, especially when enrichment of essential fatty acids is required, is not economically practical. Minimizing the use of such live feed while transitioning to an inert feed as a weaning attempt, could be. Weaning is typically defined as the gradual transition from one type of feed to another. In aquaculture, weaning is usually performed during the larval stages, with attempts to replace a species reliance on live feed to inert feed (Gisbert et al., 2018). This weaning strategy has been performed with success on other sturgeon species such as the Siberian sturgeon. While larviculture for Siberian sturgeon has been reported to be considerably more flexible than Russian sturgeon (Gisbert et al., 2018), it is possible that weaning maybe as well effective with Russian sturgeon larvae. In an effort to reduce mortality rates of Russian sturgeon larvae during their transition to exogenous feeding, while also maintaining an optimal growth, the first aim of this project was to design an adequate weaning regime, using artemia nauplii and inert feed, and test it against the previously used inert feeding regimes in Caviar de Neuvic.

Due to the extensively long culture periods of the Russian sturgeon, little research has been conducted at the post-larval stages. As previously mentioned, before the emergence of commercial sturgeon aquaculture, most cultures were only performed at the reproductive and hatching stages, with the objective of releasing larvae or fry in to wild in an effort to restore wild populations. Research regarding optimal rearing strategies for juveniles of sturgeon species has only begun to gain traction within the last 20 years. Basic parameters such as differing temperatures, stocking densities, enclosure size/enrichment and oxygen concentrations, have been tested with the intentions of perfecting rearing protocols with higher success. For example, recent studies have found that increasing stocking density of Chinese amur sturgeon juveniles (18-20 g body weight), can decrease growth rates but do not significantly affect survival (Yang et al., 2011). High stocking densities from a commercial standpoint are ideal, because they maximize the use of available water and space for production. However, if the threshold for the target species is surpassed, it may cause health problems, reductions in growth and performance, and ultimately lower quality in the final product (Fagerlund et al., 1981; Gibtan et al., 2008). Regarding Russian sturgeon, this basic parameter at this specific life stage has yet to be explored. Experiments with juvenile Russian sturgeon at much larger body sizes and scales have been performed, where juveniles were reared in cages placed in a large lake. The results from these trials indicate that stocking densities at this scale do not significantly affect growth or mortality (Celikkale et al., 2005). In an effort to find a suitable stocking density for post-larval staged juveniles, surpassing 12 grams in wet body weight, the second aim of this project was to test the effects of increasing population densities, on growth and survival of juvenile Russian sturgeon.

2. Objectives of the work

The primary objective of this project was to improve commercial protocols for Russian sturgeon production, what was achieved within two different trials. Trial 1 focused on decreasing mortality rates of Russian sturgeon larvae that commonly occur during this critical period, while also maintaining optimal growth rates. This was done through incorporating a short timeframe into the dietary regime to which live feed was provided during the onset of exogenous feeding.

Trial 2 focused on the parameter of stocking density. The expectation was to find an optimal stocking density that can increase biomass production while maintaining an optimal growth model. These potential improvements to future protocols for this species could provide higher yields for the various consumer products that sturgeon provides, such as meat and caviar at a lower production cost.

3. Methodology

3.1 Broodstocks, Gamete Quality, & Incubation

The present work was developed on January 2023 using existing broodstocks produced at the company Caviar de Neuvic. Prior to reproduction, the female individuals underwent biopsies in which oocytes were collected, placed in vitro containing a progesterone-based solution and monitored until the maturation process was complete (Figure 3.1 C). Then, 3 mature females were transferred to a basin allocated for reproduction. A total of 10 mature males were selected based on kin to prevent inbreeding and were stripped for sperm collection. Samples of sperm from each male underwent motility analysis to determine gamete quality. These tests were carried out manually using a microscope. Small samples of sperm were mixed with freshwater in a slide to activate dormant sperm cells, and quality was determined by visually recording the proportion of sperm cells within the population that exhibited high motility *vs* the proportion that did not. The four sperm samples that exhibited the highest motility rates were selected for reproduction. The 3 mature females were then placed in anesthesia (clove oil in freshwater (0.1ml L^{-1}) for the collection of eggs. Once anesthetized, unfertilized eggs were harvested, washed to remove blood, urine as well as any other potential metabolites, and weighed to determine clutch size.

Figure 3.1: Female broodstocks in concrete pond (A), Ultrasound via ethograph on female broodstock to identify egg quantity (B), and Oocytes in vitro containing a progesterone-based solution (C)

The eggs were then fertilized by mixing freshwater, sperm, and eggs altogether. Once fertilization was complete, the eggs were treated with tannin to prevent the adhesive zona radiata externa of the eggs from sticking to one another during incubation. For incubation, the fertilized eggs were transferred into Mcdonald Jars equipped with an upwelling flow (Figure 3.2 C), to ensure that the eggs remained in suspension. The eggs remained in incubation for 5 days, maintained at a temperature of 15.5-16° C to ensure adequate development and eliminate the potential of fungal growth. Once hatching occurred, the larvae were allocated in increments of 100 into 7 different troughs ranging from 200-300L maintaining a stocking density of 22 ind L^{-1} A population of 40,000 larvae were sampled for the upcoming trials (**1 & 2**), which were overseen and conducted at the research facilities of Caviar de Neuvic, during the months of January-June 2023.

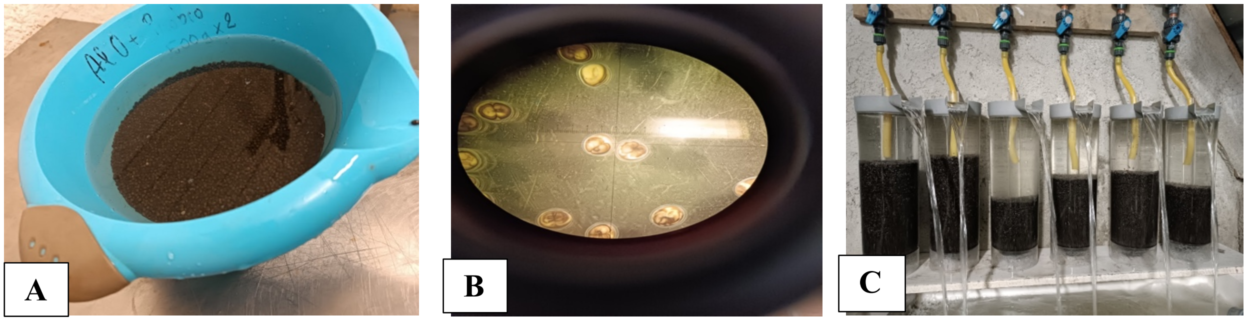


Figure 3.2: Harvested eggs undergoing insemination via mixing of freshwater and sperm (A), Fertilized eggs undergoing first discoidal cleavage (B), Fertilized eggs in incubation using Mcdonald Jars (C)

Trial 1: Measuring the effects of weaning on survival of Russian sturgeon larvae using live feed

3.2 Culture Parameters for Larvae & Experimental Setup

A population 40,000 larvae were divided and allocated into 7 troughs ranging from 200-300L in volume. Each trough (with the exception of 1, 200L), contained a stocking density of 22 ind L^{-1}, and was provided with freshwater from the Isle River in Dordogne, France. Temperature conditions were maintained at 18 ±1 °C, Oxygen concentration at 9 ±1 mg L^{-1} and pH at 7.8 ±0.03. These conditions were monitored daily using “Aqualarme” software by Teraqua and water analyses were performed via photometry whenever needed. The troughs were inserted in a semi-open recirculating aquaculture system, equipped with UV, particulate, and bio-media filtration, as well as automated water renewal to ensure optimal water quality. The flow rate of each trough was set at 8L/min to ensure adequate water renewal and optimal O_2 concentrations within each trough. Larvae were initially exposed to a 24h darkness photoperiod, for the first 3 days after hatching, to prevent stereotypic behavior, and afterwards to a 12:12 h photoperiod using fluorescent lights until the end of the trial. In this first trial, 3 experimental groups were assembled, each having 2 replicates. Each group was fed a different dietary regime, with the primary experimental group's (G1 & G3) regime using live feed (*Artemia*) cultures. These *Artemia* cultures were treated with inert feed in powdered form. This was done as an attempt to correlate the smell and taste of the artemia to the inert feed throughout the weaning process. However, the larvae in G1 were provided with a mixed diet comprised of artemia and inert feed directly upon the onset of exogenous feeding (9 dph), while the inert feed for G3 was implemented after the critical period (15 dph). The other experimental group (G2) served as a control and was only provided with inert feed throughout the whole trial. The final 200L trough was stocked with 7,000 larvae (35 ind L^{-1}) and was fed only "O.range Wean S" inert feed from INVE aquaculture, that contains a composition primarily made of rotifers and *Artemia nauplii*.

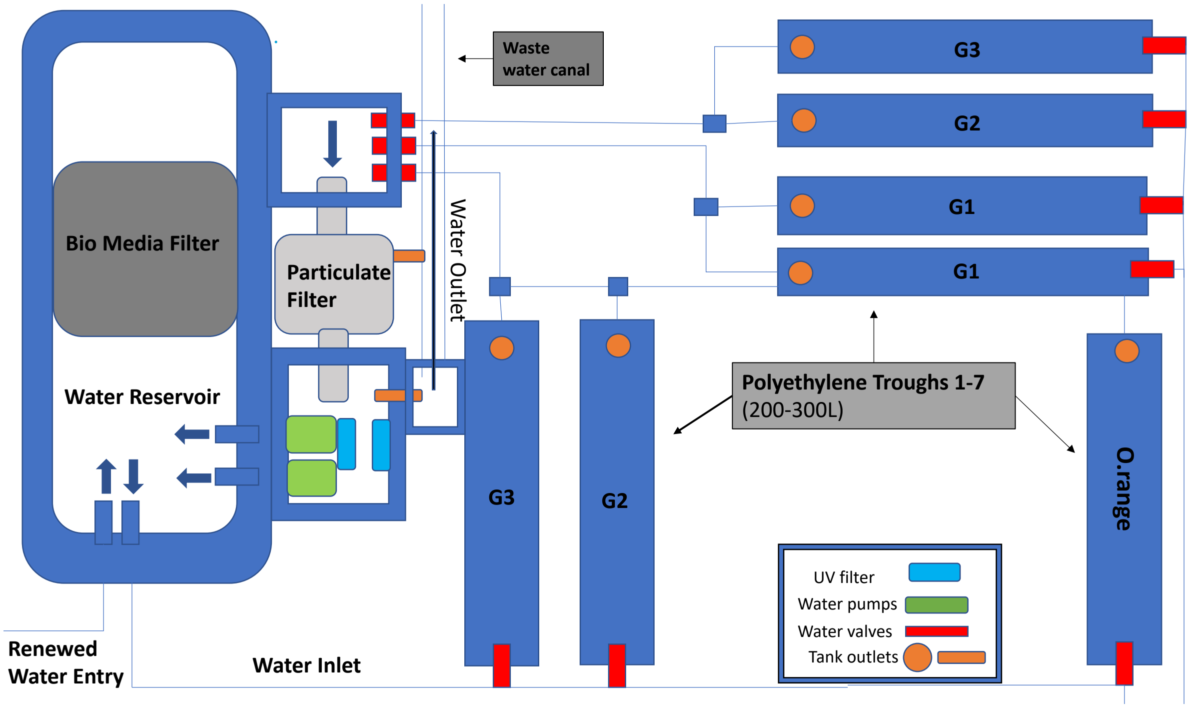


Figure 3.3: Water circuit & trough layout for hatchery with treatment group allocation for G1, G2, G3, & O.range

