UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI BIOLOGIA

Corso di Laurea magistrale in Marine Biology

TESI DI LAUREA

Larval fish transport: enhancing protocol to increase fish welfare and survival in Meagre (Argyrosomus regius)

Relatore: Prof.ssa Daniela Bertotto

Dipartimento di Biomedicina Comparata e Alimentazione

Correlatore: Dott. Constantinos C. Mylonas
Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Crete (Greece)

Laureanda: Anahita Sodagar

ANNO ACCADEMICO 2023/2024

Index

Abstract

There is an increasing interest in high-value marine finfish, with the expansion of aquaculture. The transportation of early-life stages, especially fertilized eggs, from hatcheries to aquaculture and research facilities is a commonplace procedure. However, challenges such as low hatching rates and mortality were observed during egg shipment due to factors like high stock density, extended shipping times, physical shocks, and water quality issues. Shipping yolk sac larvae may offer a potential solution to address these challenges. This research aimed to develop an accessible, user-friendly, and adaptable protocol for shipping yolk sac larvae, focusing on critical factors such as stocking density and transport duration. Three larval concentrations (4000 larvae L^{-1}, D4; 8000 larvae L^{-1}, D8; 12000 larvae L^{-1}, D12) from two spawning events of meagre (*Argyrosomus regius*) were tested in replicates at three different simulated shipping times of (24h, ST24; 36h, ST36; 48h, ST48). Post-shipping survival was monitored within incubators for 24 hours. Results indicated successful transportation for all tested densities within ST24, with average (±SD) survival rates of 69 ± 2.47% for D4, 81± 4.24% for D8, and 80 ± 10.96% for D12. Higher densities (D8 and D12) exhibited higher survival rates at unpacking during ST24. Additionally, D4 showed transport viability within all tested shipping times, with an average(±SD) survival rate of 72 ± 12.30%. Surprisingly, D4 showed the highest average (±SD) survival rate of 85 ± 1.47% at ST48. While water quality showed no significant impact on larval survival during ST24, extended transportation emphasized the importance of maintaining optimal temperature and oxygen levels. The results underscore the need for species-specific considerations in larval transportation protocols. The stock densities and shipping duration tested in this study were important steps in improving long-distance

transport protocols and pushing the limits of the early life stage of shipping marine fin fish.

1 Introduction

1.1 The importance of aquaculture

Aquaculture, the controlled cultivation of aquatic organisms, has experienced rapid global growth in recent decades compared to other livestock sectors. As the world's population increases, aquaculture plays a crucial role in enhancing global food security by supplying a consistent and reliable source of protein, essential fatty acids, vitamins, and minerals(Frankic & Hershner, 2003). Additionally, aquaculture and its related industries contribute to economic growth in communities by generating employment opportunities, boosting income, and participating in international trade through the exportation of fish and seafood products (Subasinghe et al., 2009). Moreover, aquaculture helps reduce pressure on wild fish populations by providing an alternative source of seafood and contributing to the conservation of wild stocks. The importance of aquaculture underscores the necessity to expand and implement novel technologies and methods for its sustainable growth(Anderson et al., 2017).

1.2 Meagre (*Argyrosomus regius*)

The meagre (*Argyrosomus regius*, Asso 1801)(Fig. 1) , a member of the family Sciaenidae, also known as croakers and drums (Nelson, 2016) is one of the emerging species in the European and Mediterranean aquaculture industry **(**Diken et al., 2019; Duncan et al., 2012). This specie is identified by its elongated silver-gray body, large head, and small eyes. Its mouth is yellow-gold, positioned terminally, and lacks barbels (FAO, 2005–2010b). Meagre is recognized for producing drumming sounds during the spawning period, facilitated by a specialized sonic muscle closely associated with the swim bladder (Lagardère & Mariani, 2006). Widely distributed in the Mediterranean sea , undertakes

anadromous migration and using estuaries as both breeding and nurseries areas (Trabulo et al., 2023)**.** Meagre exhibits high tolerance to a wide range of salinity (5–55‰) and temperature (13–28°C) (Fountoulaki et al., 2017; Mohammed-Geba et al., 2017). While in its natural habitat, the fish can grow up to 2 m and reach 50 kg (FAO, 2005–2010b), the optimal growth for farmed meagre is observed in the 24–29°C range, leaning towards the lower end (Kır et al., 2017; Stavrakidis-Zachou et al., 2021). Fast growth rate, high tolerance to salinity and temperature, high feed conversion ratios, and substantial nutrient content collectively position the meagre as a promising candidate specie for Mediterranean aquaculture (Duncan et al., 2013; Fountoulaki et al., 2017; Grigorakis et al., 2011).

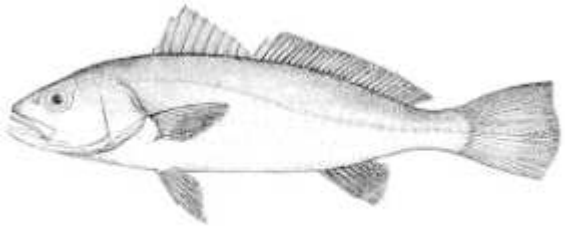

Fig. 1. Meagre (*Argyrosomus regius*, Asso 1801), (FAO, 2009)

1.3 Production and market landscape

Most of the world's meagre production, about 68% in 2019 (37,536 tonnes), comes from aquaculture, while 32% (18,024 tonnes) is caught in the wild. Over the last decade, farming meagre has increased in the main producing countries(Fig.2) (EUMOFA., 2022)**.**Egypt is the top global meagre producer, contributing 47% of the world's production in 2019, with most (96%) coming from aquaculture, followed by the EU-27 (82% from aquaculture), Angola and Mauritania (100% from wild catches each), and Turkey (98% from aquaculture) (EUMOFA., 2022).In

the EU, both aquaculture and fishery production increased significantly from 2010 to 2019, with respective growth rates of +298% and +92%. The rise in farmed meagre production is related to an increase in farmed production since 2015 (+6.323 tonnes of farmed meagre between 2015 and 2019) (EUMOFA., 2022).Meagre production is expected to grow fast in the medium term due to the high acceptance levels of consumers provided by attractive fish shape, good nutritional value, low-fat content, excellent taste, and firm texture suitable for various recipes (Duncan et al., 2013; Fountoulaki et al., 2017).

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	% total 2019	Evol. 2019 / 2010
Aquaculture	14.356	14.398	10.133	6.583	12.109	14.275	23.126	31.335	33.555	37.536	68%	+161%
Fisheries	23.397	20.523	23.167	21.719	23.207	11.204	15.820	30.717	20.463	18.024	32%	-23%
Total	**37.753**	**34.921**	**33.300**	**28.303**	**35.316**	**25.479**	**38.946**	**62.052**	**54.018**	**55.560**	**100%**	**47%**

Fig. 2. Evolution of world farmed meagre production and wild caught meagre (tonnes) (EUMOFA., 2022)

1.4 broodstock management and larval rearing

The aquacultural production of meagre has been developed successfully in last decade. In large-scale fish production, broodstock management ensures captive fish undergo reproductive maturation, spawning, and produce fertilized eggs(Mylonas et al., 2010). Despite reported reproductive dysfunctions under captivity, an effective spawning induction protocol has been established for broodstock reared in tanks. Through the application of hormone administration (Mylonas et al., 2013, 2015), either independently or in combination with thermal or photothermal control (De Mello et al., 2021; Mylonas et al., 2016), substantial quantities of high-quality eggs and sperm have been successfully obtained. Consequently, this approach has resulted in improved fertilization rates and heightened survival rates among the larvae (De Mello et al., 2021; Ramos-Júdez et

al., 2019). Meagre has an average standard length of (2.82 ± 0.37 mm) at hatching time point (Duncan et al., 2013). The pre-larval stage extends until 3 dah, with yolk reserves mostly consumed (SL = 3.25 mm) (Duncan et al., 2013; Klimogianni et al., 2013; Papadakis et al., 2013). Additionally, the liver development is observed during this time. Eyes are pigmented between 2 and 3 dahs, and a primary marginal fold from the head to the stomach appears. The pectoral fins become visible in the form of strands. (Papadakis et al., 2013). Larvae are typically fed 5–10 rotifers ml^{-1} enriched with commercial products or microalgae twice daily from 2 dah to 15–20 days after hatching (Duncan et al., 2013; Roo et al., 2010). The swim bladder, formed during the pre-larval stage, inflates at 4 to 6 dah post mouth opening. Tail flexion completion occurs at 14–15 dah, with fin differentiation and pelvic fins appearing at 18 to 19 dah. The main developmental stages conclude at 34 dah with scale appearance and final pigmentation, resembling juvenile adults (Papadakis et al., 2013). Meagre larvae can reach 16–19 mm total length by day 30 at 20–21°C, with culture conditions including a 12hL:12hD photoperiod and 500 lux light (Duncan et al., 2013). The environmental conditions for larval culture of meagre vary among different research stations and private companies, although they generally include the use of filtered sea water at temperature of (20.8-24.1ºC), salinity (27.0-40.0 g L^{-1}), oxygen (8.4-14.4 mg L^{-1}), and pH (7.5-7.9) (Diken et al., 2019; Papadakis et al., 2013; Roo et al., 2010)**.** Despite progress in different aspects of meagre production and a rising market demand, EU meagre aquaculture depends on only a few hatcheries, restricting the potential for significant development in large-scale production of this species(Duncan et al., 2018). Considering the valuable characteristics of meagre —high tolerance to fluctuations in water quality parameters attributed to its anadromous nature and remarkable resilience in

