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Arctic charr start-feeding with live artemia and preserved marine copepods: Effects on the onset of exogenous feeding, growth, and survival

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ABSTRACT

The onset of first feeding, the survival, and the growth performance were examined in Arctic charr over 121 days. Group A was fed live enriched artemia for 30 days, with weaning onto dry feed starting on day 20. Group C received preserved marine copepods exclusively for the first 20 days, followed by dry feed. Group D was fed dry feed exclusively. Most juveniles in Groups A and C began exogenous feeding by the first and third day of the experiment, respectively, whereas only about 74% of juveniles in Group D had ingested dry feed by day 15, with the remaining individuals failing to initiate feeding and succumbing to starvation. Fish in Group C were unable to effectively digest the preserved copepods and suffered the lowest growth rate and survival (60%). While early exogenous feeding in Group A improved survival (90%) and growth in the initial rearing stages, using artemia as an alternative to dry feed had no long-term growth benefits.

Keywords: Brine shrimp, dry feed, first feeding, *Salvelinus alpinus*

YFIRLIT

Fóðrun bleikjuseiða með lifandi artemíu og varðveittum krabbaflóm: Áhrif á tímasetningu frumfóðrunar, vöxt og lifun

Aldur við upphaf fæðunáms, lifun og langtímavöxtur voru rannsökuð í bleikju yfir 121 daga tímabil. Hópur A var fóðraður með lifandi, auðgaðri artemíu fyrstu 30 dagana, og aðlögun að þurrfóðri hófst á degi 20. Hópur C fékk dauðar krabbaflær í 20 daga og var í kjölfarið fóðraður með þurrfóðri. Hópur D fékk eingöngu þurrfóður allan rannsóknartímann. Flest seiði í hópum A og C hófu fæðunám á fyrsta eða þriðja degi tilraunarinnar, en einungis um 74% seiða í hópi D höfðu hafið fæðunám á degi 15. Seiði sem ekki átu þurrfóður innan þessa tímaramma drápust síðar úr næringarskorti. Fiskar í hópi C gátu ekki melt krabbaflærnar nægjanlega vel, sem leiddi til hægs vaxtar og lægsta lifunarhlutfallsins (60%) í samanburði við aðra hópa. Þrátt fyrir að snemmbúið fæðunám í hópi A hafi leitt til aukinnar lifunar (90%) og hraðari vaxtar á fyrstu stigum eldissins, hafði fóðrun með artemíu ekki marktæk langtímaáhrif á vöxt miðað við fiska sem voru eingöngu fóðraðir með þurrfóðri.

INTRODUCTION

At the end of the yolk-sac stage, the process of learning to catch prey is a determining factor for the early survival rates of teleosts. The first food of wild Arctic charr (*Salvelinus alpinus*) is somewhat variable between populations and habitats, but, as for other salmonids, the main prey types are probably insect larvae and microcrustaceans (Klemetsen et al. 2003). Arctic charr juveniles have a well-developed digestion system at the start of exogenous feeding. For farming purposes the species can be weaned directly onto dry feed at the end of the yolk-sac stage. Zooplankton are therefore generally not used as a first food of farmed Arctic charr.

Historically, difficulties with initiating exogenous feeding in salmonids have been documented since the earliest attempts at their culture. These challenges were often manifested as so-called "pin-head" mortalities, which occur when the fish fail to initiate exogenous feeding and eventually succumb to starvation (Wallace & Aasjord, 1984). Subsequent research demonstrated that survival rates could be improved through interventions, such as offering feed prior to complete yolk-sac absorption, and other refinements in husbandry practices (Alanäraä, 1993). In short-term experiments using small start-feeding tanks (20–30 L), survival rates exceeding 95% have been reported in Arctic charr juveniles that were offered dry feed (Wallace & Aasjord, 1984; Ndjonjip, 2018; Árnason et al. 2022). However, such high survival rates are typically not achieved under commercial farming conditions (personal observation), which suggests the potential value of exploring alternative feeding strategies to improve early survival.

