



SEACASE SUSTAINABLE EXTENSIVE AND SEMI-INTENSIVE COASTAL AQUACULTURE IN SOUTHERN EUROPE

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Slaughtering procedures: fish from extensive and semi-intensive production

DELIVERABLE 10

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The deliverable 10 is a description of the slaughtering processes actually used in non-intensive aquaculture systems, based in SEACASE evaluations by direct contact with farmers and also using results from an enquiry specially designed to this purpose. It also includes, in Annex, the description and conclusions of a pilot trial, performed by SEACASE in one of the farming units that collaborates with the project, in which changes in some parameters during slaughter in ice slurry were evaluated in order to improve slaughter efficacy. The main objective is to provide a picture of the current situation in terms of slaughtering and welfare, to be used as basic information for slaughtering and welfare improvement in this kind of activities, and also to contribute with practical suggestions for welfare improvement associated with slaughtering.

Slaughtering procedures: fish from extensive and semi-intensive production

Table of Contents

• Introduction	3
• Objectives	5
• Methods	5
• Results and Discussion.....	5
• Conclusions.....	7
• Recommendations	7
• Annex 1: Enquiry (English version).....	8
• Annex 2: Pilot trial (Aqualvor)	17

• Introduction

One of the most recent generalized concerns, both from fish industry and from fish consumers, is about slaughtering techniques and associated welfare, in search for an ideal “humane slaughtering”. As the main focus of SEACASE project is on non-intensive aquaculture, this and other aspects of fish quality and acceptance are of primordial importance.

Fortunately, the interest of the industry is coincident with the interest of their clients: most efficient, rapid and humane slaughtering always originates better quality products, and fish is no exception to this rule. In fact, the less the peri-mortal stress and pain, the better is the initial quality and the latter preservation capacity. An effective improvement in the current slaughtering techniques can be associated with non-intensive farming as an added value.

The main killing methods actually in use in aquaculture are:

Cerebral concussion

This method is also named as percussive stunning, knocking or blow in the head. It can be used also as a stunning method, but here it will be considered as killing method, and in this perspective this is a very common method, of worldwide spread use. It is very simple, easy to perform (although some practice is needed to achieve perfection) and it is common in all salmon farming, where several “killing machines” were developed based on this method. This is a very effective and welfare-supportive method, but only if the efficacy is verified, as sometimes fish show recover signs (so, later it becomes evident that fish was not effectively killed).

Electrocussion

This is a method normally used as a complementary method in combination with others, like CO₂ narcosis and lowering the temperature. Results depend mainly on species, water salinity and other chemical and physical properties, characteristics of the electrical current applied and other indirect factors. The fish unconsciousness or killing obtained depends highly on the intensity and length of the current applied. As in the previous method, the final killing of the fish is frequently a result from asphyxia and not the low temperature and the consequent low metabolism, but the electricity seems to be a very effective way of shortening the processing times, thus increasing welfare.

CO₂ narcosis

It is used commonly as a complementary method to fish crowding and electricity. This method helps to reduce killing times of other methods, or it can be used alone. In this last case, respiration is difficult or impossible and fish dies, within a period of time that depends of species, water characteristics and other less evident and/or known factors.

Thermal shock

Thermal shock in ice slurry or in a mixture of ice+water is used as stunning method and was described above, but the efficiency (both for stunning and for killing) is arguable as, depending on species and many other unclear factors, fish can take too long to die and welfare is then not guaranteed as it should be. But it must be emphasized that this is most common method used nowadays by the farming industry, although the final killing of the fish is frequently a result from asphyxia and not the low temperature and the consequent low metabolism.

Asphyxia

Also known as death in air, this is a very common killing method, especially when applied after a stunning method. It is very simple and easy to apply, cheap and convenient for the industry, as it only implies leaving the fish out of water. Fish will die by lack of oxygen in the tissues, showing signs of struggle or being quiet depending on species and some other factors. To increase efficiency or to avoid taking fish from the water, this can also be done by crowding the fish in a small space and by the inclusion of a high percentage of CO₂ in the same water.

Brain spiking

This method is also named pin-bolt, decerebration or brain destruction. This is a very specific method, as it is almost exclusively applied to some species like tuna and other fish of considerable dimensions. A metallic pin or special sharp-pointed hand-held instrument is used by experienced workers to achieve the brain tissues and, by lateral movements, to destroy the integrity of the organ, inducing immediate death. It is considered as an appropriate welfare method, because it is very quick (it takes only a few seconds to induce death).

Evisceration

This is considered as a non-acceptable method in terms of welfare, but is still used in some parts of the world, namely in Asiatic countries. No references were found for the use of this method within Europe (as direct killing method), but unintentionally it still can exist, as in some evisceration rooms after killing, there is no complete confidence on fish being always dead and consequently some can be eviscerated still alive.

Gill excision

Like for the previous method, this is considered as a non-acceptable method in terms of welfare. Gills are taken of by precise cuts and fish dies as a consequence of the bleeding (loss of blood, low blood pressure, heart stop).

As the stress and welfare represent the most interesting and important characteristics of these methods, some important considerations to keep in mind are:

- less time suffering and less stress, higher welfare and higher fish quality
- it is difficult to change industrial activities in a short time
- there is a controversy about the ice slurry method, the most used method
- trustable indicators of welfare or even stress have not yet been developed
- processes depend considerably on tradition, facilities, purposes, times and costs

• Objectives

The main objective is to provide a picture of the current situation in terms of slaughtering and welfare, to be used as basic information for the improvement of the slaughtering techniques and resulting welfare. A set of recommendations, that will be later developed in Deliverables 74 to 79 (dedicated to Codes of Conduct) is also an important objective.

• Methods

The methods used to achieve the results described in this deliverable can be divided in 3 main kinds:

- 1) Collection of published information about fish slaughtering methods;
- 2) Direct contact (through personal visits) with farmers from SEACASE and other farmers in all countries involved; and
- 3) Performing of an enquiry especially dedicated and designed to collect specific details on pre-slaughtering and slaughtering methods, to be filled in by farmers (SEACASE and others)

The first versions of the enquiry were tested and improved, and the final version is shown in Annex 1.

The number of enquiries filled in by farmers was 6.

• Results and Discussion

The results can be summarized as follows:

Summary of results from enquiry answers

Enquiry topics	Main answers
1) Farmed Species	seabass, seabream
2) Production system and main characteristics	4 ha, 20 tonnes/year, 5.5 ha, 70 tonnes/year, 6 ha, 170 tonnes/year, 12 ha, 400 tonnes/year Semi-intensive (mainly artificial feed)
3) Pre-slaughter data	1 to 5 days starving period, sometimes ½ tank water lowering 1 to 2 h separation time with nets
4) Catching method (removal from water)	hand nets (30 s to 5 min total) surrounding nets (5 to 120 min total) trawling nets (10 to 15 min total) water lowering (120 min)
5) Stunning method	all answers: not used
6) Killing method	all answers: ice slurry between 75 and 500 kg freshwater ice/m ³ seawater no precise idea about temperatures (initial, after adding fish and at death) 3 to 5 min to death (seabream), 5 to 10 min (seabass)
7) Flowdiagram	data and details as predicted
8) Processing	in all farms, around 10 kg of fish are ice-boxed in polystyrene boxes, with draining system and normally no lid.
9) Overall animal welfare	welfare considered as relatively good in the parameters: feed quantity, starving period, fishing and transportation, rapidity and efficiency of killing welfare considered as relatively poor in the parameters: feed quality, water pollution and eutrophication, density, fishing, washing, calibration, delays on procedures,
10) Additional information	no farmers added information (long enquiry?)

Immersion in ice slurry is by far the most used killing method. It is performed by transferring the fish from growing tanks directly to a mixture of freshwater ice + seawater. It is used in all species in which it is possible (species that die quickly in ice slurry, like seabass and seabream). Only species that cannot be killed in ice slurry (because they die very slowly), like eels, flatfish and salmonids) are killed using other methods, but even in this cases ice slurry is used and complemented with other techniques to increase rapidity.

Although considered a controversial method, it is considered acceptable and even recommended within the farming industry, for example by The Federation of European Aquaculture Producers (FEAP), composed by the National Aquaculture Associations of European countries. On the other hand, many of the independent organizations with concerns in this area claim it is unacceptable.