cultivated environments (Abdel-Rahim et al., 2020; Kır et al., 2017; Stavrakidis-Zachou et al., 2021)— developing a reliable method to transfer early-life stages, such as yolk-sack larvae, could bring potential advantages for commercial producers escaping the incubation phase and its costs and challenges. Additionally, research sections may benefit from this method by freeing up hatchery space and reducing its associated costs for experimental larviculture or conservation programs(Hayashida et al., 2023; Zink et al., 2011).

1.5 Early life stag shipping (pros and cons)

Aquaculture often requires the transportation of fish at different life stages for various purposes, including shipping eggs to facilities, transferring fry (yolk-sac, swim-up) from hatcheries to growth or fattening ponds, transporting wild animals for aquaculture facilities, releasing juveniles for stocking programs, and supporting research (Sampaio & Freire, 2016). The success of shipping for each life stage of fish depends on the duration of the shipping, water quality parameters, fish size, and fish species (Harmon, 2009; Sampaio & Freire, 2016). The activities associated with transporting, from preparation and packing to unpacking, possess the potential to induce physiological changes in the fish due to heightened stress and increase mortality post-shipping (Swann, 1993). The implementation of appropriate transport protocols for each purpose with the least stressful procedures is crucial for commercial producers. This is because it can significantly contribute to increasing the profitability of their business by boosting production, decreasing fish mortality and reducing costs(Harmon, 2009).

Shipping fish early-life stages (*i.e.* fertilized eggs, yolk-sack larvae and fingerlings) from hatcheries to aquaculture and research facilities takes place routinely (Hayashida et al., 2023; Hilomen-Garcia, 1998; Stuart et al., 2018) .

Transferring eggs is usually preferred because it offers several advantages, such as ease in counting, handling, disinfection and reduced costs due to higher carrying capacity compare to other stages(Scolamacchia et al., 2023; Stuart et al., 2018). However, some drawbacks such as low hatching rates, significant mortality and increased deformities have been noted during the extended shipping time with high density (Broach et al., 2017; Chattopadhyay et al., 2021; Endo et al., 2016; Hayashida et al., 2023). The optimal timeframe for transferring fish eggs after fertilization varies based on the specific species and the sensitivity of fish eggs to physical shock across the different stages of embryonic development.. This decreased success may stem from heightened physical shock damage to the eggs caused by an increased frequency of collisions during longer shipping durations(Hayashida et al., 2023; Hilomen-Garcia, 1998; Krise, 2001). Unexpected high occurrences of deformed larvae or those displaying apparent Iordoses upon hatching may be caused by disruption of embryonic cells during handling and transport of eggs.(Garcia & Toledo, 1988) . The greatest concern with any live-fish transport is minimizing the amount of stress. Potential stressors related to transportation include physical shock during handling, collisions among individuals, degradation of water quality, and increased bacterial load, which are considered common challenges throughout the shipping process at all life stages (Harmon, 2009; Sampaio & Freire, 2016). Although there are some more stressors, like stopping feeding and starvation due to a decrease in metabolism during fingerling transportation and post-shipping recovery. Hence, shipping yolk sac larvae may be more practical, as it avoids the challenges associated with the critical embryonic development, hatching phase and facing starvation as yolk sac larvae rely on consuming the yolk reservation.

Shipping bags can be considered a static environment where egg movement is linked to vehicle motion (Fey & Greszkiewicz, 2023). In prolonged transportation with frequent stops, demersal eggs like hilsa shad (*Tenualosa ilisha*) tend to settle at the bottom, leading to oxygen depletion, anoxia, stress, and bacterial contamination (Chattopadhyay et al., 2021). Buoyant eggs, such as milkfish (*Chanos chanos*) can form mats on the water surface in undisturbed water. This situation could cause a swift decline in the quality of the static environment (Hilomen-Garcia, 1998). For northern pike (*Esox lucius*) eggs, using a small mobile transportation system allows egg movement and reduces the risk of eggs sticking to each other during transport and is more effective over long distances and higher densities (Fey & Greszkiewicz, 2023). It is supported by the fact that hatchery operators maintain continuous water circulation during egg incubation, preventing settling at the bottom and ensuring a constant supply of oxygenated water without interruption (Moretti et al., 2005). Considering these challenges, although yolk sac larvae are weak swimmers, their movement is more independent of the water's motion, potentially making them advantageous for shipping.

1.6 limiting factor (water quality, density, time duration)

Previous studies showed the importance of maintaining adequate levels of physicochemical parameters such as temperature (T), oxygen (O_2), pH, and ammonia (NH_3) during transportation (Broach et al., 2017; Colburn et al., 2008; Gomes et al., 2003; Guo et al., 1995; Poxton & Allouse, 1982; Snow et al., 1978; Zink et al., 2011). Responses to water quality changes, both in amplitude and velocity of these changes, have been found to be diverse among different species (Poxton & Allouse, 1982). To limit these changes and improve survival, the effect of various products such as water conditioners, probiotics, polyethylene glycol and

anesthetics were evaluated (Guo et al., 1995; Hayashida et al., 2023; Stuart et al., 2015; Zink et al., 2011). Density and shipping time are crucial factors that drive and influence the entity of water quality degradation and the subsequent survival rate after shipment (Gomes et al., 2003; Hayashida et al., 2023; Scolamacchia et al., 2023; Stuart et al., 2018). To achieve this, the maximum survival rate post-shipping must come along with cost-effectiveness to transport the highest density of individuals with the lowest volume of water without increasing mortality for the most prolonged time of shipping possible (Broach et al., 2017; Stuart et al., 2018). However, a certain level of post-transportation mortality might be unavoidable, thus, efforts should be made to minimize it as much as possible (Alias & Siraj, 1988; Broach et al., 2017; Scolamacchia et al., 2023).

Water quality, hatching success, and survival rate are also linked to the bacterial load occurring naturally on the outer surface of the eggs. Egg disinfection is a compulsory preventive practice when transported eggs are introduced to a new facility (De Swaef et al., 2016). The disinfection of eggs prior to their shipment can mitigate the spread of pathogens between facilities and maintain appropriate oxygen levels during shipping by reducing microbial respiration.(De Swaef et al., 2016; Salvesen & Vadstein, 1995). However, the chemicals used for disinfection can also hamper the development of the embryos and post-hatching performance (Ben-Atia et al., 2007; Katharios et al., 2007; Salvesen & Vadstein, 1995). The optimal timeframe for disinfecting fish eggs after fertilization is species-specific, so that eggs may exhibit differential sensitivity during this operation (e.g. chemicals, exposure time), which must be performed with caution (Douillet & Holt, 1994; Katharios et al., 2007; Salvesen & Vadstein, 1995). Hence, shipping newly

hatched larvae might be more suitable for some species whose eggs display excessive sensitivity to disinfection.

When spawners are not synchronized, the hatching of eggs does not occur simultaneously. Unexpected shipping delays can lead to some eggs hatching during transit, triggering a rapid deterioration in water quality. This decline in water quality is a critical factor contributing to the decrease in survival rates and causing mortality post-transfer (Holt & Arnold, 1983). NH3 is produced as a byproduct of protein and amino acid catabolism. During embryonic development, metabolism based on amino acids causes a significant production of ammonia, which increases due to limitations in effective ammonia excretion caused by the chorion capsule in embryos and the absence of functional gills (Essex-Fraser et al., 2005; Zimmer et al., 2017).The presence of a chorion capsule causes the internal NH3 level to rise during embryonic development (Essex-Fraser et al., 2005; Zimmer et al., 2017). Therefore, shipping yolk sac larvae may be more beneficial across the extended shipment and high densities because the critical hatching phase is bypassed.