Figure 3.4: An assembled water circuit containing 7 troughs (200-300L) equipped with a reservoir, aquatic heaters, UV, particulate, & bio media filtration

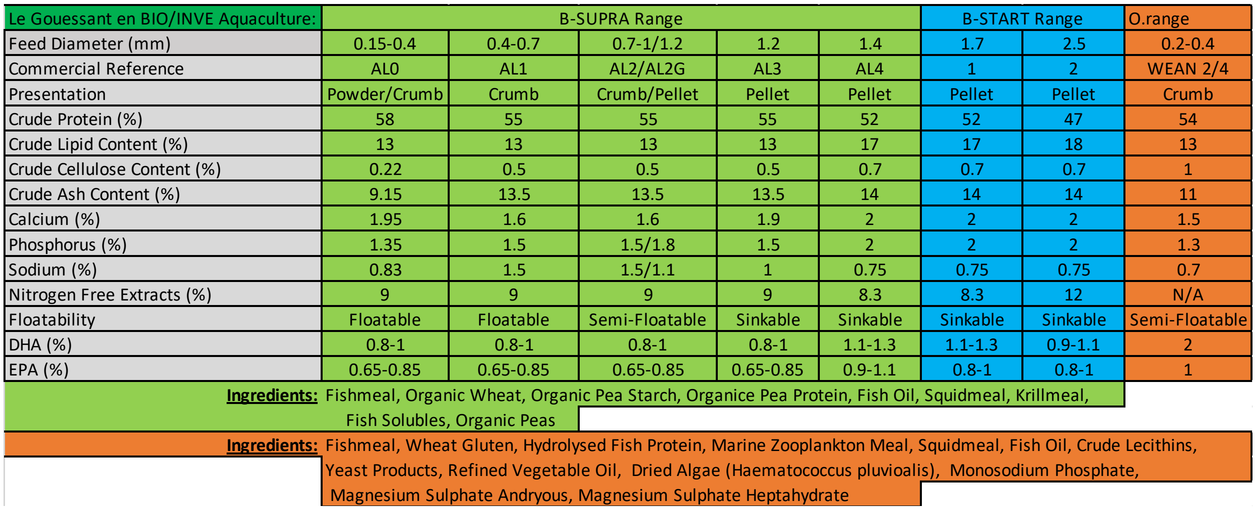
3.3 Dietary Regimes

Russian Sturgeon is a lecithotrophic species in which larvae initiate the exogenous feeding stage between 6-10 days post hatching (dph). Endogenous feeding in this species can be identified through their behavioral patterns. Initially, and until their yolk reserves are depleted, larvae present active schooling behavior. Afterwards, schooling behavior disappears along with the extrusion of the melanin plug, and larvae will begin to swim actively, typically around the bottom of the tank as well as on tank walls. This change in behavior indicates that the larvae are now ready to start exogenous feeding. In the present study, these indicators were clear by 9 dph. Larvae in G1& G3 underwent an 11-day feeding period where they were fed *Artemia* daily, at varying concentrations in a bell curve formation, with a concentration peak in the middle of the critical period (12 dph), allowing a gradual removal of artemia from the feeding regime *(Table 4.2 & 4.4)*. The underlying difference between the two groups is that G1's feeding regime was accompanied with a mixture of B-Supra AL1 inert feed by Gouessant BIO (50%) and O.range Wean S inert feed (50%) by INVE aquaculture for the first 11 days of the exogenous feeding period, while G3's was not. At this stage, larvae were fed artemia nauplii four times a day (9:30, 11:30, 13:30, & 15:30), to maximize their chance of consumption during each feeding period, given that artemia have roughly a 1-hour survival period in freshwater. The feeding regime was planned to debut at a concentration of 1,000 ind/larvae, with a peak concentration of 2,200 ind/larvae by day 4 post-endogenous feeding (pef) and gradually decrease throughout the course of the trial. However, due to a lack of adequate materials to carry out optimal artemia production, hatching rates were considerably low (36%) compared to standard average of 80%, resulting in lower artemia availability. The produced feeding regime in turn formed a bimodal curve formation with 2 peaks on days 4 and 9 pef (320 ind/larvae) (373 ind/larvae), an initial concentration of 72 ind/larvae and, an exponential reduction in daily concentrations after day 9 pef for the remainder of the trial period.

After 7 days of exogenous feeding, group G3 began the weaning process towards inert feed, by implementing B-Supra AL1 into the feeding regime. Larvae at this point were fed 277 ind/larvae in Artemia Nauplii, while the quantity of inert feed gradually increased, initially beginning at 5g/1000 larvae with O.range Wean (50%) and AL1 (50%) (***Table 3.2)***. By day 13 pef artemia was removed from the feeding regime and the larvae were fed only inert feed for the remainder of the trial. The inert feeds were administered in 24-hour cycles, with the use of automated distributors, to ensure a constant availability of feed. As days passed, inert feeds AL2 (0.07 mm) were gradually implemented, and eventually as the larvae grew, AL2 (0.08 mm) was then administered in pellet form. Every two days the quantity of inert feed increased by 1g/1000 larvae until day 17 pef, at which the feed increased by 1g/1000 larvae each day. At day 22 pef, feeding regime only consisted of AL2 feed (***Table 3.2***).

The larvae in G2 served as our control group and were fed with inert feed (B-SUPRA) & (O.range), in calculated rations that followed previous feeding protocols used by Caviar de Neuvic. Feeds were administered in 12-hour cycles, once during the day and again at night using automated distributors. These feeding regimes were maintained for a 35-day trial to which newly metamorphosized fry were then separated by size (small, medium, & large) and allocated to larger 1.1 m^3 tanks. Tables illustrating G1's, G2's, & G3's feeding regimes, as well as the details of the B-Supra & O.range Wean S inert feed are listed below ***(Tables 3.1 and 3.2)***.

Table 3.1: The range and parameters of inert feed B-SUPRA & B-START by the company Le Gouessant en Bio and O.range WEAN 2/4 by the company INVE Aquaculture.



Le Gouessant en BIO/INVE Aquaculture:	B-SUPRA Range					B-START Range		O.range
Feed Diameter (mm)	0.15-0.4	0.4-0.7	0.7-1/1.2	1.2	1.4	1.7	2.5	0.2-0.4
Commercial Reference	AL0	AL1	AL2/AL2G	AL3	AL4	1	2	WEAN 2/4
Presentation	Powder/Crumb	Crumb	Crumb/Pellet	Pellet	Pellet	Pellet	Pellet	Crumb
Crude Protein (%)	58	55	55	55	52	52	47	54
Crude Lipid Content (%)	13	13	13	13	17	17	18	13
Crude Cellulose Content (%)	0.22	0.5	0.5	0.5	0.7	0.7	0.7	1
Crude Ash Content (%)	9.15	13.5	13.5	13.5	14	14	14	11
Calcium (%)	1.95	1.6	1.6	1.9	2	2	2	1.5
Phosphorus (%)	1.35	1.5	1.5/1.8	1.5	2	2	2	1.3
Sodium (%)	0.83	1.5	1.5/1.1	1	0.75	0.75	0.75	0.7
Nitrogen Free Extracts (%)	9	9	9	9	8.3	8.3	12	N/A
Floatability	Floatable	Floatable	Semi-Floatable	Sinkable	Sinkable	Sinkable	Sinkable	Semi-Floatable
DHA (%)	0.8-1	0.8-1	0.8-1	0.8-1	1.1-1.3	1.1-1.3	0.9-1.1	2
EPA (%)	0.65-0.85	0.65-0.85	0.65-0.85	0.65-0.85	0.9-1.1	0.8-1	0.8-1	1
Ingredients:	Fishmeal, Organic Wheat, Organic Pea Starch, Organice Pea Protein, Fish Oil, Squidmeal, Krillmeal, Fish Solubles, Organic Peas							
Ingredients:	Fishmeal, Wheat Gluten, Hydrolysed Fish Protein, Marine Zooplankton Meal, Squidmeal, Fish Oil, Crude Lecithins, Yeast Products, Refined Vegetable Oil, Dried Algae (Haematococcus pluvioalis), Monosodium Phosphate, Magnesium Sulphate Andryous, Magnesium Sulphate Heptahydrate							

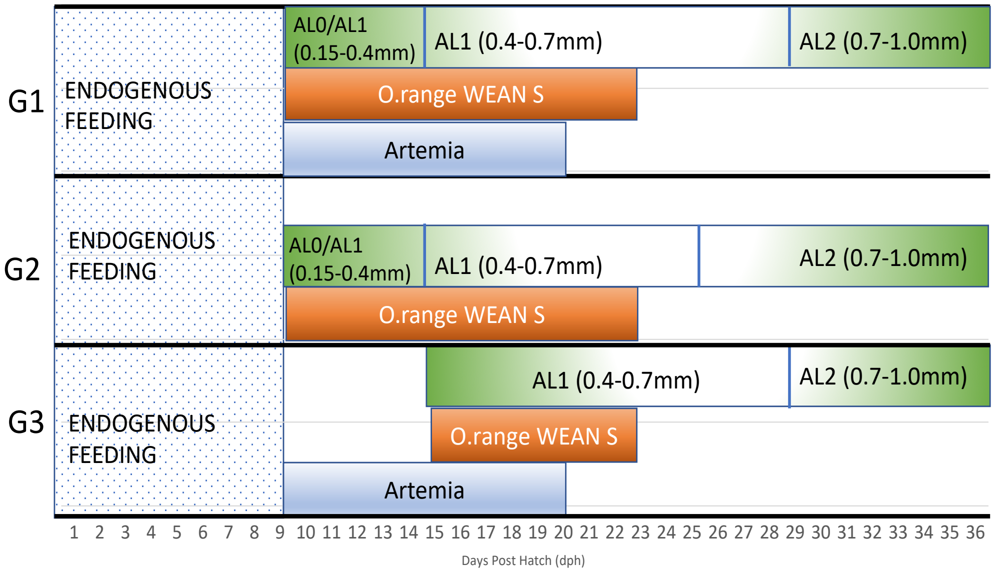


Figure 3.5: Dietary regimes for treatment groups' G1, G2, & G3 containing the following feeds: *Artemia nauplii* as live feed, AL B-SUPRA Range by Gouessant en BIO, & O.range Wean S by INVE Aquaculture

3.4 Data Collection & Statistical Analysis

Dead larvae were removed and recorded twice a day (Morning & Afternoon), so that mortality rates could be calculated accurately. This was done as tanks were cleaned via aspiration of the tank floor, while carefully avoiding stressing live larvae. Mortality rates between groups G1, G2, & G3 were compared and analyzed using ANOVA tests after being tested for normality (Shapiro-Wilk) ($p < 0.05$) as well as Tukey Post hoc test, so that variations from each group could be compared against one another. Due to the lack of replication within the O.range treatment group, the results from this trough were excluded from the overall statistical analysis of this trial. The mortality rates for this trough were still recorded to assess the feasibility of further experimentation of this dietary option.