Scientific research is still trying to prove which is best, since the major constrain is the welfare assessment. SEACASE defined and evaluated the use, among other methods, of *rigor mortis* measurements as peri-mortal stress indicators, ending with some possible improvements to the present situation, and will do efforts to spread and implement all positive results that were obtained. The SEACASE team and all participants of the fish producing chain that were contacted by the project believe that this will be certainly a very perceivable improvement on the quality and welfare of farmed fish, for the currently very well informed consumers. This will contribute strongly to improve the image, acceptance and success of farmed products.

• Conclusions

Immersion in ice slurry is presumptively almost the only method used in non-intensive seabream and seabass aquaculture. It is somewhat controversial in terms of welfare (only with more precise methods for welfare evaluation this controversy can be reduced), it is ideal as method because it is easy to perform in high fish quantities and it is relatively cheap. It is not easily applicable to some other species (some, like eels, salmonids and flat fish, that take too long to die in ice slurry or don't die at all) without combinations with other methods, like electrocussion and increased asphyxia by CO₂.

Immersion in ice slurry is a method that can be considerably improved, both from the industrial and the welfare points of view, by increasing the speed of death. This can be achieved by using less water, more ice, colder ice, less fish or a combination of these.

To achieve a more effective increase in the welfare of fish, the phase of production in which changes can be more effective and easy to apply are all pre-slaughter steps, like water quality, fish feed, calibration, starving and catching operations, crowding, transport, and handling before slaughter, as the bigger welfare problems cited by farmers and found in literature and by SEACASE direct contact with industry are almost exclusively located in the pre-slaughter phase.

• Recommendations

As a consequence of all previous considerations, the recommendations of SEACASE are in the following directions:

- Increase ice slurry rapidity (more ice, less water, less fish, combination with other methods);
- Improve welfare in all possible pre-slaughter steps;
- Developing of more clear welfare indicators and introduce changes in processes according to new findings.

- Annex 1: Enquiry (English version)

Project SEACASE
Task 2.2 - Optimizing slaughtering procedures

ENQUIRY TO PROJECT SEACASE FARMERS

OBJECTIVES

This is a first questionnaire to evaluate the current situation, as a basic knowledge collection to let us think on ways to improve the procedures and to achieve more efficient methods, with better animal welfare.

FILLING AND SENDING

We suggest it should be filled by a person deeply involved in the technical management of the farm. It might be easier to print, fill it by hand and send it back by post mail, to:

Paulo Vaz-Pires
ICBAS
Largo Prof. Abel Salazar, 2
4099-003 PORTO / PORTUGAL

An alternative is to fill it using the Word of your computer, and send it back as an e-mail attachment, as you received it. You will not be able to insert characters inside the boxes, but don't mind with formatting, just write a capital X **instead** of the squared box and write numbers or data **instead** of the underscored lines. Please choose the method you prefer.

Answers will be kept on the scope of the project and you can consider them as confidential. Obviously no judgement will be made on what is done; the actual situation will serve as a working basis and will be used to try to improve the general procedures.

Please feel free to comment the questions, to add ideas and suggestions and to give additional explanations when questions and/or answers are not enough clear.

Please answer only thinking on the major species you farm; fill another questionnaire to other important species, especially if there are noticeable differences.

1) FARMED SPECIES

(Please answer taking into account the farm where the fish for the project will be produced)

SPECIES	produced	main in quantity	main in value	choose one for this enquiry
example of marking using Word:	X	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eel (<i>Anguilla anguilla</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Rainbow trout (<i>Oncorhynchus mykiss</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Salmon (<i>Salmo salar</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sea bass (<i>Dicentrarchus labrax</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sea bream (<i>Sparus aurata</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Senegalese sole (<i>Solea senegalensis</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Brill (<i>Scophthalmus rhombus</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Turbot (<i>Psetta maxima</i>)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
()	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
()	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
()	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
()	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
()	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

2) PRODUCTION SYSTEM AND MAIN CHARACTERISTICS

PRODUCTION SYSTEM	data
Production area (water surface)	_____ ha _____ m ² _____ m ³
Production spaces (on-growing)	<input type="checkbox"/> tanks, material: _____ <input type="checkbox"/> cages, material: _____ <input type="checkbox"/> earth ponds, lakes <input type="checkbox"/> other: specify: _____
Quantity produced by year	_____ kg/year _____ tonnes/year
Yield	_____ kg/m ² /year _____ tonnes/ha/year
Feeding system	<input type="checkbox"/> Only artificial <input type="checkbox"/> Mixed, mainly artificial <input type="checkbox"/> Mixed, mainly natural <input type="checkbox"/> Only natural
Do you consider this production...	<input type="checkbox"/> Intensive <input type="checkbox"/> Semi-intensive <input type="checkbox"/> Semi-extensive <input type="checkbox"/> Extensive
...mainly based on what criteria?	Specify: _____

3) PRE-SLAUGHTER DATA

(e. g. fish density, starving period, water level changes)

OPERATIONS BEFORE CATCHING	please specify:
Maximum fish density in growing tanks, cages, ponds, etc. (charge)	biomass (kg) / water surface (m ²): _____ biomass (kg) / water volume (m ³): _____
Starving period (feed withdrawal)	total starving time (days):
Water temperature (average and limits)	average temperature (°C): lower and upper limits: between _____ and _____ °C
Water level lowering (emptying as a confinement technique)	proportion of remaining water: _____% proportion of fish caught: _____%
Physical separation of the fish	total separation time (hours): type of barrier (nets, tank transfer, etc.):
Occurrence of repeated starving	proportion of starved fish that is effectively caught: _____%
Maximum fish density in crowding spaces (charge)	biomass (kg) / water surface (m ²): _____ kg/m ² biomass (kg) / water volume (m ³): _____ kg/m ³

4) CATCHING METHOD (REMOVAL FROM WATER)

CATCHING METHOD	specify total time (min):	please specify
Hand nets		
Mechanically operated nets or rigid grids		<input type="checkbox"/> nets, material: _____ <input type="checkbox"/> grids, material: _____
Surrounding nets		material: _____
Trawling nets		material: _____
Fish pumping		describe pumping system and tubes: _____ _____ _____
Water level lowering (emptying as a catching technique)		proportion of remaining water: _____% proportion of fish caught: _____%
Exposure to air		
Occurrence of repeated catches (fish escaping from the catching net or redirected to tanks instead of slaughtering)		proportion of caught fish that is effectively slaughtered: _____%

5) STUNNING METHOD

STUNNING METHOD	total handling time between (min-min)	time fish remain quiet between (min-min)	please add information about
manual blow in the head			instrument used:
mechanical blow in the head			machine used:
electrical shock			electricity used: ___ volt ___ ampere <input type="checkbox"/> continuous current <input type="checkbox"/> alternate current
temperature lowering			initial temperature: ___ °C final temperature: ___ °C
CO ₂ introduction in the water			pressure: ___ (units:___) flow: ___ (units:___) concentration before adding fish: ___ mg/l
anaesthetics			product used: chemical compound(s) trade name
compressed air shot			___ pressure (units:___)
other (specify)			describe system: _____ _____ _____

6) KILLING METHOD

KILLING METHOD	total handling time: between (min-min)	time until all fish remain quiet: between (min-min)	please add useful information
taking fish from tank and leave in air			
manual blow in the head			instrument used:
mechanical blow in the head			machine used:
electrical shock			electricity used: ___ volt ___ ampere <input type="checkbox"/> continuous current <input type="checkbox"/> alternate current describe system: _____
temperature lowering (cold shock)			<input type="checkbox"/> mechanical refrigeration <input type="checkbox"/> freshwater ice+freshwater <input type="checkbox"/> freshwater ice+seawater <input type="checkbox"/> other, specify: _____ kind of ice: <input type="checkbox"/> flakes <input type="checkbox"/> cubes <input type="checkbox"/> other, specify: _____ proportion ice:water: _____:_____ proportion fish: (ice+water): _____:_____ volume (or mass) ice:water: ___:___ litre (or kg) volume (or mass) fish:(ice+water): __:__ litre (or kg) initial temperature (before adding fish): _____ °C temperature immediately after adding fish: _____ °C final temperature (after all fish dies): _____ °C
CO ₂ introduction in the water			pressure: _____ (units:_____) flow: _____ (units:_____) concentration before adding fish: _____ mg/l
anaesthetics			active chemical compound(s): _____ trade name: _____
compressed air shot			pressure: _____ (units:_____) describe system: _____
manual brain spiking			instrument: _____ describe system: _____
mechanical brain spiking			machine: _____ describe system: _____ _____ _____
other (specify)			describe system: _____

7) FLOWDIAGRAM

Please change the following flowdiagram according to operations of your farm. Cut inexistent steps and add information about duration times.

