EFFECT OF LIVE FEED ON GROWTH AND SURVIVAL OF *ARCTIC CHARR* (*Salvelinus alpinus)* JUVENILES

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ABSTRACT

Juveniles of Arctic Charr were raised for 20 days in buckets with a density of 10 juveniles per litre according to 5 diet treatments: artificial food as a control, microworms, artemia, enriched artemia and the combination artemia-microworms. The aim was to measure the effect of these different treatments on growth and survival. The results showed that there was a significant difference of growth performance among treatments. Fish fed on artificial feed had the highest weight 0.079 g ± 0.021 and it significantly differed from other treatments. Fish fed with enriched artemia, microworm and the combination of artemia and microworms achieved similar growth performances. Combining microworms with artemia, the growth performance of Arctic Charr juveniles was better than when fed with microworms only. Overall, the mortality rate was lower than 4%. The highest mortality rate was recorded with the artificial food, 3.4% ± 1.0 while the lowest rate was obtained with enriched artemia 0.6 % ± 0.0. The average weight of the prey used was 1.70 E-06 g ± 1.2 E-07, 1.9E-06 g ± 5.8 E-08 and 5.1 E-07 g ± 8.1 E-08 respectively for nauplii, enriched artemia and microworms. Their size was 0.50 mm ± 0.06, 0.85 mm ± 0.07 and 1.44 mm ± 0.21 respectively for nauplii, enriched artemia and microworms. The weight ratio between the nauplii and microworm is 3.3 g. Microworms could be a good candidate to replace the traditional artemia which is quite expensive, and which, probably due to osmotic stress, does not survive as long in freshwater as the microworms. The duration of this study did not permit the determination of nutritional value yet highlighted the importance of these worms. Its value in unsaturated fatty acid, amino acids and vitamins has not been determined. Future research should test the effect of microworm enrichment on juveniles and determine its adequate density to match tested fish species nutritional needs.

Key words: microworms, enriched Artemia, Arctic Charr juveniles, growth

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1 INTRODUCTION

1.1 Background

Aquaculture was introduced in Cameroon in 1948 by the colonial administration. The production system is mostly the polyculture of *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) sometimes in association with *Heterotis niloticus* (Kanga) and *Cyprinus carpio* (common carp). To date, aquaculture production in Cameroon is still low, about 10 000 tons (MINEPIA, 2016), compared to the potential of the country in terms of water resources. 420 km of coastline, 3.5 million hectares of water including rivers, craters and artificial lakes where many species of fish live that can be cultivated. There is biodiversity in the country, the climate and soil quality, for example more than 67% of southern Cameroonian soils are ferralitic with high water retention capacity (MINEPIA & FAO, 2009; Pouomogne, *et al*., 2008). Lack of seed and quality feeds have been cited as the most important constraints for aquaculture development in Cameroon (MINEPIA & FAO, 2009).

In aquaculture, the production strongly depends upon the availability of quality larvae or fingerlings. For most species, larval phase is one of the most critical during the production cycle. Larvae of most fish and crustaceans need mobile prey and encounter problems to accept inert/dry diets because of their poor enzymatic activity and their poor stomach development that does not allow them to digest the existing formulated diets (Pedersen & Hjelmeland, 1988; Pedersen, et al., 1987). Larval culture, particularly the start feeding of early larval stages, appears to be the major bottleneck for the industrial up scaling of fish farming.

In order to solve the problem of seed, the Cameroon government has created several aquaculture stations of which the main purpose is to produce fish seed to supply the producers. To date, the demand for fingerlings is increasing and is a veritable bottleneck for aquaculture development in Cameroon. The production of private farms and government stations does not satisfy the demand. A census of aquaculture farms carried out in 2012 estimated that 210 million fingerlings are needed in Cameroon (MINEPIA, 2012), while at that time the production was only at 0.2 % (MINEPIA. 2015).

This insignificant production of fingerlings is due to the high mortality which occurs during the larval phase. Poor management of the hatcheries, the poor water quality, the quality of broodstocks and especially the quality and the quantity of food can be the possible causes of this high mortality observed at this stage of rearing. Rearing fish larvae in hatcheries strongly depends on the permanent availability of a suitable food source. Many believe that the quality and quantity of food would have an effect on survival and growth of fish larvae. In the wild,

larvae of several species of fish generally feed on phytoplankton, zooplankton (Telesh, 1993; E. Agh, 2005). Thus, in a rearing situation, it is essential to feed the larvae with these types of food, unlike the inert food. These authors believe that rotifers are the most suitable for early life stages of fish.

In most cases in Cameroon, larvae are kept in the hatchery for 2 weeks after the resorption of their yolk sac. During this period, the larvae are fed with artemia. It has been observed that more than 50% of the larvae die before they leave the hatchery for the nursery tanks. Fry producers in Cameroon lose nearly 70% to 80% of their production before fish reach 5 g. Several studies show that artemia is deficient of certain nutrients such as certain essential amino acid, vitamins and poly unsaturated fatty acid which are required for larval growth and survival (Dantagnan, *et al*., 2010; Faramarzi, 2012; N. Agh & Sorgeloos, 2005). This deficiency could be one of the causes of mortality observed at the larval stage.

This work aims to develop different live feed culture techniques as well as enrichment methods, assess their nutritional value and to test their effect on growth and survival of Arctic charr larvae, a cold model fish species available in Iceland. This species is obviously different from those found in Cameroon but in the wild it also feeds on a large variety of live prey such as insect larvae, amphipod and zooplankton, mainly cladocerans (Amundsen & Knudsen, 2009).

This work will provide some results on the effect of different diets on Arctic charr survival and growth at early stage of development and will be helpful to adapt a similar protocol to the species of interest in Cameroon. In addition, the results of this study will not only benefit to the fry producers, but also an emerging group of Cameroonians who practice the aquaculture of ornamental species. The success that will certainly be generated by this research work on the performance of growth and survival of fry will enable the sellers of aquaculture products to enrich their shelves with two new products: artemia enrichers and worm starter culture. The fish aquaculture market in Cameroon is controlled by women, so the increase of production resulting from the implementation of these techniques in the production system will create more jobs for this segment of the population and improve their income. At the social level, the increase in aquaculture production will improve the supply of fresh fish that are qualitatively better compared to frozen fish.

Five types of live feed will be tested: microworm, non-enriched artemia, artemia enriched with Easy DHA Selco and artificial feed used as control. The choice of these ingredients and enricher is justified by their availability and their relatively low cost on the market. In addition, microworm (*Panagrellus sp.)* is a small nematode very rich in protein (48 %).

1.2 **The general objective:**

The overall objective of this work is to evaluate the survival and growth performance of juvenile Arctic charr under different feed types.

Specific objectives

- To optimize the culture of live preys (culture techniques, nutritional value, characteristics of preys).
- To measure the effect of microworm on growth and survival of Artic charr juveniles.
- To measure the effect of enriched Artemia on growth and survival of Artic charr juveniles.

2 LITERATURE REVIEW

2.1 Water quality for Arctic charr

Water is for fish what a house is for a man. It is in this ecosystem that it must feed, find protection, reproduce. Water is therefore of vital importance in aquaculture because its quality can affect the growth, health, reproduction and survival of fish. In waters intended for fish farming, knowledge of the physico-chemical parameter is the key of success of fish farming. Some factors such as temperature, pH, dissolved oxygen, ammoniac and carbon dioxide have a direct effect on the ability of fish to eat, grow and reproduce (Baroudy & Elliott, 1994). These factors according to Sæther, et al., (2016), influence the physiology, behaviour, and performance of the fish to a greater or lesser extent. Therefore, it is necessary to maintain good water quality to support maximum growth and reduce mortality of fish.

Effect of temperature

In fish culture, the temperature is a very important parameter because it conditions the general activity of the ecosystem. Each species of fish has its optimal range of temperature for feeding, growth and reproduction. The critical temperature limit for fish feeding and their survival must be known by the farmer to expect a good result (Baroudy & Elliott, 1994). With Arctic charr for example, Gillet, (1991) noted that ovulation was totally inhibited at 11 degrees Celsius, and when spawners were reared at 5 degrees Celsius and examined twice a week to detect ovulation, 80 to 90 percent survival to the eyed stage could be expected for fertilized eggs. For some tropical species like *Clarias gariepinus* or *Oreochromis niloticus*, ovulation appears when the temperature is above 20 degrees Celsius for *Oreochromis niloticus* and between 28-32 degrees Celsius for *Clarias gariepinus* (Pandit & Nakamura, 2010; Potongkam, 2006; Orina, P et al., 2016).

Temperature also affects growth, feed consumption and fish health. Some species eat and grow well with increasing temperature, but below or above certain limits, the fish stops eating and can even die. It is the case with *Oreochromis niloticus*, many believe that below 10 degrees Celsius it cannot survive for more than a few days (Dampin, *et al*., 2012) and its optimal growth at juvenile and fry stages is between 27- 32 degrees Celsius (Pandit & Nakamura, 2010), while growth rates for Arctic charr juveniles peak at 14–16 degrees Celsius (Siikavuopio, *et al.*, 2013), while optimal food utilization occurs at approximately 9 degrees Celsius (Supattra, 1982).