A recent short-term study indicated that Arctic charr juveniles may exhibit improved survival when offered live artemia rather than dry feed during the initial feeding period (Ndjonjip 2018). Similarly, studies on other salmonid species suggest that initial exposure to zooplankton can influence feeding behavior and growth. For example, first-feeding sockeye salmon (*Oncorhynchus nerka*) and chum salmon (*Oncorhynchus keta*) have been observed to exhibit a stronger feeding response when offered frozen zooplankton compared to dry feed (Fulton 1976). Additionally, several studies have reported better survival and growth among hatchery-reared salmonid juveniles feeding on live zooplankton when compared with conspecifics offered a pellet diet as first feed (Holm 1987, Kim et al. 1996, Akbary et al. 2010, Czerniawski et al. 2014). Exposure to live zooplankton has also been found to have a positive impact on survival, foraging skills and growth of salmonids after stocking in the wild (Brown & Laland 2001, Czerniawski et al. 2011, Czerniawski et al. 2014).

On this basis, the current study was designed to explore the effects of three distinct types of initial feeds on the commencement of exogenous feeding, as well as the subsequent survival and growth of Arctic charr throughout a four-month period from the late alevin stage.

MATERIALS AND METHODS

Experimental design

The experimental fish were of mixed parental origin and were randomly selected from a group of about 450,000 alevins kept at Samherji's Arctic charr hatchery at Núpar, South Iceland. At Núpar, the Arctic charr were maintained at 5 – 7°C during the egg and yolk-sac stage in CompHatch® hatching trays fitted with EasyHatch substrate (Alvestad Marin AS). On 4 March, 2019 (at ~600 °d post-fertilization), approximately 3600 alevins were transferred from Núpar to the MFRI Aquaculture Research Station at Grindavík on the Reykjanes peninsula, where the experiment was conducted. Upon arrival at the Aquaculture Research Station, the mean size of the fish was assessed using two methods: individual measurements of 48 fish and bulk weighing of 60 fish. These measurements indicated an initial mean weight of 0.053 ± 0.008 g and 0.056 g, respectively.

The experiment spanned 121 days, starting on 5 March and finishing on 4 July, 2019. Feed intake, growth, and survival were assessed across three distinct groups: A, C, and D. Each group was randomly divided into four rectangular fiberglass tanks (100 × 100 × 37 cm) containing 370 L of water, with each tank containing 308 alevins.

Group D was exclusively provided with dry feed throughout the experimental period. Feed was continuously offered in excess for 24 hours using an automatic belt feeder (FIAP GmbH), stocked with 15 g of feed. During the first 30 days of the study, an additional, 3 g of dry feed was hand-fed four times daily (a total of 12 g per day) at 9:00, 11:00, 13:00, and 15:30. From day 30 onward, feed was exclusively offered via automatic feeders. Between days 30 and 66, each feeder was stocked with 20 g of feed, after which feeding was adjusted to minimize leftovers in the tanks. The dry feed used in this study was Nutra from Skretting A/S, with an initial particle size of 0.3 mm, increasing to 0.7 mm by the second month of the study.

Group A was fed exclusively with live *Artemia salina* for the first 20 days. Each morning, 80 g of artemia cysts (Sep-Art®, EG artemia, INVE Ltd) were placed in a 100 L plastic silo containing vigorously aerated seawater at a stable temperature of 28°C and a salinity of 31 ppt. For disinfection, 5 ml of Pyceze™ (bronopol) was added to the tank. After a 24 hours of incubation under continuous light, the artemia were separated from the cysts, using a magnetic separator, and collected in a submerged sieve. Following gentle rinsing in 12°C seawater, the nauplii were transferred to a 100 L silo, where they were enriched with 60 g of Easy DHA Selco® (INVE Ltd) for approximately 20 hours. The enriched artemia were then washed with 7°C seawater, using a submerged sieve before being offered to the fish. Artemia were introduced into each tank twice daily, at 10:00 and 16:00. Half of the artemia were offered immediately after washing at 10:00, while the remaining half were stored in 20 L of seawater at 4°C until being offered at 16:00. Each tank received approximately 500,000 artemia per feeding. From days 20 to 30, weaning onto dry feed was carried out by hand-feeding the juveniles in the morning before offering artemia and again in the afternoon before the second artemia ration was added to the tanks. During this period, each tank received a daily ration of 6 g of dry feed. Starting on day 30, the fish in were fed exclusively with dry feed administered via an automatic belt feeder with the same ration used for Group D.