1.7 Aim of study

As the ability to trade early life stages can constitute a competitive advantage by enhancing business profitability through increased cost-effective production. Producers that possess the necessary know-how do not share it willingly with third parts, hence, excluding many potential stakeholders from utilizing it effectively. Our research aimed to provide producers and public institutions alike with a practical method well-detailed shipping method for yolk sac larvae. Therefore, the objective of the present study was to provide an efficient, accessible, and user-friendly protocol for shipping yolk sac larvae with clear steps

that can be adjusted based on specific species requirements and their tolerance due to stress. We focused on limiting factors, which might affect survival rate and water quality in a closed system. The examined parameters were stocking density and transport duration, as overloading and prolonged shipping can expose larvae to various stressors (i.e., water temperature, pH, and oxygen saturation, physical disturbances from water movement). In addition, the survival rate after a 24-hour post transportation period was evaluated to investigate short-time effect on larvae.

2 **Materials and Methods**

The experiment was held in the AQUALABS facilities of the Institute of Marine Biology, Biotechnology, and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (HCMR), Crete, Greece (Fig. 3A, B).

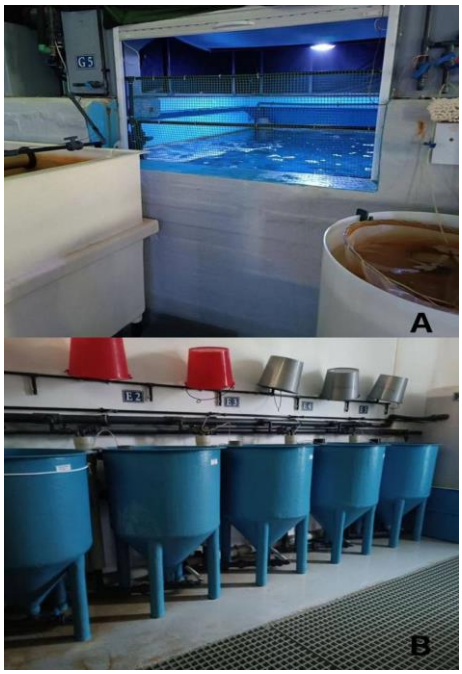

Fig. 3. Broodstock facilities of AQUALABS at the Institute of Marine biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (HCMR), broodstock.

2.1. Experimental design

Three preliminary trials were conducted to set up optimal temperature, water volume/air ratio in the shipping bag, and density of larvae as well as the best combination of them to optimize larval transportation (data not shown). In addition, the effect of using a siphon and a 2-L beaker during the transfer of larvae from

incubators to shipping container, as a causative agent of deformities, was examined. Based on the results obtained, a temperature of 19 ± 0.5 °C, a water volume of 20 L, and 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). The experiment was repeated twice, using eggs from two different spawning.

2.2 Egg production, collection and stocking

Eggs were obtained from a meagre broodstock maintained in a rectangular 40-m^3 tank supplied with aerated borehole seawater, and exposed to attenuated seasonal photothermal conditions (De Mello et al., 2021). Spawning was obtained artificially through administration of gonadotropin releasing hormone agonist (GnRHa). Females (n = 7) of mean ± SD body weight (BW) of 12 ± 3.1 kg) were given liquid injections with desGly^{10}, DAla^{6}, Pro^{9}-GnRH-NEthylamide (H-4070, Bachem, Switzerland) with an effective dose of 15 ± 0.2 μg GnRHa kg^{-1} BW; males (n= 6; 11 ± 2.5 kg BW) were treated with a GnRHa implant constructed with [Ethyl-Vinyl Acetate]-copolymer for an effective dose of 51 ± 5.1 μg GnRHa kg^{-1} BW (Fakriadis et al., 2020; Mylonas et al., 2016) (Fig. 4).

Fig. 4. Administration of solid implants loaded with GnRHa to a meagre (*Argyrosomus regius*) male.

After spawning, eggs were collected through a passive egg-collector placed at the exit of the outflow of the breeders' tank, and stored in a 10-L bucket provided with aeration. To reduce at minimum the risk of any masking effect due to the possible variable quality of the eggs among spawning events, only those with a fertilization success rate ≥ 85% were used. Eggs from two different spawning were used and the experimental conditions were tested in replicate for each spawning. In order to estimate daily relative fecundity (total fecundity x females Kg^{-1}) and fertilization success (number of viable eggs: number of total $eggs^{-1}$), a 10-ml sub-sample was obtained after vigorous agitation and examined under a stereoscope. Eggs were then left in the bucket for 5 minutes without aeration to allow the floating (live) to separate from the sinking (dead) before being transferred to the incubators.

To limit the addition of organic matter from the spawning tank, live eggs were thoroughly rinsed before being transferred to 247-L fiberglass cylindro-

conical incubators (Fig. 5). Shortly, 10-L buckets filled with clean borehole seawater were prepared, and eggs were subsequentially transferred from one bucket to another (n=6) until debris were not detectable at sight. Eggs were distributed to the incubators (~1000 gr per incubator). They were maintained in the incubators until hatching (~36h after spawning, 19 ± 0.5 °C), with a 300% daily water renewal and gentle aeration with an airstone. To remove dead eggs and shells from the incubators, aeration and water renewal were stopped for 10 minutes and the following actions were taken: i) two quick flushes were performed by opening the bottom drains and ii) 50% of the water volume was exchanged by using a syphon inserted into outflow screen (protected with 300µm mesh).

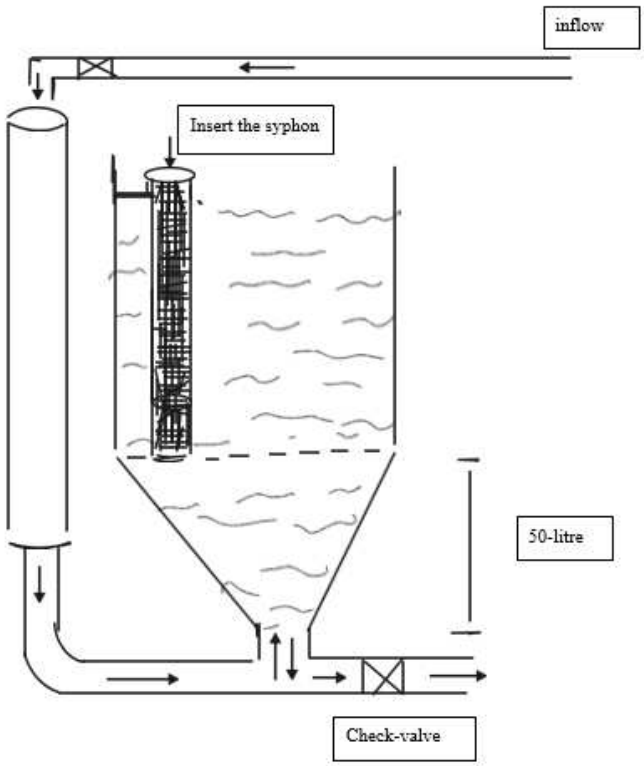

Fig. 5. cylindro-conical incubator

2.3 Preparation of larvae and shipping simulation

Two hours after hatching of all eggs was completed, yolk-sack larvae were prepared for shipping. At first, to facilitate the collection of larvae, the water level of the incubator was reduced to reach a volume of 50 L. The water removal was performed using a siphon, placed in the outflow section and protected with a 300-µm mesh to prevent larvae from also being removed; water was drained at a speed of 5 L min^{-1} (Fig. 6). As larval density increased in the incubator, dissolved oxygen (DO, %) was monitored and oxygen was provided to maintain saturation ≥ 100 %. Concentrated larvae were then transferred via a 2-L beaker in one 10-L bucket, filled previously with two liters of oxygenated borehole water, until a total volume of 10 L was reached in the bucket. Three 10-ml subsamples were taken to estimate the concentration inside the buckets (L^{-1}) as described above for the evaluation of daily relative fecundity, by counting the number of larvae using a stereoscope. Once the desired larval concentration was obtained, dissolved oxygen saturation was adjusted to reach 300–350% by introducing a wooden bubbler into two buckets: one holding 10 liters of water containing larvae, and the other with 10 liters of borehole seawater. This adjustment was made before the contents were transferred into the polyethylene bag.

Fig. 6. Removal of water with a siphon (larval concentration).

Larvae were then transferred to a polyethylene shipping bag (20 L, VALCHOS BROSS S.A. Greece) positioned into a Styrofoam box (59L X 39W X 27H cm, wall thickness: 2.5cm, FELIPACK, Greece). Firstly, 5 L of oxygenated borehole seawater were added to the empty shipping bag. Larvae were then transferred gently from the bucket to the shipping bag using a 2-L beaker and a funnel. Once all 10 L from the bucket were transferred to the bag, 5 L more of oxygenated water was added to reach the final volume of 20 L. Eventually, shipping bag was sealed with a butterfly cap following oxygen was inflated into the polyethylene bag. An ice pack was attached to the inner side of the lid to prevent the temperature from increasing drastically during transport. Eventually, each box was sealed with plastic tape and driven for ~70 Km at a speed of ~70 Km h^{-1} before being returned to the facility (Fig. 7).