Effect of pH

This parameter allows to evaluate the degree of acidity of water. In fish farming the level of pH can affect growth, survival and reproduction; the normal for most species is between 6.5 to 9.0. Several studies show that excessively high or low pH affect the growth and survival of fish. Reproduction ceases for most species when pH is below 5 or above 9.6, in addition, fish is also exposed to fungal diseases and to several physical pathologies such as bone

malformation (Lloyd, 1992). A change of pH., can have a long-term effect on the living organisms in the water body. Many factors such as CO_2 and temperature can affect the level of pH in the water. $C0_2$ is the most common cause of acidity in the water (Håkanson, 2006). Temperature and pH influence also the proportions of total ammonia nitrogen (TAN) present as ammonia and the ammonium ion. According to Sæther et al., (2016), the proportion of the most toxic form, ammonia, increases as pH and water temperature are increased. (Appendix 1: Fraction of toxic (un-ionized) ammonia in aqueous solutions at different pH values and temperatures).

Effect of dissolved oxygen

Like humans, fish also use oxygen; however, the oxygen available to them is that which is dissolved in the water. Dissolved oxygen is the level of free non-compound oxygen present in the water. It is one of the water parameters on which the producer must take care because its effect is often dramatic for the fish. In fact, the fish farmer must regularly observe any sign of oxygen deficiency and act quickly. It is found in microscopic bubbles mixed in between water molecules. It can enter into the system through direct diffusion and as a by-product of photosynthesis. This means then that the level of dissolved oxygen in the water can be increased through mechanical aeration. A normal dissolved oxygen level is approximately 7-9 mg/l in 25˚C freshwater at sea level. Most fish prefer a minimum dissolved oxygen level of 5 mg/l (R F P, 2010). But this minimal level hides some disparities, since some species like Clarias can withstand a concentration of 1mg /l for a long time and mortality starts if it is exposed for a long time to a concentration of less than 0.3mg / l (Ekubo & Abowei, 2011). Griffiths, *et al*., (2006) have observed that salmonids can survive at a concentration of 4mg / l and begin to die only if the concentration of dissolved oxygen is below 3mg / l. Many factors such as size of fish, the rate of feeding, the temperature, pressure and salinity affect the level of oxygen in the water. Generally, dissolved oxygen decreases exponentially as salinity increases, and when the pressure increases, the level of dissolved oxygen also increases (Lembi, 2001). Therefore, the oxygen level decreases with increased temperature and when the level is lower than 2 or 3 mg/l, fish die (R F P, 2010; BFAR-Philminaq, 2007; Lembi, 2001).

2.2 Size of prey and feeding behaviour of Artic charr juveniles

As well as in raising land animals so it is in fish farming, food takes up an important part of the total cost of production. In fish farming, feed represents 40-50% of the production costs (Craig & Helfrich, 2002). Food must commonly be formulated according to the nutritional needs and feeding behaviour of the species. Improved knowledge of fish feeding behaviour and digestive physiology can contribute to the optimization of diets and feeding protocols and can eventually improve growth rates and survival. The nutritional effectiveness of a food organism is in the first place determined by its digestibility and, as a consequence of its availability, size and form (Agh & Sorgeloos, 2005). This supposes that when the fish has different types of feed in its environment, it tends to select the prey which not only suits the size of its mouth, but also that which is easily digestible and which allows it to gain in energy because spending energy searching for and capturing prey can have severe consequences particularly for larvae if they are not successful at feeding. It is therefore preferable that prey

is not only adequate in size but also that they are abundant to ensure good growth. In a study of the feeding behavior of fish, Dabbadie, (1996) noted that when prey is abundant, according to the size of fish, predation is mainly on large forms, especially because they are more visible. Choice is oriented towards a large prey because the consumption of small prey is not efficient in terms of energy gain for the fish (Craig & Helfrich, 2002). Dahlhansen et al., (1994) have also observed this prey selection phenomenon with Arctic charr, which had a preference for large cladocerans in an environment dominated by copepods. But these authors note that it is due to the high mobility of copepods compared to cladoceraus. Arctic char spends less energy on capturing cladocerans. When fish search for food, they are more attracted by live than inert prey, if the size of inert prey is greater. Other factors that influence feeding behaviour included the shape of the prey, the water temperature, the colour, the daylight condition.

For several species, food is detected by using eyes, smell or by mechanical stimuli such as free neuromasts and lateral line (Craig & Helfrich, 2002; Dabbadie, 1996). Visual observation allows fish to appreciate the shape and size of the prey and compare it to the diameter of its mouth. The ability to use these different organs is not the same for all species. Some species such as Arctic charr are visual predators and use primarily their eyes to detect and appreciate prey. It has been observed that these species have a preference for benthic zone and sometimes refuse to go back to the surface to catch a prey (Jørgensen & Jobling, 1990). On the other hand, the food compartment does not constitute a barrier for certain species such as *Clarias gariepinus* (Abbas, 2010) and *Oreochromis niloticus* (Dabbadie,1996) which, although being respectively benthic and pelagic species, seeks prey out in all strata of the water body especially when they are hungry. Studies on the dietary behaviour of these species have revealed that, even at the fingerling stage, they are an omnivorous, opportunistic species, (Abbas, 2010; Dabbadie,1996) which does not hesitate to consume several varieties of prey. This is certainly one of the reasons why Clarias is the most used in polyculture to control the overpopulation of tilapia.

2.3 **Nutritional requirements for Artic charr juveniles**

In animal production as in fish farming, feed plays an essential part and has implications for the composition and the quality of the final product. By correctly feeding the fish the farmer will be able to improve production and increase his profit. Each fish species has its nutritional needs and these needs differ depending on feeding habit and the stage of growth. The challenge in fish feeding is the management of the larvae or juvenile phase where the producer must be rigorous on the nutritional quality of food because the poor stomach development and low level of enzyme production in larvae will not allow them to digest the existing formulated diets (Pedersen & Hjelmeland, 1988; Pedersen, et al., 1987). Proteins, carbohydrates and lipids appear to be the most distinct nutrient groups that the body metabolizes to produce the energy it needs for numerous physiological processes and physical activities (Gatlin, 2010). In fish farming, it is important that protein and amino acid requirements are known and matched for each of the fish species reared to maximize the growth and health of the fish. For Arctic charr which is a carnivorous species, high growth rate has been achieved on diets containing 44-54% protein (Tabachek, 1986) while the best growth for tilapia, Clarias and common carp three

tropical species is obtained with a food containing 35 % protein, 40-42% and 25-38% respectively (R F P, 2010; Verreth & Den Bieman, 1987).

The resorption of the yolk sac is a critical stage in the life of the larva because its nourishment will change from liquid to solid, generally with different nutritional composition. The adaptability to this new food is a major challenge to the young fry because its stomach is not yet ready to digest the new food. Food larvae must be the highest quality as possible and must contain all nutrients principally all essential and non-essential amino acids because they are absolutely essential for every metabolic process. Less protein can be used if farmers meet the minimum requirement for the first limiting amino acid(s) instead of meeting a minimum protein requirement. Wilson, (2003) and Mente, (2006) made the same observation and suggested that even if the requirement of protein is not exact in the fish diet, it is important to have a well-balanced composition of essential and non-essential amino acids. In both fish and terrestrial animals, essential amino acids are those which the animal or fish cannot synthesize, and which must be brought by food. The deficiency of some amino acids can affect directly the growth of most species of fish. It had been found that lysine deficiency can reduce growth and feed efficiency in most fish species (Wilson & Cowey, 1985), so satisfy the requirement of amino acids can be a challenge for feed producers.

The main function of carbohydrates is to provide energy to the fish. Therefore, it has a fundamental role in fish because it needs the energy to capture its prey and to digest it. But the requirement of carbohydrate depends on the species and their feeding habits (Rønnestad *et al*., 2013). Omnivorous fish like *Oreochromis niloticus* need a high level of carbohydrate in comparison to Arctic charr which is a carnivorous species (Roberts, 1994; Wilson, 1994; Enes *et al*., 2011). In general, fish with a long intestine have a high level of carbohydrate compared to those with short intestine because their bodies are not equipped to handle large quantities of carbohydrate. That explains the results of Roberts, (1994) which found that the amount of soluble carbohydrate included in formulated food for carnivorous species should generally be less than 20%, whereas diets for omnivorous species generally contain between 25% and 40%.

Like carbohydrate, fats also act as the body's energy stores. They are also necessary to promote healthy cell function (Klein et al., 1997). In several species, lipids facilitate the absorption of certain nutrients, especially lipo-soluble vitamins, but the need for lipids is not the same for all species. Because of the high cost of protein raw material, the trend observed in food manufacturers is to reduce protein sources in the diet for lipid substitute in order to maximize profit. Although this practice helps to increase profit, it affects quality. Fish lipids include fatty acids and the most important one, principally for fresh water fish are omega 3 and 6 (n-3 and n-6) families (Craig & Helfrich, 2002) which are the High Unsaturated Fatty Acids HUFA. Tocher, et al., (2003) reported that fresh water fish are not able to produce Essential Fatty Acids (EFA), particularly eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA:22:6n-3), so these EFA must be provided by food and the quantity of 18 carbon n-3 fatty acid, linolenic acid (18:3-n-3), required for fresh water fish range from 0.5 to 1.5% of dry diet.

