SHIPPING PROTOCOL FOR MARINE FISH YOLK-SAC LARVAE: A MODEL STUDY ON MEAGRE *Argyrosomus regius*

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Introduction

To further expand meagre (*Argyrosomus regius*) aquaculture production, it is necessary to address the need for effective transferring of eggs and larvae from broodstock to larval rearing facilities. Egg production is easy and results in high arrival survival but is limited by the short incubation time, as larvae that hatch during transport usually die. Shipping yolk-sac larvae would allow longer transfer durations, exceeding the limited duration of egg incubation.

Goal

To develop a protocol for shipping meagre (*Argyrosomus regius*) yolk-sac larvae, focusing on the influence of stocking density, transport duration, and water quality (**Fig.1**).

Methodology

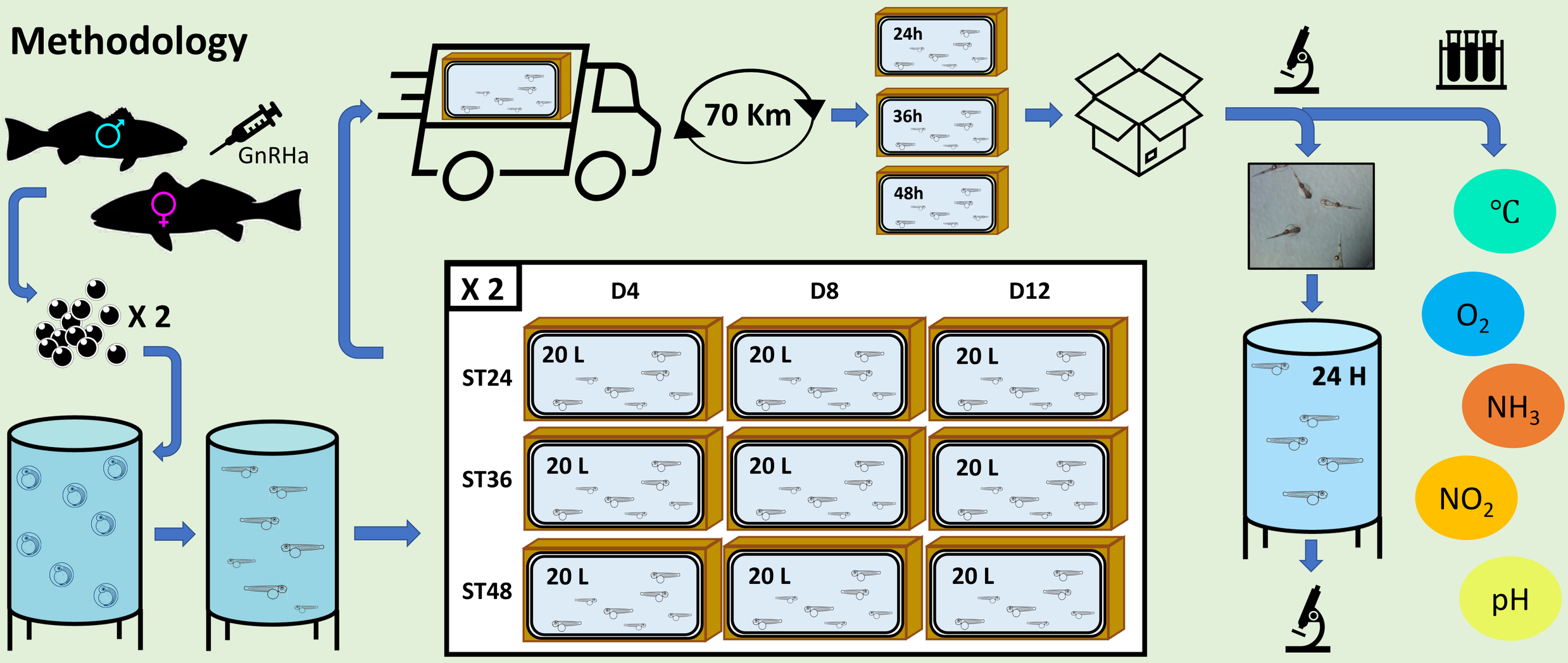

Fig.1 Meagre (*Argyrosomus regius*) eggs from different high-quality spawning (fertilization >85%, n=2) obtained through exogenous induction with GnRHa, were collected and left to hatch in fiberglass incubators. Then, larvae were concentrated and transferred to polyethylene shipping bags and underwent simulated shipping conditions. Three different larval concentrations (4000 larvae L^{-1}, D4; 8000 larvae L^{-1}, D8; 12000 larvae L^{-1}, D12) were tested in replicates at three different simulated shipping times (24h, ST24; 36h, ST36; 48h, ST48). Temperature (°C), dissolved oxygen (DO %), pH, NH_3 (mg L^{-1}), and NO_2 (mg L^{-1}), were measured to monitor water quality changes. Larval survival was assessed at the end of the simulation of shipping (unpacking). Surviving larvae were transferred to incubators to evaluate also the survival rate after a 24-hour post-transportation period (incubator).

Results

Although inter-batch differences were visible, overall, the results showed the successful transportation for all groups (D4, D8, and D12 at ST24 (**Fig.2**). Moreover, D4 exhibited transportability at all the shipping time tested (ST24, ST36, and ST48).