3.5 Culture Parameters & Rearing of Fingerlings (Post Trial 1)

By 36 dph, the entire population had surpassed their final stages of metamorphosis, entering the fingerling stage. The fingerlings from all previous treatments were weighed, sorted based on body size and average wet body weight (Head >0.8g> Tail) and allocated to 5 1.1m^3 polyethylene tanks with flow rates set at 18L/min. As days passed, the variation in growth between individuals within the population continued to increase, as well as the biomass density in each tank (day 48: 6-10 kg.m^{-3}). By 49 dph, the fingerlings were weighed and sorted again (Head: 3.7g, Middle: 2.5g, Tail: 1.7g). To account for the increase in biomass and the variation in individual size within the population, 7 more 1.35m^3 tanks were stocked with fingerlings based on their size group (Tail, Middle, or Head). This process was repeated again on 58 dph with 4 more 2.55m^3 tanks being stocked to accommodate the growing increase in biomass, while maintaining consistency in size within the populations of each tank. On 70 dph, the sampling process for trial 2 began. The fingerlings were weighed and sorted with all the individuals from the "Middle" size group being transferred out of the hatchery into 3.7m^3 polyethylene tanks to be prepared for Trial 2. The "Middle" size group was chosen for Trial 2 because they represented the largest proportion of the total population (10,000/18,210 ind.). The "Tail" and "Head" groups remained in the hatchery for 35 more days until the end of Trial 2.

Throughout this entire rearing process temperature conditions were maintained at 18 ±1 °C, Oxygen concentration 8 ±2 mg L^{-1} and pH at 7.8 ±0.03. These conditions were monitored daily using "Aqualarme" software by Teraqua and analyses of water were performed twice weekly via

Photometry to ensure nitrite, pH, and ammonium levels did not surpass lethal concentrations. In order to combat the increased consumption of O_2 as well as the increase in nitrites that occurred during the growing process, aeration tubes containing purified O_2 were inserted into each tank and 75kg of salt (3g L^{-1}) was added weekly into the water circuit. These fingerlings were fed AL2-AL4 B-SUPRA Range pellet feed in 24-hour cycles using automatic distributors throughout this 35-day process (36-70 dph).

Trial 2: Measuring the effects of increasing stocking densities on growth and survival of Russian sturgeon fry

3.6 Culture Parameters & Experimental Setup

10,000 fingerlings with an average wet weight of 12.1g were sampled, divided randomly into 3 treatment groups, and transferred into 8 3.7m^3 polyethylene tanks (**Figure 3,6 & 3.7)**. Each treatment group contained a different population density, ranging from low to high (Low: 800 ind., Medium: 1,200 ind., High: 1,600 ind.). The original protocol predicted 3 replicates per treatment, but due to insufficient number of fingerlings available for this trial, the Low treatment group only had 2 replicates. This decision was made on the expectation that variations in growth were more likely to be exhibited in the groups containing higher stocking densities. Each tank was inserted in a semi-open circulation system, with freshwater obtained from the Isle River in Dordogne, France (**Figure 3.6)**. Temperature conditions were maintained at 17 ±2 °C, Oxygen concentration at 8 ±1 mg L^{-1} with the use of aeration tubes providing purified O_2 for each tank, pH 7.6 ±0.03 and flow rates were consistently maintained at a high rate (90L/min) generating a 100% water renewal every 40 minutes. This ensured that pH, nitrite, nitrate, and ammonium levels were maintained within adequate thresholds for the species. Dissolved oxygen (DO) concentrations were stable during first two weeks of the trial, but once biomass densities within Medium & High-density tanks began to surpass 10 $kg.m^{-3}$, DO concentrations decreased below the required levels (8±1 mg L^{-1}). To overcome this situation, purified O_2 aeration was provided to these tanks. Fingerlings were exposed to the standard outdoor photoperiod for spring season in Dordogne, France (12:12 LD) and were fed B-SUPRA's Coul 2 (2mm) inert feed, at an average rate of 2.52% of their body weight, in 24-hour cycles using automatic feed distributors at night.

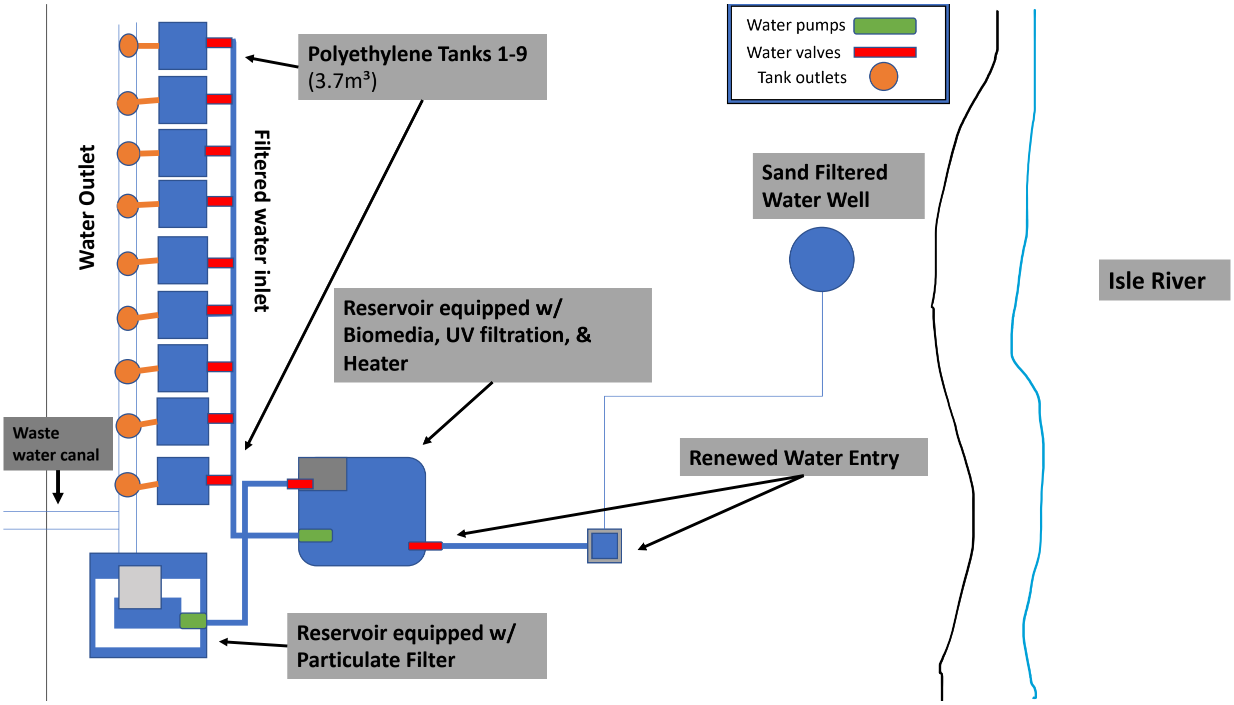


Figure 3.6: Water circuit & tank layout with water renewal source for Trial 2

Figure 3.7: Assembled water circuit containing 9 polyethylene tanks (3.7 m³) equipped with 2 reservoirs, aquatic heaters, UV, particulate, & bio media filtration

3.7 Data Collection & Statistical Analysis

Dead fry was removed and recorded daily, so that mortality rates could be calculated. In order to calculate average wet weights, weekly samples (n=50) of fry were collected from each culture and weighed using a laboratory scale (0.1g), starting from the first day of the trial, so that initial weights were recorded. These values were then used to calculate specific growth rate (SGR). SGR was calculated using an instantaneous growth rate equation with mean initial weights (W1) and final weights (W2) values paired with logarithms (ln) and the total duration time (t) of the experiment. The equation is as follows: $SGR=(\ln W2-\ln W1)/t \times 100$. Lastly, food conversion rates (FCR) were calculated using total distributed feed (DF) values from each treatment group paired with mean initial and final weight values from each treatment group. The equation is as follows: $FCR (\%) = DF/(W2-W1)*N$. Specific growth, food conversion, size deviation, mean body weights, mean weight gains, and mortality rates between groups Low, Medium, and High were compared and analyzed using ANOVA tests after being tested for normality (Shapiro-Wilk) ($p < 0.05$) and Two Way ANOVAs, so that these parameters could be compared at each sampling day.

4. **Results**

4.1 Trial 1: Measuring the effects of weaning on survival of Russian sturgeon larvae using live feed

After the 35-day trial period, the mixed diet treatment group G1, as well as the trough only being fed O.range inert feed exhibited the lowest mortality rates, with group G1 averaging at of 41.7% and trough “O.range” generating a mortality rate of 39.89%. Significant differences were found between treatment groups (ANOVA, $P<0.05$) The live feed treatment group G3 exhibited the highest average mortality rate at 57.5%, and together with G2 , which had a mortality rate of 50.8%, were significantly higher than G1’s (Tukey, $P>0.05$), indicating that the treatment provided for group G1 had the best performance in terms of survival (**Figure 4.1).**