It is fundamental to know that minerals are inorganic compounds, of which every animal and human organism needs to maintain itself in good health. There are two large groups of minerals:

the macro minerals that are required in great quantity by the organism, and the micro minerals that are present in the form of trace and which are of crucial importance for the maintenance of the health and specially the immune system (Klein et al., 1997; Craig & Helfrich, 2002). Fish also need minerals for their growth; thus, the fish feed must have a sufficient quantity of minerals because they are responsible for skeletal formation, cellular respiration, oxygen transport, immune function, and also for regulation of acid–base equilibrium. Fish have unique physiologic mechanisms to absorb and retain minerals from their diets and from the surrounding water (Halver & Hardy, 2002). An excessive intake of minerals either from the diet or from gill uptake causes toxicity, and therefore maintaining a fine balance between mineral deficiency and surplus is vital for aquatic organisms to maintain their homeostasis either through increased absorption or excretion (Lall & Tibbetts, 2009).

2.4 **Live feed culture and nutritional value**

Despite the variety and popularity of the various manufactured food that you find in the store, there is still a need to feed your fish with some varieties of live food as often as you can. In addition to adding a welcome change to their feeding routine, it also introduces a lot of nutrients that may be lacking in prepared food.

Live food organisms include all plants (phytoplankton), animals (zooplankton) and another type of microorganism which are used in fish and shellfish larval rearing systems. Live foods are able to swim in the water column and this ability to move makes them more appealing than formulated food (Bengtson, 2003).

Microworms (Panagrellus sp.) are one of the simplest live fish food to culture. Fish fry and fingerlings will find in this feed an excellent choice. They are prolific small nematodes rich in protein and energy, that are great for feeding small fish fry, (Schlechtriem et al., 2004; JJ, 1988). It has been found that they have a high reproductive rate and one female can release 40 young for each 1 or 1.5 day; the optimum yield will result at 20°C to 27°C and they are 40- 50 microns in diameter and 1.5 mm in length (Chappell & Fisheries, n.d.). This type of nematodes is easy to produce in tropical areas. Their small size is an advantage and also an inconvenience. The advantage is that their size allows them to swim in the water for more than twelve hours (Ozaydin-Ince, et al., 2011; Chappell & Fisheries, n.d), so it would be easy for fish to feed before reaching the base. In addition, because of their size and shape, they can be successfully fed to fish that are small. But the main inconvenience is that they would not be visible enough and the fish would spend a lot of energy in capturing them. However, the analysis of the nutritional value of microworms suggests that these nematodes are an extra food for fish and especially fish larvae after absorption of their yolk sack. Their high rate of protein and amino acid and their low production cost make these nematodes a potential substitute for Artemia or rotifers (Majdi & Traunspurger, 2015; Kumlu & Fletcher, 1997). It is possible to improve their nutritional value, especially in poly-unsaturated fatty acids through an enrichment process (see Appendix 2. The comparison between protein and amino-acids of penagrellus and artemia Biedenbach, et al., (1989) concluded that nematodes appear to be a promising food source for commercial penaeid.

Nutritional Value banaworm:

- Protein: 48%
- Fat: 21%
- Glycogen: 7%
- Organic Acids: 1%
- Nucleic Acids: 1%

Source: (Schlechtriem et al., 2004)

Artemia has been currently recognised as a high value food. It represents almost the totality of live prey used in most hatcheries. With 61.6 % of protein content (Rouse, *et al.,* 1992), it tends to be the most practical food for early stages of fish and crustacean larvae of several species (De Groot, 1974). Its average length of 400-500 μm (Merchie, 2014. Amarouayache & Kara., 2010) is ideal for the small mouth of the larvae; but using them as a larval food is not fool proof, because some nutrients of the Artemia can lose their value after a certain time. This is the case for its dry weight and its protein and calorific content which can decrease by 25% within 24 h after hatching when kept to 25 degrees Celsius (De Groot, 1974). This means content that the nauplii must be fed to the larvae as soon as possible after hatching or be stored at low temperatures to decrease their rate of metabolism. Another drawback is that although containing a high level of protein, they have been found to be nutritionally deficient, especially in the long chain polyunsaturated fatty acids (PUFAs), and this deficiency tend to reduced growth and increased the mortality of larvae. (Treece, 2000; Hawkyard, *et al*., 2011) . However, several studies show that this deficiency can be corrected by enrichment of the Artemia nauplii.

3 MATERIALS AND METHODS

3.1 Experimental units

Juveniles used in this study came from the fertilization of eggs produced at the Hólar aquaculture station in charge of the Arctic charr breeding selection program in Iceland They were brought on 19th December 2017 to Verið, Sauðárkrókur, the research facility of Hólar University College where the experiment was conducted. The eggs were then counted and spread on a rack and placed inside an incubation tank after acclimation to avoid thermal shock. A few hours later, they started hatching and 24 hours after, the hatching rate was estimated at 80%. The water temperature was taken every day at 10 am (picture 1a), and it ranged between 6.3 and 8.2°C. After 171.7 degree-days, 80 % of the yolk sacs were depleted and the fish were then randomly split into ten cylindrical flow-through tanks (15 L). Each tank contained 150 individuals (picture 1b). A sample of juveniles (10%) was taken to be weighed. The average weight was obtained by dividing the total weight by the number of individuals. The individual length was not measured due by the fragility of the fry at this stage of development.

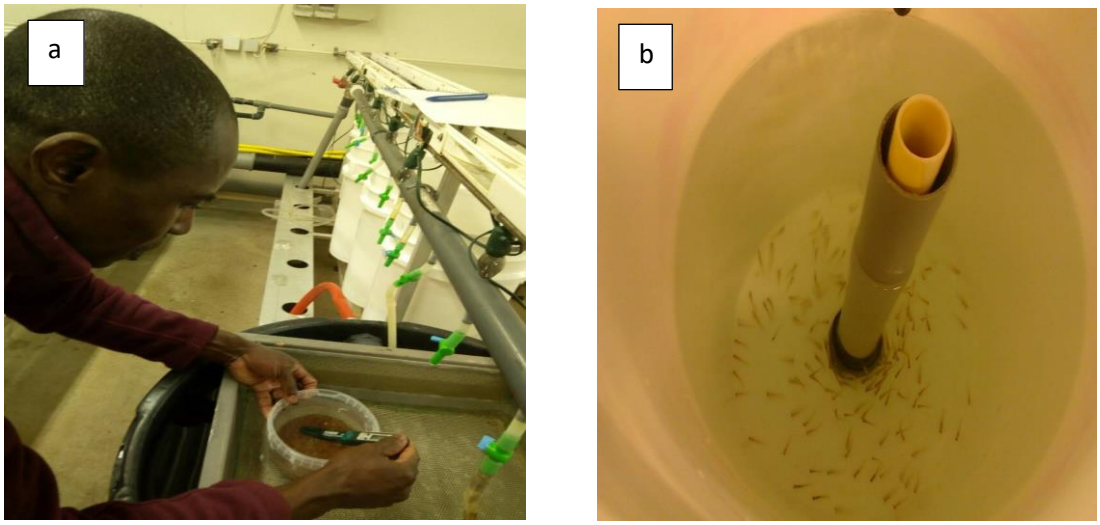

Figure 1: a) Taking the temperature of the eggs on their arrival, b) Rearing tank with juveniles

3.2 Experimental set up

The experiment consisted of comparing five diet treatments in duplicates. The draw of the various treatments was as follows:

- Artificial feed which is the control
- Microworms
- Microworms + Artemia (Ao)
- Artemia (Ao)
- Enriched Artemia (A1)

3.3 **Optimization of live prey cultures**

Production of nauplii Ao

They were obtained from cysts of Artemia provided by INVE. Several techniques were tested in order to obtain the best hatching rate. Whatever the technique, the cysts were first hydrated in freshwater for one hour (picture 2a).

Figure 2: a) Filtration of cysts after hydration, b) Incubation of artemia cysts and enrichment of nauplii (white colour)

Technique using only chlorine

The cysts were decapsulated with chlorine without NaOH. After hydration, the cysts were collected in the bowl containing 0.5 liter of freshwater, then 12 ml of bleach was added in the bowl and the solution was stirred for 10 minutes. The cysts were flushed with freshwater to remove any chlorine remnants. The cysts were decapsulated with chlorine without NaOH. After hydration, the cysts were collected in the bowl containing 0.5 liter of freshwater, then 12 ml of bleach was added in the bowl and the solution was stirred for 10 minutes. The cysts were then flushed with freshwater to remove any chlorine remnants. After this, the cysts were transferred into the incubation cone containing 1.3 L of sea water at 28 degrees Celsius for 24 hours with aeration and 40 W fluorescent light. The incubation cone was bottles of mineral water cut at their base. These bottles were placed in a Bain Marie (picture 2b). Two 50 W heating resistances were used to maintain the temperature at 28°C .

Technique using chlorine and NaOH

After the one-hour hydratation period, the cysts were collected in a 63-micrometer sieve and flushed with freshwater. The decapsulating solution prepared with 7.5 g of NaOH plus 50 ml of sea water at 16 degrees and 82.5 ml of chlorine was added in the bowl containing the cysts with 0.4 litter of sea water at 16 degrees. The next step consisted of stirring it until the color of

the cysts turned from brown to orange. Different timings were tried: 5 minutes, 8 minutes, 10 minutes and 15 minutes.