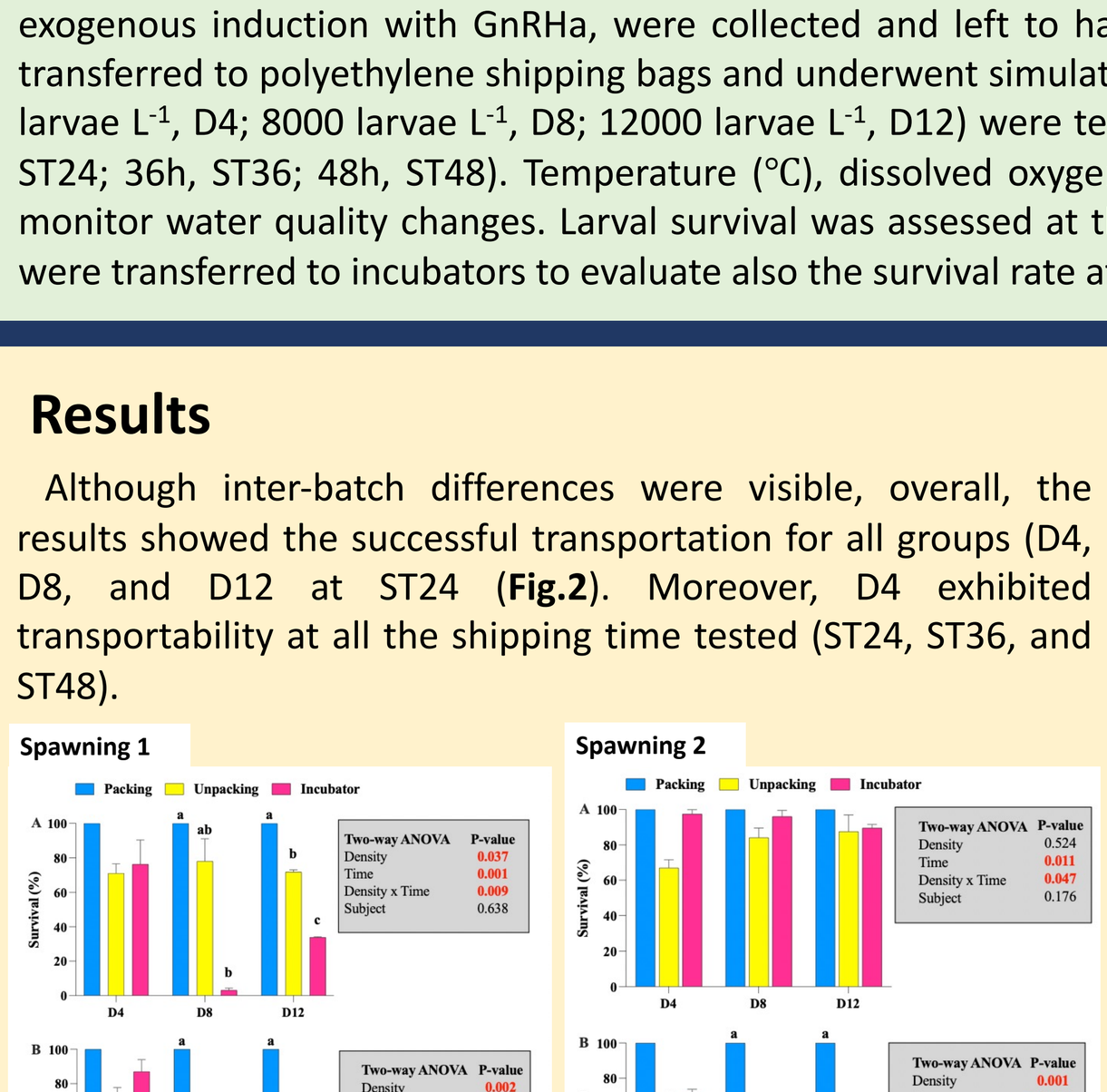

Fig. 2 Mean (±SEM) larval survival (%) density during the simulation of shipping of meagre (*Argyrosomus regius*) yolk-sack larvae obtained from spawning 1 (left) and 2 (right). The shipping time imposed on the larvae during the experiment were **A.** ST24 **B.** ST36 and, **C.** ST48. Superscript letters above columns indicate differences between different time points (packing, shipment, incubator) for each given experimental group (D4, D8, and D12) (two-way ANOVA, Tukey HSD, $P < 0.05$).

The susceptibility of larvae to the degradation of water quality and the extent of time larvae were exposed to it, was also tested.