Fig. 7. The boxes containing the prepared larvae for shipping.

2.4 Larval survival evaluation

Boxes were opened at the end of the time established for the simulated shipping and the content of each shipping bag was transferred into two 10-L buckets, and subsamples of 10 ml (n = 3) were collected from each to estimate the mean larval survival (%). Subsequentially, live larvae were passed into a cylindro-conical incubator and monitored further for 24h. Due to changes in pH, oxygen levels and increase in temperature during the time of simulated transport, larvae were acclimatized by slowly adding water from the incubator equivalent to one fifth of the bucket volume before being released into the incubators.

2.5 Monitoring of environmental parameters

Prior to packing and after transportation, physico-chemical parameters such as temperature (T, °C), dissolved oxygen (DO, %) (H01P OXYGUARD, HANDY, POLARIA, Denmark), pH (PHC101, NACH, LANGE, USA), ammonia (NH3, mg L^{-1}) and nitrite (NO2, mg L^{-1}) (DR 2800™ Portable Spectrophotometer, NACH, USA) were monitored (Fig. 8).

Fig. 8. Measuring the ammonia and nitrite

2.6 Statistical analysis

Post-shipping larval survival dependency from larval density and duration of shipping was evaluated in each spawning individually using a two-way ANOVA followed by Tukey's test. Correlation between changes of physicochemical parameters during transportation and survival of larvae in relation to shipping time was assessed using a Spearman's correlation. In all the statistical tests performed, p-values below 0.05 were considered statistically significant. Statistical analyses were run using GraphPad Prism 9.4.1 for Mac OS, GraphPad Software, San Diego, California USA, www.graphpad.com.

3 **Results**

3.1 Effect of larval density and time of shipment on larval performances

Despite all the experimental conditions (i.e., larval density and duration of simulated shipment) being the same for larvae obtained from two spawning, the results were analyzed separately to assess the possibility of individual differences arising from variations in the quality of each spawning. In larvae from both spawning events that were used during the shipping experiment, a significant effect on survival due to larval density (larvae L^{-1}) was evident at every shipping time (two-way ANOVA, Tukey's HSD, $P < 0.05$, Fig. 9 and 10), except for larvae obtained from spawning 2 and exposed to ST24 (Fig. 10A). Precisely, in spawning 1, larvae from the D4 group did exhibit significant mortality only 24h after the longest shipping time (ST48) when reallocated in the incubator (Fig. 9C). On the other hand, significant mortality was recorded in larvae from the D8 and D12, exposed to any of the shipping time (Fig. 9). While, at ST24 larvae from D8 and D12 groups had a gradual decrease of survival and reached the minimum of 3.2 ± 1.6 and 33.8 ± 0.6 % when monitored in the incubator (Fig. 9A), larvae did not survive shipment and survival percentage dropped to zero at the unpacking stage of the experiment after being exposed to ST36 and ST48 (Fig. 9 B, C). On the contrary, in larvae obtained from spawning 2, survival of larvae from all experimental groups did not show any significant effect due density of individuals at ST24 (Fig. 8A), and for larvae from the D4 group, the same lack of negative effect was also recorded at ST36 and ST48 (Fig. 10B, C). Moreover, although a small percentage of larvae from the D8 group (7.8 ± 2.8 %) was found alive during the unpacking phase (Fig 10B), survival of larvae from D8 and D12 groups reached 0% at both ST36 and ST48 (Fig. 10B, C).

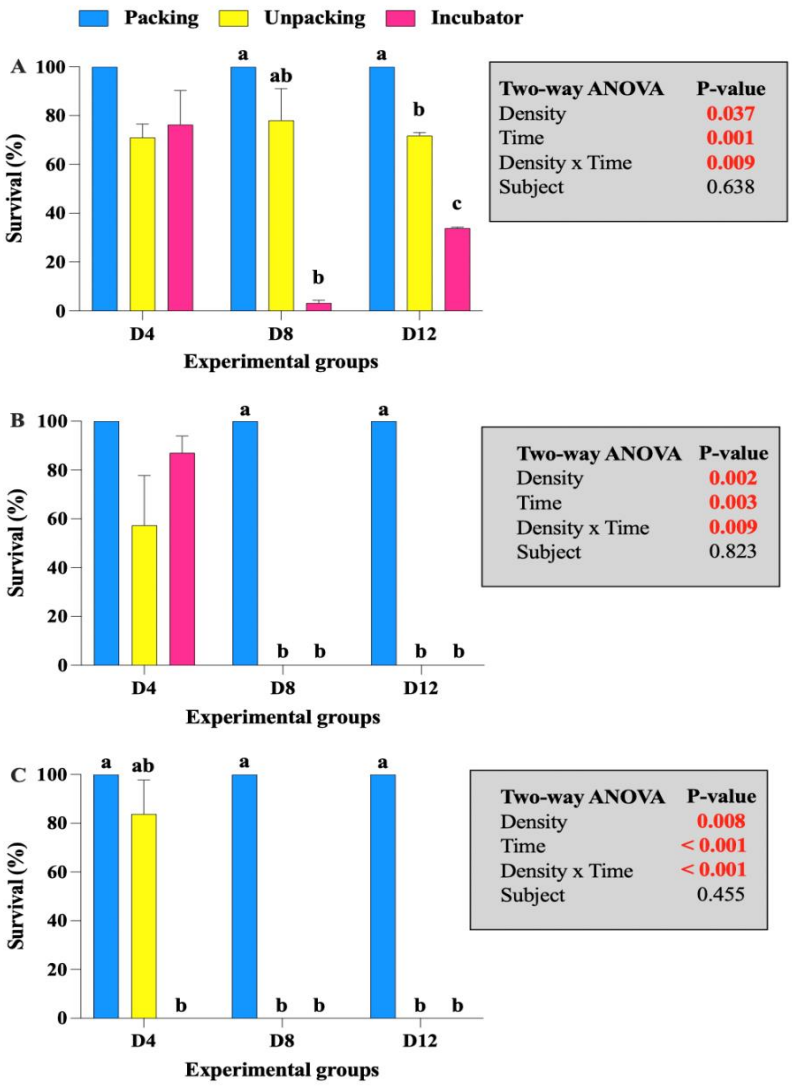

Figure 9. Mean (±SEM) larval survival (%) density during the simulation of shipping of meagre (*Argyrosomus regius*) yolk-sack larvae obtained from spawning 1. The shipping time imposed on the larvae during the experiment were **A.** 24h (ST24) **B.** 36h (ST36) and, **C.** 48h (ST48). Superscript letters above columns indicate differences between different time points (packing, shipment, incubator) for each given experimental group (4000larvae L^{-1}, D4; 8000 larvae L^{-1}, D8; 12000 larvae L^{-1}; D12) (two-way ANOVA, Tukey HSD, $P < 0.05$).