Group C was exclusively fed preserved copepods for a period of 20 days and then transitioned directly to dry feed. The copepods were preserved in liquid and supplied in sealed bags by the manufacturer, Planktonic AS (Trondheim, Norway). Prior to use, the feed was stored at 4°C and rinsed in freshwater before feeding. The copepods consisted of a mixture of several calanoids, including *Temora longicornis*, *Centropages typicus*, *Pseudocalanus* spp., *Microcalanus* spp., and *Acartia* spp. These copepods were harvested off the coast of Norway and subsequently processed by Planktonic AS. The preserved copepods were added manually to the tanks four times daily, during the operational hours from 9:00 to 16:00. Beyond these hours, dosing pumps (Grundfos®, DME) were used to introduce the copepods to each tank from 5 L cylindrical containers, at four-hour intervals from 20:00 – 4:00. In these containers the copepods were immersed in vigorously aerated seawater (~7°C). In each tank, the fish received 10 g of copepods every 24 h. From day 20 onward, the dry feed was offered in the same manner as described for Group D.

All groups were reared in a flow through system, in slightly brackish water (3.6 ± 0.2 ppt) and at a temperature of 9.9 ± 0.05 °C. Throughout the study, the fish were reared on a flat bottom without shelters under constant photoperiod (LD 24:0) with a light intensity of 200 lux at the surface. For the first month the waterflow was maintained at 80 l/h and was roughly doubled thereafter. The waterflow was sufficient to maintain oxygen saturation at 90 – 100% throughout the study. During the first two months, when excessive feeding was maintained, the tanks were cleaned with a broom twice daily at 8:30 and 15:30. Thereafter, the feeding was regulated to minimize leftovers, and the tanks cleaned manually once every 24 h.

Measurements and data analysis

Foraging success was evaluated during the first 15 days of the study. On days 1, 3, and 6, this was assessed by visually examining the digestive systems of four randomly selected fish per tank for the presence of feed. On day 9, the sample size increased to 10 fish per tank, and on day 15, up to 40 fish per tank. All fish examined for feed presence were also individually weighed to the nearest 0.01 g. On days 1, 3, and 6, the sampled fish were sacrificed with an overdose of tricaine methane sulfonate anesthesia (Finquel® vet) and photographed before analysis.

On subsequent days, analysis and measurements were conducted under anesthesia (0.05 g/L, Finquel® vet) in a 5L bucket. The fish were then caught from the bucket using a 5 cm diameter plastic strainer. Before weighing, the strainer was carefully dried with paper to remove excess water from the strainer as well as the fish. Because the fish have translucent bellies, feed presence in the digestive tract could be visually assessed during this process.

After day 15, measurements focused exclusively on growth and were conducted on several occasions throughout the 121-day study. These involved individual measurements from each tank on days 30, 66, 90, and 121, with sample sizes of 30, 23, 40, and 38 fish per tank, respectively. Dead fish were collected and counted daily.

To evaluate differences between treatment groups on each sampling day, foraging success was first calculated as the percentage of fish that had clear indication of food in their digestive tract in each replicate tank. A Kruskal-Wallis rank sum test was then conducted separately for each day to determine if significant differences in foraging success existed among groups. The replicate tanks were treated as independent statistical units. For days where a significant overall group effect was detected, Dunn's multiple comparison test was conducted as a post-hoc analysis to identify specific pairwise differences between groups. The Bonferroni method was applied to adjust for multiple comparisons.

To evaluate differences in survival among groups at the end of the study, the total numbers of dead and surviving fish were summed across replicate tanks within each group. Fisher's exact test was then used to assess whether the proportion surviving fish differed significantly among groups.

