João Filipe Macedo Pereira

Internship in Safiestela S.A.: Implementation of Fish Welfare Assurance System (FWAS) and Effect of light spectrum in the larvae development of the *Solea senegalensis*

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

Orientador – Prof. Doutor José Fernando Magalhães Gonçalves
Categoria – Professor auxiliar
Afiliação – Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

Co-orientadora – Drª. Renata Maria Neto Serradeiro
Categoria – Administradora da AQUACRIA PISCÍCOLAS, S.A., Diretora Geral & de ID da AQUACRIA PISCÍCOLAS, S.A e da SAFIESTELA, S.A.
Afiliação – Sea8.

Dedico esta tese aos meus pais, à minha irmã, à minha namorada e a todos os meus amigos.

Agradecimentos:

A toda a equipa da Sea8, em especial ao pessoal da Safiestela S.A. por me terem recebido tão bem, são pessoas fantásticas e que me integraram melhor do que poderia imaginar.

Um especial agradecimento ao Prof. Doutor José Fernando e à Drª. Renata Serradeiro por terem aceite este desafio e por me terem realmente orientado durante este tempo (espero não ter sido muito difícil). Esta etapa foi uma das que nunca me arrependerei de ter ingressado, pois, com esta experiência, consegui crescer a nível académico e pessoal.

Ao Dr. JW (Hans) van de Vis que foi de grande ajuda em momentos mais difíceis deste projeto.

Aos meus colegas, e acima de tudo, amigos, que me acompanharam durante este ano: Filipe Furtado, Luis Baião e Tiago Sá. Sem o vosso poderia ter feito o mesmo, mas não era a mesma coisa.

À minha namorada, Rafaela Santos, que me acompanhou e aturou, e muito, durante esta etapa. Ajudaste-me a encontrar o rumo deste-me a estabilidade emocional necessária para realizar este trabalho.

Por último, mas não menos importante, agradeço aos meus pais e à minha irmã que me apoiaram para que pudesse alcançar este patamar e que me ajudaram a pensar de outra forma para ultrapassar problemas encontrados nesta etapa, espero ter-vos deixado orgulhosos.

Resumo

Atualmente, e cada vez mais, a sociedade preocupa-se com as condições de bem-estar animal em produção, com o aumento da aquacultura esta preocupação é cada vez mais direcionada para este sector. Devido a isso, existem agora autoridades especializadas em bem-estar animal em produção, que criam certificações que levam a uma vantagem competitiva. Tais vantagens levaram a que a direção da Safiestela, S.A. estivesse interessada na implementação de um plano *Fish Welfare Assurance System (FWAS)*, com o intuito de melhorar as condições de produção para salvaguardar o bem-estar animal, este plano irá ajudar a instalação na obtenção de uma futura certificação para o bem-estar e na competição em mercados internacionais.

A aquacultura de *Solea senegalensis* é algo relativamente recente. Vários estudos foram realizados, e outros ainda estão em progresso, para ultrapassar alguns desafios na produção desta espécie. Neste contexto, o desenvolvimento larvar desta espécie é um destes desafios. Devido a tal parte deste trabalho foi focado no experimento de novas condições de produção, para tentar melhorar o desenvolvimento larvar do linguado-do-Senegal. Assim, neste trabalho foram realizados 2 experimentos, para tentar avaliar os efeitos de 2 espectros diferentes (um usado conforme os protocolos de desenvolvimento larvar da Sea8 e outro com um comprimento de onda de 435-500 nm, que corresponde à luz azul) no desenvolvimento larvar.

Resultados deste primeiro experimento não foram conclusivos, no entanto alguns parâmetros diferiram significativamente ($p<0,05$) entre grupos: despigmentação, peso seco, estádio da metamorfose e intensidade luminosa. Em relação ás taxas de despigmentação, o grupo de tratamento apresentou valores mais altos (41%), comparativamente com o grupo de controlo (11%). Outro parâmetro que diferiu entre grupos foi o peso seco; que foi maior nos grupos de tratamento (0,241 mg e 0,747 mg no 7º e 12º dia após eclosão, respectivamente) comparativamente com o grupo de controlo (0,205 mg e 0,671 mg no 7º e 12º dia após eclosão, respectivamente). Foi também avaliado o estádio de metamorfose; as larvas do grupo de tratamento apresentaram um desenvolvimento mais rápido (o estádio 1 foi observável no segundo dia da experiência, 8% das larvas, enquanto que no grupo de controlo este estádio foi apenas observável no sexto dia da experiência, 10% das larvas). Em relação à intensidade luminosa, o grupo de tratamento apresentou menores valores (621 lux, em média), comparando com o grupo de controlo (média de 1027 lux, em média).

Os resultados do segundo experimento mostraram que alguns parâmetros ainda apresentavam diferenças significativas ($p<0,05$) entre grupos (intensidade luminosa e despigmentação). A intensidade luminosa continuou a ser menor no grupo de tratamento (760 lux, em média), comparativamente com o grupo de controlo (1167 lux, em média), apesar dos melhoramentos do protocolo. Em relação à despigmentação, este parâmetro apresentou maiores percentagens no grupo de tratamento (0%, 80% e 50% no 2º, 5º e 12º dia após eclosão, respectivamente), enquanto que o grupo de controlo as percentagens foram significativamente menores (2,5%, 15% e 7,5% no 2º, 5º e 12º dia após eclosão, respectivamente).

Estes experimentos revelaram que a luz azul pode melhorar as condições em cativeiro, no entanto não há evidências de que, sob condições de produção, o desenvolvimento larvar seja melhorado.

Ao longo destes trabalhos, havia também o objetivo de realizar um estágio nesta instalação, com a finalidade de perceber como é o trabalho rotineiro numa aquacultura.

Abstract

Currently, the society is concerned about the animal welfare conditions in production. This problematic is increasingly more focused on aquaculture. Due to that, there are now authorities specialized in animal welfare in production, these create certifications that potentially lead to a competitive advantage. Such advantages led the direction of Safiestela, S.A. to be interested in the implementation of a Fish Welfare Assurance System (FWAS) plan, in order to improve the production conditions, to safeguard the animal welfare. This plan will help the facility to acquire a future welfare certification and compete in international markets.

The aquaculture of *Solea senegalensis* is relatively recent. Various works were done, and others are still in progress, in order to surpass some challenges in the production of this species. In this context, one of the production challenges is the larvae development. Consequently, part of this work was focused in experiment new holding conditions. In order to attempt to improve the larvae development; 2 experimental works were conducted, in order to evaluate the effects of 2 different light spectrums (one used according to the Sea8 protocols and another with a wavelength of 435-500 nm that correspond to a blue light) in the larvae development.

Results of this preliminary experiment were not conclusive, although some parameters significantly differ ($p<0.05$) between groups: depigmentation, dry weight, metamorphosis stage and light intensity. Regarding the depigmentation rates, the treatment group presented higher values (41%) comparatively with the control group (11%). Another parameter that differed between groups was the dry weight, that was greater in the treatment groups (0.241 mg and 0.747 mg in the 7 and 12 dph, respectively), comparatively with the control group (0.205 mg and 0.671 mg on the 7 and

12 dph, respectively). Was also assessed the metamorphosis stage: the larvae from the treatment group presented faster development (the stage 1 was observable on the second day of experiment, in 8% of the larvae, while this stage in the control group only was observable on the sixth day of experiment, in 10% of the larvae). Regarding the light intensity, the treatment group presented lower values (average of 621 lux), comparing to the control group (average of 1027 lux).

The results from the second experiment showed that some parameters presented significant differences ($p<0.05$) between groups (light intensity and depigmentation). The light intensity continued to be lower in the treatment groups (760 lux), comparatively with the control group (mean of 1167 lux), despite the protocols improvements. Regarding the depigmentation, this parameter presented higher percentages in the treatment group (0%, 80%, and 50% in the 2, 7 and 12 dph, respectively), while in the control group these values were significantly lower (2.5%, 15% and 7.5% in the 2, 7 and 12 dph, respectively).

These experiments revealed that the blue light can improve the captivity conditions, however, there is no evidence that, under production conditions, the larval development is ameliorated.

Along with these works, there was also the objective to realize an internship in this facility, in order to integrate the routine work in an aquaculture.

Index

List of Figures

List of tables

List of Abbreviations

DOM – Dissolve organic matter

FAA - Food anticipatory activity

FCR - Feed conversion ratio

FWAS - Fish Welfare Assurance System

GAP - Good aquaculture practices

HACCP - Hazards analysis and critical control points

INE - Instituto Nacional de Estatística

OWI - Operational welfare indicator

POM – Particulate organic matter

RAS - Recirculating aquaculture system

RSPCA - Royal Society for the Prevention of Cruelty to Animals

TL - Total length

TMC - Tropical Marine Center

UV - Ultraviolet

Chapter 1: Introduction

1.1 State of world aquaculture

For the past five decades, the production of aquatic organisms has been expanding due to the increase of demand for such products, but also due the decrease of wild stocks among the planet.

In 2012, the aquaculture production was 90.4 million tons (figure 1); in that amount, 66.6 million tons correspond to fish production and 23.8 million tons to algae (FAO, 2014). Regarding the variety of species produced worldwide, in total there are 567. Of these, 20 species represent 74% of the whole production. Similarly of what happened with the terrestrial animals production, it is expected that the aquaculture production narrows to a limited number of species (Bostock et al., 2010, Diana et al., 2013, FAO, 2014).

In 2007, the production in aquaculture handled 43% of the consumption of aquatic products, and, currently, it is expected thatthis percentage exceed 50% (Bostock et al., 2010, Volpe et al., 2013, FAO, 2014).

In 2012, the worldwide average consumption *per capita* of such products was about 19.2 kg while, in 1960 these value was 9.9 kg (Volpe et al., 2013, FAO, 2014).

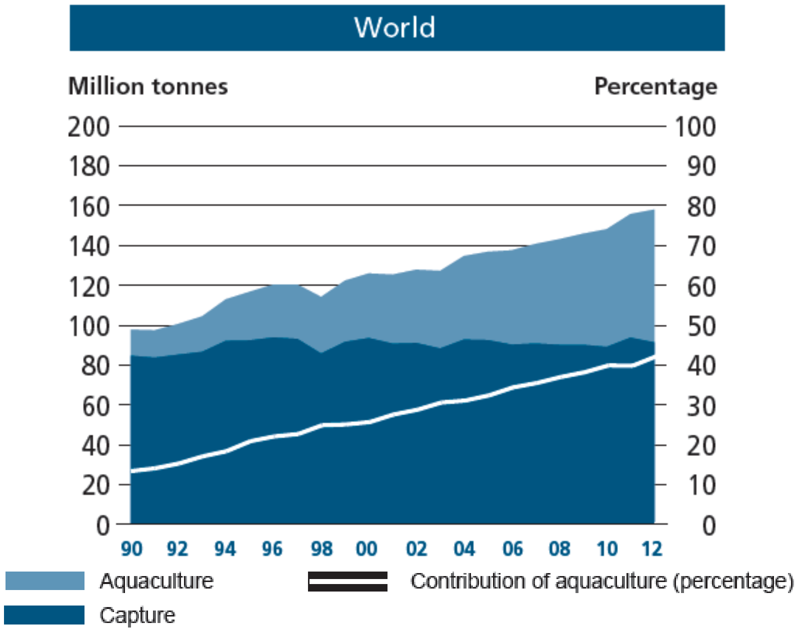


Figure 1 - World aquaculture production between 1990 and 2012 (from FAO 2014).

According to FAO (2014), the larger aquaculture producer in 2012 was China; this country alone produced 42 million tons, which correspond to 88% of the entire production of the Asia continent and 62% of the world (table 1). Although, part of China aquaculture production is for exportation, internal demand is increasing. When compared the average consumption, *per capita*, of these products in 1990 (13.4 kg) and 2010 (35.1 kg) it is noticeable this tendency.

This information shows that the production is not balanced among the globe and that inequity create instability in economic and environment systems.

In table 1 it is described the aquaculture production of continents and it is observable that China production alone is higher than Europe, Africa and North America combined. Nonetheless, the region with the higher increase, among 2000 and 2012, was Africa with 11.7%; this percentage is greater than 5.5% from China in the same period. These last values can be interpreted as the stability of China production and the increase of investment in Africa in this activity (Bostock et al., 2010, Volpe et al., 2013).

Despite the general rise in aquaculture production, some countries reported opposite trends, such as Spain, United States of America, Japan and France. One of the causes of this is due to the inability of these markets to compete with others, which provide cheaper products due to low-cost labour (Volpe et al., 2013). Another possible cause for this decrease in production may be due to punctual problems. Such as

Table 1- Aquaculture production in main countries and continents (from FAO 2014).

Selected groups and countries		1990	1995	2000	2005	2010	2012
Africa	**(tonnes)**	**81 015**	**110 292**	**399 688**	**646 182**	**1 286 591**	**1 485 367**
	(percentage)	***0.62***	***0.45***	***1.23***	***1.46***	***2.18***	***2.23***
North Africa	(tonnes)	63 831	75 316	343 986	545 217	928 530	1 030 675
	(percentage)	*0.49*	*0.31*	*1.06*	*1.23*	*1.57*	*1.55*
Sub-Saharan Africa	(tonnes)	17 184	34 976	55 702	100 965	358 062	454 691
	(percentage)	*0.13*	*0.14*	*0.17*	*0.23*	*0.61*	*0.68*
Americas	**(tonnes)**	**548 479**	**919 571**	**1 423 433**	**2 176 740**	**2 581 089**	**3 187 319**
	(percentage)	***4.19***	***3.77***	***4.39***	***4.91***	***4.37***	***4.78***
Caribbean	(tonnes)	12 169	28 260	39 704	29 790	37 301	28 736
	(percentage)	*0.09*	*0.12*	*0.12*	*0.07*	*0.06*	*0.04*
Latin America	(tonnes)	179 367	412 650	799 234	1 478 443	1 885 965	2 565 107
	(percentage)	*1.37*	*1.69*	*2.47*	*3.34*	*3.19*	*3.85*
North America	(tonnes)	356 943	478 661	584 495	668 507	657 823	593 476
	(percentage)	*2.73*	*1.96*	*1.80*	*1.51*	*1.11*	*0.89*
Asia	**(tonnes)**	**10 801 531**	**21 677 062**	**28 420 611**	**39 185 417**	**52 436 025**	**58 895 736**
	(percentage)	***82.61***	***88.90***	***87.67***	***88.46***	***88.82***	***88.39***
China	(tonnes)	6 482 402	15 855 653	21 522 095	28 120 690	36 734 215	41 108 306
	(percentage)	*49.58*	*65.03*	*66.39*	*63.48*	*62.22*	*61.69*
Central and Western Asia	(tonnes)	72 164	65 602	122 828	190 654	259 781	311 133
	(percentage)	*0.55*	*0.27*	*0.38*	*0.43*	*0.44*	*0.47*
Southern and Eastern Asia (excluding China)	(tonnes)	4 246 965	5 755 807	6 775 688	10 874 073	15 442 028	17 476 296
	(percentage)	*32.48*	*23.61*	*20.90*	*24.55*	*26.16*	*26.23*
Europe	**(tonnes)**	**1 601 649**	**1 581 359**	**2 052 567**	**2 137 340**	**2 548 094**	**2 880 641**
	(percentage)	***12.25***	***6.49***	***6.33***	***4.83***	***4.32***	***4.32***
European Union (Member Organization) (28)	(tonnes)	1 033 857	1 182 098	1 400 667	1 269 958	1 280 236	1 259 971
	(percentage)	*7.91*	*4.85*	*4.32*	*2.87*	*2.17*	*1.89*
Other European countries	(tonnes)	567 792	399 261	651 900	867 382	1 267 858	1 620 670
	(percentage)	*4.34*	*1.64*	*2.01*	*1.96*	*2.15*	*2.43*
Oceania	**(tonnes)**	**42 005**	**94 238**	**121 482**	**151 466**	**185 617**	**184 191**
	(percentage)	***0.32***	***0.39***	***0.37***	***0.34***	***0.31***	***0.28***
World	**(tonnes)**	**13 074 679**	**24 382 522**	**32 417 781**	**44 297 145**	**59 037 416**	**66 633 253**

pathogen outbreaks or the closure of establishments; nonetheless, these causes are momentary, and the production numbers can recover in the future.

The global growth rate of aquaculture, between 2000 and 2012, was 6.2% per year, which is lower than the 10.8% in the period of 1980 to 1990. This trend is expected, since aquaculture production have a tendency to stabilize (Bostock et al., 2010, Volpe et al., 2013).

1.2 Aquaculture in Portugal

Globally aquaculture is conditioned by a group of factors and their implications. These are: (1) existent technology; countries or regions with scarce resources are unable to produce at the same tecnological leve, when compared with more resourceful countries; (2) institutional system; since aquaculture is a novelty in some countries and, this system may present lacunas; (3) environmental conditions; the production will be less expensive and simplest if the species are comfortable with the range of environment parameters existing in the region; (4) human resources; in some areas the specialized manpower is scarce; (5) the market demand; which is different according to the region, since each region have its traditions and preferences regarding to the consumption of aquatic products.

In Europe, the aquaculture has been challenged in the last years, and that affected the production, some countries showed a decrease in their productivity (such as Spain and France). This summarize the incapability of Europe to compete with another markets (figure 2) (Bostock et al., 2010, Volpe et al., 2013, FAO, 2014, Troell et al., 2014).

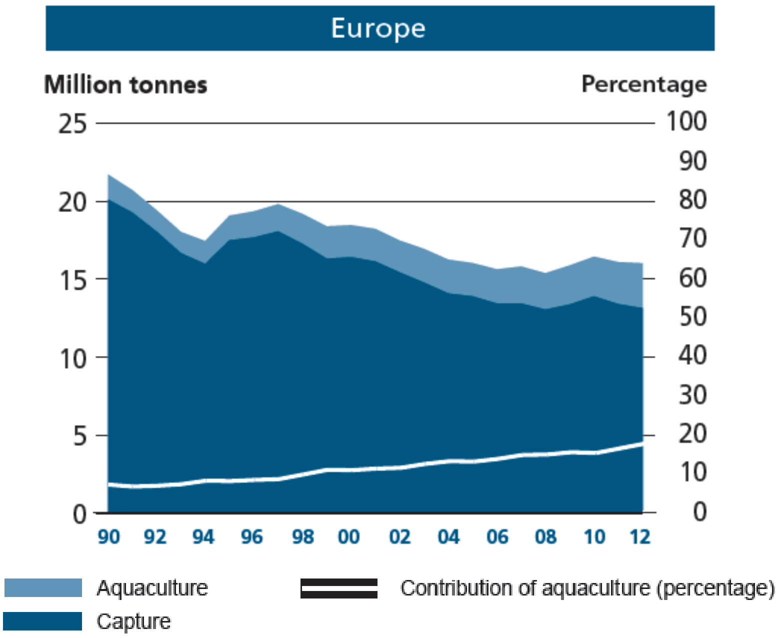


Figure 2 -European aquaculture production between 1990 and 2012 (FAO 2014).

The socio-economic crisis that Europe is going through affected many activities, including aquaculture. In the Portugal case, this crisis has been particularly severe, and as consequence, the aquaculture production decreased. According to INE (Instituto Nacional de Estatística) in 2013 the aquaculture sector decreased 9.0%, comparing with the year before (figure 3), this decline was due to the reduction in *Scophthalmus maximus* production (-46.6%). This reduction produced an opportunity in the market for other species, such as sole. Nonetheless, the value of the aquaculture products presented an increase of 3.1% (figure 3).

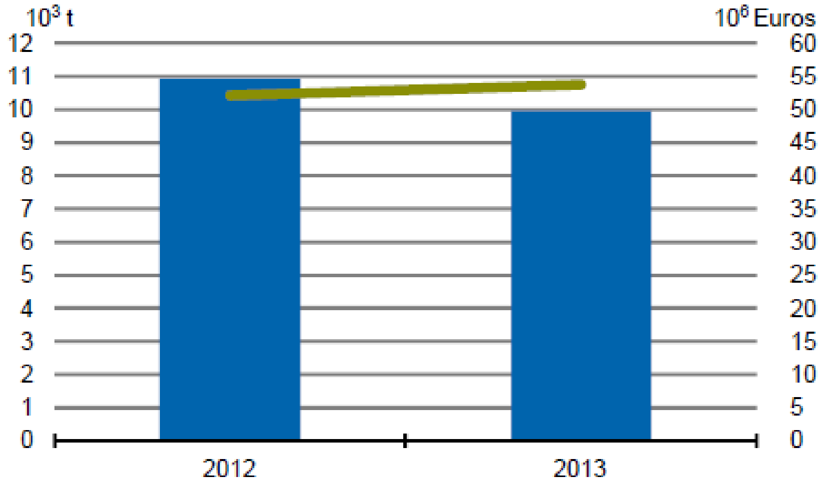


Figure 3 - Portugal aquaculture in 2012 and 2013 (INE, 2014).

The Portuguese aquaculture has a significant focus on marine and brackish species. The main fish species produced in 2012 were turbot (*Scophthalmus maximus)* and gilthead sea bream (*Sparus aurata*), representing 85% of the entire national production in marine and brackish waters.

Half of aquaculture production in Portugal is due to molluscs, where clam (*Ruditapes decussatus*) and mussel (*Mytilus edulis*) are the most produced species.

Regarding the fresh water production, in 2013 and 2012 Portugal only produced one species, rainbow trout (*Oncorhynchus mykiss)* (INE, 2014).

1.2.1 Establishments and productions systems

Comparing 2013 with the previous year, aquaculture facilities in Portugal increased to a total of 1,522. This increase led to a rise of area used for this sector of 62.6%, this significant increase was also due to the authorization for offshore production.

The majority of the facilities (88.2%) are intended for the production of molluscs. Regarding the fish production, only 9.2% of the establishments were intended for that purpose.

Concerning to the type of production system used in Portugal, in marine and brackish waters 54.7% of the facilities produce in extensive systems while 34.7% of the facilities operate in the intensive system. The entire fresh water production comes from intensive production systems (figure 4) (INE, 2014).

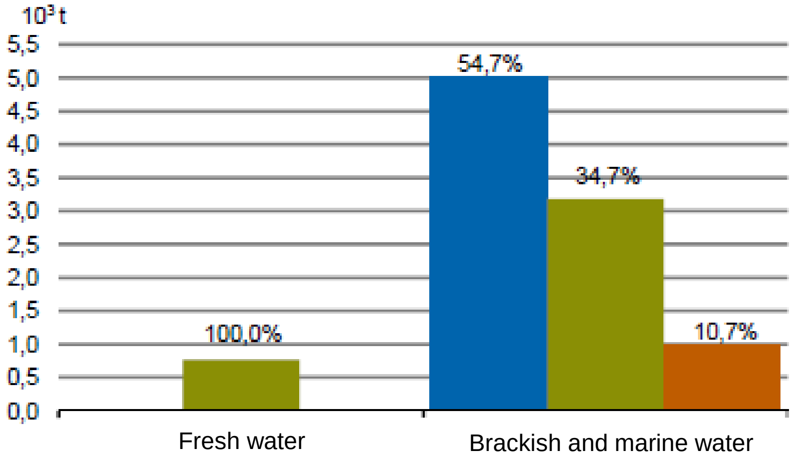


Figure 4 - Aquaculture production according to the production system and type of water. (INE, 2014).

1.3 Animal welfare in aquaculture

The increase of production in aquaculture sector is higher than any other animal production (FAO, 2014). In recent years,The rise in production was followed by the rise of some concerns, namely, the conditions of culture and, consequently, the animal welfare. (Frewer et al., 2005, Huntingford et al., 2006, Ingenbleek et al., 2012, van de Vis et al., 2012, Bovenkerk and Meijboom, 2013, Huntingford and Kadri, 2014, Mellor, 2014, Mustapha, 2014).

There are now authorities that inspect these aspects in different kinds of production, including aquaculture. One example, in the United Kingdom, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) (King, 2009, Bovenkerk and Meijboom, 2013). This type of associations promote actions amongst producers, with the objective of improving the animal welfare in production. Additionally, the general public is also creating, increasingly, more pressure in this sector, due to the increase about this ethical concern. Nowadays, in some cases, people prefer to pay more for a product that is certified in welfare. This pressure from the public made appear the certification processes in aquaculture, namely in countries in the North of Europe (Diana et al., 2013, Harvey and Hubbard, 2013, Ingenbleek et al., 2013, Rossi and Garner, 2014).

The European population is one of the most aware about the animal welfare, in particular, the people from United Kingdom and Nordic countries. Thus, it is most likely that this kind of certification processes will also appear in other countries as well, such as Portugal.

Though, the certification process requires certain points. Firstly it is necessary to define what welfare is, how to measure it, how to improve the culture conditions and also how to improve practices for each species production (van de Vis et al., 2012, Ingenbleek et al., 2013, Rossi and Garner, 2014, Colson et al., 2015, Ellingsen et al., 2015).

1.3.1 Definition of animal welfare

In fish farming the welfare is complicated to define; this concept creates complex questions between normative and ethical actions. Fish welfare can be defined in different ways, depending on the perspectives (function-based, feeling-based and nature-based) (van de Vis et al., 2012, Bovenkerk and Meijboom, 2013, Huntingford and Kadri, 2014).

The definition of welfare has changed through the time: firstly, the welfare was safeguarded if the physiological equilibrium of the animal was reached. However recently this concept has been defined in numerous ways. One of the most used references to measure animal welfare in production is through the five freedoms (freedom from hunger, freedom from pain, disease or injury, freedom from discomfort, freedom from fear and stress and freedom to express their natural behavior) of the Farm Animal Welfare Council (Bovenkerk and Meijboom, 2013). The majority of the authorities use this definition; however, there are authors who claim that this definition is too static and cannot measure the welfare effectively in all cases (McEwen and Wingfield, 2003, Bovenkerk and Meijboom, 2013). The five freedoms concept assume that welfare can only be assessed if the production guarantees all the freedoms. However in some authors perspective the freedoms can be antagonistic. In the example used in Ohl and van der Staay (2012), it is explained that a natural behavior, such as exploring the environment can implicate stress in the animal, and not all of the five freedoms statements can be assured at the same time. Such critics has conducted to the appearance of a new concept, allostasis (McEwen and Wingfield, 2003, Schreck, 2010, Ohl and van der Staay, 2012, Turnbull and Huntingford, 2012). In this concept, the main modification in the definition of welfare was, the capacity to change and adapt to new conditions.

Accordingly to Fraser (2003), there are mainly 3 ways to define animal welfare, these are: (1) function-based (the main question of this view is if the fish can adapt to the farming conditions); (2) feeling-based (in this perspective the central bottleneck is if the fish have feelings, assuming that they have, the welfare can only be achieved if the feelings of the fish are take into account, in order to avoid any distress or pain to the animal); (3) nature-based (in this view, welfare of fish only can exist if they can express their natural behavior).

Other complex questions are implicated in the definition of welfare in fish, such as: What is the natural behavior? This issue is highly difficult to answer, since in animal production, usually, there is sustainability. In other words, the animals in production come from selected broodstock; this selection of specific traits can indirectly or directly change the animal behavior through generations (Teletchea and Fontaine, 2014). According to Bovenkerk and Meijboom (2013), in 2009 it was conducted an inquiry, by Wageningen UR Livestock Research, in order to know the opinion of part of the population related to cow production. The conclusion of this investigation was that, for the general opinion the best way to produce cow was in open field, however welfare specialists defend that indoor production can promote better welfare. At least in some matters, for example in this type of production, the pathogenic spread is highly controlled, in relation to outdoors production. Another point defended by the specialists, is that the cows are domesticated animals and the definition of what is their natural behavior can be different from the general assumption.

These inconsistencies in the definition of animal welfare are complicated in terrestrial animal productions but in fish production are even more complex. The knowledge regarding to this group of animals presents many gaps, for example in *Solea senegalensis* the optimal parameters in culture are not all defined. Despite of that, the culture of this species is already undergoing, and many researchers efforts have being done in the recent years (Villamizar et al., 2011, van de Vis et al., 2012).

In conclusion, there are several definitions of what welfare is, and there are mainly three ways to analyze it: function-base, emotion-base, and nature-base.

The other major problem is how welfare should be quantified objectively. Generally in aquaculture is performed the register some parameters that could affect the fish welfare, such as: water quality parameters and animals numbers with tissue damages (van de Vis et al., 2012). However, the fish farmers only observe the effects of poor welfare conditions and not the cause, so in this matter it is important to act before the appearance of side effects. To achieve this, it is important to implement a system that safeguard the fish welfare and promote preventive actions (van de Vis et al., 2012).

In this work, it was tried to take into account all these perspectives to implement a Fish Welfare Assurance System (FWAS), to improve animal welfare in Safiestela S.A. This system will be explained in detail further in this document.

1.4 Welfare and husbandry

Nowadays, the concept of animal welfare is increasingly associated with the production of animals for consumption, but in many cases it is targeted to mammalians, such as pig farming and cow farming. Nonetheless, in the past decade studies concerning about this thematic in fish farming, appeared. This fact is caused by the increase of this sector in meat production and because of the popularization of aquaculture in general (Frewer et al., 2005, Mesquita, 2011).

On the other hand, pressure of the public to apply husbandry systems that improve animal welfare has been caused by the increase of ethical and moral concerns; either due to the higher educational qualifications, either by the increasing in the access to information (Ingenbleek et al., 2013, Van Loo et al., 2014). This pressure is reflected at economic level, thus, a new market demand appeared (particularly in North of Europe), which is, products that are certified in animal welfare (Frewer et al., 2005, Ingenbleek et al., 2012, Ingenbleek et al., 2013, Mellor, 2014) .

In fish farming, there are routines that can compromise the animal welfare (transport, slaughter, grading). To mitigate this, it is important to know the species requirements and natural behaviors. In extensive productions, usually, a welfare friendly husbandry system exists (Håstein et al., 2005, Håstein, 2007, Roberts et al., 2008).

Husbandry systems that promote welfare are being studied, planned and implemented in intensive aquaculture. One example of that is the relatively new system to improve animal welfare in aquaculture, the FWAS plan. This is an ongoing plan that can be applied in any aquaculture, to reduce and eliminate various problems that affect the welfare of fish, such as in the slaughter process, the environmental conditions and stockmanship (van de Vis et al., 2012).

Improved husbandry systems in intensive aquaculture can be a challenge. The production methods have a strict dynamic and any minor alteration require a detailed study of the consequences in the production chain and profitability (Huntingford and Kadri, 2014).

In the literature, there are authors that defend that the concern about animal welfare shall come in the planning of the facility and with the choice of the species to produce. Since, different species have different requirements and behaviours, for example, there are species that are more resilient to handling than others, and it is more likely to produce these more resilient species (Jarman et al., 1976, Håstein, 2007, Roberts et al., 2008, Mesquita, 2011, Huntingford and Kadri, 2014).

Huntingford et al. (2006) describes a series of husbandry operations that can compromise the animal welfare in aquaculture facilities, these are: (1) Stocking densities;

(2) water quality; (3) photoperiod; (4) stockmanship; (5) transport; (6) disease and subsequent treatment; (7) slaughter; (8) food administration; and (9) crowding.