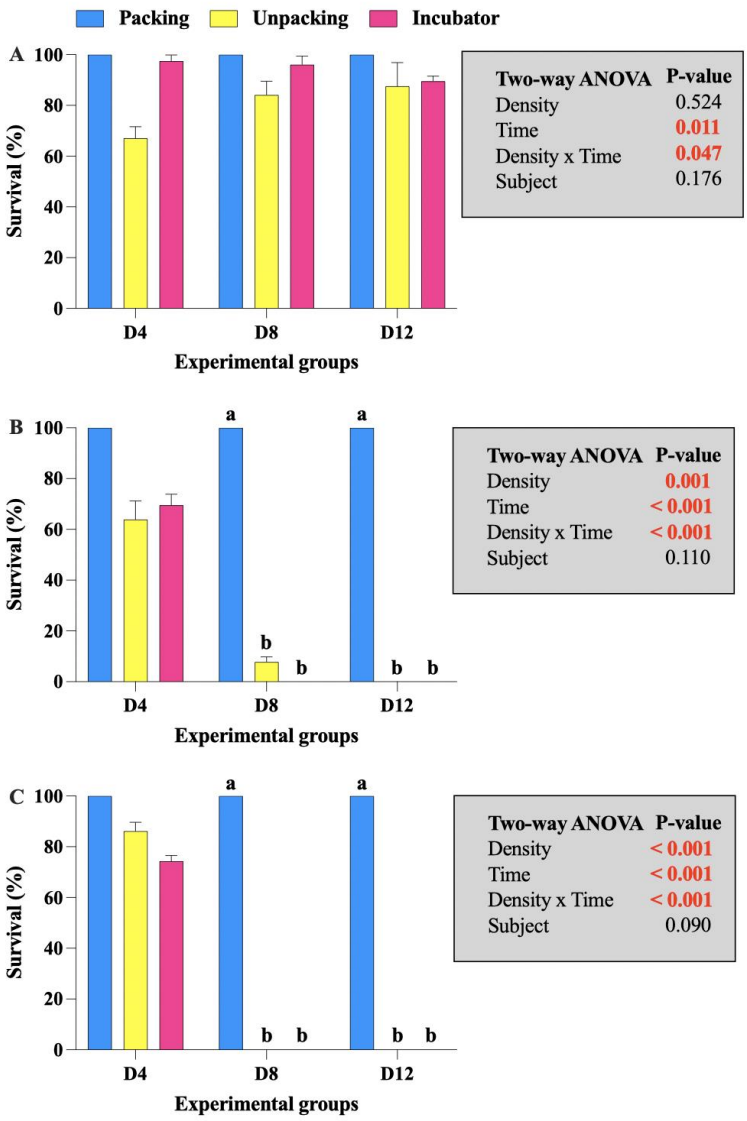

Figure 10. Mean (±SEM) larval survival (%) density during the simulation of shipping of meagre (*Argyrosomus regius*) yolk-sack larvae obtained from spawning 2. The shipping time imposed on the larvae during the experiment were **A.** 24h (ST24) **B.** 36h (ST36) and, **C.** 48h (ST48). Superscript letters above columns indicate differences between different time points (packing, shipment, incubator) for each given experimental group (4000larvae L^{-1}, D4; 8000 larvae L^{-1}, D8; 12000 larvae L^{-1}; D12) (two-way ANOVA, Tukey HSD, $P < 0.05$).

3.2 Correlation between water quality and shipping time on larval survival

Postulating that independently by the larval density (i.e. D4, D8, D12) used during the experiment, the survival of larvae was dependent on the degradation of water quality and the extent of time that larvae were exposed to it, its correlation with different environmental proxies was evaluated.

No significant effect of any of the parameters considered in the analysis was found to be correlated with the survival of larvae (Fig.11, 12). The increased temperature during transportation appeared to be negatively correlated to the percentage of larval survival after the simulation of shipment at ST48 (Spearman's correlation, $P < 0.05$, Fig. 11A). On the other side, a positive strong correlation was found between larval survival and proxies such as DO and pH after ST36 and ST48 shipping time (Fig. 11B, C), almost being monotonic between pH and survival at ST36. Moreover, nitrogenous compounds negatively influenced in a moderate to strong trend the survival of larvae (Fig. 12). Precisely at ST36 only the levels of NO_2 significantly decreased the percentage of survival, while at ST48, the increase of both NH_3 and NO_2 levels significantly hampered survival (Spearman's correlation, $P < 0.05$).

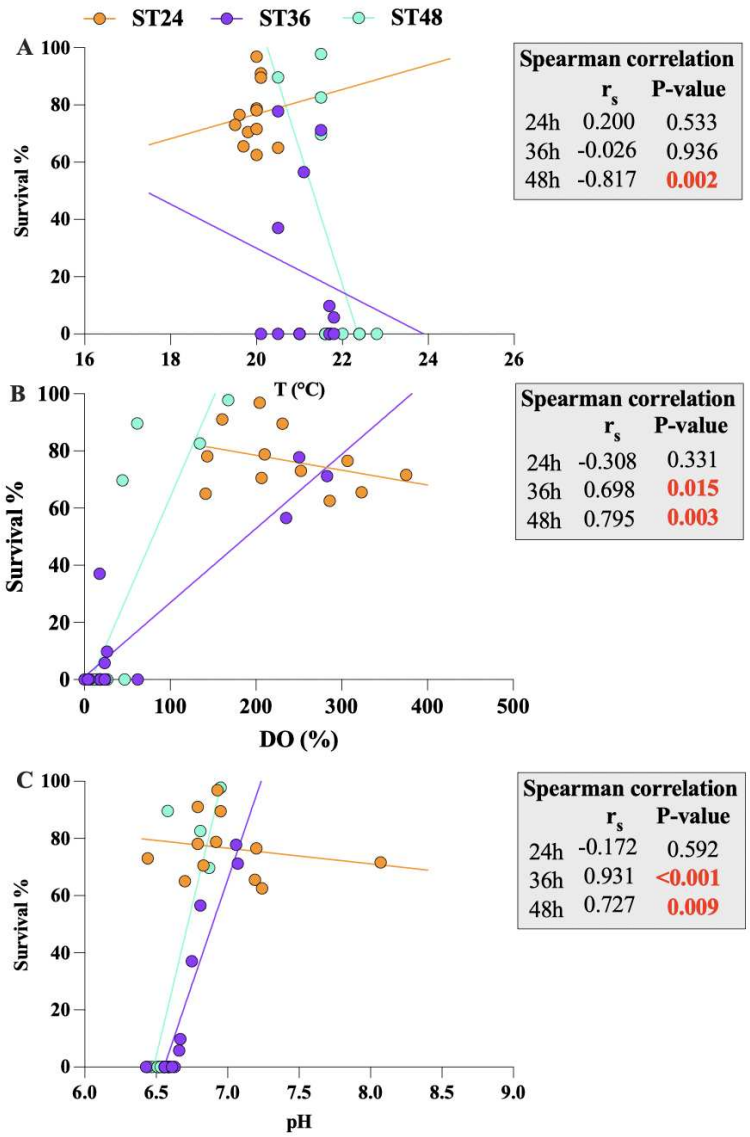

Figure 11. Correlation plots. Spearman correlation scatter plot for survival (%) of meagre (*Argyrosomus regiuis*) yolk-sack larvae after shipment at the opening of the shipping containers (Unpacking phase) and **A.** Temperature (T °C), **B**. dissolved oxygen (O_2 %), and **C.** pH. All experimental 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 (24h, ST24; 36h, ST36 and 48h, ST48) were grouped together. Solid lines are correlation lines of each given group ($P < 0.05$).