The cysts were then filtered and thoroughly flushed to remove any chlorine remnants. To neutralize the chlorine residuals, a solution prepared with 50 ml of vinegar and 300 ml of sea water at 16 degrees was added to the cysts. Then the cysts were transferred into the incubation cone containing 1.3 L of sea water at 28 degrees Celsius for 24 hours with aeration and 40 W fluorescent light. The incubation cone was bottles of mineral water cut at their base. These bottles were placed in a bain Marie. Two 50 W heating resistances each were used to maintain the temperature at 28°C .

Technique without decapsulation solution

After hydration in the water for one hour, the cysts were transferred directly into the incubation cone containing 1.3 L of sea water at 28 degrees Celsius for 24 hours. Incubation and hatching occurred under the same conditions as described above.

Estimated number of cysts per gram

Estimation was carried out by using the bio-volume method which consisted of weighing a certain quantity of cysts and mixing them in 100 ml of water. After stirring, 1 ml of this solution was sampled and diluted with 9 ml of water. Then, 0.1 ml was finally sampled and put in a petri dish to count with the help of a binocular magnifier. This counting was done in quadruple to reduce the margin of error. The average of the four counts was calculated and multiplied by 10,000 to arrive at the number (**C**) of cysts and this number was divided by the weight of the cysts used to get the number of cysts per gram.

Harvesting and determination of hatching rate

To harvest the nauplii, the incubation cone was covered with black tissue and the top of the bin was illuminated. The opening of the cone allowed the harvest of the nauplii. The nauplii are attracted by the light. The non-hatched cysts sank to the bottom and were eliminated by opening the valve. Then the nauplii were collected in the sieve making sure to stop the flow as soon as it was close to the water surface where empty shells were floating.

After harvesting, the nauplii were stored in a beaker containing 100 ml of water. The number of nauplii was estimated following the same method as described above (bio-volume) and the number N of nauplii was obtained by multiplying by 10,000 the average of the counts in quadruple of the contents of 0.1 ml of the solution, but with the technique using NaOH and chlorine. The nauplii were stored in the beaker containing 50 ml. and the average number was multiplied by 5,000.

To estimate the hatching rate by type of techniques used, we performed seven tests. Three tests for the technique using chlorine and NaOH, three tests for the one without chlorine or NaOH and a single test for the technique using only the chlorine. For each technique we averaged the hatching rate.

The hatching rate is given by the relation: $HR = \frac{N}{\text{Ci}} \times 100$

Where Ci is the number the cysts.

The opening of the cone allows the nauplii to be harvested. The nauplii are attracted by the light. The non-hatched cysts sank to the bottom and were eliminated by opening the valve. Then the nauplii were collected (picture 3) in the sieve making sure to stop the flow as soon as it was close to the water surface where the empty shells were floating.

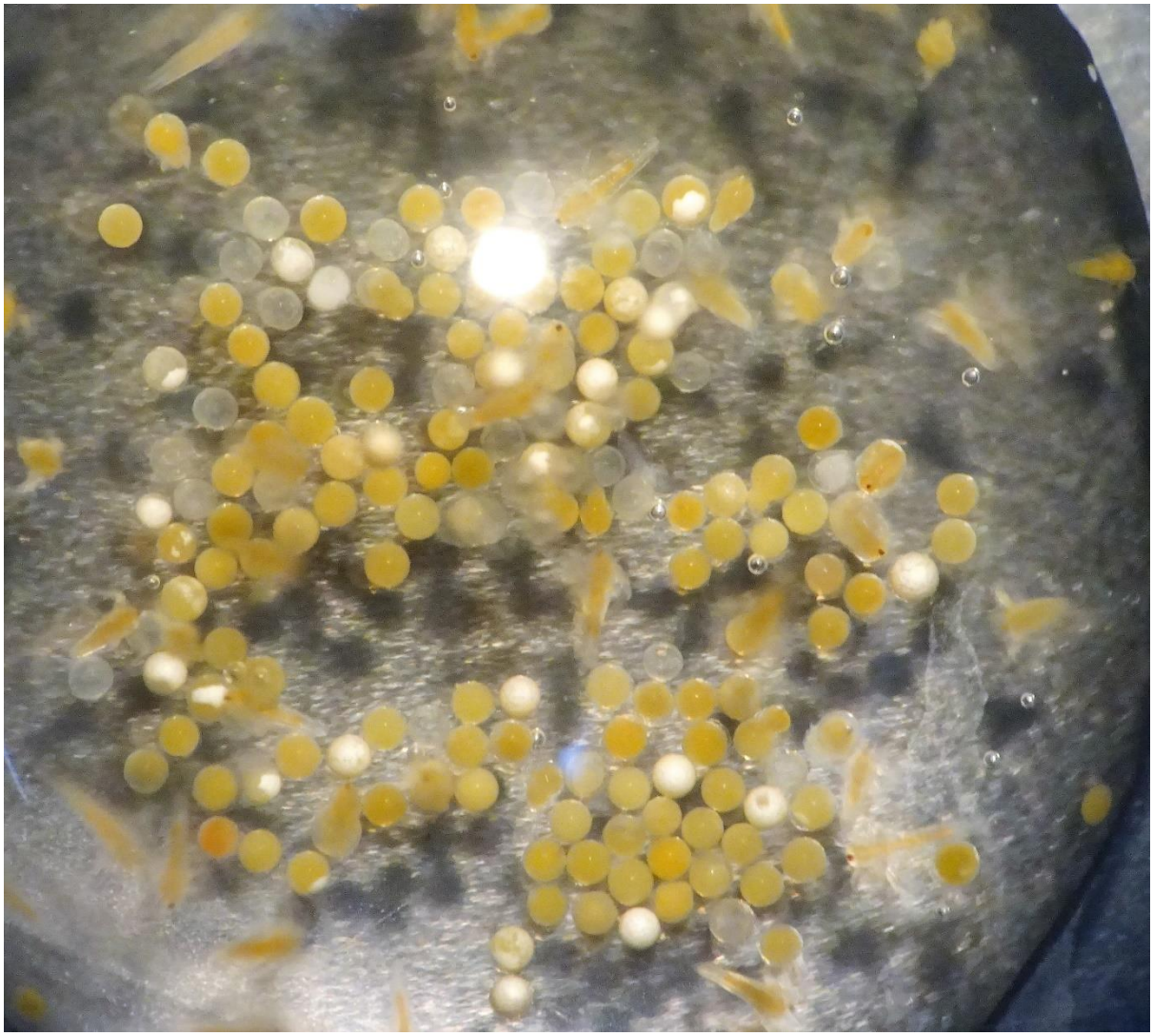

Figure 3: Type of products obtained after incubation of cysts: dead eggs are in white, in yellow are the decapsulated eggs and fusiform are the Nauplii

Calculation of Artemia ration

The experimental design provided 2 tanks where the juveniles were fed on non-enriched Artemia (Ao), 2 tanks where the juveniles were fed on enriched Artemia (A1) and 2 tanks where given half non-enriched artemia and half microworms (2Ao, 2A1, 1Ao). Every day, the number of nauplii produced was counted by the bio-volume technique described above. The

amount of nauplii necessary to feed larvae daily was obtained by splitting the total amount of nauplii produced into several portions according to the treatment.

We began the experiment with a daily ration of 50 Nauplli (Ao) per individual. After three days of feeding, we sampled a volume of water in the tanks to evaluate the food consumption by the larvae. To do this, we used a control tank without fish, but with preys (Nauplii and microworms). In each tank we had 15 ml at a rate of 5ml on the surface, 5ml in the middle and 5ml at the bottom. These samples were observed with a magnifying binocular. In the tanks containing the juveniles, there were no prey while in the control tank several microworms and nauplii were found dead. These preys were not accounted for because they were already very decomposing and fragmented. From the 5th day of feeding, we doubled the daily ration.

Enrichment of nauplii

The number of nauplii harvested was divided into 5 parts since we had 5 treatments where the nauplii were used as food. Each day 2/5 of these new nauplii were enriched for 24 hours in a 1.3 litre cone bottle.

The enhancer we used was Easy DHA Selco at a dose of 1g per litre according to the manufacturer's recommendation. For the 1.3 litre conical bottle, 1.3 g of enhancer was used.

The product was mixed with 1 litre of seawater in a bottle of mineral water. After that, the solution was transferred to the conical bottle containing the nauplii and filled up with 0.3 l of sea water. The culture medium was constantly aerated and illuminated by a 40 W light. The nauplii were kept in the culture medium for 24 hours. After 24 hours, the enriched metanauplii were harvested and counted as previously described.

Microworm culture

Microworm (*Panagrellus sp.)* is a small nematode very rich in protein (48 %) and measuring 40 to 50 microns in diameter and 1.5 mm in length.

Production of microworms

The production was made in a plastic container 20 cm high and 12 cm in diameter. The culture medium consisted of oats, yeast and the starter culture. The production process consisted of mixing 400g of oats with 300ml of freshwater. Then, the starter culture and yeast were added in the culture. The container was covered with a perforated lid to facilitate the respiration of the microworms. To maintain the temperature up to 24°C, a 40 W light was used during the production cycle. Under such temperature conditions, the microworms began to develop in the culture medium 3 days later. On the 4th and 5th days, colonies of microworms were observed on the walls of the container (picture 4).