1.4.1 Rearing densities

In aquaculture, some operations can affect the production and the animal welfare, as described above. Thus, stocking density requires a well-established management plan, to maximize the profitability and to maintain the welfare conditions in acceptable levels.

This thematic has been studied in a number of species (salmonids, turbot, and Senegalese sole, and Atlantic cod) (Iguchi et al., 2003, van de Nieuwegiessen et al., 2009, Wunderink et al., 2011, Sánchez et al., 2013, Villanueva et al., 2013, Creţu et al., 2014, Andrade et al., 2015, Menezes et al., 2015). The effects of rearing densities are different in accordance with the species, the phase of the life cycle, season, type of exploration and type of water circulating system. In extensive and semi-intensive aquacultures the stocking densities, usually, do not affect negatively neither growth rate, neither welfare. That's because these type of aquaculture systems depends on environmental conditions, favors the natural behavior of animals, and the rearing densities are quite low when compared with intensive systems.

On the other hand, intensive aquaculture, by definition, is a type of production that has high stock densities and tend to control the production parameters. For instance, in the recirculating aquaculture system (RAS), it is possible to manipulate several water parameters, such as temperature and oxygen, required for the production. On the contrary, in flow through less parameters can be manipulated. These different systems l influence the rearing densities (Zhang et al., 2011, Kolarevic et al., 2014, Colson et al., 2015).

Regarding stock density in fish farming, no legislation determine the minimum area that each animal requires, which could generate unhealthy husbandry conditions. Nevertheless, the profitability of an aquaculture, namely in those that use the intensive exploration, is strictly connected to the proper husbandry conditions. Stocking densities correlate with many of these conditions, so it is imperative to define the proper rearing density. Thus, is in the best interest of the producer to improve the holding conditions (Iguchi et al., 2003, Saillant et al., 2003, Richard and Flemming, 2007, Andrade et al., 2015).

According to the species and to the life stage, there must be a careful plan, based on investigation studies, to implement the rearing density. In literature, it is described species that are not affected by high stocking densities and other species that prefer lower stocking densities (Daniels et al., 1996, Iguchi et al., 2003, Saillant et al., 2003, Turnbull et al., 2005, Salas-Leiton et al., 2011, Luo et al., 2013, Andrade et al., 2015, Menezes et al., 2015). Commonly high rearing densities are connected to detrimental welfare. However some studies prove that, depending on the species and life stage, high rearing densities improve the animal welfare. For example, in juveniles of African catfish high stocking densities improve welfare conditions, which is reflected by the increase in growth rate and decrease in aggression between specimens (van de Nieuwegiessen et al., 2009). Another species that do not appear to be damaged with high stocking densities is the Senegalese sole (Sánchez et al., 2010, Wunderink et al., 2011, Sánchez et al., 2013, Andrade et al., 2015). In table 2 there are some examples of species reared at different densities and their effects.

Table 2 -Examples of stocking densities studies of different species.

Species	Initial Densities	Measure	Conclusion
Senegalese sole *(Solea senegalensis)*	7, 17, 24 kg/m^2	Growth performance and stress hormones	High stocking densities did not affect growth performance or levels of stress hormones (Andrade et al., 2015).
Jade perch *(Scortum barcoo)*	120, 180, 270 fish/m^3	Physiological	Best performance at the density of 180 fish m^3 (Luo et al., 2013).
Ayu sweetfish *(Plecoglossus altivelis)*	100, 400, 1250 fish/m^3	Stress hormones	Moderate stocking densities are better for the production of this species (Iguchi et al., 2003).
Silver catfish *(Rhamdia quelen)*	8, 16, 32 kg/m^3	Physiological	The moderate and the higher density are more suitable for aquaculture of this species (Menezes et al., 2015).

1.4.2 Grading

Grading is also an operation that can compromise the productivity and the animal welfare. Nevertheless, this operation is fundamental in intensive aquaculture, since in high stocking densities, commonly found in these systems, there are an increasing competition for food between animals, spread of diseases, accidental (damage due to the contact with other individuals or with equipment), aggressiveness and space competition (Saillant et al., 2003, Qu et al., 2009, Overton et al., 2010, González et al., 2011, Petrović et al., 2011, Slavík et al., 2011, Daly et al., 2012, Barron et al., 2013). All of these problems can be ameliorated when a frequently grading processes is implemented, which regulate the size of the specimens within cages, tanks or ponds.

These consequently affect the profitability of the aquaculture (Daly et al., 2012, Barron et al., 2013). Another productivity benefit in grading is the size control in each compartment, this regulation allows to improve other husbandry operations, like feeding, disease treatment and transport. Grading also help the management of the facility to estimate more accurately the biomass in the production, since along with the grading process it is possible to recalculate the biomass that is going through that process (Saillant et al., 2003, Petrović et al., 2011, Barron et al., 2013, Lekang, 2013).

In this operation, disadvantages are also incremented, for instance, grading requires handling, this can cause damages along with stress issues, and this can lead to disease spread and consequently decrease in productivity (Qu et al., 2009, Slavík et al., 2011, Mustapha, 2014).

On the other hand, according to Slavík et al. (2011), energy consumption of graded European catfish were higher when they were exposed to unfamiliar individuals, than when they were exposed to familiar individuals. This can compromise not only the welfare but also the growth rate since the energy of the animal is used to other physiological processes than growth. This study concludes that to improve welfare in aquacultures, it is necessary to take into account the path and social connections of each species. In another study to improve animal welfare through grading, Barron et al. (2013) showed that in larvae of *Lota lota maculosa*, the survival in this life stage was increased with regular grading. The results of this study showed a survival of 74.3% when grading was performed and 59.3% when not graded. Salas-Leiton et al. (2010) conducted another experiment regarding how grading benefits depend on which species is analyzed. In this case the species studied was *Solea senegalensis* and it was concluded that the growth was higher when there was heterogeneity in sizes. This is explained due to social relationships and hierarchy in this species, this is, the size distribution is caused by the hierarchy established. This might suggest that it is necessary to change the way this species is produced, to improve profitability and the welfare.

In conclusion, these studies remark the importance and the dangers of husbandry operations. It is important to know the species requirements, to improve the animal welfare and also productivity.

1.4.3 Feeding

One of the significant bottlenecks in aquaculture of any species is the feeding. This operation not only require the knowledge of what are the feed requirements, but also to know the physiologic processes of each species (Mesquita, 2011, Boglino et al., 2012, López-Olmeda et al., 2012, Turnbull and Huntingford, 2012, Luo et al., 2013, Marinho et al., 2014, Trejchel et al., 2014).

This husbandry operation is highly related to welfare, since in farming conditions one of the first signs that farmers usually are aware, when the welfare is compromised, is the lack of feed intake by the animals or the increased amount of wasted feed. Thus, this operation, like other husbandry operations, is related and it is crucial to know how these relations work for each species production, in order to improve productivity and welfare conditions (Mesquita, 2011, Turnbull and Huntingford, 2012, Liu et al., 2014, Mustapha, 2014).

Feed intake is regulated by the hypothalamus; this portion of the brain is responsible for receiving and interpreting inner and outer stimulus and then transmit the appropriate response. These responses, include the regulation of hormone secretion, hormones that regulate hunger, temperature and others physiological processes (Kulczykowska and Sánchez-Vázquez, 2010).

Therefore, the endocrine system not only is controlled by internal stimulus but external ones also regulate it. Roughly, all species, including the ones in the aquaculture, are controlled by their circadian cycle. Thus, this cycle controls a series of behaviors, such as feeding rhythms. In practically all species, this particular behavior occurs only at day or at night, depending on the species. However in many species, the circadian cycle is flexible and can be modified in farming conditions through time, when environment conditions are manipulated (Kulczykowska and Sánchez-Vázquez, 2010, López-Olmeda et al., 2012, Ganzon-Naret, 2013, Liu et al., 2014). The food anticipatory activity (FAA) is a behavior that affect several species under farming conditions, this behavior require that the animal stays in aquaculture long enough to synchronize their cycle with the implemented feed regime (Kulczykowska and Sánchez-Vázquez, 2010).

In aquaculture, namely in intensive systems, the feed intake is conditioned by some husbandry operations, including the artificial feed. These operations can potentially interfere in the animal welfare, and consequently inhibit the feed intake and ultimately the productivity. On the other hand, the feed residues left in a tank, can potentially alter the water quality to a point which will be adverse to the animals health and welfare (Kulczykowska and Sánchez-Vázquez, 2010, Nilsson and Torgersen, 2010, Mesquita, 2011, López-Olmeda et al., 2012, Turnbull and Huntingford, 2012, Ganzon-Naret, 2013, Liu et al., 2014, Marinho et al., 2014, Bonaldo et al., 2015, Summerfelt et al., 2015).

In the majority of the aquaculture facilities, the manual feeding distribution is used because is cheaper, since it is required a minor investment than in automatic systems, although it requires more labour. This feeding system can be less effective, once it depends on the regularity of the distributions, and the amount of feed and the knowledge of the operator (Mesquita, 2011).

For the reasons mentioned above, more and more facilities are investing in automatic systems. These can deliver feed when the fish requires (on demand system) or when it is predicted by the management (timed releasing feeding system). This system demands an excellent management plan, since optimal feeding can be compromised by miscalculating the biomass of the tank, due to high dispersion of the fish sizes in the tank or an error in the weight sampling method. It adds that if the amount of feed deliver by this system is too high, this can cause the decrease of water quality and an increase in suspended solids, leading to a risk for the fish health. Despite those disadvantages, the automatic systems are less labour-expensive than the manual systems (Bostock et al., 2010, Nilsson and Torgersen, 2010, Mesquita, 2011, Atoum et al., 2015).

In some species it is known that external stimulus can influence the feeding behavior. Learning from other animals, such as mammals and birds, some of the farmers nowadays invest in self-demanding food systems. This alternative operates because of the learning capacity that some species present. For example, the experiment of Nilsson and Torgersen (2010) compared two groups of *Gadus morhua*, where all fishes were introduced to a trigger system but only one of the groups was rewarded with food. The results showed that, despite both groups presented high rates of contact with the trigger, at the beginning of the work, both groups decrease these rates. In the final, the group that were rewarded showed higher rate of contacts with the trigger. This kind of learning is known as operate conditioning. These self-demand systems, can potentially decrease the costs of aquaculture and improve the welfare. Since, the rhythm of feed intake is determined by the animal itself which consequently will decrease the wasted feed (Bostock et al., 2010, Nilsson and Torgersen, 2010, Atoum et al., 2015). Despite of the benefits of the self-demanding feed systems, in many facilities, the feed regime is done manually or automatically (time releasing feeding system), this is because not all species can be easily conditioned to use a self-demanding system and because it is less expensive to use one of this system instead of a self-demanding one.

In conclusion to improve the animal welfare in any aquaculture operation, it is important to know the how's, what's and when's of the feeding aspects.

1.4.4 Transportation of live fish

Transport is indispensable in aquaculture. However, this practice induces considerable stress in the animals. This operation allows that fish can be transferred between (external transport) and within (internal transport) production facilities, or between aquaculture and retailer, or even between aquacultures and rivers for restocking.

Transportation is seen as a welfare threatening during and after the operation, and is likely to observe a rise in mortality, lesions and deterioration of quality in the animals (Barton and Peter, 1982, Dobsikova et al., 2009, Iversen et al., 2009, King, 2009, Lekang, 2013, Mustapha, 2014, Tacchi et al., 2015). A lot has been done to control these consequences of transportation. Nowadays there are vehicles specialized in this type of operation, that ameliorate the conditions of transportation and in some countries legislation is applied, such as: fish densities ranges, design of the equipment involved and water quality parameters. These regulations are intended to protect the animal welfare in this delicate operation (Håstein, 2007, King, 2009, Lekang, 2013, FAO, 2014, Ignacio Martín and Rasines, 2014).

Before this operation, the animals pass through fasting that last, ate least, 24 hours, to reduce the metabolism. With this technique less oxygen is consumed and less metabolic wastes are release, this help to maintain for longer time the water parameters in acceptable levels. Another important requirement is the preparation of the fish for transport, to prevent, any pathology or other signals of debilitation in the animals (Dobsikova et al., 2009, King, 2009, Lekang, 2013, Mustapha, 2014).

In order to proceed to transport, the animals need to be graded, this operation, as described above, may induce stress. Due to that, the animals need to recover before transportation to prevent damages and even mortality during and after the operation (Lekang, 2013).

Although it is possible to do transportation without water (Ignacio Martín and Rasines, 2014), in many cases the transportation of aquatic animals requires the use of water. Thus, the water used in the vehicle or compartment is another critical point that must be taken into account for the animal welfare. Therefore, the water used for transportation must be the same as utilized in the aquaculture facilities, this will prevent stress in the fish. Despite that, it is important to reduce the water temperature, this, if not exaggerated, will benefit the animal welfare (Tang et al., 2009, Farrell et al., 2010, Lekang, 2013, Daniel et al., 2014). Lower temperatures decrease metabolism and consequently decrease the metabolic wastes and the oxygen consumption.

In the case of transport without requiring water, the animal skin must be moisted to ensure the animal welfare. Since the first barrier against pathogens is the mucous that covers the epidermis. This was studied in *Solea senegalensis* and it was concluded that it is safe to transport adult animals in a spatial time of 28 hours (Ignacio Martín and Rasines, 2014).

During transport, additives in the water can be used, these can prevent some complications during this operation. For example: salt (prevent osmoregulatory issues) and sodium chloride (prevent foam forming in fresh water) (Lekang, 2013, Tacchi et al., 2015). There are other compounds that can be used, in order to improve the welfare during transport and to preserve the freshness and quality of the animal, these are sedatives, like clove oil or oil of *Aloysia triphylla* (Iversen et al., 2009, Daniel et al., 2014).

There are different ways to transport live animals, these are: by land, by sea, by air, and there are other methods such as plastic bags, plastic containers and tractor-trailers(Lekang, 2013), however, for the interest in this work this last three methods will not be discussed any further.

In the case of air transportation, this type is rarely used, since the cost of the operation is quite high, usually, it is used for restocking or commercial purposes, but only when land and sea transportation are not applicable because of geographic difficulty to access to the destination area. In this case, may be used airplanes or helicopters and in both vehicles it is required the use of pure oxygen (Lekang, 2013).

The transport by land is the most common, can be done in trucks or another vehicle properly equipped. Normally, these vehicles have a tank or tanks that can be made of fiberglass, stainless steel or aluminum.

The air (especially for flatfish) and oxygen supply must be guaranteed, in order to protect the welfare. In this case the air can be provided by oxygen bottles attached to the vehicle or even by the air itself (Lekang, 2013, FAO, 2014). However, there are problems that need to be taken into account, such as: (1) air distribution, it is required a good air distribution to guarantee that the oxygen reach all animals; (2) air bubbles, if the air is distributed only from the bottom of the tank this will create an agglomerate of bubbles in that region, which will stress the fish, and consequently induce adverse effects during and after transportation, as mortality; (3) it is required an airlift pump or other equipment capable of distributing the air; (4) supersaturation with nitrogen is not recommended (King, 2009, Tang et al., 2009, Lekang, 2013).

The transport by land in long distances can also require the exchange of water. Is also a crucial point where good routines must be implemented, to minimize the stress in the fish it is required that this new water have similar properties, such as temperature to avoid shock. In some cases, the vehicles are equipped with a water treatment unit, to recycle the water.

The biomass in each tank must also be taken into account, the density of transportation cannot exceed a maximum of fish biomass per volume of water to achieve the best results. The proper density is dependent on fish species.

When the transportation vehicle does not have an alarm system for information of unfavorable water parameters, a stop procedure must be considered to evaluate these, such assessment can be performed with portable devices (such as oximeter and thermometer). Thus, this operation requires a good management plan. Generally, in most transportations it is usual to stop after 15 minutes, subsequently these stops are done every 2 hours, allowing the driver to make a safer travel (Lekang, 2013).

The type of vehicle involved in the transport by sea are well boats or boats that can convey compartments where the fish are (such as cages or rafts). In this case, the velocity must be low, to not crash the animals against the walls of the compartments. The well boats allow the transport a much larger amount of fish compared to other methods (up to 150 tons) (Lekang, 2013). To ensure that the water quality remains favorable during the transportation, the water in the well is renovated constantly through valves placed in the back and in the front of the boat, this flow is only active if the boat is in movement.

When the boat is in movement, the masses of water can be a stressor factor, to monitor this properly there must be an alarm system in place.The water parameters evaluation can be done manually or automatically.

During sea transportation, some species are sensitive to the variation of pressure caused by waves, also creating a stressor factor for the animals. Since they will try to oppose this variation through physiological mechanisms. Thus, in this type of transport it is important to know the weather conditions before transport (Lekang, 2013).

It is essential that the method used in each transport be adequate for the species in question, their life stage, and the destination.

1.4.5 Breeding

Breeding in aquaculture is one of the areas with most studies done. In many cases, selective breeding has been shown that can improve the productivity, through: feed conversion ratio (FCR), higher resistance against diseases and less aggressive behaviors (Pottinger and Carrick, 1999, Gjedrem et al., 2012, Lind et al., 2012, Moss et al., 2012, Teletchea and Fontaine, 2014).

To improve the production there are processes that must be take into account: (1) the selection of breeders and maturation, (2) the spawning, (3) the fertilization, and (4) the harvest and incubation of the eggs. Along with these processes, animal welfare can be compromised, since handling of the fish and manipulation of environment conditions may be required, depending on the process used and the species (Pottinger and Carrick, 1999, Lind et al., 2012, Fernández et al., 2014, Mustapha, 2014).

In breeding operations, it is necessary to know the requisites for each species, to obtain maximum yield. Therefore, it is necessary to know the proper ratio between genders, the duration of the spawning and the fecundity. The origin of the selected breeders for the aquaculture can be from wild stocks or aquaculture. Moreover, in all cases, caution is needed when introducing these animals in another environment. Thus, a quarantine and acclimation process is required, to make sure that the animals do not have any pathologies or lesions that can compromise the production itself. It adds, that the input of animals require that this, must go through a acclimation process, to habituate to the new environmental conditions and husbandry operations (Pottinger and Carrick, 1999, McEwen and Wingfield, 2003, Blonk et al., 2010, Fernández et al., 2014, Teletchea and Fontaine, 2014).

The maturation of the individuals can be challenging depending on the sexual maturation process that each species have. There are two principal groups of animals: the ones who have the same gender along the life cycle, and those who can have both sexes (hermaphrodite), in simultaneous or sequentially (protandry or protogyny, respectively). In nature the specimens are stimulated by environment variations (photoperiod, salinity, and temperature are some examples) to maturate and consequently reproduce. This occur due to the endocrine system that increase the concentration of some hormones responsible for the maturation (gonadotropin release hormone, gonadotropin release inhibitors factors and gonadotropins) (Saillant et al., 2003, Guzmán et al., 2009, Schreck, 2010, Fernández et al., 2014). In aquaculture, this environment conditions can be manipulated to assure the production cycle. Another way to assure that, is by the use of hormonal manipulation, in this case, hormones are injected directly into the animal. Various factors limit the success of this technique, such as; maturation phase of the animal, type and dosage of hormone used and the application time. Both techniques can compromise the welfare, due to handling or due to variations in environment conditions (Guzmán et al., 2009, Gjedrem et al., 2012).

Regarding fertilization there are two methods to achieve that: the artificial method, which requires human labour to extract gametes; the other method is the natural one, in which the animals copulate (Linhart et al., 1995, Guzmán et al., 2009, Kohara et al., 2010, Neufeld et al., 2011, Weingartner et al., 2015). The artificial method can compromise more the animal welfare, compared to the other method, since it is required the handling of the animal to be removed from water and extract the gametes. This extraction can be done in several ways, such as: by compressing the animal manually, by surgery methods (that may implicate death), and by injection of air into the abdominal cavity (Saillant et al., 2003, Guzmán et al., 2009, Kohara et al., 2010, Neufeld et al., 2011, Weingartner et al., 2015).

When the animal fertilization is done without the direct handling, described above, the harvest of the fertilized eggs is usually done using a automated method. Since, in several marine species, the eggs have a floatability capacity that makes it possible to harvest the eggs using a filtration system. Consequently, it is necessary to analyze the viability of the eggs and, therefore, proceed to incubation. In numerous cases, the unviable eggs sink in water with salinity superior to 32 ‰, which allow the farmers to access which eggs are viable and ready for incubation and those who can be discarded (Linhart et al., 1995, Kohara et al., 2010, Neufeld et al., 2011, Mizuno et al., 2012).

Regarding incubation, three different methods are widely used: (1) The direct method, in which the eggs are incubated in the larvae tanks; (2) the indirect method, in which the eggs are incubated in isolated tanks specialized for this process; and (3) the incubation in a container inside of a tank with high renovation, this method is used to access the hatching rate and other properties of the eggs in the direct method (FAO, 2015).

The success of breeding, quality of eggs and consequently the quality of larvae, will be dependent on the good physiologic condition of the breeders. Thus, the feed administered to these animals must fulfill their requisites, to safeguard the productivity of the facility. Nowadays, one of the bottlenecks in aquaculture is the quality disparity of the eggs, this influence the development of the fish and the profitability of the facility. Thus, it is usually used natural feeding (fresh or frozen) when the nutritional requisites of the fish are not fully understood, since this type of food will most likely fulfill the requirements of the animals. Once known the nutritional requirements, it is possible and it is more efficient to administrate artificial feed, because it is easier to control the feed composition along the year, and consequently it is possible to improve the breeding process (Kulczykowska and Sánchez-Vázquez, 2010, Morais et al., 2014, FAO, 2015).

1.4.6 Slaughter

Regarding the welfare, one of the most controversies themes is the slaughter of animals in aquaculture. The process of slaughter is usually studied for animals that are for human consumption. There are a various methods that can be implemented, depending on the species, these are: (1) Asphyxiation in air or ice; (2) bleeding; (3) carbon dioxide; (4) stunning; (5) percussive stunning; (6) decapitation; (7) electrocution; (8) spiking; (9) live chilling; in this process the animals are placed in water with ice (Håstein et al., 2005, Lines and Spence, 2012, Huntingford and Kadri, 2014, Lines and Spence, 2014, Mustapha, 2014).

In the case of slaughter in animals that are for human consumption it is required that the process used will maintain the properties and appearance of the animals. Otherwise, the value of the product will decrease significantly. Although, every process needs to be adjusted to each species (Håstein et al., 2005).

The slaughter process usually needs to be well planned, due to that the fish pass. To prevent contamination and to maximize the shelf life the animals need to starve, noemally ate least twenty four hours. The animals can also be subject to sedation (this method is prohibited from the European Union), or a stunning method, to avoid unecessary disconfort or pain (King, 2009, Lines and Spence, 2012, Lines and Spence, 2014, Mustapha, 2014, Bermejo-Poza et al., 2015). The processes that are commonly used are, the death by hypothermia (using the live chilling process) or by bleeding (Håstein et al., 2005). Nonetheless, according by several studies none of these process is considered to be human, that is, this methods does not protect the animal welfare, avoiding unnecessary pain and stress (Håstein et al., 2005, Bovenkerk and Meijboom, 2013, Lines and Spence, 2014, Mustapha, 2014). In fact, in this sector little was done to improve this process regarding the welfare. According to the literature the only produced species that altered significant the husbandry alterations was the Atlantic salmon aquaculture (Lines and Spence, 2014).

The various works regarding this thematic link the slaughter methods to the quality of the final product (Lines and Spence, 2012, Lines and Spence, 2014, Mustapha, 2014).

Since the work presented was integrated entirely in an aquaculture nursery, the slaughter for consumption is not a process implemented in the husbandry operations of this facility. Nonetheless, another less studied type of slaughter present in this facility is the elimination of selected animals (Håstein et al., 2005). The selected fish in aquaculture is a process where the animals with illness, lower growth rates, or any damage that compromise the commercial value are discarded, this will improve the productivity by various ways like: disease control, less expense in food, and increasing the average economic value of the biomass (Håstein et al., 2005).

This theme is much less studied than the previously mentioned because the animals are not for consumption and to cut expenses, usually, the slaughter is done in a most inexpensive way. This careless for the slaughter of selected animals will reflect in the welfare, since this methods, most probably, do not avoid distress and pain.

Concluding, the best method of slaughter should inflict the lesser stress and pain possible, for that it is considerate by some authors that sedation or stunning before slaughter, when properly applied, are the best possibilities to guarantee a friendly-welfare operation (Håstein et al., 2005, Bovenkerk and Meijboom, 2013, Mustapha, 2014).

1.5 Good aquaculture practices

As already referred, the aquaculture is an activity in a fast expansion across the globe. Thus, the implementation of good aquaculture practices is, more and more, important, to respond to the society demands regarding food quality, animal welfare, and ecological concerns.

The aquaculture practices will influence: (1) the site selection of the facility; (2) the water quality, since this factor is depending on the local that the facility is constructed; (3) the source of animals, the source of animals will depend on the management plan implemented; (4) and the husbandry operations (Khuraibet and Al Attar, 2002, Boyd, 2003, Sapkota et al., 2008, Broughton and Walker, 2010, Bosma and Verdegem, 2011, Moss et al., 2012, Diana et al., 2013, Mondal et al., 2013, Boison and Turnipseed, 2015).

1.5.1 Site selection

The location of an aquaculture is dependent of parameters, such as: (1) what species is going to produce; (2) what production system is going to be used (for example offshore aquaculture will need specific environment conditions, although, inland aquacultures have other requisites); (3) the access to natural resources (water); (4) access to the aquaculture facility (for example: roads); (5) surrounding activities that can compromise the production (for example: agriculture leakages). For these reasons, the site selection of an aquaculture facility must be careful deliberated to assure its success (Soto et al., 2013).

1.5.2 Water quality

Usually, the aquaculture products are for human consumption. Thus, the safety protocols of these are more rigorous. In this activity, one of the most control factors is the water quality control, in which the animal develops. This factor is even more relevant when it comes to intensive aquaculture, namely, in facilities that use RAS, in this case, the water treatment system must guarantee the control of the water quality within the favorable levels for the species produced. Although, this system complexity can create management problems, nowadays there are more and more facilities that implement these systems, due to the advantages (more efficient, more ecological, safeguard the animal welfare and increase productivity).

Despite the system used, the water must be treated within the appropriate levels for the produced species. This control is fundamental to prevent any diseases and outbreaks in the facility and subsequently safeguard the productivity and animal welfare.

The water source shall be free from any contamination, either by other production activities, either by urban effluents (Yang et al., 2001, Bossù et al., 2013, Ababouch, 2014, Colson et al., 2015).

1.5.3 Source of animals

In the aquaculture the import/transfer of animals from others facilities, or the wild is widely common. However, this is one step that could endanger the production, since any introduction of live biomass can translate to an outbreak of pathogens in the facility, thus, any introduction of animals should pass through the quarantine facilities (Broughton and Walker, 2010, Ababouch, 2014).

1.5.4 Husbandry operations

Regarding the good practices in the husbandry operations, these were already covered in the sections above. In summary, there are a number of practices that must be implemented, in order to safeguard the productivity and the animals: (1) good hygiene practices, this should cover the disinfection and sanitary practices in the holding facilities, equipment, and staff; (2) the production design should be adequate for the species in production; (3) minimize the stress in the animals, the stress can be caused by the handling in the routine operations or by other factor such as water quality or transportation; (4) use exclusively feeds that are certified by an authority, this can prevent diseases dissemination and contaminations in the facility; (5) protect the fish in production from any predators or other animals that can harm the fish in production, this can also be a source of pathogens; (6) control holding conditions, in order to increase the animal welfare; and (7) do not exceed the carrying capacity of the aquaculture (Khuraibet and Al Attar, 2002, Frewer et al., 2005, Sapkota et al., 2008, Broughton and Walker, 2010, López-Olmeda et al., 2012, Vázquez-Rowe et al., 2012, Ababouch, 2014, Huntingford and Kadri, 2014, Boison and Turnipseed, 2015).

All of these practices will depend on the species that is produce, the live stage and the type of production system used.

1.6 Biology of *Solea senegalensis*

Regarding the distribution of this species, it is commonly found from north to south in the Atlantic east, has a high market value and, for that reason, it is considered a species of great interest to produce. This rise promoted an increase in the number of studies with this species inthe last decades, with the objective to know therequirements for aquaculture (Bedoui, 1995, Dinis et al., 1999, Imsland et al., 2003).

Solea senegalensis is a benthonic fish, regarding the morphology it is a flatfish with the eyes on the right side. It presents an asymmetric oval shape and has the anal and dorsal fins connected with the caudal one. It reaches maturity approximately at 30 cm of length, which corresponds to 4-5 years of age. The reproduction season initiate in May-June when the waters warm up to 18-20º C (Dinis et al., 1999).

This animal normally inhabits littoral ecosystems with sandy or muddy bottoms in depths inferiors to 100 m. In nature, this species feeds upon polychaetes, small crustaceans, and other benthonic invertebrates.

1.6.1 Aquaculture

The production of this species has encountered various challenges, such as, high susceptibility to pathologies, technological limitation and captivity reproduction (Dinis et al., 1999).

The time that is required to this species achieve the commercial size lasts, usually, two years (125 g). Concrete or fiberglass can compose the production tanks, depending on the production process that is used, this will also influence the density used (Dinis et al., 1999, Dias et al., 2004, Salas-Leiton et al., 2008, Andrade et al., 2015).

For the production, the environmental parameters vary according to the management plan, however regarding the temperature this can range between the 22-27º C (Dinis et al., 1999). The feed administered can be natural (fresh or frozen polychaetes and other invertebrates) or artificial (commercial pellets) (Bedoui, 1995, Dinis et al., 1999, Imsland et al., 2003, Dias et al., 2004, Salas-Leiton et al., 2008, Marinho et al., 2014).

In farming, the reproduction is controlled by temperature and photoperiod, to produce eggs according to the management plan, each female can produce 140,000-200,000 eggs/kg (Imsland et al., 2003), and the incubation time is normally of 800 degrees-hours at 19º C (Dinis et al., 1999). Depending on the characteristics of the gametes and the environmental conditions, the fertilization rate can range between 50-100% (Bedoui, 1995) and the hatching between the 30-80% (Dinis et al., 1999).

The larvae are pelagic, and begin metamorphosis at 2,2-2,9 mm (Dinis et al., 1999, Blanco-Vives et al., 2010, Blanco-Vives et al., 2011). Normally, the provided feed in this stage is live feed from 3 dph until the 35 dph (Dinis et al., 1999, Pedro Cañavate et al., 2006). Posteriorly the larvae stage, the animals pass through a weaning phase to acclimatize to inert feed, this process can produce mortality between the 40-80% (Dinis et al., 1999, Imsland et al., 2003).

The market demand for this species is due, namely, to his organoleptic properties and due to their nutritional benefits (Dinis et al., 1999, Imsland et al., 2003).