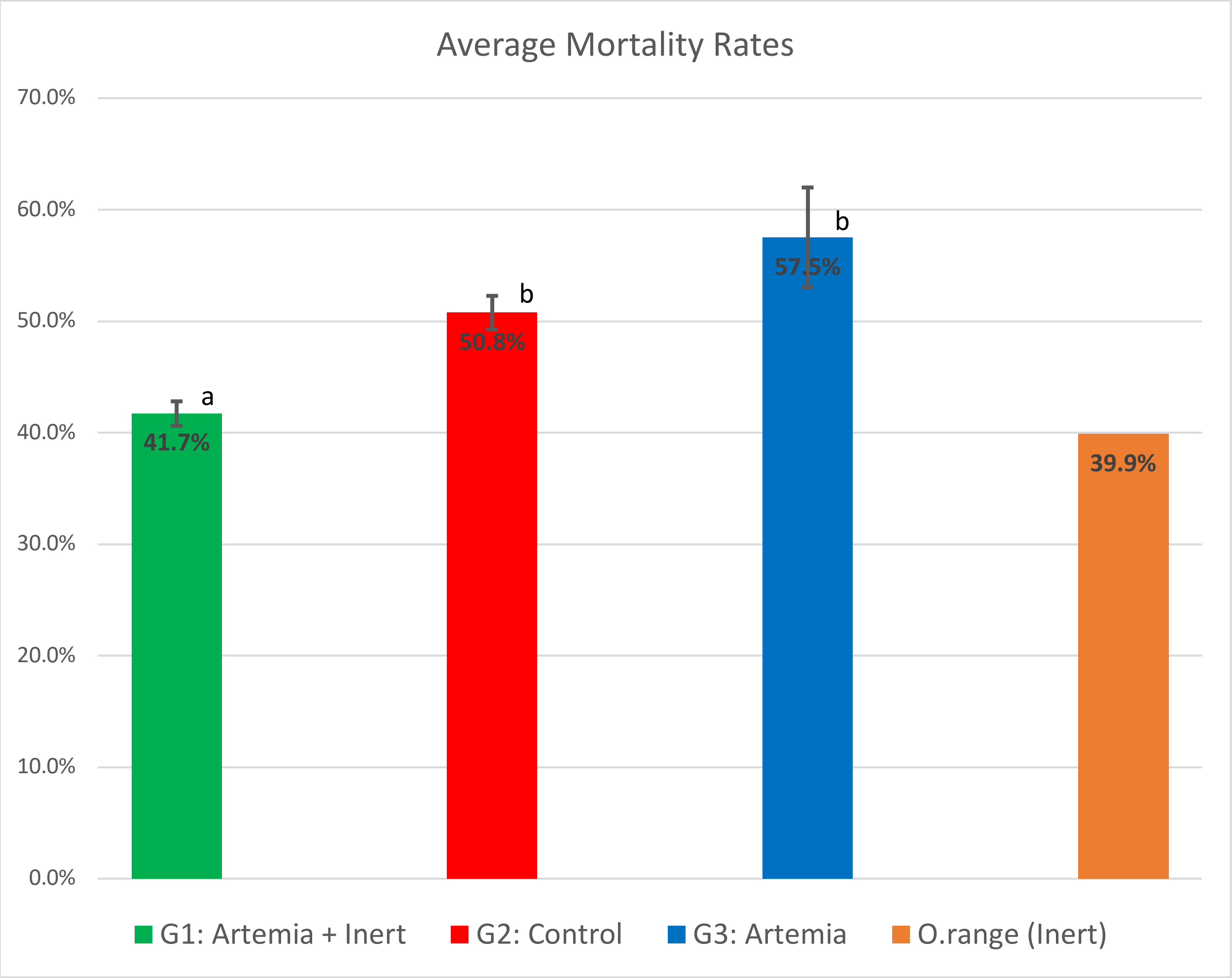


Figure 4.1: Average mortality rates of Russian sturgeon larvae over a 35-day trial period under different feeding regimes. Letters a,b indicate groups with statistically significant differences (Anova, Tuckey, $p<0.05$). Last treatment, O.range did not enter statistical analysis due to a lack of replication

While mortality rates significantly differed between treatment groups, the distribution of daily mortality appears to be parallel within all treatments. No important mortality was recorded until day 10 dph, where all treatment groups exhibited minimal counts (<20), except for G3 which exhibited no mortality until the 11th dph. Daily mortality rate remained quite consistent until 15 dph, after which all treatment groups experienced an exponential increase in daily counts, peaking on the 16th & 17th dph and marking the end of the critical period. These values are shown in **Figure 4.2**. By the 19th dph, daily mortality greatly decreased and stabilized throughout the rest of the trial period. The live feed treatment group G3 experienced the highest mortality peak on 16th dph, losing 14.68% of its population on the same day. Control treatment group G2 also experienced high mortality counts on the peak day 16th dph losing 9.65% of its population. The mixed diet treatment group G1 exhibited the best survival on the peak day 16th dph losing 6.4% of its population. The O.range trough also exhibited better survival then groups G2 & G3, experiencing its peak in mortality on the 17th dph losing only 6.89% of its population. The distribution of daily mortality along the trial is shown in **Figure 4.2**.

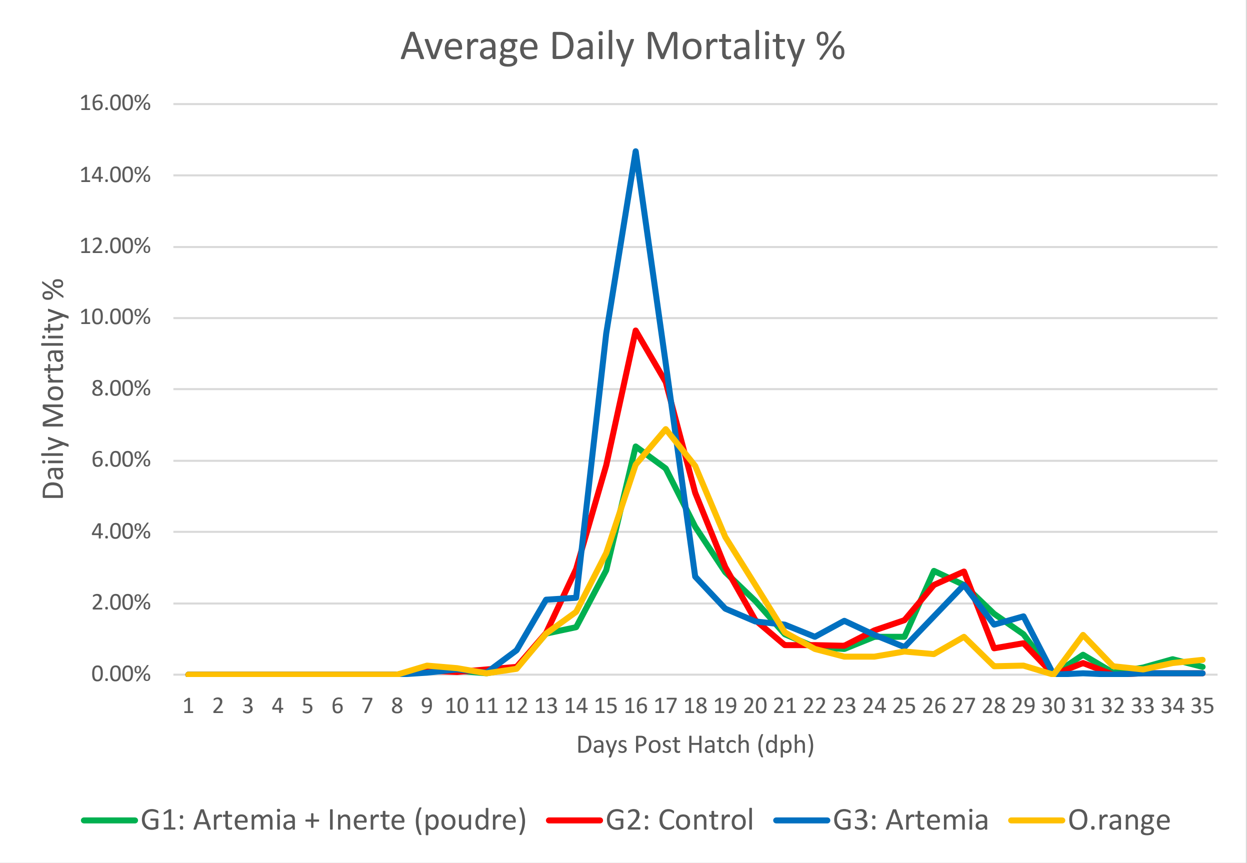


Figure 4.2: Daily mortality percentages for each treatment group in Trial 1 starting from 9th dph to 30th dph

4.2 Trial 2: Measuring the effects of increasing stocking densities on growth and survival of Russian sturgeon fry

The mean initial and final body weights, as well as mean SGR, FCR, and weight gain of the fry reared under different stocking densities, are shown in ***Table 4.1***. The SGR (Low: 3.73±0.03, Medium: 3.76±0.07, High: 3.69±0.06), final body weights (Low: 40.1± 2.77g, Medium: 43.3± 0.38g, High: 41.21± 1.37g), weight gain (Low: 28.4± 2.06g, Medium: 30.84±0.39g, High: 29.05±1.18g), and FCR values among all stocking densities (Low: 0.66±0.03, Medium: 0.64±0.01, High: 0.67±0.02) were not statistically significant (ANOVA, P>0.05 **Figure 4.4** shows the mean body weights of the fingerlings on each sampling day, from the three stocking density,. **Figure 4.5** shows the weekly mean FCR of the fingerlings on each sampling day, for the three stocking densities tested. No significant differences were found in weekly mean FCR values or weekly mean body weight values between each sampling day for all stocking densities (Two-Way ANOVA, P>0.05). **Figure 4.6** shows the weekly mean weight gain of the fingerlings on each sampling day, from each stocking density. No significant differences were detected in the weekly weight gain values or size deviation (ANOVA, P>0.05) between stocking densities as well. There was no mortality registered throughout the entire 35-day trial period, for any of the stocking densities tested. The mean stocking densities (Low: 2.53-8.67 kg.m^{-3} , Medium: 4.05-14.05 kg.m^{-3} , High: 5.26-17.81 kg.m^{-3}), though significantly different between treatment groups, generated little to no variation for any of the measured parameters.

Table 4.1: The effect of stocking density on mean initial/final body weights, overall mean SGR, FCR, and weight gain of Russian sturgeon fry from groups containing stocking densities: Low, Medium, High

Treatment	**Low**	**Medium**	**High**
Stocking Density (ind/tank) (3.7m³)	800	1200	1600
Initial Biomass Density (kg.m^{-3})	2.53	4.05	5.26
Final Biomass Density (kg.m^{-3})	8.67	14.05	17.81
Initial Body Weight (g)	11.69±0.71	12.46±0.30	12.17±0.25
Final Body Weight (g)	40.1± 2.77	43.3± 0.38	41.21± 1.37
Weight Gain (g)	28.4± 2.06	30.84±0.39	29.05±1.18
Feed Conversion Rate (%)	0.66±0.01	0.64±0.03	0.67±0.02
Specific Growth Rate (% bw $^{-1}$)	3.73±0.03	3.76±0.07	3.69±0.06