Figure 4: A colony of microworms around the bucket

Microworm harvesting and counting

To harvest the microworms, we used a small paint brush that we passed every morning around the container. Then the paint brush was dipped into small buckets containing 100 ml of water to collect the harvested microworms. To estimate the number of microworms, 1ml of this solution was diluted with 9 ml of water. Then we took 1 ml of the new solution to count in quadruple. The average of these 4 counts was multiplied by 1.000 to ascertain the number of microworms in 100 ml of the start solution.

Calculation of the ration of microworms

To estimate the ration of microworms, we made a volume ratio with the artemia because both organisms have a cylindrical shape. The volume of a cylinder being $V = \pi r^2 h$. The diameter of the microworm is 40 microns for a length of 1.5 mm. The Artemia has a length of 470 micro for a diameter of 200 microns. According to this, nauplii can be equivalent to 6 microworms. So, the number of microworms per tank is 6 times the number of nauplii.

Artificial food

The starter INICIO Plus feed was used as a control. It is specially formulated for juveniles after the yolk sac stage. This feed emphasizes nutritional stability with boosted vitamins and immune-modulating ingredients and a high digestibility to promote good health and growth of

fry. Its protein content according to the producer is 63%, the fat rate is 11 % and the size 0.4mm. The amount of artificial food was calculated on the basis of total biomass. During the 21 days of the experimentation, each tank concerned by this ration received 5% of the total biomass.

3.4 **Estimation of prey weight and length**

To estimate prey weight, we filtered a known volume using 20-µm Whatman filter (110 mm diameter). This filter was dried for 24 hours at 66°C in an incubator to have a dry weight and then put in a desiccator for one hour before being weighed. After filtration (picture 5a), the filter containing the prey was put back to the incubator for drying at 66 °C for 24 hours (picture 5b), then to the desiccator for one hour before weighing. This weighing gives the dry weight (W_2) of the filter containing the prey. The total weight (Wt) of the prey was obtained by calculating the difference between the two weights of the filter. The individual weight (Wi) was obtained by dividing this total weight by the number (N) of prey.

So, $Wi = \frac{Wt}{N}$ where Wt= W_2-W_1 and N is the number of prey. It can be nauplii (No), metanauplii (N_1) or microworms (Nw).

For each prey type, the experiment was repeated three times. At each weighing, the amount of prey filter was estimated by the method of bio-volume. Four counts were carried out each time.

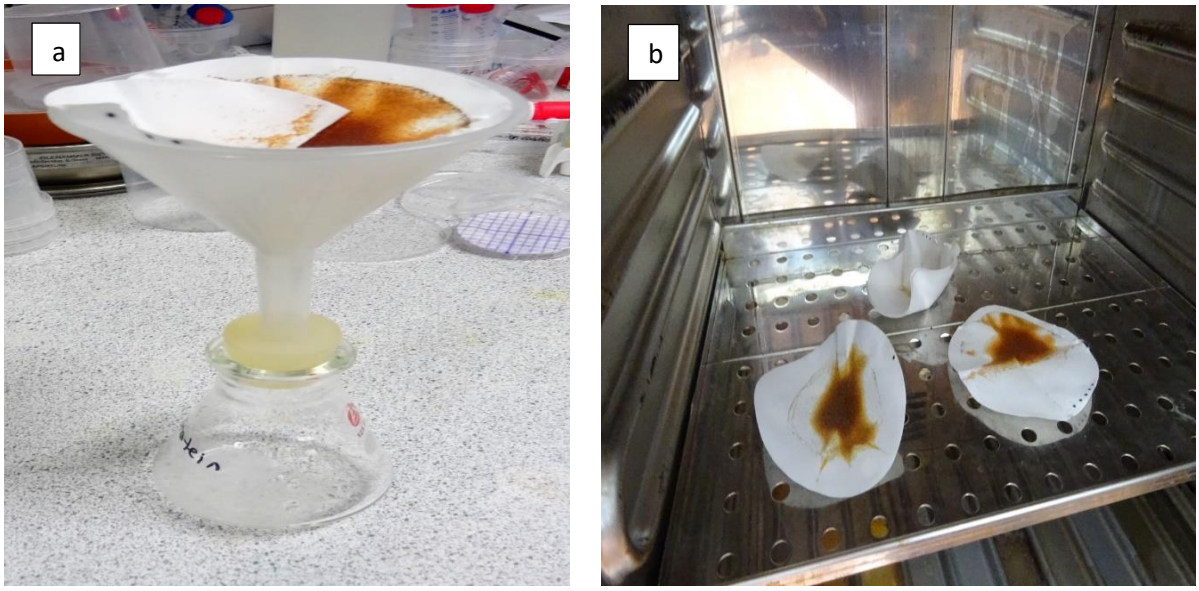

Figure 5: a) Filtration of prey with Whatman filter paper, b) Drying prey after filtration

For the length measurement, the preys were pictured using a camera on a magnifier binocular and the image J software was used to measure them. We measured six microworms, fourteen nauplii Ao and eigth metanauplii A_1.

3.5 **Nutritional analysis**

Each type of food was analyzed at the nutritional laboratory of Iceprotein in order to determine their biochemical constituents such as moisture for dry food, total protein, total lipid, ash and carbohydrate.

The moisture content:

It was determined by drying a 5 g sample at 110°C overnight followed by cooling it in a desiccator before reweighing.

Crude protein

Crude protein was deduced from total nitrogen content from a 1.0 g sample which was determined in a Kjeldahl system following acid digestion and titration of sample distillate according to the ISO standard (ISO 5983, 2005). Crude protein content was calculated as N×6.25 (Nordic Committee on Feed Analysis, 1976).

Total lipid

Total lipid was determined gravimetrically following ethyl-ether extraction from a dried sample according to Ba 3-38 (AOCS, 1998) in a Soxhlet extractor.

Ash

Ash content was determined as total inorganic matter by incineration of a 10 g sample at 550°C overnight followed by cooling it in a desiccator before reweighing according to ISO standard (ISO 5984, 2002). Since time was not available to collect 10 g of microworms which was the minimum that could be analyzed, the fat and ash rate could not be determined for the microworms.

Carbohydrates

Carbohydrate rate was deduced from other nutrients.

Some formula:

Carbohydrate= 100- (ash+water+H protein+fat)

Dry weight= 100-water

Protein in dry weight=H protein*100/Dry weigh

3.6 Conduct of the study

Before putting them in the water, a sample of juveniles was weighted in order to determine their initial weight. To obtain this initial weight, a sample of 25 larvae was randomly taken in each tank. These juveniles were weighed and the value obtained was divided by 25 to obtain the average weight.

The study lasted 21 days, the temperature and dissolved oxygen were measured every day at 10 am.

A digital electronic probe (Multi meter) was used to measure different water quality parameters such as water temperature and dissolved oxygen (DO).

The fish were fed four times per day at 11 am, 1 pm, 3 pm and 4. 30 pm.

Every day before feeding, the dead larvae, non-consumed food was removed and noted in the daily register.

- ❖ After 21 days, all the fish from each tank, were collected to be weighed. Based on this measurement and according to the number of fish in each tank, we estimated the average weight. We also sampled 20 fish in each tank that were individually weighed and sized (total length). These two measurements were compared using a linear regression method that enabled an estimation of the individual average weight at the beginning of the experiment. The average weight calculated from the method where we collected all the fish was indeed systematically higher than the one where the fish are individually weighed (weight of the water when collecting all the fish together).

Wet weight of juveniles was measured to the accuracy 0.0001g sensitive balance and graded ruler.

Growth rates were calculated according to Forence & Harrisson, (2012).

- Specific Growth Rate (SGR) will be calculated and converted into percentage thus:

$$\boldsymbol{SGR}\ (\%\ day) = \frac{100 \times [In\ (\ final\ body\ weight) - In\ (initial\ body\ weight)]}{Rearing\ period\ in\ days}$$

Where "In" = natural logarithm

- Survival Rate (SR) will be calculated and converted into percentage thus:

$$\boldsymbol{SR}\ (\%) = \frac{100 \times total\ number\ of\ fish\ harvested}{total\ number\ of\ fish\ stocked}$$

3.7 **Statistical analysis**

Total length, weight and condition factors measured at the end of the experiment were compared using a nested ANOVA with diet treatment as a fixed factor and tank as a random factor nested to treatment.

4 RESULT

4.1 Optimization of live prey culture

Estimation of the number of cysts per gram

Table 1: Estimation of the number of cysts per gram

	Cyst Weight	Dilution Volume (ml)	Total Number of cysts	Number cyst/g
Incubation 1	0.484	100	230000	475010.33
Incubation 2	0.597	100	210000	351699.88
Incubation 3	0.597	100	282500	473120.08
Average				**433277**
STDEV				70653.9

This table shows that the average number of cysts per gram is approximately 433277 ± 70653.9. It is also observed that for the same quantity of cysts and diluted.