Mean body weights are presented as mean ± S.E. The normality of the W distributions was assessed using the Shapiro-Wilk test. A two-way mixed model analysis of variance (ANOVA), with replicate tanks as random factor and feed as fixed factor, was used to test for possible differences in mean weight among groups. Significant differences in the ANOVA analysis were followed by Tukey HSD tests. All statistical analyses were conducted using R version 4.3.0 (R Core Team, 2023)

On day 121, a total of 144 fish from each group were randomly selected and tagged. These fish were then used in a separate study, spanning 17 months, which focused on monitoring their responses in different salinity regimes (unpublished). A brief mention of the differences in size among the groups at the conclusion of the extended study period will be presented in this paper. This mention serves to complement the findings obtained on day 121 in the current study.

RESULTS

The onset of first feeding

During the first 9 days of the experiment, there was a gradual increase in the number of fish that started consuming dry feed in Group D. However, on day 15 the weaning onto dry feed was successful in only about 74% of the fish within this group. Preserved copepods were more palatable for the fish in comparison to dry feed, as all fish had initiated copepod consumption within just six days. Furthermore, the fish in group C exhibited significantly higher foraging success than those in Group D on all sampling days during the first two weeks of the experiment (Dunn's multiple comparison test, $p < 0.05$). Based on careful observations and video recordings during the first week of the study, it became evident that the juveniles mostly remained at the bottom and showed a strong preference for feed that was either drifting or sinking slowly in close proximity. Thus, the relatively slow sinking rate of the preserved copepods seemed to be advantageous in terms of facilitating the onset of exogenous feeding.

While preserved copepods proved appealing, their palatability was lower when compared with live artemia. In Group A, the fish demonstrated

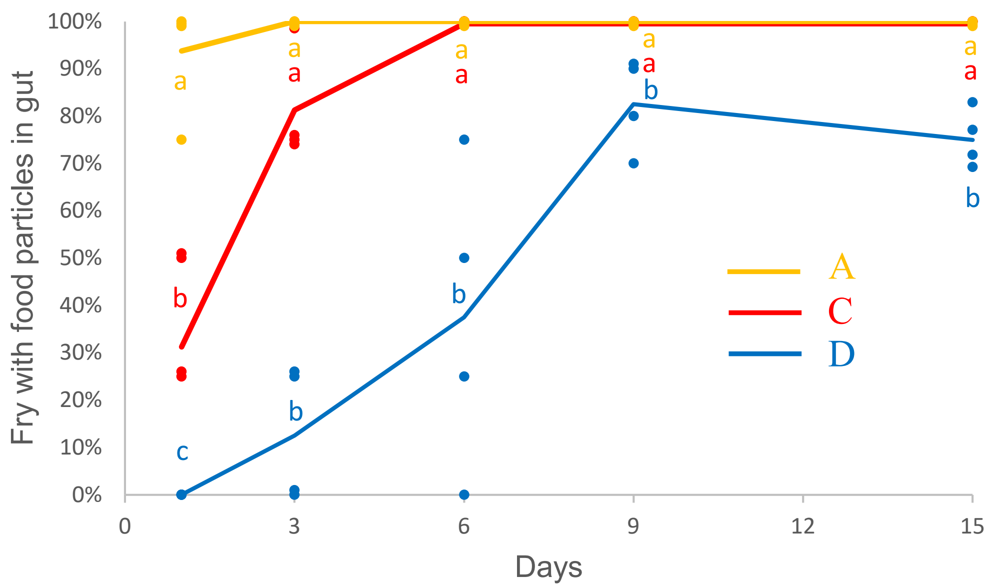

Figure 1. Proportion of juveniles with visible artemia (A), copepods (C) or dry feed (D) in digestive tract during the initial two weeks of start-feeding. Data represent the percentage of individuals observed with each feed type over time, with each point representing an individual replicate and lines indicating the mean trend. Different lower case letters indicate significant differences between groups at the 5% level

