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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

Sparus aurata larvae production in mesocosm: evaluation of abiotic and biotic parameters

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Dissertação de Mestrado em Ciências do Mar e Recursos Marinhos Especialidade em Aquacultura e Pescas

2012

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Dissertação de Candidatura ao grau de Mestre em Ciências do Mar e Recursos Marinhos – Especialidade em. Aquacultura e Pescas submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto.

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Categoria – Chefe de Divisão

Afiliação – Centro de Maricultura da Calheta, Direcção Regional de Pescas, Governo Regional da Madeira

Resumo

Nas últimas três décadas a aquacultura tem-se afirmado como uma das principais formas de produção de alimento para a população mundial. O incremento dos valores desta produção foi conseguido com a melhoria das técnicas e com o aumento do número de instalações de produção.

O avanço no desenvolvimento de metodologias de cultura têm permitido a reprodução em cativeiro e o domínio dos estádios larvares de diferentes espécies de interesse comercial, fundamentais para a diversificação de espécies e para a sustentabilidade da actividade.

Uma das metodologias que tem apresentado melhores resultados na produção larvar de novas espécies de peixes marinhos é o mesocosmos, de características semi-intensivas. Isto é, metodologias de produção larvar intermédias entre o intensivo e o extensivo, que utiliza tanques de cultura de grande volume entre 30 e 100 m^3, e uma densidade larval de 2-8 indivíduos por litro.

Os sistemas de produção larvar são sistemas dinâmicos, sujeitos a diferentes variáveis – físico-químicas e biológicas, que variam ao longo do período de cultura e influenciam o desenvolvimento larvar.

Existe pouca literatura a incidir sobre o padrão dos parâmetros ambientais bióticos e abióticos durante a produção de larvas de peixes marinhos em mesocosmos e como os mesmos se distribuem no interior do tanque. Neste trabalho propomo-nos realizar a descrição espacial e temporal da evolução dos parâmetros ambientais durante a cultura de larvas de dourada (*Sparus aurata*) em mesocosmos de metodologias semi-intensivas. Procuramos ainda descrever o comportamento das larvas desta espécie ao longo do período de fase larvar até à fase juvenil.

As larvas de dourada apresentaram uma taxa de eclosão elevada (98±0.1%), bem como uma elevada taxa de insuflação da bexiga-natatória (90%). A taxa de sobrevivência das larvas registou valores esperados para esta metodologia (60%). Um dos parâmetros utilizados para assegurar o bem-estar animal, com possibilidade de ser utilizado como parâmetro de análise de produção, é o comportamento. Na pesquisa efectuada sobre este tema, o registo existente incide sobretudo sobre a população juvenil ou adulta, com particular interesse nos comportamentos de alimentação e agressivos. Neste

sentido o trabalho apresentado é pioneiro ao descrever alguns comportamentos de larva de dourada em mesocosmos. É descrita a distribuição larval na superfície e profundidade. Foram também descritos 7 comportamento, dos quais se destaca a baixa incidência de canibalismo, sendo que ocorrem comportamentos agressivos (dia 35).

Foram analisados vários parâmetros e descrita a sua variação ao longo do tempo. São descritos os padrões hidrodinâmicos (velocidade e direcção da corrente), assim como de luz, pH, temperatura, oxigénio, nos momentos em que ocorram alteração do meio de cultura.

Quanto aos padrões hidrodinâmicos no tanque as correntes apresentaram uma direcção paralela à parede lateral, sendo os desvios relacionados com a posição e presença de arejadores. A velocidade das correntes (entre 0.69 e 1.38 cm/s) esteve relacionada com o fluxo de entrada de água, sendo que a sua variação foi regulada de acordo com o estado de desenvolvimento larval.

A temperatura é um parâmetro homogéneo no interior do tanque (20.1 ±0.1), sendo que o padrão da sua variação é igual a da água do mar. O padrão de distribuição do oxigénio (6,06±1,35 mg/L) não é homogéneo em todo o tanque de cultura. Ocorreram variações espaço - temporais deste parâmetro ambiental, com menores valores, na parte central do fundo do tanque. A salinidade (36±0.05‰) e o pH 8.6 (±0.09) foram constantes ao longo do período analisado. A luz é um dos parâmetros que apresenta maior variação (1514 ± 1444 lux), associada à fase do protocolo de cultura e ao local de amostragem.

Espera-se que este trabalho permita um novo olhar sobre o mesocosmos de metodologias semi – intensivas. A compreensão da distribuição dos parâmetros ambientais, no interior do tanque de cultura, associada ao estabelecimento de padrões normais de comportamento larvar, poderão melhorar a produção em termos qualitativos e quantitativos e, no futuro servir de base para outros estudos.

Palavras – Chave: Mesocosmos metodologia semi-intensiva; *Sparus aurata larvas*; parâmetros abióticos; parâmetros bióticos; padrões; comportamento.

Abstract

Aquaculture is one of the growing food supply sources in the world. Over the last years aquaculture production has grown at steady pace. This growth was achieved by improving and development of larval rearing methodologies, as well as with the increase of the number of production facilities.

In the case of the rearing methodologies, the mesocosm or semi-intensive methodologies has provided juveniles of higher quality of well-known species, as well as being a methodology that is suited for the culture of "new" species, contributing for species diversification.

Mesocosm of semi-intensive methodologies is a rearing methodology that is situated between intensive and extensive methodologies. Mesocosm methodologies make use of large rearing tanks, with volumes between 30 and 100 m^3, and rearing densities between 2 and 8 individuals per litter.

Rearing systems are dynamic due to being under the influence of several physical – chemical and biological parameters, which vary during the rearing period and with direct influence on larval development.

Literature concerning the distribution of the parameters -light, dissolved oxygen, temperature, salinity, pH - in aquaculture production systems is scarce and is almost null when referring to larval stages. In this paper we propose to make the description of the spatial and temporal evolution of environmental parameters during the culture of sea bream (*Sparus aurata*) larvae in mesocosm of semi-intensive methodologies. We also aim to describe larvae behaviour in those culture conditions.

Sparus aurata hatching rate was high (98 ± 0.1%), and the larvae presented a high rate of inflation of the swim bladder (90%). Survival rate was within the expected values for these rearing methodologies (60%).

Larvae distribution patterns and behaviour in the rearing tank can be used to assess animal welfare. The distribution of larvae on the surface and depth are described. This work presents new data for *Sparus aurata* larvae behaviour in mesocosm.

Seven behaviours are described for the entire larval phase. Despite the common aggressive behaviour of larvae from day 35 post hatching, there was low incidence of cannibalism.

The different abiotic parameters showed highly variable patterns. Temperature presents a uniform pattern throughout the larval development. Dissolved oxygen presented an irregular pattern in both time and space with the existence of multiple areas of different concentrations. Light was extremely variable at surface, while in depth it varied according to the culture stage.

Regarding the hydrodynamics of the larval rearing tank, the water current was parallel to the lateral wall, with alterations due to the presence of aerators. Water velocity in the rearing tank was related to the water exchange rate, according to the larvae stage and was consistently below the critical limits to the larval species.

Temperature was homogenous for the entire rearing tank (20.1 ± 0.1ºC), and the variation pattern was similar to the sea water inlet. Dissolved oxygen (6.6±1.35 mg/L) presented significant differences between stations. The lowest values for this environmental parameter were registered at the central part of the bottom of the tank due to the accumulation of wastes. Both salinity (36±0.05‰) and pH (8.6 ±0.09) were constant along the rearing period. Light was the parameter that presented the highest variation (1514 ± 1444 lux), in both time and space.

It is expected that this work provides a new look on mesocosm of semi-intensive methodologies. A better understanding of the rearing tank environmental parameters, as well as of larvae behaviour will be useful to enhance production quality and quantity and provide a base line for further studies.

Key- words: Mesocosm of semi intensive methodologies; Environmental parameters, Behaviour; *Sparus aurata*; Biotic and Abiotic Parameters; Patterns

Agradecimentos

Quero agradecer a Direcção Regional de Pescas da Madeira, por ter permitido efectuar a minha tese de dissertação no Centro de Maricultura.

Ao Doutor Carlos Andrade pela ideia, orientação, confiança e conhecimentos, a sua sabedoria foi essencial para a elaboração desta tese.

A todo o pessoal do Centro de Maricultura da Calheta, especialmente ao António Abreu "James", pelo auxílio na elaboração e execução das tarefas... não me esqueci que te devo uma (s) poncha (s). Ao Emanuel Pinto, pela ajuda na construção dos materiais de apoio, ao António Branco e a Maria João pelos conhecimentos partilhados.

A Lurdes Ferreira, pela companhia inesperada.

Ao Bernardo Sumares, pela companhia ao longo desta jornada.

A Doutora Délia Canha Departamento de Matemática e Engenharia, ao Doutor. Miguel Sequeira do Centro de Ciências da Vida, ambos da Universidade da Madeira, a Doutora Antonieta Amorim da Direcção Regional de Pescas, pelo tempo e ajuda disponibilizados para me auxiliar aspectos técnicos da tese.

A minha turma de mestrado, pela amizade, brincadeira, que o contacto não se perca.... I can't take it take it take no more\Never felt like felt like felt like this before...

Ao Carlos Marques, Ana Beça, Amelia Miguel-Gilmore pelas correcções e pela amizade. Carlos vai mais um café?

Para terminar aos meus pais, irmão e a Cristina sem o vosso apoio incondicional esta jornada não teria acontecido. Esta tese no fundo é-vos dedicada.

Funchal, Outubro de 2012

Índex

Acronyms and abbreviations

CMC - Centro Maricultura da Calheta

Dah - day after hatching

SB1 and SB2 – Bottom Squares

SK – Surface Skimmer

SS – Surface Square

TB – Total bottom

Wi - Water inlet

Wo - Water outlet

Wos - Surface water outlet

Figure Index

General Introduction

1. INTRODUCTION

Aquaculture can be defined as the farming of aquatic organisms - plants or animals - using techniques that increase production above the natural capacity of the system. Aquaculture production has been growing since the 1980's, at an average increase of 8.8% per year, with marine aquaculture representing 30% of the total value *(FAO, 2010, 2012b)*

Animal aquaculture production can be divided according to the type of animal reared. There are three major aquaculture activities: marine shellfish, freshwater finfish and marine finfish. Aquaculture of plants is marginal in European aquaculture production, and appears separated in aquaculture production sheets (FAO, 2010).

The growth in aquaculture was achieved with the increase in the number of production facilities, as well as with the optimization and creation of rearing protocols and the use of new technology (Giménez & Estévez, 2008; Shields, 2001)

Aquaculture production is based on a wide range of systems and species – 600 species in over 190 countries worldwide (FAO, 2012b) - mostly high value carnivorous species, with good growth, high prices and increasing market demand (Luis Alvarez-Lajonchère & Pérez-Roa, 2012)

There is a good knowledge for most of the species used in aquaculture production. Understanding a species development requires knowledge on keeping a viable broodstock, knowing when they are sexually mature and spawn, as well as knowledge of the proper conditions for eggs and larvae to develop and to become juveniles. Once this knowledge is gathered and the culture is technically achieved, producers try to increase production to the highest number of individuals possible, in a process of intensification. Intensive rearing in aquaculture was only accomplished for a few species (Shields, 2001).

Aquaculture production must solve several bottlenecks at hatchery level, such as improving the rearing methodologies, have a better understanding of the rearing environment, and a better knowledge of larvae nutritional requirements (Giménez & Estévez, 2008; Kolkovski, Curnow, & King, 2004).

Advances in these areas of research will certainly increase the number of species that are intensively reared (F. J. Roo, Hernandez-Cruz, Socorro, Fernandez-Palacios, & Izquierdo, 2010). Concurrently, aquaculture is expected to take a more ecological approach (COMMUNITIES, 2009; FAO, 2012b).

Mesocosm or semi-intensive production is one the fish rearing methodologies that has provided better results for aquaculture diversification, for the production of high quality juveniles and with a low ecological footprint (Shields, 2001). Mesocosm makes simultaneous use of extensive and intensive larval rearing methodologies (Divanach & Kentouri, 2000).

Mesocosm methodologies are widely used for larval rearing and production trials of different marine fish species (C. A. P. Andrade, Abreu, et al., 2012; Ben Khemis, Zouiten, Besbes, & Kamoun, 2006; Gyllenhammar, Håkanson, & Lehtinen, 2008; Katharios, Papadaki, Papandroulakis, & Divanach, 2008; Papandroulakis, Kentouri, Maingot, & Divanach, 2004; Papandroulakis, Mylonas, Maingot, & Divanach, 2005; F. J. Roo et al., 2010).

Much of the success of mesocosm in larvae rearing derives from the stable environment due to the use of large culture tanks (C. A. P. Andrade, Abreu, et al., 2012). Rearing tanks are not static environments, being highly dynamic with a wide variety of parameters that can influence larval development. However, the size of the larval rearing tanks represents limitations to the control of culture conditions and larval stocks (Shields, 2001).

Only the understanding of the relationship between abiotic and biotic parameters and larval development will allow the improvement of the management of the tank to achieve higher production and quality of juveniles. In this regard several questions about the mesocosm methodologies may arise:

Is the mesocosm rearing tank a homogeneous environment for the development of fish larvae?

How does the environment (abiotic and biotic parameters) perform inside the rearing tank throughout the larval rearing cycle?

How does this affect larval development and behaviour?

How can we analyse it?

Do we have methodologies suited to analyse the system or do we need to develop new approaches?

Can the system be improved? If so how?

It is also one of the aims of this work, to provide knowledge of *Sparus aurata* behaviour in mesocosm of semi intensive methodologies.

These are just a few questions about the methodologies, but others certainly could be formulated. In order to answer these questions it is necessary to introduce the description of fish larvae development, the different types of rearing methodologies and rearing facilities that uses mesocosm of semi-intensive methodologies, with particular attention to the rearing tank.

1.1 GILTHEAD SEA BREAM (Sparus aurata)

Sparus aurata, is a perciform teleost of the Sparidae family. It is demersal, eurythermal and euryhaline species present in a wide range of habitats, from the South of England to Mauritania.

Sexually *S. aurata* is a protandrous hermaphrodite, achieving maturity in 2 years and, males transforming into females between 2-3 years old. During the spawning season, under good conditions, a single female can lay between 20 000 to 80 000 eggs per day (FAO, 2012a).

Gilthead sea bream aquaculture began in the 1970's, and nowadays is one of the most farmed species in European marine aquaculture (Shields, 2001).

1.1.1 Sparus aurata LARVAL DEVELOPMENT

Fish larvae development can be defined as the phases a fish goes through between hatching and becoming a juvenile (Howell, Day, Ellis, & Baynes, 1998).

Larval development can be divided in three main stages and two transitional stages, according to Kendal (1984) and Howell (1999):

1. Egg stage – which embraces the entire process from the fecundation until hatching.

2. Larval stage – which includes the period between, hatching and the beginning of squamation. During this stage larva undergoes transformations in terms of body shape, locomotion. There are several sub stages:

    a. Yolk - sac larvae – Developmental stage beginning with hatching and ending with exhausting of yolk sac;

    b. Preflexion larva – From the exhaustion of the yolk sac until the upward flexion of the notochord;

    c. Flexion larva – After the notochord has terminated its flexion until the plural bones assuming a vertical position;

d. Post flexion larva - Larvae acquires the formation of the caudal fin (hypural elements vertical), as well as full external meristic complements (fin rays);

3. Juvenile stage: Completion of fin ray counts and beginning of squamation until fish enters adult population or attain sexual maturity.

S. aurata larvae at hatching has 3 mm total length, the eyes become functional at the end of the stage and larvae acquire mobility (Moretti, Fernandez-Criado, Cittollin, & Guidastri, 1999). During this stage the digestive tract starts differentiation (Kendall, Ahlstrom, & Moser, 1984; Sarasquete, Polo, & Yufera, 1995)

There are two moments that define the second phase of fish larval development: first feeding and the swim bladder inflation.

Not being able to feed at this stage is the cause of high mortality among fish.

In culture conditions *S. aurata* larvae are first fed rotifers of about 100 µm, which is the size of the mouth of larvae at this stage (Moretti et al., 1999). At older stages bigger size prey such as *Artemia* can be provided, since larvae have bigger mouth and more developed digestive tract (Fernández-Diaz & Yufera, 1995).

The non-inflation of the swim bladder can be responsible for problems in buoyancy and eventually death. In *S. aurata* swim bladder initiates differentiation at 2 - 3 days after hatching (dah) and it is positioned dorsally between the digestive tract and the spleen (Sarasquete et al., 1995). The first inflation of the swim bladder ends around day 15, with the process being complete around 40-50 dah (Moretti et al., 1999).