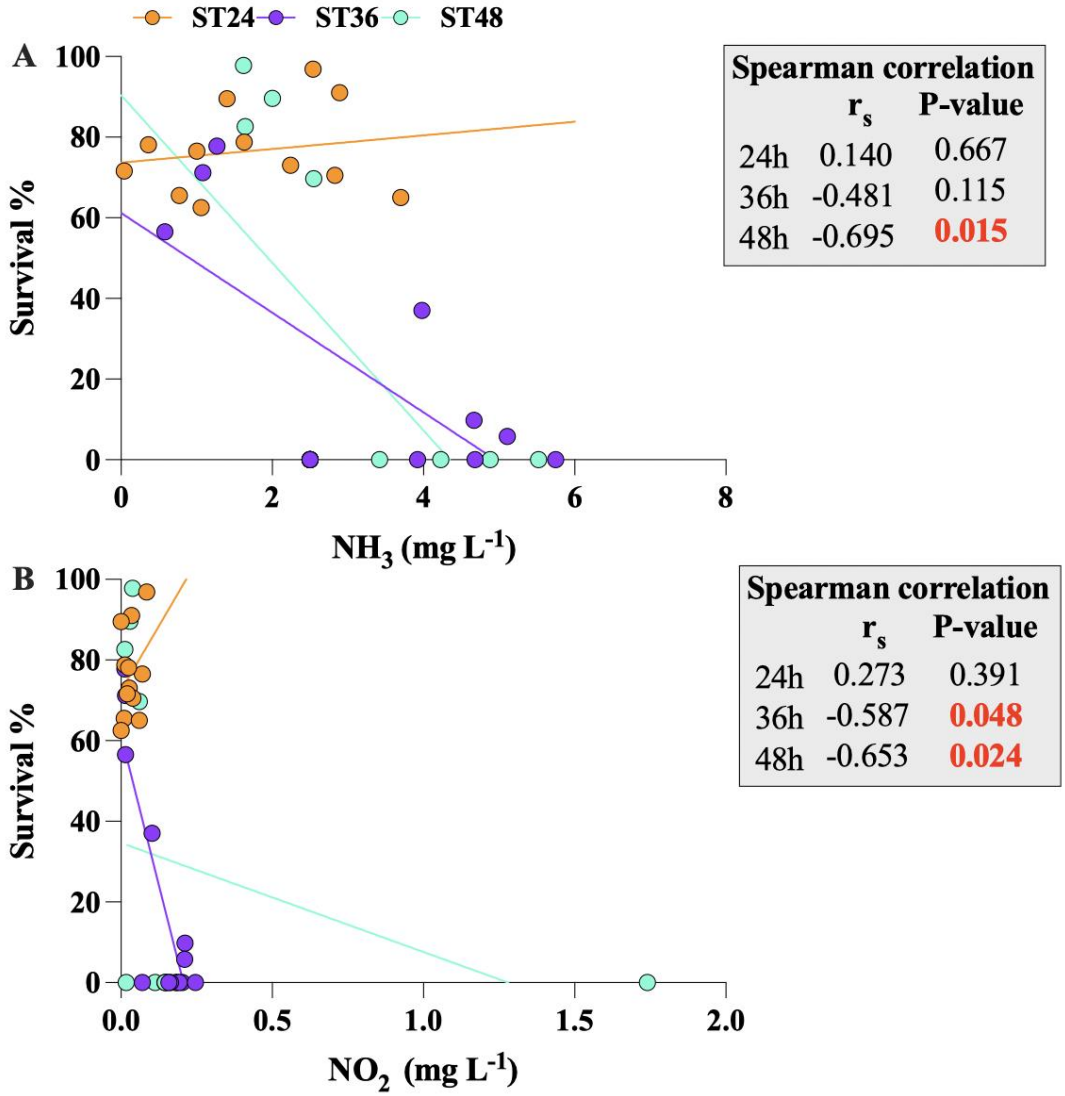

Figure 12. Correlation plots. Spearman correlation scatter plot for survival (%) of meagre (*Argyrosomus regiuis*) yolk-sack larvae after shipment at the opening of the shipping containers and **A.** ammonia (NH_3-N, mg L^{-1}) and **B**. nitrite (NO_2, mg L^{-1}). Larvae were grouped and assigned to one of the three shipping times: (24h, ST24; 36h, ST36; 48h, ST48). Solid lines are correlation lines of each given group ($P < 0.05$).

4 **Discussion**

The present study aimed to provide a practical and detailed protocol for transporting yolk sac larvae that can be adjusted to different species. We specifically examined factors such as stocking density and transport duration, focusing on their impact on survival rates and water quality in closed systems during the shipping process.

Although the number of studies on shipping the early life stages of marine fish is still limited, various post-shipping survival rates have been reported. 60-day-old juvenile grouper(*Epinephelus striatus*) achieved over 95% survival at 23°C and a stock density of 50-100 L^{-1} after 8 h (Estudillo & Duray, 2003).Yellowfin tuna (*Thunnus albacares*) yolk sac larvae, at a density of 871 L^{-1}, exhibited 88% survival rate after 24 h, including the addition of Bacillus probiotic to shipping bags; however, this survival rate did not show a significant difference compared to the results of the control group (Zink et al., 2011). California yellowtail (*Seriola lalandi*) and white seabass (*Atractoscion nobilis*) shipped for 24 h at a density of 6750 and 3000 L^{-1}, respectively, while using a pH buffer, demonstrated over 90% survival (Stuart et al., 2015). Moreover, the survival rates for yellowtail tuna (*Thunnus albacares*) and California yellowtail (*Seriola lalandi*) were mentioned, based on unpublished data, indicating a range of 70% to 95% after 24 hours of air shipping at 1000–2000 L^{-1}. These survival rates were observed using the same concentration of pH buffer (Stuart et al., 2015). One-day yolk sac larvae of hilsa (*Tenualosa ilisha*) showed over 90% survival after being transported for 120 km over 5 hours on the road at a density of 1000 L^{-1}, and six-day-old yolk sac hilsa (*Tenualosa ilisha*) achieved over 95% survival at 400 L^{-1} after 2 h of shipping (Chattopadhyay et al., 2021). Juvenile milkfish (*Chanos chanos*) at optimal

densities of 60 L^{-1}, 40 L^{-1}, and 20 L^{-1} for 12, 16, and 20 h, respectively, demonstrated a survival rate exceeding 95% at temperatures of 20°C to 22°C in combination with the anesthetic (2-phenoxyethanol) (Failaman et al., 2022). Therefore, it is evident that variations in post-shipment survival rates are influenced by a multiplicity of factors such as species, age, shipment duration, density, water quality, and the techniques employed, highlighting the complexity of these interactions in the transport of early life stages of marine fish.

Our study showed that newly hatched, meagre yolk sac larvae could be successfully transported at three tested densities within ST24 period. The average (±SD) survival rates were 69 ± 2.47% for D4, 81± 4.24% for D8, and 80 ± 10.96% for D12. These results suggest that the appropriate duration for transporting meagre yolk sac larvae, ensuring survival in both unpacking and incubators, was ST24.

Although immediate survival at unpacking was not affected in the first spawning following ST24 duration, D8 and D12 experienced a decline post-shipping in the incubators. In contrast, during the second spawning event, all densities exhibited high survival rates at both time points within ST24. Therefore, conclusions regarding observed survival must be made with caution, considering post-trial survival.

During the ST48 shipping duration, D4 was successfully transported while maintaining its survival within the incubators. D4 was successfully transported during this period while maintaining its survival within the incubators. Surprisingly, D4 exhibited the highest average (±SD) survival rate of 85 ± 1.47% at ST48despite the reduced water quality during the shipping time in compression to ST24 and ST36. Moreover, in another study, Yolk Sac Larvae of Yellowfin tuna exhibited high survival rates after 24 h of simulated shipping, despite the degradation of water

quality, especially in control bags where no water conditioner was employed (Zink et al., 2011). Even though we do not have a clear explanation for this now, it opens the door for more research opportunities in the future.

In the present study, D4 demonstrated successful transfer across all shipping times (ST24, ST2, and ST48) without water regulation. Post-transfer survival was observed within the incubators for 24 h, except for the first spawning after ST48, which resulted in total mortality. In addition, D12, which had the highest stock density, was successfully transferred within ST24 without water parameter regulation, showing an average (±SD) survival rate of 80 ± 10.96% while maintaining survival within the incubator.

The stock density of D12 and transport duration of ST48tested in this study significantly surpassed those in prior research, pushing the limits of marine fish larval shipping.

Maintaining water quality during larval shipping is influenced by factors such as density, transit duration, and regulation of physicochemical parameters in water. The current study avoided any adjustment of water quality factors to better understand their impact on larval survival and limitations during simulated shipping.

Throughout the ST24 shipping period during unpacking, water quality parameters such as T, pH, O2, NH3, and NO2 showed no significant effect on larval survival. The results of the statistical analysis indicate that the water quality parameters examined may not be deemed the primary concern for larval survival during the ST24 in the present study. Additional investigation is required to ascertain whether there are any long-term effects on larvae following shipment.

By extending the shipping duration to ST36and ST48, the influence of water quality parameters on survival became significantly pronounced, emphasizing their impact over a prolonged shipping period. Furthermore, an increase in T can lead to a decrease in pH, reduced O2, and increased NH3 and NO2 toxicities (Kir et al., 2015, 2015, 2016). The strong correlation observed between T and O2 on survival, along with pH, NH3, and NO2, during the ST48, suggests that maintaining an optimal T and O2 during transit may significantly influence overall water quality and survival.

Our research revealed a surprising observation in contrast to other studies that emphasize high stocking density as a primary factor limiting survival during fish transportation, attributing it to heightened stress and increased mortality (Barton, 2002; Urbinati et al., 2004). In this study, following ST24 shipping period, at the unpacking time point, higher densities (D8 and D12) exhibited higher survival rate than the lowest density (D4).

This finding may align with a previous study, which reported that *A. regius* juveniles showed superior growth rates and enhanced feed utilization when raised at high stocking densities compared to those at low stocking densities (Millán-Cubillo et al., 2016). Moreover, elevated plasma cortisol, glucose, and triglyceride levels at the lowest stocking density indicate an activated stress response in meagre juveniles under the low stock density conditions (Millán-Cubillo et al., 2016). The closely related species *Argyrosomus japonicus* exhibited similar observations as well. (Pirozzi et al., 2009). However, similar findings in both species (Millán-Cubillo et al., 2016; Pirozzi et al., 2009) differ from the established pattern observed in other teleost species (Barton, 2002), leading us to suggest that low stocking densities might induce stress in *A. regius* yolk-sac larvae, causing higher mortality.

Additionally, in the present study, during both preliminary and final experiments, upon unpacking, we observed yolk-sac larvae tending to gather at the corners of the plastic bags. This personal observation aligns with the behavior of juveniles of this specie, commonly observed to form large groups in brackish waters (Sobrino et al., 2005). Our results, coupled with these characteristics, indicate promising potential for the successful transfer of meagre yolk sac larvae at high density during real shipments in the future.