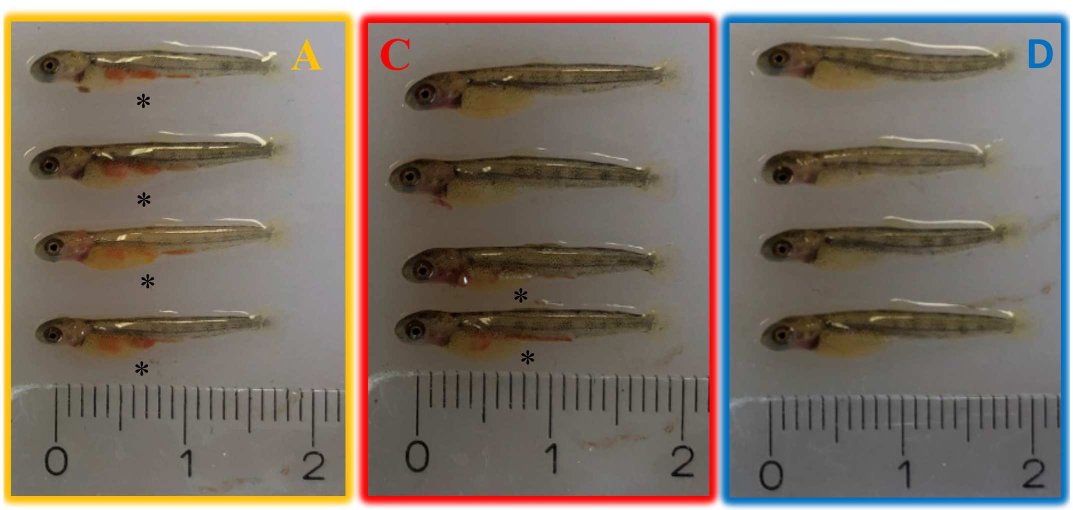

Figure 2. Photographs of representative Arctic charr from the experimental groups. Groups A, C, and D were fed with artemia, copepods, and dry feed, respectively. The images were captured one day after the initiation of first feeding, at approximately 600 degree-days post-fertilization. At this stage, the fish were transitioning from alevins to juveniles and had not yet fully depleted their yolk sacs. Asterisks indicate the presence of food within the digestive systems of the fish.