Hatching rate per technique

Table 2: Estimation of the hatching rate of Artemia

Technique used	**Cyst weight**	**Number cyst/g**	**cyst incub**	**Number Ao**	**Hatching Rate**
Chlorine	1.0049	433277	437133	387500	**88.64%**
Chlorine+NaOH	2.0407	433277	884188	60000	**6.78%**
without chlorine and NaOH	0.9835	433277	426149	280000	**65.45%**

It is clear from this table that the decapsulation of cysts with chlorine gives the best rate of hatching (88.64 %), but when chlorine is associated with soda, the hatching rate is very low (6.78 %) on the contrary to the incubation.

4.2 Estimation of the length

(See appendix 3 for length measurements of different preys.)

The average length of preys as well as the number measured is shown in the table below.

Table 3: Estimation of the average length of nauplii, metanauplii and microworms.

	Nauplii Ao	Metanauplii A1	Microworms
Number of preys measured	14	8	6
Average length	**0.50 mm ± 0.06**	**0.85 mm ± 0.07**	**1.44mm ± 0.21**

In terms of length, microworms are the longest (**1.44 mm ± 0.21)** of the three live prey while the naupli are smallest in size (**0.50 mm ± 0.06).**

4.3 **Estimation of the weight**

The average weight of prey as well as the number of prey filter is shown in the table below.

Table 4: Estimation of the average weight of nauplii, metanauplii and microworms

	Nauplii Ao	Metanauplii A1	Microworms
Number of filtrations	3	2	2
Average number per filtration	221,66	184,37	219,37
Average Weight	**1.7E-06 ± 1.E-07**	**1.8E-06 ± 5.8E-08**	**5.1E-07 ± 8.2E-08**

This result shows that microworms are the lightest of the three types of prey while metanauplii are the heaviest. The weight ratio between the nauplii and microworm is 3.3 g, which means that a nauplii weights 3.3 times more than a microworm**.**

4.4 **Nutritional value of different feed**

The analysis of the nutritional value of the rations used is shown in the table below.

Table 5: Nutritional value of food

Type	Sample	Ash	Water	Protein	Fat	Total	C	DW (g)	DW Protein	BE (kj/g)
a	1		75.5±0.22	10.7±0.07		75.5	13.8	21.5	43.6	12.7
b	3	0.7±0.07	89.4±0.06	7.0± 0.13	1.6±0.09	98.9	1.1	10.5	66.8	16.6
c	4	0.5±0.12	93.4±0.00	4.1±0.07	1.3±0.02	99.2	0.8	6.6	61.8	15.2
d	5	10.5±0.01	5.5±0.01	59.5±0.2	7.9±0.08	83.4	16.6	94.5	62.9	20.9

Note: The types of feed indicated above are as follows a) worms, b) A0, c) Al and d) artificial feed. DW denotes dry weight, C is the carbohydrates, BE is brute energy.

Nauplii are richer in protein (66.8 %) than all other rations used in this experiment while microworms are less rich (43.6). On the other hand, the metanauplii are less rich in carbohydrate and fat (0.8 and 1.3±0.02 respectively**)** than nauplii. In addition, it is noted that the artificial feed provides more carbohydrate (**13.8)** and fat (**7.9±0.08)** than other feeds. The estimation of the amount of energy provided per kilo Joule was found to be higher in nauplii than in metanauplii.

4.5 Temperature and oxygen

(The daily value of the temperature and oxygen is presented in Appendix 4.)

During the experiment, the temperature and oxygen were stable, and the average was about 7.6 ^{o}C ± 1.4 while the average level of oxygen was about 102.4 % ± 4.5.

4.6 Growth weight

Estimation of the individual weight at the beginning of the experiment.

By the correlation between the average weight estimated from individual weight measurement and the average weight estimated from total weight measurement (figure 6), we obtained an estimation of individual weight at the beginning of the experiment (table 6).

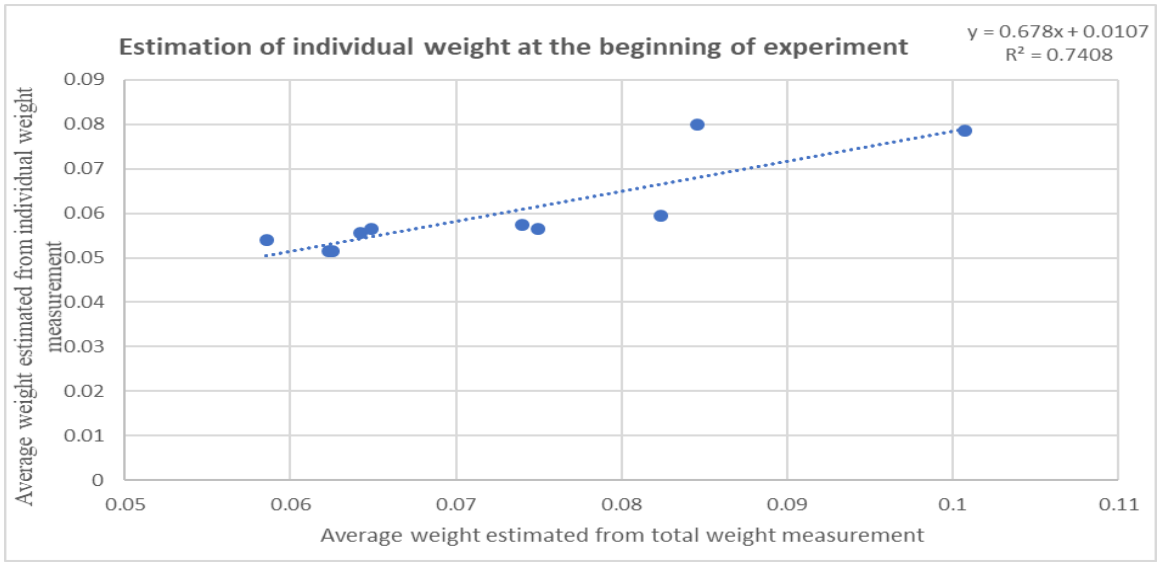

Figure 6: Correlation between the average weight estimated from individual weight measurement and the average weight estimated from total weight measurement

Table 6: Estimation of the individual weight at the beginning

Tank	Average weight (g)	Individual weight estimation(g)
1	0.07	0.060
2	0.060	0.050
3	0.050	0.045
4	0.07	0.060
5	0.060	0.051
6	0.054	0.050
7	0.051	0.045
8	0.034	0.034
9	0.043	0.040

No tank (treatment) effect was shown for any of the tested variables (Figure 7).

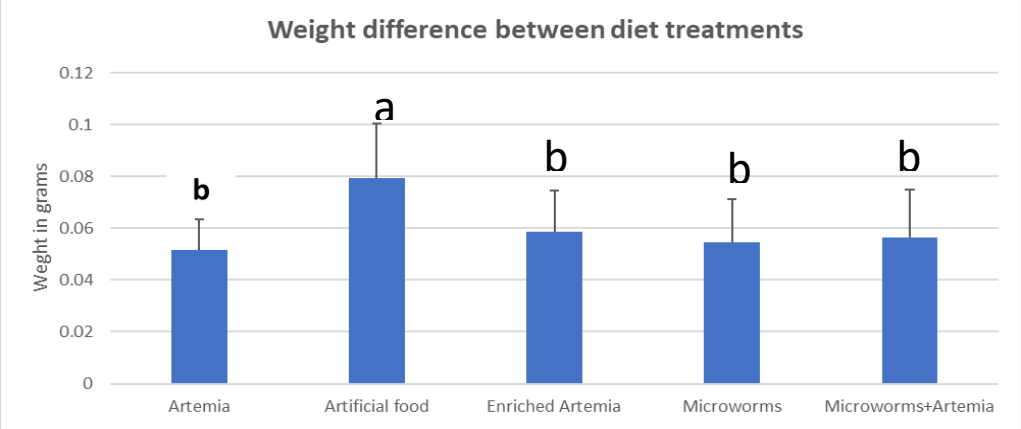

Figure 7: Growth weight between different treatments. Two different letters indicate a significant difference between treatments (Newman-Keuls post hoc test, $p < 0,001$)

At the end of the experiment, there was significant difference of growth performance among treatments, Fish fed on artificial feed had the highest weight 0.079 g ± 0.02 and it significantly differed from the other treatments ($F(4,190)=285,2$, $p < 0,0001$). Fish fed with Enriched Artemia, microworm and the combinaison of Artemia and microworms showed similar weight. By combining nauplii and microworms, weight gain is improved.

4.7 Growth length

The effect of the different treatments on the total length is represented by the graph below.

Total length was significantly different between treatments (($F (4,190) =62,8$, $p < 0,001$), Figure 8).