Despite the larvae being exposed to uniform experimental conditions with identical densities and durations of simulated shipment, those from the second spawning event showed higher survival inside the incubator after all shipping times. This superiority was particularly observed during the ST24 and ST48.

A possible explanation for these results might be associated with transgenerational effects arising from elevated maternal stress (Eriksen et al., 2006; Sloman, 2010; Wassink et al., 2020) due to artificial induction processes, such as transfer to an anesthetic bath, ovarian biopsies, and hormone injection during the first spawning.

A previous study on Atlantic salmon revealed effects on offspring due to elevated prespawning maternal cortisol levels, including increased post-hatch mortality and a reduction in yolk sac size (Eriksen et al., 2006), which might be linked to heightened metabolic rates (Sloman, 2010). Similarly, an experiment conducted on brown trout found that subjecting eggs to a short (3-hour) physiologically relevant cortisol concentration before fertilization induced significant changes in larvae, leading to increased rates of oxygen consumption and ammonia excretion (Sloman, 2010). Furthermore, exposure to cortisol in the ovarian fluid resulted in altered physiology and behavior, including increased

metabolic rates and higher post-hatch aggression (Sloman, 2010). Likewise, elevating egg cortisol levels pre-fertilization in lake sturgeon (*Acipenser fulvescens*) to simulate maternal stress demonstrated the capacity of maternal stress to influence physiological and behavioral traits in their larvae (Wassink et al., 2020).

Although the mechanism of the impact of maternal stress on meagre larvae has not yet been investigated, it can represent an exciting challenge for future studies. Moreover, considering and addressing potential stressors that may arise in earlier stages before preparing larvae for shipping can be beneficial. This approach aims to mitigate the impact of additional stressors on larval survival rates beyond shipping-related stressors.

5 **Conclusion**

This study aimed to establish an effective and accessible protocol for transporting yolk sac larvae. This involved a detailed exploration of the effects of stocking density, transport duration, and water quality in closed systems, with the goal of addressing and bridging the existing gaps in knowledge.

The results showed the successful transportation for D4 (4000 $L^{-1)}$, D8 (8000 L^{-1}), and D12 (12000 L^{-1}) within ST24 (24-h). Moreover, D4 (4000 L^{-1}) exhibited transportability during ST24 (24-h), ST36 (36-h), and ST48 (48-h) of shipping time. Our findings showed that higher stocking densities, particularly at the unpacking time point, had higher survival rates within ST24(24-h). This result challenges the prevailing belief that increased density negatively affects larval

survival during shipping. These results emphasize the necessity of species-specific considerations in larval transportation protocols. Although water quality parameters showed no significant impact on larval survival during ST24 (24-h) of shipping, likely due to short-term exposure, we recommend monitoring post-shipping survival until the first feeding. The influence of water quality becomes more pronounced during extended shipping durations, emphasizing the importance of maintaining optimal temperature and oxygen levels throughout transit.

Moreover, the results pointed out additional stressors beyond those associated with shipping might potentially impact larval performance. Larvae from the second spawning exhibited higher survival rates in the incubator, suggesting a possible correlation between maternal stress and larval performance. This aspect introduces a novel dimension to our understanding of the factors influencing larval survival during shipping, and highlights the need for further research to explore and comprehend the mechanisms involved.

In conclusion, our research provides valuable insights into the transportation of early life stages of marine fish, with a specific focus on meagre yolk sac larvae. The developed protocol, along with insights into the factors influencing larval survival, provides a practical and user-friendly approach that addresses existing knowledge gaps for both producers and public institutions. This study offers guidelines applicable and adaptable to other desired marine species in aquaculture.

The present study encourages a reconsideration of the recognized pattern observed in stocking density and additional stressors during shipping, and highlights the importance of species-specific approaches in larval transportation protocols.

6 References

Abdel-Rahim, M. M., Lotfy, A. M., Toutou, M. M., Aly, H. A., Sallam, G. R., Abdelaty, B. S., & Helal, A. M. (2020). Effects of salinity level on the survival, growth, feed utilization, carcass composition, haematological and serum biochemical changes of juvenile Meagre (*Argyrosomus regius*) (Asso, 1801) grown in ground saltwater. *Aquaculture Research*, *51*(3), 1038–1050. https://doi.org/10.1111/are.14449

Anderson, J. L., Asche, F., Garlock, T., & Chu, J. (2017). Aquaculture: Its Role in the Future of Food. In *World Agricultural Resources and Food Security* (Vol. 17, pp. 159–173). Emerald Publishing Limited. https://doi.org/10.1108/S1574-871520170000017011

Barton, B. A. (2002). Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. *Integrative and Comparative Biology*, *42*(3), 517–525. https://doi.org/10.1093/icb/42.3.517

Chattopadhyay, D. N., Chakraborty, A., Ray, P. K., Mandal, R. N., & Das, A. (2021). Transportation of fertilized eggs and yolk-sac larvae of hilsa shad, Tenualosa ilisha (Hamilton, 1822) in different transportation systems. *Aquaculture*, *532*, 736042. https://doi.org/10.1016/j.aquaculture.2020.736042

De Mello, P. H., Lancerotto, S., Fakriadis, I., Tsoukali, P., Papadaki, M., & Mylonas, C. C. (2021). The importance of thermoperiod for proper gametogenesis and successful egg and sperm production in meagre (Argyrosomus regius) breeders in aquaculture. *Mediterranean Marine Science*, *22*(2), 218. https://doi.org/10.12681/mms.25806

Diken, G., Demir, O., & Naz, M. (2019). THE INHIBITORY SITUATIONAL ANALYSIS OF SOME FEED INGREDIENTS FOR MEAGRE, Argyrosomus regius (Asso 1801) LARVAE AND EVALUATION FOR DIET FORMULATIONS. *Aquatic Research*, 41–52. https://doi.org/10.3153/AR19006

Duncan, N., Estévez, A., Porta, J., Carazo, I., Norambuena, F., Aguilera, C., Gairin, I., Bucci, F., Valles, R., & Mylonas, C. C. (2012). Reproductive development, GnRHa-induced spawning and egg quality of wild meagre (Argyrosomus regius) acclimatised to captivity. *Fish Physiology and Biochemistry*, *38*(5), 1273–1286. https://doi.org/10.1007/s10695-012-9615-3

Duncan, N. J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C. M., Roo, J., Schuchardt, D., & Vallés, R. (2013). Aquaculture production of meagre (Argyrosomus regius): Hatchery techniques, ongrowing and market. In *Advances in Aquaculture Hatchery Technology* (pp. 519–541). Elsevier. https://doi.org/10.1533/9780857097460.3.519

Duncan, N. J., Mylonas, C. C., Milton Sullon, E., Karamanlidis, D., França Nogueira, M. C., Ibarra-Zatarain, Z., Chiumento, M., & Aviles Carrillo, R. O. (2018). Paired spawning with male rotation of meagre Argyrosomus regius using GnRHa injections, as a method for producing multiple families for breeding selection programs. *Aquaculture*, *495*, 506–512. https://doi.org/10.1016/j.aquaculture.2018.06.017

Eriksen, M. S., Bakken, M., Espmark, Å., Braastad, B. O., & Salte, R. (2006). Prespawning stress in farmed Atlantic salmon *Salmo salar*: Maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. *Journal of Fish Biology*, *69*(1), 114–129. https://doi.org/10.1111/j.1095-8649.2006.01071.x

Estudillo, C. B., & Duray, M. N. (2003). Transport of hatchery-reared and wild grouper larvae, Epinephelus sp. *Aquaculture*, *219*(1–4), 279–290. https://doi.org/10.1016/S0044-8486(02)00413-1

European Commission. Directorate General for Maritime Affairs and Fisheries. & EUMOFA. (2022). *Meagre in the EU.* Publications Office. https://data.europa.eu/doi/10.2771/28936

Failaman, A. N., Traifalgar, R. F. M., & Corre, V. L. (2022). Survival of nursery-reared juvenile milkfish, *Chanos chanos* , at different transport density and temperature. *Journal of Applied Aquaculture*, *34*(4), 938–952. https://doi.org/10.1080/10454438.2021.1910605

Fakriadis, I., Zanatta, E. M., Fleck, R. P. D. S., Sena Mateo, D. L., Papadaki, M., & Mylonas, C. C. (2020). Endocrine regulation of long-term enhancement of spermiation in meagre (Argyrosomus regius) with GnRHa controlled-delivery systems. *General and Comparative Endocrinology*, *297*, 113549. https://doi.org/10.1016/j.ygcen.2020.113549