For gilthead *Sparus aurata* the development milestones of the larval stages were already established for intensive rearing at 17-18 ºC (Moretti et al., 1999) Figure 1:

Day	Size (mm)	Characteristics
1	3	Hatching
2	3.5	Pectoral fins appear
3	3.8	Exotrophy starts
4		Eyes pigmented 60% of yolk sac absorbed 40% of oil drop absorbed
5	4	Primary swim bladder inflation 100% of the yolk sac absorbed 70% of oil drop absorbed
15	5	End of the primary swim bladder inflation 100% of the oil drop reabsorb Caudal fin
17	7	Anal fin
20	7.5	Stomach starts developing
45	11	Second dorsal fin
50	15	First dorsal and ventral fin
60-70	20	Scales
90	30	Definite morphology

Figure 1 - Larval development of *Sparus aurata* at 17-18ºC according to Moretti, *et al*. (1999).

1.1.2 Sparus aurata BEHAVIOUR

Behaviour represents a reaction to the environment as fish perceive it and is therefore a key element of fish welfare (Martins et al., 2012).

One of the most common observed behaviours of larvae fish is vertical migrations, which are a form to control light intensity and escape predators (Fiksen, Jorgensen, Kristiansen, Vikebo, & Huse, 2007).

There is limited information available regarding *S. aurata* larvae behaviour. Moretti *et al*. (1999) described two types of major behaviours for *S. aurata* larvae in culture conditions, in 6-10 m^3 tanks:

1) At hatching, yolk – sac larvae have passive floating with slow and infrequent body movements without a clear posture, they sink slowly, head first and then every second swim upwards;

2) As larvae, they have a pronounced tendency to sink, with almost complete passivity accounting for a uniform dispersion in the water body.

Behaviour is influenced by rearing conditions, therefore the importance of understanding the culture environment (Monk, Puvanendran, & Brown, 2008).

1.2 ENVIRONMENTAL REQUIREMENTS FOR LARVAL DEVELOPMENT

Water quality is of the major importance to provide a healthy environment to fish (Moschou et al., 2000). If the right parameters and environmental conditions are not met it is possible that survival of larvae is at risk or that larval development will be compromised (Andrades, Becerra, & Fernández-Llebrez, 1996; Yúfera & Darias, 2007).

Environmental factors play an important role in all aspects of a fish life cycle. Parameters can be divided accordingly to their type of action: 1) if they act directly over larva development or 2) if they are limiting, if larvae require a certain range to survive (Boeuf & Payan, 2001). Temperature, salinity and light intensity were the parameters studied as part of the former category of parameters. Whereas the dissolved oxygen and pH, also analysed in the course of this thesis, need to be within a certain range for the proper development of larvae.

1.2.1 Hydrodynamics

Water dynamics is responsible for water quality and for the distribution and welfare of fish (J. Oca, Masalo, & Reig, 2004; Ross, Watten, Krise, Dilauro, & Soderberg, 1995).

The water exchange is responsible for assisting fish achieve a proper development, through providing oxygen and a suitable water velocity for swimming (Divanach, Papandroulakis, Anastasiadis, Koumoundouros, & Kentouri, 1997; Timmons, Summerfelt, & Vinci, 1998; Valverde, Mendiola Lopez, & de Costa Ruiz, 2005). Water current has been manipulated and used to improve feeding behaviour of sea bass (Valverde et al., 2005).

The longer is the residence period of rearing water, the lower is the quality of the water (Good et al., 2009; Hougham & Moran, 2007).

However, the higher the exchange rate, the higher is the velocity of the water in the rearing tank. High water velocities are responsible for fish spending more energy swimming and thus, requiring energy that otherwise could be used for growth (C. A. P. Andrade et al., 2011). Higher water velocities may also decrease the probability of larvae to feed (Canavate & Fernandez-Diaz, 2001) and is frequently the source of skeletal abnormalities (Divanach et al., 1997; J. Roo, Socorro, & Izquierdo, 2010).

Aerators are commonly used in larval rearing tanks. The aerators increase water mixing improving water quality, as well as causing alterations on the water flow pattern (Shiotani, Hagiwara, Sakakura, & Chuda, 2005).

According to Moretti *et al.* (1999) for *Sparus aurata* culture water velocity should be kept below 10 cm/s.

1.2.2 Water Temperature

Temperature is one of the most decisive environmental variables affecting all the stages of a fish cycle, from induction of reproduction to regulating larval development.

Larval stages are the most sensitive to variations of this parameter (D. Stewart Fielder, Bardsley, Allan, & Pankhurst, 2005; Green & Fisher, 2004; Koumoundouros, Divanach, & Kentouri, 2001).

Inadequate temperatures can be the responsible for several abnormalities on larval development, for example: lordosis or absence of gill covers (Sveinung Fivelstad, Bergheim, Hølland, & Fjermedal, 2004) and in extreme cases cause mortality of fish (Bermudes & Ritar, 1999). Still there is a narrow range of temperatures that provide a normal development – in terms of organogenesis- only affecting growth (Bermudes & Ritar, 1999)

For *S. aurata* larvae development water temperature should be within 14-22ºC, with optimal interval for larval development between 16-22ºC (FAO, 2012a).

1.2.3 Salinity

Salinity can be defined by the amount of salts dissolved in the water (Castro & Huber, 2003).

Salinity is one abiotic parameter that is exclusive of the aquatic environment (Boeuf & Payan, 2001)

Osmoregulation is demanding in terms of energy. Fish species prefer conditions that allow a reduction in this process, allocating energy to other processes, like growth (Sampaio & Bianchini, 2002).

Osmoregulation is carried out through ion and water regulation at different parts of the fish body. To keep homeostasis fish drink sea water and excrete the excess of salt (Tandler, Anav, & Choshniak, 1995).

Salinity can affect buoyancy of eggs and larvae, as well as the ability to inflate the swim bladder. Problems with the swim bladder inflation result in high mortality reduced larval growth and skeletal deformities (D. Stewart Fielder et al., 2005; Tandler et al., 1995). Salinity is also one of the main factors influencing fish distribution (Bodinier, Sucre, Lecurieux-Belfond, Blondeau-Bidet, & Charmantier, 2010; Moustakas, Watanabe, & Copeland, 2004).

S. aurata is able to tolerant a wide range of salinities, from 15 to 40‰ (Boeuf & Payan, 2001), with optimal salinity for larvae at 25‰ (Tandler et al., 1995)

1.2.4 pH

pH is determinant in establishing the acid-base relations of several reactions in aquatic environment (Piedrahita & Seland, 1995)

pH may have effects on fish health or be responsible for environmental problems, in either natural or controlled environments. pH is not a constant value, but is often restricted to a narrow range, despite the buffer ability of the water (Parra & Yufera, 2002; Piedrahita & Seland, 1995).

S. aurata larvae have been proved to be able to develop within pH ranging from 4,88 – 9,57 (Parra & Yufera, 2002).

1.2.5 Dissolved Oxygen

Oxygen is a fundamental variable in aquaculture production, being one of the most important factors that should be taken into account, when designing a facility. The first oxygen source is the water inlet (Merino, Piedrahita, & Conklin, 2009).

In the culture environment it is not only fish who consume oxygen. The bacteria, algae and live feeds (rotifer and *Artemia)* also require oxygen to survive, and should be taken into account when calculating oxygen requirements (Merino et al., 2009).

Oxygen requirements vary according to depth, stock density, feed and larval stage (S. Fivelstad et al., 1999; Merino, Conklin, & Piedrahita, 2011; Merino, Piedrahita, & Conklin, 2007).The effects of this variation has so far not been subject to investigation (Thetmeyer, Waller, Black, Inselmann, & Rosenthal, 1999).

Low oxygen availability will constrain feeding and consequently development (Merino et al., 2011).

The distribution of oxygen inside the rearing tank should be the most homogenous possible. The use of aerators besides increasing the oxygen levels, contributes to a good water mixing and prevents hypoxia situations – oxygen value below 2,0 mg/L – or anoxic situations – 0,0 mg/L (Diaz & Rosenberg, 1995; Wu, 2002).

In aquaculture the parameter measured is dissolved oxygen (mg/L), which is a good indicator of water quality, due to its use in biological and chemical reactions (Mustapha, 2008).

Despite differences in the adopted methodologies it is general consensus that oxygen uptake is directly proportional to water temperature and feeding ration, and inversely to fish size (Merino et al., 2009).

A proper oxygen supply provides correct amounts of oxygen without compromising water velocity as it is referred to by several authors in Merino *et. al.* 2009.

Dissolved oxygen should be within the interval of 6.4- 8.2 mg /L for a proper development of *S. aurata* larvae (Navarro & Sarasquete, 1998).

1.2.6 Light

Light is one of the principal parameters that help regulate fish life cycle, and is one of the most studied parameters for larval development (Boeuf & Le Bail, 1999).

Light intensity can be responsible for larvae initiating feeding, since larvae require a minimum of light to feed (Boeuf & Le Bail, 1999; D. S. Fielder, Bardsley, Allan, & Pankhurst, 2002; Monk, Puvanendran, & Brown, 2006). The photoperiod, can be defined as the amount of time light is available. The more extensive is the photoperiod, the longer the time a fish may feed, allowing an increase in energy which can be used, for example for growth. The duration of day also seems to be related to swim bladder inflation (D. S. Fielder et al., 2002).

Light is a highly variable parameter, varying during the day, season and medium where it interacts. In the aquatic environment light is affected by the

suspended materials that alter the depth light can reach, as well as its reflexion (Boeuf & Le Bail, 1999; Karakatsouli et al., 2010).

Alterations in light can be used to alter diets, namely alterations of prey, according to their transparency (Naas, Naess, & Harboe, 1992).

Light is not only beneficial for a fish, as problems related to hatching and buoyancy of eggs have been reported (Downing & Litvak, 2002). Long photoperiods can affect the larvae ability to inflate the swim bladder (D. S. Fielder et al., 2002). These effects of light are species specific (Monk et al., 2006).

Light is one of the aquaculture parameters that can be manipulated, with the use of artificial sources. Light is also influenced by the colour of the tank, presence of algae in the rearing environment and is dependent of the rearing methodologies used.

Sparus aurata larvae should be reared under a light intensity of at least 50-150 lux (Boeuf & Le Bail, 1999) and with optimal light intensity from 1000 to 3000 lux (Moretti et al., 1999).

1.3 REARING METHODOLOGIES

The economic viability of an aquaculture unit depends on hatcheries, which are responsible for supplying the correct quantities of juveniles at a requested moment, with reasonable prices (L. Alvarez-Lajonchère, Reina Cañez, Camacho Hernández, & Kraul, 2007).

There are several types of hatcheries and these are classified according to tank volume, rearing density, prey source (Shields, 2001).

One of the most common classifications is proposed by Divanach and Kentouri (2000) (Figure 2).

Parameters	Methodologies		
	Extensive	Mesocosm	Intensive
Rearing enclosures	Ponds or bags	Thanks or bags	Tanks
Localization	Outdoor	Indoor	Indoor
Rearing volume (m^3)	>100	30-100	<20
Rearing density (individuals per liter)	0.1-1	2-8	30-200
Food chain	Endogenous	Mixed	Exogenous
Infrastructures	Light	Medium	Sophisticated
Environment	Natural	Mixed	Controlled
Dependence on man and technique	Light	Medium	High to very high
Need for specific biological knowledge	Light	Medium	High to very high
Validity for new species	Very high	High	Medium to low

Figure 2- Rearing methodologies according to Divanach *et al.* (2000)

Every methodology presented has advantages and disadvantages. The extensive approach, provides a better higher knowledge of the biology and requirements of the species, but lacks control over the production system and has limited production capacity.

The intensive approach on the other hand, allows more production with higher economical costs, due to all the technology and knowledge required.

Another disadvantaged of choosing this rearing method is that there are few species that can be reared in intensive systems, as it is the case of *Dicentrarchus labrax, Sparus aurata, Diplodus sargus, Solea solea* and *Scophtalmus maximus* (Divanach & Kentouri, 2000).

So far the natural process for aquaculture development has been to first understand the basic culture requirements and mastering the farming process (extensive methodology), then intensifying the rearing process (Shields, 2001)

Mesocosm is an intermediate rearing methodology between extensive and intensive method. It uses large volumes tanks from 30 to 100 m^3, and larval densities between 2-8 larvae per/L.

Mesocosm also uses a partial control of the rearing parameters and a diet regime with daily adjustments.

More than 20 species have been successfully reared in this methodology with different approaches, increasing the number of current commercial aquaculture species. Survival success rates reach a minimum of 20% independently of the biological knowledge of the species (Divanach & Kentouri, 2000).

Divanach and Kentouri (2000) sustain that mesocosm or semi-intensive, can be used with any kind of water technique, as long as the optimization of the rearing process concerning natural and artificial conditions is achieved. This optimization allows for a reduction of economic costs and can help avoid or minimize seasonal or geographical changes.

In enclosed facilities as is the case of hatcheries, larval development is meant to achieve the highest growth and survival rates, due to the application of optimal conditions for the culture of the species (Andrades et al., 1996).

2. Centro de Maricultura da Calheta (CMC).

2.1 General layout of the hatchery

Larviculture takes place in hatcheries. They are the basis of the aquaculture industry, for they provide a safe environment for the development of high quality juveniles at the right moments, at the desired quantities and at reasonable prices(L. Alvarez-Lajonchère et al., 2007).

In 2001 a fish hatchery was installed in Calheta, Madeira by the Regional Government to support the development of marine aquaculture (C. A. P. Andrade, Abreu, et al., 2012).

The hatchery installed has different culture areas as described below:

- Breeders area – where the broodstock is kept. There are 8 tanks of 10m^3, where several species are kept, among them *S. aurata*.
- Green-house – the larval area. There are four - 40 m^3 tanks, on which eggs are placed to hatch and larval development takes place for the first two months.
- Weaning area – there are eleven 10m^3 tanks, in which juvenile fish grow until reaching 5-10 g before transfer to growing facilities.
- Auxiliary production areas – the areas for algae, rotifer and *Artemia* production.

2.2 Mesocosm larval rearing tank

Larval rearing tanks have to provide the maximum production capacity, as well as taking minimal advantage of space and resources. The tank has to assure correct conditions to enhance fish development and welfare (Duarte, Reig, Masaló, Blanco, & Oca, 2011; Joan Oca & Masaló, 2007; Timmons et al., 1998)

The great majority of larval rearing tanks have a circular shape, with a conical bottom. This design together with the lateral placement of water inlet (Wi), confers the tank homogenous distribution of water and fish, favours the accumulation of waste on the central area of the cone for cleaning and the control of water velocity (Joan Oca & Masaló, 2007; Ross et al., 1995). A bad

design of tank influences rearing parameters and contributes for a bad performance of the fish (Lunger, Rasmussen, Laursen, & McLean, 2006).

The tanks used at CMC are cylinder shape with a conical bottom and with a volume of 40 m^3 (r=2.5m; h= 2.2m Figure 3). The water inlet (Wi) is placed laterally in the side wall. There are three water outlets: one opposite to the water inlet (Wo), another one placed at the centre and one placed perpendicular to Wi at, the surface to aid the cleaning of water surface (Wos).

The tanks are built of fibreglass, which provides a soft surface facilitating cleaning procedures and not harming larvae. The tanks have a black colour. The use of dark colours relates to the illusion provided by these colours of the natural environment, which is mimicked. This increases the probabilities of larvae feeding by capturing prey (Planas & Cunha, 1999).

