OPTIMIZING *Artemia* HARVEST TIMING/ BALANCING BIOMASS YIELD AND NUTRITIONAL QUALITY DURING EARLY INCUBATION

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Introduction

The first 36 hours of incubation of dried A*rtemia* cysts represent a metabolically intensive phase of development, during which embryonic growth is entirely supported by nutrient reserves stored within the yolk sac. As development progresses through early instar stages, these internal reserves are gradually depleted, directly influencing the nutritional value of the resulting live feed.

For fish and shrimp hatcheries, A*rtemia* production is often approached with a focus on harvesting specific developmental stages, such as early Instar I nauplii, due to their perceived higher nutritional quality. However, this approach may overlook the broader production dynamics. Extending incubation time allows a greater proportion of cysts to hatch and develop into a harvestable population, increasing overall biomass output. Rather than targeting a single developmental stage, hatchery efficiency can benefit from an optimized balance between total biomass production and preservation of nutritional quality. This implies managing incubation conditions and harvest timing to maximize overall yield while minimizing depletion of endogenous reserves. In this context, harvesting a mixed population of Instar I and II nauplii can represent a practical and efficient strategy, provided that system conditions are optimized to ensure high output and controlled nutritional decline.

The objective of this study is therefore to evaluate the evolution of overall nutritional composition, average nauplius dry weight (IDW), and total nauplius biomass (IWW) during the first 36 hours of *Artemia* incubation, with a focus on defining optimal production windows that maximize total feed availability while maintaining adequate nutritional value.

Materials and methods

EG SEP-Art D-FENSE™ cysts sourced from the Great Salt Lake (Utah, USA) were incubated under standardized hatching conditions (25 ppt salinity, 29 °C, pH 8.25). Samples were collected periodically over 36 h of incubation in six replicate hatching tanks to assess hatching kinetics, individual dry weight (IDW), individual wet weight (IWW), fatty acid composition, and proximate nutritional profile.

Samples were taken for each hatching tank replicate. The cyst shells were removed with a magnet. The nauplii were collected on a 118µm sieve and rinsed with RO water. A weighed portion of the nauplii were then reintroduced to a 1L Imhoff cone, which was filled to 1L with RO water. 250µL of sample from each cone replicate was pipetted into each well of a 3x4 cell culture well plate, with one plate sampled for each replicate. Each well was then treated with a fixing solution and counted using the SnappArt L-SENSE. Results from the SnappArt portal were averaged for each replicate of the given sampling time. Once the well plate samples were taken, 100mL samples from each replicate were taken and collected on pre-weighed dried 118µm sieves in triplicate. The sieves were then dried at

60°C for 24h and weighed to determine the total dry weight of the animals. The IDW and IWW were then calculated and averaged according to replicate.
The remaining nauplii were gathered and analyzed using fatty acid methyl ester (FAME) analysis, and proximate composition was measured using standard nutritional assays.

Results

Results showed a significant increase in individual wet weight (IWW) from 10.99 µg nauplius^{-1} at 20 h to 17.74 µg nauplius^{-1} at 36 h of incubation. In contrast, individual dry weight (IDW) declined over the same period, decreasing from 2.18 µg nauplius^{-1} at 20 h to 1.90 µg nauplius^{-1} at 36 h. This reduction in IDW was primarily driven by losses in carbohydrates, which declined from 0.5994 to 0.3719 µg nauplius^{-1}, and fatty acids, which decreased from 0.3853 to 0.2808 µg nauplius^{-1}.
A notable decline in IDW occurred during the transition from the freshly hatched umbrella stage to instar I, while smaller losses were observed during development from instar I to instar II. The IDW loss was the largest between 30 h and 36 h of incubation. Fatty acid utilization was selective, with polyunsaturated fatty acids exhibiting the greatest reductions over the hatching period, particularly 18:3(n-3) (α-linolenic acid). Among monounsaturated and saturated fatty acids, 18:1(n-9) (oleic acid) and 16:0 (palmitic acid) showed the largest decreases respectively.

Discussion

These findings suggest that early *Artemia* harvesting (18–21 h) may not always represent the optimal strategy for commercial hatcheries. While instar I nauplii are often considered nutritionally superior to later stages, this advantage may be outweighed by increased nauplius availability under longer incubation.
Importantly, the commonly held view that Instar II nauplii are inherently inferior to Instar I should not be interpreted as a strict or binary distinction. In practice, it is often assumed that all A*rtemia* hatched beyond ~22 hours (e.g., 22–48 h) represent a uniform Instar II population with similar nutritional characteristics. However, this work clearly demonstrates that early Instar II nauplii differ significantly from later developmental stages that are often broadly, and sometimes incorrectly, grouped under the same label.
Specifically, early Instar II nauplii remain nutritionally very close to late Instar I, with only limited depletion of internal reserves. In contrast, at extended incubation times (>30 h), the population increasingly consists of more advanced Instar II and even later stages, where metabolic activity has led to a substantial consumption of endogenous reserves. It is within this later phase, rather than the transition from Instar I to early Instar II, that the most pronounced decline in nutritional quality occurs. At these later stages, where reserve depletion becomes more pronounced, enrichment strategies should be considered to compensate for nutritional losses and optimize the feed quality. Therefore, rather than focusing strictly on stage-specific harvesting, optimizing *Artemia* production should aim at balancing total biomass output with controlled nutrient depletion, allowing the efficient use of mixed-stage populations while maintaining overall nutritional performance.