COOP-CT-2004-512991

StunFishFirst

Development of prototype equipment for humane slaughter of farmed fish in industry

Cooperative Research (CRAFT)

Instrument: **Cooperative Research (CRAFT)**

Thematic Priority: **Food Quality and Safety** area **5.4.6 Safer and environmentally friendly production methods and technologies and healthier foodstuffs**

StunFishFirst Final Report
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Project coordinator name: dr. J.W. van de Vis
Project coordinator organisation name: IMARES Revision: [draft]

Summary

General objectives

In the European Union demands for humane methods to convert live farmed fish into food are increasing.
The overall objective of the project is therefore to develop prototype equipment for humane slaughter of the selected farmed fish species, namely eel (*Anguilla anguilla*), nile tilapia (*Oreochromis niloticus*), sea bass (*Dicentrarchus labrax*) and turbot (*Psetta maxima*). Humane slaughter consists of stunning (rendering unconscious without avoidable stress prior to killing). Feasibility criteria rule out individual handling of the concerned species. The aim is therefore to develop prototype equipment for electrical stunning, as this can be applied to batches of fish. In order to achieve the overall objective, the following sub-objectives are envisaged for the project:

- To model the physical processes involved in electrical stunning of fish to predict parameters for electrical stunning of various fish species
- To characterize requirements for electrical stunning of the selected species with respect to effectiveness of stunning and product quality.
- To design and build prototype equipment on basis of the established requirements
- To study power saving techniques to facilitate implementation of electrical stunning at small SMEs

To assess prototype equipment with respect to effectiveness of stunning, product quality and operational characteristics required by the SMEs which farm the selected species.
The following partners were involved in the project.

Partic. Role*	Partic. Type**	Partic. no.	Participant name	Participant short name	Country	Date enter project***	Date exit project***
CO	RTD	B1	Nederlands Instituut voor Visserijonderzoek (RIVO) BV New name in 2006 Wageningen IMARES BV	RIVO IMARES	Nl	1	24
CR	SMEP	A1	Rijpelaal b.v	Rijpelaal	Nl	1	24
CR	SMEP	A2	J.G. Janssen	Janssen	Nl	1	24
CR	SMEP	A3	Seafarm BV	Seafarm	Nl	1	24
CR	SMEP	A4	Zeeland Vis BV	Zeeland	NL	7	24
CR	SMEP	A5	Viveiro Vilanova S.A.	VVN	P	1	24
CR	SMEP	A6	Noordzee Su Urünleri	NSU	TR	1	24
CR	SMEP	A7	Ace Aquatec	Ace	UK	1	24
CR	SMEP	A8	Specialist Welding Services Ltd.	SWS	UK	1	24
CR	OTH	C1	Viskwekerij Royaal B.V.	Royaal	Nl	1	24
CR	OTH	C2	ANOVA FOOD BV	ANOVA	Nl	1	24
CR	OTH	C3	Waitrose Ltd.	Waitrose	UK	1	24
CR	OTH	C4	Isidro de la Cal-Fresco S.L.	Isidro	E	1	24
CR	RTD	B2	ID-Lelystad, Instituut voor Dierhouderij en Gezondheid BV New name in 2006 ASG Veehouderij BV	ID Veehouderij	Nl	1	24
CR	RTD	B3	The University of Bristol	Bristol	UK	1	24
CR	RTD	B4	Silsoe Research Institute Silsoe Livestock Systems	SRI SLS	UK UK	1 10	9 24

*CO = Coordinator
CR = Contractor

To achieve the overall objective experimental work was performed in three workpackages. A fourth workpackage was needed for overall coordination. Workpackage 1 was finished in the first year of the project. Results obtained in Workpackage 1, 2 and 3 are presented beneath.

Modelling of electrical stunning

Electric field strengths in water required to eel, tilapia, sea bass and turbot were investigated experimentally. The results show how the electric field depends on water conductivity. The electric fields in water required to generate immediate insensibility in the fishing following an exposure duration of 1 s were identified. A mathematical model of the electric field in the stunning tank was constructed and shown to produce results consistent with the experimental results. This simulation indicates that the electric field that occurs in the fish will vary little with variation of the fish position in the tank and fish size but that there may be a significant variation in the electric field experienced by individual fish in a high density cluster of fish if water conductivity is below 50 μS/cm or greater than 500 μS/cm.

Technical file on characteristics demanded by SMEs

Characteristics of prototype equipment, as required by the SMEs and end-users, was established for all fish species. The characteristics concern product quality, required throughput (slaughter rate), prices, ease of use, safety for workers in the company and compatibility with existing processing lines.

Conditions to provoke immediate loss of consciousness without recovery, using available laboratory-scale equipment

At the end of the first year of the project we decided to use a pulsed square wave current only for stunning of all selected fish species in the project, as it was observed for tilapia that power could be saved by a factor of 1.9, compared to applying a 50 Hz sinusoidal current. Similarly, to our results obtained with a 50 Hz sinusoidal current EEG measurements revealed that immediate loss of consciousness and insensibility can be provoked, provided that sufficient current is passed through the brains of each fish. For eel we assumed that the same current density of the square wave current had to applied as for tilapia, as it was observed during assessment of electrical stunning by applying a 50 Hz sinusoidal current that current densities needed for instantaneous stunning of eel and tilapia were the same. However, EEG data are lacking.
In the project stunning of sea bass and turbot in water fresh water (1000 μS/cm conductivity) was also investigated, in order to study the possibility to save power to a greater extent. Power can be saved as the conductivity of the fresh water used is 48 to 53 times lower, compared to sea water. As EEG registrations are lacking for stunning of sea bass and turbot in fresh water (1000 μS/cm conductivity) no firm conclusions can be drawn about the immediate loss of consciousness and sensibility in sea bass and turbot by using the pipeline stunners. Conditions to achieve an instantaneous stun in sea bass and turbot in seawater, which is their natural environment at the farms, were established by using EEG data.
EEG registrations revealed that electrical stunning of tilapia followed by gill cutting only resulted in signs of recovery during bleeding for 3 out of 5 tilapia. Recovery can be prevented by bleeding the stunned tilapia in a mixture of crushed ice and water. For eel, it appeared that the electrical stun in combination with desliming in a saturated aqueous $Ca(OH)_2$ solution could prevent recovery, as judged from the EEG registrations. EEG registrations in both sea bass and turbot revealed that an electrical stun followed by chilling of the unconscious and insensible fish in an ice slurry is sufficient to prevent recovery during for instance gutting and filleting. For turbot we observed that a temperature increase during gutting of the animal should be prevented, as the animal may

still recover. Therefore, gutting should be performed immediately after taking the chilled fish from the tank or the cold chain should not be interrupted for turbot.