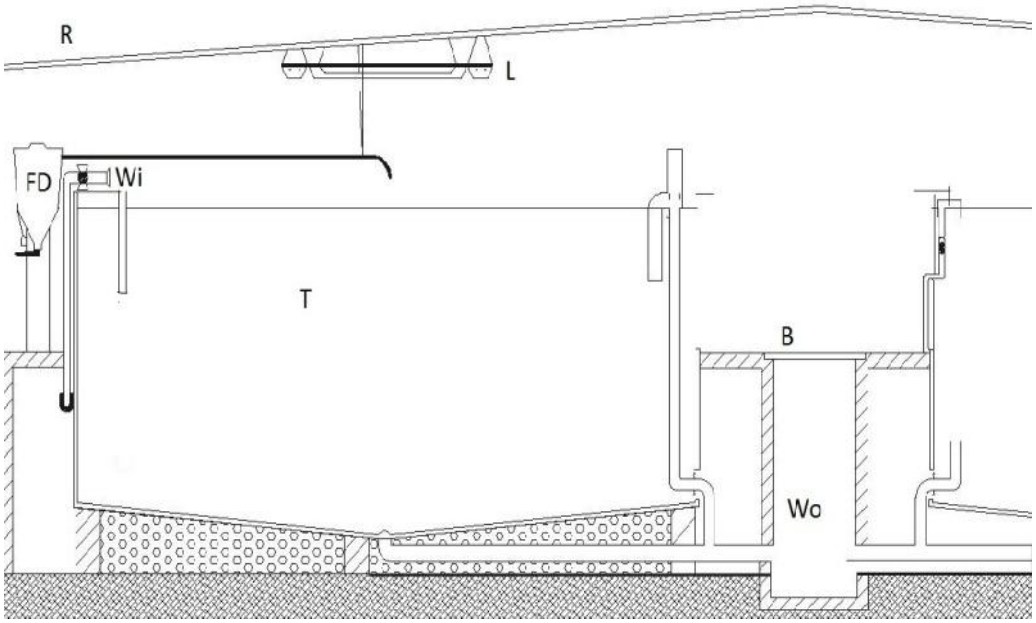
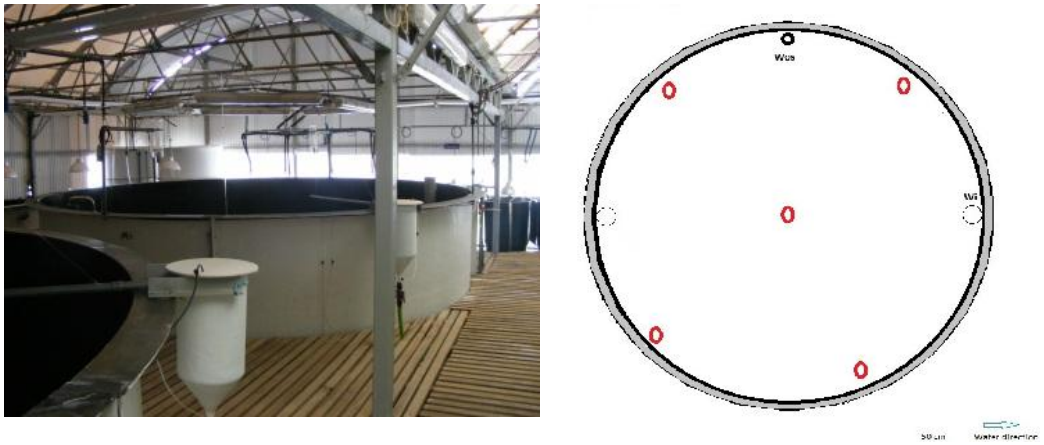


Figure 3 – Transversal view of mesocosm larval rearing facilities (adapted from (Interactt, 1997)). T – rearing tank; L- Light; FD – Automatic feeding dispenser; Wi – water inlet; Wo – Water outlet; R – Roof \polycarbonate cover; B – Boardwalk.

The existence of a polycarbonate cover provides protection from abnormal climacteric conditions. The polycarbonate plaque does not have a uniform colour, being composed by several plaques with different degrees of opacity. The darker plaques are place in the East part of the green house (Figure 4A).

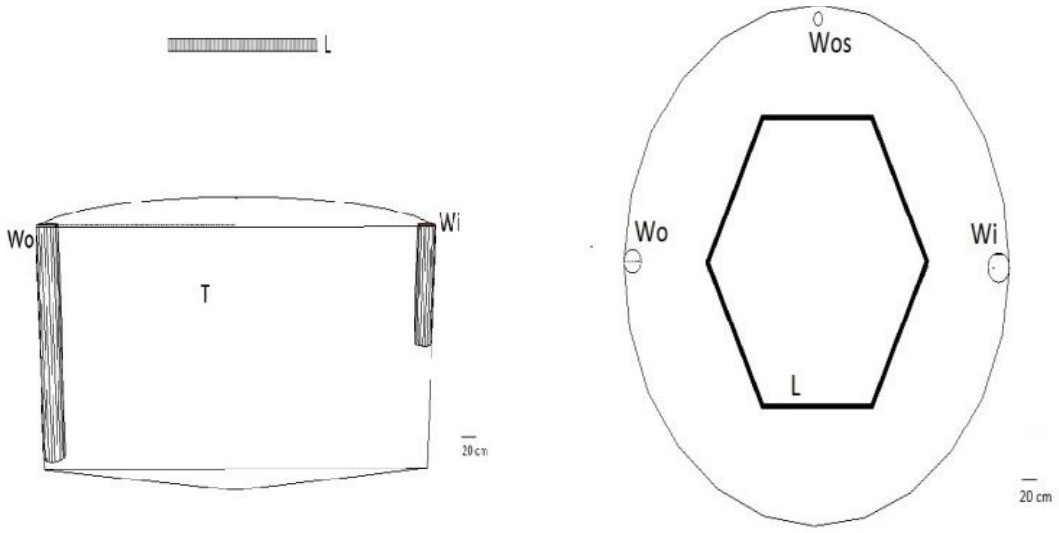


A B

Figure 4 - (A) Photograph of the larval rearing thank used in this work. (B) Position of the aerators red circles in the tank.

There are several aerators present inside the tank to proportionate a homogenous distribution and increase oxygen availability in the rearing environment (Figure 4B).

Light is provided by natural conditions, as well as by artificial light. Lamps are positioned in a polygon at a distance of 1, 5 m of the tank water surface (Figure 5 A and B). Each side of the polygon has two 60W fluorescent lamps; in a total of 12.The light is switched at the same moment that microalgae and rotifers are added to the rearing tank and kept 24h per day, until the end of the larval rearing period.



A B

Figure 5 - Light position in relation to the rearing tank. (A) Transversal view; (B) Longitudinal view. L – Light; T – rearing tank; Wi – water inlet; Wo – water outlet; Wos ; Water outlet surface.

3. Methodology

3.1 Larvae source and culture methodology

Sparus aurata eggs were collected from broodstock tanks at Centro Maricultura da Calheta (CMC) and disinfected with 0.06% formalin for 5 minutes. Approximately 374 000 eggs were placed into the 40m^3 mesocosm tanks for incubation and larval rearing. Production was carried between 30 of April and 19 of July 2012.

A green water technique was applied, using a mix of frozen and live phytoplankton produced at CMC. The algae species used were *Nanochloropsis spp* (250X10^3 cell/mL) and *Isochrysis spp* (200X10^3 cell/mL). Filtered seawater (10µm) was supplied at an increasing daily exchange rate. Light was kept constant (2000lux) from 3dah. The rearing methodology followed standard methodology use at CMC for the culture of *S. aurata* larvae (C. A. P. Andrade, Nascimento, et al., 2012) and is represented in Figure 6.

Rotifers and artemia were the live feed supplied to larvae. Enriched rotifers were added two to three times a day, in order to maintain a density of 2-4 ind/mL. *Artemia* naupli density was kept at a density of 12 ind./L, increasing to 300 ind/L and *Artemia* metanaupli was kept at 250 ind/L.

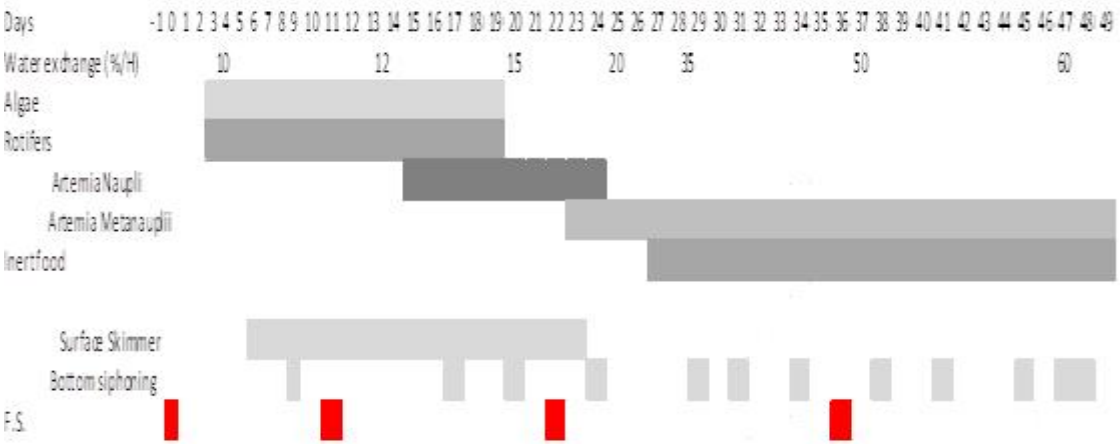


Figure 6 – Mesocosm larval rearing procedures for gilthead sea bream adapted from Andrade et al 2011. F.S – Full sampling, - days that all the parameters were measured in the rearing tank.

Surface cleaners (wooden skimmers) were used to remove oil film, in order to facilitate swim bladder inflation. Permanent surface skimmers were placed simultaneously to *Artemia* introduction. These skimmers were cleaned once a day.

3.2 Egg and larval growth performance

Egg and larval performance were evaluated taking into account egg hatching rate and larval growth, mortality rate and swim bladder inflation rate respectively

Egg hatching rate was calculated using 2 baskets of 500 ml placed floating at the surface of the mesocosm tank. Each basket was inoculated with 100 eggs of the same batch as the culture tank (C. A. P. Andrade, Abreu, et al., 2012). Mean hatching rate was calculated as the percentage of larvae hatched at 1 day after first hatching as follows.

$$Hatching\,rate = \frac{(Total\,eggs - Eggs\,that\,did\,not\,hacth)}{Total\,of\,eggs} X100$$

One of the parameters more commonly used to characterize the larvae production quality is larvae growth. The sampling and measurements followed the established procedures at CMC (C. A. P. Andrade, Abreu, et al., 2012).

Twenty larvae were collected from the central area of the rearing tank and total body length (TL) measured under the microscope (Setmi SV11; Carl Zeiss Microimaging Gmbh) on a petri dish with milimeter (mm) paper in the bottom. Photographs were taken using Canon Power Shot.

As larvae size were measured, swim bladder inflation success was also registered.

Total survival was calculated at fish transfer from mesocosm tank at 49 dah.

Larvae were trapped with a net in a reduced area of the rearing tank, and collected with a shrimp net. Five buckets were filled with juvenile fishes from a single shrimp net collected at different times of larvae harvesting – beginning, middle and end. The larvae in each bucket were counted and the mean value of the 5 buckets used as the number of fish per net. To calculate total number of fish transferred this number was multiplied by the total number of shrimp nets used for harvesting. Survival was calculated based on the procedure used by (Alves, Cerqueira, & Brown, 2006) as follows.

$$Survival = \frac{Transfered\,larvae}{Total\,number\,of\,larvae} X\,100$$

During larval rearing daily mortality was calculated in two areas of the rearing tank, at the surface and at the bottom of the tank.

Daily surface mortality was calculated by two methods. In one method a plastic square of 0.25m^2 was placed at the surface of the rearing tank and designated SS (Figure 7Erro! A origem da referência não foi encontrada.). A second method was tested using surface skimmers (SK) to collect all dead larvae from the water surface of the entire tank. For this method a collecting basket with a 50µm mesh net was placed at the exit of the water outlet surface (Wos).

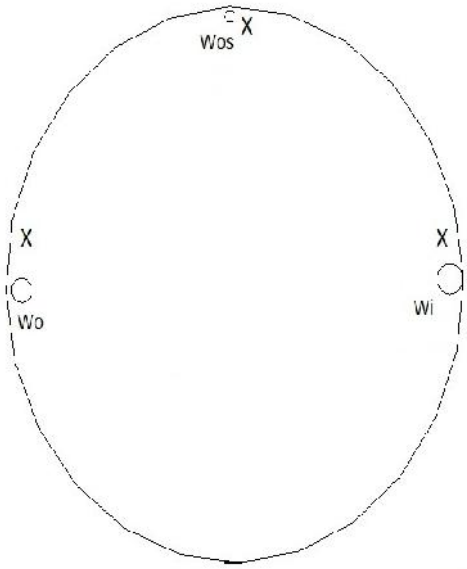


Figure 7 - Dead larvae counting devices. The X marks the positions of the squares at the surface.

Daily mortality at the bottom of the tank was registered by siphoning two squares (0.25m^2) painted with a white line at the bottom of the rearing tank (Figure 8). The squares (BS1 and BS2) were placed opposite each other. The squares were painted in the axel Wi – Wo, with the center at equal distance between the outlet and the center of the tank.

Daily total mortality based on this method was extrapolated from the mean value of the 2 squares, as the area of each square was approximately 79 times smaller than the area of the rearing tank.

The mortality rate was also calculated when siphoning of total bottom of the tank was done, as daily routine allowed.

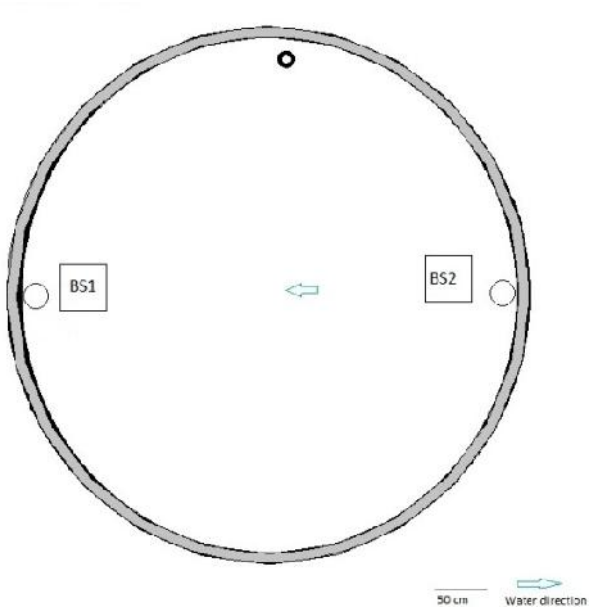


Figure 8 - Location of the squares (BS1 BS2), at the bottom of the tank, siphoned daily to collect dead larvae.

A tube with a flat end was used to reach the tank bottom squares and proceed to its siphoning. The material siphoned was collected with a 50 µm mesh net. In the case of data provided by total siphoning 1 ml counts of dead larvae was done in triplicate.

The formula used to calculate mortality rate was:

$$Mortality\,rate = \frac{Number\,of\,dead\,larvae}{Total\,number\,of\,larvae} X100$$

3.3 Behavioural observations

Behavioural observations were conducted, with the adoption of a protocol similar to the one used by Andrade *et al.* (2012a) which is based on the method described by Puvanendran *et al.* (2008).

The observer placed himself on the edge of the tank and all movements and social interactions of larvae were registered for 5 minutes.

Other observations were done during the daily routine around the rearing tank. Regarding larvae distribution at the water surface (Figure 9) and water column these were related to paint marks on the rearing tank (Figure 13).

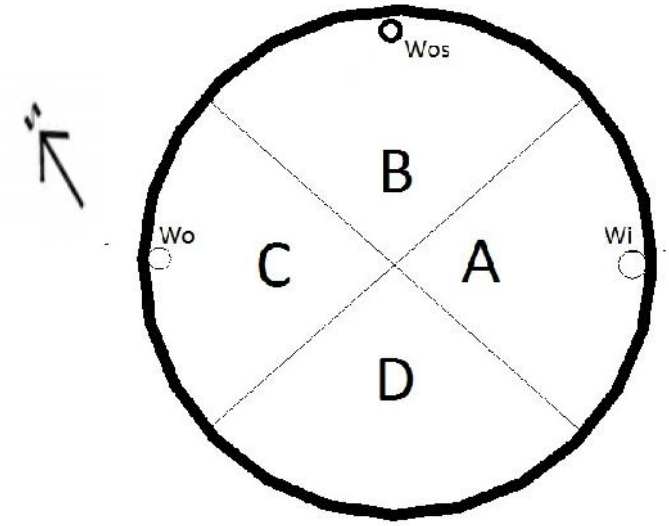


Figure 9 –Tank divisions and references marks used to evaluate larvae distribution at the water surface.

The criteria used for larvae distribution at surface was to register the tank quarter with the highest number of larvae. For distribution regarding depth, the criteria was to register the first time a larvae was seen near one of the side wall marks. The position of the marks are described below in Figure 14

3.4 Mesocosm environmental analysis

Faced with the task of the characterization of the rearing environment a major question arised: How do we analyse a tank of this size?

In order to establish the methodologies to be used, it was first necessary to decide what and when to sample. This decision requires good planning, in order to provide the less possible stress to larvae.