FLOWDIAGRAM	RELEVANT DETAILS	ADDITIONAL COMMENTS
Farming	production and handling conditions	
↓		
Starving period	time	
↓		
Selection	market demands	
↓		
Separation	physical barriers	
↓		
Pre-catching treatment	temperature lowering	
↓		
Catching	emptying, nets, pumps	
↓		
Transportation	time, handling	
↓		
Post-catching treatment	low temperature	
↓		
Stunning	blow, thermal shock, electricity, CO ₂ , anaesthesia	
↓		
Slaughtering	blow, thermal shock, electricity, CO ₂ , brain spiking	
↓		
Death	time	
↓		
Boxing	time	
↓		
Icing	time, ice quality and quantity	
↓		
Dispatching	time	
↓		
Consumption	time, temperature and ice	

8) PROCESSING

PROCESSING	mark with X if normally done	please add useful information
Evisceration	<input type="checkbox"/>	time (for 1 fish): ___ min
De-heading	<input type="checkbox"/>	
Gill removal	<input type="checkbox"/>	
Washing	<input type="checkbox"/>	
Scaling	<input type="checkbox"/>	
Filleting	<input type="checkbox"/>	
Skining	<input type="checkbox"/>	
Icing	<input type="checkbox"/>	
Boxing	<input type="checkbox"/>	approximate size ___ x ___ x ___ (cm) material _____ <input type="checkbox"/> lid <input type="checkbox"/> draining system nr. fish/box ___ fish weight/box: ___ (kg) quantity of ice/box ___ (kg) ___ (%)
Refrigeration (room)	<input type="checkbox"/>	___ time (min, for 1 fish) ___ temperature (°C)
Transport in isolated truck	<input type="checkbox"/>	___ time (hour) ___ temperature (°C)
Transport in refrigerated truck	<input type="checkbox"/>	___ time (hour) ___ temperature (°C)
Freezing in forced air tunnel	<input type="checkbox"/>	___ time (min, for 1 fish) ___ final temperature (°C)
Freezing in nitrogen tunnel	<input type="checkbox"/>	___ time (min, for 1 fish) ___ final temperature (°C)
Packaging	<input type="checkbox"/>	packing material(s) shape approximate size atmosphere: <input type="checkbox"/> normal air <input type="checkbox"/> vacuum <input type="checkbox"/> modified if modified, indicate % gas composition CO ₂ : O ₂ : N ₂ _____ : _____ : _____ (%)
	<input type="checkbox"/>	
	<input type="checkbox"/>	

9) OVERALL ANIMAL WELFARE

Please rate the welfare conditions in your farm, in the several indicated situations.

WELFARE	rate from 0 (poor) to 10 (excellent)	main reason for not being excellent	possible improvements
Production - water quality			
Production - density			
Production - feeding quantity			
Production - feeding quality			
Production - _____			
Handling - weighing			
Handling - feeding operations			
Handling - vaccination			
Handling - calibration			
Handling - _____			
Catching - starving period			
Catching - confinement			
Catching - fishing			
Catching - transportation			
Catching - _____			
Stunning - time to stun 1 fish			
Stunning - efficacy (all fish stunned?)			
Stunning - method (fish seems to suffer?)			
Stunning - _____			
Killing - time to perform			
Killing - efficacy (fish always killed?)			
Killing - method (fish seems to suffer?)			
Killing - _____			
_____ - _____			
_____ - _____			
_____ - _____			

10) ADDITIONAL INFORMATION

Please add any information that could be useful to understand and improve your slaughtering process and/or overall welfare in your farm.

Name of the person who wrote the answers:

Name of the farm considered:

Adress:

E-mail:

Fax:

Thank you very much!

END OF THE ENQUIRY

- Annex 2: Pilot trial (Aqualvor)

Project SEACASE

Workpackage 2, task 2.2 - Optimize Slaughtering Procedures

Pilot trial: improvement of ice slurry temperature

Results and conclusions - May 2009

Introduction

Ice slurry is the most common technique used for stunning/killing of seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*), two of the most important species in modern aquaculture.

Although some of the reasons that farmers use to justify this method are obviously plausible and scientifically supported as correct, in terms of welfare there are still some less clear points.

Until clarification of these points occurs, one possible solution to improve the welfare is to reduce the time necessary to kill the fish, and this is achievable by several approaches. Reducing the temperature of the mixture ice/seawater (by using cooler types of ice or by increasing the amount of ice in relation to the seawater) and/or limiting the temperature rise caused when fish is added to the mixture (by increasing the volume of ice:seawater mixture in relation to the fish quantity added) are simple but effective ways of achieving lower temperatures and shorter times to complete killing. One must note that, if the amount of freshwater ice is increased, water salinity will be lower and this will contribute to create a higher osmotic differential between fish and external environment, which probably contributes to accelerate the killing process.

In this pilot trial, the common procedure adopted by the farm was used as control, and two different conditions were tested: one corresponding to a slight increase in temperature, the other corresponding to a slight decrease. Quality of the resulting fish (kept iced and boxed until rejection) was followed using several different quality-related parameters.

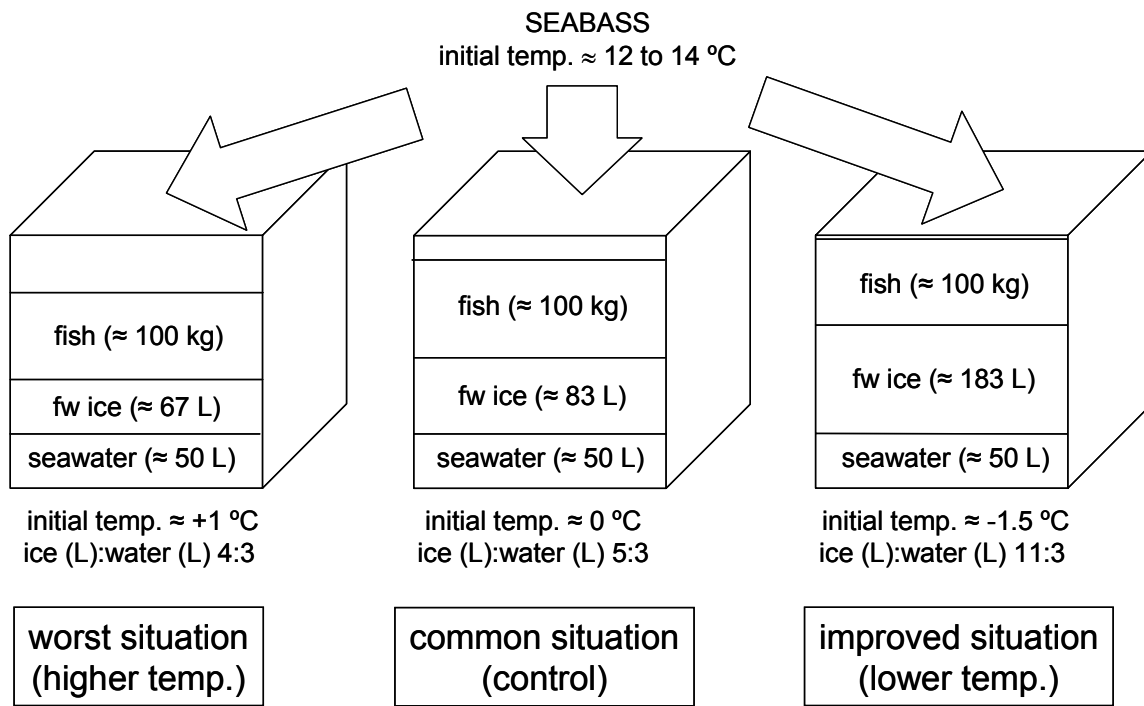
Materials and Methods

The following schematic diagrams (Fig 1 a) and b)) show the normal procedure and the two additional trial conditions performed in this pilot study at the farm.