Assessment of stunning by observation of behaviour

Results obtained from behavioural measures have to be interpreted with caution. Therefore, the use of EEG recordings as well as evoked responses on the EEG is recommended for an unequivocal assessment of the level of brain function in fish. Nevertheless, EEG recordings cannot be carried under all circumstances and therefore observation of behaviour and responses to administered stimuli is used to assess the prototype stunners. In case fish are able to swim immediately in coordinated way post stunning there is little doubt that these animals are still conscious.

Electrical stunning and carcass damage

Downgrading in eel, tilapia, sea bass and turbot was not observed when a pulsed square wave alternating current was used. The was not observed either when the selected fish species were stunned by a 50 Hs sinusoidal current.

Study power saving techniques

During the first year of the project it became clear that power had to be saved, so that existing three phase mains, ranging from 16 to 32 A, which are available at the companies of the StunFishFirst Consortium, may provide sufficient electrical power needed for stunning of the selected fish species. Our experiments showed that the use of a pulsed square wave alternating current resulted in a reduction of needed power by a factor 1.9 for tilapia, compared to the use of a 50 Hz sinusoidal current for stunning of the fish species.

Design and build prototype equipment

In order to generate a square wave alternating current of we designed and built three amplifiers.
Three prototypes for continuous stunning of all fish species were developed in the last year. A pipeline stunner was designed for use with sea bass, one continuous stunner for use with turbot, and one open pipeline stunner for use with tilapia and eel. The open pipeline stunner was not closed, as this facilitated assessment of stunning in the peak stun and maintenance stun area. For eel, sea bass and turbo we also construct a tank for stunning of the species in batches, as this was needed to tune the prototype to needs of some of the SMEs.

Assessment of prototype equipment by observation of behaviour at industrial partners

The electrically stunned fish species emerged from the stunner immobile and showed no escape behaviour as they dropped into the ice slurry of the harvest bin or the aqueous salt solution that was used to deslime eels. Fish that were taken out of the stunning tank were also motionless.

Assessment of prototype equipment by analysis of product quality at industrial partners

In the continuous stunners the water is reused. Thus, food safety an issue. In the pipeline stunner that were tested with sea bass no microbial growth was detected due to the low temperature of the water. However, more detailed analysis is necessary for drawing firm conclusions. Microbiological analysis of water in the open pipeline stunner, which was reused for 6 hours at room temperature, showed that the total plate count was increased by a factor of 20. Coliforms, which were analysed as indicator for pathogens, were not detected. For the open pipe line stunner more detailed analysis is required for conclusions about food safety.
For all fish species downgrading could be prevented. Product quality was similar to that obtained by current industrial methods. Slaughter rates for eel, tilapia, sea bass and turbot that were in accordance with the wishes of the SMEs (1 to 4 tonners/hour) were obtained.

In conclusion we can state that all fish species can be stunned instantaneously in the prototype stunners. Recovery during killing can be prevented. Carcass downgrading can be prevented in all species. By using the prototypes slaughter rates can be obtained that were in accordance with the demands of the industrial partners in StunFishFirst.
Regarding food safety, the commercial stunners, which will be built and sold beyond the lifetime of the project, have to meet standards are laid down in EU directives and national legislations concerning the Hazard Analysis Critical Control Points approach, which is routinely used nowadays in the European food industry.

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Introduction

Current slaughter methods for farmed fish (e.g. death in air, death in an ice slurry, freezing, bleeding, carbon dioxide narcosis in combination with bleeding) do not provoke unconsciousness immediately until death occurs or, if unconsciousness is not induced immediately, without avoidable suffering (Robb and Kestin, 2002; Van de Vis et al., 2003).
When a fish is effectively stunned (immediate and permanent loss of consciousness) prior to killing the slaughter process is considered humane. We have established that percussive stunning (a blow to the head by using an instrument, captive needle stunning (injection of air under pressure into the brains) and the application of electrical current (provided that appropriate conditions are used) can provoke immediate and permanent loss of consciousness in fish (Robb and Kestin, 2002; Lambooij et al., 2002; Van de Vis et al., 2003). All RTD performers reported for various species that they recovered from the application of an electrical current for 1 second (Lambooij et al., 2002, Lines et al., 2002; Robb and Kestin, 2002, Van de Vis et al., 2003b).The authors observed that prolonging the exposure of fish to electricity or applying electricity in combination with e.g. rapid cooling results in permanent loss of consciousness until death occurs.
In Australia a chemical substance eugenol, is added to the water to stun fish prior to killing. Eugenol is a food grade substance, which is based on clove oil. Barriers to its use in for instance the UK include the cost of overcoming the legislative requirements to introducing a new medication and the possible public response to eating fish that could be perceived as having been poisoned (Lines et al., 2003).
It is anticipated that electrical methods for stunning will be the most feasible, as the large numbers of individual fish make individual percussion or captive needle stunning and bleeding uneconomic. Percussive stunning of Atlantic salmon is an exception, as equipment for automated percussive stunning was developed especially for this species by the Australian company Sea Food Innovations in collaboration with Marine Harvest, a large farmer of Atlantic salmon in Scotland. In 2002 Marine Harvest won a welfare award for introducing percussive stunning to protect A. salmon at slaughter (Anonymous, 2002). For other fish species, which differ substantially in anatomy and morphology, high-speed machinery to singulate, orientate, percuss and bleed them would be complex and expensive (Lines et al., 2003), as the developed equipment for salmon is not adapted to other species such as turbot. Moreover, for fish species such as gilt-head seabream and eel percussive stunning is not feasible without severe damage to the head which is not acceptable for industry (Van de Vis et al., 2003). In case of fish species with a thick scull such as African catfish the application of percussive stunning does not result in immediate loss of consciousness and is therefore unfit for use (Van de Vis and Lambooij, unpublished results).