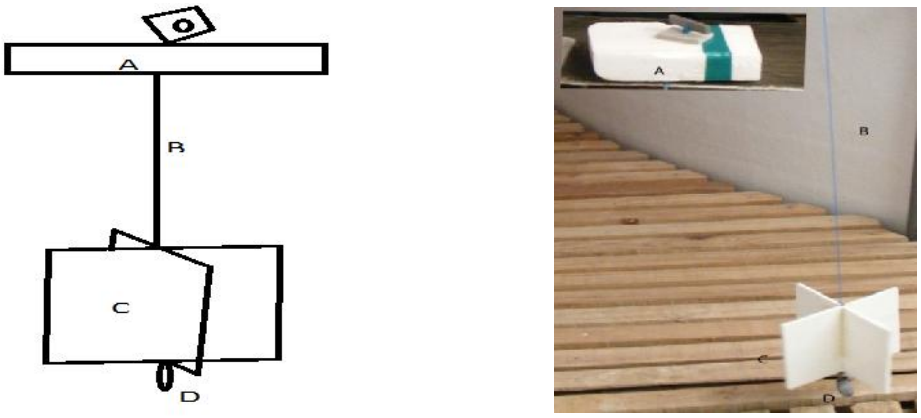
3.4.1 Hydrodynamics

The methods used to determine flow pattern and water velocity, were the use of drogue floats and dyes. Compared to the use of sensitive flow meters, these methods require low man power, little preparation, they allow for alterations in the trials with short notice and are inexpensive (Hughes, 2002).

The disadvantage of using both methods in natural conditions is the limited scope and spatial coverage, as well as the almost null probability of repeating trials (Dugan & Piotrowski, 2003). However, this is not a problem in the hatchery, as we deal with smaller masses of water and some level of control of for the environmental parameters.

The drogue float used in order to establish the flow pattern was designed and built for this purpose according to Hughes, (2002) (Figure 10). The drogue float was placed on the rearing tank, at 4 depths: -10 cm, 50 cm, 100 cm and 200 cm (Figure 11). Surface measurements were considered at 10 cm, since the floats at the surface were only 50% immersed.

The drogue was always placed at the water inlet, at the planned depth. The depths were achieved by increasing the length of the rope. The drogues positions were recorded at established time intervals (5 min) by photograph using Fujifilm FinePix 5700.



A) foam; B) Rope: C) plaques; D) Weight

1 2

Figure 10 - Drogue float device 1) scheme; 2) picture of the used object.

To evaluate water velocity we have used a method based on particle transport velocity (PTV), described by Oca et al (2004). A lime solution (25g/L) was placed at the water inlet (4L) and the water valve opened to reach the established water exchange rate. The entire process was video recorded using Fujifilm FinePix 5700.

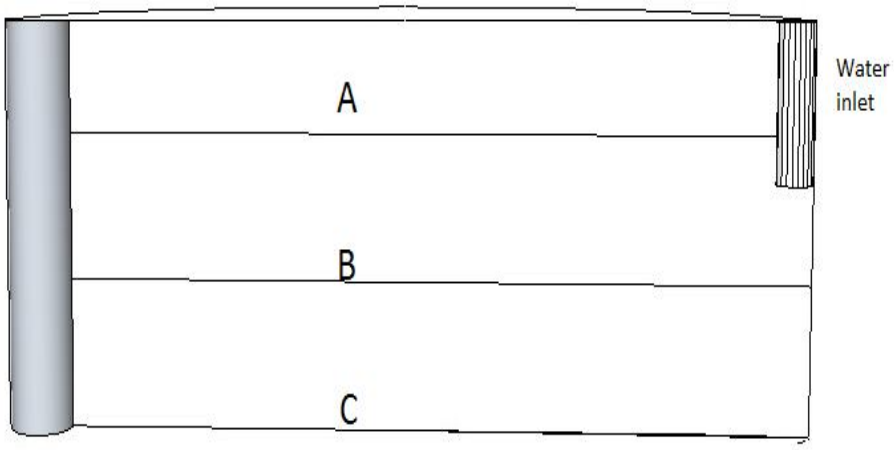


Figure 11 - Scheme of the division of the water collumn, for evaluation the watter flow pattern. A- top of the surface layer (50 cm); B- Middle of the water collumn (100 cm); C - bottom of the water collumn (200cm). Lime was placed on the water inlet, on the right side of the drawing.

The use of PTV derives from the fact that appropriate dyes were not available at the local market. The cost and time needed for ordering made the option of PTV more viable.

3.4.2 Culture sampling stations

Sampling stations were selected to gather data about the different abiotic and biotic parameters of the entire rearing tank.

Two transects were considered for the sampling stations. One transect covering the full diameter of the tank, from the inlet to the outlet of the seawater. The second transect covering the radius, from the centre of the tank to the tank border, at an equal distance from the inlet to the outlet of water. The radius transect was perpendicular to the diameter transect (Figure 12).

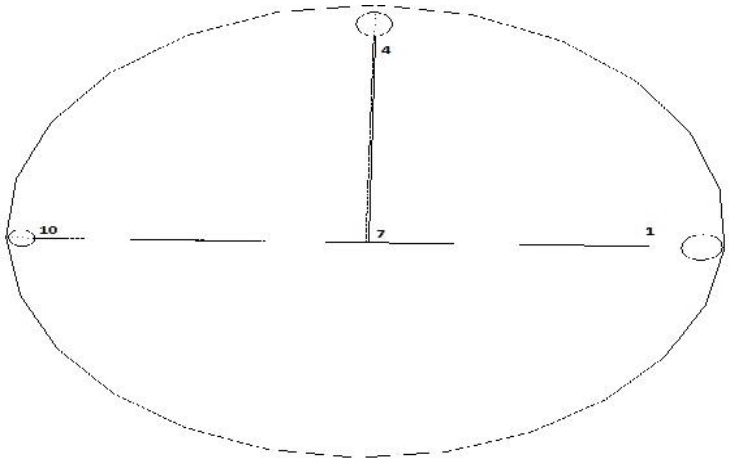


Figure 12 – Position of the two transect with the sampling stations used for measurements. (1 water inlet; 7 centre of the tank; 10 – water outlet; 4 border of the tank).

Along the transect the water sampling and measurements were made at selected stations at the surface (10-15 cm), at 50 cm depth, middle of the water column (100 cm) and bottom of the water column (200 cm) (Figure 13).

It was considered that these sampling stations provided a complete description of the environment, covering the entire rearing tank.

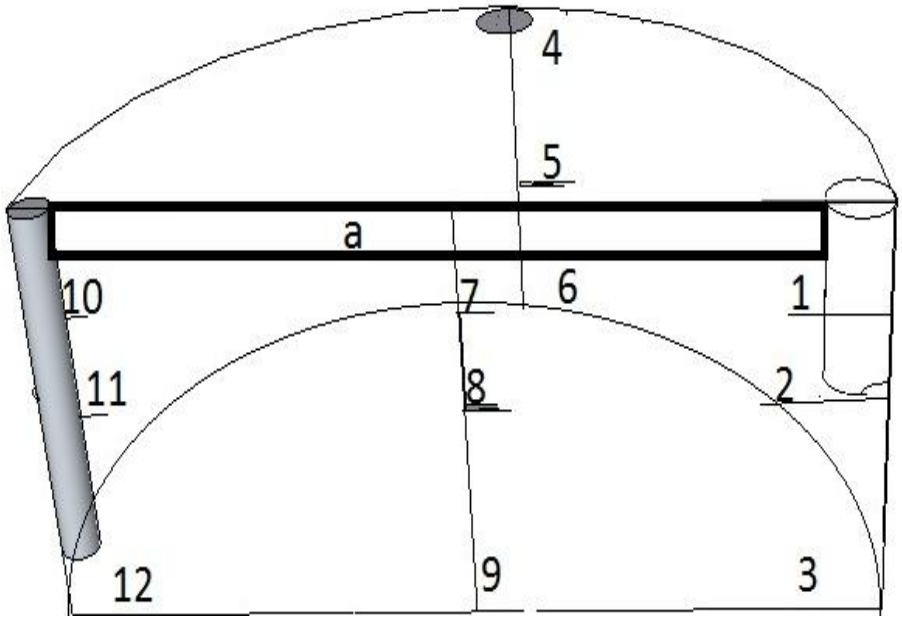


Figure 13 – Sampling stations: the superficial layer; points at 50 cm (1,7,10,4); points at 100 cm (2,8,11, 5); points at 200 cm (3,6,9,12) points 1, 2 and 3 are next to the water inlet.

A pole was used to reach the centre sampling points of the tank. At the extremity of the pole a flat base was used to install the light meter. On the side of the pole, a hose was attached for the collecting water by siphoning 250 ml sample to a plastic cup, for salinity and pH measurements. An oxygen and temperature probe was also attached to the pole to measure central station points.

Sampling points on the edge of the tank were marked with white paint in the dark background (Figure 14), before filling the rearing tank. This was done well in advance the beginning of the rearing cycle. The water was renewed for several days to wash away any harmful substances that could have any toxic effect on the larvae.

Figure 14 - Marks on the wall of the rearing thank representing the depths measured. In the picture it is also possible to see, the water inlet and one of the aerators.

The water collection hose and the probe to sample oxygen, temperature, pH and salinity were submerged to the appropriate depth and station.

A shorter pole with a flat base was used for measuring light intensity under water with a probe, in order to obtain a more stable reading. Since the probe was not water proof, all measurements were done with the probe wrapped in a plastic sheet. The measurements were corrected from the plastic as follow, lux at any depth – lux at surface free probe – lux at surface with plastic. Preliminary tests indicated that data provided by the probe with and without the plastic, had a differences of just one or two lux. The effect of the plastic was not considered.

The daily routine measurements at surface were made near sampling point nr. 10, in area C near the water outlet. The probe is placed at a depth of 10-15 cm and the value registered assumed to be for the entire surface layer of the tank.

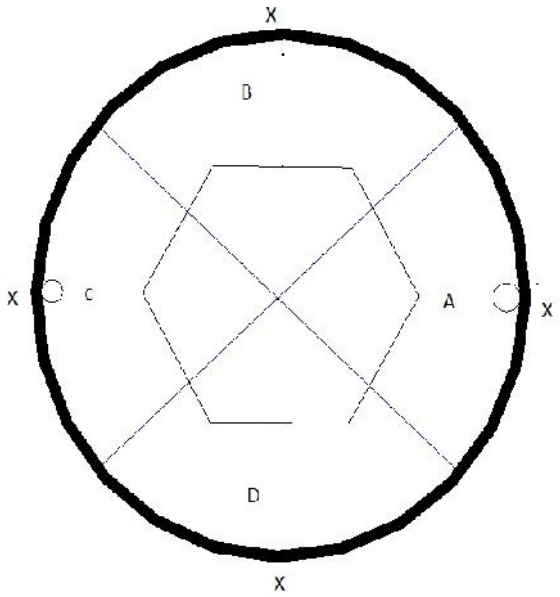


Figure 15 - Sampling points (X) for surface light and measurements.

Daily surface measurements were made around 12.00h according to Figure 15.

Light measurements of the light distribution inside the rearing tank, were made at 22.00h. The reason for the late hour was to assure that there was only one light source, the fluorescent lamps.

The following devices were used for the physical and chemical measurements:

- Water temperature and dissolved oxygen - Handy Polaris; Oxy Guard International A/S.
- Salinity – Refractometer Atago, S-10E
- pH – pH meter Hanna HI-96196 Champ pH Testes
- Light intensity– Testo^{c} 540

Data collection was done in a daily basis, however at the weekends there was a shortage of staff and only oxygen and temperatures were recorded.

3.3 Statistical analysis

Results are presented as mean ± standard deviation (SD)

Oxygen, temperature, light and pH assumption of normality adjustment and homogeneity for variance were verified using Kolmogorov-Smirnov's and Leven's tests, respectively with a significance level of 0.05 (Zar, 1996). If results of Kolmogorov-Smirnov were not verified, a non -parametric test was applied, (Kruskal-Wallis) to test the homogeneity along the rearing period. A mean pairwise analysis of variance was used for determining missing data.

Statistical analysis was completed using IBM SPSS Statistics V.20, (Chicago Illinois, USA).

4. Results

4.1 Egg and larvae performance

Egg hatching was 98%, for the floating baskets method. The hatching rate based on first siphoning of the tank (9dah) provides a slightly higher hatching rate of 99 %.

Total survival rate at the end of the rearing period was 61%. The rate of normal swim bladder inflation was 90%.

Larval growth performance is shown in Figure 16.

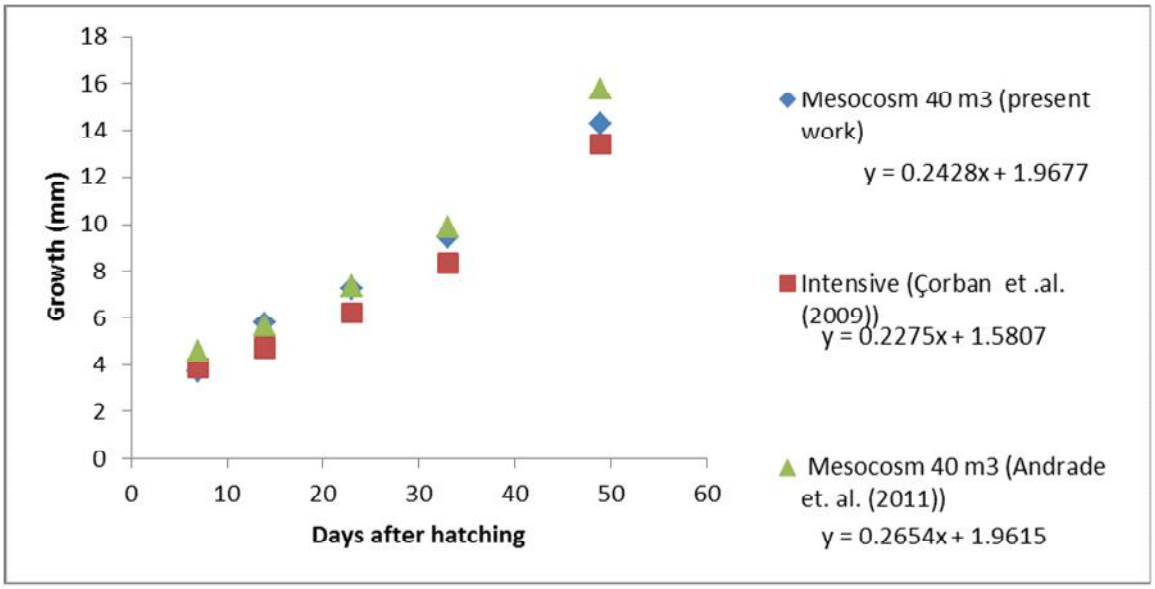


Figure 16 - - Larvae growth along the rearing period. Also presented data from Andrade et al. (in Press) using similar mesocosm (40 m3) and Çoban et al (2009) in intensive production.

Mortality rate was registered at surface and at the bottom of the tank (

Figure 17). Mortality decreases along time. Several peaks of mortality occurred at, 15 dah, 33 dah and 40 dah.

Figure 18 presents a comparison of the values recorded and expected for the different methods to evaluate mortality. Methods SB and TB presented estimates similar to the final larvae mortality registered at the transfer to the juvenile rearing tanks.

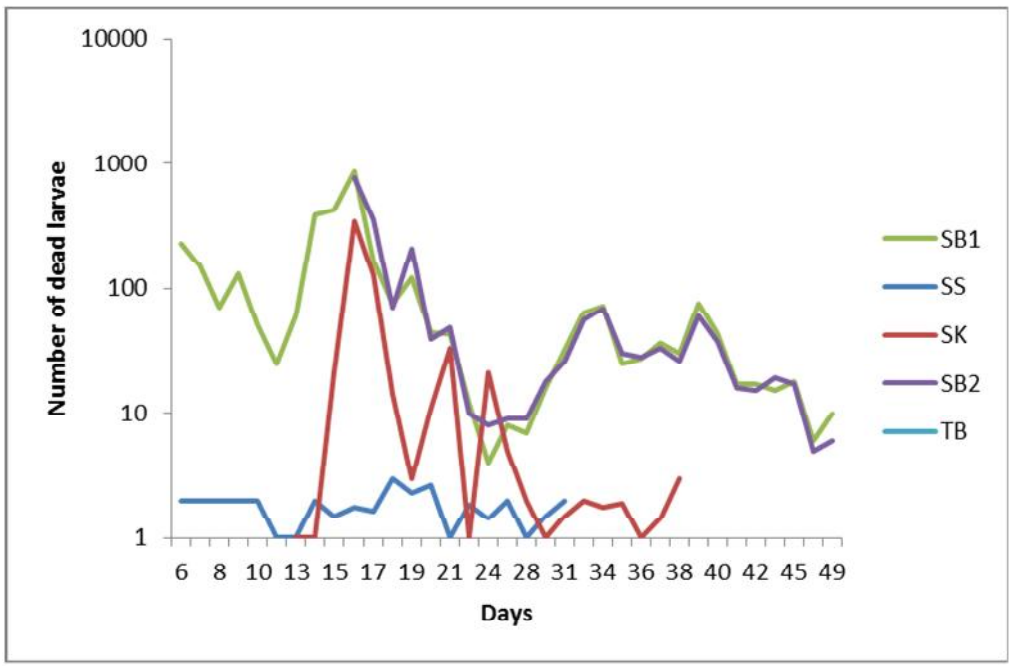


Figure 17 - Mortality data from all methods used (SS – surface squares; SK – surface skimmer; SB1 and SB2 – bottom squares, TB – total bottom).