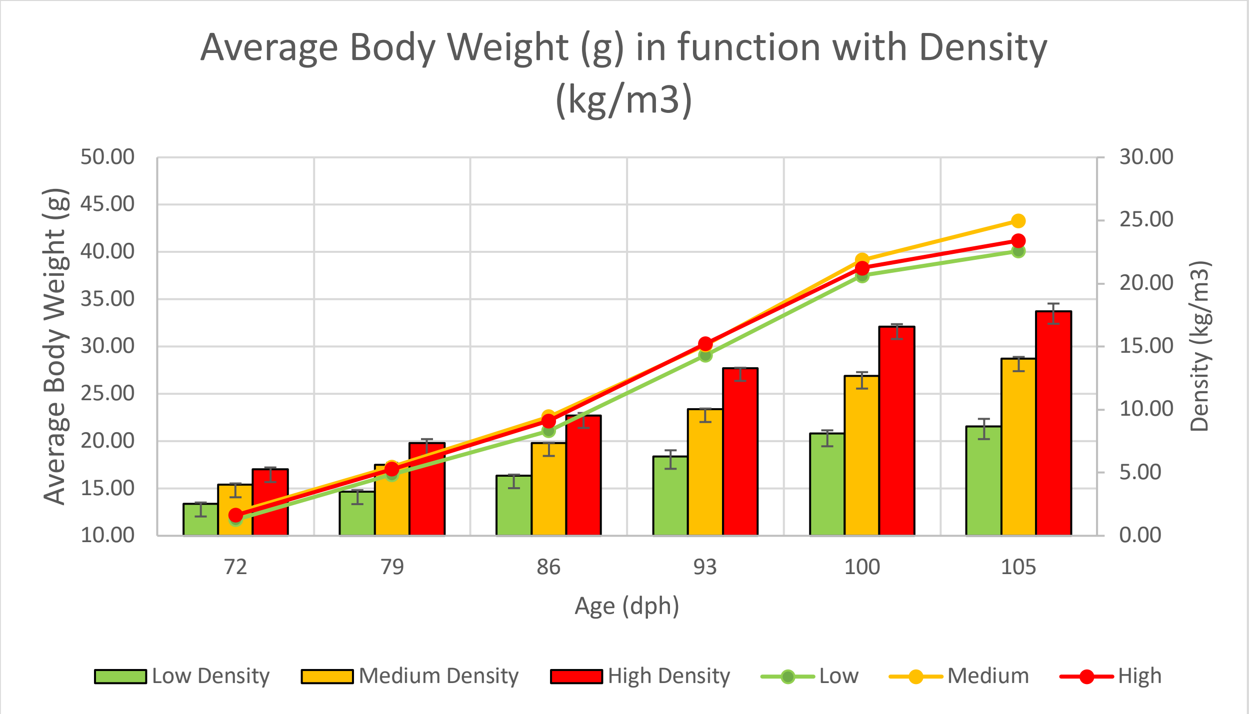


Figure 4.4: Mean body weights (line plot) of the Russian sturgeon fry in function with its given biomass density (bar graph) on each sampling day from each stocking density (Low in green, Medium in yellow, & High in red)

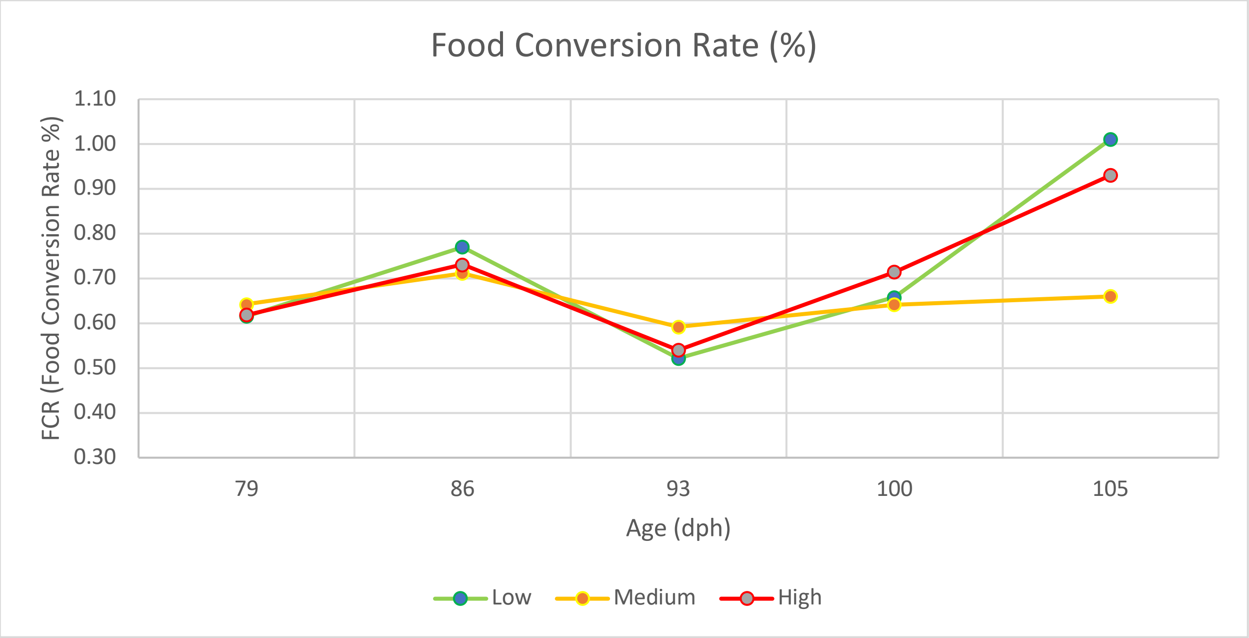


Figure 4.5: Weekly mean FCR of Russian sturgeon on each sampling day from each stocking density (Low in green, Medium in yellow, & High in red)

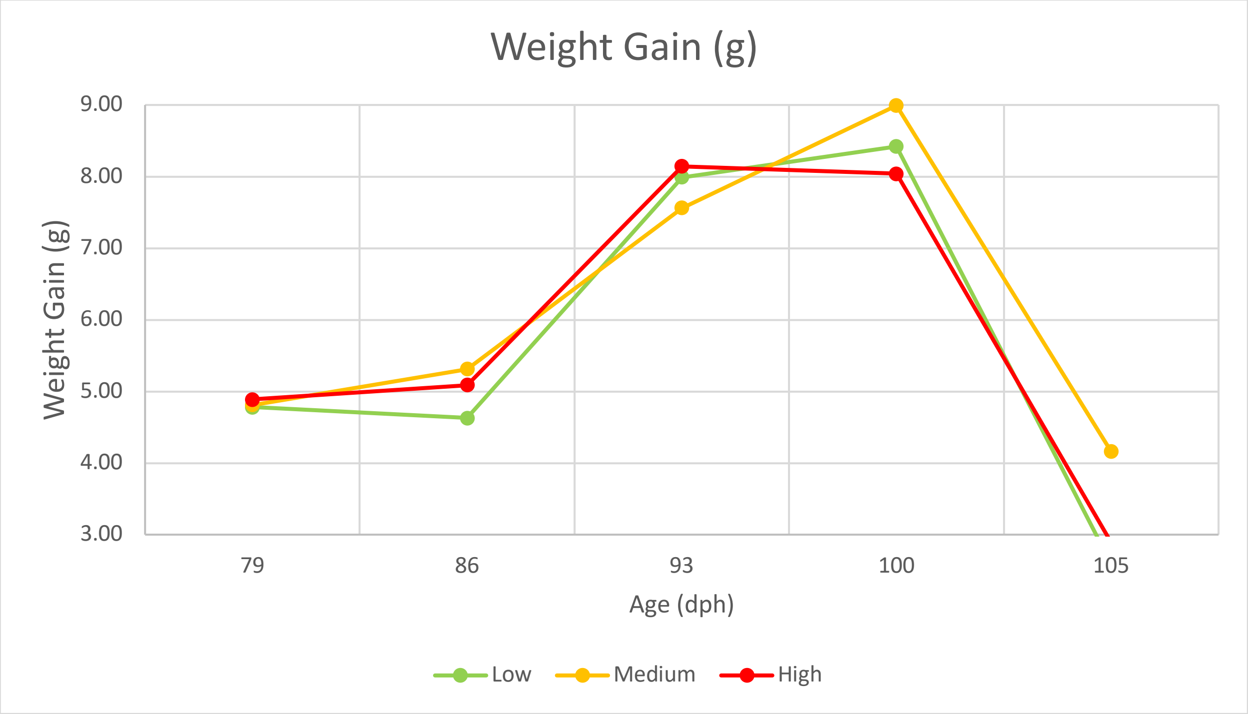


Figure 4.6: Mean values of weekly weight gain of Russian sturgeon fry on each sampling day from each stocking density (Low in green, Medium in yellow, & High in red)

5. Discussion

5.1 Trial 1: Measuring the effects of weaning on survival of Russian sturgeon larvae using live feed

This study revealed a favorable effect of G1's mixed diet containing both live and inert feeds on the survival of Russian sturgeon larvae, similarly as in the study conducted by Didieu et al. (2011), where diets comprised of both live and inert feed significantly outperformed a commercial inert diet for Russian sturgeon larvae. After 36 days, the treatment composed of both commercial and inert feed (G1) generated a significantly lower mortality rate of 41.7%, while the treatments composed of only live feed (G3) and inert feed (G2) generated mortality rates of 57.5% and 50.8% respectively. The underlying differences between this study and that of Didieu et al. (2011) is that this present study only provides live feed for 11 days, as a weaning attempt where diets in the study conducted by Didieu et al. (2011) were constant throughout the entire trial (36 days). Both studies generated similar survival rates, but in our study costs related to labor and production were reduced. The use of artemia nauplii during the first 11 days of exogenous feeding appears to provide larvae with a form of stimulation that significantly prevents mortality, when compared with only inert feed from B-SUPRA Range paired with O.range WEAN S. It has been previously stated that Russian sturgeon larvae often rely more on olfactory then on visible senses when detecting and consuming food (Kynard et al., 2002). In this sense, it is probable that these larvae preferred odors generated by *Artemia* nauplii, the same being possible for other zooplanktonic species. This is also supported by the results generated by treatment group O.range, that was fed only on O.range Wean S inert feed, whose ingredients are mainly composed of artemia nauplii and rotifers. While no replication was provided to treatment group O.range, its trough generated the highest survival rate in this trial, making this dietary regime worthy of future testing. It is also important to note that feed distribution is improved when using live or mixed diets, due to increased dispersion generated by the movement of live feed, increasing individual's chance of detecting food particles (Rosenlund et al., 1997).

The results from Didieu et al. (2011) revealed that live or mixed diets generate significantly higher feeding efficiency rates than artificial feeds. During the onset of exogenous feeding, the digestive tract is still under development, with digestive enzymes activity being an effective indicator of development. The low feeding efficiency rates observed by Didieu et al., (2011), indicated that most larvae being fed on inert diets held digestive systems that were not mature enough or morphologically developed to digest hydrolyzed inert feed, thus increasing mortality. Mixing inert feeds with live preys can be a solution to this problem (Munilla-Moran et al., 1990; Holt, 1993; Person Le Ruyet et al., 1993), as live prey contains exogenous enzymes and pancreatin that improve the digestion of inert hydrolyzed diets. In gilthead seabream, it has been reported that mixing diets improved the digestion of inert diets by 30% (Kolkovski et al., 1993).