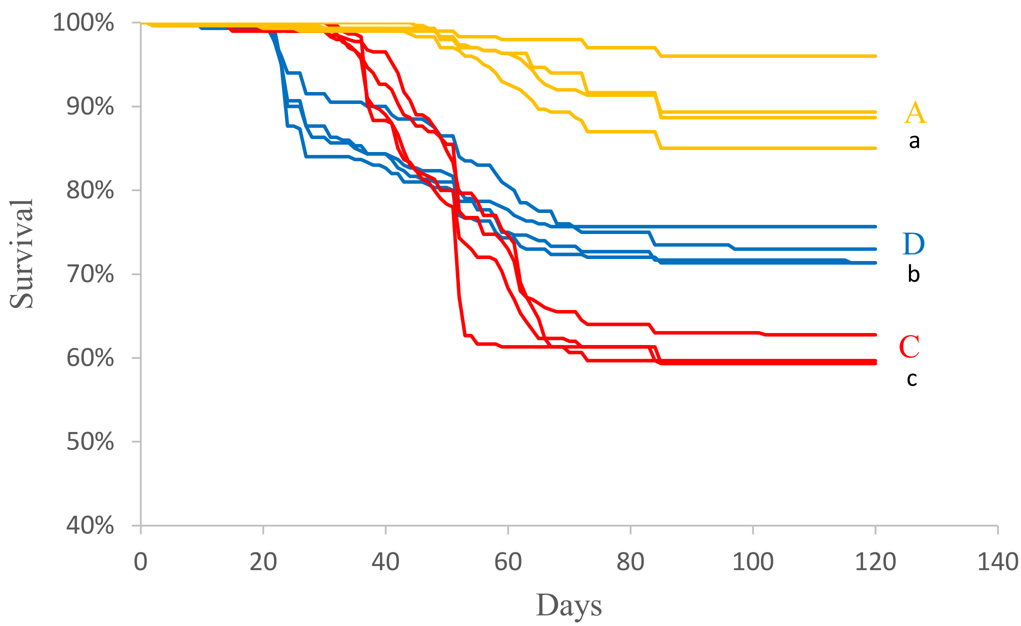

Figure 3. The percentage survival of Arctic charr spanning 121 days from the commencement of first feeding. For each group, there are four distinct lines denoting survival rates within individual replicates. Group A was fed with artemia for the first 30 days, Group C with preserved copepods for the first 20 days, and Group D with dry feed throughout. Different lowercase letters indicate significant differences in survival rates between groups on day 121 ($p < 0.05$)

clear foraging behavior immediately after artemia was first offered, even before the yolk-sac was fully depleted. By day 3, fish in group A had significantly higher foraging success than those in groups C and D (Dunn's multiple comparison test, $p < 0.05$), with 94 – 100% of the sampled fish having already ingested live artemia (Fig. 1, Fig. 2).

Survival

In groups D and C, the appearance of the fish that died during the initial half of the study suggested that starvation was the main cause of mortality. In Group D, the 72 – 76 % survival rate shown in Figure 3 corresponds with the feeding rate on day 15 in Figure 1. This, however, was not the case for Group C, which, unlike Group D, showed a strong appetite for the first weeks of the study, but nonetheless, suffered the lowest survival rates (59 – 63 %). Start-feeding with artemia (Group A) produced the best results, with 86% to 96% survival over the 121-day experiment (Fig. 3). In contrast to Groups C and D, mortalities in Group A occurred later in the study and primarily involved fish that appeared to be in good physical condition at the time of death. There was a significant difference in survival rates between all groups at the end of the study (pairwise Fisher's Exact Tests, $p < 0.001$).

Growth

Initially, the Arctic charr fed on enriched artemia exhibited the highest growth rate, and on day 30 there were significant differences in mean weight among all groups (Fig. 4). However, approximately two months into the study, Group D had caught up with Group A, as no significant difference in body weight was detected between these groups on day 66 (Tukey HSD, $p > 0.05$, Fig. 4). While Group C displayed slow growth during the first month, the growth rate increased

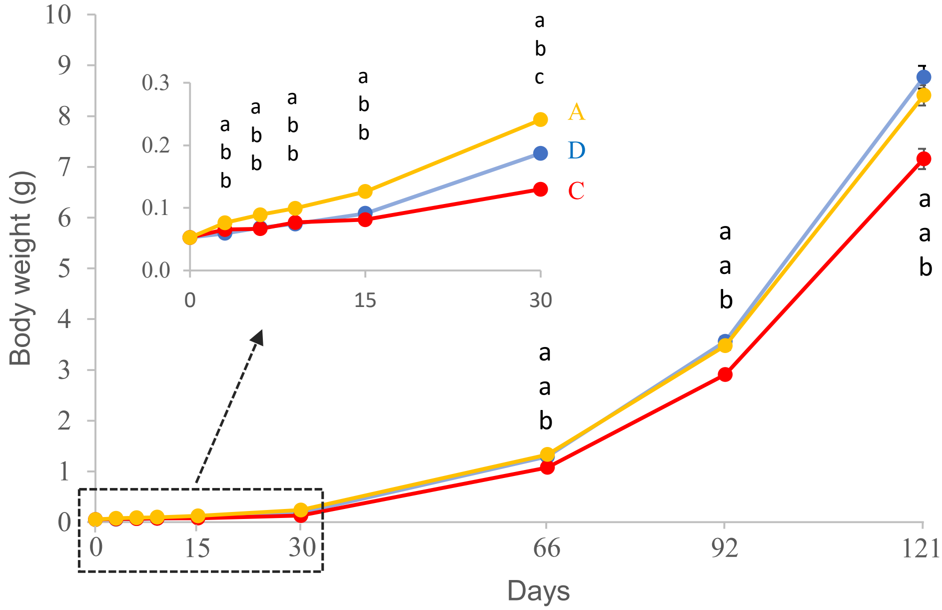

Figure 4. Mean weights of Arctic charr subjected to three distinct initial feeding methods and reared for 121 days post first feeding. Group A was fed with artemia for the first 30 days, Group C with preserved copepods for the first 20 days, and Group D with dry feed throughout. Different lowercase letters indicate significant differences between groups at the 5% significance level