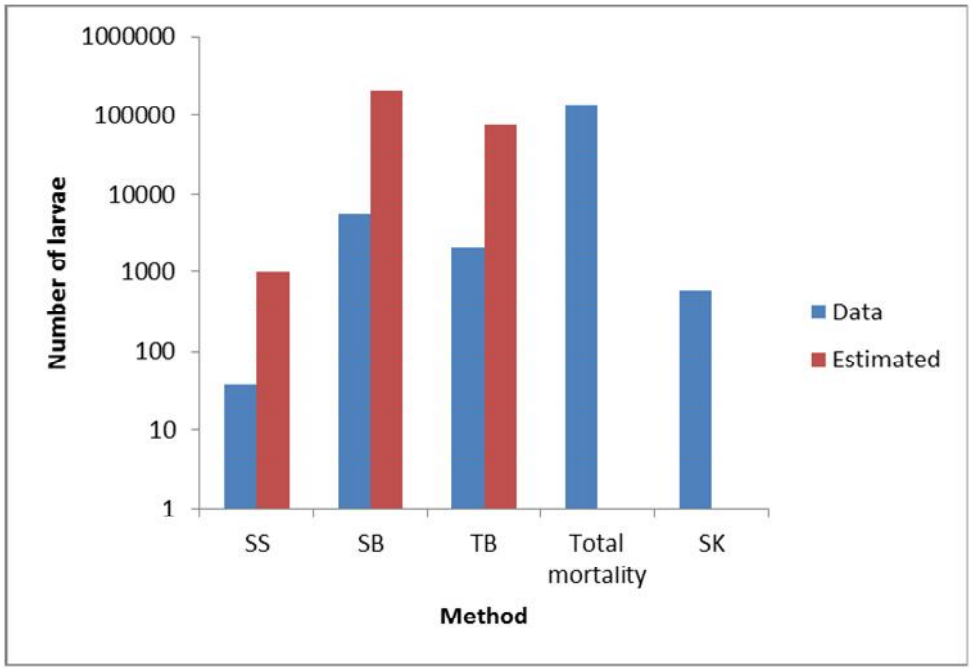


Figure 18 - Comparison of the methods used to gather mortality information, the value for total mortality is based on the difference between the number of larvae hatched and larvae transferred to weaning tanks. (SS – surface squared; SK - surface skimer; SB1 e SB2 – bottom squares; TB – total bottom.

4.1.1 Larvae distribution

Eggs displayed an almost uniform distribution on the side walls of the tank (Figure 19). From 4 dah forwards most larvae occupied the D area of the tank. Larvae occupied the full surface of the tank at later stages of development.

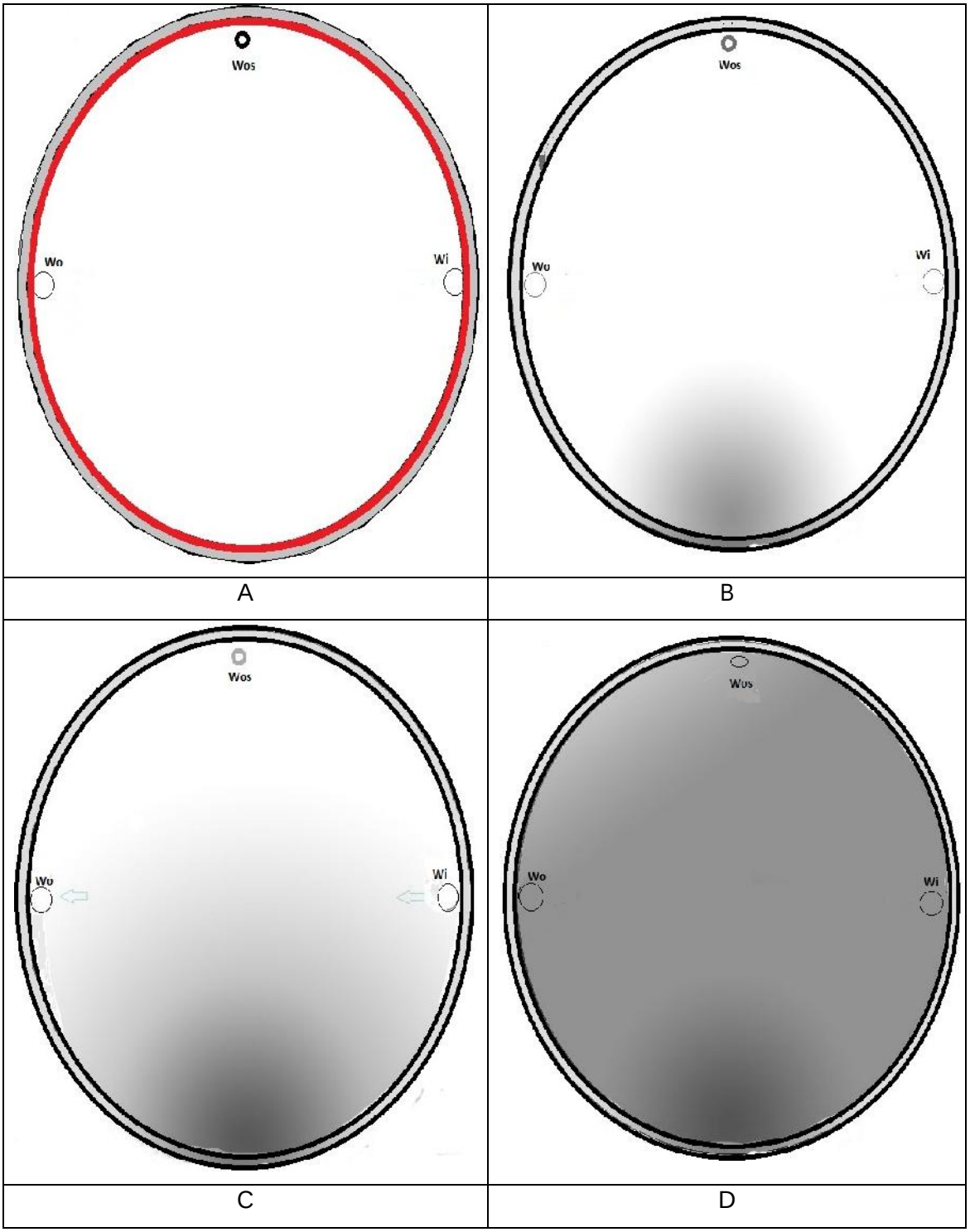


Figure 19 - Larvae distribution at the surface of the rearing tank during the larval rearing period: A- O- 3 dah; B 4-20 dah; c- 21-32 dah; D – 32-49dah.

During most of the larval cycle the larvae were situated at the surface layer, above 50 cm depth (Figure 20 and Figure 21).

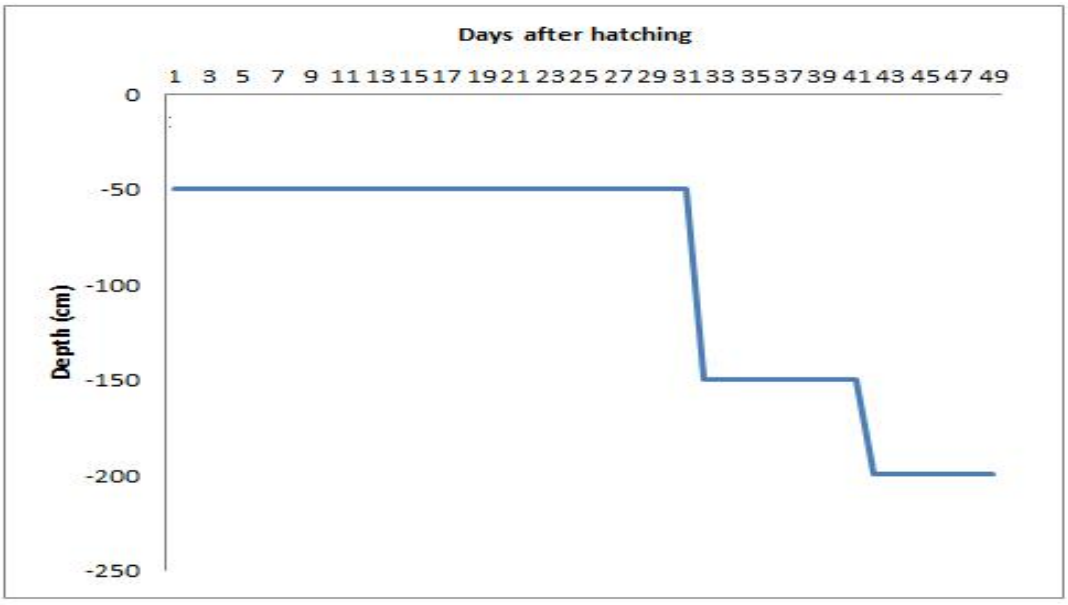


Figure 20 - Larvae distribution in the water column during the 50 days rearing trial.

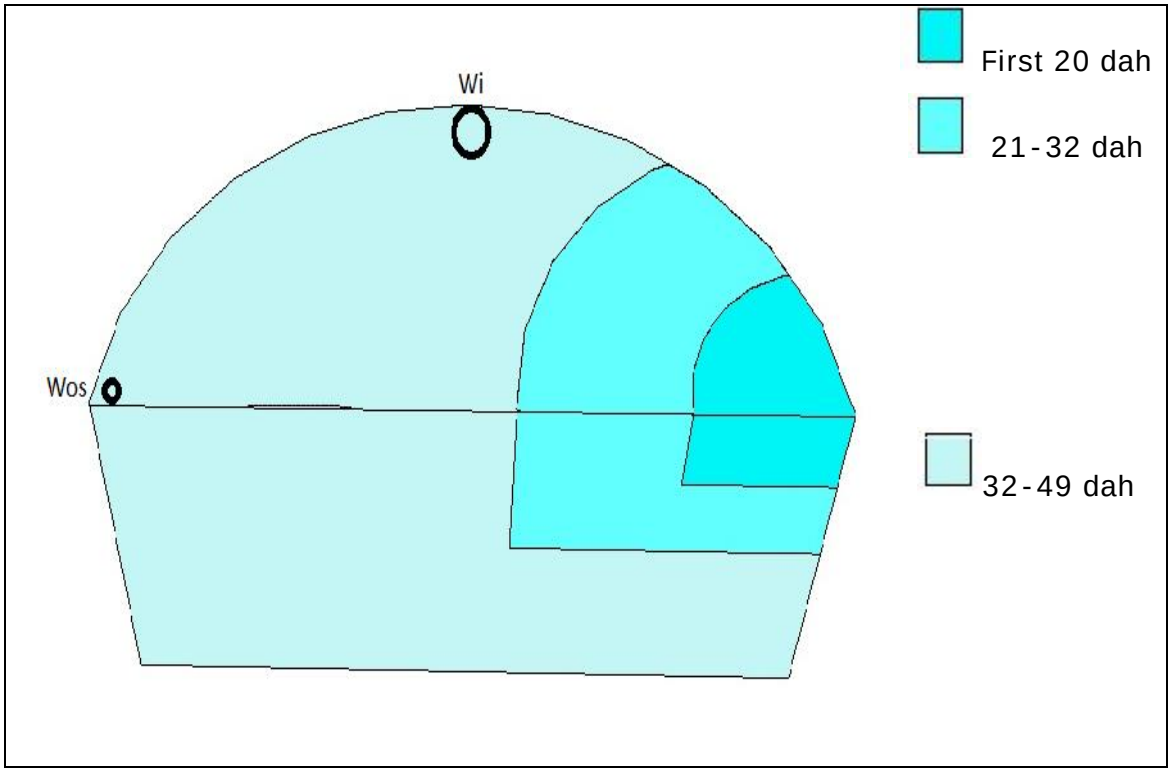


Figure 21 - 3D representation of larvae distribution along the rearing period.

4.1.2 Larvae behaviour

A wide range of larvae behaviours were registered (Table 1 and Figure 22) during the culture trial

Behaviour		Description	Days after hatching
1	Standing still	Larvae standing still without movement	1 to 15
2	Active feeding with low movement	Short feeding movements.	4 – 10
3	Active feeding with high movement	High active feeding	11- 49
4	Aggressive behaviour	Larvae would swim against other larvae	35 - 49
5	Cannibalism	Isolated episodes of larvae engulfing another larva	
6	Sprint	Increasing swimming activity	29 - 49
7	Schooling	Direction change to follow nearby larvae	32- 49

Table 1 - Description of the registered larvae behaviours in time (days after hatching).

Figure 22 – Representation of the observed behaviors of *S. aurata* larvae during the present trial

When larvae were transferred from the mesocosm tank 90% of the larvae had the tail fin nipped (Figure 23).

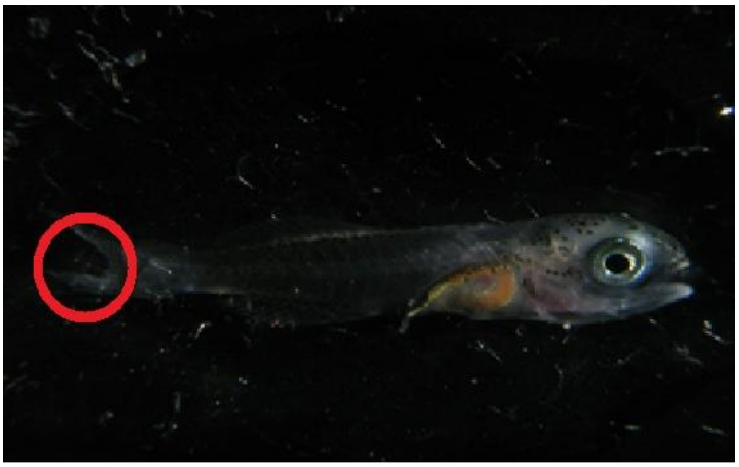


Figure 23 - Photograph of one of the larvae transferred from mesocosm with fin tail nipped.

4.2 Environmental parameters

4.2.1 Water flow patterns

The observed flow patterns using drogue floats are presented in Figure 24.