Another bottleneck in the aquaculture of the Senegalese sole is, the susceptibility to pathologies, normally these can be treated and the fish can survive, but the physical damages caused by the pathologies result in an economical losses, for example the

Photobacterium damselae ssp *piscicida* causes an erosion in the caudal fin and even if the fish are treated the economic value will decrease, because the retailers and consumers have certain esthetic standards (Dinis et al., 1999, Imsland et al., 2003). Other blockages include the formulation of adequate feeds, which usually translate in a poor FCR and consequent poor growth, and the need to capture wild breeders to assure the production cycle, because the animals that are produced do not have the best qualities to became breeders (for example: low production of sperm) (Dinis et al., 1999, Brown, 2002, Imsland et al., 2003).

The increase of investigation works and the holding conditions improvement can reduce and solve these problems.

1.7 Thesis objectives

The goals of this work were:

1. Integration in the routine practices in Safiestela S.A., to apply theoretical knowledge in a realistic context;
2. Implementation of a FWAS plan in the facility;
3. Realization of an experiment in the larval stage, to compare holding conditions at a commercial level.

Chapter 3: Implementation of a Fish Welfare Assurance System (FWAS) in Safiestela S.A.

3.1 Introduction

Following other animal productions, aquaculture has a need to implement a plan that safeguard the animal welfare and the production itself. Since 2006, all food producers that compete in the European market are obliged to implement HACCP (hazards analysis and critical control points) into their production safety plan, this regulation created an increase in the concern about the products quality from animal production (Ingenbleek et al., 2012, Ingenbleek et al., 2013). Another increasing concern among the generally population is, the holding conditions that the animals are subject under production, in other words, the animal welfare (van de Vis et al., 2012).

These concerns together generated pressure on the market for aquaculture products, creating a demand for products that are animal welfare certified. This demand were first applied in terrestrial animal productions, nonetheless, with the increase of aquaculture, these concerns are, nowadays, more focused on this sector.

To supply the demand generated, it was created a welfare assurance system for fish farming, FWAS. This system follows the same seven principles of HACCP. Nonetheless, each step was altered to be applied to animal welfare in aquaculture.

In order to create a strong and simple FWAS plan, it is necessary to have already established a code of good aquaculture practices (GAP) and a biosecurity plan in the aquaculture, this underlies that exist good hygiene, good maintenance and control of other operation practices. If these pre-requirements are established, it is possible that minor adjustments are enough to reach the main goal. If not, greater changes are needed, consequently the production could be affected. Because new practices need to be implemented, and the workers need time to adjust to these, which could influence the productivity of the company.

For the reasons mentioned above it has been implemented a FWAS plan in Safiestela S.A. In this chapter it is presented part of the work done in this facility, the complete flow diagram and FWAS plan are present in the annex one and two, respectively.

The United Kingdom was one of the first countries to implement this certification and to create associations, such as, RSPCA, to acknowledge and come to agreement of which rules need to be applied to the fish farmers (Bovenkerk and Meijboom, 2013).

The FWAS diverge from the common system, HACCP. This last system allows the production strengthen, not only by improving the product quality, but also by optimization of the production (Fernández-Segovia et al., 2014, Psomas and Kafetzopoulos, 2015). The HACCP is based on seven principles, with 13 steps within them, and according to van de Vis et al. (2012) it is possible to adapt these to fish welfare in aquaculture, these steps are: (1) Assemble the FWAS team; (2) Describe the product; (3) Identify intended use; (4) Construct a flow diagram; (5) Assess on site; (6) Identify hazards and preventive measures; (7) Identify critical control points; (8) Determine target and critical levels for each critical control point; (9) Establish monitoring system; (10) Establish corrective measures; (11) Establish verification procedures; (12) Establish a record keeping system; (13) Review the FWAS plan.

These steps or stages can differ depending on the type of production and on it is implemented. For example, in United Kingdom there are 14 stages instead of the 13 presented above, the difference in the UK system is the addition of a first step (define the terms of the reference, in other words, define what the plan is going to focus). This first step is often implemented to prevent an exaggerated plan that probably is not going to be fully implemented, therefore, will be ineffective (Dillon et al., 1996).

In this case, the first step is to assemble a team. Depending on the size of the company, several members can compose this group, normally never exceeding six members, or only by one person (Dillon et al., 1996). The team selection can be done by one external identity specialist in HACCP plans, or by an internal one. Nevertheless, the knowledge of the team regarding the quality control, routine works, HACCP plan and identify hazards is always essential (Dillon et al., 1996, Jahncke et al., 2002, Miget, 2004).

The next stage, describes the product, is essential that the team is provided with the information about the production, to evaluate in the best way possible all the hazards and consequently improve the quality.

The third stage referred is, the identification of the intended use, in other words, identify the target population of the product and if there are any danger concerning the product.

In the elaboration of a flow diagram (step four), is crucial understand all the production process and to analyze possible hazards along the process, allowing an overview of the production cycle. There is a group of rules to create a flow diagram, each symbol represents one feature, component or piece of the production cycle (figure 5). In figure 6 is represented an example of a part of the flow diagram from the Safiestela S.A., that was result from the implementation of the FWAS plan. (Annex1).

The next step will be confirming on site the information provided by the flow diagram. Thus, it is needed that every member of the team assesses the events described by the flow diagram, at different times and with different workers in the same event. This will provide a more realistic evaluation of the production routines and consequently a more accurate and better plan (van de Vis et al., 2012). In the point 3.9 it is specified the work done regarding this stage in, Safiestela S.A.

The sixth stage is, to identify any hazard along the production and implement preventive measures, this step in the plan is crucial, and it needs to be thorough. At this step, an inexperienced person will have difficulties to identify the hazards. Therefore, a person who has experience in this plans is required on the team, to guide the other members. The identification of hazards normally is done by categories, for example, in a FWAS plan it is common to use four categories to identify hazards: biotic, abiotic, managerial and environmental (van de Vis et al., 2012).

Posteriorly the identification of all hazards present in the production cycle it is required to assess which are critical control points (CCP). A CCP is a point where the control is essential, to guarantee the objective of the plan, either if the aim is food safety or animal welfare (Dillon et al., 1996, van de Vis et al., 2012). To identify these CCP it is used a scheme that guide through a series of questions, to separate these critical control points from others problems (figure 7).

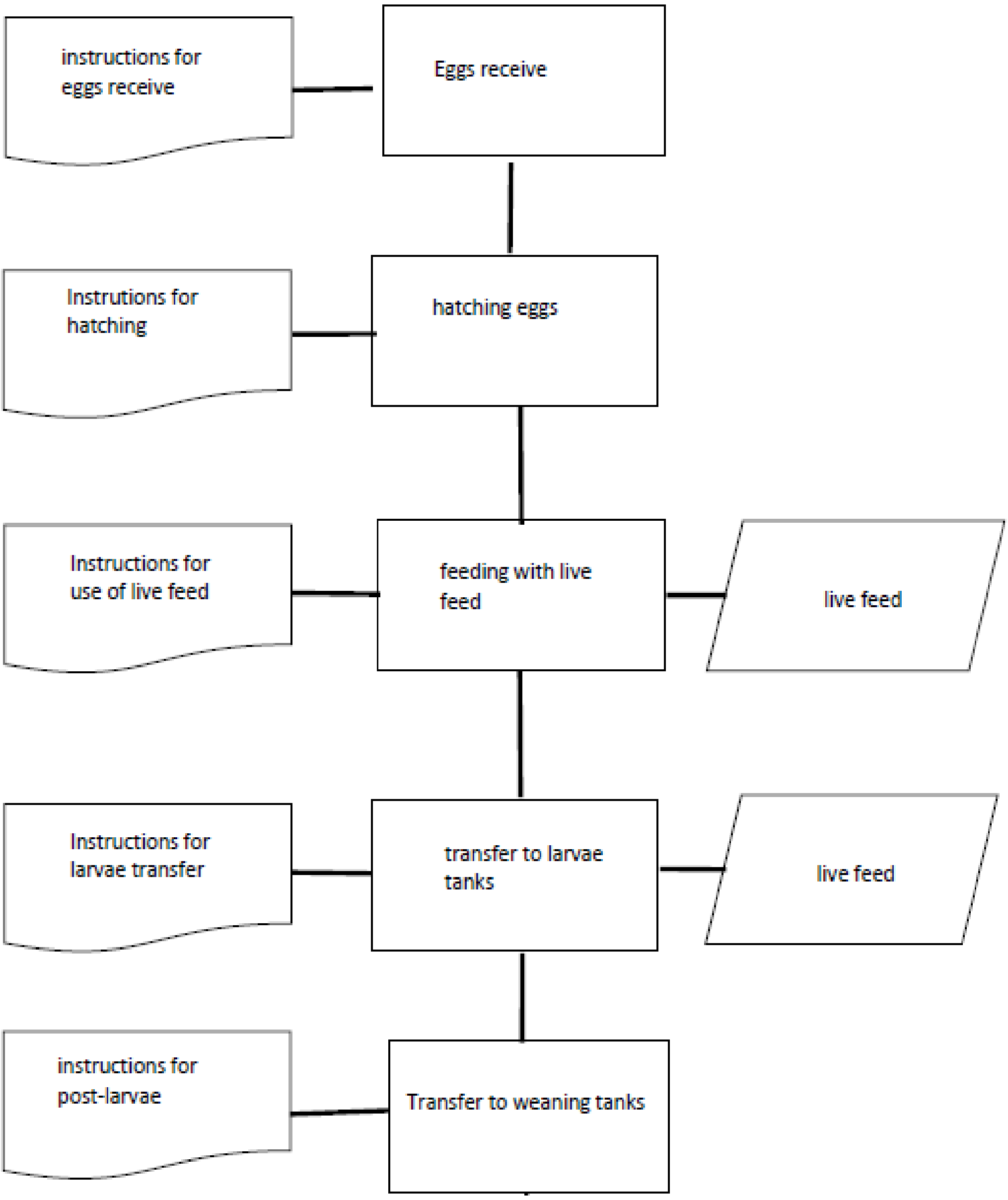


Figure 5 - Example of a flow diagram, regarding the incubation and larvae production, developed for Safiestela S.A. Annex 1.

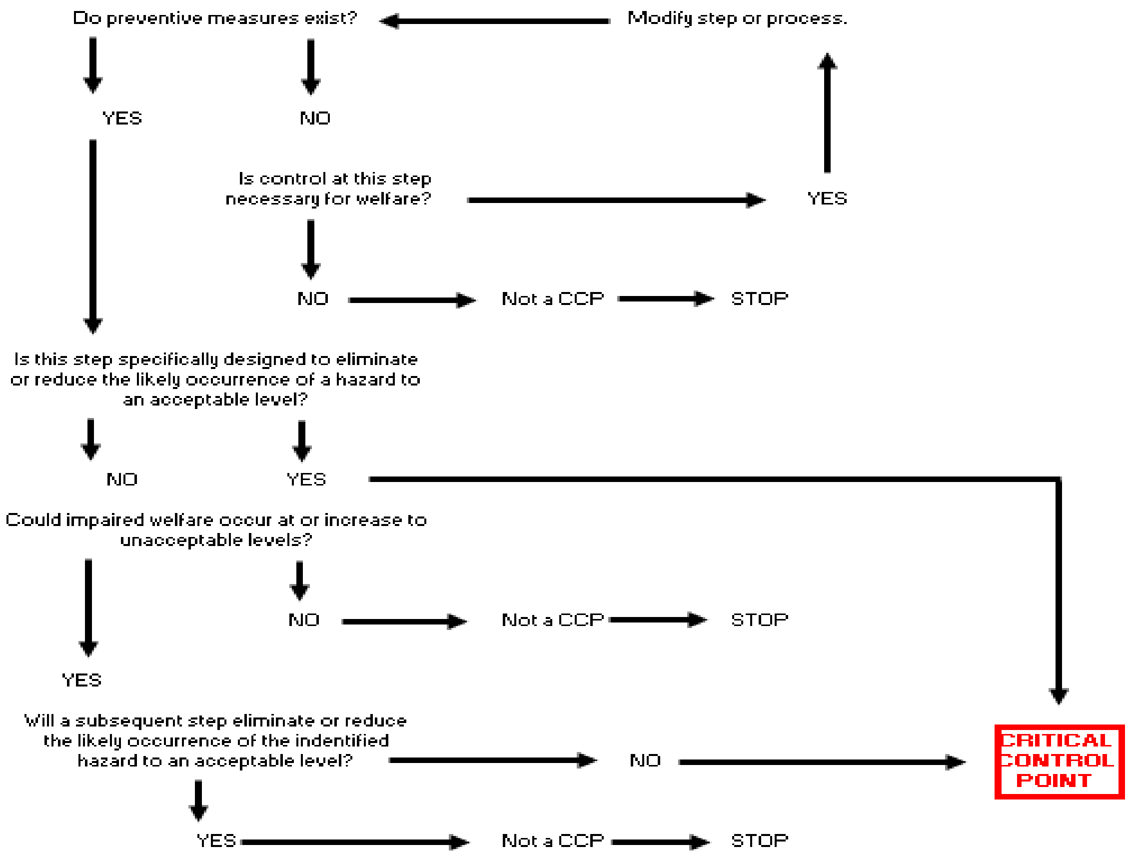


Figure 6 - Decision tree to identify critical control points (CCP) (van de Vis et al, 2012).

The next stage is to establish target and limit levels for each CCP. Although, this process can be relatively straightforward in some productions, regarding the food safety and quality, in a FWAS plan this can be challenging. Due to the subjectivity of the concept of animal welfare, nonetheless, in either case, this step must be implemented. In the case of a FWAS plan, the target or limit levels can be defined in some ways, depending on the point that is being analyzed. For example, in aquaculture, acceptable levels , as well as limit levels, are determined for various parameters, such as the concentration of ammonia levels in the tanks of production, to protect the animal welfare. Nonetheless, these boundaries will differ from the species produced, life stage, and the production system.

In this stage is fundamental analyze a significant period, to collect enough data to recognize any pattern of the variation of these levels, because any pattern can be an evidence of a problem in the production process.

The monitoring system will be a tool to prevent the origin of a CCP and will help in the control of any CCP in production. The monitoring system needs to specify what is monitoring, by whom, the frequency of the monitoring and how the monitoring can be done (table 3) (Dillon et al., 1996, van de Vis et al., 2012). Establishing corrective measures for the CCP, consist in establishing a plan to prevent that the levels surpass the critical limits. One example of corrective actions can be the training of the workers, in order to change their routines in a way that these levels will not be exceeded, and subsequently reduce or eliminate the stress factor (van de Vis et al., 2012). (Table 3).

The next stage (step eleven), consist in the confirmation that the plan is being implemented, and to fulfill the objective the verification procedures must be described (table 3 and annex 2). Often in the food industry a laboratory analysis are performed in the products to assess if the critical limits are being respected. These verification procedures are periodical and help to identify any pattern, and consequently contribute to associate other parameters and determine a possible cause (Dillon et al., 1996, van de Vis et al., 2012). (Table 5).

The record keeping system will provide information about the product and it is a form of confirm if the HACCP/FWAS plan is working or if it needs any improvement (Dillon et al., 1996, Miget, 2004, van de Vis et al., 2012). The final step is the review of the whole plan, process by process and it is complementary to the verification procedure. This review must be periodical and must be placed into action when any part of the process is subject to change. This stage will determine if any CCP can be eliminated, or if any was created. Therefore, the HACCP/FWAS plan is in constant self-improvement, due to its cycle structure (Dillon et al., 1996, van de Vis et al., 2012). (Table 6).

These steps determine the chronological implementation of the FWAS/HACCP plans and they are within seven basic principles, common to the two plans (HACCP and FWAS), these are: (1) Conduct a Hazard Analysis and Risk Assessment; (2) Determine Critical Control Points (CCP); (3) Establish Target Levels and Critical Limits for CCP; (4) Establish monitoring procedures at each CCP; (5) Establish Corrective Actions; (6) Establish Verification Procedures; (7) Establish a Record-Keeping System.

Table 3 - Example of how to implement a monitoring system developed for Safiestela S.A. (van de Vis et al., 2012). Annex 2

Step in process	Hazard	Monitoring			
		What	How	Frequency	Who
Hatching eggs	Biotic				
	Disease	Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager
	Abiotic				
	Fish exposed to deteriored water quality	Monitor water quality as specified in the instructions	Inspect work done	Every hatching of eggs	Hatchery manager
	Managerial				
	Eggs/larvae handled roughly during hatching	Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager
	Environmental				
	Intake of polluted water to holding tanks	Assess that instructions are followed	Inspect work done	Daily	Hatchery manager

3.2 Conduct a hazard analysis and risk assessment

In a normal HACCP plan the prime objective is headed for the improvement of the management system, in order to increase food safety (Dillon et al., 1996, Khuraibet and Al Attar, 2002, Miget, 2004, Psomas and Kafetzopoulos, 2015). In the case of a FWAS plan, the objective is to improve the animal welfare in production. Nonetheless, this goal is linked with the food quality produce (van de Vis et al., 2012).

Since the FWAS plan derives from the HACCP plan, there are many similarities between them, as for the principles that these plans have. Before the implementation of the first principle some steps are needed to execute the FWAS plan, this steps are: (1) Assemble the HACCP/FWAS team; (2) describe the product; (3) Identify intended use of the product; (4) construct a flow diagram; (5) confirm the flow diagram on site.

The first principle is the analysis of possible hazards for the animal welfare. These hazards in aquaculture can be separated into different categories, depending on their origin: biotic, abiotic, managerial and environmental (van de Vis et al., 2012).

Nonetheless, this categorization is used only to facilitate the assessment of the hazards, since the factors that can compromise the animal welfare are, usually, related. For example, the concentration of the dissolved oxygen in a tank is influenced by treatments and the amount of feed administered.

In this principle, the identification of hazards in aquaculture will depend on: (1) what species is produced, (2) what kind of production system (RAS or other) and (3) what life stages are in the production cycle (nursery or ongrowing stage).

Regarding the species produced there are different requirements. Thus, it is essential to know the various parameters that will constrain the production and welfare of each species. For example, what range of temperatures is suitable to produce the species and what range is optimal for that species. Optimal ranges will offer to the animal better welfare conditions, and that is the prime objective of this plan. However, quantify the welfare is challenging, due to its subjective concept (Grigorakis and Rigos, 2011, Bovenkerk and Meijboom, 2013, Huntingford and Kadri, 2014, Bermejo-Poza et al., 2015).

The hazards encountered in Safiestela S.A. are described in the table 6 and in the annex two.

3.3 Determine critical control points (CCP)

The identification of a CCP by the team is facilitated by a scheme or a decision tree (figure 6). In this scheme, there is a group of questions that help to guide the team to identify correctly a CCP (van de Vis et al., 2012).

The CCP found in Safiestela S.A. are described in the table 6 and in the annex two.

3.4 Establish critical and target limits

Posteriorly to the CCP determination is fundamental to create the critical limits and target limits for each CCP. A critical limit is determined by a maximum and a minimum value. If a CCP presents a value that is outside of this range, then the animal welfare is compromised, if the value does not exceed these limits then the conditions are created to improve the animal welfare (van de Vis et al., 2012).

As mentioned above, to implement a FWAS plan there are four categories to separate the hazards. Depending on the produced species, life stage and type of production, these hazards have different target and limit levels, nonetheless, depending on the category it can be more suitable apply only one type of limit. For example, for managerial hazards the application of critical limits is not adequate for the application of target limits, as explained by van de Vis et al. (2012). In this work the example given is, the necessity to remove the Atlantic salmon of the water when it is vaccinated and in that case, the target limit referred to remove the fish is when it is strictly necessary. Thus, no critical limits are adequate, only was determined the target limit for when it is right to remove the fish from the water. If it were other species that could resist to lower oxygen levels, it would be possible to determine the critical and target levels. (van de Vis et al., 2012).

One of the main focus to determine the animal welfare in aquaculture is done by the evaluation of the water parameters, since there are a series of relations within those parameters and between them and pathologies. There are tolerance levels for the water parameters according to each species, and it is essential for an aquaculture facility to know and maintain these levels to guarantee the production cycle and the animal welfare. This tolerance range can be narrow or wider depending on the species. Nevertheless, all species have a level of tolerance, to maintain stability over environmental changes, and this is known as allostasis (McEwen and Wingfield, 2003, Schreck, 2010). Under farming conditions this tolerance levels should be narrow to prevent any complications, so two limits were created two limits: one narrower, operating limit; and other wider, critical limit (van de Vis et al., 2012). The operating limit is determined by a limit that is minor than the critical limit, and give the operator some time to adjust the levels to more favorable values. Is like an alarm before anything is going out of control (figure 7). There are situations where the critical limit is the same as the operational one, thus in this cases there are no space for errors (van de Vis et al., 2012).

In table 6 there are some limits (in the column “Criteria”) created for the CCP encountered in Safiestela S.A., in the annex two it is specified all of the limits created for each CCP.

Figure 7 - Scheme to explain the critical limit (M) and operational limit (m). (van de Vis et al., 2012).

3.5 Establish monitoring procedures

To assess if the FWAS is implemented through time, it is required to create monitoring systems. This allows the identification of any possible complications in the production cycle at any time and to recognize any pattern, by evaluating the historic of recorded levels from each CCP, and consequent identification of the cause. To assess the animal welfare some indicators can be applied in a production cycle, operational welfare indicators (OWI). One example of these indicators is the record of the mortality in the facility (Huntingford and Kadri, 2014). These indicators need to be practical to not complicate the work of operators to a point that is counterproductive. In the FWAS plan is required OWI, which allows the detection of anything that compromise the animal welfare, for example, observation of lesions.

In table 3 there is part of the monitoring procedures implemented in the Safiestela S.A., to assess all these procedures is necessary to analyze the annex two.

3.6 Establish corrective actions

To control the CCP it is required to implement corrective actions, these allow to regain control whenever the levels of the CCP are beyond the critical or operational limit. To apply these actions, it is necessary to understand what caused the problem, and register the corrective actions taken. In the FWAS plan, the corrective actions are determined and help the operators to implement them (Dillon et al., 1996, van de Vis et al., 2012).

In table 4 it is demonstrated some corrective actions developed for Safiestela S.A., all the corrective actions are described in the annex two.

Table 4 - Example of corrective actions developed for Safiestela S.A. Annex 2.

Step in process	Monitoring				Corrective actions
	What	How	Frequency	Who	
Hatching eggs					
	Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers
	Monitor water quality as specified in the instructions	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers
	Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers

3.7 Establish verification procedures

As already mentioned, verification procedures assure that the conditions are within the critical limits. In the case of animal welfare, these procedures can be more challenging because ascertain welfare is complex. Despite that, welfare can be assessed in various ways: for example by observing the feeding behaviors, if the animal does not feed it is most likely that the welfare is compromised (van de Vis et al., 2012) (table 5). The frequency of this procedures must be regular and unexpected, to catch the most diversity possible events and therefore assess the most realistic scenario possible in the production cycle (Dillon et al., 1996, van de Vis et al., 2012).

In table 5 there are some examples of verification procedures resulted from the FWAS plan applied in Safiestela S.A., all of the verification procedures are described in annex two.

Table 5 - Example of verification procedures developed for Safiestela S.A. Annex 2.

Step in process	Monitoring				Corrective actions	Verification
	What	How	Frequency	Who		
Hatching eggs						
	Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	A sampling plan is used to monitor the fish
	Monitor water quality as specified in the instructions	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	A sampling plan is used for water analysis

3.8 Establish a record keeping system

A record keeping system is essential to guarantee that the FWAS plan is working properly. Another advantage of a record keeping system is to create dynamism in the FWAS plan, to reduce the CCP over time and find others problems that could only be identified through a significant period. According to van de Vis et al. (2012), there are a list of points that help to understand if the record keeping system is effective or not, these are: (1) Point the person responsible; (2) Due diligence requirements; (3) Pre-requisite programs controlled and monitored; (4) Documentation of the procedures; (5) Records of sanitary procedures in a manner to control CCP; (6) Corrective actions documented; (7) Document to determine when, by who and how the FWAS plan is reviewed.

In the next image is part of the FWAS plan from Safiestela S.A., while in annex 2 there is the whole plan.

Table 6 - Example of fish welfare assurance system (FWAS) plan for hatching eggs of *Senegalese sole,* developed for Safiestela S.A. Annex 2.

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Hatching eggs	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor egg/larvae quality; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	A sampling plan is used to monitor the fish	keep records of instructions, analysis and corrective actions
	Abiotic												
	Fish exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of system are followed	Operating limits for operation in instructions	Critical limits for water quality in instructions	Monitor water quality as specified in the instructions	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Eggs/larvae handled roughly during hatching	Bad management of hatching conditions	Instruct the workers	Use of instructions for hatching of the	Here m=M / avoid rough handling of		Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	Verify the ecclosion rate	keep records of the ecclosion rate
	Environmental												
	Intake of polluted water to holding tanks	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions

3.9 Verification on site

The critical control points (CCP) identification was done primarily by assessing the production cycle and then on the site. Nonetheless, this first verification in the local was done simultaneously with the integration in the routines of the production, and further attention was needed to confirm the CCP. To complete this task, it was elaborated several verification lists (one per each CCP) with a series of parameters that were discussed by the FWAS team.

These lists are checklists that needed to be measured,and posteriorly registered (e.g. time that the fish were exposed to air or the time of the cleaning of the tank). In order to create an average for each point, this average take into account the operator that was in the particular task. The results are posteriorly compared to the limits, which were pre-established by the FWAS team, to indicate if the operations are respecting the limits or not. These verifications create a record to, not only, percept eventual problems in the production but, as well to assure that the previous CCP were, in fact, well identified. Since, some of them can be eliminated or subject to alteration after this thorough analysis on site.

Next it will be presented some examples of this verification procedures taken in Safiestela S.A. Not all of the processes were verified due to the time limitations. Nonetheless, the verification templates were done.

3.9.1 Receiving of breeders

In the process of bringing new broodstock to the aquaculture, it is usually captured new individuals from the wild or extensive and semi-intensives aquacultures. During this process the animals can suffer damage, due to the potential rough handling, other problems may include parasitic contamination, so analysis must be done to ensure the health state. Regarding the transportation this is done with the animal out of the water, this method do not compromise the animal welfare (Ignacio Martín and Rasines, 2014).

The next tables are templates that were created for Safiestela S.A., each one as a checklist necessary in this CCP. Firstly it is recorded the date, if there are any visible damages, the origin of the animals, this allow to the manager to follow the animals and prevent receiving animals from facilities where there are historic of diseases. Finally the operator responsible for the record must sign, this is important because people perception of visible damages may differ. Because the analysis are only performed after acclimation, it was needed to create two separate tables at this point. In table 8, it is recorded the date, the animal (in this step the breeder is identifiable by introducing an electronic tag), and the operator responsible for the record.

At this stage the animals are in good conditions or not, there is not a medium scenario.

Table 7 - Template required in the integration of new income breeders developed for Safiestela S.A.

Date	Visible damages OK/Not OK	Origin	Operator

Table 8 - Template required in the integration of new income breeders developed for Safiestela S.A.

Date	Animal	Analysis OK/Not OK	Operator

3.9.2 Broodstock in RAS and in flow through system

The maintenance of broodstock in aquaculture must be regulated thoroughly. Since, in this part the animals are induced to different thermoperiods (according to which room: Spring, summer, autumn and winter) to control the spawning according with the facility needs. Due to that, a template was created to verify if the parameters in production are the best ones to the animals, according to the FWAS plan. To guarantee the animal welfare, limits regarding oxygen concentration and temperature were created, to assess if there were anomalies that could compromise the welfare.

The next table represents part of the verification record in the broodstock area, in this table it is only presented the room (spring), however, for the other rooms the verification is similar. In this record must be specified the date, the limits that were established by the FWAS team, and finally the actual values of temperature and oxygen concentration in each tank.

Table 9 - Template for broodstock developed for Safiestela S.A.

				<Temperature> | <Oxigénio> | Comportamento OK/Not OK					
Date	Operator	Limits		Spring					
				1		2		3	
				Temperature	Oxygen	Temperature	Oxygen	Temperatura	Oxygen
		>Temperature>*	>Oxygen>*						

*Limits according to the Sea8 protocol

3.9.3 Transference of larvae to the weaning area

In order to confirm if this step was actually a CCP it was necessary to assess on local some parameters, like the time that the larvae were subject to handling, the temperatures of the origin and destiny tanks and which worker was responsible for the operation (table 10). In this case, it was followed ten operations to assess if the limits, established by the team, were respected or if this was, in fact, a critical control point. The results are demonstrated in the next graph (figure 8). Because normally the transference is done in two days (the first day is transferred half of the tank and the next day the other half) in the results there are two separate moments. It is noticeable that in the second transference the average time that the fish is handled is higher (1361 seconds), than in the first moment (719 seconds). This difference was caused by the husbandry operation itself, since in the second half of each tanks, the operator must be more thorough to guarantee that all the larvae are transferred.

Nevertheless, it seems that the larvae were not affected by the time of transference, since there was no mortality in the next two days, and the feeding behavior was normal.

Table 10 -Template for transference of larvae to the weaning area developed for Safiestela S.A.

Date	Operator	Origin tank	Destiny tank	Time of handling (s) Limit max *	Temperature		DAH
					Origin	Destiny	

*Limits according to the Sea8 protocol

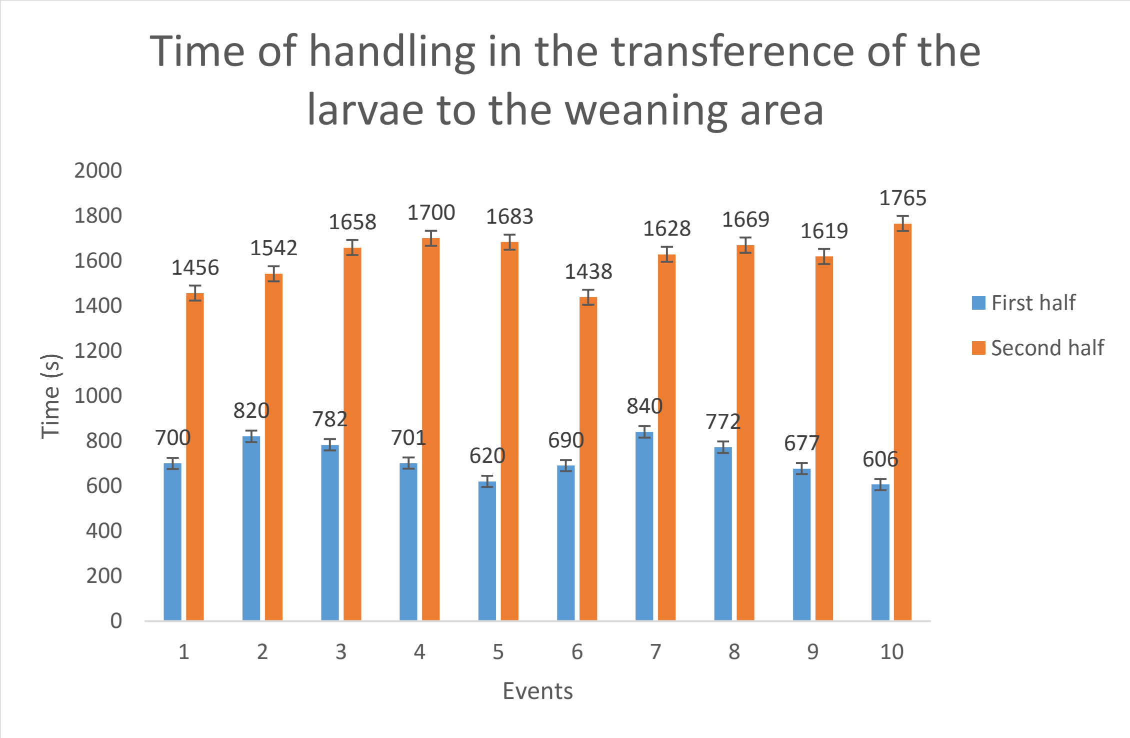


Figure 8 -Time for each moment of the larvae transference to the weaning area.