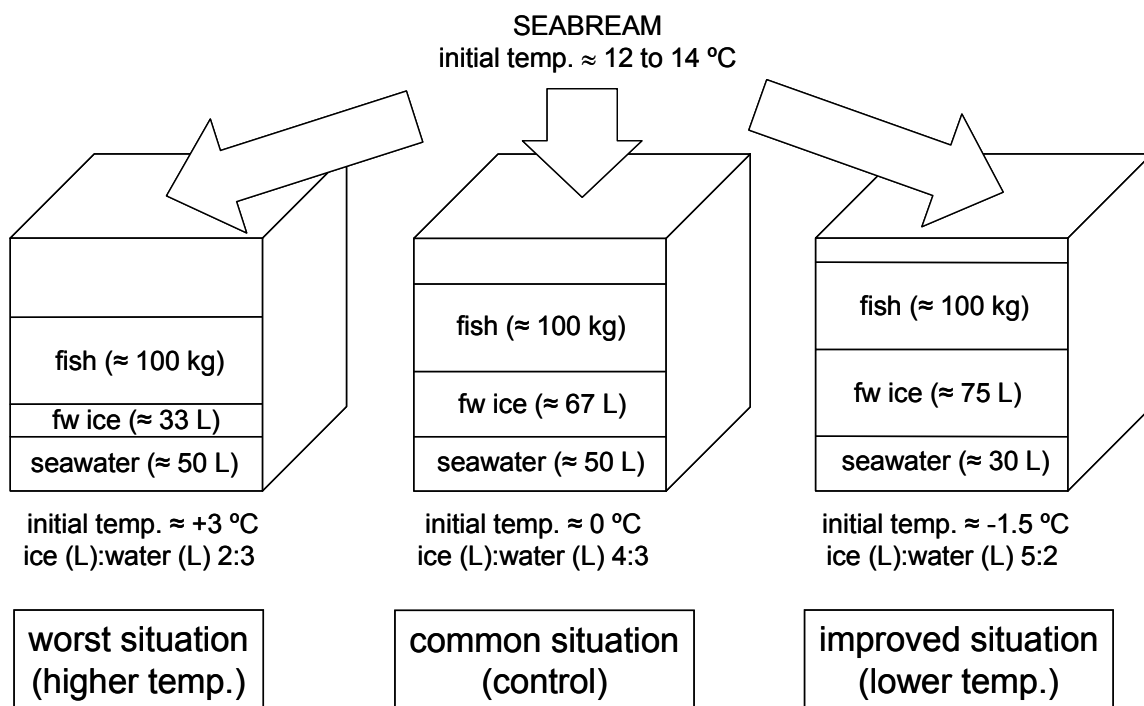
The amount of water was in all cases the same (50 L) except for seabream lower temperature treatment, in which water was reduced to 30 L to help reduce the temperature, but even taking this option the minimum temperature obtained was only -1.5 °C and not the -3 °C that were the ideal predicted at the beginning; the amount of ice was changed to achieve the different temperature conditions, and the amount of fish added was also kept approximately constant (around 100 kg).

In all quality evaluations, 6 fish from each treatment were randomly sampled and used for analysis. The total was therefore 6 fish x 3 treatments x 2 species = 36 fish. All results represent the mean of 6 fish.

For sensory evaluations, the Quality Index Method (QIM) was used (Table for seabass from Alasalvar *et al.*, 2002 and seabream from Huidobro *et al.*, 2000).



a)



b)

Fig. 1 - Schematic representation of the normal procedure (at center) and the two additional trial conditions (left and right sides) performed in this pilot study:

a) method for seabass; b) method for seabream.

Rigor mortis was measured according to Azam et al. (1990) but with a modification in the registration method (visually done, instead of registered by a video camera).

Freshmeter (Rafagnatækni Electronics, Reykjavík, Iceland) was used in both fish sides, touching each side of each fish for approximately 3 seconds.

Torrymeter (Distell Industries Ltd, West Lothian, Scotland) was used taking 3 individual measurements at each of both sides of all fish.

Microbial counts were performed in skin samples, taken by swabbing with cotton swabs of an area of around 12 cm² near the operculum, above the lateral line. Samples were appropriately diluted and plated on Iron agar (which allows counts of the total number of microorganisms and the number of specific spoilage organisms, *Pseudomonas* agar (for counts of *Pseudomonas* species) and McConkey agar (for counts of Enterobacteriaceae).

Results

Results are presented, in graphic form, in the following figures (Fig. 2 to 15). Results of McConkey agar counts are not shown, as they were not consistent. But the low numbers found were always close to the minimum detectable limit, which is normally associated with low precision. All numbers reveal an adequate low microbiological load of *Enterobacteriaceae*.

Discussion

Results for seabass and seabream are quite similar and can be analysed together.

Rigor mortis measurements show the common expected pattern of pre-rigor / rigor / post-rigor, but the maximum rigor is not shown as it occurred somewhere between 6 and 24 h after death. Although the differences in the *rigor* results in this trial were expected to be small and difficult to observe, the methodology can be further developed to allow higher precision, and the logistic planning could be improved in order to avoid the transportation of the samples (first hours done at the farm, following days done at a laboratory, with 24 hours in between with no measurements). From the results obtained, however, the slight differences detected between the 3 temperatures during slaughter are not related with the treatments and seem to be induced by other parameters, like differences between *rigor* process of individual fish, fish size, muscle chemical or physical characteristics and others, not defined.

Quality indicators (QIM, *rigor mortis*, Freshmeter and Torrymeter measurements) do not show perceptible differences between treatments with different temperatures, so temperatures (both higher and lower) did not induce visible differences in fish quality over time. However, in the first 2 days on ice (which is the most interesting period of high quality) some slight differences particularly for sensory scores (QIM) can be observed, the treatment with lower temperatures (0 °C and -1.5 °C) showing consistently better quality scores (lower demerit points). This beneficial effect seems to be lost after 3 days in ice.

The Freshmeter and Torrymeter values show expected patterns (general shape of the curves) but no considerable differences between treatments. The extremely small differences induced, if any, are responsible for these results.

Microbiology results also show an expected increase in numbers with time, both for the total number of microorganisms and for *Pseudomonas* counts, with a lag phase of approximately 2 days in ice. Numbers of specific spoilage organisms were not countable in many of the plates. No differences seem to exist between treatments.

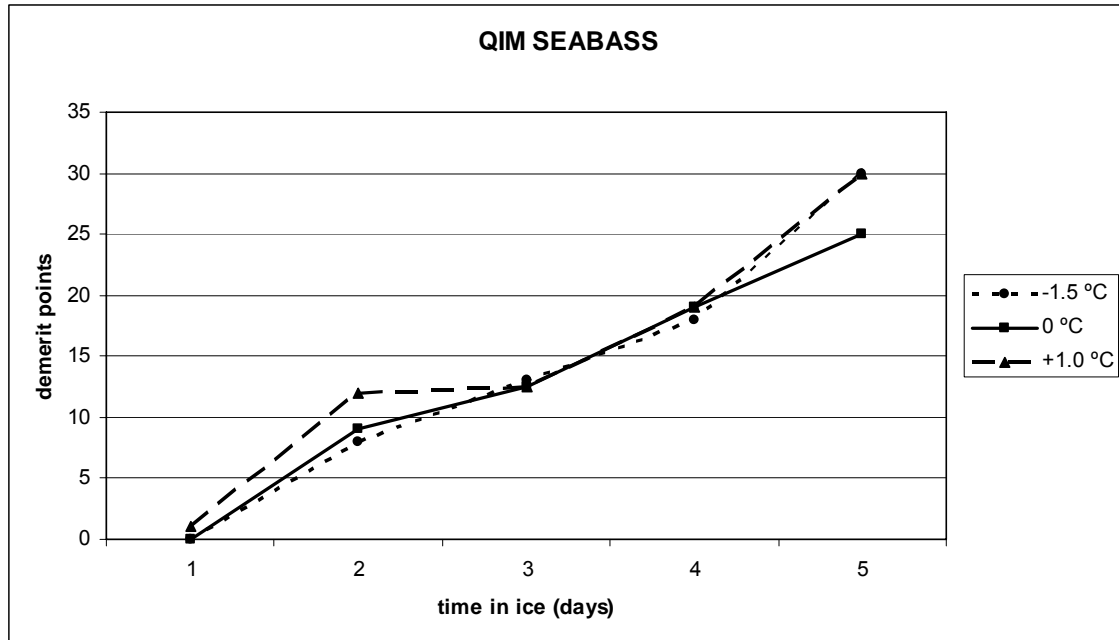


Fig. 2 - Results from sensory quality scores (QIM demerit points *versus* time in ice) of farmed seabass, slaughtered in ice slurry at different temperatures.