To date, at least one device is available commercially for electrical stunning of Atlantic salmon (Roth, pers. comm.). For rainbow trout a prototype for electrical stunning was developed (Lines et al., 2003). However, the stunners were designed primarily for Atlantic salmon and trout, respectively. It is unlikely that these stunners are suitable to use for the selected fish species (eel, tilapia, sea bass and turbot) in the proposed study.

The reason is that optimal conditions for electrical stunning with respect to immediate loss of consciousness and prevention of carcass damage depend on the fish species. Laboratory-scale trials revealed for instance that the optimal conditions for eel are a electric field strength of 1250 V/m for 1 s followed by 313 V/m for 5 min in combination with removal of oxygen from the water (Lambooij et al., 2002, Morzel and Van de Vis, 2003), whereas the established optimal conditions for Atlantic salmon and trout are stunning for 6-12 s in a field of 75 V/m using sinusoidal 1000 Hz current (Robb and Roth, 2002; Roth, et al., 2002) and 60 s in a field strength of 250 V/m, using a sinusoidal 1000 Hz current (Lines et al, 2003), respectively. When a 50 Hz sinusoidal current is applied for salmon or trout, this will cause carcass damage, contrary to eel (Morzel and Van de Vis, 2003) and African catfish (Van de Vis et al., 2003b). Similar to salmon and trout, we found that applying a 50 Hz current for stunning of turbot also causes carcass damage (Morzel et al., 2003). We expect that the conditions for effective stunning of the other selected species in this study, i.e. tilapia, sea bass and turbot, with high product quality standards differ from the ones already established for other species in the reported studies.

In Germany, only electrical stunning is permitted as a slaughter method of eels since April 1999. However, the RTD performers have established that the method is not humane, using the prescribed conditions in the German legislation, as the eels are not rendered unconscious immediately (Lambooij *et al.*, 2002; Van de Vis *et al*., 2003) and most animals are stunned slowly during application of the current, which can be very painful. These authors have also established that eel cannot be rendered unconscious permanently when only electricity is used, as prescribed in the German legislation. Equipment available for electrical stunning of eel on a commercial scale, which is designed to meet the demands of the German legislation, is therefore not suitable for humane slaughter. The current method for slaughtering of eel in the Netherlands consists of desliming live eels by the use of salt followed by gutting (envisceration). It was established that is not a humane method (Verheijen and Flight, 1997; Van de Vis *et al.*, 2001). Current industrial methods applied to the other species selected in the proposed research (tilapia, sea bass and turbot) are described by Robb and Kestin, 2002). For sea bass and tilapia, the use of an ice/water slurry is the first step in the slaughter process. The rapid cooling does not result in an immediate loss of consciousness and is most probably stressful, as demonstrated in salmon, eel and gilt-head seabream (Lambooij et al., 2002b; Skjervold et al., 2001; Van de Vis et al., 2003). Tilapia are also killed by removing the fish from water, which results in asphyxiation. Temporarily removal from the water is widely used as an experimental stressor in studies of the stress response of fish. Turbot are killed by gutting the gills or the caudal vein and subsequently placing the fish in an ice/water slurry. During the procedure vigorous activity occurs, which may indicate stress (Morzel et al., 2003).

Stressful slaughter methods can generally lead to fish flesh prone to gaping, with an unappealing colour, a soft texture and a low water holding capacity. More humane handling at slaughter, with reduced stress and physical activity immediately prior to death, results in better carcass quality (Robb and Kestin, 2002). For various farmed fish species it has been observed that reduced-stress at the process of slaughter has a positive influence on the flesh quality (Azam et al., 1989; Berg et al., 1997; Byrne, 2002; Kestin et al., 1995; Roth et al.., 2002; Sigurgisladottir, 2001; Morzel and Van de Vis, 2003).

Reduction of stress at slaughter by applying electrical stunning under specific conditions have been found to be humane and generally promote better fish quality (Kestin et al., 1995; Lines et al., 2003; Morzel and Van de Vis, 2003; Robb and Kestin, 2002).
In 2005 a Craft proposal focused on turbot started (Turpro 508070). RIVO is one of the partners in the project. Within the scope of Turpro no research will be undertaken to develop and test prototype equipment for humane slaughter of turbot. In the granted Integrated Project Seafoodplus (FQS-506359) research of RIVO and ID is also focused on turbot. In Seafoodplus development and testing prototype equipment will not be performed.
Finished research work, which has been undertaken on electrical stunning of fish, resulted in a few patents in the area (Van de Vis, 2001, 2001b; Moller and Roth., 2000). However, the parameters associated with electrical stunning are complex and a patent is not a protocol for effective electrical stunning with high standards of product quality for fish species. The patents do not contain details for optimal electrical stunning of eel, tilapia, sea bass and turbot under various conditions. Major factors, which determine whether electrical stunning is optimal (immediate loss of consciousness and high product quality)are the size, shape of the fish, fish species and the electrical conductivity of the fish and of the water). Hence, no satisfactory electrical stunning equipment exists for stunning of eel, tilipia, sea bass and turbot on commercial scale.
The overall objective of the project was to develop prototype equipment for humane slaughter of the selected farmed fish species, namely eel (*Anguilla anguilla*), nile tilapia (*Oreochromis niloticus*), sea bass (*Dicentrarchus labrax*) and turbot (*Psetta maxima*). Humane slaughter consists of stunning (rendering unconscious without avoidable stress prior to killing). Feasibility criteria rule out individual handling of the concerned species. The aim is therefore to develop prototype equipment for electrical stunning, as this can be applied to batches of fish. In order to achieve the overall objective, the following sub-objectives are envisaged for the project:

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To assess prototype equipment with respect to effectiveness of stunning, product quality and operational characteristics required by the SMEs which farm the selected species

Materials and Methods

To achieve the overall objective, four workpackages were used.