3.9.4 Grading in weaning area

The grading is a common operation in the aquaculture sector and helps to improve the productivity as mentioned in the 1.4.2 chapter. However, this induces stress in animals due to the handling and, can be a critical point that can potentially compromise the productivity. It is important to define limits, such as time of handling, to avoid any decrease in productivity.

In the weaning area it was followed this operation for six times and established the average duration that the fish were handled (time that the fish was packed, plus the time that the fish were out of the water). Other records were made: (1) tank that were graded, (2) destiny tanks, (3) tanks that were potentially disturbed by the operation, (4) operators involved, (5) date of the operation, and (6) mortalities in the day of operation and the next day (table 11).

The averages times are presented in figure 9, and it is clearl that the animals are out of the water less time (20.4 seconds, in average), than when they are packed (200.4 seconds, in average). Because the grading process in this area is performed with equipment with a screen and the animals are easily separated by size. The animals exposed to air did not present any signs of distress. Thus, grading in this area is normally rapid and seems not to influence the fish negatively. Nonetheless, further studies are needed to define a limit time for the operation.

Table 11- Template for grading in weaning area developed for Safiestela S.A.

Date	Operators	Packed time (s)	Time out of the water(s)	Origin tank	Destiny tanks	Mortality	
						In that day	Next day

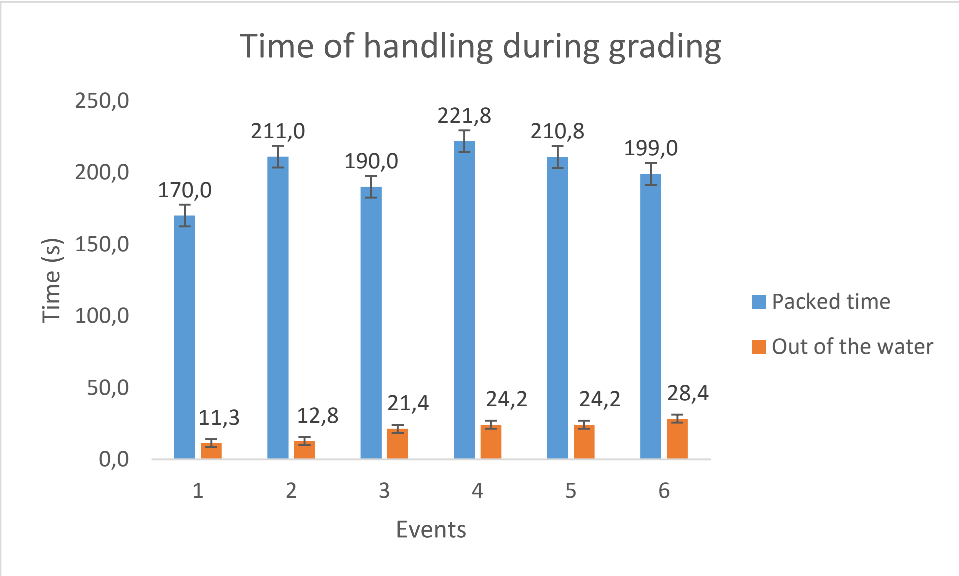


Figure 9 - Time of handling during grading in weaning area.

3.9.5 Fish transference from the weaning to the pre-ongrowing area

When the fish reach the appropriated size (around 1 g) these are transferred to the pre-ongrowing area, where there are different husbandry conditions. This operation needs to be done rapidly to avoid any decrease in productivity.

In this step it was measured: (1) handling time (packed time plus time out of the water), (2) origin tank, (3) destiny tank, (4) operators involved and (5) mortalities in the day of operation and the next day (table 12). These measurements were taken in five moments.

The averages time of handling showed that the time out of the water was lower than the time packed (figure 10). Regarding the effects of this operation, it seems that the fish are negatively affected. This was concluded when analysed the mortalities (figure 11), these were higher in the day after the operation (average of 45 fish), than in the day of the operation (average of 15 fish).Thus, a limit must be defined to established, and further studies are needed to assess if the animal welfare is compromised.

Table 12 - Template for the transference from weaning to pre-ongrowing area developed for Safiestela S.A.

Date	Operators	Origin tank	Destiny tank	Packed time (s)	Time out of the water (s)	Mortality	
						In that day	Day after

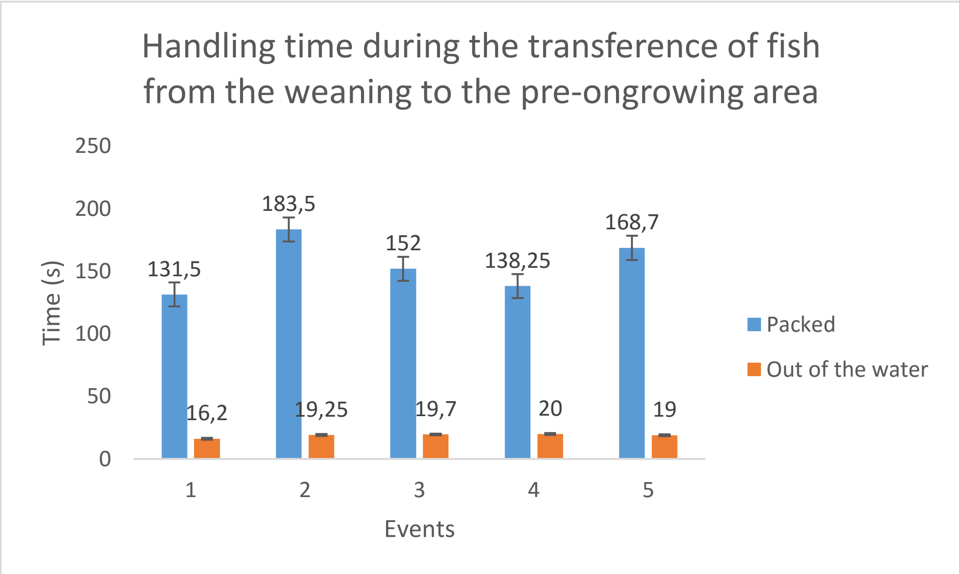


Figure 10 - Average time of handling during transference.

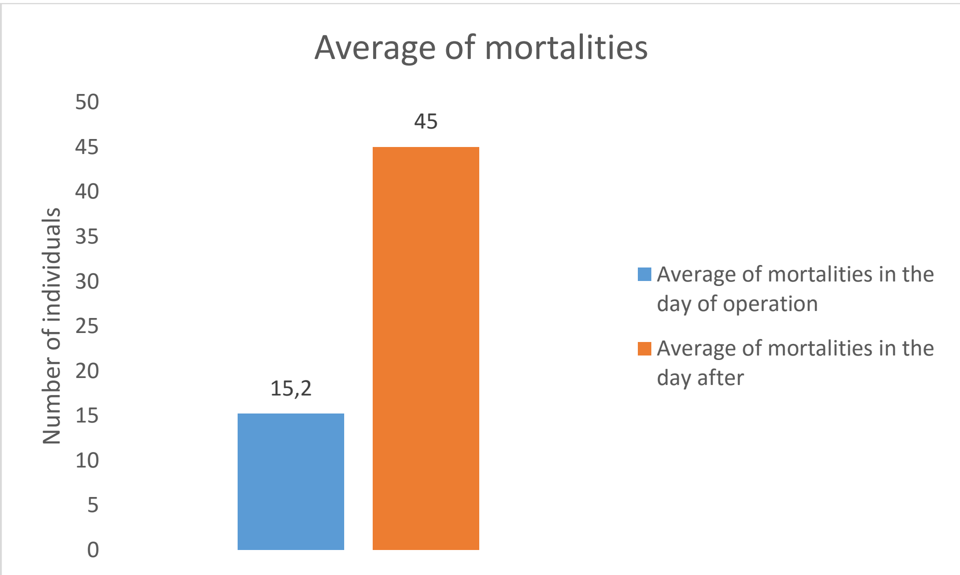


Figure 11 - Average of mortalities in the day of transference and in the day after..

3.9.6 Grading in pre-ongrowing area

As mentioned before, grading can potentially decrease productivity. In this case, it was followed 3 events, and it were registered the next parameters: (1) date, (2) operators involved, (3) handling time, (4) origin tank, (5) destiny tanks, (6) tanks that were potentially disturbed by the operation and (7) mortalities on the day of operation and the next day.

In this case, it was not differentiated the time out of the water with the packed time. Because this operation is done using an automatic calibrator, which complicated the following of each fish when it is out of the water. Nonetheless, the time of handling is this case was, in average, 110 seconds. The average mortalities were slightly higher on the following day (52 fish on the day of operation and 60 fish in the next day). Thus, in this case, it is not clear if the grading is affecting the fish negatively or not. Nonetheless, a extended study must be done to reach conclusions.

Table 13 – Template of grading in pre-ongrowing are developed for Safiestela S.A.

					Mortality	
Date	Operators	Time of handling	Origin	Destiny	In that day	Next day

3.9.7 Water quality in weaning and pre-ongrowing areas

In aquaculture, water parameters need to be in optimal values, to increase productivity and to safeguard the animal welfare. In this case, it was performed a compilation of data from six months (from November of 2014 to April of 2015) that had values outside the limits established. It was concluded that in Safiestela S.A., there was only one parameter that exceed these limits, this was the ammonia concentration, for this parameter it was defined an operating limit of 0.45 mg/L. Other parameters were analyzed, and limits were established, such as: temperature, oxygen concentration, nitrites and bromine (table14).

In figure 12 is represented the percentage of days, in each month analyzed, that the ammonia concentration was higher than the limits established by the FWAS team. It is clear that the optimal levels were not reached between this months. However, this does not seem to affect the productivity negatively. One explanation is that the operating limit established, according with the literature, did not corresponded to the best ranges for this species. Nevertheless little work with this species was done, to discover which ammonia concentration levels are beneficial and which are harmful. Additionally, the ammonia values were tested for correlation with the temperature levels. This was an attempt to find a pattern that could lead to the cause of the non-optimal levels. The results of this correlation showed that the temperature fluctuations does not were the cause for the higher levels of ammonia (the correlation value was 0.116 for the weaning area and 0.41 for the pre-ongrowing area, and $p>0.05$).

Table 14 – Template, in which was compiled the water quality data, developed for Safiestela S.A.

Date	Temperature Limits	OK/ Not OK	Temperature Limits	OK/ Not OK	Temperature Limits	OK/ Not OK	Temperature Limits	OK/ Not OK
	18º C < Temp <22º C		0.45 mg/L		1.5 mg/L		0.08 mg/L	

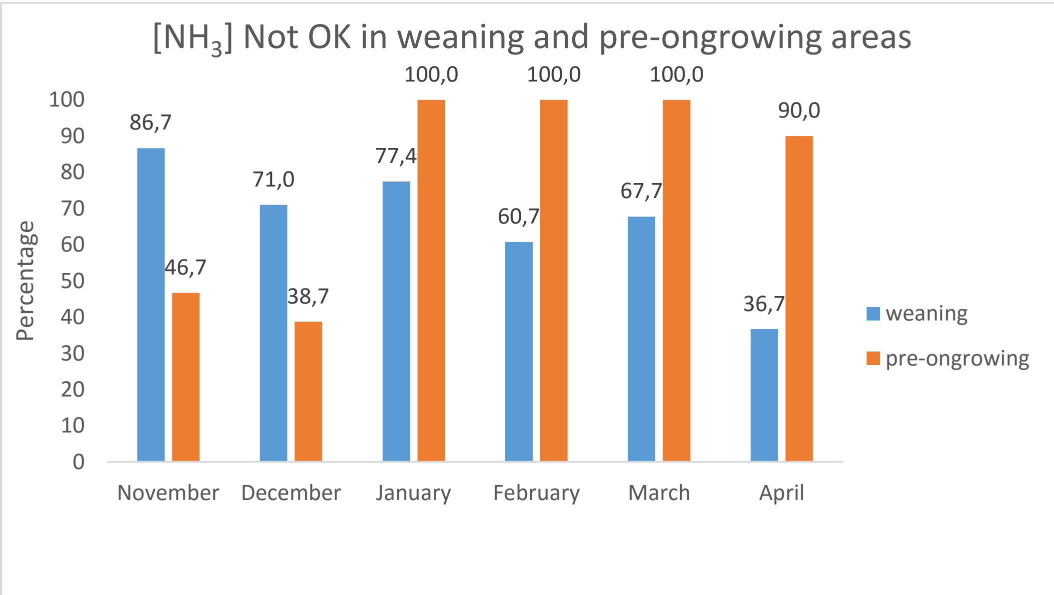


Figure 11 - Percentage of days, per month, that ammonia levels were higher than 0.45 mg/L.

3.9.8 Conclusion

With this verification process, it was possible to eliminate some of the initial established CCP. Since, it was confirmed that some of these points did not compromise the animal welfare. Nonetheless, this species is very challenging to work in the context of welfare, due to the limit knowledge that still exist. Hopefully, future works will contribute to improving the welfare conditions of *Solea senegalensis* aquaculture.

The FWAS plan is an ongoing work, and it is possible that in the future more CCP will be eliminated, and others will appear.

Chapter 4: Effect of light spectrum in the larvae development of the *Solea senegalensis*

4.1 Introduction

The production of Senegalese sole has been studied in the last 2 decades. This species is considered one of the most interest at a commercial level (Bedoui, 1995, Dinis et al., 1999, Brown, 2002, Imsland et al., 2003, van de Vis et al., 2012, Menezes et al., 2015). Nowadays, the production of this species is mainly focused in Portugal and Spain and in the last years there were accomplish important advances, in order to surpass the productions bottlenecks. In production, the objective is to achieve the commercial size in the shortest time possible, to magnify the productivity, so any alteration in production must be carefully studied and fundament at a scientific level. Thus, this work has the objective to confirm, in commercial conditions, some results provided by previous studies (Villamizar et al., 2011).

One of the common practices in aquaculture, namely, in intensive production systems is the light manipulation. This allow an aquaculture facility to increase productivity, either by the rise of FCR or by the increasing the reproduction cycles (Fernández-Díaz et al., 2001, Szisch et al., 2002, Pedro Cañavate et al., 2006, Blanco-Vives et al., 2010, Blanco-Vives et al., 2011, Villamizar et al., 2011).

However, this manipulation is normally focused by the light intensity and photoperiod; regarding to spectrum of light, few studies were made. Thus, insufficiently is known about the spectrum requirements among the species that are produced in aquaculture nowadays (Szisch et al., 2002, Blanco-Vives et al., 2010, Blanco-Vives et al., 2011, Villamizar et al., 2011). Water filter a great variety of spectrums with the increase of depth. At deeper levels (60 m), the remaining spectrum available has the wavelength of 435-500 nm, which correspond to the blue light. So in aquaculture conditions it is expected that the light spectrum can influence the productivity as well, depending on the species (Szisch et al., 2002, Blanco-Vives et al., 2010, Villamizar et al., 2011).

The physiological importance of the light during development stages, such as larval stage, it is not entirely understood. However, it is known that light properties play a fundamental role in many physiological aspects. In larval stages, light properties influence the development, even when the retinal photosensors are under development. Another organs that are sensitive to these properties, like the pineal organ and deep brain photoreceptors. These organs interfere with the secretion of hormones, like melatonin, and eventually influence the development of the animals. Thus, these aspects can be used towards the improvement of aquaculture production (Blanco-Vives et al., 2010, Blanco-Vives et al., 2011, Villamizar et al., 2011, López-Patiño et al., 2013).

In the case of aquaculture of benthonic species, such as *Solea senegalensis*, this factor can be even more relevant. Since, in the natural habitat this animals receive light that has been greatly filtered. Thus, the alteration of the induced spectrum in production in benthonic species can implicate the increase of productivity.

The larvae of *Solea senegalensis* are pelagic until they perform metamorphosis, which normally occur until 15 days post hatching (Dinis et al., 1999, Pedro Cañavate et al., 2006, Blanco-Vives et al., 2010, Blanco-Vives et al., 2011). In this species there are studies that related light properties with the development at this stage, for example in one study made by Pedro Cañavate et al. (2006) larvae of *Solea senegalensis* had a survival rate of 81% when implemented a photoperiod of 10D:14L (10 hours dark: 14 hours light). Another study, related with egg incubation and larvae development, showed that under a spectrum of blue light (463 nm) the incubation rate and larvae development had better performances comparing to other spectrums (Blanco-Vives et al., 2011). Thus, the objective of these experiments was to assess the development of larvae of *Solea senegalensis* under two different spectrums, in commercial conditions in Safiestela S.A.: Blue (435-500 nm) and the spectrum used according the company protocols. Two experiments were performed. The second one was attempted to confirm the results by the preliminary experiment, so in the next chapters to facilitate the analysis these will be separated.

The preliminary experiment has served to test the experimental design and the techniques inherent to the experiment.

4.2 Materials and methods of the preliminary experiment

For the preliminary experiment, there were four tanks with 2.7 m^3 of volume and with approximately 45,000 larvae in each. There were two groups, one was the control (spectrum used according to Sea 8 protocol) and was constituted by two of the four tanks, and the other two tanks were under the treatment light (blue light 435-500 nm). The holding conditions for all tanks were in accordance with the Sea8 protocols, with the exception of the light properties. The tanks of each group were separated in different rooms. However, the treatment group was in a room with other production tanks with other light spectrums, and the tanks were not isolated due to production restrictions.

Nonetheless, this first experiment was carried on, in order to adjust the techniques and the experimental design.

The larvae used were from distinct breeders. For the control group, the larvae were from the same spawning and the larvae used in the treatment group were from two different spawnings, from different breeders.

Regarding the light properties induced, the brand of led lights used for the treatment group were AQUARAY® model AquaBeam 1000. These were obtained through the Tropical Marine Center (TMC) and was installed one lamp for each tank, at 80 cm from the water surface. While the lights used for the control treatment were defined by the protocol of Sea8 for larvae development. In each tank, it was assessed the light intensity every day with a photometer.

This experiment lasted 13 days, the first measures were in larvae of 3 day post hatching (dph) and the final measures were on larvae with 15 dph. In order to evaluate the larvae development was conducted a sampling of 20 larvae everyday in each tank and consequent assessed their: (1) total length (TL), (2) stage of metamorphosis according to Fernández-Díaz et al. (2001), (3) stomach content and (4) depigmentation. On the 7 and 12 dph it was conducted a supplementary sampling for dry weight analysis, 60 larvae of each tank were collected, then these were passed through hydrogen peroxide to remove any remain salt water, and finally it was crio-preserved in eppendorfs for posterior lyophilization.

The statistical analysis was done with the software IBM SPSS Statistics 22.

4.3 Results

4.3.1 Total length

A linear regression was performed, to compare the group of control and the group of treatment, the next graph (figure 13) show the growth rate of the two groups related to the days post hatching of the larvae. The statistical analysis revealed that, in the control group, the R square was 0.956, and for the treatment group it was 0.941, this result is regarding the evolution of the total length along the time of the experiment, and confirm that the growth of the larvae is higly linked with the passage of time, more than

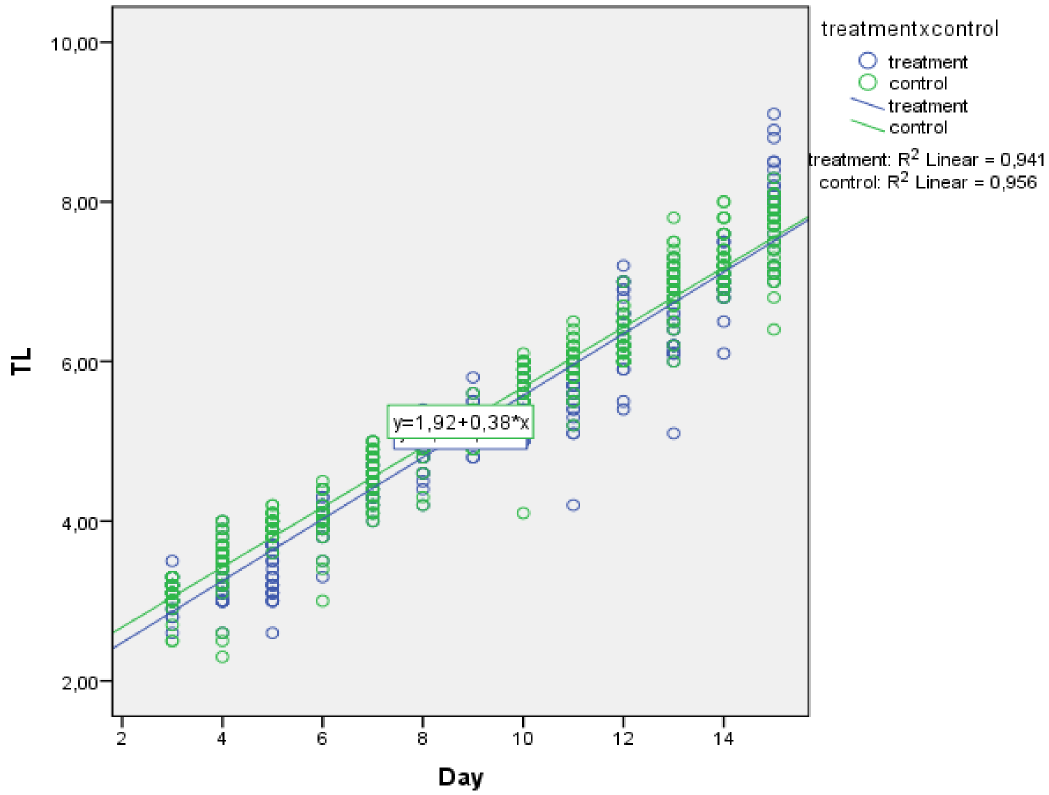


Figure 12 – Relation between the total length and the group.

with any other parameter. Although in the treatment group it seems that this is less correlated.

.

4.3.2 Dry weight

Regarding the dry weight, there were significant differences between the control group and the treatment group ($p<0.05$), as can be observed in the next graph (figure 14). The larvae belonging to the control group showed lower average values regarding the dry weight (0.205 mg and 0.671 mg on the 7 and 12 dph, respectively) comparatively with the treatment group (0.241 mg and 0.747 mg on the 7 and 12 dph, respectively). The R square of the control group was 0.900, and this value for the treatment group was 0.805, these values explain the linkage between the dry weight with the passage of time. For this parameter, the larvae from the treatment group appear to have been more influenced by the environmental conditions, than the larvae from the control group.

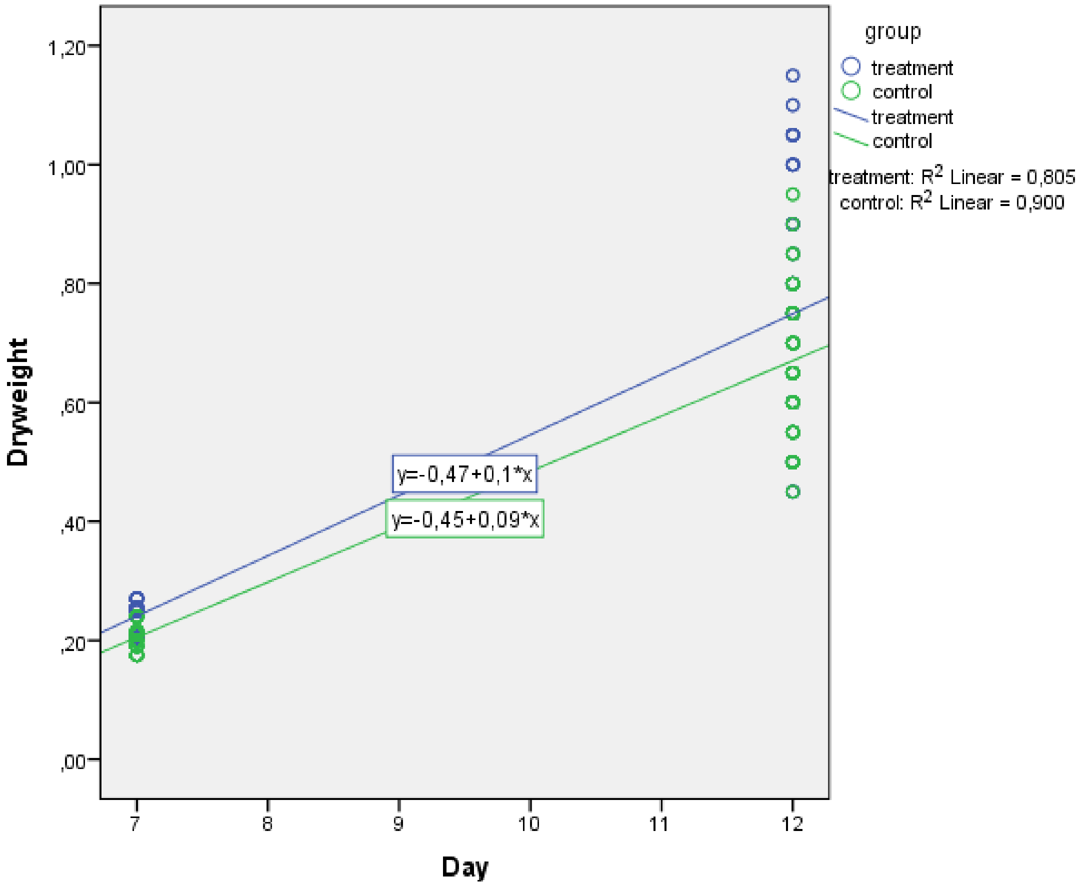


Figure 13 - Dry weight of the larvae from the two groups.

4.3.3 Depigmentation

Another parameter that was assessed during the experiment was the amount of larvae that shown anomalies in pigmentation, namely, depigmentation, it was evident that the larvae under the treatment presented a higher percentage of anomalies (41%), comparatively with the control group (11%). Nevertheless, the difference between groups had the tendency to dissipate, with time (figure 15). The results from the ANOVA test revealed that this difference was significant ($p<0.05$).

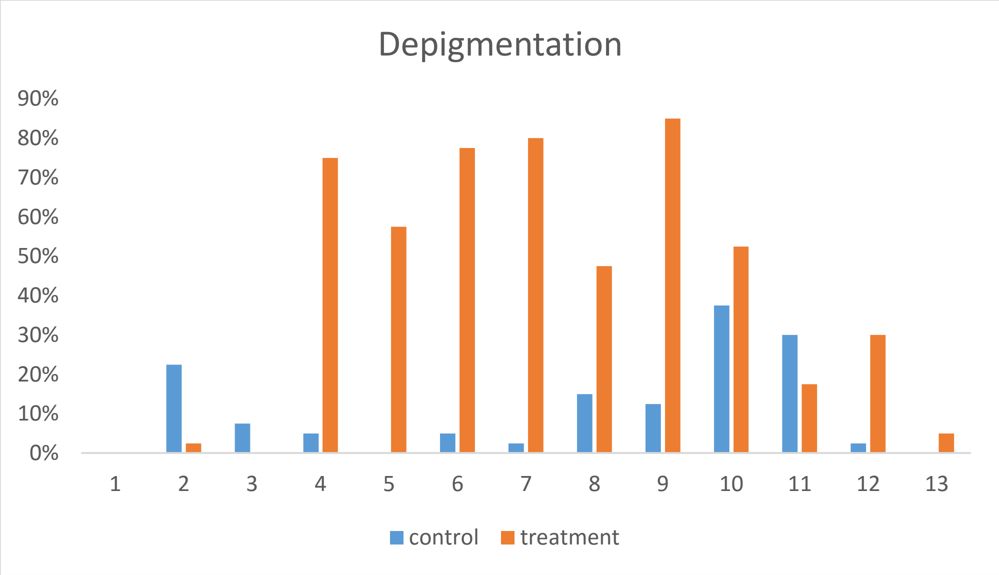


Figure 14 - Percentage of depigmentation from each group.

4.3.4 Metamorphose stage

The stage of metamorphosis between the two groups was significantly different ($p<0.05$) when performed the ANOVA test. Tanks with the treatment light had a faster development, comparing with the tanks in the control group (figure 16, 17, 18, 19 and 20). This is more marked in figure 17 related with the stage 1, in this case, was observable larvae from the treatment group at the second day of the experiment. In the control group the same stage of development appeared on the sixth day of the experiment.

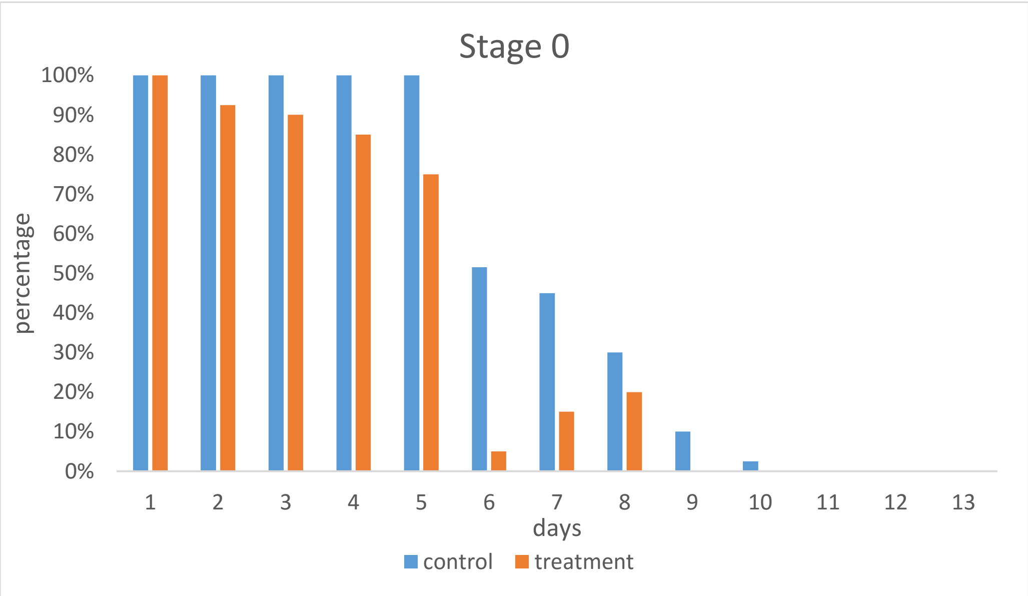


Figure 15 – Percentage of larvae on the stage 0 of metamorphosis.