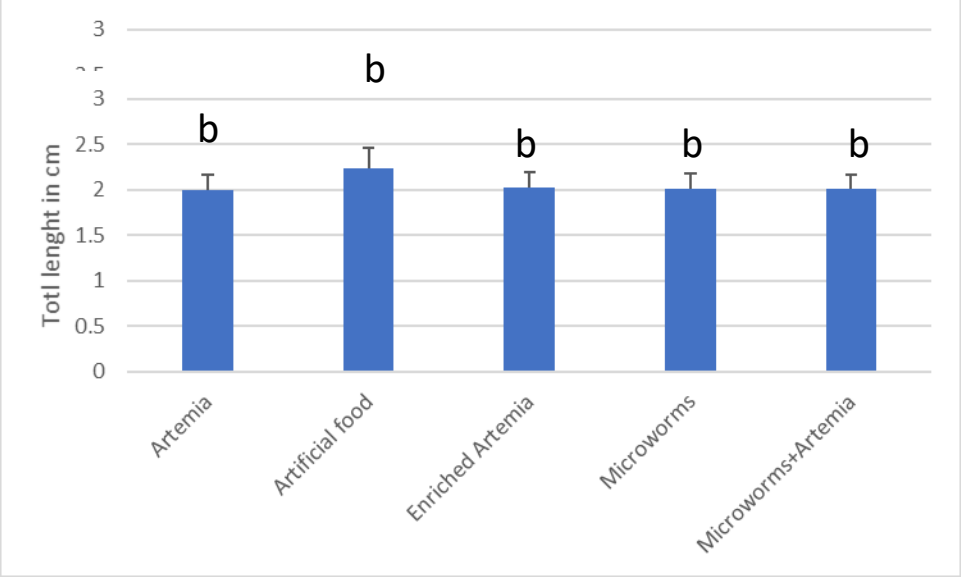

Figure 8: Length of fish between different treatments. Two different letters indicate a significant difference between treatments (Newman-Keuls post hoc test, $p < 0,001$)

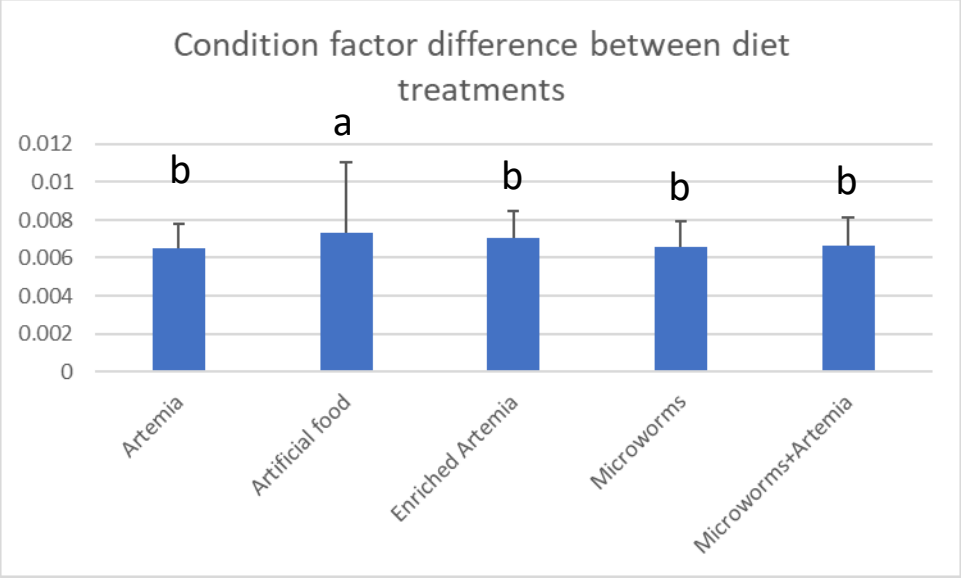

Figure 9: Condition factor difference between diet treatments

The condition factor was not significantly different between diet treatments.

4.8 **Specific growth rate SGR**

The SGR is illustrated in the graph in Figure 10 below.

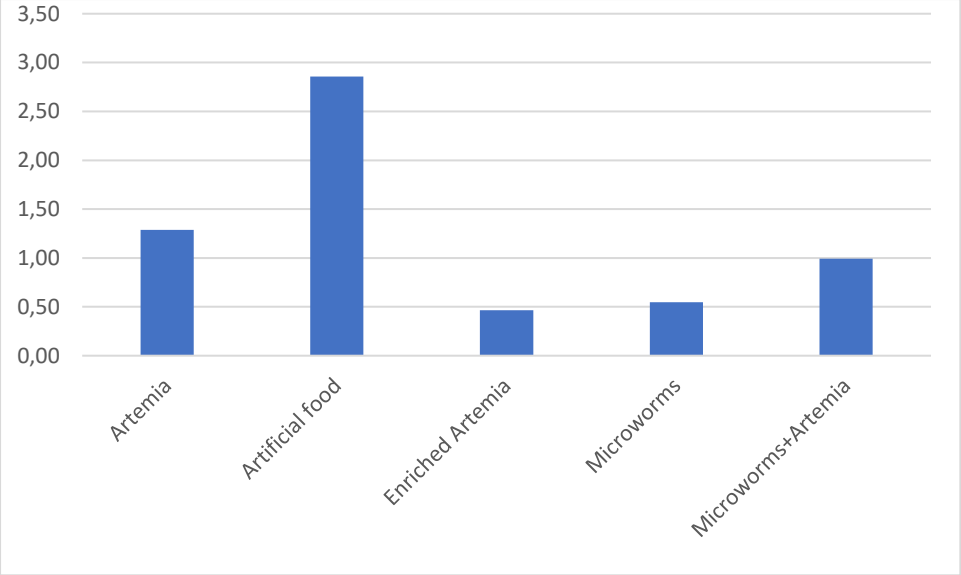

Figure 10: The specific growth rate

This graph shows that the best specific growth is obtained with artificial feed (0.030 ± 0.010) while the poor one is observed with fish fed with enriched Artemia (0.012 ± 0.010).

4.9 **Survival rate**

The survival rate is deduced from the table below.

Table 7: Survival rate of fish per treatment

Treatment	**% dead**	**STDEV**
Worms	2.6	2.8
Artificial food	3.4	1.0
Worm + artemia	2.0	1.9
Enriched artemia	0.6	0.0
Artemia	2.0	1.9

It is noted that the mortality rate is low for all treatments, less than 5%. The highest mortality was obtained with the artificial feed, 3.4% ± 1.0 while the lowest rate was obtained with enriched Artemia 0.6 ± 0.0

5 DISCUSSION

The objectives of this work were to optimize the culture of live prey by testing different culture techniques, characterizing the preys used and determining the nutritional value of different feeds. We then assessed the effect of different diets including microworms, artemia, enriched artemia and a combination of microworms and artemia on the growth and survival of Arctic charr juveniles.

5.1 Average number of cysts per gram

The average number of cysts obtained in one gram of cysts is approximately equal to 433.277 ± 70.659. This average is much higher than the 250 000 ± 12.021 found by Bio & Pham, (2004) comparing the cysts of American origin and Russia. The origin of the cysts, the size of the cysts (Bio & Pham, 2004; Amarouayache & Kara., 2010) and the quality of sampling can influence the results of counting. Indeed, the values we have obtained in our different collection and counting are higher than this average of Bio and Pham.

5.2 Average hatching rate

We tested three cyst incubation techniques and noted that the effectiveness of the hatching rate varies considerably depending on the technique used. The best hatching rate (88.64%) is obtained when the chlorine is used to facilitate the decapsulation of cysts and mixed for 10 minutes. However, when the action of the chlorine is combined with NaOH, the hatching rate drops down considerably (6.8%) although it was observed that the majority of cysts were decapsulated compare to when chlorine acts alone or when incubation occurs immediately after hydration without chlorine and NaOH. This method gave a quite interesting hatching rate, 65.4%, much better than the 6.78% obtained with the mixture chlorine-NaOH. This 65.4% hatching rate obtained by incubating without decapsulation of the cysts is close to 69% obtained by Amarouayache & Kara, (2010) which hydrated the cysts in a hydrogen peroxide (H_2O_2) solution at 3 ^{O}C for 30 minutes with a continuous aeration, but less than when the cysts are treated with chlorine.

If incubation without chlorine or NaOH is less time consuming, it is however inconvenient to have more than 30% of non-hatched cysts, which can be detrimental to fish if they ingest them. Treatment with chlorine when dosages are respected is best because it reduces the risk of mortality due to ingestion of cysts. In addition, the chlorine allows the deactivating of the embryonic diapause, improving thus the hatching rate, compare to the incubation without decapsulation.

5.3 Average size of nauplii. metanauplii and microworm

This study aimed at comparing the efficiency of three types of prey. The prey used in this study were the nauplii, the metanauplii and the microworms. The mean sizes of these preys were 0498 mm ± 0.06, 0853 mm ± 0.07, and 1,444 mm ± 0.21, respectively, for nauplii, metanauplii, and microworms. Microworms are the largest in terms of length, unlike nauplii, which are small prey. The size of these nauplii is higher than the value obtained by Bio & Pham, (2004) but much smaller than 667 ± 32,7 µm obtained by Amat *et a*l., (2004) on some Mexican strains. Several studies have shown that prey size has an effect on fish growth. Due to the low

nutritional value of microworms compared to nauplii, a strong difference in growth between the two treatments would be expected; this is not the case, the treatment effect between the two prey types is not significant in this study.