Workpackage 1. In the first workpackage electrical stunning will be modelled, so that the required stunning parameters (e.g. voltage field, frequency of the current, exposure duration, field direction, etc.) can be predicted for eel, tilapia, sea bass and turbot. The model will be build, using the available data for trout.
These predictions of the required stunning parameters will be used to guide the design of animal experiments (in workpackage 2 and 3) and so reduce the number of animals required. It will enable humane slaughter parameters to be optimized for a range of situations more efficiently, and enable early predictions of carcass quality. Workpackage 1 will assist us in our responsibility to Reduce, Refine and Replace animal experimentation (The 3 Rs of animal experimentation)

Workpackage 2. The work the second workpackage will be performed with eel, tilapia and sea bass and turbot to establish requirements for electrical stunning of the selected fish in water with respect to product quality and welfare. The experiments will be designed on basis of the results obtained in workpackage 1. Trials with the selected fish species are foreseen to validate the model, which is build in workpackage 1.The following tasks have been identified: Establishment of a technical file on characteristics demanded by SMEs and on the health and safety requirements, which are demanded by the EU: measurement of brain function to assess loss of consciousness until death occur, as well as assessment of electrical stunning by observation of behaviour and analysis of product quality.

Workpackage 3. On basis of the results in workpackages 1 and 2 prototype equipment for stunning fish will be designed and build. It is expected that three prototypes (one for both freshwater fish species and two for the saltwater species) is made. The prototype equipment will be tested at the SMEs which farm the fish species. During testing it will be evaluated whether the prototype equipment meet the required characteristics, demanded by the SMEs which farm and process fish. Power saving techniques will studied to facilitate implementation of electrical stunning at small SMEs. The manufacturers of the equipment tune the prototypes to the needs of the SMEs, which farm and process the selected species. At the SMEs the prototypes will be evaluated with respect to the ability to render the fish species unconscious immediately until death occurs and with regard to product quality. For each fish species two slaughter trials will be performed. The farmers and end-users will assess carcass quality (including downgrading),using the specifications available at these companies. The work in this Workpackage will be performed on eel, tilapia and after this seabass and turbot.

Workpackage 4. In the last workpackage the overall coordination of the proposed research will be performed.

Methodology used in Workpackage 1

The main parameters that characterise an electric stun are the duration for which the voltage is applied, the voltage across the animal and the electric waveform used. Electrical stunning, especially with alternating current and pulsed direct current, has been historically associated with a certain degree of carcass damage. Similar results have been shown in red meat animals (e.g. cattle) stunned by electricity. Damage is manifested as haemorrhages within the flesh and spinal rupture depending on the degree. It has been found that by changing the parameters of electrical output, the degree of damage to pig carcasses can be greatly reduced without compromising the welfare (Simmons, 1989) Increasing the frequency of the applied current for stunning was found to have the greatest effect on reducing carcass downgrading. Both RTD partners from the UK have established that effective electrical stunning of trout with high standards carcass quality can be achieved (Lines et al., 2003)
This workpackage is dived into the following four tasks.

Task 1.1 Build model

The electric field that exists in the water surrounding a fish is different to that which is induced in the fish. The determining factors are the shape of the fish, the electrical conductivity of the fish and of the water. This work will build on the successful application of this modeling approach for the humane slaughter of trout where it enabled the electric fields required for humane slaughter to be calculated for a wide range of water conductivities (Lines, unpublished results)
Measurements of fish shape, brain location and the bulk conductivity of the fish species will be made by examination of recently killed fish. Finite element techniques will be used to build a model of the electric field in a tank and the fish during stunning. This will be based on available software packages. The model will allow the nominal electric field in the fish brain to be calculated for a range of fish sizes, shapes, fish orientations and water conductivities. It will also allow prediction of electric fields where more than one fish is being held in the stunning tank. Where possible a 2 dimensional model will be used for simplicity and speed, however in seawater a 3 dimensional model may be necessary because of the wide discrepancy between the conductivity of the fish and that of surrounding water. The internal structure of the fish will not be modeled. Phantoms will be used to provide validation of the model.

Task 1.2. Apply model to species, validate and generate predictions

Existing data on the electric fields and exposure durations required to achieve immediate and permanent unconsciousness in fish until death will be used with the model to calculate the required electric field in the fish brain. It is foreseen that experimental data are necessary (to be obtained in Workpackage 2). This data will be in turn be used to provide initial estimates of the required electric field in the water for other physical arrangements (e.g. water conductivity, fish size, field orientation, fish density and alternative species.
High accuracy in not required because this model does not attempt to address the biological variability of the fish. It will however provide initial estimates to guide the

design of trials, and provide an interpretation of the causes of variability where these have a physical rather than biological origin.
This process will be iterative since predictions will be used to identify the starting points for experimental tests (to be done in Workpackage 2), and the results of these tests will be used to validate or correct the model. The use of this approach will therefore build up during the research project and enable the results of the research to be applied to a wider species after the project is completed.

Task 1.3. Develop carcass quality predictive indices

An important problem associated with electric stunning is carcass quality problems associated with broken bones and blood spotting. This is widely thought to be associated with high levels of muscle tension during the stunning process. An in-situ technique for measuring muscle tension on recently killed fish will be developed. This will probably be based on direct measurement of compression in the spine. This will be used to determine empirical relationships between muscle tension, electric field strength and the frequency and wave form of the electric field. Muscle tension measurements will then be correlated with carcass quality measurements, particularly bone breakages and blood spotting. If successful, this approach will enable predictions to be made of the carcass quality implications of various approaches and so enable the best approaches to be rapidly identified and optimised. The value of this predictive technique will be assessed by experiments in Workpackage 2.