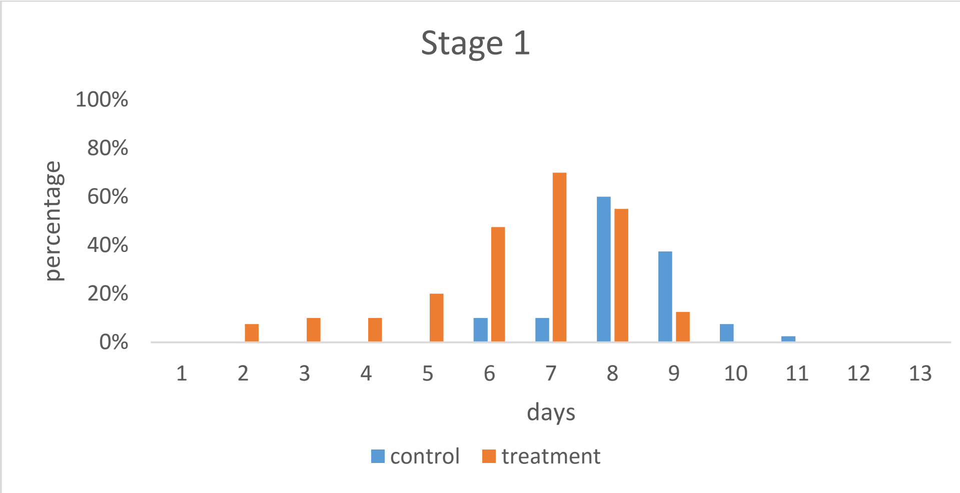


Figure 16 - Percentage of larvae on the stage 1 of metamorphosis.

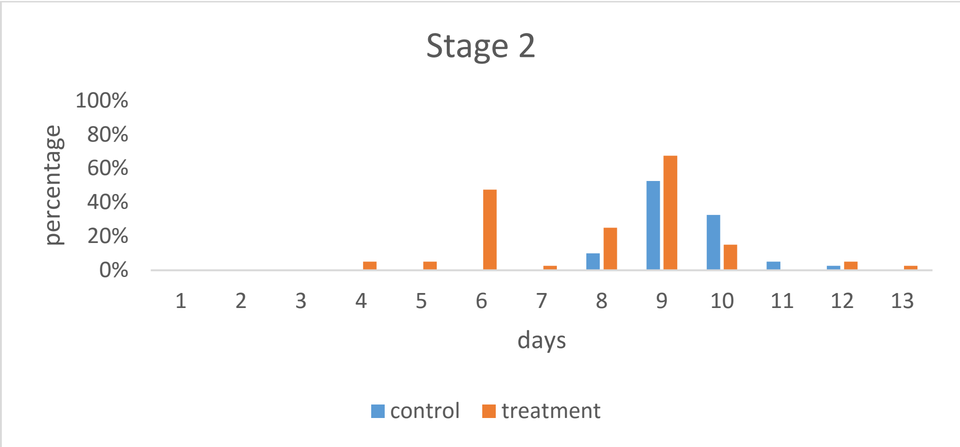


Figure 17 - Percentage of larvae on the stage 2 of metamorphosis.

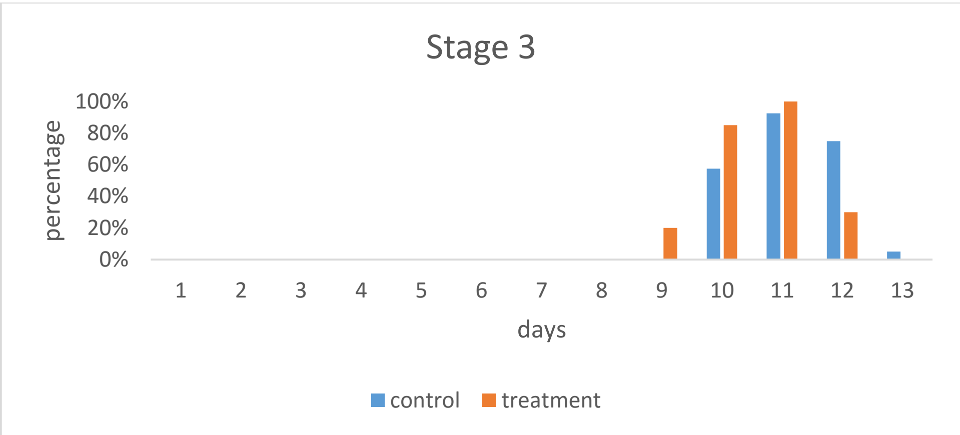


Figure 18 - Percentage of larvae on the stage 3 of metamorphosis.

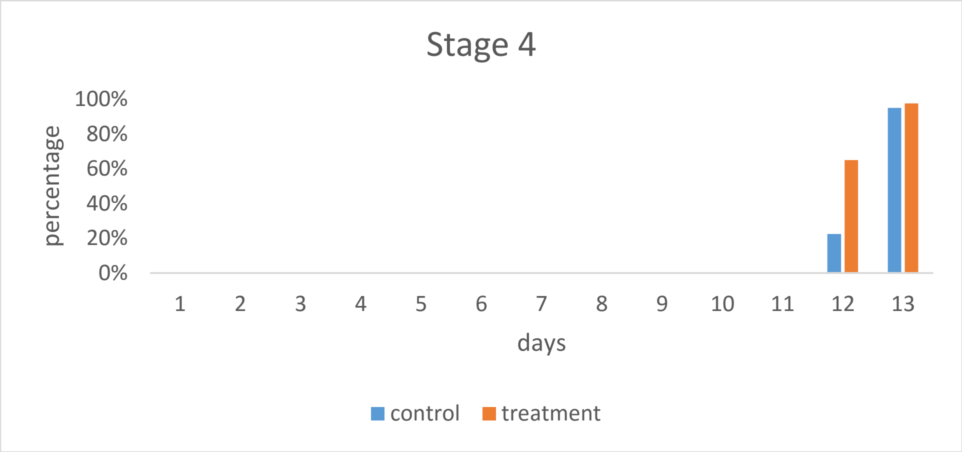


Figure 19 - Percentage of larvae on the stage 4 of metamorphosis.

4.3.5 Stomach content

In all tanks it seems that the presence of stomach content fluctuated with the same pattern. Although, the treatment group showed a delayed (figure 21). The statistical analysis did not reveal any significant differences between the groups, according by the ANOVA test ($p>0.05$).

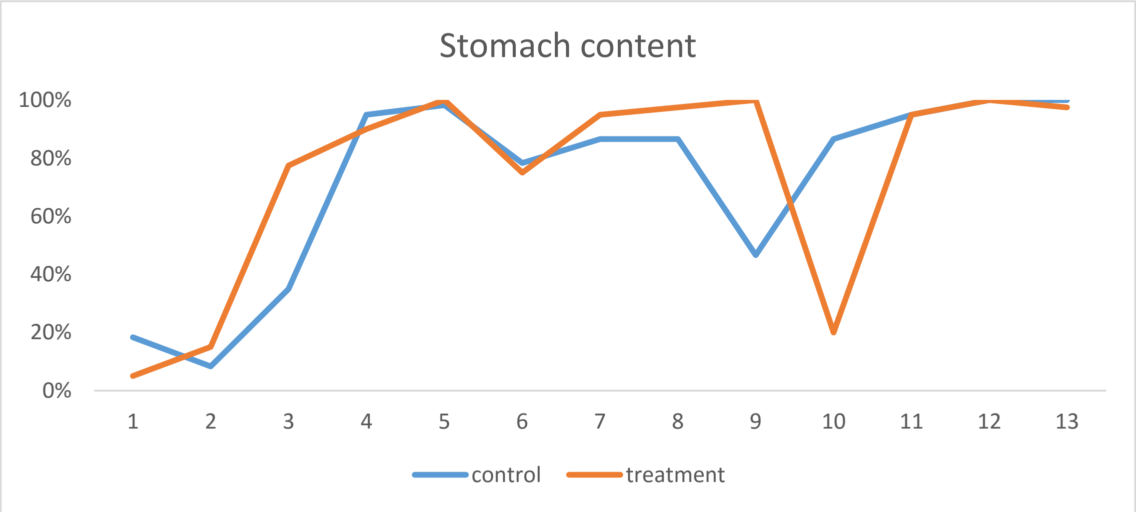


Figure 20 - Percentage of larvae with stomach content, comparing the two groups.

4.3.6 Light intensity

There were significant differences between the two groups when performed ANOVA test, regarding the light intensity ($p<0.05$). The tanks from the control group had a light intensity higher (average of 1027 lux), than the tanks from the group of treatment (average of 621 lux).

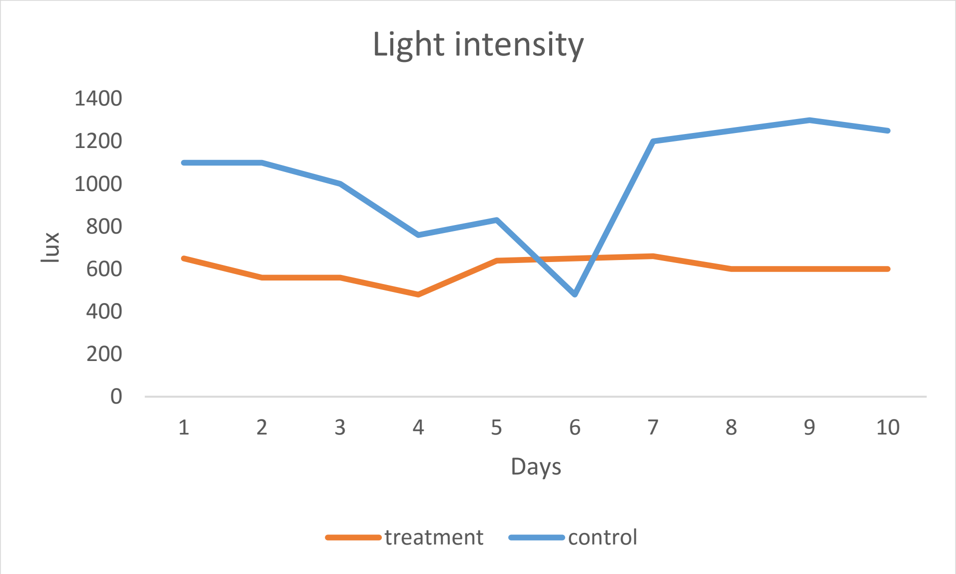


Figure 21 - Light intensity in each tank during the experiment.

4.4 Discussion

This experiment was an attempt to replicate some results described by Villamizar et al. (2011), and serve as well to amend the experimental design.

In the experimented presented in this work the results were not conclusive, due to the number of variables that the experiment had. For instance the light intensity between the control group and the treatment group differ, and the results were probably, influenced by this. The treatment group had low light intensity because it was used only one lamp per tank; additionaly, the type of lamps used was designed for tanks with small diameters (one lamp per 0.2-0.3 m^2), and the production tanks had a diameter larger than the recommended, this affected the distribution of light and consequently the distribution of the live feed. Since, the *Artemia spp,.* that was used as feeding during part of the experiment, has a positive phototaxis and were concentrated in the center of the tank where the light intensity was higher. This may induced an additional energy expenditure for the larvae, in order to find the feed.

Another fact that may have affected the development of the larvae in the tanks under the blue spectrum, was the light interference, due to the ack of isolation in the tanks under blue spectrum, since these tanks were in the same room with other tanks of the production. Hence, other light spectrums may have interfered in the tanks under treatment.

It was not possible to amend this problem during the experiment, because, under production, it is quite challenging manipulate any holding conditions without affecting the productivity of the facility.

Since, larvae from the two groups were not from the same breeders and spawning, this was another factor that influenced the results. Because different breeders, and even different spawning from the same breeders, can influence the quality of the eggs and larvae (Dinis et al., 1999, Guzmán et al., 2009).

It was not possible to amend this problem during the experiment, because, under production it is very challenging manipulate any holding condition without affecting the productivity of the aquaculture facility.

Despite these facts described above, in general, seems that the development of the larvae was positively affected by the blue spectrum, namely, when analyzed the dry weight and the metamorphosis stages.

Regarding the depigmentation, this parameter presented the highest percentages in the tanks under the blue spectrum. And as in other species like in *Pagrus pagrus*, this can be related to better holding conditions and better welfare conditions (Szisch et al., 2002, López-Patiño et al., 2013). Although, this fact is not confirmed for the *Solea senegalensis*, the larvae under blue spectrum presented a faster development in the metamorphosis stages, possibly corroborating the better holding conditions in these tanks. In both groups the larvae transited from pelagic to benthonic stage at the 11 dph, and were then transferred to other tanks with different light regime, so the measure of the light intensity ended on that day.

To confirm the results from this experiment, it was important to revise the protocol, in order to amend some flaws from this preliminary work.

Nonetheless, in this experiment seems like the blues spectrum beneficiate the development of the larvae, due to the faster development (figure 16, 17, 18, 19 and 20) and the higher dry weights (figure 14). These results can be due to better holding conditions that promoted higher synthesis rate of hormones (Daniels et al., 1996, Szisch et al., 2002, Blanco-Vives et al., 2011, Villamizar et al., 2011, Wunderink et al., 2011, López-Patiño et al., 2013), and/or by higher rates of feeding. Since, the live feed administered could be more visible in tanks under blue light, however, no significant differences were found when analyzed the stomachs content (figure 21).

4.5 Materials and methods of the second experiment

In this second experiment, it were used three tanks with a volume of 2.7 m^3 each, the same type of tanks as the preliminary experiment. However, in this case, two of the three tanks represent the control group, and the third one was the group of treatment. This last tank was properly isolated to avoid any light interference from other tanks in the production and, this time it was used two lamps with blue spectrum, (435-500 nm) from the same brand and model (AQUARAY® AquaBeam 1000), these were installed at 80 cm from the water surface.

In the tanks under control, the spectrum used was according to the protocol from the Sea8. The density in each tank differed from the first experiment. There were 36,000 larvae per tank, and the larvae in all tanks were from the same breeders. The rest of the holding conditions were similar to the first experiment (according to the Sea8 protocol).

In this experiment the larvae were monitored since the day two post hatching until the 12 dph, 3 samplings were done during this time: On the first day (2 dph); on the fifth day (7 dph); and on the tenth day (12 dph). In each sampling 60 larvae per tank were collected and crio-preserved in eppendorfs for posterior lyophilization. However, in this sampling it was measured the total length and assessed anomalies in pigmentation, thestomach content and the metamorphosis stage from 20 larvae from each thank.

The statistical analysis was realized with the software IBM SPSS Statistics 22.

4.6 Results

4.6.1 Total length

The total lengths evaluation, as for the other parameters, was done in 3 distinct phases: 2 dph, 7 dph and 12 dph. Thus, it was assessed the evolution in each tank through a linear regression analysis (figure 23). The next graph shows the total lengths of all larvae from each tank in every sampling point.

No significant differences were found ($p>0.05$), between the control group (2.84 mm at 2dph, 5.12 mm at 7 dph and 7.26 mm at 12 dph) and the group of treatment (2.81 mm at 2dph, 4.97 mm at 7 dph and 7.35 mm at 12 dph). The R square values are 0.952 (treatment) and 0.954 (control), they are very similar and explain the evolution of total lengths through time, revealing that the development was highly correlated with the passage of time.

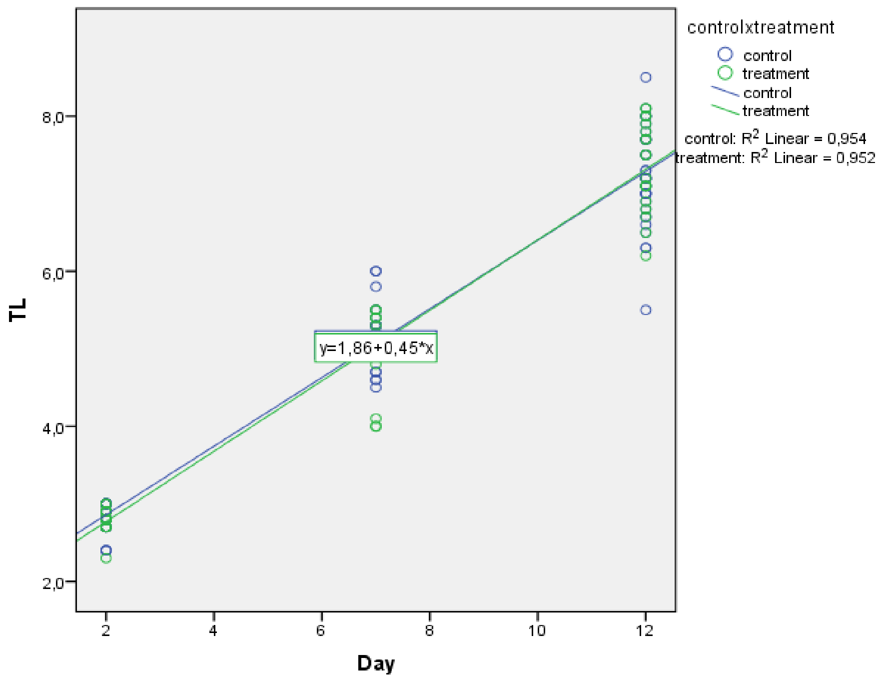


Figure 22 - Linear regression of total lengths (TL) from each group.

4.6.2 Dry weight

For statistics analysis of the dry weight, it was also done a linear regression analysis (figure 24). There were no significant differences between the dry weights of the larvae from the two groups (means of the control group were: 0.035 mg at 2 dph, 0.428 mg at 7 dph and 0.738 mg at 12 dph; means of the treatment group were: 0.035 mg at dph, 0.427 mg at 7 dph and 0.747 mg) (p>0.05). The value of R square is higher in the treatment group (0.885) while this value in the control group was 0.835.

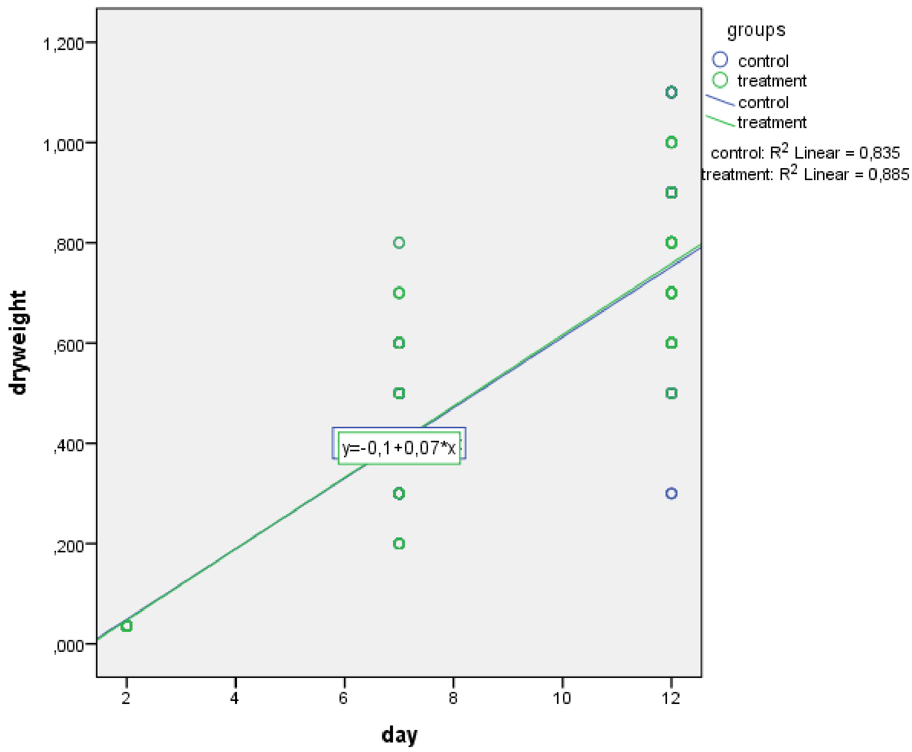


Figure 23 - Linear regression of the dry weights from each group.

4.6.3 Depigmentation

Regarding depigmentation, there were significant differences between the control group and the treatment group ($p<0.05$). In this case, it was done an ANOVA test to compare both groups and tanks. In figure 25 it is noticeable that the larvae that were under treatment presented higher rates of depigmentation: 0% at 2 dph, 80% at 7 dph and 50% at 12 dph. While, the control group had lower percentages: 2.5% at 2 dph, 15% at 7dph and 7.5% at 12 dph.

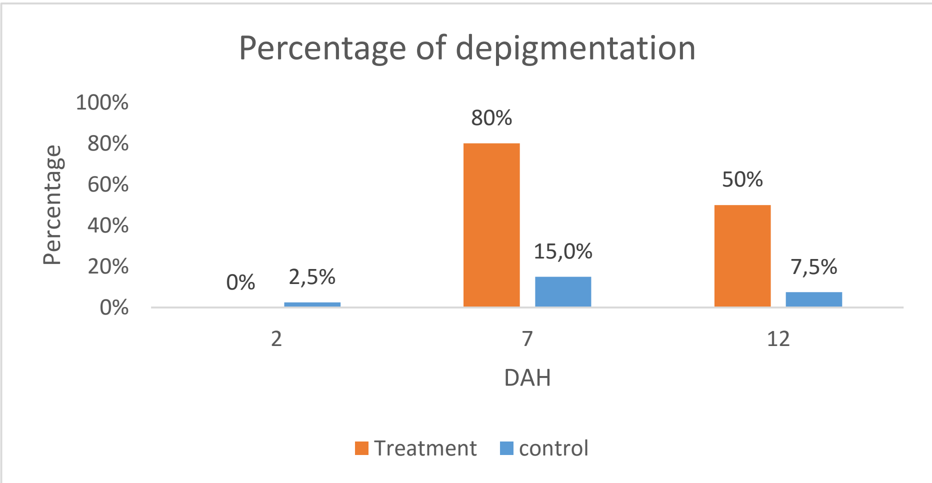


Figure 24 - Percentages of depigmentation of each tank.

4.6.4 Metamorphose stage

In this experiment, there were no significant differences between the larvae from the two groups ($p>0.05$), when performed the ANOVA test. It appears that the blue light had not effect, in this parameter, when comparing to the control group (figures 26, 27 and 28).

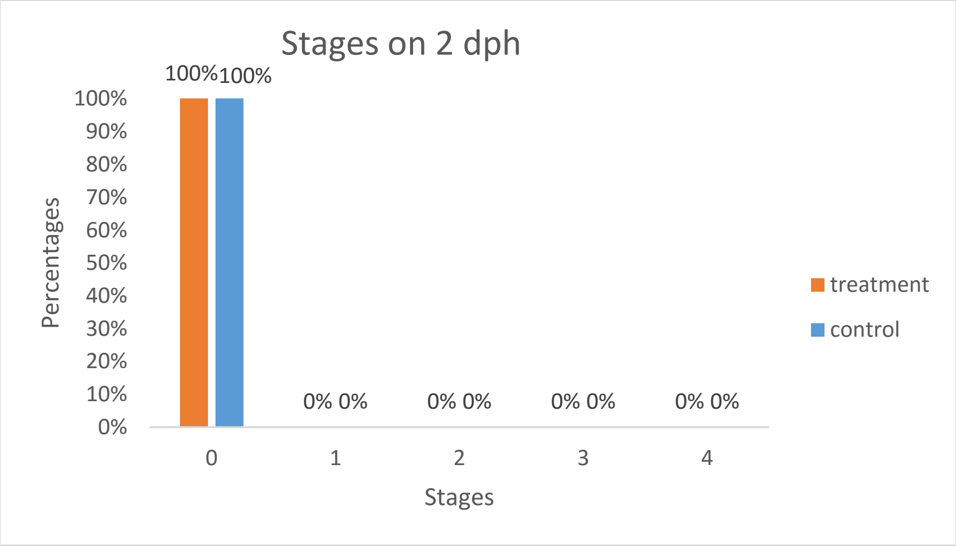


Figure 25 – Percentage of larvae from each group in each stage of metamorphose, from each group at 2 dph.

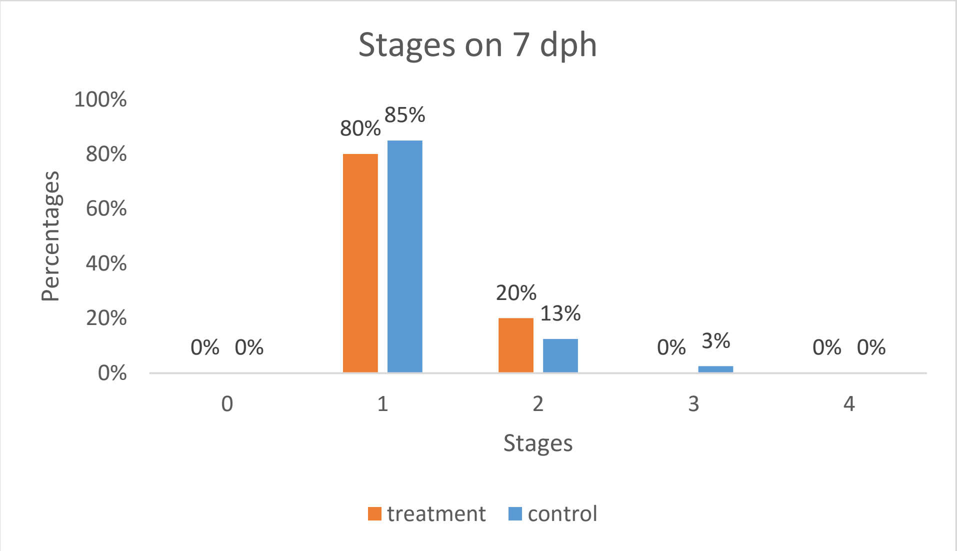


Figure 26 - Percentage of larvae from each group in each stage of metamorphose, from each group at 7 dph.

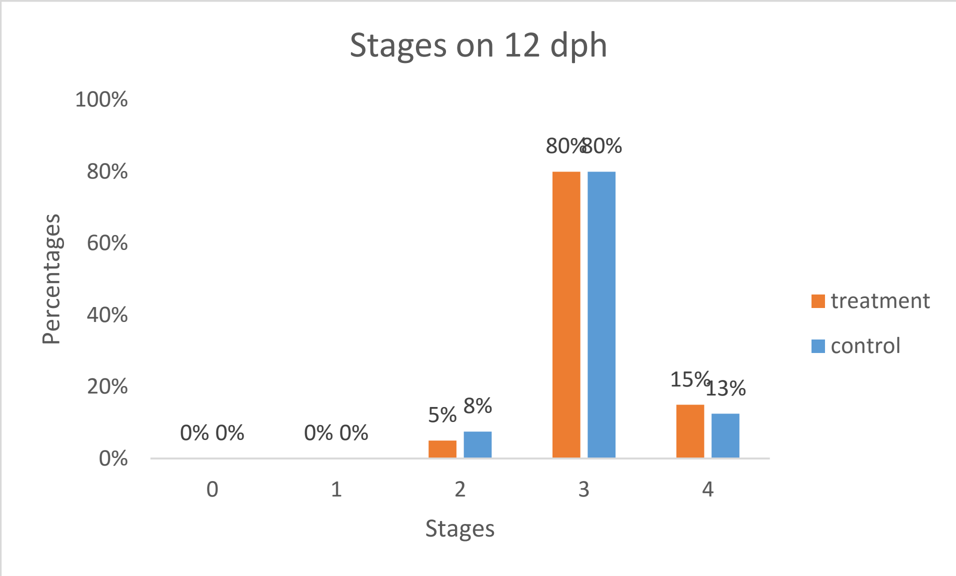


Figure 27 - Percentage of larvae from each group in each stage of metamorphose, from each group at 12 dph.

4.6.5 Stomach content

Regarding the stomach content, no significant differences were present between the control and treatment groups (p>0.05). On the first day of experiment (2 dph) the larvae were not feed, so there were any stomach content (figure 29). On the other days of sampling the stomach content was present in the majority of the larvae from the both groups (the percentages at 7 dph were, 100% in the treatment group and 98% in the control group; in the 12 dph they were 100% in both groups).

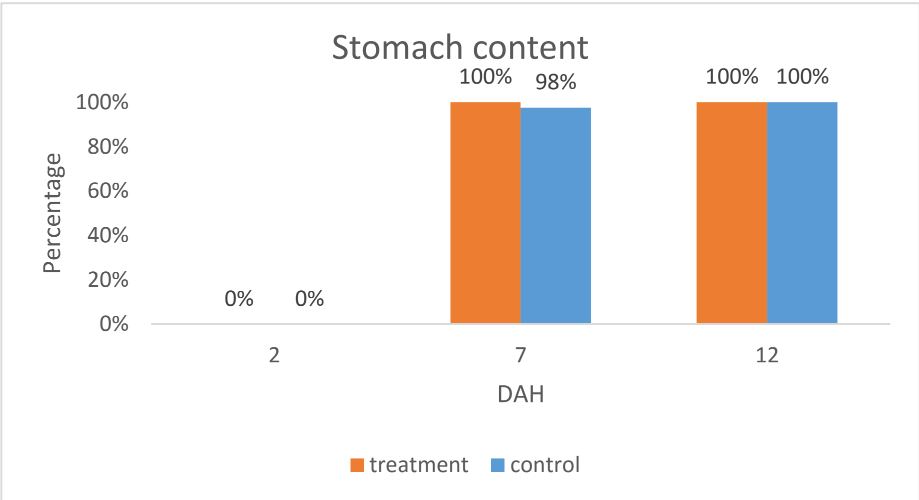


Figure 28 - Percentage of larvae with stomach content in each group at the time of the samplings.

4.6.6 Light intensity

During the experiment, the light intensity was measured in the same days as the samplings, and there were significant differences between the two groups (p>0.05), when performed the ANOVA test, in figure 30 this is also noticeable. The group of control had higher light intensity (mean of 1667 lux), as in the first experiment. However, in the treatment group the light distribution was more homogenous and the intensity slightly higher too (760 lux), than in the preliminary experiment (621 lux in average).

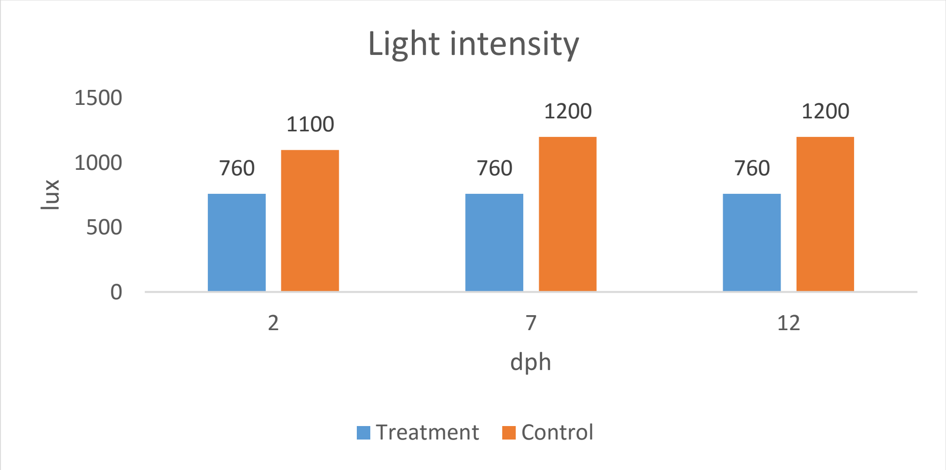


Figure 29 - Light intensity at the time of each sampling in each group.