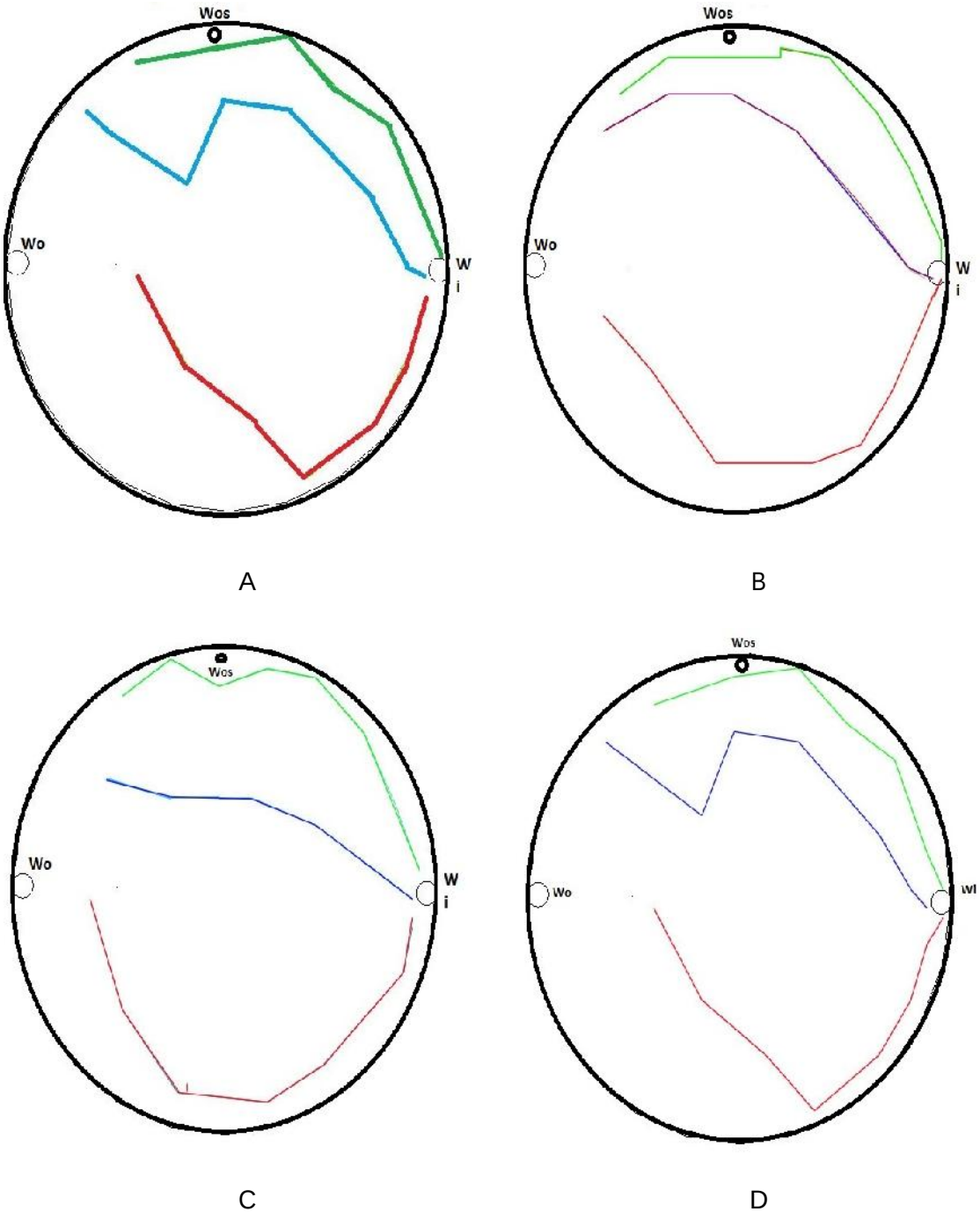


Figure 24 - Flow pattern observed at the different depths: A -10 cm; B - 50 cm; C - 100cm; D -200 cm. Each colour represents a drogue float device.

The pattern of lime dispersion in the rearing tank is presented in Figure 25. The water current calculated by the lime method, varied between 0.69 cm/s and 1.38 cm/s (Figure 26).

The highest velocity was recorded by lime dispersion, with the maximum water renewal.

The lowest speed was achieved by the drogue at the lowest water renewal rate. The central area of the tank presented consistently slightly higher water velocity.

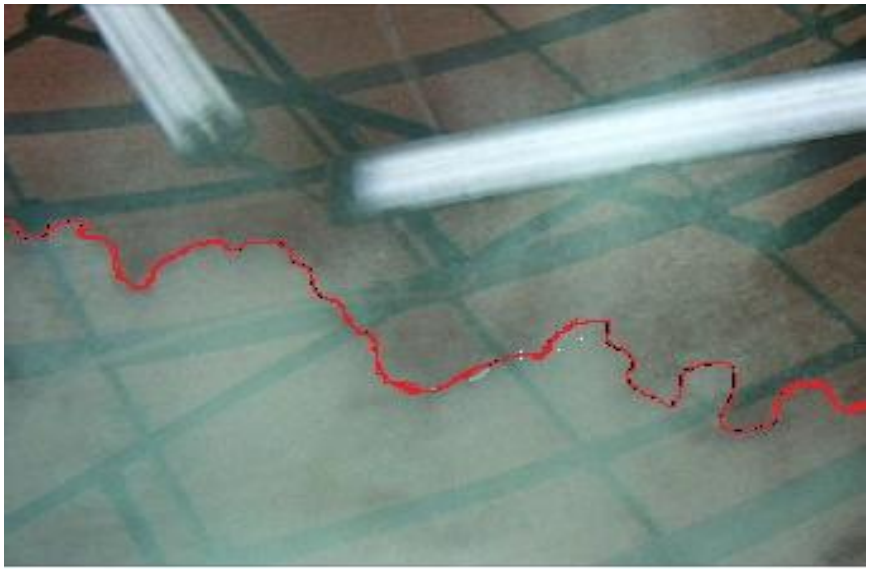
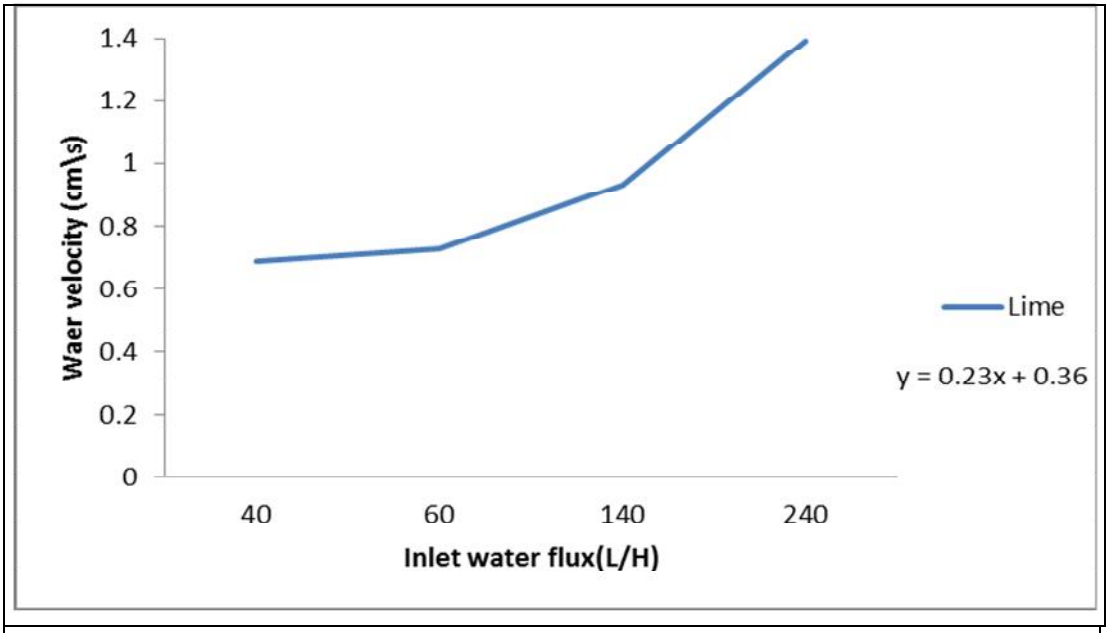
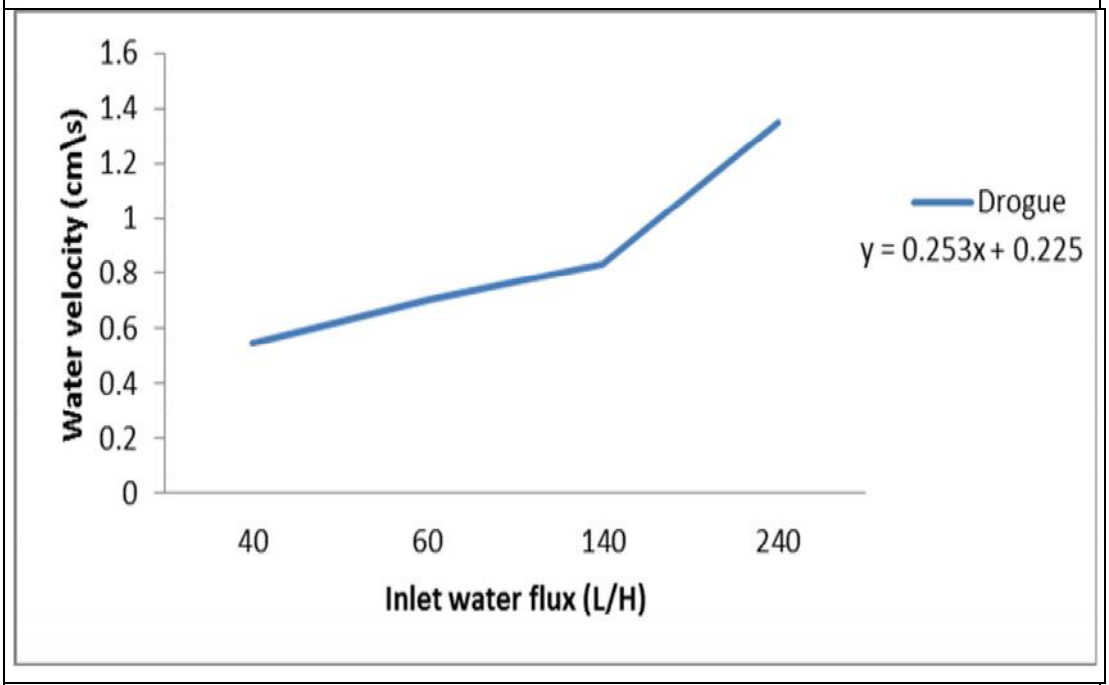


Figure 25 - Lime dispersion (red line) inside the rearing tank.



A



B

Figure 26 - Water velocity calculated at different water renewal rates (% per day) A – Lime; B Drogues.

4.2.2 Salinity

Salinity had a mean value of 36 ±0.05‰ for all stations and for the entire duration of the rearing period.

4.2.3 Temperature

Mean temperature was 20.1 ± 0.1ºC, increasing for the duration of the rearing period from 17.8ºC to 22.8ºC. The bottom of the water column was colder than the surface.

Temperature had no significant variations in terms of depth or surface stations during the rearing period (Kruskal-Wallis test; $\rho < 0.05$) (Figure 27).

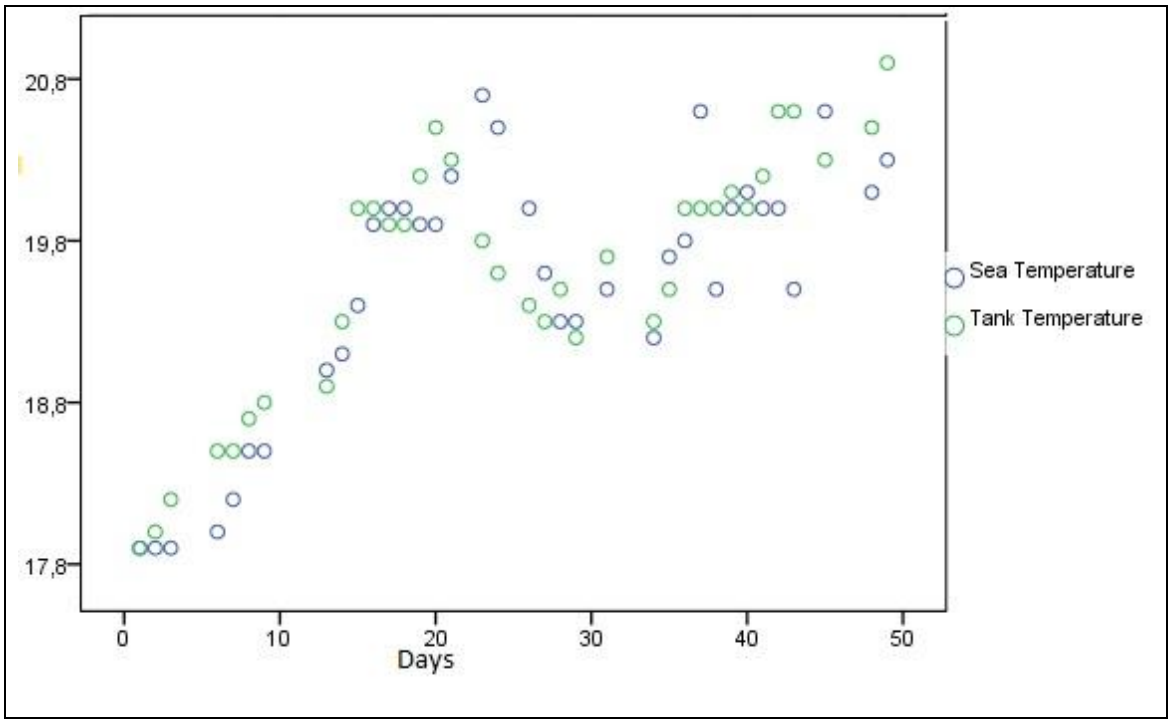


Figure 27 - Sea and Tank temperature along the rearing period.

Temperature was registered daily. Particular emphasis was given to three moments, which corresponded to alterations of the live feed regime (Figure 28). Temperature presented a slight stratification pattern to 1.0 m depth until 21 dah. As time passed the different temperature layers became more distinct. The same pattern of distribution is observed on the lateral profiles of Figure 28 from the surface to the bottom of the tank.

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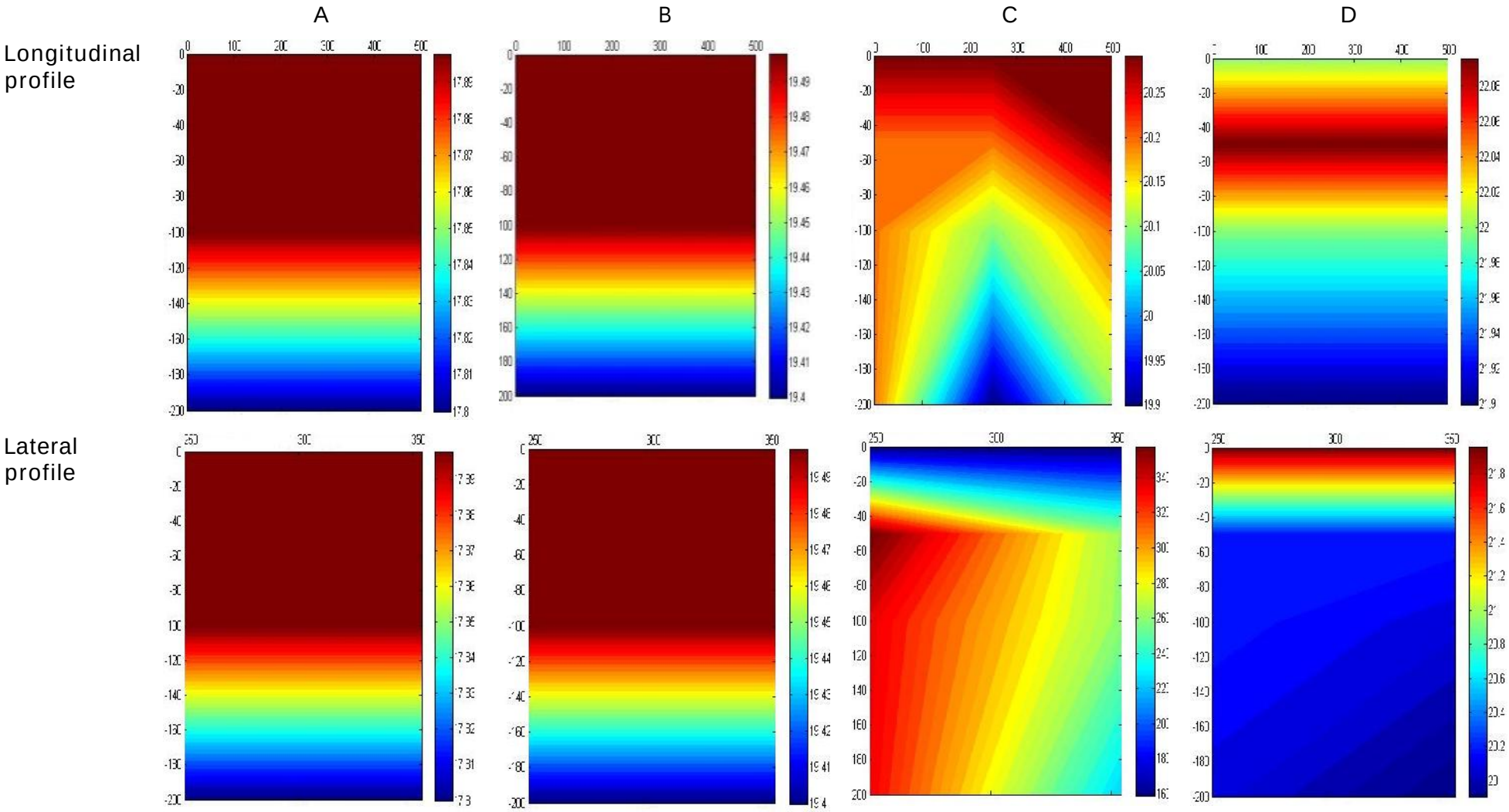


Figure 28 – Temperature (ºC) registered on the days that . A - Blank Tank; B - 10 dah; C - 21 dah; D -35 dah.