Task 1.4. Assessment of the variation in electric field between individual fish

Simulations with trout show that the presence of fish in a tank distorts the electric field which other fish in the tank are subjected to. This can lead to significant variations in the electric field in individual fish. The variations increase with the discrepancy between water and fish conductivity and with the density of fish in the water and so are likely to be particularly significant in sea water stunning tanks. Stun requirements need to be based on the minimum electric field received by individual fish rather than the mean field, so an understanding of this variation is important for translating stun requirements from ideal test situations to industrial situations. The model will be used to determine the magnitude of this affect and explore how it can be mitigated. These results will be used in the design of commercial scale equipment in Workpackage 3.

Methodology used in Workpackage 2

In workpackage 2 several existing laboratory-scale equipment at the RTD performers will be tested for the four farmed fish species at the SMEs which farm the species. Trials of the laboratory-scale equipment, which will be designed on basis of the outcome of Workpackage 1, at the SMEs will allow us to establish the SME’s demands on the characteristics of the slaughter equipment.
For tilapia and eel, a freshwater the stunning tank will be filled with tapwater. For the selected seafishes sea bass and turbot the stunning tank will be filled with salt water.

For electrical stunning it is essential that, whatever the equipment used, a homogeneous electrical field is obtained in the tank thereby ensuring that sufficient current is passed through the head of each animal to provoke immediate loss of consciousness. At the research partners, several pieces of equipment are available, ranging from power supplies capable of delivering 50 Hz a.c. from 0 to 250 V, 0-20 A, to high frequency apparatus capable of delivering variable frequencies (up to 1000 Hz a.c.) and 30 A. For electrical stunning a power supply with adjustable frequency will be used, as various frequencies may influence downgrading (Robb et al., 2002).

Task 2.1 Establisment of a technical file on characteristics demanded by SMEs

Characteristics of the slaughter equipment, as required by the SMEs, have to be established. The characteristics concern product quality, required throughput (slaughter rate), prices, ease of use, safety for workers in the company and compatibility with existing processing lines.
With respect to safety of equipment for electrical stunning, the stunner has to be constructed (to be done in workpackage 3) in accordance with the EU Directive on machinery (Council Directive n^{o} 98/37/EC, 1998).
It is expected that the prototype equipment meets the health and safety requirements, as electricity is applied safely for humane slaughter of pigs and poultry in industry. Nevertheless in the case of fish the amounts of water, especially salt water have to be taken into account.
All relevant on characteristics demanded by SMEs and EU Directive on machinery will be compiled in a Technical File (responsible Ace, one of the manufacturers of the prototype).

Task 2.2 Measurement of brain function

The state and duration of unconsciousness in fish will be assessed by measurement of brain function. The brain function can be measured by recording the EEG and evoked responses in the EEG. Measurement of the EEG and evoked responses was performef on one of the selected species, eel (Lambooij et al., 2002)
The observation of behaviour only is not sufficient for assessment of electro-narcosis, because ineffective electrical stunning can be very painful and paralysis may occur without loss of consciousness (Croft, 1952). Therefore, the use of EEG recordings in combination with registration of the evoked responses on the EEG are recommended (Hoenderken, 1978). On basis of the measurement of brain function the monitoring of behaviour at slaughter can be validated.
To establish whether the parameters for stunning render a fish species unconscious immediately until death the brain function of 20 animals will be determined. In case the application of an electrical current does not result in an irrecoverable stun, a combination of electrical stunning and killing method e.g. rapid cooling, will be studied.

Task 2.3 Observation of behaviour

A standardised protocol to monitor fish behaviour during slaughter has been developed (Kestin et al, 2002). The protocol includes observation of self initiated behaviours (e.g. swimming), responses to stimulation, e.g. handling, and breathing and the vestibulo-ocular response.

Task 2.4 Product quality assessment

Quality of the fish flesh will be assessed to establish whether the use of the laboratory-scale equipment results in a satisfactory quality, i.e. at least similar to that obtained by the application of current slaughter methods. Standard methods to assess the quality parameters described below are available at the RTD performers.

Chemical methods. The proximate composition will be determined by measurement of total fat, protein and water content. For determination of the proximate composition 5 animals from each fish species will be analysed at every slaughter trial performed.

Physical methods. There is evidence that stress at the time of slaughter may influence a number of physical characteristics of fish products, for example pH, colour of the flesh, rigor mortis and gaping. As a consequence, the quoted parameters will be assessed following instrumental methods in which the RTD performers have expertise.
Because it is of interest for the fish industry, filleting yield will also be determined. Finally, the appearance of the whole gutted fish will be assessed visually for the extent of downgrading, loss of scales and the occurrence of bruises and fin damage. Fillets will be assessed for bruises and haemorrhages.
During storage trial of the gutted fish in ice, samples of 10 fish will be taken at regular time intervals for physical analysis. For the assessment of downgrading a sample of 20 fish will be analysed at the start of the storage trial.

Sensory methods. Sensory analysis on the whole fish will be performed by using schemes available at the RTD performers. The schemes will be used to assess changes in sensory quality. During the storage trial samples of 10 fish will be taken at regular time intervals.

Methodology used in Workpackage 3

Task 3.1 Study power saving techniques for electrical stunning

In-water electrical stunning of fish may require huge currents to provoke immediate loss of consciousness. It is known that the application of a short duration huge current is not sufficient to induce permanent loss of consciousness in fish and therefore the current is maintained to render the stunned fish unconscious permanently. For African catfish (a freshwater species) we established that for batch stunning currents of more than 60 A 50 Hz a.c. may be needed (Van de Vis et al., 2003b). Especially in case of in-water stunning of salt water fish species the required currents are likely to be higher than 50 A due to the high conductivity of salt water (Robb and Roth (2002). These reported results clearly show the need to study power saving techniques.