4.6.7 Relation between dry weight and total length

Due to an improvement in the protocol, in this experiment, another measurement was performed. In this work, a correlation test between the total length and the dry weight was conducted, in order to understand the relation between the two parameters. Therefore, a Pearson's correlation test with these variables was done, and the results were similar (in the treatment group it was 0.931, and in the control group was 0.926). To perform such evaluation it was necessary to identify the larvae and their TL from each day of sampling, and consequently measure their dry weight.

4.7 Discussion and conclusions

In this second experiment, it was attempted to improve the protocol, in order to surpass the interferences that were noticeable in the preliminary experiment. The improvements covered: (1) the isolation of the tank under the blue spectrum, to avoid light interference from other production tanks ; (2) the installation of two lamps in the tank with blue spectrum, to improve the light distribution and increase the light intensity; (3) Use of larvae from the same breeders and the same spawning; (4) Reduce of samples to 3 moments, this was implemented since it was determined that this would not compromise the statistic value; (5) And in each sampling 20 larvae were measured and it was assessed all other parameters described already. Posteriorly, it was assessed the relation between the dry weight and total length, in order to provide an explanation to the results in the preliminary experiment, which the t groups had no significant differences in the total length, but there were significant differences in the dry weight.

In this second experiment, the majority of larvae started metamorphosis on the 12 dph.

The total lengths of the larvae did not showed significant differences ($p>0.05$) between the groups. Other parameters that did not differ, were: (1) the dry weight; (2) the metamorphosis development and (3) the stomach content. These can be the result of the alterations in the protocol for the experiment, which eliminated part of the variables that were present in the first experiment.

On the other hand, despite these alterations, in the other parameters there were significant differences between groups (depigmentation and light intensity). As explained in the preliminaryexperiment, the depigmentation could be an indication that, under blue spectrum, the larvae are in better holding and better welfare conditions. Which allow a better development either by endocrine production of hormones, either by better rate of feeding (Daniels et al., 1996, Szisch et al., 2002, Blanco-Vives et al., 2011, Villamizar et al., 2011, Wunderink et al., 2011, López-Patiño et al., 2013). Since, the blue spectrum is the one that can penetrate deeper into the water column, this could provide an enhancement in the visualization of the preys by the larvae at some point of the larvae development.

Some problems with the design experimental were noticed. Perhaps in this work, it was important to implement more tanks in each group, to increase the value of the statics results. However, a good experimental design it is always very challenging under production conditions, since the productivity of the company is probably affected by minor alteration in the facilities due to the fragile dynamics.

Concluding, this second experiment had some results that are corroborated by the first study. However, the implementation in production of these light regime is not justified, according with the results from these two works. Despite that, it is required further works to understand how different spectrums could affect the larvae development in this species. As suggestion, these studies could evaluate the hormonal production to assess if different spectrums influence the sinthesys of hormones related with growth and welfare.

Chapter 5: Internship report in the Safiestela S.A.

5.1 Introduction

The Safiestela, S.A. belongs to the international group Sea8, with the Aquacria Piscicolas, S.A., and it is located in Estela, Póvoa do Varzim. This facility is responsible for producing juveniles of *Solea senegalensis*, while the ongrowing part is performed in Aquacria Piscicolas, S.A. located in Torreira, Aveiro.

Although, primarily, these facilities were building to produce *Psetta maxima* (turbot), due to a recent restructure the production of Senegalese sole is now the only species that is produced. This restructure in the facilities was owed to a market saturation in the production of turbot. Thus, this create an opportunity to produce a species that has a great value, but its production was scarce. The restructure allowed the facilities to implement new technologies that improve productivity, creating one of the most advanced facilities in Portugal.

One of the Sea8 goals is to be the main producer of Senegalese sole in the Iberian Peninsula, to achieve that, it is required to close the production cycle.

The help of investigation works is crucial to increase productivity, relying on that, the Sea8 is permanently investing on projects, one example of that is the implementation of the FWAS plan mentioned already in this work.

To improve the production and to close the production cycle the majority of the projects are realized in the nursery (Safiestela, S.A.). This part of the company produce juveniles in RAS and in raceways, this allows a higher control of the environment conditions.

The production of eggs is performed during all year by regulated the thermo and photo periods. The fertilized eggs are only produced by wild stocks, and this represents one of the bottlenecks in the production of this species.

Regarding the structure of the company, this is divided into several parts: (1) broodstock area; (2) incubation room; (3) larvae room; (4) live feed room; (5) Weaning area; (6) and pre-ongrowing area. These different areas are described in the sections bellow, as long the procedures performed in each one. In annex one it is a scheme describing all the production cycle.

5.2 Broodstock area

In the case of this company, the broodstock is divided into four different sub-parts or rooms, in each one it is manipulated the photoperiod, water temperature and the feed. These manipulations create a specific environment that simulate a season. Thus, the rooms are labeled as: summer, autumn, winter and spring. All rooms are integrated into the RAS, with the exception of the summer room, which is the only one that is in flow through.

The breeders are obtained from wild stocks or extensive and semi-intensive aquacultures, outside of the spawning season, due to that it is difficult to the animals obtained to acclimatize to the new environment. For that, and for sanitary reasons, it is required that the animals pass through a quarantine and acclimation phase.

This phase is done in a particular part of the company, where the animals are in square tanks in a flow through system, to avoid any possibilities of contamination on the production. At this stage neither the photoperiod, neither the thermoperiod are controlled. The acclimation process normally last six months, during these it is made a sampling (to assess the fishes health state), tagging (this allow traceability) and habituation to inert food pellets (nonetheless, while the animals are not habituated to the artificial feed it is necessary administrate live feed, such as polychaets worms and molluscs). To identify the gender of each animal two methods can be used: (1) it can be assess manually or (2) through hormonal tests.

In *Solea senegalensis* aquaculture, the necessity to capture wild animals for broodstock represent a bottleneck. During the past years there were done efforts to surpass this obstacle, but with no success yet. The inability to produce fertilized eggs from animals that are descendent from the wild breeders represent the main cause, as well as the lower production of sperm by the males (Dinis et al., 1999, Imsland et al., 2003, Guzmán et al., 2009, Morais et al., 2014).

When the breeders pass the acclimation phase, in other words, when the animals survive and are in good conditions after the six months, they can be integrated into the production rooms (mentioned above). Each room is composed by three a five square tanks, each one has an egg collector that harvests the eggs automatically. The egg collector is installed at the exit of the tank and restrain the eggs.

To assess if the eggs are fertilized it is done a floatability test, in which the eggs are placed in a recipient with salt water (with approximately 32‰ of salinity), the eggs that float are viable, the eggs that sink are unviable (Dinis et al., 1999, Neufeld et al., 2011). The viable eggs are going to the incubation room, as for the others, these are discarded.

There are others husbandry operations that are fundamental to the animal welfare and productivity, these are: (1) feeding, which required a good feeding management, to guarantee the productivity of the company. It is admnistered live (molluscs and polychaete worms) and inert feed, the formulation of the inert feed is done in this part of the facility, according with the Sea8 protocol; (2) cleaning, this is a significant step, since cleaning the tanks and equipment is crucial. However, the cleaning of the tanks with animals must not take too long, because this can increase stress in the animals to unacceptable levels and compromise the productivity (van de Vis et al., 2012, Mondal et al., 2013, Volpe et al., 2013, Boison and Turnipseed, 2015). The cleaning of the rooms and equipment it is done everyday when all the other routines are performed. Ocasionally, the sand filters (correspondent to this area) need to be wash, this is done by reversing the water flow, which will remove particles and decompress the filter to maintain its functionality. Regarding the rest of the recirculating system, it is done routines checks to assess the proper function of the ozone generator, UV (ultraviolet) filters, water pumps and heat exchangers; (3) the assessment of the animal behavior and condition, this it is done mainly through observation to cause the minimum disturbance on the animals (van de Vis et al., 2012, Mondal et al., 2013, Volpe et al., 2013, Boison and Turnipseed, 2015). In this case, it is important to evaluate the feeding behavior, to guarantee that the animals are expressing normal behavior under production. The reproduction behavior is assessed during this step, this help the management to improve the protocol to manipulate the environment conditions. The observation of the fish condition can help the production to assess if the animal is healthy or if there are anything that compromise their health (like pathogens). Additionally, this observation helps to evaluate if the fish are with gametes or not (this case is only observable in the female, where the production of gametes produce a swelling on the lateral side); and (4) water analysis, this englobes the verification of temperature, salinity, oxygen, ammonia and nitrites.

5.3 Incubation room

To initiate a production cycle, it is necessary that the breeders produce enough fertilized eggs. Thus, it is necessary two conditions: (1) the breeders need to produce fertilized eggs; (2) the management plan needs to be synchronize (the fertilized eggs production with the productions cycles already ongoing in the facility). These two conditions help the aquaculture to maintain productivity and to safeguard the animal welfare.

If everything goes as expected, the viable eggs will go to the incubation room, where they will be distributed to provide each tank with the expected biomass (according with the Sea8 protocol), this estimation is done through the weight of the eggs (according to the Sea 8 protocol).

The tanks are cylindrical with a cone bottom to facilitate the posterior remove of the eggs and larvae, at the transfer to the larvae room. Regarding the environmental conditions, the tanks are integrated in a flow through system and need constant air circulation. This is provided by air diffusers, and the air caudal needs to be sufficient to provide a current, to prevent the accumulation of eggs or larvae in the bottom of the tanks. However, if the flow is too high, there could be damages in the larvae.

The temperature of each incubation tank needs to be, as close as possible to the tanks where the eggs came from, in order to avoid any thermal shock, normally the temperature is about 20º C. Eggs are incubated in light absence, since during this period the light can be harmfull.

The hatching of the eggs occur passed 48h, and the larvae remain in the incubation room until the 2 dph, after this time the animals are transferred to the larvae room.

Despite the high resilience of the Senegalese sole larvae, the transference procedure needs to be careful to avoid any harm. This process is done firstly by harvest the larvae that are nearest the surface, since the larvae are pelagic until perform metamorphosis, with a beaker like recipient and then by the filtering the reminiscent water in the tank. This filtering is done with a bucket like equipment with a filter in the middle,to prevent the fouling of the filter it is required to provide turbulation, this is done with a air diffuser that helps to detach any larvae or debrist.

Each incubation tank normally provides larvae for two larvae tanks. In order to distribute an equal number of larvae between tanks it is required to transfer an equivalent volume for each tanks. For example, when the worker transfer one beaker of larvae to the larvae tank it needs to transfer the

beaker to the other tank.

5.4 Larvae room

Once the transference is complete, the larvae will have different holding conditions from the incubation room. For start, the volume of tanks in this room is higher (2.7 m^3 each) and the amount of larvae per tank is lower (according with the Sea8 protocol). The tanks are cylindrical with a conic bottom.

Regarding the light regime, the larvae will be under a photoperiod of 8D:16L with higher light intensity in the first days (as estipulated by the protocols of Sea 8). In each tank the temperature will vary between the 18-20º C and the oxygen saturation between the 90-100%. In order to maintain the oxygen levels in each tank an air diffuser is permanently operating. The concerns about this rely in the caudal that shall be used, as with what happens in the incubation tanks, if the air flow is too much it will damage the larvae, while if it is too low it will not provide the turbulence necessary to distribute the larvae and the live feed homogeneously, leading to poor environmental conditions and compromising the production cycle.

About feeding concerns, the larvae present diurnal behaviors so they are fed when the light conditions are favorable. Firstly, they are fed with rotifers, and after a few days they are fed with brine shrimps (according with the Sea8 protocols). At this stage, it is crucial to administrate feed that will fulfill the larvae requirements. Since, the larvae rely on visual perception to predate the live feed, it is fundamental to provide the proper environment conditions, this can be achieve by produce larvae in green waters (this enhance the contrast of the preys with the surround environment) (Villamizar et al., 2011). Another requirement rely on the size of the preys, these need to have the proper size for the mouth size of the larvae (Villamizar et al., 2011).

The quality of feeding is essential to provide proper development and to increase ultimately the productivity. Since, it is at this point that the future development will be more influenced, in other words, if the larvae are in good conditions, this will promote an increase in productivity, if not, the productivity will face more challenges, and their future development will be slower and more challenging. Due to that, the larvae development must be followed, every day three to six larvae are sampled per tank, in order to assess their total length, stomach content and anomalies. These samplings provide valuable information to the production management, such as: if the larvae are feeding properly (since adaptation to the feed can be problematic), if there is any tank that has pathogens or any other problems in the holding conditions.

Another routine that is essential in this stage is the water parameters control, namely, the temperature and oxygen. Regarding other parameters, these include ammonia, nitrites, salinity and pH. To ensure the proper levels in the various parameters it is periodically done the filters maintenance (backwash of the sand filter and verification of the UV filters).

The tanks cleaning is performed in a non-intrusive way, since, the tanks are integrated into a flow through system the metabolites and any dead animal are eliminated with the water flow (the caudal used is stipulated by the company protocols, and it has to be higher enough to properly renovate the water and lower enough to not disturb the larvae).

When the larvae starts to perform metamorphosis (10-13 dph) they will sink in the tank and became benthonic, posteriorly it will be transferred to the weaning area. This process is very similar to the transfer between the incubation room and the larvae room. Thus, in this case, the larvae will be siphoned from the bottom of the tank, to a bucket like equipment with a filter in the center. When all the larvae are restrained, they are distributed between, usually, two weaning tanks. To guarantee that the distribution is equivalent between weaning tanks, half of the volume of the bucket like equipment shall be distributed in each tank (since the larvae are homogenously distributed in the bucket, due to the turbulence provided by an air diffuser).

5.5 Live feed room

Regarding the live feed production, this aquaculture is equipped with the necessary means. Since, the larvae are fed with live feed it is required that this production is synchronized with the larvae cycle, to fulfill the larvae feeding requirements (such as the size of the feed). The feeding of larvae is done manually and it is required to prepare enriched live feed in the morning for the rest of the day.

This production area is sub-divided in two parts/rooms, being one focused in the rotifers production and the other in the artemia production.

5.5.1 Rotifers room

Rotifers are produced in cylindrical tanks with a conic bottom to facilitate husbandry operations. Concerning about the parameters that need to be followed, the rotifers are maintained at a temperature of 28º C with a salinity level similar to the fish production conditions and an oxygen saturation of 80%. The oxygen is provided by air diffusers that, as in the larvae and incubation room, need to have a particular flow (according to the Sea8 protocol), to maintain the homogenous distribution of animals and feed.

The feed of rotifers is composed of algae and yeast and it is administrated several times per day (Lavens and Sorgeloos, 1996). However, when the rotifers achieve the proper size for feeding the larvae, it is required to enrich them. This is done by a commercial formula (according to the Sea8 protocol), which has high levels of fatty acids (Lavens and Sorgeloos, 1996).

Routines tasks that need to be done to maintain the cycle of production. It is required: (1) the control of oxygen levels in intervals of four hours; (2) cleaning, equipment and room disinfection and cleaning is required to prevent the development of pathogens that can contaminate the rotifer and fish productions (rotifers are, mainly, affected by bacteria and parasites) (Mondal et al., 2013, Volpe et al., 2013, Boison and Turnipseed, 2015); (3) every day is necessary to assess the biomass, this is done by collecting a sampling and make a count through a magnifier, and estimating the population for the volume of the tank. In this process it can be assessed if the rotifers have any parasite that can compromise the population (Lavens and Sorgeloos, 1996); (4) when the rotifers reach the 3 dph it is necessary to harvest them by filtering, this process is similar to what is done in the incubation and larvae rooms, and (5) conserve the recent enriched rotifers in a cold place (an adapted milkmaid equipment), this lower the metabolism and avoid the loss of properties through metabolization.

5.5.2 Artemia room

The *Artemia spp.* has unique properties that allowed it to become the most used type of feed for larvae in many species.

The production of brine shrimp is done under similar circumstances comparing with the rotifers production. They are produced in cylindrical with a conic bottom tanks, oxygen is provided by the same air system as what happens in the rotifers room, with a saturation of 80%. There are other parameters that are in the same values, when comparing with the rotifers production, these are: the temperature and the salinity . However, there are some differences between the two productions, for instance, in order to maintain the pH at the adequate level (pH = 8), it is administrated sodium hydroxide (NaOH), which prevent the pH level to decrease to unfavorable levels (Lavens and Sorgeloos, 1996).

Normally the hatching of artemia happens in 24h (Lavens and Sorgeloos, 1996) and, to separate the cysts from the brine shrimp it is directed the water through a filtering system that will restrain the cysts, due the action of a magnet field. Since, the cysts obtained were passed through a technique that make possible to restrain them with this method (Naessens-Foucquaert et al., 2009).

Once the filtering is complete, the brine shrimp are relocated in other tank, in these they will develop until they reach the proper size, to feed the larvae. In the preparation to feed the larvae, it is required enrich the brine shrimp with a commercial formula (according the Sea8 protocol) rich in fatty acids (Lavens and Sorgeloos, 1996). This enrichment is crucial to increase the nutritional value of the brine shrimp and, therefore, satisfy the larve nutritional requirements.

Regarding the routines for the production of brine shrimp, these are quite similar to what happens in the production of rotifers: It is required (1) the control of oxygen levels every four hours; (2) cleaning, disinfection and cleaning of the room is required to prevent the development of pathogens that can contaminate the artemia and fish productions; (3) Separate the cysts from the recent incubated artemia, through the technique mentioned previously; and (4) conserve the recent enriched artemia in a cold place (an adapted milkmaid equipment), this lower the metabolism and avoid the loss of properties through metabolization.

5.5 Weaning area

The weaning area is the part with the higher area, and it is composed of 49 square tanks (each tank has a small water column) and another small room (laboratory), where all the water parameter tests from the facility are performed. The area where the tanks are implanted is subdivided into two minor areas (although there is none physical barrier) weaning one and weaning two.

The main difference between this two areas is the type of system in which each one is integrated, the weaning one area is in flow through and the weaning two is in a recirculating system.

The transference from the larvae room is usually done to the weaning one area, this allows the production to make sure that recently transferred fishes will not transmit pathogens to the rest of the production.

It is called weaning area, due to the acclimation to the artificial feed, this is done between the 25-30 dph, and it is a crucial phase in the production cycle. Since, if the larvae, for some reason, do not habituate to this new feed all the production is compromised, or if there is a problem during this transition, the condition of the fish could be affected for the rest of the cycle, and therefore, compromise the productivity.

In this area there are a numbers of routines that need to be done every day: (1) water parameters analysis, these analysis are regarding the temperature, oxygen, redox, ammonia, salinity, and nitrites. The temperature is measured at the water entry of a tank with a thermometer and the oxygen with a oximeter (this is measured in every tank, near to the water exit, where the value is lower and where it could be more problems related to lower and high levels of oxygen). While, the rest of the parameters is measured by collecting water samples (normally these samples are from a water entry from one tank), for each parameter there are a protocol to follow, which normally involve adding a reagent and posteriorly pass the sample in a spectrophotometer. The limits for each level are established according to the Sea8 protocols; (2) cleaning, the cleaning and disinfection of equipment and this part of the facility is essential, these are practices that are integrated in the GAP (Mondal et al., 2013, Volpe et al., 2013, Boison and Turnipseed, 2015), every day it is done the cleaning of the tanks (while is doing that, it is simultaneously assessed visible damages in the fish), with a brush for each tank to avoid contamination. The disinfection of the floor and footbaths is also done every day; (3) feeding, this operation is made in an automated way, since, in this area, there is a feeder in each tank, these are connected to a silo like machine that is programmed to deliver an pre-determine amount of feed regarding the biomass of the tank. Nonetheless, in tanks were it is required live feed this operation is done manually; (4) and assess the general condition of the fish in the culture tanks, this is done through observation when the tanks are cleaned or during grading. Any damage or ill fish is removed from the tank and register in the production register system. Tanks with excessive rates of illness/damage fish are treated according to Sea8 protocol.

Additionally, there are other operations that need to be done with other periodicity, these are: (1) grading, this operation must be done regularly to separate fishes by size and to evaluate the health state, since this species present a great disparity in size of fish with the same age (Blonk et al., 2010, Overton et al., 2010). Normally, this husbandry operation, at this life stage, is made manually with the help of a screen that have a specific mesh, the fishes that pass this mesh are the ones that did not grow as expected and are discarded, the rest of the fishes are distributed according to the management plan; (2) estimate the biomass of a tank, this process consist in weight a determined amount of fishes and estimate the mean weight, this provide to the management an approximate biomass and allow the increase of productivity, since the quantity of feed is determined by the estimation of biomass in each tank, on the other hand, it helps in the traceability process ; (3) and another operation that is done in the weaning area, is the transfer to the pre-ongrowing area, this operation require an excellent management, since the fishes need to be in perfect conditions (to not contaminate the animals in the pre-ongrowing area). When the transference is schedule the daily routines need to be done in the fastest way possible, without compromise the welfare of the animals. The transference, usually, is made when the animals reach the proper size (according with the Sea8 protocol).

This company promotes the GAP to increase the productivity.

5.6 Pre-ongrowing area

This area is composed by 56 raceway tanks, there are four levels, and each is divided into two parts (north and south) with seven tanks each (figure 31).

After the transference from the weaning area to this part of the aquaculture, it is expected that the fish will grow until 40 g and therefore, transferred to the ongowing unit of the Sea8, Aquacria Píscicolas S.A., located in Estarreja.

In this area similar processes as what happen in the weaning area are done. For instance the daily routines are the same (water parameter analysis, cleaning, feeding and assess the fish condition) with the difference, that is, the larger proportions that are implied in this area. For example, it is needed to provide higher amounts of feed, due to the superior dimensions of the fish and due to the higher biomass presented in each tank.

The holding conditions of the animals are also similar to the weaning area, with the temperature between the 18-20º C, the salinity between 30-35 ‰ and oxygen above the 80% of saturation.

There are other operations that are required, in order to maintain the productivity, these are also similar to what happens in the weaning area (grading and transport). However, as mention before, the larger dimensions of the animals and the higher biomass present in each tank in this area, results in higher duration of these operations.

Regarding the grading process, this is done with the aid of a calibrator that split the fishes by three sizes (small, medium and larger). Normally, the smaller fishes are discarded.

The transportation management plan required that the fishes pass through a starvation phase (24-48h). This time is in accordance with what is found in the literature (Lekang, 2013), and this will prevent the deterioration of the water during transportation. This process, usually is done once per month by land (truck) and, depending on the biomass that is needed to transport, can be performed on two consecutive days. In the day when the transportation occurs, the personal of the pre-ongrowing area have to expedite the daily routines, without compromise the production and the welfare. For that, it is required an excellent management plan to alleviate, for that day, these routines. This operation must be performed in the fastest way possible, without compromising the animal welfare.

The fish distribution in the vehice shall be equivalent between the several compartments. Thus, the nursery manager calculate the biomass during the loading.

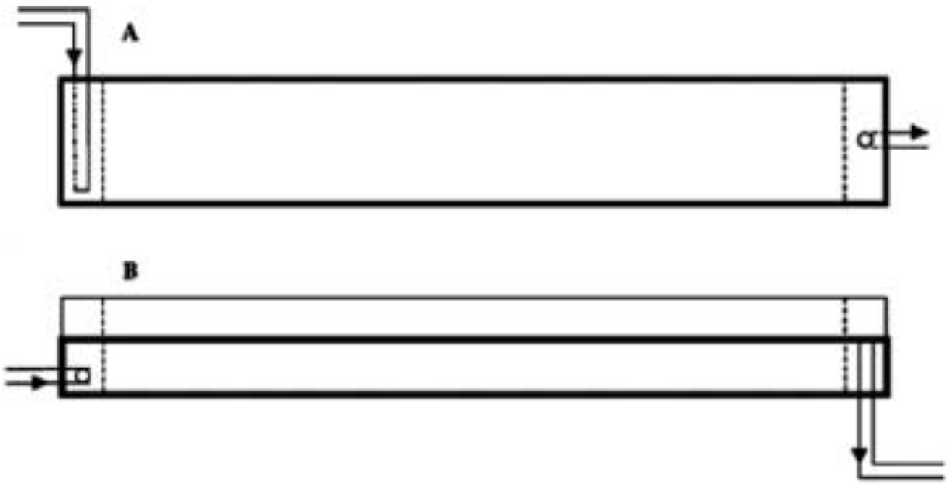


Figure 30 - Example of a raceway tank (Imsland et al., 2003).

5.7 Daily routines in Safiestela S.A.

Since this facility is composed of six parts (broodstock area, incubation room, larvae room, live feed room, weaning area, and pre-ongrowing area), it is natural that group of workers are established to work in different areas. Nonetheless, there are parts of this facility that are not used continuously (larvae room and incubation room), since in these areas the biomass is transferred quite rapidly (in some days).

The description of one day of routine work in this facility englobes the areas in which is needed workers permanently.

5.7.1 Weaning area

As mentioned already, this part is separated into three sub-parts (weaning one, weaning two and laboratory). The weaning one receive animals from the larvae room and, for that, it is required to administrate live feed in some of the tanks, until the animals reach the proper age to acclimatize to the inert feed. In the tanks that live feed is administered, the tanks cleaning routines and the animal health state assessment are not performed, since the fishes are too small and could be damaged during this procedure, additionally, during feeding the remain brine shrimps are wash with the water flow. Otherwise, in the rest of the tanks it is required the cleaning and health state assessment of the animals.

Thus, on a normal day the chronological routines are: (1) first it is assessed if the recirculating system is working properly (if the filters working properly, it is assessed the skimmer level, and verified the water pumps, the heat exanchers and the ozone generator) ; (2) If there are larvae in the correspondent room it is necessary to feed and take samples of these from each tank, to posterior evaluation for the parameters described in the point 5.4; (3) clean the tanks and assess the health state of the fishes (during this operation it is necessary to remove any ill or dead fish from each tank and report the amount in the register board, for posterior record in the database); (4) the next task that is done is the water analysis, there are parameters that are analyzed in all tanks (oxygen saturation and temperature) and other parameters that are assessed at only one point (redox, ammonia and nitrites), the methods to evaluate these parameters have been already described in the point 5.5; (5) there are a number operations that can be performed after, like grading and consequent distribution, assessing the biomass by calculate the mean weight in each thank, the transference of animals to the pre-ongrowing area, apply treatment in tanks were disease was confirmed during the assessment of the health state of the fishes, and the cleaning and disinfection of the weaning area.

Due to the fish age dispersion, grading is a common practice, while the biomass estimation and the transference to the pre-ongrowing area are performed with another periodicity (in accordance with the Sea8 protocols).

The cleaning and disinfection are performed every day, nonetheless, these can be performed more or less thoroughly depending on the time spent on other operations. However, there are minimal tasks in this step that need to be performed to guarantee that the biosecurity protocol is respected; (6) verification of the recirculating system and their components (as already described in the point one); and (7) register the data in the facility digital database.

5.7.2 Live feed rooms

In the production of live feed there are two separated rooms, one is for artemia production, and the other is for rotifers production. Although, the production of larvae is not a continuous process, the production of live feed needs to be continuous. Since, even after the metamorphosis the animals require live feed, until they reach the proper age to pass through the weaning process (according to the Sea8 protocols).

The daily routines described include the production of both live feeds, and can happen simultaneously.

Regarding the artemia production these are the chronologic tasks: (1) in order to provide the larvae first feed of the day, it is required that the previously enriched artemia are filtered, and then put them in the cold (milkmaid); then (2) is necessary to estimate the amount of brine shrimp cysts that is going to be needed for the next 48 hours; (3) in the artemia that need to be enriched it is added sodium hydroxide ever six hours. Every time that this is added it is required to register this in the correspondent sheet; the next task (4) is the calculation of the biomass in each tank, this is done by taking a sampling from the production tank. Posteriorly, it is counted the number of individuals from 1 mL from the diluted sample, knowing this, it is estimated the number of individuals for the volume where the sample was taken. These estimations of biomass allow the personal of this area to calculate the volume needed for each feeding (according with the Sea8 protocols). Thus, any other worker can feed the larvae with the proper instructions from the live feed personal, this is more relevant for the night turns workers; and (5) clean and disinfection of the area, this is performed everyday, in accordance with the Sea8 protocol.

Otherwise, the tasks to produce rotifers are these: (1) place the previoulsy enriched rotifers in a filtering system, to admnisterate the first feed of the larvae; (2) initiate a new batch in a clean tank, according with the Sea8 protocols; (3) it is required to calculate the biomass in each tank. To perform this, it is collected a sample from each tank, then to calculate the number of rotifers present in this volume it is collected 100 µL from the sample, and it is counted the number of individuals. Like what happen in the artemia counting, it is estimated the biomass from this count; and (5) clean and disinfect the area, this is performed everyday, in accordance with the Sea8 protocol.

5.7.3 Broodstock area

Regarding the broodstock daily routines, these are quite simple and normally they are performed in a rapid way, in order to not disturb the animals.

The person in charge of the breeders have to: (1) firstly it is assessed if the RAS system and the components are working properly; then (2) it is assessed the state of the animals and verify if there is any eggs in the collectors; (3) clean the collectors and the tanks (this is done according with the Sea8 protocols). While the tanks are cleaned it is assessed the health state of the fishes, as well as, if there are any indications that the fishes are producing gametes; (4) then it is verify the water parameters (referred in the point 5.2); (5) next it is admnistrate feed to each tank. The feeding is usually composed of inert feed, and to administrate it is done a calculation (this is made by the broodstock manager in accordance with the Sea8 protocol), that have to take into account the biomass in each tank, then it is distributed the feed manually; (6) it is performed the cleaning and the disinfection of the area. This step can be done more thoroughly or not depending on the time that the worker have. Nonetheless, there are cleanning procedures that need to be performed to respect the biosecurity protocol; (7) it is registered in the database the water parameters and any other valuable information (like mortalities or presence of gametes, on the animals or collectors); at the end (8) it is verify if the recirculation system is working properly, as described in the point 5.2.

The management of the environments parameters allow to manipulate the breeders spawning timing, normally, this operation involves the adjustment of the water temperature (according with the Sea8 protocol).

5.7.4 Pre-ongrowing area

As mentioned already in the point 5.6, the pre-ongrowing area has four levels. The daily routines in this area, generally, take more time than in any other part in the production.

Thus, at the beginning of the day the workers have to: (1) proceed to inspection of the recirculating system, similarly to what happens in the weaning area; then (2) the workers need to clean all of the tanks, this operation is performed in the lower and upper levels at the same time. While the cleaning is performed it is required to remove any ill or dead fish, and register on the board the amount of fishes that were removed. After the tanks cleaning it is remove particles or any fish from the floor; (3) transport of animals to the ongrowing facility when it is needed; (4) grading if necessary, (5) water analysis, (6) cleaning and disinfection, (7) treatment in tanks where it was verified disease outbreaks, (8) verification of the recirculating systems and (9) it is realized the preparations to receive animals from the weaning area, when necessary.