G3's dietary regime, which was comprised of only *Artemia nauplii* during the critical period, generated a significantly higher mortality rate in comparison to G1's mixed diet, which contradicts the results from Didieu et al. (2011). This significant difference is a result of feed quantity, as Didieu et al. (2011) provided much higher concentrations of live feed to their populations, increasing food availability for the individuals within their cultures. The feed quantity was also higher in treatment group G1 as the same concentrations of *Artemia* were used in the co-feeding diet. Positive and significant correlations have been found between feed quantity and larval survival for Siberian sturgeon (Charlon & Bergot, 1991). The same can be suggested for Russian sturgeon larvae when comparing results from this present study to Memis et al. (2009). Daily mortality rates peaked around the same time (15-17 dph) and generated similar mortality rates (6.4-6.6%) for co-feeding treatments, where the live feed treatment (G3) in our study generated a mortality rate of 14.68%. It was evident during the trial that feed quantity for the live feed treatment (G3) was inadequate, due to the inability to produce the targeted quantities of artemia and the lack of unconsumed feed found in the troughs after feeding.

The results from this trial have proven to be beneficial towards improving protocols for larviculture at Caviar de Neuvic, as mortality rates were much lower in this years' generation compared to the generation that was hatched in 2022. The larvae in the generation of 2022 were reared under similar conditions, except the populations were fed a diet that consisted of only B-SUPRA Range from Gouessant en BIO for the entirety of the larval life stage (35 days) and generated a mortality rate of 68.9%. The application of co-feeding has a favorable effect on survival when comparing the mortality rates of G1 (mixed diet) to the generation of 2022. When suggesting modifications to a protocol intended for commercial production that may lead to higher production and labor expenses, it is essential to ensure that the improvements are evident and will ultimately result in profitability. In this case, improving survival rate by 27.2% would offset the increased costs of producing live feed for 11 days. The addition of O.range WEAN S by INVE Aquaculture to the dietary regime also revealed to have a favorable effect toward survival as G2's (control) mortality rates were improved as well when compared to the generation 2022. The reasoning for this trend is still unclear as further testing regarding palatability, digestion, and growth would be required, to determine if the effect is of any significance.

Studies have shown that artificial diets can be successfully implemented at the larval stage during the onset of exogenous feeding, with low mortality associated, only for a few species of sturgeon such as the *Acipenser transmontanus* and the *Acipenser baerii* (Gisbert & Williot

1997). While this has yet to be proven possible for the Russian sturgeon, the results from this trial do reveal that the implementation of live feed into the dietary regime is not necessary for the entirety of the larval life stage. The reduction in mortality can be achieved by providing a short co-feeding period directly on the onset of exogenous feeding, as opposed to the other studies that have explored this species who typically implement live or natural feed until the metamorphosis process is complete (33-36 dph) (Memis et al., 2009; Didieu et al., 2011; Kamaszewski et al., 2014).

5.2 Trial 2: Measuring the effects of increasing stocking densities on growth and survival of Russian sturgeon fry

Increasing stocking density allows farmers to efficiently maximize their water source as well as their available space. However, it is important to describe a threshold to avoid any potential loss in biomass gain, that may occur due to social stress (Brown et al., 1992), reduction in water quality, and/or reductions in feed consumption or conversion efficiency (Yang, et al., 2011). In this study, when increasing stocking density, all these factors were accounted for. The initial size deviation between individuals was minimal to reduce feeding competition and minimize the possibility of large deviations in fish weight within the population. Adequate water quality parameters were maintained through the entire trial. After 35 days, the highest biomass density achieved from a tank was 18.3 kg.m^{-3} (1600 ind./tank) and the lowest was 8.24.kg m^{-3} (800 ind/tank). Even though, no significant variation in growth, survival, or size deviation was detected between the tanks containing High, Medium, or Low stocking densities. Previous research with other species of sturgeons, such as the Amur sturgeon (Yang et al., 2011), have observed that stocking densities that surpass 5 kg.m^{-3} will begin to have negative effects on conversion efficiency, feeding intake, and ultimately growth. These effects were believed to be a result of social stress, due to a lack of available space to move, which in turn affects the metabolic rate of the fish and also increases competition for food. While this has been the case for other aquaculture species, (e.g. rainbow trout *Oncorhynchus mykiss*, Zoccarto et al., 1993), based on the results produced in this study, social stress due to limited available space was likely not a factor, as biomass densities were more than 3 times higher in this study. The parameter of focus for the effects of stocking density on the Amur sturgeon was O_2 concentration (Yang et al., 2011), which varied considerably when compared with the present trial. Mean O_2 concentrations at the highest density descended to values of 3.74±0.81 mg L^{-1} and at the lowest density were 5.27± 0.78 mg L^{-1} (Yang et al., 2011), while the average O_2 concentrations in this study never fell below 8±1 mg L^{-1}. Exposing fish to low concentrations of "DO" can disrupt many essential physiological functions, as well as increase cortisol production which in turn inhibits growth (Frank et al. 2006; Ingebrigt et al., 2009; Emilio et al., 2010). It is highly plausible that inadequate concentrations of O_2 is what caused the significant variation in growth between stocking densities for Yang et al. (2011) as consumption of DO increases with increased biomass density. This same trend was reported by Dicu et al. (2013) where stellate sturgeon *Acipenser stellatus* fingerlings were reared in high stocking densities (initial density: 4.58 kg.m^{-3} , final density: 24 kg.m^{-3}), with no losses in growth rates, where water quality, specifically oxygen concentration, was maintained at adequate concentrations.

Nevertheless, Feshalami (2017) reported that high stocking densities (18.4 kg.m^{-3}) do negatively influence growth for beluga and ship sturgeon, even when water conditions are adequate. This disturbance in growth was believed to be a result of uneven feed distribution between individuals, generating significant deviations in individual size within the population as ingested feed values were significantly lower in the high-density groups while FCR values remained constant between all stocking densities. The hematological analysis also reported no significant differences in plasma cortisol concentration, indicating that stress was not a factor in the results. Uneven feed distribution seems to be the leading cause for loss in growth. It is possible that this occurrence could have been avoided if a smaller size pellet was used and a continuous feeding strategy had been applied in the study conducted by Feshalami (2017). Pellet size and the time frame in which feeds are administered will affect feed intensity and distribution between individuals in a population (Bailey & Alanärä, 2006). The size of the pellet used in the study conducted by Feshalami (2017) was 6.5 mm in diameter, for fingerlings containing initial weights 143.0±0.3g (beluga) and 93.7±0.9g (ship). Russian sturgeon with average body weights ranging between 100-200g are fed pellets of 3-4 mm in diameter at Caviar de Neuvic, to promote high dispersity and increase the chance of an equal feed distribution amongst the individuals in the population. Using pellets that are roughly half the size in diameter means a substantially higher quantity of available pellets to feed on. While the mouth size of the beluga sturgeon is larger than that of the Russian sturgeon, this is not the case for the ship sturgeon, which was fed the same size pellet as the beluga

(Vecsei & Peterson, 2004) and produced similar results. The feeds in the study conducted by Feshalami (2017) were also administered four times a day, while in the present study feed was constantly administered in a 24-hour cycle using automated distributors, this way increasing food availability. In the case of the stellate sturgeon (Dicu et al., 2013), feed availability was also higher, with feed being administered six times a day.

When exploring stocking density thresholds, it is important to focus on all culture parameters and ensure they are optimal, as they could be the primary factor affecting growth performance, rather than the density of biomass itself. This includes water quality, habitat formulation, habitat enrichment, as well as feeding strategies. An example of this has been reported with the halibut, where biomass density thresholds depend mostly on tank surface area due to the benthic behavior exhibited by this species (Bjornsson, 1994). It is also possible to obtain increased yields of biomass by breaking thresholds. Slight losses in average body weights, for example, may still be beneficial when assessing yields of biomass per unit of cost. This has been reported with the sutchi catfish, while mean weights of fish can be inversely related to increasing stocking density, the SGR, FCR, and survival remain unaffected. This increases the net revenue of biomass positively, producing improved farm economics (Bosworth, 2015). In the case of the Russian sturgeon, this may be applicable if the stocking threshold is high enough to produce enough biomass to which the loss in average body weight will not outweigh the net yield in biomass.

Overall the results from this experiment were beneficial for the improvement of stocking density protocols for Russian sturgeon fry at Caviar de Neuvic. Previous protocols ensured that biomass densities in the hatchery could never surpass 12 $kg.m^{-3}$, assuming that negative effects on growth would occur. Nevertheless, the results from this study indicated that this threshold can be extended to at least 16 $kg.m^{-3}$, assuming the RAS system can filter the increased waste products generated by the increase in biomass. The question that still remains is identifying the highest stocking density that produces the maximum biomass, while still being commercially profitable when applying the increased costs of energy that derive from increased water (flow rate) & oxygen consumption (Purified O_2 aeration). This would require further testing to which biomass densities higher than 18 $kg.m^{-3}$ would be tested while also calculating the increased costs of energy, water, and DO consumption that come with maintaining stocking densities of this magnitude.

6. **Conclusion**

- The results from Trial 1 revealed that a co-feeding period (artemia + inert feed) of 11 days starting from the onset of exogenous feeding, and coinciding with the critical period, can successfully reduce mortality in Russian sturgeon larval culture.
- The presence of artemia in the tanks, co-fed with inert feeds seemed to contribute for an equal feed distribution within the population and facilitate Russian sturgeon larvae's capacity to digest and assimilate nutrients from artificial feeds.
- O.range WEAN S by INVE Aquaculture seems to be a promising feed for larvae as its implementation into the regime generated lower mortality rates when compared to previous years at Caviar de Neuvic
- The results from Trial 2 were beneficial for the improvement of stocking density protocols for Russian sturgeon fry at Caviar de Neuvic. This trial indicated that the threshold can be extended to at least 16 kg.m^{-3}, when water quality and an equal feed distribution are maintained adequately.
- The densities tested showed no decrease in any of the measured parameters (SGR, FCR, Mean body weight, weight gain, survival, and size deviation.

7. **References**

Arndt G., Gessner J., & Raymakers C. 2002. *Trends in farming, trade and occurrence of native and exotic sturgeons in natural habitats in Central and Western Europe*. Journal of Applied Ichthyology 18, 444–448.

Bailey J., & Alanärä A. 2006. *Effect of feed portion size on growth of rainbow trout, Oncorhynchus mykiss (Walbaum), reared at different temperatures.* Aquaculture 253, 728–730.

Birstein V.J. 1993. *Sturgeon and paddlefishes: threatened fishes in need of conservation*. Conservation Biology 7, 773-787.

Björnsson B. 1994. *Effects of stocking density on growth rate of halibut (Hippoglossus hippoglossus L.) reared in large circular tanks for three years.* Aquaculture 123, 259-270

Bondarchuk M. 2017. *Status and trends of the world market of caviar*. Problems and prospects of economics and management 4 (8), 133-139.