4.2.4 Dissolved Oxygen

Mean oxygen was 6.06 (±1.35) mg/l, for all stations and the entire duration of the larvae rearing period. Dissolved oxygen decreased as time passed (Figure 29). Oxygen presented significant differences (Kruskal – Wallis; $\rho<0.05$) in distribution, either in stations or depth (Figure 30 A and B).

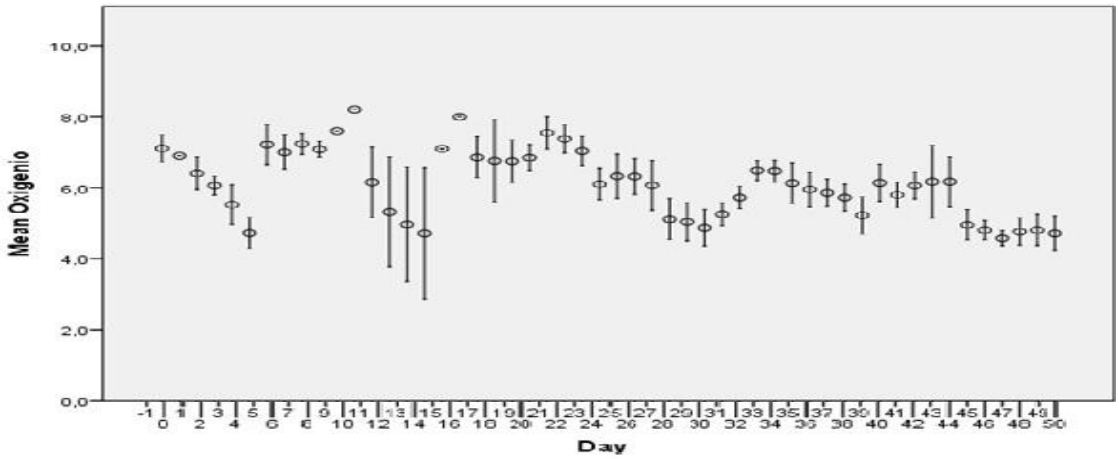
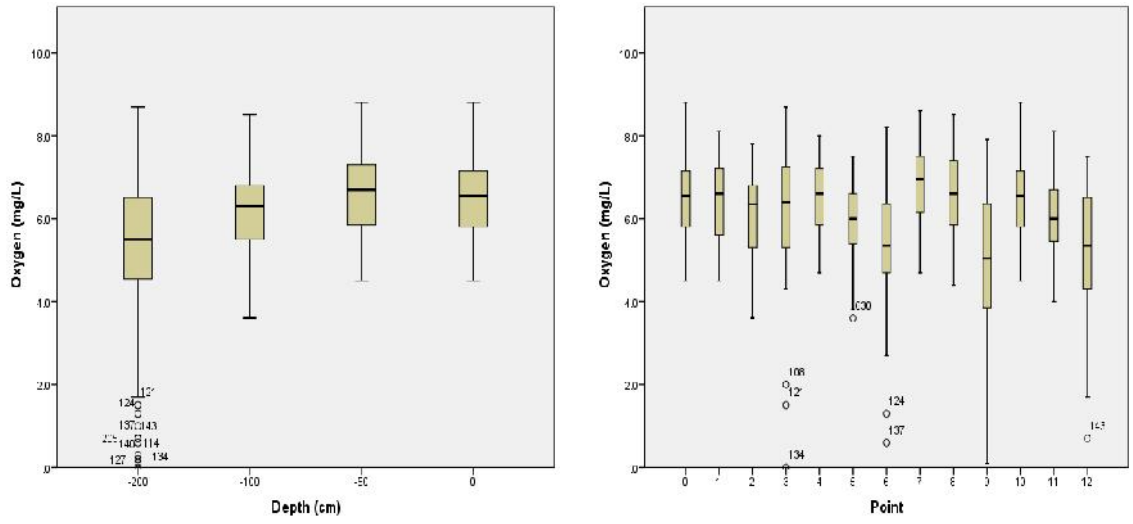


Figure 29 - Mean dissolved oxygen variation along the rearing period



A B

Figure 30 - Dissolved oxygen variation regarding depth (A) and sampling points (B)

Oxygen displayed two patterns: before and after larval hatching (Figure 31). In the pre-culture moment (Figure 31A), the pattern displayed was highly variable, with two areas near the side walls with lower dissolved oxygen concentrations. After larval hatching this parameter register similar patterns with concentration decreasing as time passed (Figure 31B-D). The lateral profile evidenced that concentration decreased from the surface to the bottom of the tank.

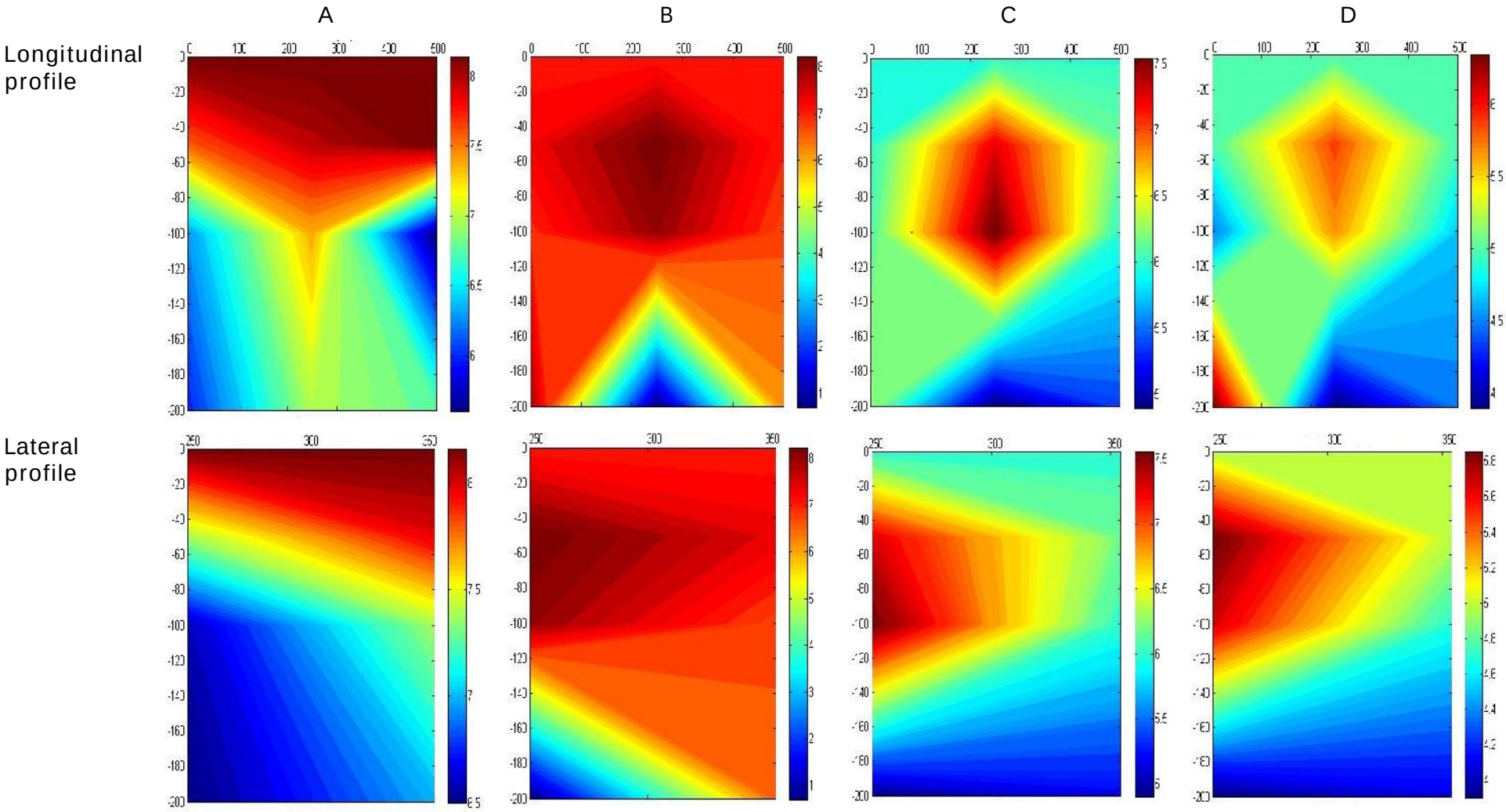


Figure 31 – Dissolved oxygen variation (mg/L) at different moments of the rearing period . A – Blank tank; B – 10 dah; C – 21 dah; D – 35 dah

4.2.5 pH

pH determined at different stages registered a mean value of 8.6 ±0.09. pH was homogenous for the entire tank, that is no significant differences were registered between stations (Kruskal-Wallis; ρ>0.05).

pH pattern increased from water inlet to water outlet (longitudinal profile (Figure 33). It also increased from the bottom to the surface (lateral profile).

4.2.6 Light

Light intensity at the surface of the rearing tank was 1514 ± 1444 lux (Figure 32). Light intensity for all stations varied between 7594 lux and 99 lux. Light presented significant differences between sampling stages (Kruskal-Wallis; ρ <0.05) Figure 34.

At pre - hatching of larvae, the light intensity decreases from surface to bottom and from the center to the tank walls (Figure 34).After hatching (15, 21 and 36 dah), the pattern is similar, decreasing from the center to the tank wall.

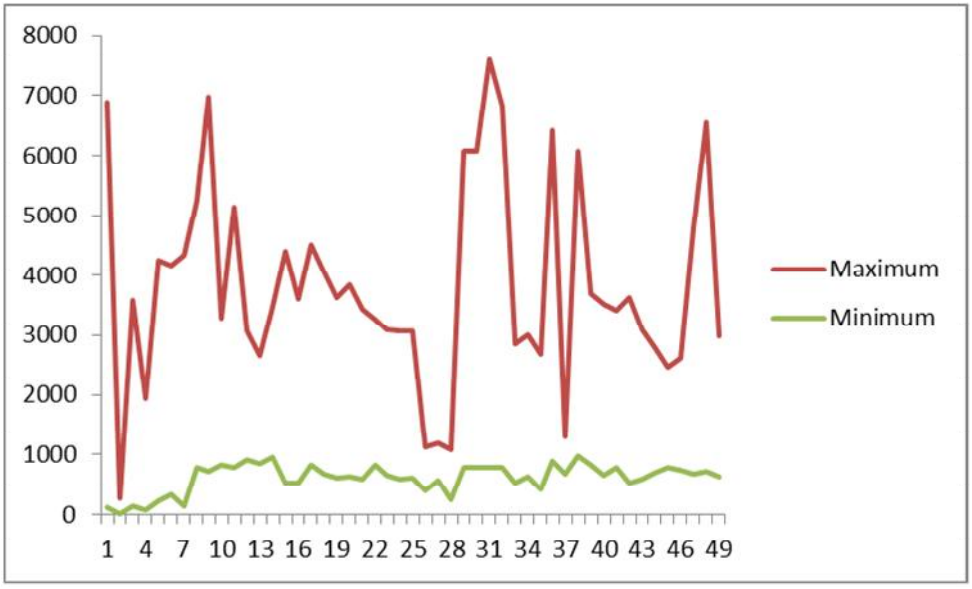


Figure 32 - Light intensity (lux) at the surface of the rearing tank, during the entire rearing period.

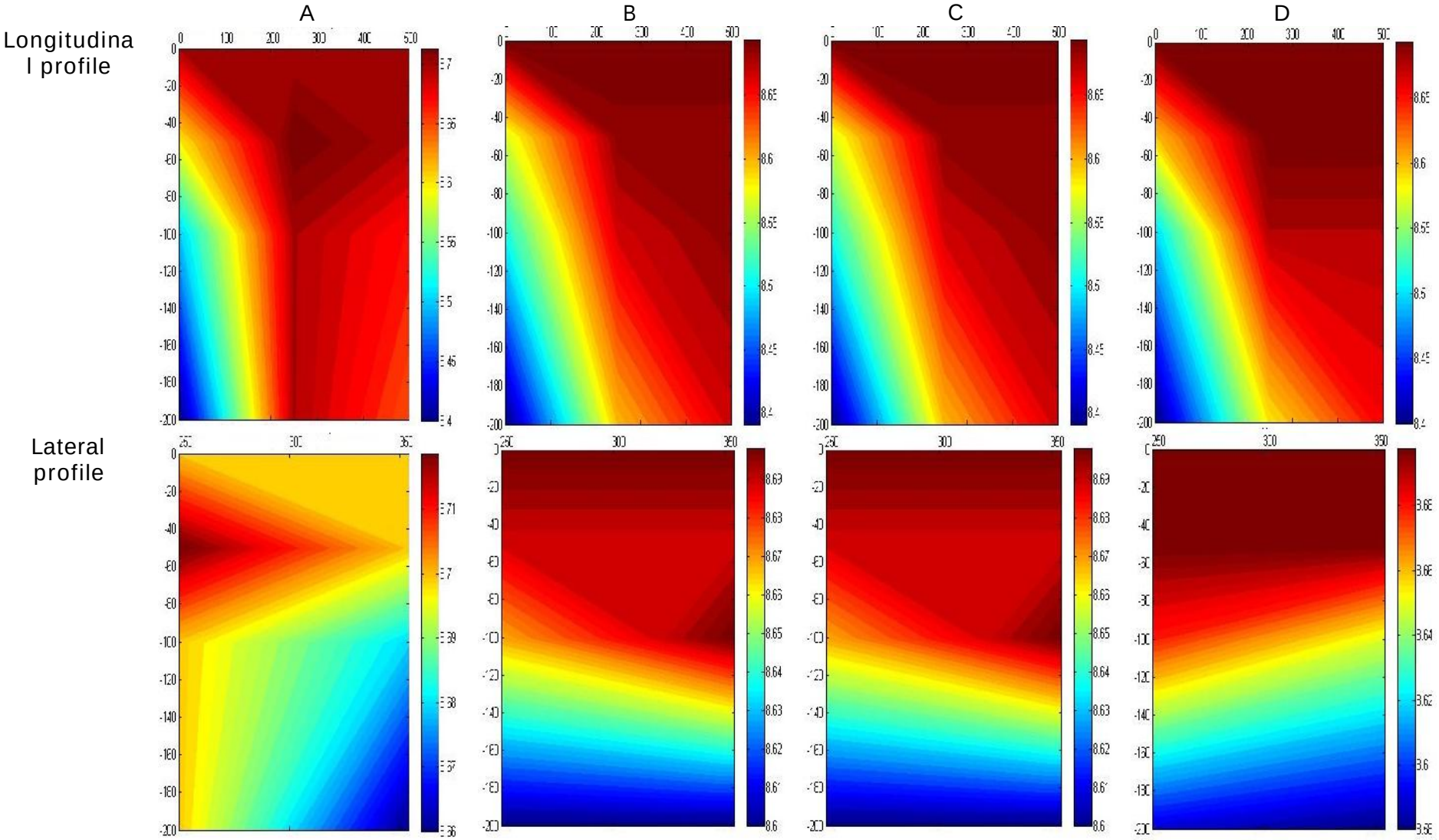


Figure 33 - Variation of pH at different rearing moments . A – Blank tank; B – 10 dah; C – 21 dah; D – 35 dah.