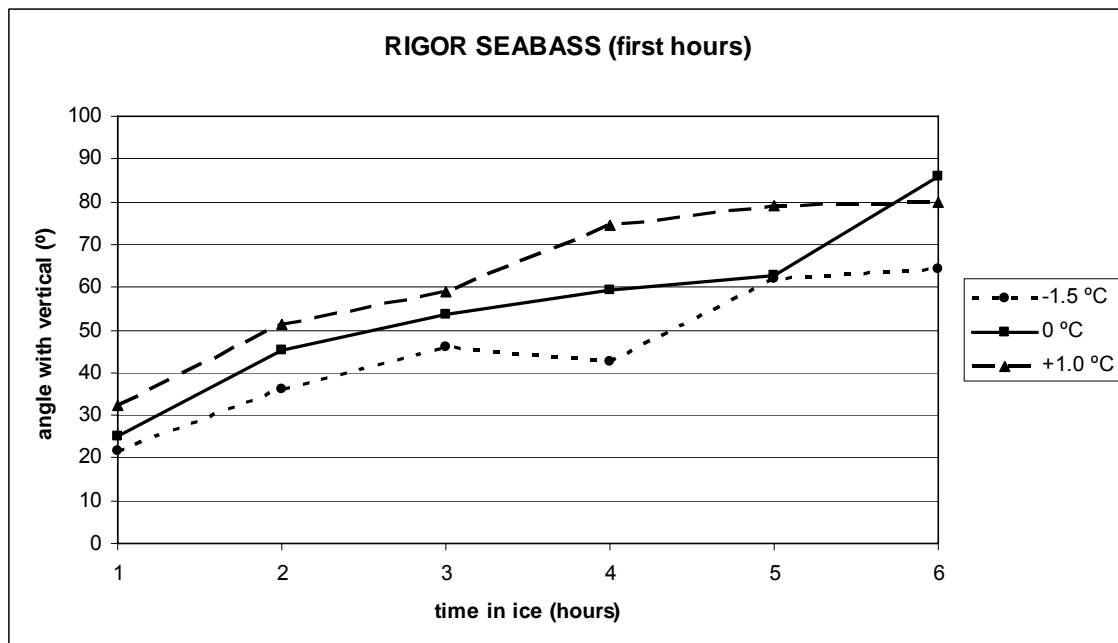


Fig. 3 - Results from *rigor mortis* analysis (angle with vertical *versus* time in ice) of farmed seabass, slaughtered in ice slurry at different temperatures (first 6 hours after death).

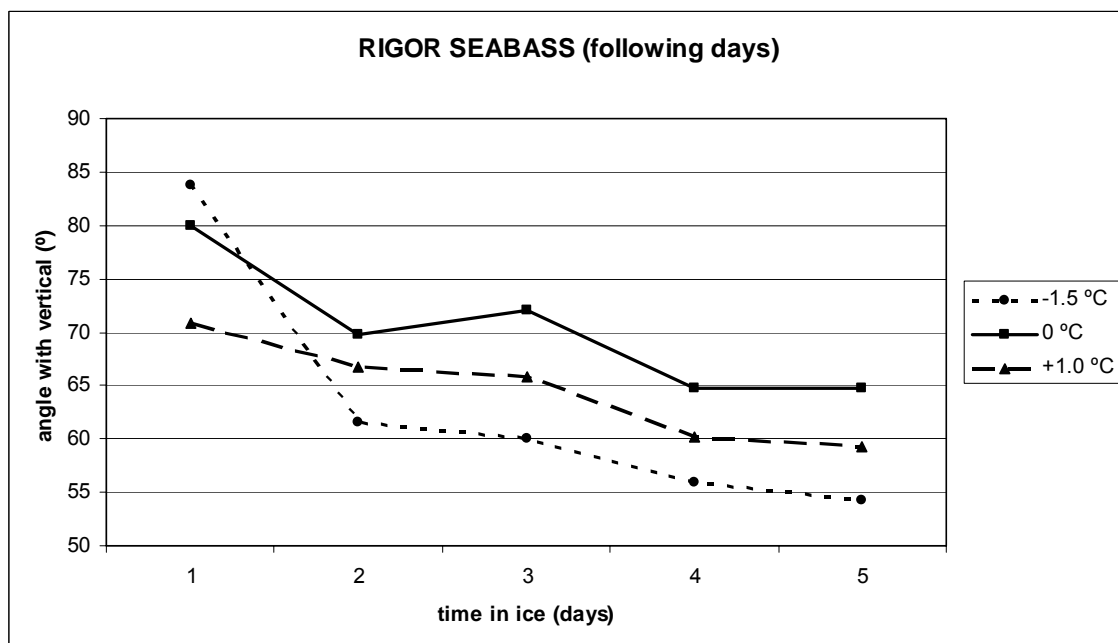


Fig. 4 - Results from *rigor mortis* analysis (angle with vertical *versus* time in ice) of farmed seabass, slaughtered in ice slurry at different temperatures (first 5 days after death).

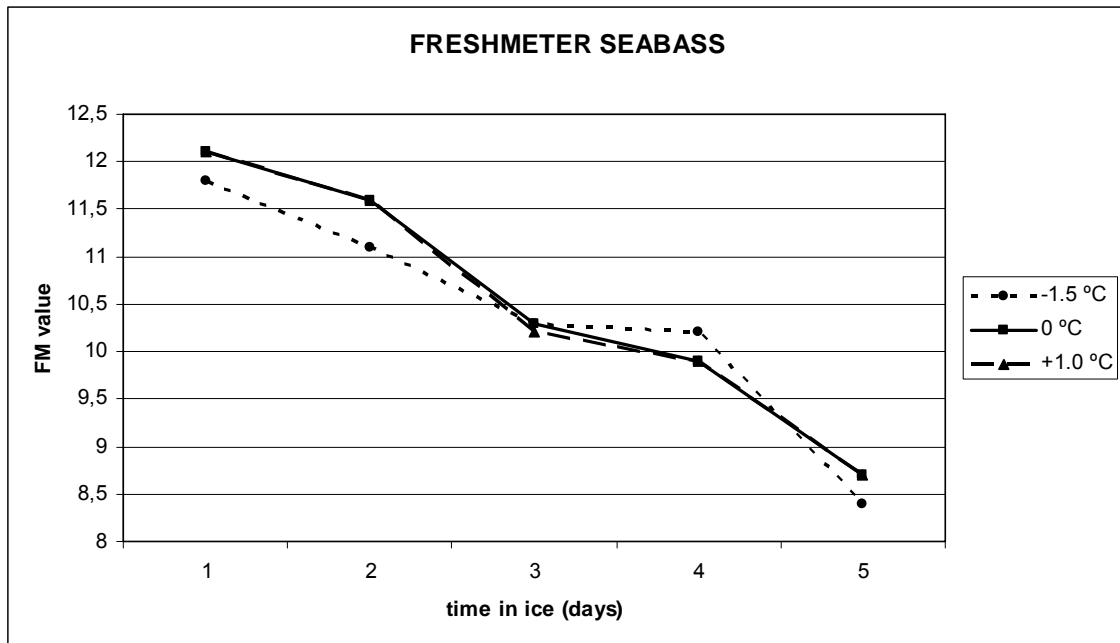


Fig. 5 - Results from Freshmeter measurements (Freshmeter values *versus* time in ice) of farmed seabass, slaughtered in ice slurry at different temperatures.