Due to the large proportions and the fish age dispersion present in this part, the grading operation is performed regularly. However, this operation lasts a longer time than in the weaning area. During this operation, some fishes are selected, due to the presence of illness, damage or because they are slow growers.

Regarding the water analysis, there are parameters that are measured in every tank (oxygen saturation and temperature) and parameters that are measured at one point (redox, ammonia and nitrites).

The disinfection and cleaning of this area are performed similarly as what take place in the weaning area, which is conditioning by the available time. Nonetheless, this step needs to guarantee the sanitary conditions defined by the biosecurity protocol.

At the end of the day, it is verified the recirculating system as already described in the point 5.7.1.

There are operations that are performed sporadically, these are: the internal transport of animals within the facility (from the weaning to the pre-ongrowing area) and the external transport (that is, animals that are transported to the ongrowing installation, Aquacria Piscicolas, S.A.). Both of this transports are described previously, in the points 5.5 and 5.6, respectively.

5.8 Recirculating aquaculture systems (RAS)

In animal production, the environmental contamination it is one of the greatest threats, and aquaculture is no exception. In this sector, there are different systems such as flow through and RAS that offer different levels of protection against pathogen outbreaks.

In the last decades aquaculture experienced the greatest rise in production, this brought a series of concerns and problems, for example: access to fresh water; effluent discharges; and land resources (Zhang et al., 2011, Badiola et al., 2012, Dalsgaard et al., 2013, Powell et al., 2015, Schroeder et al., 2015, Summerfelt et al., 2015).

The RAS is considered the most ecological and the most efficient system for this activity (Zhang et al., 2011). This system helps to control the water parameters that could potentially improve the efficiency and could improve the welfare of the animals within the facility. In a study comparing flow through and RAS, relatively to welfare in rainbow trout

(*Oncorhynchus mykiss*) culture, it was concluded that the RAS do not play a harmful role in that species (Colson et al., 2015), and this is likely to be similar to other species as well. Additionally, this system helps the production to be more independent from the weather and climate conditions, in the case of Nordic countries, aquaculture has been challenged by the climate and weather, which make it hard to access to fresh water (Dalsgaard et al., 2013).

Recent reports reveal that aquaculture production is stagnating in some developed countries (such as Spain and France), due to the concurrence inability with other markets. To oppose this tendency the technological advances are crucial, and RAS is one example of that, since improves productivity, when compared with other systems. (Zhang et al., 2011, Badiola et al., 2012, FAO, 2014, Yuqing et al., 2014, Rabassó and Hernández, 2015).

The RAS was created to increase efficiency and decrease the harmful impacts of aquaculture. To accomplish that, this system needs to treat the water, to reutilize the majority of it, and minimize the incoming water to the production. This treatment requires mechanical, biological and chemical processes that help maintain the water parameters at adequate levels, which depend on what species is produced. Additionally, RAS produce lower discharges volumes (Gowen, 1994).

5.8.1 Maintenance of RAS

In every aquaculture, regardless the type of water circulation used, there are concerns to take into account, to maintain the water quality in optimum levels for the culture. These levels can be conditioned by, mostly, metabolites and by particles of feed that were not consumed. These compounds are for example: POM (particulate organic matter), DOM (dissolve organic matter), nitrogen and phosphorus.

The aquaculture has greatly evolved in the recent decades, which brought new technologies and new types of culture, which allow to improve the efficiency. For example in 1975, the FCR in salmonids was about 3.5 and dropped to 1.1 in 20 years, so problems regarding the waste of feed in aquaculture were minimized (Piedrahita, 2003). However, the example used is not representative to other species produced around the world, because the salmonids are well-known fishes in aquaculture and one of the first being produced.

RAS led to a way to produced intensively fish, with minimum water intake and outtake by conserving and reutilizing the maximum of water within the culture. However, RAS has the disadvantages: (1) difficulty in treating diseases, (2) the management of the system, and (3) high monetary costs in implementation of such system.

5.8.2 RAS in the Safiestela S.A.

In the facility the RAS is divided into two parts, one treat the water from the pre-ongrowing area, and other treat the water from the weaning and broodstock areas. In each part, there are biologic and mechanic filters, one skimmer in each, heat exchangers, an ozone system and UV filters. Additionally, in each part there are pumps responsible for the water circulation within the facility, in case of damage there are pumps of reserve. Nonetheless, to guarantee that every pump function properly it is regularky alternated the pumps in use.

Regarding to the Safiestela S.A. there are a number of procedures that allow the proper functioning of the RAS, these are: (1) assess regularly the level of water in the filters; (2) verify the caudal in the skimmer; (3) check if the ozone has the proper flow; (4) check the redox values; and (5) verify regularly the filters in the filter systems, in the case of fouling this will compromise the entire facility (all these procedures are perfromed according with the Sea8 protocols).

The verification of some parameters is done every day (in the morning and in the afternoon), such as the verification of caudal in the filter systems and in the skimmer, the flow of ozone and the levels of redox.

In case of energy collapse the facility is equipped with a diesel generator, to safeguard the production. Occasionally, this equipment is used to guarantee its proper functioning.

References

Ababouch, l. 2014. Good Practices In Fisheries and Aquaculture. *Food Safety Assurance Systems,* 4**,** 159-167.

Andrade, T., Afonso, A., Pérez-Jiménez, A., Oliva-Teles, A., De Las Heras, V., Mancera, J. M., Serradeiro, R. & Costas, B. 2015. Evaluation of different stocking densities in a Senegalese sole (*Solea senegalensis*) farm: Implications for growth, humoral immune parameters and oxidative status. *Aquaculture,* 438**,** 6-11.

Atoum, Y., Srivastava, S. & Liu, X. 2015. Automatic feeding control for dense aquaculture fish tanks. *IEEE Signal Processing Letters,* 22**,** 1089-1093.

Badiola, M., Mendiola, D. & Bostock, J. 2012. Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. *Aquacultural Engineering,* 51**,** 26-35.

Barron, J. M., Jensen, N. R., Anders, P. J., Egan, J. P. & Cain, K. D. 2013. Suppression of Cannibalism during Larviculture of Burbot through Size Grading. *North American Journal of Aquaculture,* 75**,** 556.

Barton, B. A. & Peter, R. E. 1982. Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia, and cold shock. *Journal of Fish Biology,* 20**,** 39-51.

Bedoui, R. 1995. *Solea senegalensis* (Kaup, 1958) rearing in Tunisia. *Options Mediterraneennes (CIHEAM)* 16**,** 31-39.

Bermejo-Poza, R., De La Fuente, J., Pérez, C., Lauzurica, S., González, E., Diaz, M. T. & Villarroel, M. 2015. The effect of intermittent feeding on the pre-slaughter fasting response in rainbow trout. *Aquaculture,* 443**,** 24-30.

Blanco-Vives, B., Aliaga-Guerrero, M., Cañavate, J. P., Muñoz-Cueto, J. A. & Sánchez-Vázquez, F. J. 2011. Does Lighting Manipulation During Incubation Affect Hatching Rhythms and Early Development of Sole? *Chronobiology International: The Journal of Biological & Medical Rhythm Research,* 28**,** 300-306.

Blanco-Vives, B., Villamizar, N., Ramos, J., Bayarri, M. J., Chereguini, O. & Sánchez-Vázquez, F. J. 2010. Effect of daily thermo- and photo-cycles of different light spectrum on the development of Senegal sole (*Solea senegalensis*) larvae. *Aquaculture,* 306**,** 137-145.

Blonk, R. J. W., Komen, H., Kamstra, A. & Van Arendonk, J. A. M. 2010. Effects of grading on heritability estimates under commercial conditions: A case study with common sole, *Solea solea*. *Aquaculture,* 300**,** 43-49.

Boglino, A., Darias, M. J., Estévez, A., Andree, K. B. & Gisbert, E. 2012. The effect of dietary arachidonic acid during the Artemia feeding period on larval growth and skeletogenesis in Senegalese sole, *Solea senegalensis*. *Journal of Applied Ichthyology,* 28**,** 411-418.

Boison, J. O. & Turnipseed, S. B. 2015. A review of aquaculture practices and their impacts on chemical food safety from a regulatory perspective. *Journal of aoac international,* 98**,** 541-549.

Bonaldo, A., Parma, I., Mongile, F., Gatta, P. P., Di Marco, P., Petochi, T., Marino, G., Finoia, M. G., Fontanillas, R. & Koppe, W. 2015. Feeding turbot juveniles *Psetta maxima*. With increasing dietary plant protein levels affects growth performance and fish welfare. *Aquaculture nutrition*.

Bosma, R. H. & Verdegem, M. C. J. 2011. Sustainable aquaculture in ponds: principles, practices and limits. *Livestock science,* 139**,** 58-68.

Bossù, T., Lanni, L., Nocera, F. D., Marrozzi, S., Amiti, S. & Boria, p. 2013. Evalutation of the correct applications of good manufacturing practices in aquaculture: preliminary data. *Italian journal of food safety***,** 59.

Bostock, J., Mcandrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I. & Corner, R. 2010. *Aquaculture: global status and trends*.

Bovenkerk, B. & Meijboom, F. B. 2013. Fish welfare in aquaculture: explicating the chain of interactions between science and ethics. *Journal of agricultural and environmental ethics,* 26**,** 41-61.

Boyd, C. E. 2003. Guidelines for aquaculture effluent management at the farm-level. *Aquaculture,* 226**,** 101-112.

Broughton, E. I. & Walker, D. G. 2010. Policies and practices for aquaculture food safety in china. *Food policy,* 35**,** 471-478.

Brown, N. 2002. Flatfish farming systems in the atlantic region. *Reviews in fisheries science,* 10**,** 403-419.

Colson, V., Sadoul, B., Valotaire, C., Prunet, P., Gaumé, M. & Labbé, L. 2015. Welfare assessment of rainbow trout reared in a recirculating aquaculture system: comparison with a flow-through system. *Aquaculture,* 436**,** 151-159.

Creţu, M., Cristea, V., Dediu, L. & Petrea, S. M. 2014. The influence of different stocking densities on biochemical composition of rainbow trout meat reared in a recirculating aquaculture system. *Scientific papers: animal science & biotechnologies / lucrari stiintifice: zootehnie si biotehnologii,* 47**,** 200-204.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K. & Pedersen, P. B. 2013. Farming different species in RAS in nordic countries: current status and future perspectives. *Aquacultural engineering,* 53**,** 2-13.

Daly, B., Swingle, J. S. & Eckert, G. L. 2012. Increasing hatchery production of juvenile red king crabs (*Paralithodes camtschaticus*) through size grading. *Aquaculture,* 364-365**,** 206-211.

Daniel, A. P., Veeck, A. P. L., Klein, B., Ferreira, L. F., da Cunha, M. A., Parodi, T. V., Zeppenfeld, C. C., Schmidt, D., Caron, B. O., Heinzmann, B. M., Baldisserotto, B. & Emanuelli, T. 2014. Using the essential oil of aloysia triphylla (l'her.) Britton to sedate silver catfish (*Rhamdia quelen*) during transport improved the chemical and sensory qualities of the fish during storage in ice. *Journal of food science,* 79**,** s1205-s1211.

Daniels, H. V., Berlinsky, D. L., Hodson, R. G. & Sullivan, C. V. 1996. Effects of stocking density, salinity, and light intensity on growth and survival of southern flounder *paralichthys lethostigma* larvae. *Journal of the world aquaculture society,* 27**,** 153-159.

Diana, J. S., Egna, H. S., Chopin, T., Peterson, M. S., Cao, L., Pomeroy, R., Verdegem, M., Slack, W. T., Bondad-Reantaso, N. G. & Cabello, F. 2013. Responsible aquaculture in 2050: valuing local conditions and human innovations will be key to success. *Bioscience,* 63**,** 255-262.

Dias, J., Rueda-Jasso, R., Panserat, S., Conceição, L. E., Gomes, E. F. & Dinis, M. T. 2004. Effect of dietary carbohydrate-to-lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegalese sole (S*olea senegalensis*, Kaup). *Aquaculture research,* 35**,** 1122-1130.

Dillon, M. C., Dillon, M. & Griffith, C. 1996. *How to HACCP: an illustrated guide*, m.d. associates.

Dinis, M. T., Ribeiro, L., Soares, F. & Sarasquete, C. 1999. A review on the cultivation potential of *Solea senegalensis* in spain and in portugal. *Aquaculture,* 176**,** 27-38.

Dobsikova, R., Svobodova, Z., Blahova, J., Modra, H. & Velisek, J. 2009. The effect of transport on biochemical and haematological indices of common carp (C*yprinus carpio* l.). *Czech journal of animal science - uzei (czech republic)*.

Ellingsen, K., Grimsrud, K., Nielsen, H. M., Mejdell, C., Olesen, I., Honkanen, P., Navrud, S., Gamborg, C. & Sandøe, P. 2015. Who cares about fish welfare? *British food journal,* 117**,** 257-273.

FAO 2014. The state of world fisheries and aquaculture.

FAO. 2015. *Seed production - an overview* [online]. http://www.fao.org/docrep/field/003/ac182e/ac182e01.htm. [accessed june 2015].

Farrell, A., Tang, S., Nomura, M. & Brauner, C. 2010. Toward improved public confidence in farmed fish: a canadian perspective on fish welfare during marine transport. *Journal of the world aquaculture society*.

Fernández-Díaz, C., Yýfera, M., Cañavate, J. P., Moyano, F. J., Alarcón, F. J. & Díaz, M. 2001. Growth and physiological changes during metamorphosis of senegal sole reared in the laboratory. *Journal of fish biology,* 58**,** 1086-1097.

Fernández-Segovia, I., Pérez-Llácer, A., Peidro, B. & Fuentes, A. 2014. Implementation of a food safety management system according to iso 22000 in the food supplement industry: a case study. *Food control,* 43**,** 28-34.

Fernández, J., Toro, M. Á., Sonesson, A. K. & Villanueva, B. 2014. Optimizing the creation of base populations for aquaculture breeding programs using phenotypic and genomic data and its consequences on genetic progress. *Frontiers in genetics,* 5**,** 1-13.

Fraser, D. 2003. Assessing animal welfare at the farm and group level: the interplay of science and values. *Animal welfare,* 12**,** 433-443.

Frewer, L. J., Kole, A., Kroon, S. M. A., & De Lauwere, C. 2005. Consumer attitudes towards the development of animal-friendly husbandry systems. *Journal of agricultural & environmental ethics,* 18**,** 345.

Ganzon-Naret, E. S. 2013. Influence of different feeding rates using commercial dry pellets on growth, feed efficiency and survival for hatchery produced sea bass lates calcarifer. *Animal biology & animal husbandry,* 5**,** 241-248.

Gjedrem, T., Robinson, N. & Rye, M. 2012. Review: the importance of selective breeding in aquaculture to meet future demands for animal protein: a review. *Aquaculture,* 350-353**,** 117-129.

González, R., Celada, J. D., Carral, J. M., García, V., Sáez-Royuela, M. & González, Á. 2011. Intensive rearing of juvenile crayfish (P*acifastacus leniusculus*, *astacidae*) during the first 6 months: effects of size grading. *Aquaculture research,* 42**,** 1385-1392.

Gowen, R. J. 1994. Managing eutrophication associated with aquaculture development. *Journal of applied ichthyology,* 10**,** 242-257.

Grigorakis, K. & Rigos, G. 2011. Review: aquaculture effects on environmental and public welfare – the case of mediterranean mariculture. *Chemosphere,* 85**,** 899-919.

Guzmán, J. M., Ramos, J., Mylonas, C. C. & Mañanós, E. L. 2009. Spawning performance and plasma levels of gnrha and sex steroids in cultured female Senegalese sole (*Solea senegalensis*) treated with different gnrha-delivery systems. *Aquaculture,* 291**,** 200-209.

Harvey, D. & Hubbard, C. 2013. Reconsidering the political economy of farm animal welfare: an anatomy of market failure. *Food policy,* 38**,** 105-114.

Håstein, T. 2007. Oie involvement in aquatic animal welfare: the need for development of guidelines based on welfare for farming, transport and slaughter purposes in aquatic animals. *Developments in biologicals,* 129**,** 149-161.

Håstein, T., Scarfe, A. D. & Lund, V. L. 2005. Science-based assessment of welfare: aquatic animals. *Revue scientifique et technique (international office of epizootics),* 24**,** 529-547.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandøe, P. & Turnbull, J. F. 2006. Current issues in fish welfare. *Journal of fish biology,* 68**,** 332-372.

Huntingford, F. A. & Kadri, S. 2014. Defining, assessing and promoting the welfare of farmed fish. *Revue scientifique et technique (international office of epizootics),* 33**,** 233-244.

Ignacio Martín, J. R. G., Juan Antonio Martos-Sitcha, Inmaculada & Rasines, C. R., Juan Miguel Mancera, Olvido Chereguini 2014. Is it possible to transport *Solea senegalensis* specimens without water? *Aquaculture europe.* Donostia - san sebastian, spain.

Iguchi, K. I., Ogawa, K., Nagae, M. & Ito, F. 2003. The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). *Aquaculture,* 220**,** 515-523.

Imsland, A. K., Foss, A., Conceição, L. E., Dinis, M. T., Delbare, D., Schram, E., Kamstra, A., Rema, P. & White, P. 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. *Reviews in fish biology and fisheries,* 13**,** 379-408.

INE, I. P. 2014. Estatísticas da pesca. *In:* instituto nacional de estatística, I. P. (ed.) *Instituto nacional de estatística, I.p.* lisboa - Portugal.

Ingenbleek, P. T., Harvey, D., Ilieski, V., Immink, V. M., De Roest, K. & Schmid, O. 2013. The european market for animal-friendly products in a societal context. *Animals (2076-2615),* 3**,** 808-829.

Ingenbleek, P. T., Immink, V. M., Spoolder, H. A., Bokma, M. H. & Keeling, L. J. 2012. EU animal welfare policy: developing a comprehensive policy framework. *Food policy,* 37**,** 690-699.

Iversen, M., Eliassen, R. A. & Finstad, B. 2009. Potential benefit of clove oil sedation on animal welfare during salmon smolt, *Salmo salar* l. Transport and transfer to sea. *Aquaculture research,* 40**,** 233-241.

Jarman, M. R., Clark, G., Grigson, C., Uerpmann, H.-P. & Ryder, M. L. 1976. *Early animal husbandry [and discussion]*.

Khuraibet, A. M. & Al Attar, F. 2002. Eia, HACCP and ISO 14000 as three pillars for sustainable and environmentally friendly aquaculture projects: advantages and concerns.

King, H. R. 2009. 2008 Australian RSPCA welfare symposium: fish transport in the aquaculture sector: an overview of the road transport of atlantic salmon in tasmania. *Journal of veterinary behavior: clinical applications and research,* 4**,** 163-168.

Kohara, M., Ogawa, S., Kasai, H. & Yoshimizu, M. 2010. Effecto of egg-washing with isotonic solution on elimination of bacteria and viruses before fertilization in salmon aquaculture. *Suisanzoshoku (Japan)*.

Kolarevic, J., Baeverfjord, G., Takle, H., Ytteborg, E., Reiten, B. K. M., Nergård, S. & Terjesen, B. F. 2014. Performance and welfare of atlantic salmon smolt reared in recirculating or flow through aquaculture systems. *Aquaculture,* 432**,** 15-25.

Kulczykowska, E. & Sánchez-Vázquez, F. J. 2010. Neurohormonal regulation of feed intake and response to nutrients in fish: aspects of feeding rhythm and stress. *Aquaculture research,* 41**,** 654-667.

Lavens, P. & Sorgeloos, P. 1996. *M*anual on the production and use of live food for aquaculture, food and agriculture organization (FAO).

Lekang, O.-I. 2013. Transport of live fish. *Aquaculture engineering.* John wiley & sons.

Lind, C. E., Ponzoni, R. W., Nguyen, N. H. & Khaw, H. L. 2012. Selective breeding in fish and conservation of genetic resources for aquaculture. *Reproduction in domestic animals = zuchthygiene,* 47 suppl 4**,** 255-263.

Lines, J. A. & Spence, J. 2012. Safeguarding the welfare of farmed fish at harvest. *Fish physiol biochem,* 38**,** 153-62.

Lines, J. A. & Spence, J. 2014. Humane harvesting and slaughter of farmed fish. *Rev sci tech,* 33**,** 255-64.

Linhart, O., Kudo, S., Billard, R., Slechta, V. & Mikodina, E. V. 1995. Morphology, composition and fertilization of carp eggs: a review. *Aquaculture (Netherlands)*.

Liu, Z., Li, X., Fan, L., Lu, H., Liu, L. & Liu, Y. 2014. Measuring feeding activity of fish in RAS using computer vision. *Aquacultural engineering,* 60**,** 20-27.

López-Olmeda, J., Noble, C. & Sánchez-Vázquez, F. 2012. Does feeding time affect fish welfare? *Fish physiology & biochemistry,* 38**,** 143.

López-Patiño, M. A., Conde-Sieira, M., Gesto, M., Librán-Pérez, M., Soengas, J. L. & Míguez, J. M. 2013. Melatonin partially minimizes the adverse stress effects in Senegalese sole (*Solea senegalensis*). *Aquaculture,* 388-391**,** 165-172.

Luo, G., Liu, G. & Tan, H.-X. 2013. Effects of stocking density and food deprivation-related stress on the physiology and growth in adult S*cortum barcoo* (mc culloch & waite). *Aquaculture research,* 44**,** 885-894.

Marinho, G., Peres, H. & Carvalho, A. P. 2014. Effect of feeding time on dietary protein utilization and growth of juvenile senegalese sole (*solea senegalensis*). *Aquaculture research,* 45**,** 828-833.

Mcewen, B. S. & Wingfield, J. C. 2003. The concept of allostasis in biology and biomedicine. *Horm behav,* 43**,** 2-15.

Mellor, D. J. 2014. Animal welfare: focusing on the future. *Revue scientifique et technique (international office of epizootics),* 33**,** 21-32.

Menezes, C., Ruiz-Jarabo, I., Martos-Sitcha, J. A., Toni, C., Salbego, J., Becker, A., Loro, V. L., Martínez-Rodríguez, G., Mancera, J. M. & Baldisserotto, B. 2015. The influence of stocking density and food deprivation in silver catfish (R*hamdia quelen*): a metabolic and endocrine approach. *Aquaculture,* 435**,** 257-264.

Mesquita, F. O. 2011. Coping styles and learning in fish: developing behavioural tools for welfare-friendly aquaculture.

Miget, R. J. 2004. HACCP seafood program and aquaculture.

Mizuno, K., Shimizu-Yamaguchi, S., Miura, C. & Miura, T. 2012. Method for efficiently obtaining fertilized eggs from the black scraper *Thamnaconus modestus* by natural spawning in captivity. *Fisheries science,* 78**,** 1059-1064.

Mondal, S., Rahman, M. M., Saha, D., Adhikary, R. K. & Belal Hossain, M. 2013. Present status of good aquaculture practices (GAP) in shrimp farms of south-western coastal area, bangladesh. *Middle east journal of scientific research,* 14**,** 873-878.

Morais, S., Mendes, A. C., Castanheira, M. F., Coutinho, J., Bandarra, N., Dias, J., Conceição, I. & Pousão-Ferreira, P. 2014. New formulated diets for *solea senegalensis* broodstock: effects of parental nutrition on biosynthesis of long-chain polyunsaturated fatty acids and performance of early larval stages and juvenile fish. *Aquaculture*.

Moss, S. M., Moss, D. R., Arce, S. M., Lightner, D. V. & Lotz, J. M. 2012. Minireview: the role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture. *Journal of invertebrate pathology,* 110**,** 247-250.

Mustapha, M. K. 2014. Aquaculture and fish welfare: are the rights of fish compromised?/ akwakultura i dobrostan ryb: czy prawa ryb są szanowane? *Zoologica poloniae,* 59**,** 49.

Naessens-Foucquaert, E. M. J., Rombaut, G. & Van, N. L. A. J. 2009. *Method for treating artemia cysts*.

Neufeld, M. D., Davis, C. A., Cain, K. D., Jensen, N. R., Ireland, S. C. & Lewandowski, C. 2011. Evaluation of methods for the collection and fertilization of burbot eggs from a wild stock for conservation aquaculture operations. *Journal of applied ichthyology,* 27**,** 9-15.

Nilsson, J. & Torgersen, T. 2010. Exploration and learning of demand-feeding in atlantic cod (G*adus morhua*). *Aquaculture,* 306**,** 384-387.

Ohl, F. & Van Der Staay, F. J. 2012. Animal welfare: at the interface between science and society. *The veterinary journal,* 192**,** 13-19.

Overton, J. L., Steenfeldt, S. J. & Pedersen, P. B. 2010. The effects of grading on the growth and survival of juvenile dover sole (S*olea solea L.*). *Aquaculture research,* 42**,** 31-39.

Pedro Cañavate, J., Zerolo, R. & Fernández-Díaz, C. 2006. Feeding and development of Senegal sole (*Solea senegalensis*) larvae reared in different photoperiods. *Aquaculture,* 258**,** 368-377.

Petrović, S., Bavčević, L., Pasarić, Z. & Mihovilović, A. 2011. Effects of successive size grading on the growth of juvenile european sea bass (*Dicentrarchus labrax l.*) At three temperatures. *Aquaculture research,* 42**,** 277-287.

Piedrahita, R. H. 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture,* 226**,** 35-44.

Pottinger, T. G. & Carrick, T. R. 1999. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. *Gen comp endocrinol,* 116**,** 122-32.

Powell, A., Chingombe, P., Lupatsch, I., Shields, R. J. & Lloyd, R. 2015. The effect of ozone on water quality and survival of turbot (*Psetta maxima)* maintained in a recirculating aquaculture system. *Aquacultural Engineering,* 64**,** 20-24.

Psomas, E. L. & Kafetzopoulos, D. P. 2015. HACCP effectiveness between ISO 22000 certified and non-certified dairy companies. *Food Control,* 53**,** 134-139.

Qu, Y., Li, X., Yu, Y., Vandepeer, M., Babidge, P., Plarke, S., Bott, K. & Li, H. 2009. Effect of different grading equipment on stress levels assessed by catecholamine measurements in Pacific oysters, *Crassostrea gigas* (Thunberg). *Aquacultural engineering*.

Rabassó, M. & Hernández, J. M. 2015. Bioeconomic analysis of the environmental impact of a marine fish farm. *Journal of Environmental Management,* 158**,** 24-35.

Richard, R. & Flemming, L. 2007. Fin condition and growth among rainbow trout reared at different sizes, densities and feeding frequencies in high-temperature re-circulated water. *Aquaculture International,* 15**,** 97-107.

Roberts, R., Peeler, E., Sures, B. & Vis, J. W. V. 2008. Scientific report of EFSA prepared by Working group on Eel welfare on Animal Welfare Aspects of Husbandry Systems for farmed European Eel.

Rossi, J. & Garner, S. 2014. Industrial Farm Animal Production: A Comprehensive Moral Critique. *Journal of Agricultural & Environmental Ethics,* 27**,** 479-522.

Saillant, E., Fostier, A., Haffray, P., Menu, B., Laureau, S., Thimonier, J. & Chatain, B. 2003. Effects of rearing density, size grading and parental factors on sex ratios of the sea bass (*Dicentrarchus labrax L*.) In intensive aquaculture. *Aquaculture,* 221**,** 183-206.

Salas-Leiton, E., Anguis, V., Manchado, M. & Cañavate, J. P. 2008. Growth, feeding and oxygen consumption of Senegalese sole (*Solea senegalensis*) juveniles stocked at different densities. *Aquaculture,* 285**,** 84-89.

Salas-Leiton, E., Anguís, V., Rodríguez-Rúa, A. & Cañavate, J. P. 2011. High stocking density and food restriction have minimum impact on size dispersal of cultured Senegalese sole (*Solea senegalensis*, Kaup 1858) juveniles. Evidence for individual growth being regulated by population structure. *Aquacultural Engineering,* 45**,** 43-50.

Salas-leiton, E., Anguís, V., Rodríguez-Rúa, A. & Cañavate, J. P. 2010. Stocking homogeneous size groups does not improve growth performance of Senegalese sole (*Solea senegalensis*, Kaup 1858) juveniles: Individual growth related to fish size. *Aquacultural Engineering,* 43**,** 108-113.

Sánchez, P., Ambrosio, P. P. & Flos, R. 2010. Stocking density and sex influence individual growth of Senegalese sole (*Solea senegalensis*). *Aquaculture,* 300**,** 93-101.

Sánchez, P., Ambrosio, P. P. & Flos, R. 2013. Stocking density affects Senegalese sole (*Solea senegalensis*, Kaup) growth independently of size dispersion, evaluated using an individual photo-identification technique. *Aquaculture Research,* 44**,** 231-241.

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., Mckenzie, S., Walker, P. & Lawrence, R. 2008. Aquaculture practices and potential human health risks: Current knowledge and future priorities. *Environment International,* 34**,** 1215-1226.

Schreck, C. B. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. *Gen Comp Endocrinol,* 165**,** 549-56.

Schroeder, J. P., Klatt, S. F., Schlachter, M., Zablotski, Y., Keuter, S., Spieck, E. & Schulz, C. 2015. Impact of ozonation and residual ozone-produced oxidants on the nitrification performance of moving-bed biofilters from marine recirculating aquaculture systems. *Aquacultural Engineering,* 65**,** 27-36.

Slavík, O., Pešta, M. & Horký, P. 2011. Effect of grading on energy consumption in European catfish *Silurus glanis*. *Aquaculture,* 313**,** 73-78.

Soto, D., Aguilar-Manjarrez, J., Falconer, L., Ross, L. G., Telfer, T., Food & Agriculture Organization of the United Nations. 2013. *Site Selection and Carrying Capacities for Inland and Coastal Aquaculture,* Rome, FAO.

Summerfelt, S. T., Zühlke, a., Kolarevic, J., Reiten, B. K. M., Selset, R., Gutierrez, X. & Terjesen, B. F. 2015. Effects of alkalinity on ammonia removal, carbon dioxide stripping, and system pH in semi-commercial scale water recirculating aquaculture systems operated with moving bed bioreactors. *Aquacultural Engineering,* 65**,** 46-54.

Szisch, V., Van Der Salm, A. L., Wendelaar Bonga, S. E. & Pavlidis, M. 2002. Physiological colour changes in the red porgy, *Pagrus pagrus*, following adaptation to blue lighting spectrum. *Fish Physiology and Biochemistry,* 27**,** 1-8.

Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E. T. & Salinas, I. 2015. Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* 435**,** 120-127.

Tang, S., Brauner, C. J. & Farrell, A. P. 2009. Using bulk oxygen uptake to assess the welfare of adult atlantic salmon, *Salmo salar*, during commercial live-haul transport. *Aquaculture,* 286**,** 318-323.

Teletchea, F. & Fontaine, P. 2014. Levels of domestication in fish: implications for the sustainable future of aquaculture. *Fish & Fisheries,* 15**,** 181-195.