It is foreseen that power saving can be achieved by

1. Power saving using a low voltage maintenance stun. For eel we established that unconsciousness, which was provoked immediately by applying a high voltage (a peak stun to provoke immediate loss of consciousness) could be prolonged by applying a low voltage stun. The stun is irrecoverable when the water in the tank was flushed with nitrogen gas to remove oxygen. The use of the low voltage stun for eel resulted in a 4 fold reduction in the electrical power demand (Lambooij et al., 2003; Morzel and Van de Vis., 2003)
2. Power saving using hybrid techniques. For African catfish we observed that by applying a peak stun in combination with fast chilling in ice slurry resulted in an immediate and permanent loss of consciousness (Van de Vis et al., 2003b), as concluded from the measurement of brain function. We estimate that the use of the hybrid technique results in a 20 fold reduction in the electrical power demand.
3. Build an amplifier for pulsed square alternating currents. The use of different waveforms for electrical stunning may lead to a reduction in power needed for stunning.

Task 3.2 Design and build prototype equipment

The most suitable approach to applying an electric stun to fish for any particular farm depend on the quantities of fish being harvested, the rate at which they must be delivered, the conductivity of the water, and the availability of equipment, capital and labour (Lines et al., 2003).
The simplest approach to applying an electric stun is the use of a batch system, where the fish are placed in a tank and exposed to an electric field until they have lost consciousness permanently. Another approach is to reduce the volume of the system (and thereby the power demand for stunning) by stunning small batches of fish (or even individual fish) immediately in a small reservoir using a peak stun. Subsequently, the unconscious fish are collected at a high density for exposure to the maintenance stun to induce permanent loss of unconsciousness. In case of eel we established that is it is necessary to flush the latter reservoir with nitrogen during stunning to prevent recovery. Recently, we established that exposure of unconscious fish to the maintenance stun can be replaced by chilling in an ice slurry (Van de Vis et al., 2003b).
The motivation for reducing the volume of the system is the reducion of the capital cost of the equipment required to generate high-frequency sinusoidal voltage, since the power required to sustain an electrical field in a volume of water is proportional to the volume of water (Lines et al., 2003)

Task 3.3 Assessment of prototypes by observation of behaviour

A standardised protocol to monitor fish behaviour during slaughter will be used, as described in task 2.4. For the four fish species the behaviour of 20 animals will be monitored at each slaughter experiment.

Task 3.4 Assessment of prototypes by product quality assessment

Quality of the fish flesh will be assessed to establish whether the use of the prototype equipment results in a quality, at least similar to that obtained by the application of current slaughter methods. Standard methods to assess the quality parameters described below are available at the RTD performers. The RTD performers will use chemical, physical and sensory methods (details are given in task 1.5).
During the storage trial samples of 10 fish will be taken at regular time intervals.

Results and Discussion

Work performed in WP 1 and results achieved

Modelling of electrical stunning

Electric field strengths in water required to eel, tilapia, sea bass and turbot were investigated experimentally. The results show how the electric field depends on water conductivity. The electric fields in water required to generate immediate insensibility in the fishing following an exposure duration of 1 s were identified. A mathematical model of the electric field in the stunning tank was constructed and shown to produce results consistent with the experimental results. This simulation indicates that the electric field that occurs in the fish will vary little with variation of the fish position in the tank and fish size but that there many be a significant variation in the electric field experienced by individual fish in a high density cluster of fish if water conductivity is below 50 μS/cm or greater than 500 μS/cm.

Develop carcass predictive indices

The current results suggest that drop in pH and onset and resolution of rigor mortis can be useful to predict whether downgrading occurs. It is known that the application of stressful methods can result in a sharp drop in pH of muscle tissue and a rapid depletion of ATP. This may cause gaping and rapid onset of rigor mortis, which can be very intense.
Chilling of sea bass in crushed ice should be prevented as this may lead to blood spots on the belly, which reduces the value of the fish. The fish farming SMEs and the end-user provided the RTD performers information for development of the predictive indices. For their advice they used their own data bases and experience on quality of whole fish and fillets.

Work performed in WP 2 and results achieved

Technical file on characteristics demanded by SMEs

Characteristics of prototype equipment, as required by the SMEs and end-users, was established for all fish species. The characteristics concern product quality, required throughput (slaughter rate), prices, ease of use, safety for workers in the company and compatibility with existing processing lines.

Conditions to provoke immediate loss of consciousness without recovery, using available laboratory-scale equipment

In order to protect welfare of animals (including fish) at slaughter stunning has to be applied without avoidable stress prior to killing, as stated in the current EU legislation to protect welfare of animal at slaughter (Council Directive 93/119/EC, 1993). For fish no specific methods are prescribed for stunning and therefore only the general provision, which states that an animal has to be rendered unconscious without avoidable stress and suffering until death occurs, applies. During assessment of stunning it is required that an animal is unconscious and insensible. Insensibility is required to avoid that methods applied in industry, such as gill cutting or evisceration after the stun, lead to recovery of a fish.
Results obtained in experiments with our laboratory-scale equipment are presented in the table beneath. EEG measurements revealed that immediate loss of consciousness and insensibility can be provoked, provided that sufficient current is passed through the brains of each fish. We would like to emphasize that a 1 second electrical stun does not lead to permanent loss of consciousness and sensibility. Tilapia and sea bass for instance may recover 16 $\pm$ 26 and 48 $\pm$ 34 s post stun, respectively. It is foreseen that this period is too short for application of a killing method to prevent recovery until death. For the application of bleeding as killing method the period of loss of consciousness and sensibility needs to be longer than the values reported for a 1 second stun, as in a reported study it was established that that bleeding is a slow killing method for a conscious fish. Atlantic salmon lost consciousness and sensibility after approximately 5 min. The conditions for eel have been established in a previous study. Observation of behaviour of stunning of sea bass in fresh water (1000 μS/cm) revealed that the applied conditions (see table beneath) may lead to an instantaneous stun. However, EEG data are lacking, so no firm conclusions can be drawn.