as the juveniles transitioned from copepods to dry feed. However, by day 121, Group C still had significantly lower mean body weight compared to Groups A and D (Tukey HSD, $p < 0.05$). At the same time, the difference in mean weight between Groups A and D remained non-significant (Tukey HSD, $p > 0.05$).

A subsequent study involving 144 tagged fish from each group (432 in total), reared until reaching a harvest size of around 1.2 kg under two different salinity regimes, largely confirmed these findings at day 623 post first feeding. At this point, the size difference between groups A and D was minimal (less than 2.6%). Although the harvest weight in Group C was 4–9% lower than in the other groups, no significant difference in harvest weight was observed among the feed groups (one-way ANOVA, $p > 0.05$).

DISCUSSION

In the present study, Arctic charr fed on enriched artemia exhibited the highest growth rate during the initial month of the study. While this outcome aligns with earlier short-term studies involving other salmonid species where artemia replaced dry feeds (Kim et al. 1996; Akbary et al. 2010), the current study did not show any long-term growth benefit from using artemia. However, the study clearly shows that the feeds differed significantly in palatability, as the fish approached artemia and copepods voraciously, while showing considerably less interest in dry feed during the early rearing stages.

Although the preserved copepods used in the current study have been used effectively to raise adult ornamental fish, emerging evidence raises concerns about the preservation technique in the context of start-feeding. Specifically, there is a possibility that the preservation process may have altered the biochemical composition of the copepods, making them challenging for the small juveniles to digest. After the completion of this study, notable advancements have been implemented by the company Planktonic in refining their preservation techniques. These enhancements have resulted in substantial improvements in the digestibility of the preserved copepods (Nils Tokle, Planktonic AS. Pers. Comm.).

Previous studies have shown that introducing dry feed before complete yolk sac absorption may improve survival and growth rates in Arctic charr (Wallace & Aasjord, 1984; Alanäraä, 1993). In the study by Alanäraä (1993), alevins that were start-fed upon emergence from gravel, with approximately 14% of their yolk reserves remaining, had a low survival rate (47%). In contrast, survival increased markedly to 80% when dry feed was introduced earlier, while 55% of the yolk remained. According to Alanäraä (1993), mortality was primarily due to starvation, as alevins with minimal yolk reserves at first contact with food had a shorter window to transition to dry feed and were more vulnerable to starvation than individuals with greater yolk reserves.

In the present study, Arctic charr alevins exhibited varying degrees of yolk-sac depletion at the start of the experiment, with some individuals approaching complete depletion (Fig. 2). Given the mean survival rate of 73% in Group D, it seems likely that earlier dry feed introduction would have improved survival. However, studies carried out in small tanks (20 – 30 L) have reported survival rates exceeding 95%, even among Arctic charr introduced to dry feed close to yolk-sac exhaustion (Wallace & Aasjord, 1984; Árnason et al., 2022). In contrast to the studies reporting survival rates above 95%, both the present study and Alanäraä's (1993) study were carried out in tanks with volumes of 250 – 370 L and with a bottom area of 1 m^2. This suggest that the different survival rates among studies may to some extent be due to difference in tank sizes. As mentioned previously, observations from the current study indicate that alevins primarily remained at the bottom of the tank and only responded to feed as it descended through the water column in close proximity. These observations highlight the critical importance of feed distribution for successful weaning onto dry feed. Feed distribution is more manageable in small tanks with limited bottom surface area, whereas in larger tanks, achieving even feed dispersion becomes increasingly challenging. This challenge is further amplified in commercial Arctic charr production, where tanks are typically many times larger than those used for experimental purposes (personal observation). One potential solution to improve feed dispersion and start-feeding success is the use of live feed or adequately preserved zooplankton, which remain suspended in the water column longer and are more palatable than commercial dry feed.