Fountoulaki, E., Grigorakis, K., Kounna, C., Rigos, G., Papandroulakis, N., Diakogeorgakis, J., & Kokou, F. (2017). Growth performance and product quality of meagre (*Argyrosomus regius*) fed diets of different protein/lipid levels at industrial scale. *Italian Journal of Animal Science*, *16*(4), 685–694. https://doi.org/10.1080/1828051X.2017.1305259

Frankic, A., & Hershner, C. (2003). Sustainable aquaculture: Developing the promise of aquaculture. *Aquaculture International*, *11*(6), 517–530. https://doi.org/10.1023/B:AQUI.0000013264.38692.91

Harmon, T. S. (2009). Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: A review of the basics. *Reviews in Aquaculture*, *1*(1), 58–66. https://doi.org/10.1111/j.1753-5131.2008.01003.x

Hayashida, T., Higuchi, K., Hashimoto, H., Kazeto, Y., & Takashi, T. (2023). Optimization of stocking density and shipping duration for transportation of Pacific bluefin tuna, *THUNNUS ORIENTALIS* (Temminck et Schlegel), eggs, and experimental verification of polyethylene glycol treatment to reduce collision damage. *Journal of the World Aquaculture Society*, jwas.12972. https://doi.org/10.1111/jwas.12972

Kir, M., Topuz, H., Sunar, M. C., & Topuz, M. (2015). Effect of Temperature on Acute Toxicity of Nitrite to Meagre, *Argyrosomus regius* (Asso, 1801). *Journal of the World Aquaculture Society*, *46*(5), 564–568. https://doi.org/10.1111/jwas.12214

Kir, M., Topuz, M., Sunar, M. C., & Topuz, H. (2016). Acute toxicity of ammonia in Meagre (*Argyrosomus regius* Asso, 1801) at different temperatures. *Aquaculture Research*, *47*(11), 3593–3598. https://doi.org/10.1111/are.12811

Kır, M., Sunar, M. C., & Altındağ, B. C. (2017). Thermal tolerance and preferred temperature range of juvenile meagre acclimated to four temperatures. *Journal of Thermal Biology*, *65*, 125–129. https://doi.org/10.1016/j.jtherbio.2017.02.018

Klimogianni, A., Pagoulatou, M., Trageli, M., & Hotos, G. N. (2013). Investigation on early development, the feeding ability and larval survival under starvation in common meagre, Argyrosomus regius (Asso, 1801). *Journal of Aquatic Science*, *1*(1), 1-6.Lagardère, J. P., & Mariani, A. (2006). Spawning sounds in meagre *Argyrosomus regius* recorded in the Gironde estuary, France. *Journal of Fish Biology*, *69*(6), 1697–1708. https://doi.org/10.1111/j.1095-8649.2006.01237.x

Millán-Cubillo, A. F., Martos-Sitcha, J. A., Ruiz-Jarabo, I., Cárdenas, S., & Mancera, J. M. (2016). Low stocking density negatively affects growth, metabolism and stress pathways in juvenile specimens of meagre (Argyrosomus regius, Asso 1801). *Aquaculture*, *451*, 87–92. https://doi.org/10.1016/j.aquaculture.2015.08.034

Mohammed-Geba, K., González, A. A., Suárez, R. A., Galal-Khallaf, A., Martos-Sitcha, J. A., Ibrahim, H. M., Martínez-Rodríguez, G., & Mancera, J. M. (2017). Molecular performance of Prl and Gh/Igf1 axis in the Mediterranean meager, Argyrosomus regius, acclimated to different rearing salinities. *Fish Physiology and Biochemistry*, *43*(1), 203–216. https://doi.org/10.1007/s10695-016-0280-9

Mylonas, C. C., Fostier, A., & Zanuy, S. (2010). Broodstock management and hormonal manipulations of fish reproduction. *General and Comparative Endocrinology*, *165*(3), 516–534. https://doi.org/10.1016/j.ygcen.2009.03.007

Mylonas, C. C., Salone, S., Biglino, T., De Mello, P. H., Fakriadis, I., Sigelaki, I., & Duncan, N. (2016). Enhancement of oogenesis/spermatogenesis in meagre Argyrosomus regius using a combination of temperature control and GnRHa treatments. *Aquaculture*, *464*, 323–330. https://doi.org/10.1016/j.aquaculture.2016.07.006

Papadakis, I. E., Kentouri, M., Divanach, P., & Mylonas, C. C. (2013). Ontogeny of the digestive system of meagre Argyrosomus regius reared in a mesocosm, and quantitative changes of lipids in the liver from hatching to juvenile. *Aquaculture*, *388–391*, 76–88. https://doi.org/10.1016/j.aquaculture.2013.01.012

Pirozzi, I., Booth, M. A., & Pankhurst, P. M. (2009). The effect of stocking density and repeated handling on the growth of juvenile mulloway, Argyrosomus

japonicus (Temminck & Schlegel 1843). *Aquaculture International*, *17*(2), 199–205. https://doi.org/10.1007/s10499-008-9190-x

Ramos-Júdez, S., González, W., Dutto, G., Mylonas, C. C., Fauvel, C., & Duncan, N. (2019). Gamete quality and management for in vitro fertilisation in meagre (Argyrosomus regius). *Aquaculture*, *509*, 227–235. https://doi.org/10.1016/j.aquaculture.2019.05.033

Roo, J., Hernández-Cruz, C. M., Borrero, C., Schuchardt, D., & Fernández-Palacios, H. (2010). Effect of larval density and feeding sequence on meagre (Argyrosomus regius; Asso, 1801) larval rearing. *Aquaculture*, *302*(1–2), 82–88. https://doi.org/10.1016/j.aquaculture.2010.02.015

Sampaio, F. D. F., & Freire, C. A. (2016a). An overview of stress physiology of fish transport: Changes in water quality as a function of transport duration. *Fish and Fisheries*, *17*(4), 1055–1072. https://doi.org/10.1111/faf.12158

Sampaio, F. D. F., & Freire, C. A. (2016b). An overview of stress physiology of fish transport: Changes in water quality as a function of transport duration. *Fish and Fisheries*, *17*(4), 1055–1072. https://doi.org/10.1111/faf.12158

Sloman, K. A. (2010). Exposure of ova to cortisol pre-fertilisation affects subsequent behaviour and physiology of brown trout. *Hormones and Behavior*, *58*(3), 433–439. https://doi.org/10.1016/j.yhbeh.2010.05.010

Stavrakidis-Zachou, O., Lika, K., Michail, P., Tsalafouta, A., Mohamed, A. H., & Nikos, P. (2021). Thermal tolerance, metabolic scope and performance of meagre, Argyrosomus regius, reared under high water temperatures. *Journal of Thermal Biology*, *100*, 103063. https://doi.org/10.1016/j.jtherbio.2021.103063

Stuart, K., Losordo, M., Olin, P., & Drawbridge, M. (2015). Effects of stocking density and water conditioners on yolk-sac larvae of two marine finfish

during simulated air transport. *Aquaculture Research*, *46*(9), 2124–2132. https://doi.org/10.1111/are.12368

Subasinghe, R., Soto, D., & Jia, J. (2009). Global aquaculture and its role in sustainable development. *Reviews in Aquaculture*, *1*(1), 2–9. https://doi.org/10.1111/j.1753-5131.2008.01002.x

Trabulo, R., Amorim, M. C. P., Fonseca, P. J., Vieira, M., Matos, A. B., Marin-Cudraz, T., Lemos, M. F. L., Moutinho, A. B., Novais, S. C., Pousão-Ferreira, P., Candeias-Mendes, A., & Faria, A. M. (2023). Impact of anthropogenic noise on the survival and development of meagre (Argyrosomus regius) early life stages. *Marine Environmental Research*, *185*, 105894. https://doi.org/10.1016/j.marenvres.2023.105894

Urbinati, E. C., De Abreu, J. S., Da Silva Camargo, A. C., & Landinez Parra, M. A. (2004). Loading and transport stress of juvenile matrinxã (Brycon cephalus, Characidae) at various densities. *Aquaculture*, *229*(1–4), 389–400. https://doi.org/10.1016/S0044-8486(03)00350-8

Wassink, L., Huerta, B., Li, W., & Scribner, K. (2020). Interaction of egg cortisol and offspring experience influences stress-related behaviour and physiology in lake sturgeon. *Animal Behaviour*, *161*, 49–59. https://doi.org/10.1016/j.anbehav.2020.01.001

Zink, I. C., Benetti, D. D., Douillet, P. A., Margulies, D., & Scholey, V. P. (2011). Improvement of Water Chemistry with Bacillus Probiotics Inclusion during Simulated Transport of Yellowfin Tuna Yolk Sac Larvae. *North American Journal of Aquaculture*, *73*(1), 42–48. https://doi.org/10.1080/15222055.2011.544622