Stunning of fish, using laboratory-scale equipment

Fish species	Vpeak/cm	Vrms/cm	type of current	current density (A/dm2)	conductivity (uS)	assessment of stunning
eel		12.5	50 Hz ac	0.64	512	EEG and behaviour
tilapia		12.5	50 Hz ac	1	703	EEG and behaviour
sea bass		1	50 Hz ac	4.3	41000	EEG and behaviour
turbot		1	50 Hz ac	3.4	32000	EEG and behaviour
sea bass		1.6	50 Hz ac	0.15	1000	behaviour
sea bass		3.2	1000 Hz ac	0.2	1000	behaviour

A 5 second electrical stun (which is instantaneous when applied for 1 second) followed by chilling for 15 min in ice slurry result appears to be sufficient to prevent recovery of tilapia during chilling, as judged from EEGs. The present data suggest that this may also be true for turbot. As we assessed 5 turbots, more EEGs measurements are needed to confirm the this. For eel it was observed that after a 15 min electrical stun recovery may be prevented by desliming the unconscious animal in a hot aqueous salt solution, which is normally used by industry to deslime the live fishes prior to gutting.

At the end of the first year of the project we decided to use a pulsed square wave current only for stunning of all selected fish species in the project, as it was observed for tilapia that power could be saved by a factor of 1.9, compared to applying a 50 Hz sinusoidal current. Similarly, to our results obtained with a 50 Hz sinusoidal current EEG measurements revealed that immediate loss of consciousness and insensibility can be provoked, provided that sufficient current is passed through the brains of each fish. For eel we assumed that the same current density of the square wave current had to applied as for tilapia, as it was observed during assessment of electrical stunning by applying a 50 Hz sinusoidal current that current densities needed for instantaneous stunning of eel and tilapia were the same. However, EEG data are lacking.
In the project stunning of sea bass and turbot in water fresh water (1000 µS/cm conductivity) was also investigated, in order to study the possibility to save power to a greater extent. Power can be saved as the conductivity of the fresh water used is 48 to 53 times lower, compared to sea water. As EEG registrations are lacking for stunning of sea bass and turbot in fresh water (1000 µS/cm conductivity) no firm conclusions can be drawn about the immediate loss of consciousness and sensibility in sea bass and turbot. For stunning of sea bass and turbot in seawater, which is their natural environment at the farms, the conditions to achieve an instantaneous stun were obtained by registration of EEGs.

We would like to emphasize that a 1 second electrical stun by applying a pulsed square wave alternating current does not lead to permanent loss of consciousness and sensibility. Tilapia (n=14) and sea bass (n=21) for instance recover 51 ± 37 s and 23 ± 11 s post stun, respectively. It is foreseen that this period is too short for application of a killing method to prevent recovery until death. Therefore, we prolonged the period of loss of consciousness and insensibility by combination of a 1 second peak stun that is followed immediately by a maintenance stun at 25% of the field strength and current density of the peak stun.
EEG registrations revealed that electrical stunning of tilapia followed by gill cutting only resulted in signs of recovery during bleeding for 3 out of 5 tilapia. Recovery can be prevent by bleeding the stunned tilapia in a mixture of crushed ice and water. For eel, it appeared that the electrical stun in combination with desliming in a saturated aqueous $Ca(OH)_2$ solution could prevent recovery, as judged from the EEG registrations. EEG registrations in both sea bass and turbot revealed that an electrical stun followed by chilling of the unconscious and insensible fish in an ice slurry is sufficient to prevent recovery during for instance gutting and filleting. For turbot we observed that a temperature increase during gutting of the animal should be prevented, as the animal may still recover. Therefore, gutting should be performed immediately after taking the chilled fish from the tank or the cold chain should not be interrupted for turbot.

Assessment of stunning by observation of behaviour

Results obtained from behavioural measures have to be interpreted with caution. Therefore, the use of EEG recordings as well as evoked responses on the EEG is recommended for an unequivocal assessment of the level of brain function in fish. Nevertheless, EEG recordings cannot be carried under all circumstances and therefore observation of behaviour and responses to administered stimuli is used to assess the prototype stunners. In case fish are able to swim immediately in coordinated way post stunning there is little doubt that these animals are still conscious.

Electrical stunning and carcass damage

Downgrading in eel, tilapia, sea bass and turbot was not observed when a 50 Hz sinusoidal current was applied.
In the second year downgrading in eel, tilapia, sea bass and turbot was not observed when a pulsed square wave alternating current was used. However, assessment of sea bass that was stunned in batches showed that the eyes were less convex and dark, compared to live chilling.

Work performed in WP 3 and results achieved

Study power saving techniques-construction of first version prototype

A first prototype for continuous stunning of all fish species was developed in the first year. The stunner consists of pipe to apply a peak stun followed by a maintenance stun to prolong the period of loss of consciousness and sensibility to prevent recovery during the application of a killing method. The fish is fed into the pipe through a funnel and falls into the peak stun area to achieve loss of consciousness and sensibility instantaneously. It is foreseen that the total length of the pipe is approximately 2 m, as required by the SMEs that farm fish. At the end of the pipe the water is fed into a box, which is covered by a grill to dewater the fish and to facilitate the application of a killing method (chilling in ice or bleeding) The water is recirculated by a pump. The pump takes the water from the box and brings into the pipe. With regard to safety of the workers, we would like to emphasize that it is not possible to be electrocuted unintentionally. The distance between the entrance of the funnel and the electric fields in the peak stun area and the end of the pipe and the maintenance stun area are both longer than the length of an arm of a human being. Moreover, the applied electricity is floating, which means that a living organisms can only be stunned when it is between two electrodes or it is touching both at the same time.
In addition to the pipe stunner, a first version prototype a control and power unit for electrical stunning was developed.The developed power unit generated a pulsed direct current. It appeared that with this power unit tilapia might be stunned instantaneously by applying 100 Hz direct current pulses of at least 3 ms duration. The application of a pulsed direct current may lead to saving of power. In future trials we will determine to which extend power can be saved. The experiments are scheduled at the beginning of the second year of the project to confirm by measuring EEGs whether power can be saved by