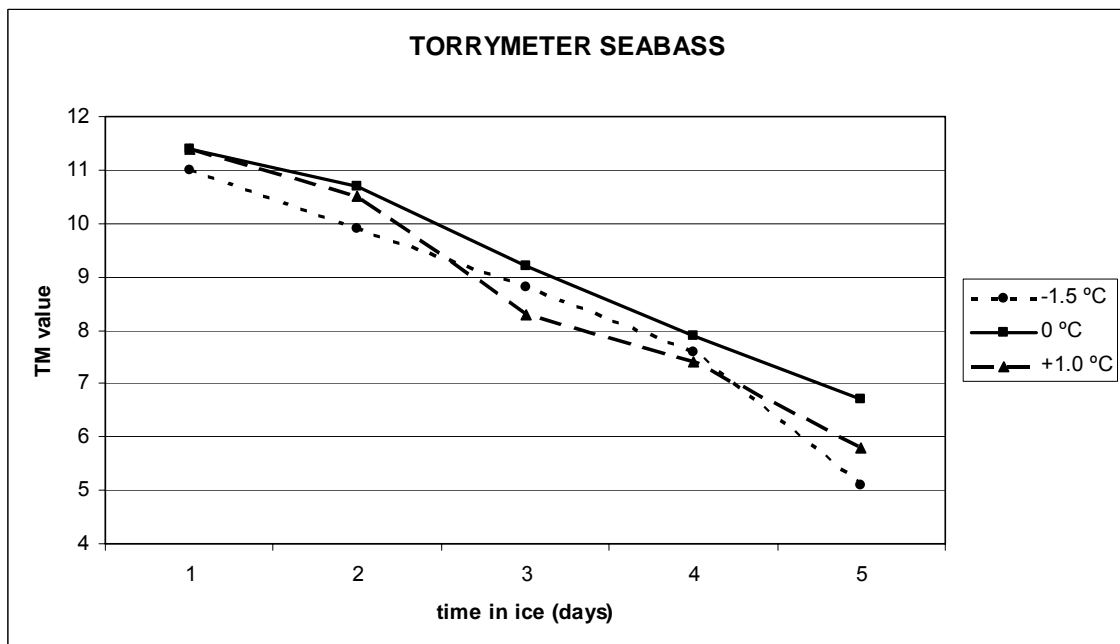


Fig. 6 - Results from Torrymeter measurements (Torrymeter value *versus* time in ice) of farmed seabass, slaughtered in ice slurry at different temperatures.

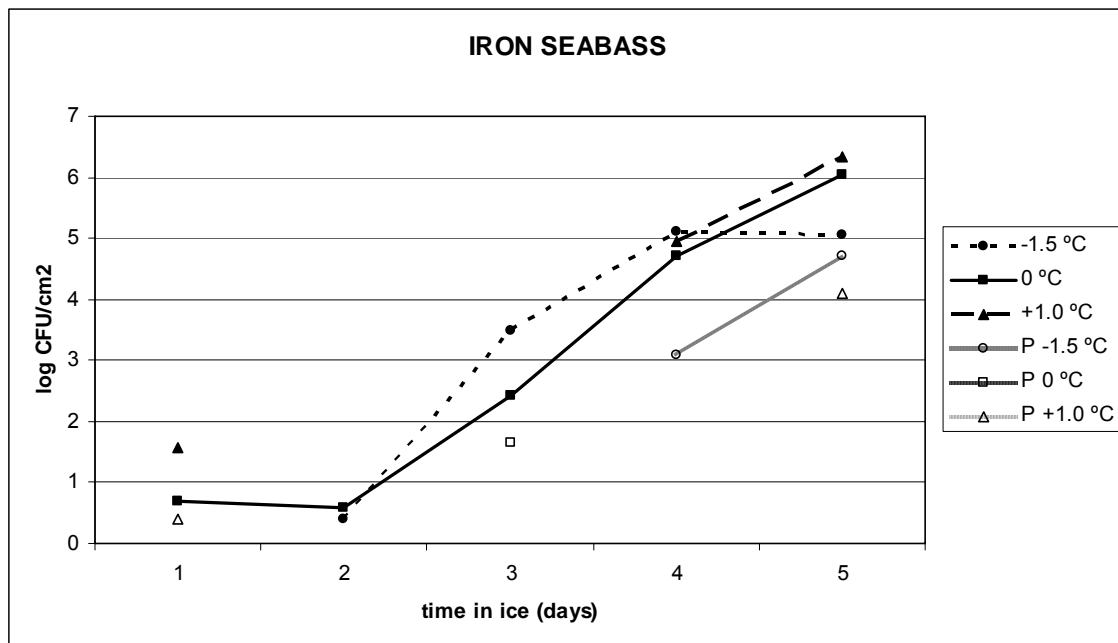


Fig. 7 - Results from microbial counts on Iron Agar (log of colony forming units per square cm versus time in ice) of farmed seabass, slaughtered in ice slurry at different temperatures. P lines are black colonies (counts of specific spoilage organisms), other lines represent the total viable count.

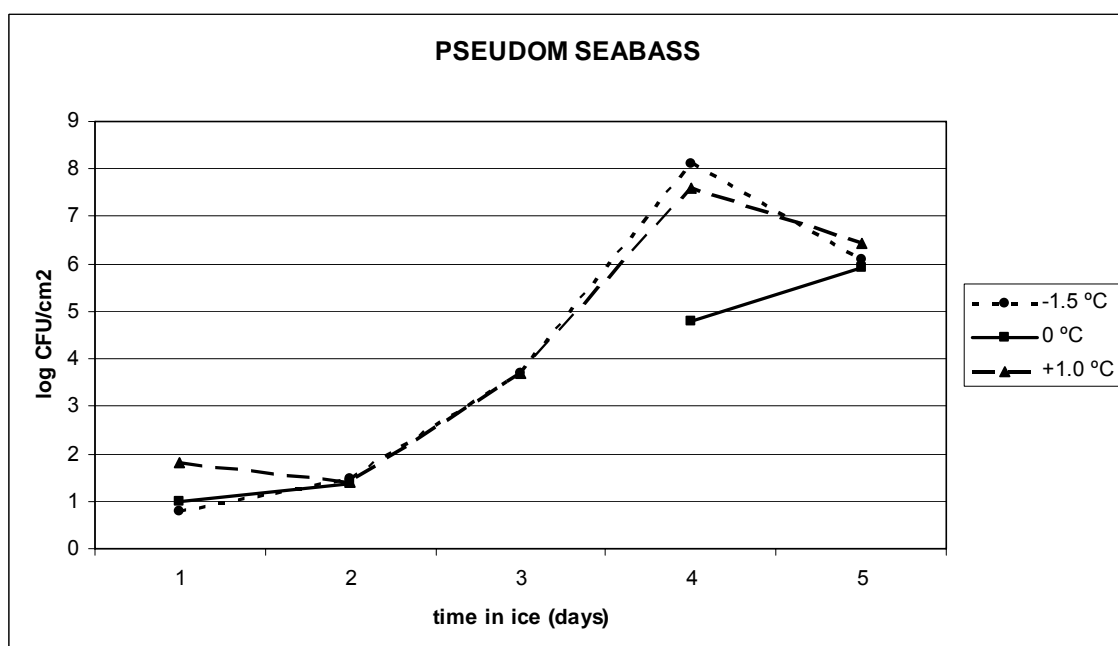


Fig. 8 - Results from microbial counts on *Pseudomonas* Agar (log of colony forming units per square cm versus time in ice) of farmed seabass, slaughtered in ice slurry at different temperatures.

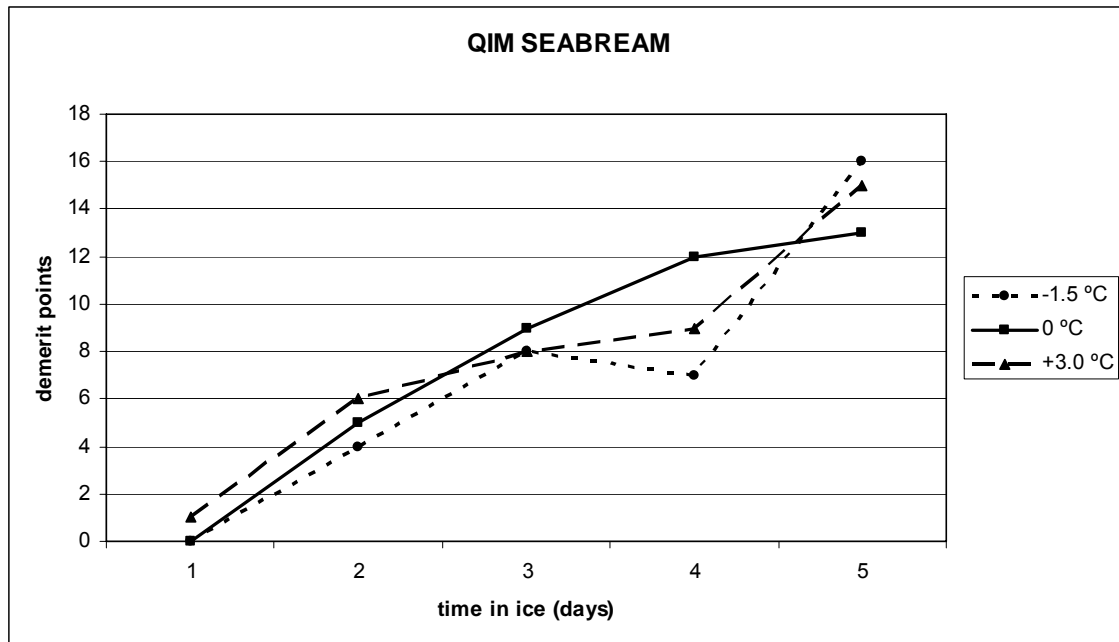


Fig. 9 - Results from sensory quality scores (QIM demerit points *versus* time in ice) of farmed seabream, slaughtered in ice slurry at different temperatures.