Bosworth B., Ott B., & Torrans L. 2015. *Effects of Stocking Density on Production Traits of Channel Catfish×Blue Catfish Hybrids.* North American Journal of Aquaculture 77(4), 437–443

Brown, G.E., Brown J.A., & Srivastava, R.K., 1992. *The effect of stocking density on the behavior of arctic charr (Salvelinus alpinus L.).* Journal of Fish Biology 41, 955-963

Cahu C. & Zambonino Infante, J. 2001. *Substitution of live food by formulated diets in marine fish larvae.* Aquaculture 200(1-2), 161–180.

Celikkale M. S., Memis D., Ercan E., & Cagiltay F. 2005. *Growth performance of juvenile Russian sturgeon (Acipenser gueldenstaedtii Brandt & Ratzenburg, 1833) at two stocking densities in net cages.* Journal of Applied Ichthyology 21(1), 14–18.

Dicu M., Cristea V., Maereanu M., Dediu L., & Petrea S. 2013. *The Effect of Stocking Density on Growth Performance and Hematological Profile of Stellate Sturgeon (A. stellatus, Pallas, 1771) Fingerlings Reared in an Industrial "Flow-through" Aquaculture System*. Bulletin UASVM Animal Science and Biotechnologies 70(2), 244-254

Didieu L., Maereanu M., Cristea V., & Maereanu D. 2011. *Effect of Formulated Diet versus Live Food on Growth and Survival of Russian Sturgeon (Acipenser guldenstaedtii) Larvae Starting Exogenous Feeding*. Bulletin UASVM Animal Science and Biotechnologies 68(1-2), 130-136

Doroshov S. I. 1985. *Biology and culture of sturgeons, Acipenseriformes*. In Recent Advances in Aquaculture Vol. 2. J. F. Muirs and R. J. Roberts, eds. Westview Press: Boulder, Colorado.

Emilio S.L., Victoria A., Beatriz M.A., Diego C., Josep V., Plana, Carlos I., Jose P. C., & Manuel M. 2010: *Effects of stocking density and feed ration on growth and gene expression in* *the Senegalese sole (Solea senegalensis): potential effects on the immune response*. Fish Shellfish Immunology 28, 296–302.

European Market Observatory for Fisheries & Aquaculture Products. 2021. *The Caviar Market: Production, Trade, and Consumption In and Outside the EU.* An update of the 2018 report, European Commission

Fagerlund U., McBride J., & Stone E. 1981. *Stress-related effects of hatchery rearing density on Coho salmon*. Transatlantic American Fishery Society 110, 644–649.

Feshalami M., Nikpey Farokh, & Mortazavizadeh S. 2017. *Influence of Stocking Density on Growth and Physiological Responses of Beluga, Huso huso (Brandt, 1869) and Ship Sturgeon Acipenser nudiventris (Lovetsky, 1828), Juveniles in a Flow-through system.* Journal of the World Aquaculture Society 48(4), 611-621

Frank, K., Emanuel, C. K. H., & Patrick, W. 2006. *Feeding experiments with the European Atlantic sturgeon, Acipenser sturio L., 1758 to accustom large juveniles to a new feed item and the influence of tank size and stocking density on growth*. Journal of Applied Ichthyology 22, 307– 315.

Gibtan A., Getahun A., & Mengistou S. 2008. *Effect of stocking density on the growth performance and yield of Nile tilapia (Oreochromis niloticus) in a cage culture system in Lake Kuriftu, Ethiopia*. Aquaculture Research 39, 1450–1460.

Gisbert E. & Williot P. 1997. *Larval behaviour and effect of the timing of initial feeding on growth and survival of Siberian sturgeon (Acipenser baerii) larvae under small scale hatchery production.* Aquaculture 156, 63-76.

Gisbert E. & Williot P., 2002. *Advances in the larval rearing of Siberian sturgeon.* Journal of Fish Biology 60, 1071-1092

Gisbert E., Solovyev M., Bonpunt E., & Mauduit C. 2018. *Weaning in Siberian sturgeon larvae.* Farming 2

Hanaee J., Agh N., Hanaee M., Delazar A., & Sarker S.D. 2005. *Studies on enrichment of Artemia urmiana cysts for improving fish food value*. Animal Feed Science and Technology 120, 107-112.

Holt, G.J., 1993. *Feeding larval red drum on microparticulate diets in a closed recirculating water system*. Journal of World Aquaculture Society 24, 225-230.

Ingebrigt, U., Elin, K., Gruven, K., & Anders, L. 2009. *Behavioural variation in cultivated juvenile Atlantic cod (Gadus morhua L.) in relation to stocking density and size disparity*. Applied Animal Behavior Science 117, 201–209.

Ivakhnenko R. 2001. *A new method of sturgeon conservation in Russia*. CITES World Newsletter 8, 13

Kamaszewski M., Ostaszewska T., Prusinska M., Kolman R., Chojnacki M., Zabytyvskij J., & Kasprzak, R. 2014. *Effects of Artemia sp. Enrichment with Essential Fatty Acids on Functional and Morphological Aspects of the Digestive System in Acipenser gueldenstaedtii Larvae*. Turkish Journal of Fisheries and Aquatic Sciences, 14, 4

Kim E., Park C., & Nam Y. 2018. *Effects of incubation temperature on the embryonic viability and hatching time in Russian sturgeon (Acipenser gueldenstaedtii)*. Fisheries and Aquatic Sciences 21, 23

Kim E., Park C., & Nam Y. 2019. *Ontogenetic behavior of farm-bred Russian sturgeon (Acipenser gueldenstaedtii) prelarvae in a diel photoperiodic cycle: behavioral modifications in response to light intensity*. Fisheries and Aquatic Sciences 22, 4

Kynard B., Zhuang P., Zhang L., Zhang T., & Zhang Z. 2002. *Ontogenetic Behavior and Migration of Volga River Russian sturgeon,Acipenser gueldenstaedtii, with a Note on Adaptive Significance of Body Color.* Environmental Biology of Fishes 65(4), 411–421.

Lu X. & Rasco B., 2013. *Sturgeon (Acipenser transmontanus) sexual maturation and caviar quality.* Reviews in Aquaculture 5, 1-11

Memis D., Ercan E., Celikkale M., Timur M., & Zarkua Z. 2009. *Growth and Survival Rate of Russian Sturgeon (Acipenser gueledenstaedtii) Larvae from Fertilized Eggs to Artifical Feeding.* Turkish Journal of Fisheries and Aquatic Sciences 9, 47-52

Morais S., Conceição L., Rønnestad I., Koven W., Cahu C., Zambonino Infante J. & Dinis M.T. 2007. *Dietary neutral lipid level and source in marine fish larvae: Effects on digestive physiology and food intake.* Aquaculture 268, 106-122.

Munilla-Moran, R., Stark, J.R., & Barbour A. 1990. The role of exogenous enzymes in digestion in cultured turbot larvae (*Scothalmus maxiumus L.).* Aquaculture 88, 337-250

Nathanailides C., 2002. *Hatching time and post-hatch growth in Russian sturgeon Acipenser gueldenstaedtii.* Journal of Applied Ichthyology, 651-654

Person Le Ruyet, J., Alexandre, J.C., Thebaud, L., & Mugnier, C. 1993. *Marine fish larvae feeding: Formulated diets or live prey?*. Journal World Aquaculture Society 24, 21 l-224.

Rafatnezhad, S., Falahatkar, B., & Tolouei Gilani, M. H. 2008. *Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (Huso huso) juveniles.* Aquaculture Research 39(14), 1506–1513.

Raymakers C. 2002. *International trade in sturgeon and paddlefish species the effect of CITES listing*. International Review of Hydrobiology 87, 525-537.

Rosenlund G., Stoss J., & Talbot C. 1997. *Co-feeding marine fish larvae with inert and live diets.* Aquaculture 155(1-4), 0–191.

Ryabova G., Klimonov O., Afanas'ev I., Vyshkvartsev I., Moskaleichik F., & Rubtsova A. 2006. *Variation in morphometric and genetic characteristics of stellate sturgeon juveniles raised at different densities.* Russian Journal of Genetics 42(2), 182–191.

Stappen G. 1996. *Manual on the production and use of live food for aquaculture.* Fisheries Technical Paper: Laboratory of Aquaculture & Artemia Reference Center, 4

Treece G. 2000. *Artemia Production for marine larval fish culture.* Southern Regional Aquaculture Center 702

Tzankova, Z. 2007. *U.S. Farmed Sturgeon, TZANKOVA, Z.* Seafood Report, Monterey Bay Aquarium

UN. 2019. Growing at a slower pace, world population is expected to reach 9.7 billion in 2050 and could peak at nearly 11 billion around 2100: UN report. https://www.un.org/development/desa/en/news/population/world-population- prospects-2019

Vecsei P., & Peterson D., 2004. *STURGEON ECOMORPHOLOGY: A DESCRIPTIVE APPROACH.* Sturgeons and Paddlefish of North America, 5, 103-133

Wei Q., He j., Yang D., Zheng W., & Li L. 2004. *Status of sturgeon aquaculture and sturgeon trade in China: a review based on two recent nationwide surveys*. Journal of Applied Ichthyology 20, 321-332.

Wei Q., Zou Y., Li P., & Li L. 2011. *Sturgeon aquaculture in China: progress, strategies and prospects assessed on the basis of nation-wide surveys (2007-2009).* Journal of Applied Ichthyology 27(2), 162–168.

Williot P., Sabeaub L., Gessnerc J., Arlatid G., Bronzie P., Gulyasf T., & Berni P. 2001. *Sturgeon farming in Western Europe: recent developments and perspectives*. Aquatic Living Resources 14, 367-374.

Williot, P. Bronzi P., Benoit P., Bonpunt E., Chebanov M., Domezain A., Gessner J., Gulyas T., Kolman R., Michaels J., Sabeau L. & Vizziano D. 2009. *Cultured Aquatic Species Information Programme - Acipenser baerii. Cultured Aquatic Species Fact Sheets*. FAO Inland Water Resources and Aquaculture Service (FIRI).