Trejchel, K., Zarski, D., Palińska-Żarska, K., Krejszeff, S., Dryl, B., Dakowski, K. & Kucharczyk, D. 2014. Determination of the optimal feeding rate and light regime conditions in juvenile burbot, *Lota lota* (L.), under intensive aquaculture. *Aquaculture international*.

Troell, M., Naylor, R. L., Metian, M., Beveridge, M., Tyedmers, P. H., Folke, C., Arrow, K. J., Barrett, S., Crépin, A.-S., Ehrlic, P. R., Gren, Å., Kautsky, N., Levin, S. A., Nyborg, K., Österblom, H., Polasky, S., Scheffer, M., Walker, B. H., Xepapadeas, T. & De Zeeuw, A. 2014. Does aquaculture add resilience to the global food system? *Proceedings of the National Academy of Sciences of the United States of America,* 111**,** 13257.

Turnbull, J., Bell, A., Adams, C., Bron, J. & Huntingford, F. 2005. Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. *Aquaculture*.

Turnbull, J. F. & Huntingford, F. A. 2012. Welfare and aquaculture: where benefish fits in. *Aquaculture Economics & Management (Taylor & Francis),* 16**,** 433.

Jahncke, M.L., C.L Browdy, M.H. Schwarz, A. Segars, J.L. Silva, D.C. Smith, A.D. Stokes. 2002. Application of hazard analysis critical control point (HACCP) principles as a risk management tool to control viral pathogens at shrimp aquaculture facilities.

Van De Nieuwegiessen, P. G., Olwo, J., Khong, S., Verreth, J. A. J. & Schrama, J. W. 2009. Effects of age and stocking density on the welfare of African catfish, *Clarias gariepinus* Burchell. *Aquaculture,* 288**,** 69-75.

van de Vis, J. W., Poelman, M., Lambooij, E., Begout, M. L. & Pilarczyk, M. 2012. Fish welfare assurance system: initial steps to set up an effective tool to safeguard and monitor farmed fish welfare at a company level. *Fish Physiol Biochem,* 38**,** 243-57.

Van Loo, E. J., Caputo, V., Nayga, R. M. & Verbeke, W. 2014. Consumers' valuation of sustainability labels on meat. *Food Policy,* 49, Part 1**,** 137-150.

Vázquez-Rowe, I., Hospido, A., Moreira, M. T. & Feijoo, G. 2012. Best practices in life cycle assessment implementation in fisheries. Improving and broadening environmental assessment for seafood production systems. *Trends in Food Science & Technology,* 28**,** 116-131.

Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S. & Sánchez-Vázquez, F. J. 2011. Effects of light during early larval development of some aquacultured teleosts: A review. *Aquaculture,* 315**,** 86-94.

Villanueva, R. R., Araneda, M. E., Vela, m. & Seijo,J. C. 2013. Selecting stocking density in different climatic seasons: A decision theory approach to intensive aquaculture. *Aquaculture,* 384-387**,** 25-34.

Volpe, J. P., Gee, J. L. M., Ethier, V. A., Beck, M., Wilson, A. J. & Stoner, J. M. S. 2013. Global aquaculture performance index (GAPI): The first global environmental assessment of marine fish farming. *Sustainability (Switzerland),* 5**,** 3976-3991.

Weingartner, M., Volpato Zanandrea, A. C. & Filho, E. Z. 2015. Cryopreserved sperm for oocyte fertilization of dourado *Salminus brasiliensis*. Sêmen criopreservado na fertilização de ovócitos de dourado *Salminus brasiliensis.,* 45**,** 892-897.

Wunderink, Y. S., Engels, S., Halm, S., Yúfera, M., Martínez-Rodríguez, G., Flik, G., Klaren, P. H. M. & Mancera, J. M. 2011. Chronic and acute stress responses in Senegalese sole (*Solea senegalensis*): The involvement of cortisol, CRH and CRH-BP. *General and Comparative Endocrinology,* 171**,** 203-210.

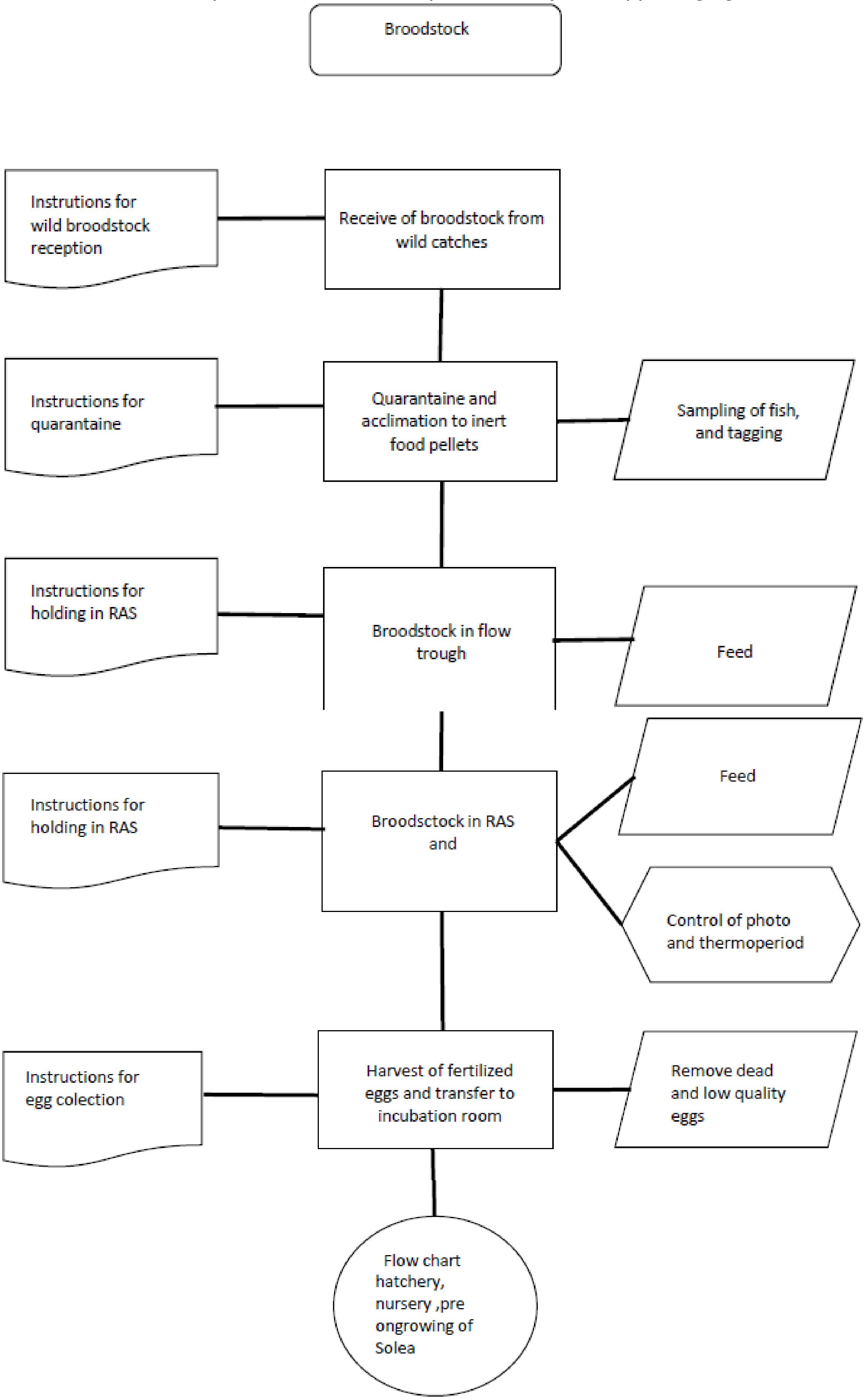
Yang, L., Chou, L.-S. & Shieh, W. K. 2001. Biofilter treatment of aquaculture water for reuse applications. *Water Research,* 35**,** 3097-3108.

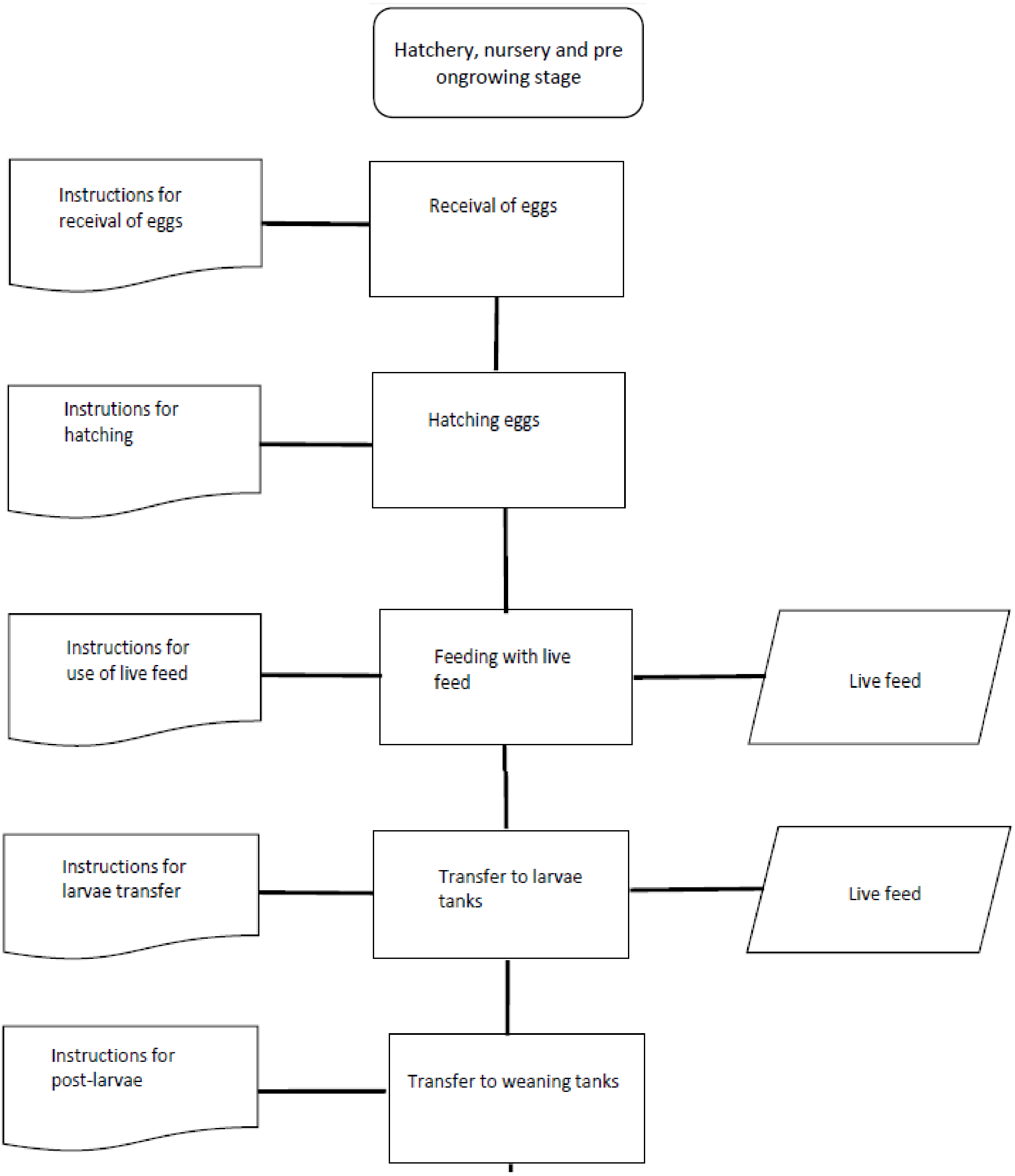
Yuqing, L., Yanxiang, W. & Liling, C. 2014. The Design of Automatic Control System for Industrialized Recirculating Aquaculture. *Advanced Materials Research***,** 246.

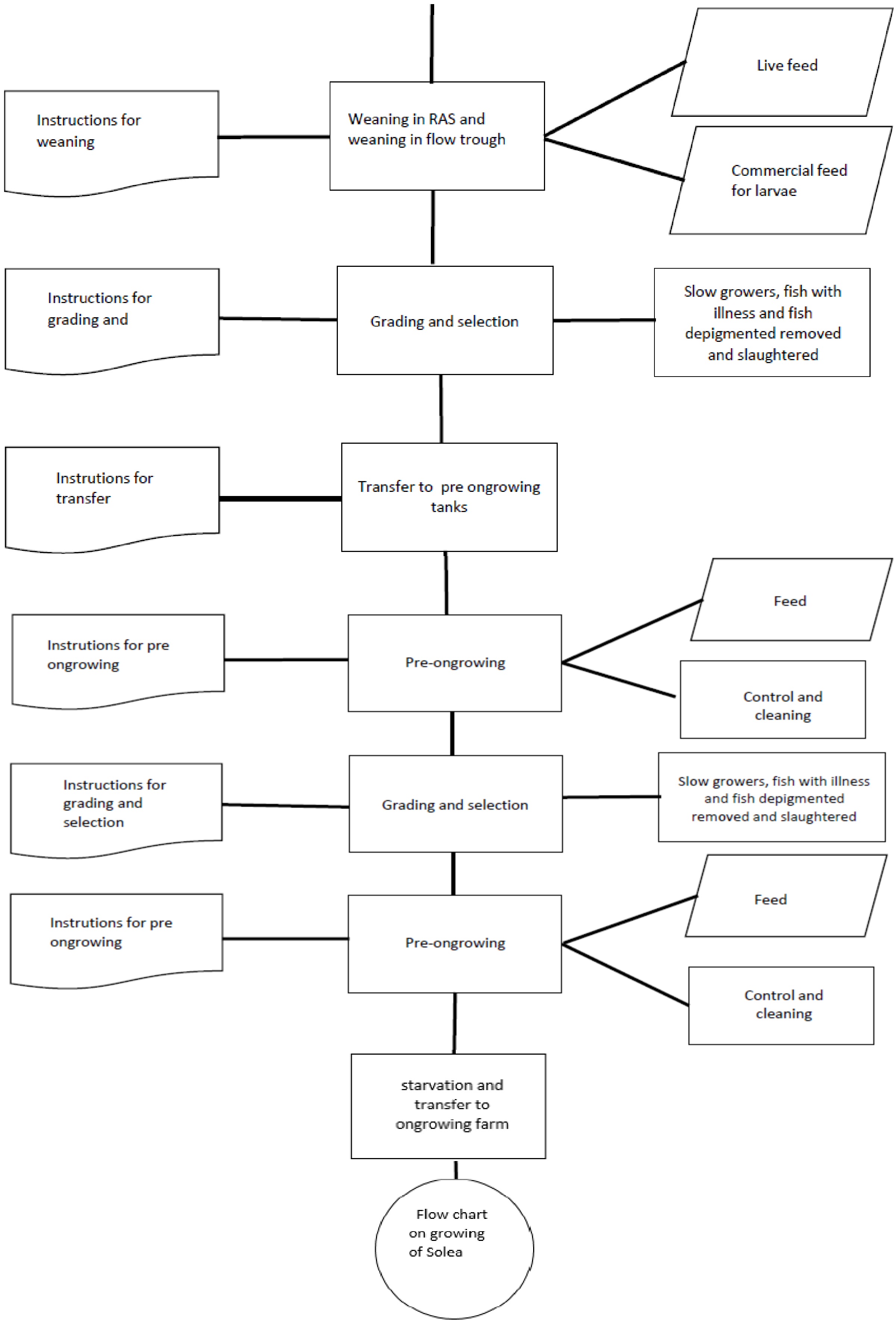
Zhang, S.-Y., Li, G., Wu, H.-B., Liu, X.-G., Yao, Y.-H., Tao, L. & Liu, H. 2011. An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. *Aquacultural Engineering,* 45**,** 93-102.

Annexes

Annex 1: Complete flow chart of the production cycle, supporting figures 5 and 6.




Hatchery, nursery and pre ongrowing stage
Instructions for receival of eggs
Receival of eggs
Instrutions for hatching
Hatching eggs
Instructions for use of live feed
Feeding with live feed
Live feed
Instructions for larvae transfer
Transfer to larvae tanks
Live feed
Instructions for post-larvae
Transfer to weaning tanks


Live feed
Instructions for weaning
Weaning in RAS and weaning in flow trough
Commercial feed for larvae
Instructions for grading and
Grading and selection
Slow growers, fish with illness and fish depigmented removed and slaughtered
Instrutions for transfer
Transfer to pre ongrowing tanks
Feed
Instrutions for pre ongrowing
Pre-ongrowing
Control and cleaning
Instructions for grading and selection
Grading and selection
Slow growers, fish with illness and fish depigmented removed and slaughtered
Feed
Instrutions for pre ongrowing
Pre-ongrowing
Control and cleaning
starvation and transfer to ongrowing farm
Flow chart on growing of Solea

Annex 2: Entire FWAS plan supporting tables 3, 4, 5 and 6

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Receive breeders from the wild	**Biotic**												
	Diseases	Natural environment	Monitor animals at source	Selection of fishing site	Here m=M / Health condition (fish without illness/damage or fish with illness/damage)		Assess that the fish is healthy	Health condition analysis, swmiming, outer appearance response to feed given	At each purchase	Broodstock manager	Purchase from another site/season	Report of labaratory analisys	keep records of analysis from each supplier
	Fish may be contaminated with pollutants in previous holding	Natural environment	Monitor animals at source	Analysis result	Here m=M / Health condition (fish without illness/damage or fish with illness/damage)		Assess that the fish is healthy	Perform water analysis	At each purchase	Broodstock manager	Purchase from another site/season	Report of labaratory analisys	keep records of analysis from each supplier
	Abiotic												
	Transport conditions	Badly managed on transportation	Monitor transport parameters	Use of Good manufacturing practices to use transport equipment	Here m=M / Make sure Good manufacturing practices are being used		Assess that Good manufactuing practices are being followed	Inspect work done	Every transport	Broodstock manager	Train workers	Assess knowledge and verify if transport plan is followed	monotoring equipment during transportation
	Managerial												
	Rough handling during catch	Badly managed catching	Instruct the workers	Intructions for handling during catching are followed	Here m=M / Fish condition (fish without damage or fish with damage)		Assess that instructions are followed	Inspect work done	At each capture or purchase	Broodstock manager	Train workers	Check external damage and long term survival (2 weeks)	keep records of sample analysed, corrective actions
	Environmental	not applicable											

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Quarantine and acclimation to inert food pellets	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health in case it has not been done at catching site	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that the fish is healthy	Check if the workers are following the biosecurity plan properly	Daily	Broodstock manager	Train the workers	A sampling plan is used to monitor the fish	keep records of analysis, corrective actions
	Abiotic												
	Fish exposed to deteriorated water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of quarantine holding tanks are followed	$O_2 < 60\%$	$O_2 > 160\%$	Monitor water quality as specified in the instructions	Inspect work done	Daily	Broodstock manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Adapt fish to artificial feed	Badly managed transition to artificial feed	Instruct the workers; feed of special composition (atractants)	Use of instructions given to adapt fish to inert feed	Here m=M/ Make sure the instruction are followed		Asses that instruction for inert feed adaptation are followed	Inspect work done	Daily	Broodstock manager	Train the workers; change feed type/supplier	Assess knowledge and use of instructions; verify feed used	keep records of analysis, instructions and corrective actions
	Disturbance	Caused by visitors and/or workers	Sound management; Instruct workers/visitors how to aproach the area	Use of Instructions given for handling of fish and quarantine area	Here m=M/ Fish behaviour (fish is swimming constantly or fish is settle at bottom)		Assess that instructions are followed	Inspect work done	When visitors/workers approach the fish	Broodstock manager	Train the workers/Plan proper instructions to visitors	Verify the disturbance when workers/visitors approach the fish	Keep records of instructions and assessments
	Environmental												
	Intake of polluted water to holding tanks	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Broodstock in flow trough	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Daily	Broodstock manager	Train the workers	A sampling plan is used to monitor the fish	keep records of instructions, analysis and corrective actions
	Abiotic												
	Fish exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of flow trough system are followed	O2 < 60%	O2 >160%	Monitor water quality as specified in the instructions	Inspect work done	Daily	Broodstock manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Disturbance	Caused by visitors and/or workers	Sound management; Instruct workers/visitors how to aproach the	Use of Instructions given for handling of fish and quarantine area	Here m=M/ Fish behaviour (fish is swimming constantly or fish is settle at bottom)		Assess that instructions are followed	Inspect work done	When visitors/workers approach the fish	Broodstock manager	Train the workers/Plan proper instructions to visitors	Verify the disturbance when workers/visitors approach the fish	Keep records of instructions and assessments
	Environmental												
	Intake of polluted water to holding tanks	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Broodstock in RAS	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Daily	Broodstock manager	Train the workers	A sampling plan is used to monitor the fish	keep records of instructions, analysis and corrective actions
	Abiotic												
	Fish exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of flow trough system are followed	O_2 < 60%; ammonia 0,75mg/l; nitrite 1,5 mg/l	O_2 >160% ; ammonia 1mg/l; nitrite 1,8 mg/l	Monitor water quality as specified in the instructions	Inspect work done	Daily	Broodstock manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Disturbance	Caused by visitors and/or workers	Sound management; Instruct workers/visitors how to aproach the area	Use of Instructions given for handling of fish and quarantine area	Here m=M/ Fish behaviour (fish is swimming constantly or fish is settle at bottom)		Assess that instructions are followed	Inspect work done	When visitors/workers approach the fish	Broodstock manager	Train the workers/Plan proper instructions to visitors	Verify the disturbance when workers/visitors approach the fish	Keep records of instructions and assessments
	Environmental												
	Intake of polluted water to holding tanks	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective	Verification	Records
					m	M	What	How	Frequency	Who			
Harvest of fertilized eggs and transfer to incubation room	**Biotic**												
	Abiotic												
	Managerial												
	Rough handling of the eggs	badly managed	Instruct the workers	Make sure instructions are followed	Here m=M/ Make sure egg hadling instructions are followed		Assess that instructions are followed	Inspect work done	Every egg collecting	Broodstock manager	Train the workers	Verify the eclosion rate	keep records of the ecclosion rate
	Environmental												
	Temperature difference between broodstock tanks and incubation tanks	badly managed	Instruct the workers	Make sure instructions are followed	Here m=M/ Maximum temperature difference 1ºC		Assess that the temperature difference does not exceed 1°C	Inspect work done	Every egg transfer	Broodstock manager	Trains the workers	Verify measurements are made	keep records of temperatures in every transfer

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Hatching eggs	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor egg/larvae quality; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	A sampling plan is used to monitor the fish	keep records of instructions , analysis and corrective actions
	Abiotic												
	Fish exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of system are followed	Operating limits for operation in instructions	Critical limits for water quality in instructions	Monitor water quality as specified in the instructions	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Eggs/larvae handled roughly during hatching	Bad management of hatching conditions	Instruct the workers	Use of instructions for hatching of the eggs	Here m=M / avoid rough handling of eggs/larvae		Assess that instructions are followed	Inspect work done	Every hatching of eggs	Hatchery manager	Train the workers	Verify the ecclosion rate	keep records of the ecclosion rate
	Environmental												
	Intake of polluted water to holding tanks	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Feeding of larvae with live feed	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used to monitor the larvae	keep records of instructions, analysis and corrective actions
	Abiotic												
	Inadequate live feed quality/regime	Bad management of live feed productions	Produce good quality and properly enriched live feed	Use of live feed production instructions	Here m=M/ Make sure instructions is followed		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used to monitor live feed quality	keep records of instructions, analysis and corrective actions
	Larvae exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of flow trough system are followed	O2 < 60% ?	O2 >160% ?	Monitor water quality as specified in the instructions	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Larvae are handled roughly	Badly managed	Instruct the workers	Instructions for handling/feeding the larvae are followed	Here m=M/ larvae behaviour and condition		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	Verify a sampling plan is planned to monitor larvae condition	Keep records of instructions and assessments
	Disturbance	Caused by visitors and/or workers; light regime	Sound management; light regime; Instruct workers/visitors how to aproach the area	Use of Instructions given for handling of larvae	Here m=M/ larvae behaviour and condition		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers/Plan proper instructions to visitors	Verify the disturbance when workers/visitors approach the fish	Keep records of instructions and assessments

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Feeding of larvae with live feed	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used to monitor the larvae	keep records of instructions, analysis and corrective actions
	Abiotic												
	Inadequate live feed quality/regime	Bad management of live feed productions	Produce good quality and properly enriched live feed	Use of live feed production instructions	Here m=M/ Make sure instructions is followed		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used to monitor live feed quality	keep records of instructions, analysis and corrective actions
	Larvae exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of flow trough system are followed	Oxygen < 60%	Oxygen >160%	Monitor water quality as specified in the instructions	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Larvae are handled roughly	Badly managed	Instruct the workers	Instructions for handling/feeding the larvae are followed	Here m=M/ larvae behaviour and condition		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	Verify a sampling plan is planned to monitor larvae condition	Keep records of instructions and assessments
	Disturbance	Caused by visitors and/or workers; light regime	Sound management; light regime; Instruct workers/visitors how to aproach the area	Use of Instructions given for handling of larvae	Here m=M/ larvae behaviour and condition		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers/Plan proper instructions to visitors	Verify the disturbance when workers/visitors approach the fish	Keep records of instructions and assessments
	Environmental												
	Intake of polluted water to live feed production tanks	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
	Environmental												
	Intake of polluted water to live feed production tanks	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions
Weaning	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used to monitor the larvae	keep records of instructions, analysis and corrective actions
	Abiotic												
	Inadequate inert feed quality	Irregular feed quality supplied	Selection of supplier based on quality standards	Acquisition of tested weaning diets	Here m=M/ only tested diets to be used		Assess feed quality	Check fish respons e to feed; check fish	Daily	Hatchery manager	Change supplier	Verify feed quality, fish response and growth	Keep records of feed quality and corrective actions
	Fish exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of weaning system are followed	O2 < 60%; ammonia 0,75mg/l; nitrite 1,5 mg/l	O2 >160% ; ammonia 1mg/l; nitrite 1,8 mg/l	Monitor water quality as specified in the instructions	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
	Managerial												
	Innapropriate feed regime	Bad management of feed regime	Instruct the workers	Use of feeding instructions	Here m=M/ Make sure the instructions are		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	Assess knowledge and use of instructions;	Keep records of instructions and assessments
	Elimination of selected fish	Selection of bad growers and defective fish	Instruct the workers	Use of instructions to kill selected fish	Here m=M/ Make sure the instructions are		Asses the instructions are followed	Inspect work done	Every time fish is eliminated	Hatchery manager	Train the workers	Assess knowledge and use of instructions;	Keep records of instructions and assessments
	Disturbance	Caused by visitors and/or workers; light regime	Sound management; light regime; Instruct workers/visitors how to aproach the area	Use of Instructions	Here m=M/ larvae behaviour and condition		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers/Plan proper instructions to visitors	Verify the disturbance when workers/visitors approach the fish	Keep records of instructions and assessments
	Environmental												
	Intake of polluted water to weaning system	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions
	Live feed grown in polluted water	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
Grading, selection and transfer	**Biotic**	not applicable											
	Abiotic	not applicable											
	Managerial												
	Elimination of selected fish	Selection of bad growers and defective fish	Instruct the workers	Use of instructions to kill selected fish	Here m=M/ Make sure the instructions are		Asses the instructions are followed	Inspect work done	Every time fish is eliminated	Hatchery manager	Train the workers	Assess knowledge and use of instructions;	Keep records of instructions and assessments
	Rough handling of the fish or exposed to air for too long	badly managed	Instruct the workers	Use of instructions	Here m=M/ Make sure the instructions are		Asses the instructions are followed	Inspect work done	Every time fish is handled	Hatchery manager	Train the workers	Assess knowledge and use of instructions;	Keep records of instructions and assessments
	Environmental	not applicable											
Pre ongrowing	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is followed		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used to monitor the larvae	keep records of instructions, analysis and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
	Abiotic												
	Inadequate feed quality	Irregular feed quality supplied	Selection of supplier based on quality standards	Acquisition of tested diets	Here m=M/ only tested diets to be used		Assess feed quality	Check fish response to feed; check fish growth	Daily	Hatchery manager	Change supplier	Verify feed quality, fish response and growth	Keep records of feed quality and corrective actions
	Fish exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of pre-ongrowing are followed	O2 < 60%; ammonia 0,75mg/l; nitrite 1,5 mg/l	O2 >160% ; ammonia 1mg/l; nitrite 1,8 mg/l	Monitor water quality as specified in the instructions	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions
	Managerial												
	Innapropriate feed regime	Bad management of feed regime	Instruct the workers	Use of feeding instructions	Here m=M/ Make sure the instructions are		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	Assess knowledge and use of instructions;	Keep records of instructions and assessments
	Elimination of selected fish	Selection of bad growers and defective fish	Instruct the workers	Use of instructions to kill selected fish	Here m=M/ Make sure the instructions are		Asses the instructions are followed	Inspect work done	Every time fish is eliminated	Hatchery manager	Train the workers	Assess knowledge and use of instructions;	Keep records of instructions and assessments
	Disturbance	Caused by visitors and/or workers; light regime	Sound management; light regime; Instruct workers/visitors how to aproach the area	Use of Instructions	Here m=M/ larvae behaviour and condition		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers/Plan proper instructions to visitors	Verify the disturbance when workers/visitors approach the fish	Keep records of instructions and assessments

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
	Environmental												
	Intake of polluted water to weaning system	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions
Starvation and transfer to ongrowing facilities	**Biotic**												
	Disease	Environment and bad management of holding conditions	Monitor fish health; verify if the UV treatment is working properly	Use of biosecurity plan	Here m=M/ Make sure the biosecurity plan is		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used to monitor the larvae	keep records of instructions, analysis and corrective actions
	Abiotic												
	Fish exposed to deteriored water quality	Bad management of holding conditions	Instruct the workers	Instructions for operation of pre-ongrowing are followed	O2 < 60%; ammonia 0,75mg/l; nitrite 1,5 mg/l	O2 >160% ; ammonia 1mg/l; nitrite 1,8 mg/l	Monitor water quality as specified in the instructions	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for water analysis	keep records of analysis, instructions and corrective actions

Step in process	Hazard	Source	Preventive mesures	CCP	Criteria		Monitoring				Corrective actions	Verification	Records
					m	M	What	How	Frequency	Who			
	Managerial												
	Rough handling of the fish	badly managed	Instruct the workers	Make sure instructions are followed	Here m=M/ Fish shoud not reveal excessive physical		Assess that the handling is gentle	Inspect the fish in ongrowing facility and inspect	Every transfer	Hatchery manager	Train the workers	verify the condition of the fishes in ongrowing facility	keep record the condition of the fishes, instructions and
	Environmental												
	Intake of polluted water to weaning system	Bad management of water intake	Instruct the workers	Make sure instructions are followed	Here m=M/ Avoid intake water if polluted		Assess that instructions are followed	Inspect work done	Daily	Hatchery manager	Train the workers	A sampling plan is used for analysis of intake water	Keep records of analysis, instructions and corrective actions