5.4 **Average weight of nauplii, metanauplii and microworm**

Despite its long length, microworms are the lightest, 5.11 e-07 ± 8.160 e-08 compared to Metanauplii 1.876 e-06 ± 5.837 E-08 and Nauplii 1.703 e-06 ± 1.245 e-07. The weight ratio between the nauplii and Microworm is 3.3 g, which means that a nauplius weights 3.3 times more than a microworm. This was a first attempt to optimize the live prey rations, especially for the microworms that is largely unknown. This provides a new insight into the live prey requirements for Arctic charr juveniles in terms of quantity (link between dry weight and number of prey). To improve this estimation, we needed to analyse the nutritional value of the different preys which has been partly done in this study.

5.5 **Nutritional value of feed**

Nauplii were the richer preys in protein, 66.8% compared with 62.9% for artificial food. The lowest protein (43.6%) levels were in the microworms. It would be expected to observe the highest weight gain on fish subjected to nauplii treatment, which is not the case. The highest gain is rather observed on fish subjected to the artificial food 0.034 g + 0.0, compared with 0.011 G + 0.01 for Artemia and 0.005 + 0.03 for microworms.

Comparing the fat and carbohydrate values of the Artemia and the artificial food, there is a significant difference between the two treatments. The artificial food contains 7.9% of fat and 16.6% of carbohydrate, compared with 1.65% of fat and 1.12% of carbohydrate in nauplii.

The analysis of the different raw energy shows the treatment artificial feed is the one that provides the most energy to the fish (20.9 KJ/g).

This result shows that the energy value of the prey has more effect on growth than its protein content. But a more complete analysis of the nutritional value of these different foods, noting the estimation of their value in amino acid, vitamins and essential fatty acid will confirm or reverse this conclusion

5.6 **Growth parameters**

Analysis of growth differences between treatments showed that the fish subjected to the artificial food performed better growth compared to all other treatments where a similar growth rate were observed. These results suggest that fish were either underfed on live prey treatments, or that these preys were deficient in certain nutrients such as amino acids, vitamins and essential fatty acids. On the other hand, we could have expected better growth in fish fed on enriched artemia which was not the case. The lowest growth rate was observed in fish fed on enriched artemia. The quality of the enrichment is likely to be the cause of this weak growth performance. In terms of brute energy, the results showed that nauplii provided more energy (16.6 KJ / g) than enriched metanauplii (15.2 KJ / g) which should not be the case.

The experiment was conducted with a density of 10 juveniles per litre which is low compared to other studies. In Arctic Charr juveniles, better growth performances have been shown at high density. Wallace, *et al*., (1988) found that fish raised at densities of 25 to 50 juveniles/L performed lower growth than those reared at higher densities (70 to 250 juveniles/L).

Overall, the mortality rate was lower than 4%. The highest mortality rate was recorded with the artificial food, 3.4% ± 1.0 While the lowest rate was obtained with enriched Artemia 0.6 ± 0.0.

6 CONCLUSION AND RECOMMENDATIONS

During this experiment, we were able to estimate the growth and survival performance of Arctic Charr juveniles under the live prey effect. Overall this species accepts the different prey they were subjected to. Overall, the mortality rate was lower than 4%. The highest mortality rate was recorded with the artificial food, 3.4% ± 1.0, while the lowest rate was obtained with enriched Artemia 0.6 ± 0.0. But the specific growth rate was low overall except in juveniles fed with artificial food. Plausible causes for this low growth could be that juveniles were underfed, or/and the feeding frequency was not sufficient.

In spite of these limitations, this work contains sufficient information to serve as a basis for future research. Microworms could be a good candidate to replace the traditional artemia which is quite expensive, and which does not survive long enough in freshwater as the microworms probably because of the osmotic stress.

This preliminary test of the effect of microworms on juvenile growth and survival of Arctic Charr juveniles can be adapted to other species.

The size, weight and nutritional value have been determined and can be the basis for future research. As a priority, we recommend an estimate of the adequate density of live prey for different species. In addition, it is important to see the effect of enriching these worms on the growth of juveniles.

The duration of this study did not permit time to determine some nutritional values which is very important for these worms. The value in unsaturated fatty acids, amino acids and vitamin has not been determined. Future research should test the effect of microworm enrichment on juveniles.

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APPENDICES

Appendix 1: Fraction of toxic (un-ionized) ammonia in aqueous solutions at different pH values and temperatures.

To determine the amount of un-ionized ammonia present, get the fraction of ammonia that is in the un-ionized form for a specific pH and temperature from the table. Multiply this fraction by the total ammonia nitrogen present in a sample to get the concentration in ppm (mg/L) of toxic (un-ionized) ammonia (table 9).

Table 1: Fraction of toxic (un-ionized) ammonia in aqueous solutions at different pH values and temperatures. Calculated from data in Emerson, et al. (1975)

	Temperatures (oC)												
pH	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**
7.0	.0013	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080
7.2	.0021	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.0126
7.4	.0034	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198
7.6	.0053	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0236	.0271	.0310
7.8	.0084	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0423	.0482
8.0	.0133	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0654	.0743
8.2	.0210	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0880	.0998	.1129
8.4	.0328	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1495	.1678
8.6	.0510	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422
8.8	.0785	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362
9.0	.1190	.1368	.1565	.1782	.2018	.2273	.2546	.2836	.3140	.3456	.3783	.4116	.4453
9.2	.1763	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599
9.4	.2533	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685
9.6	.3496	.3868	.4249	.4633	.5016	.5394	.5762	.6117	.6456	.6777	.7078	.7358	.7617
9.8	.4600	.5000	.5394	.5778	.6147	.6499	.6831	.7140	.7428	.7692	.7933	.8153	.8351
10.0	.5745	.6131	.6498	.6844	.7166	.7463	.7735	.7983	.8207	.8408	.8588	.8749	.8892
10.2	.6815	.7152	.7463	.7746	.8003	.8234	.8441	.8625	.8788	.8933	.9060	.9173	.9271

Source: Emerson, Russo, Lund, & Thurston, (1975). *Aqueous ammonia equilibrium calculations: effect of pH and temperature*.
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Appendix 2: Comparison between protein and amino-acids of penagrellus and artemia.

Table 2: The comparison between protein and amino-acids of penagrellus and artemia (Rouse et al., 1992).

	P. redivivus	Artemia
Protein	48.3	61.6
Amino acids		
ILE	5.1	3.8
LEU	7.7	8.9
MET	2.2	1.3
PHE	4.7	4.9
TYR	3.2	5.4
THR	4.7	2.5
TRY	1.5	
VAL	6.4	4.7
LYS	7.9	8.9
ARG	6.6	7.3
HIS	2.9	1.9
ALA	8.8	6.0
ASP	11.0	11.2
GLU	12.8	12.9
GLY	6.4	5.0
PRO	5.4	6.9
SER	3.7	6.7

Appendix 3: Length and weight measurement

Table 3: Length measurement of fourteen nauplii Ao

	Area	Mean	Min	Max	Angle	Length
1	0.002	158.242	141.333	180.35	103.392	0.432
2	0.003	160.16	123.333	180.778	-93.814	0.601
3	0.002	143.777	135.73	156.169	-106.928	0.481
4	0.003	145.234	96.548	162.534	-26.565	0.537
5	0.002	128.993	102.744	154.916	-38.29	0.484
6	0.003	119.005	91	129.334	-59.744	0.556
7	0.003	144.071	100.048	166.698	-64.359	0.555
8	0.002	187.997	162.912	228.333	-93.013	0.381
9	0.002	158.063	144.339	170.686	-77.4	0.418
10	0.003	156.294	108.741	166.178	-73.856	0.566
11	0.003	120.711	91.407	134.041	-60.505	0.543
12	0.002	117.338	61.913	143.61	-145.008	0.468
13	0.002	166.295	114	246.06	133.386	0.483
14	0.002	142.606	115.758	154.597	24.201	0.469
Average						**0.498143**

Length measurement of eight metanauplii A_1

	Area	Mean	Min	Max	Angle	Length
1	0.005	168.834	118.667	210.333	0	0.957
2	0.004	198.227	140	225.669	36.254	0.792
3	0.005	195.903	155.625	226.333	-4.289	0.853
4	0.005	205.144	172.369	231.826	-73.301	0.889
5	0.004	208.248	181.553	232.398	-96.17	0.792
6	0.005	203.564	128.41	234.023	-95.315	0.919
7	0.004	166.973	145.324	191.056	-18.435	0.74
8	0.005	215.029	197.378	232.61	160.253	0.882
Average						**0.853**

Length measurement of six microworms

	Area	Mean	Min	Max	Angle	Length
1	0.008	31.747	20.107	86.878	74.219	1.542
2	0.008	45.46	22.367	93.36	63.997	1.472
3	0.009	52.834	21.879	90.438	66.501	1.618
4	0.008	43.354	23.429	83.458	60.69	1.485
5	0.009	28.012	22.847	39.68	-52.595	1.529
6	0.006	59.876	15.333	125.755	-126.469	1.021
Average						**1.4445**

Appendix 4: Average temperature (°C) and dissolved oxygen (% of saturation) per tank

Tank	Temperature (°C)	O2 (% of saturation)
Microworm	7.7	101.5
Artificial feed	7.6	102.8
Microworm+ Artemia	7.5	102.7
Enriched Artemia	7.5	102.4
Enriched Artemia	7.5	102.7
Microworm+ Artemia	7.6	102.4
Artemia	7.6	102.3
Artemia	7.5	102.4
Microworm	7.5	102.4
Artificial feed	7.5	102.3