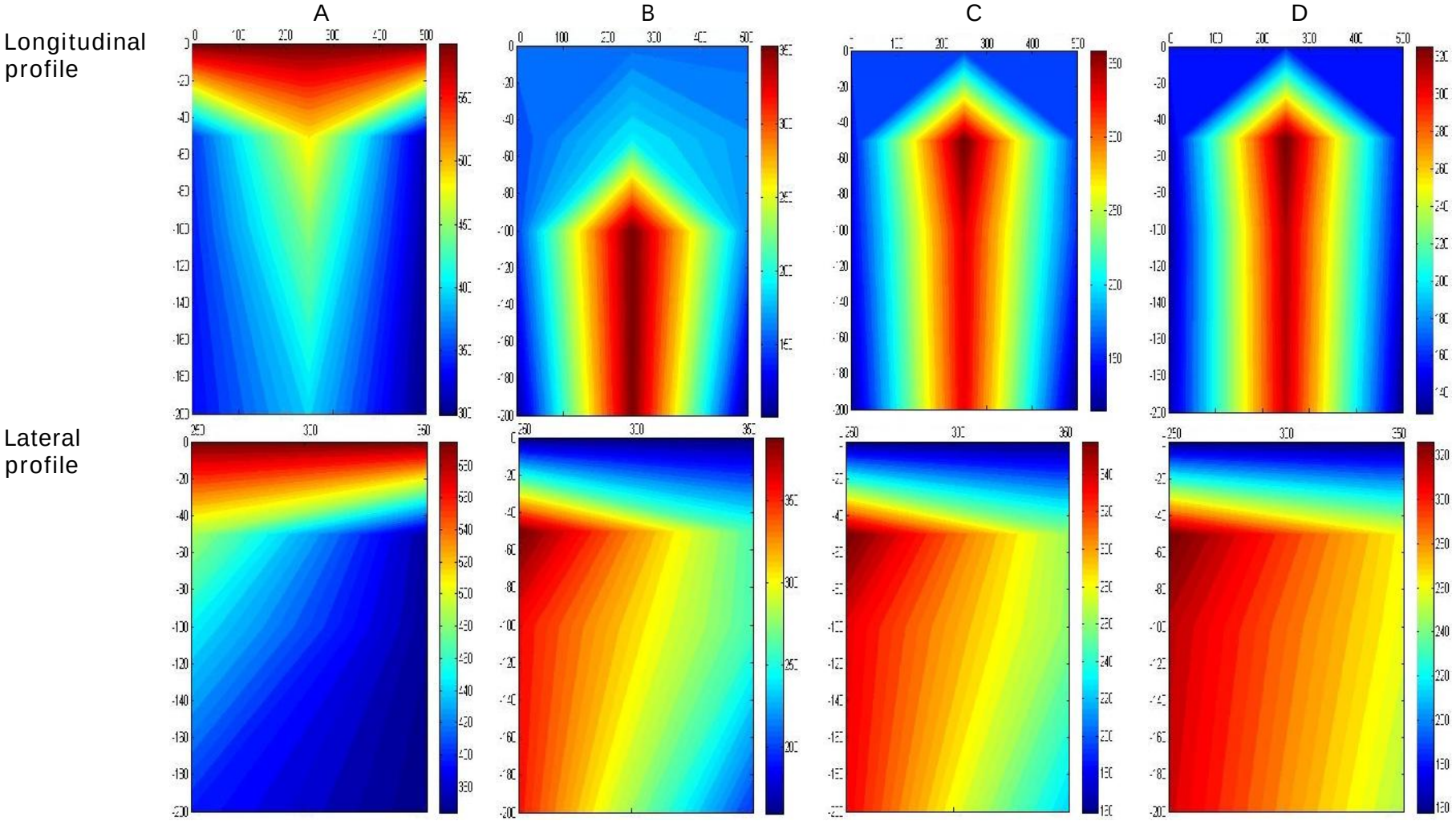


Figure 34 - Light variation (Lux) at different rearing moments. A – Blank tank; B – 10 dah; C – 21 dah; D – 35 dah.

5. Discussion

Mesocosm of semi-intensive methodologies is widely used for larval trials, especially for the diversification of aquaculture species (Divanach & Kentouri, 2000), providing higher quality juveniles (Shields, 2001).

The aim of this work was to widen the knowledge on mesocosm based hatcheries for *S. aurata* production, by providing information about the rearing environment and larvae behaviour.

5.1 Larvae performance

The larvae hatching rate of this trial was high with a value of 98%.This value is higher than the 91% from (Polo, Yufera, & Pascual, 1991). Hatching rate is even higher than the reference values for this methodology which is situated between 40-90% with a mean value of 60% (Divanach & Kentouri, 2000).

Larvae demonstrated a higher growth rate than *S. aurata* larvae reared in intensive methodology by Çorban *et al.* (2009), and similar to previously mentioned by Andrade *et al.* (in press) for the same species and methodologies. The growth demonstrated by larvae can be considered a sign of higher quality (Shields, 2001)

The slow growth rate observed at the beginning of larval development can be related to the shortage of rotifer production. The requirement of high nutritive feed is essential in the first 15 dah. The rate of swim bladder inflation was high (87%), similar to the registered for larval rearing of red porgy in mesocosm (C. A. P. Andrade, Abreu, et al., 2012).

Larvae survival at the end of the rearing period (61%) was within the expected values for this methodology, that is 40-90%, according to (Divanach & Kentouri, 2000). This survival is higher than obtained for other species, e.g., 16% for red porgy (C. A. P. Andrade, Abreu, et al., 2012) and around 6% for *Dentex dentex (Giménez & Estévez, 2008).*

Despite being a commonly used parameter, there is little information regarding daily mortality in larval rearing. Alves *et. al.* (2006) mentioned that daily mortality for fat snook (*Centropomus parallelus*) was evaluated, but no data was reported. Andrade *et al.* (2012a) in their mesocosm trial with red porgy provided information about mortality with changes of diet regime.

The first data for mortality were collected on the 7 dah. This occurred since it was not possible to clean the tanks. Larvae are extremely sensitive at this stage and cleaning procedures are a source of agitation of the rearing environment

For the different methods the estimated mortality was higher until 10 dah and then decreased significantly. This pattern can be due to starvation or for larvae not having yet completely exhausted their yolk - sac (Yufera, Pascual, Polo, & Sarasquete, 1993).

Mortality values were high at the bottom of the tank, but this was expected as larvae sunk as they died.

The two methods used to register larvae mortality at the surface did not provide viable information. The methods used to register mortality at the bottom of the tank, however, proved that they can provide more accurate information concerning the total larvae mortality that occured in the rearing tank.

In terms of surface dispersal, larvae at early stages tended to occupy the area next to the walls in a more or less homogenous distribution. As larvae grew older they tended to occupy the area of the rearing tank of higher incidence of light. This distribution is consistent with one of the most common behaviour of fish, positive phototropism, that is, fish tend to school and move towards the light (Marchesan, Spoto, Verginella, & Ferrero, 2005).

Regarding larvae distribution in depth, *S. aurata* larvae first occupied the top quarter of the tank. The same distribution was not observed in *S. aurata* larvae reared in 6-10 m^3 tanks with intensive methodologies, in which larvae occupied the entire water column (Moretti et al., 1999).

The observed occupation of the water column in the present trial, is in concordance with the observed behaviour for *Pagrus pagrus* (C. A. P. Andrade et al., 2011) and for *Melanogrammus aeglefinus* (Downing & Litvak, 2002) as well as for sea bass larvae, that occupied the first 10 cm of the water column (Conides & Glamuzina, 2001). Larger larvae where the ones found deeper in the water column, similarly as observed for herring by (Catalán, Vollset, Morales-Nin, & Folkvord, 2011). The same authors demonstrated that herring distribution in the water column had more relation to light than to any other parameter.

There was a wide range of larvae behaviours, most of them remaining until the end of the rearing period. This persistent pattern of behaviours throughout the larval period suggests that fish larvae are well adapted to the culture conditions (Ashley, 2007).

Schooling in *S. aurata* appeared around 32 dah, much later than in *Chelon labrosus* larvae, that displays this behaviour around 19 dah (Ben Khemis et al., 2006).

Behaviours similar to the behaviour described as sprint, small burst of increased swimming activity have been described for fat snook (Alves et al., 2006) and red porgy (C. A. P. Andrade et al., 2011).

Rearing density will influence behaviour, with higher densities groups displaying higher swimming activity and low densities groups displaying agnostic behaviours (Canario, Condeca, Power, & Ingleton, 1998). The lack of information regarding *S.aurata* larvae behaviour does not allow the classification of the displayed behaviours.

The lack of quantification of behaviours was due to the accumulation of larvae in one single area. This increase in density is often an obstacle for quantification (Alves et al., 2006).

Fin nipping was observed towards the end of the rearing trial. As reported in *Gadus morhua* larval culture (Puvanendran, Laurel, & Brown, 2008), *S. aurata* larvae presented this behaviour regardless of its size, in what could be considered a precursor behaviour of cannibalism. Another common reason for the appearance of fin nipping is food deprivation (Andrew, Holm, Kadri, & Huntingford, 2004).

Only a few cases of cannibalism were observed with full engulfment of prey. This low cannibalism incidence in *S. aurata* larvae is also reported by Andrew *et al*. (2004). Probable causes for cannibalism can be heterogeneity of size and low feed availability (Kestemont et al., 2003). According to Puvanendran *et al* (2008) only larvae with age difference higher than 10 days would originate cannibalism in Atlantic cod.

5.2 Abiotic Parameters

If appropriate rearing conditions are not provided for larvae, their influence on larval development can represent an elevate cost, due to high mortality (Yoseda et al., 2008).

5.2.1 Rearing tank hydrodynamics

Hydrodynamics was studied in terms of flow pattern and velocity.

The flow pattern in the mesocosm tank described a circumference route, with slight alterations due to the effect of aerators. The flow pattern i was similar to the one described by Duarte *et al.* (2011), despite the tanks used by this author presenting a water volume of just 11 L.

The pattern described at the four depths was similar. Therefore the water movement may be considered homogenous for the entire column.

The use of floating drift seemed a good choice to evaluate water currents in rearing tanks. They can provide reliable information, while allowing replication (Duck, McManus, & Charlton, 1985). The number of drogues used in this work was considered adequate. They covered the entire water inlet area and if more drogues were used, chances were that strings could become wrapped with each other influencing results.

For this kind of studies it is more appropriate to build the drogues than buying from the trade, that are designed to use in wider spaces (Duck et al., 1985). The drogues that were built are simple to construct and served its purpose.

The visualization of the pattern described by the drogues in the rearing tank can be quite a challenge in closed facilities, like the one of CMC. The photograph camera should be in a position to provide total coverage of the rearing tank.

Water velocity at the centre part of the tank was slightly higher than the water velocity by the tank walls. Water velocities were far below the limit of 10cm\s for the full production cycle of *S. aurata* (Moretti et al., 1999). Still there was no indication for which phase of the rearing period this limit is used. Also, water velocity are lower than the ones mentioned by Divanach *et al.* (1997) for the culture of sea bass larvae, at 2 cm/s. Different water velocities have been reported from the periphery to the centre of the rearing tanks

(Divanach et al., 1997).In the present trial velocity seems to be uniform inside the rearing tank, as lime dispersion was homogenous.

Another evidence of water velocity within acceptable ranges for larvae was the fact that there were no skeletal deformities or slow growth of larvae (J. Roo et al., 2010).

The two methods used to determine water velocity, seemed suited for the mesocosm environment. The use of PTV (J. Oca et al., 2004), proved to be a good method to determine velocity. The use of dissolving substances allows the evaluation of dispersion simultaneous with velocity. There are some disadvantages of using this method in the rearing environment due to characteristics of the tank. The size of the tank requires high amounts of particles in order to reach the total diameter of the tank, as followed in our method.

The observation of the dispersion of lime in the water column was not possible due to the non-existence of transparent areas on the tank wall. Placing an underwater a camera in the rearing tank could be tested, although it would be difficult to establish a reference point in the water column.

5.2.2.pH

The average pH of this work (8.6 ±0.09) is within the tolerance range of S. aurata, which is situated between 4.5 and 9 (Parra & Yufera, 2002).

pH was stable in the present trial, while in the trial of Cañavate *et. al.* (2001) pH presented a small decrease over time. Comparison between trials is difficult due to the different methodologies used (intensive versus semi-intensive).

According to Parra *et al.* (2002), *S. aurata* larvae is more tolerant to low than to high pH values.

5.2.3 Salinity

Salinity was within the range of acceptable values for *S. aurata* development and survival (PILLAY & KUTTY, 2005). *S. aurata* presents a wide capacity to survive in different salinities (Bodinier et al., 2010), but presents better growth at 25 ‰ (Boeuf & Payan, 2001).

Salinity was similar to values registered previously in the same facilities(C.A.P. Andrade, Nogueira, Silva, Dinis, & Narciso, In press).

5.2.4 Temperature

Registered temperature (17ºC to 22ºC) was within the optimal range of temperatures for the development of S. aurata, which is 16-22ºC (Polo et al., 1991)

Temperature was stable in the rearing environment and displayed the same pattern as sea water inlet. As it was expected for the season (May), water temperature increased naturally during the rearing period.

Temperature presented a slight stratification, but not significant, in a very narrow range of values. The stratification pattern changed in days 21 and 35 dah. This change could be related with the increasing water exchange rate and with the higher amplitude of water temperature between the water in the rearing tank and water inlet.

5.2.5 Dissolved Oxygen

Dissolved oxygen values were within the average for *S. aurata* larvae, 6.4-8.2 mg/L (Navarro & Sarasquete, 1998). Dissolved oxygen values presented a high variation in terms of depth and sampling point, along the rearing period.

In a separate analysis of the different rearing phases the dissolve oxygen decreased as it was expected due to the larvae higher oxygen consumption. The central part of the tank presented higher oxygen levels, due to the presence of aerator and higher water renewal as evidenced by the higher water velocity in this area.

The self-cleaning nature of the tank allows the concentration of wastes in the central bottom of the tank and microbial life increases the consumption of oxygen (J. Oca et al., 2004).

5.2.6 Light

Light is one of the parameters that presented higher variation during the culture trial. The facility has a cover that despite not obstructing totally the light does alter the distribution of sunlight in the tank area. The roof above the tank is opaque, while the areas surrounding it are transparent.

This parameter high variation is due to seasonal and daily position of natural light source in relation to the rearing tank. Larvae require a minimum of light of 50 -150 lux to begin feeding, in order to be able to better visualize

feed (Boeuf & Le Bail, 1999), and values inside the rearing tank were always above the light requirements.

The distribution pattern of light intensity is related to the position of the artificial light and is similar to observed by Yoseda *et al.* (2009) and Naas *et al.* (1996). However, neither Yoseda *et al*. (2009) nor Naas *et al.* (1996) artificial light source was similar to the one used by at CMC. Naas *et al*. (1996) used light all around and parallel to the wall of the rearing tank, and Yoseda *et al.* (2009) tested different light sources positions in the central area of the tank.

Light intensity weakens as it reaches the sides and bottom of the tank. The presence of algae is bound to affect light dispersion (Boeuf & Le Bail, 1999), and this was demonstrated by the high dispersion pattern during this phase, particularly from 3- 23 dah.

Light distribution at the surface of the tank presented a great variation, due to the influence of sun light.

6. Conclusions

When we first began this work several questions needed to be addressed in order to describe the major abiotic and biotic parameters influencing *S. aurata* larvae performance and distribution in mesocosm tanks.

The homogeneity of abiotic and biotic water parameters in the large mesocosm tank will depend on the parameter analysed. Temperature, salinity and pH have an even distribution in the entire tank. In opposition, the light is dependent on the position of light source and dissolved oxygen depends on water renewal rate and aerators positions.

Considering the group of biotic and abiotic parameters studied there were little variations inside the rearing system, therefore it can be concluded there is a single environment.

The current methodologies in use for water quality control and others developed in the course of this study do provide capable answers, allowing the analysis of the selected parameters. However, methodologies can be improved for better accuracy with minor adjustments, e.g., the size of the bottom square used to measure mortality, could be complemented with more sampling stations or increase of the size of the square. It also appears necessary to improve the siphoning method. At one point no data was gathered since the high algae concentration did not allow the visualization of tank bottom.

Larvae do not seem to be affected by the rearing environment, with the exception of the influence of light during the early stages of larval development. Larval behaviour is persistent during the entire rearing period, suggesting larvae were in the absence of stressful environmental factors.

The size of the rearing tank is the first obstacle to overcome in a monitoring programme. It is not possible to visualize the entire tank volume from one single point. In fact, for most of the rearing period the central area is “invisible” territory due to long distance from the margin and the dark colour of the tank.

The methods used in this work are easy to reproduce and allow the easy comparison with other studies.

One of the limitations that can be reported to this work is the lack of duplicates. However, the tank in the present study has a big volume, with

particular entail in several parameters. Other studies using mesocosm often provide vague data and less environmental parameters. The gathering of data of one single tank was time consuming, duplicates or triplicates, despite enriching the present work would not allow for such a complete analysis.

There are few works published concerning the importance of the abiotic and biotic parameters on larval development, as well as on mesocosm of semi-intensive methodologies. In addition there is a need to establish standard protocols, in order to facilitate comparison among larval rearing methodologies (Villamizar *et al*. 2011).

It is expected that this work may contributed to increase the knowledge of the mesocosm of semi-intensive methodologies. In future works it would be interesting to deepen the relation between the abiotic and biotic parameters and larvae biology, for *S. aurata* and other species.

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