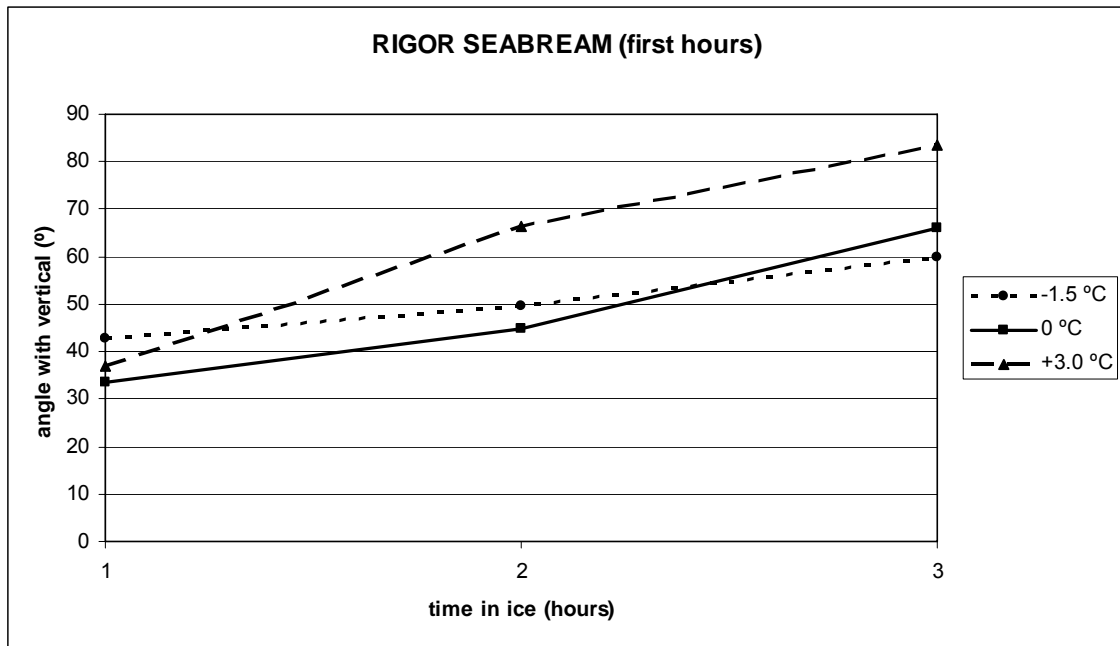


Fig. 10 - Results from *rigor mortis* analysis (angle with vertical *versus* time in ice) of farmed seabream, slaughtered in ice slurry at different temperatures (first 6 hours after death).

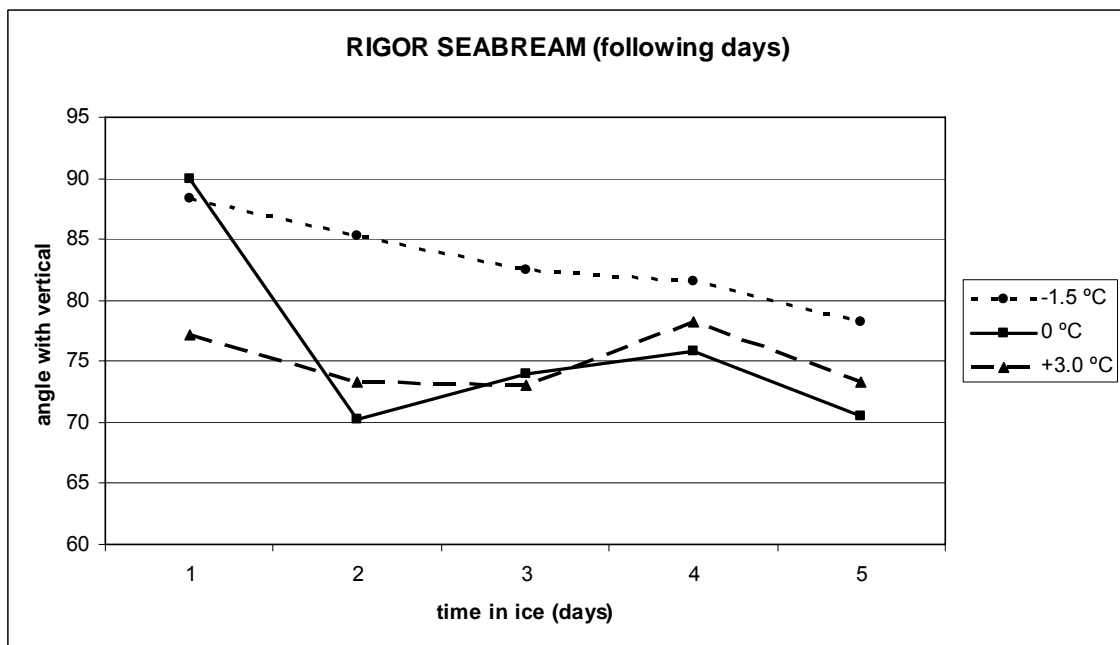


Fig. 11 - Results from *rigor mortis* analysis (angle with vertical *versus* time in ice) of farmed seabream, slaughtered in ice slurry at different temperatures (first 5 days after death).

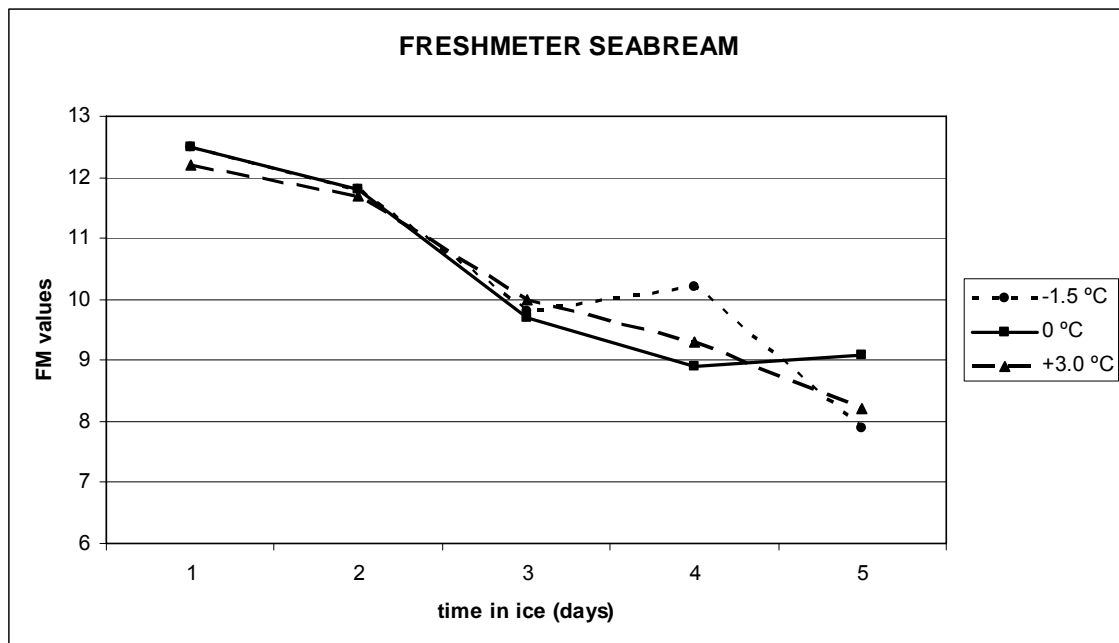


Fig. 12 - Results from Freshmeter measurements (Freshmeter values *versus* time in ice) of farmed seabream, slaughtered in ice slurry at different temperatures.