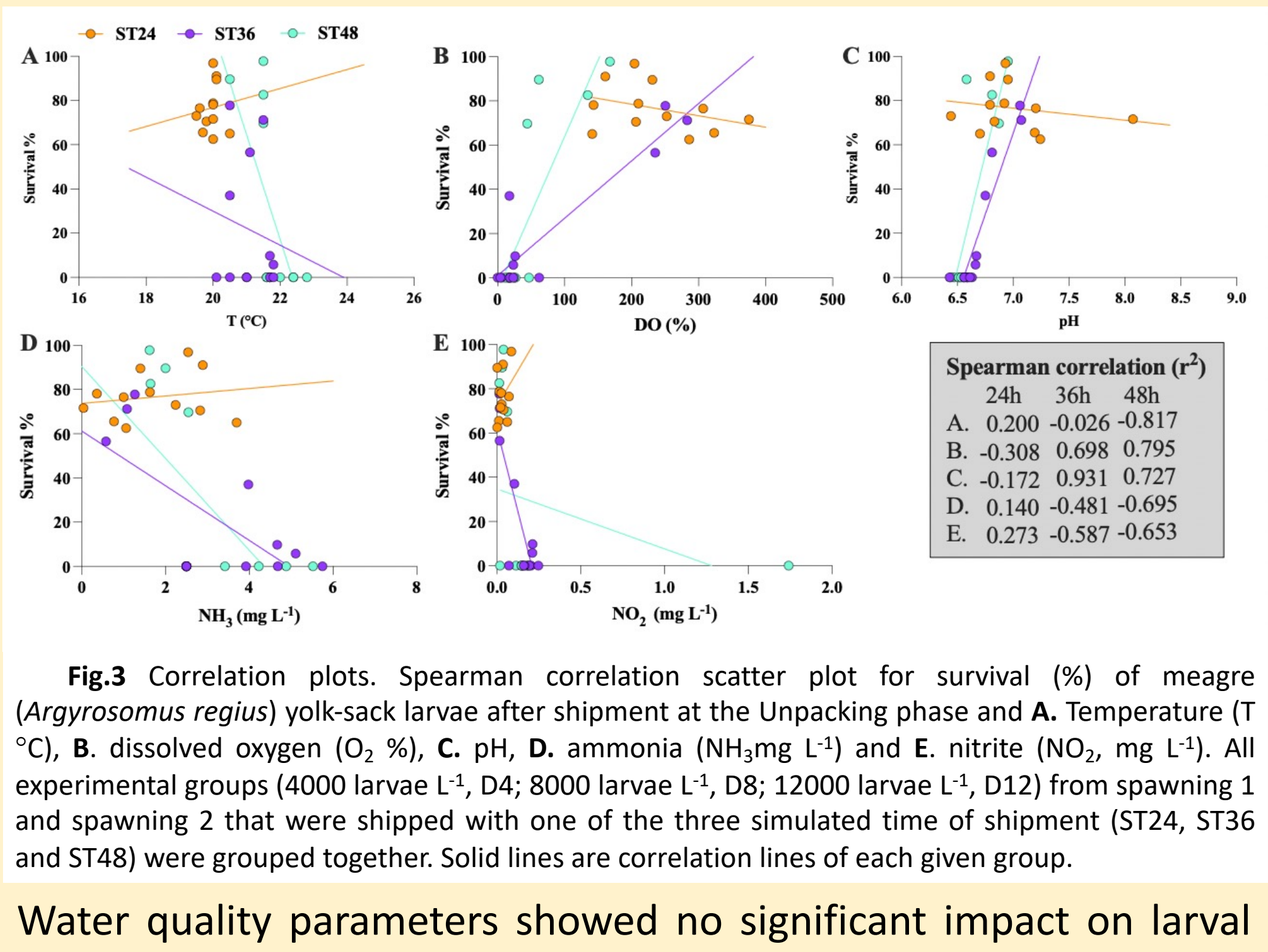

Fig.3 Correlation plots. Spearman correlation scatter plot for survival (%) of meagre (*Argyrosomus regius*) yolk-sack larvae after shipment at the Unpacking phase and **A.** Temperature (T °C), **B**. dissolved oxygen (O_2 %), **C.** pH, **D.** ammonia (NH_3mg L^{-1}) and **E**. nitrite (NO_2, mg L^{-1}). All experimental groups (4000 larvae L^{-1}, D4; 8000 larvae L^{-1}, D8; 12000 larvae L^{-1}, D12) from spawning 1 and spawning 2 that were shipped with one of the three simulated time of shipment (ST24, ST36 and ST48) were grouped together. Solid lines are correlation lines of each given group.

Water quality parameters showed no significant impact on larval survival during ST24 (24-h) of shipping (**Fig.3**). On the other side, temperature appeared to be negatively correlated to the percentage of larval survival after the simulation of shipment at ST48. Moreover, nitrogenous compounds influenced negatively with a moderate to strong trend the survival of larvae. Precisely at both ST36 and ST48, the increase of both NH3 and NO2 levels significantly hampered survival.

Conclusions

If transportation of meagre (*Argyrosomus regius*) eggs exceeds 16 h, eggs start to hatch in the shipping container and high mortality is frequently recorded. The present work showed that it is possible to extend this window of time using yolk-sac larvae.

Within 24h, successful transportation of yolk-sack larvae can be obtained at a concentration of 4000, 8000, and 12000 larvae L^{-1}, and with survival at arrival between 65 and 90%. Moreover, the time of shipment could be further protracted to 48h if the concentration of larvae is maintained at 4000 larvae L^{-1} without a decrease in larval survival percentages.