Although preserved copepods and artemia were administered less frequently than dry feed in this study, their high palatability and ability to remain suspended in the water column supported relatively high feed intake. This was evident in Groups A and C, where fish still had undigested feed in their digestive tract the following morning, 16 and 4 hours after receiving the last ration, respectively. In Group A, the presence of feed in the digestive tract, 16 hours post-feeding, may also be explained by the fact that not all artemia were flushed from the tank. Some artemia settled at the bottom and accumulated in areas with minimal water flow, remaining available for consumption for extended periods.

In the present study, the initial stocking density was approximately one juvenile per liter, which is substantially lower than densities typically used in commercial Arctic charr farming (personal observation). A previous study by Wallace et al. (1988) reported significantly improved growth in Arctic charr juveniles reared at high densities (70–250 juveniles/L) compared to lower densities (25–50 juveniles/L), following the onset of exogenous feeding. While the density in the present study was considerably lower than that used by Wallace et al. (1988), it is unclear whether this had a significant impact on growth, as the juveniles in the current study exhibited growth rates many times higher than those reported by Wallace et al., despite being reared at similar temperatures (6–9°C). Moreover, the growth performance observed in the present study also exceeded that reported in a more recent study under comparable thermal conditions (Árnason et al., 2022).

In this study, the long-term growth potential of Arctic charr was not significantly affected by the start-feeding methods tested. Even fish in Group C, which were unable to effectively digest and extract nutrition from preserved copepods for 20 days, still exhibited relatively high long-term growth performance and did not have significantly lower harvest weights 17 months after the yolk-sac stage, compared to fish in Groups A and D. This contrasts sharply with findings from studies on marine fish species that produce small eggs and fragile larvae, such as Atlantic cod (*Gadus morhua*) and ballan wrasse (*Labrus bergylta*), where both the type of first feed and the duration of zooplankton feeding have been shown to have long-term effects on growth (Baskerville-Bridges & Kling, 2000; Øie et al., 2015). These findings highlight the resilience of Arctic charr juveniles and suggest that survival could be improved by developing more palatable alternatives to dry feed at first feeding, without compromising long-term growth potential.

While assessing the economic feasibility of alternative start-feeding methods is beyond the scope of this study, live artemia stands out as a promising option. The results suggest that, although artemia is more expensive than dry feed, its cost could be offset by improved survival rates. Higher survival would mean that farmers would not need to purchase or produce as many eggs to compensate for early rearing losses. Additionally, while using artemia is more labor intensive than stocking dry feed on automatic feeders, its use could reduce labor costs associated with tank cleaning, as live artemia does not foul the water as much as dry feed. If survival rates improve, the labor required for removing dead fish would also decrease. Ultimately, the use of alternative feeds, such as artemia, may be valuable from an animal welfare perspective, as they can help reduce unnecessary mortality.

Since the present study, the start-feeding of Arctic charr and Atlantic salmon alevins at the Aquaculture Research Station has on numerous occasions been conducted using newly hatched artemia nauplii (non-enriched). Start-feeding with artemia is typically initiated at the end of the yok-sac stage and is offered in combination with dry feed for two weeks, by which time the fish are exclusively offered dry feed. This approach is cheaper and much less labor-intensive than the extended use of enriched artemia described in this study, while still ensuring high survival rates during the early stages of feeding.

Conclusion

Without prior exposure to food, nearly all alevins in this study instinctively recognized artemia as prey and began consuming them upon first encounter at approximately 600°d from fertilization. Although juveniles started feeding on preserved copepods slightly later than live artemia, preserved copepods proved more palatable than dry feed. Early initiation of exogenous feeding with artemia did not result in improved long-term growth performance. However, from an experimental standpoint, this study suggests that artemia offers several practical advantages: it accelerates the onset of exogenous feeding, reduces the risk of starvation during start-feeding, and minimizes maintenance demands by preventing the accumulation of uneaten dry feed in tanks.

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