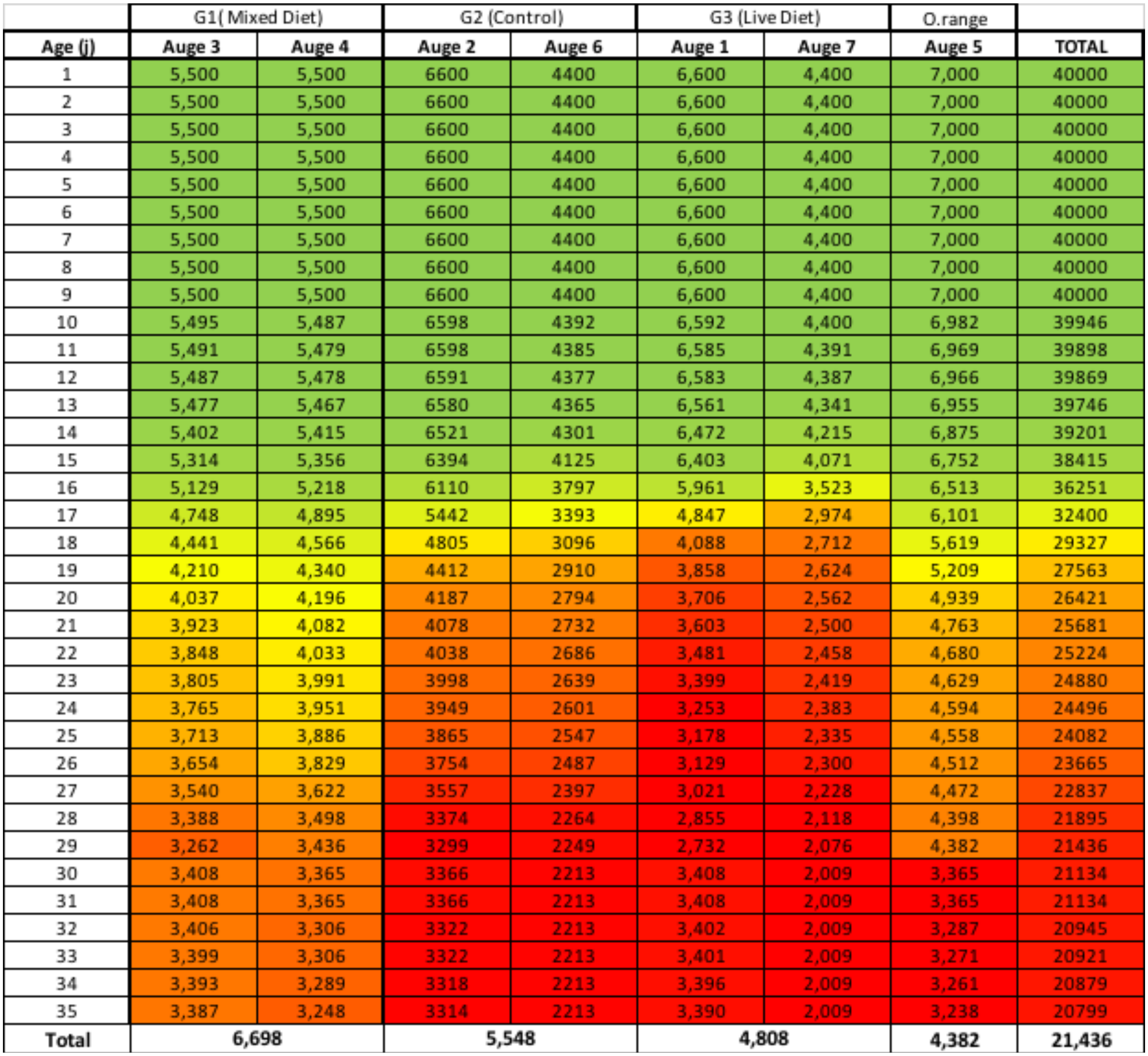
Yang D., Zhu Y., Luo Y., Zhao J., & Chen J. 2011. *Effects of stocking density on growth performance of juvenile Amur Sturgeon (Acipenser schrenckii).* Journal of Applied Ichthyology 27, 541-544

Zhu Y., Wei Q., Yang D., Wang K., Chen X., Liu J., & Li L. *Large-scale cultivation of fingerlings of Chinese Sturgeon Acipenser sinensis for re-stocking*. Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences 1-5

Zoccarato I., Benatti G., Bianchini M. L., Boccignone M., Conti A., & Palmegiano G. B. 1993. *The effect of density and feeding level on performances and body composition in Oncorhynchus mykiss. In: Production, environment and quality: Proceedings of the International Conference Bordeaux Aquaculture Ô92, Bordeaux, France, March 25–27, 1992. Barnabe ́ G. et al., (Ed.).* EAS Special Publication 18, 223–226.

8. **Appendices**

Table 1: Accumulated mortality of Russian sturgeon larvae for all treatment groups (G1, G2 & G3)



	G1(Mixed Diet)		G2 (Control)		G3 (Live Diet)		O.range	
Age (j)	**Auge 3**	**Auge 4**	**Auge 2**	**Auge 6**	**Auge 1**	**Auge 7**	**Auge 5**	**TOTAL**
1	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
2	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
3	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
4	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
5	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
6	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
7	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
8	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
9	5,500	5,500	6600	4400	6,600	4,400	7,000	40000
10	5,495	5,487	6598	4392	6,592	4,400	6,982	39946
11	5,491	5,479	6598	4385	6,585	4,391	6,969	39898
12	5,487	5,478	6591	4377	6,583	4,387	6,966	39869
13	5,477	5,467	6580	4365	6,561	4,341	6,955	39746
14	5,402	5,415	6521	4301	6,472	4,215	6,875	39201
15	5,314	5,356	6394	4125	6,403	4,071	6,752	38415
16	5,129	5,218	6110	3797	5,961	3,523	6,513	36251
17	4,748	4,895	5442	3393	4,847	2,974	6,101	32400
18	4,441	4,566	4805	3096	4,088	2,712	5,619	29327
19	4,210	4,340	4412	2910	3,858	2,624	5,209	27563
20	4,037	4,196	4187	2794	3,706	2,562	4,939	26421
21	3,923	4,082	4078	2732	3,603	2,500	4,763	25681
22	3,848	4,033	4038	2686	3,481	2,458	4,680	25224
23	3,805	3,991	3998	2639	3,399	2,419	4,629	24880
24	3,765	3,951	3949	2601	3,253	2,383	4,594	24496
25	3,713	3,886	3865	2547	3,178	2,335	4,558	24082
26	3,654	3,829	3754	2487	3,129	2,300	4,512	23665
27	3,540	3,622	3557	2397	3,021	2,228	4,472	22837
28	3,388	3,498	3374	2264	2,855	2,118	4,398	21895
29	3,262	3,436	3299	2249	2,732	2,076	4,382	21436
30	3,408	3,365	3366	2213	3,408	2,009	3,365	21134
31	3,408	3,365	3366	2213	3,408	2,009	3,365	21134
32	3,406	3,306	3322	2213	3,402	2,009	3,287	20945
33	3,399	3,306	3322	2213	3,401	2,009	3,271	20921
34	3,393	3,289	3318	2213	3,396	2,009	3,261	20879
35	3,387	3,248	3314	2213	3,390	2,009	3,238	20799
Total	**6,698**		**5,548**		**4,808**		**4,382**	**21,436**

Table 2: Feeding Regime of Experimental Group G1 with previously targeted and actualized concentrations of artemia for Trial 1

Age (dph)	**Rations (g/1000 larvae)**	**Target Artemia ind./Larvae**	**Produced Artemia ind./Larvae**	**O.range**	**AL 0 (%)**	**AL 1 (%)**	**AL 2 Crumb (%)**
1	0				-	-	-
2	0				-	-	-
3	0				-	-	-
4	0				-	-	-
5	0				-	-	-
6	0				-	-	-
7	0				-	-	-
8	0				-	-	-
9 (Day 1 pef)	2	1,000	71.4	50%	25%	25%	-
10	3	1,200	65.1	50%	25%	25%	-
11	3	1,800	229.1	50%	25%	25%	-
12	4	2,200	319.7	50%	20%	30%	-
13	4	2,000	278.0	40%	15%	45%	-
14	5	1,500	257.1	30%	10%	60%	-
15	5	1,000	277.1	20%	5%	75%	-
16	6	800	352.0	10%	0%	90%	-
17	6	600	373.3	50%	-	50%	-
18	7	400	235.8	50%	-	50%	-
19	7	200	58.7	50%	-	50%	-
20	8	100	21.5	40%	-	60%	-
21	8			30%	-	70%	-
22	9			20%	-	80%	-
23	9			10%	-	90%	-
24	10				-	100%	-
25	10				-	90%	10%
26	11				-	75%	25%
27	15				-	50%	50%
28	16				-	25%	75%
29	17				-	10%	90%
30	18				-	-	100%
31	19				-	-	100%
32	20				-	-	100%
33	21				-	-	100%
34	22				-	-	100%
35	23				-	-	100%

Table 3: Feeding regime of Control Group G2

Age (dph)	Rations (g/1000 larvae)	Artemia ind./larvae	O.range	AL 0 (%)	AL 1 (%)	AL 2 mi (%)
1	0			-	-	-
2	0			-	-	-
3	0			-	-	-
4	0			-	-	-
5	0			-	-	-
6	0			-	-	-
7	0			-	-	-
8	0			-	-	-
9 (Day 1 pef)	2	0	50%	25%	25%	-
10	3	0	50%	25%	25%	-
11	3	0	50%	25%	25%	-
12	4	0	50%	20%	30%	-
13	4	0	40%	15%	45%	-
14	5	0	30%	10%	60%	-
15	5	0	20%	5%	75%	-
16	6	0	10%	0%	90%	-
17	6	0	50%	-	50%	-
18	7	0	50%	-	50%	-
19	7	0	50%	-	50%	-
20	8	0	40%	-	60%	-
21	8		30%	-	70%	-
22	9		20%	-	80%	-
23	9		10%	-	90%	-
24	10			-	100%	-
25	10			-	90%	10%
26	11			-	75%	25%
27	15			-	50%	50%
28	16			-	25%	75%
29	17			-	10%	90%
30	18			-	-	100%
31	19			-	-	100%
32	20			-	-	100%
33	21			-	-	100%
34	22			-	-	100%
35	23			-	-	100%

Table 4: Feeding regime of Control Group G3

Age (dph)	Rations (g/1000 larvae)	Target Artemia ind./Larvae	Produced Artemia ind./Larvae	O.range	AL 0 (%)	AL 1 (%)	AL 2 mi (%)
1	0				-	-	-
2	0				-	-	-
3	0				-	-	-
4	0				-	-	-
5	0				-	-	-
6	0				-	-	-
7	0				-	-	-
8	0				-	-	-
9 (Day 1 pef)	2	1,000	71.4		0%	0%	-
10	3	1,200	65.1		0%	0%	-
11	3	1,800	229.1		0%	0%	-
12	4	2,200	319.7		0%	0%	-
13	4	2,000	278.0		0%	0%	-
14	5	1,500	257.1		0%	0%	-
15	5	1,000	277.1	50%	0%	50%	-
16	6	800	352.0	50%	0%	50%	-
17	6	600	373.3	50%	-	50%	-
18	7	400	235.8	50%	-	50%	-
19	7	200	58.7	50%	-	50%	-
20	8	100	21.5	40%	-	60%	-
21	8			30%	-	70%	-
22	9			20%	-	80%	-
23	9			10%	-	90%	-
24	10				-	100%	-
25	10				-	90%	10%
26	11				-	75%	25%
27	15				-	50%	50%
28	16				-	25%	75%
29	17				-	10%	90%
30	18				-	-	100%
31	19				-	-	100%
32	20				-	-	100%
33	21				-	-	100%
34	22				-	-	100%
35	23				-	-	100%