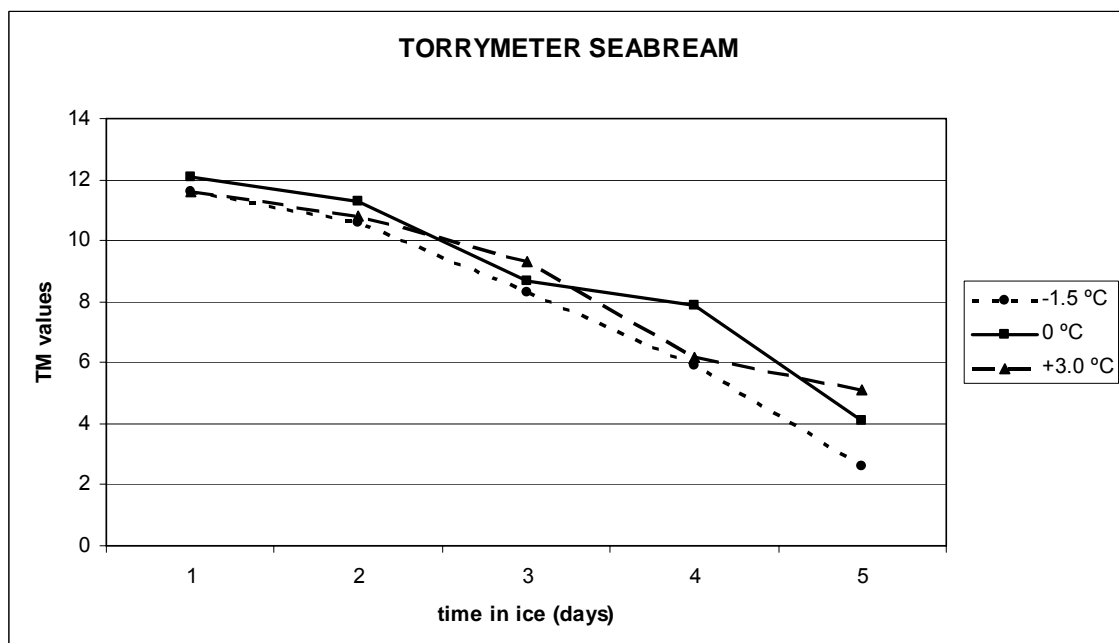


Fig. 13 - Results from Torrymeter measurements (Torrymeter value *versus* time in ice) of farmed seabream, slaughtered in ice slurry at different temperatures.

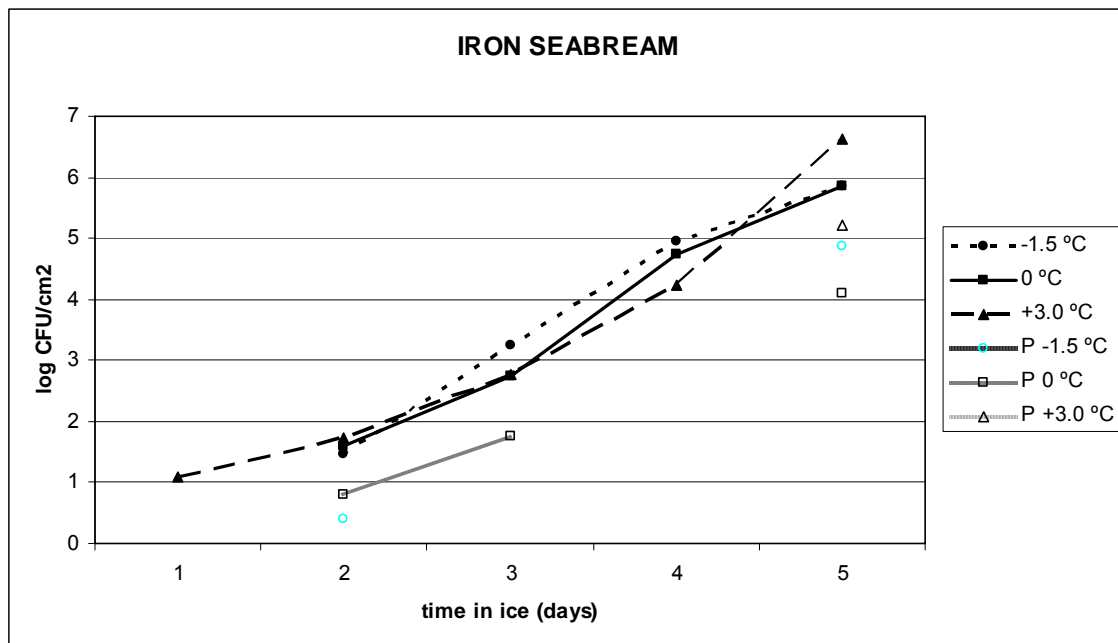


Fig. 14 - Results from microbial counts on Iron Agar (log of colony forming units per square cm versus time in ice) of farmed seabream, slaughtered in ice slurry at different temperatures. P lines are black colonies (counts of specific spoilage organisms), other lines represent the total viable count.

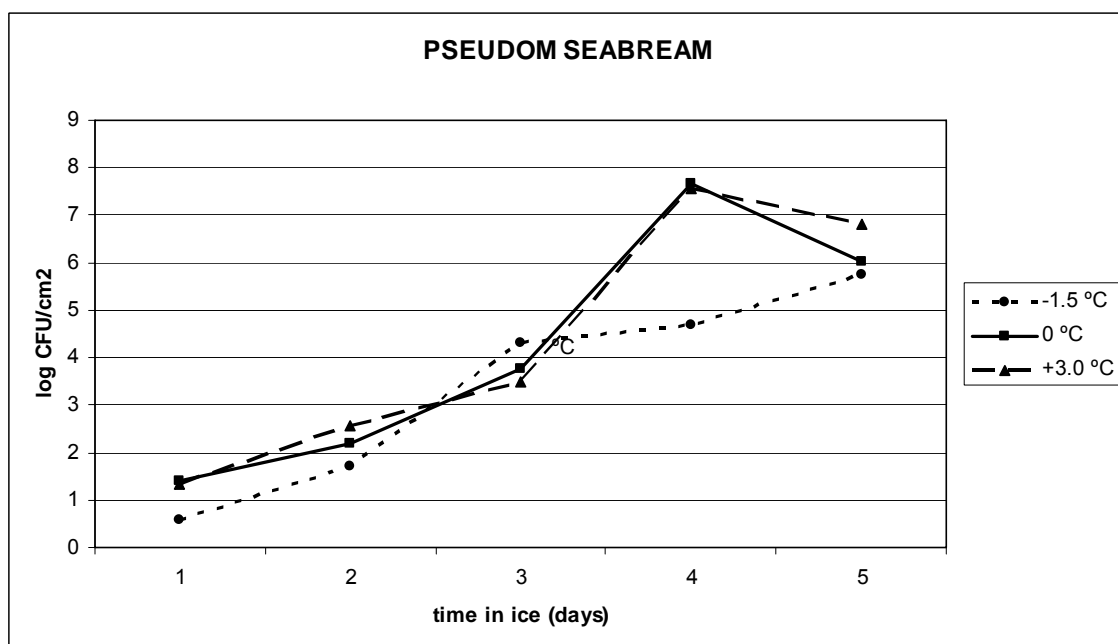


Fig. 15 - Results from microbial counts on *Pseudomonas* Agar (log of colony forming units per square cm versus time in ice) of farmed seabream, slaughtered in ice slurry at different temperatures.

Conclusions and recommendations

Taking into account the results of the fish quality indicators of this trial, there are no perceptible differences in the quality of the fish when killed in ice slurry at -1.5, 0 and approximately +2.0 °C. An exception is a slightly higher quality of both species in general sensory attributes, revealed by the QIM, when lower temperatures of around -1.5 °C were used (visible during the first 2 days).

However, although not measured with precision, fish dies quicker at lower temperatures (in this trial, around 30 min was the maximum time, obtained at higher temperatures) and this represents a considerable improvement in the welfare associated with this phase.

The quality seems to be unchanged or slightly positively changed, however if farmers can use lower temperatures they will contribute to a welfare improvement, here shown by shorter times to death, which is in accordance with the most recent demands from consumer and recommendations by scientific work.

In order to improve methodology and increase precision, in future trials it is recommended that small containers and lower volumes should be used, which allow a more homogeneous temperature and make easier to perform realistic temperature measurements. Another recommendation is to use a higher temperature range (for example, treatments at -2 °C, +2 °C and + 6 °C) to obtain effects that are more detectable and probably more correspondent to real conditions throughout the year seasons.

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