applying pulsed currents. The 400 l tank was also used to stun turbot. EEG measurements are planned to confirm whether consciousness is lost immediately.
The stunned tilapias and turbots were killed by chilling in ice for 15 min. After chilling the fishes were inspected for blood spots and broken bones. No blood spots and broken bones could be detected.
In another experiment with sea bass it was established by measurement of EEGs that it is possible to induce a general epileptiform insult, which is indicative for loss of consciousness, in fish that was stunned instantaneously by applying a pulsed direct current. Thus, pulsed direct currents can be applied to induce instantaneous loss of consciousness and sensibility in fish.
During the first year of the project it became clear that power had to be saved, so that existing three phase mains, ranging from 16 to 32 A, which are available at the companies of the StunFishFirst Consortium, may provide sufficient electrical power needed for stunning of the selected fish species.
Literature on electroconvulsive therapy, electrosleep and electroaneasthesia showed that power could be saved when a pulsed square wave alternating current is used. Our experiments showed that the use of a pulsed square wave alternating current resulted in a reduction of needed power by a factor 1.9 for tilapia, compared to the use of a 50 Hz sinusoidal current for stunning of the fish species.

Design and build prototype equipment

In order to generate a square wave alternating current of we designed and built three amplifiers.
Three prototypes for continuous stunning of all fish species were developed in the last year. A pipeline stunner was designed for use with sea bass, one continuous stunner for use with turbot, and one adjusted pipeline stunner for use with tilapia and eel. The adjusted pipeline stunner was not closed, as this facilitated assessment of stunning in the peak stun and maintenance stun area. For eel, sea bass and turbot we also construct a tank for stunning of the species in batches, as this was needed to tune the prototype to needs of some of the SMEs.
With regard to safety of the workers, we would like to emphasize that it should not possible to be exposed to electricity, while using the prototype stunners. In the pipeline stunner for sea bass the distance between the entrance of the funnel and the electric fields in the peak stun area and the end of the pipe and the maintenance stun area are both longer than the length of an arm of a human being. Moreover, the applied electricity is floating, which means that a human being can only become part of the electrical circuit by touching two electrodes at the same time.
Regarding food safety, the commercial stunners, which will be built and sold beyond the lifetime of the project, have to meet standards that are currently used in food industry. The prototypes that were designed by us did not meet these standards, as we had to give priority to designing stunners for instantaneous stunning of the selected fish species at slaughter rates, which were in accordance with the wishes of the industrial partners in StunFishFirst.
The food safety standards comprise a hygienic design of the stunner and pump to reuse the water, a cleaning and disinfection plan, monitoring the food safety aspects of a

stunner that is being used. To guarantee food safety a HACCP (Hazard Analysis Critical Control Points) has to be used. We would like to emphasize that the HACCP approach is laid down in EU directives and national legislation. This implies that the food safety issue is routine for the food industry (including fish slaughter houses). We therefore do not expect any insurmountable problems in adjusting the current prototype stunners, so that current standards for food safety are met.
A HACCP plan, which will be here used to monitor food safety aspects of a stunner that is being used, has to set up for a stunner that is installed at each company. The reason for a separate HACCP plan at each company is for monitoring food safety the design of the premises at each company, training of personnel, etc have to be taken into account.

Assessment of prototype equipment by observation of behaviour at industrial partners

The electrically stunned fish species emerged from the stunner immobile and showed no escape behaviour as they dropped into the ice slurry of the harvest bin or the aqueous salt solution that was used to deslime eels. Fish that were taken out of the stunning tank were also motionless.
During the experiments with the continuous stunner and the pipeline stunners it became clear that orientating the fish should be given attention. We want to prevent that the fish enters the electrified water with tail first, as loss of consciousness and sensibility may not be within 1 second due to exposure to the current.
Another is issue is that a dosing system has not been developed in StunFishFirst. For industry this is needed to miminize labour. In addition, avoidable stress in the fish should be avoided.

Assessment of prototype equipment by analysis of product quality at industrial partners

In the continuous stunners the water is reused. Thus, food safety a matter of concern. In the pipeline stunner that were tested with sea bass no microbial growth was detected due to the low temperature of the water. However, more detailed analysis is necessary for drawing firm conclusions.

Eel

<u>Open pipeline stunner</u>. Visual inspection of hot smoked eel by the Dutch SMEs and end-users revealed that the product quality was satisfactory. The required slaughter rate of 1-2 tonnes per hour could be obtained. Microbiological analysis of water, which was reused for 6 hours at room temperature, showed that the total plate count was increased by a factor of 20. Coliforms, which were analysed as indicator for pathogens, were not detected. More detailed analysis is required to draw firm conclusions about food safety.
<u>Batch stunner.</u> Visual inspection of hot smoked eel by the Dutch SME A1, which processes on average 50 kg eels per day, revealed that the product quality was satisfactory. In addition, the stunner was tuned to the needs of this SME After a hot smoking the outer appearance of the skin was more yellowish and the texture was firmer, compared to eels that were killed by placing them conscious in salt bath.

Tilapia

Open pipeline stunner. Visual inspection of hot smoked eel by the Dutch SMEs and end-users revealed that the product quality was satisfactory. The required slaughter rate of 1-2 tonnes per hour could be obtained.
The experimental slaughter method may result in persistently lighter fillets at the visceral side, compared to the batch that was killed by bleeding the fish in fresh water. The differences between the L* values of both batches during storage was higher than 2 units and therefore the difference were visible. Contrary, to our expectations higher a* values, which were visible for human beings, were not obtained, compared to killing by bleeding in fresh water. For the b* the differences during storage of both batches was similar than 1 to 2 units for both the visceral and skin side and therefore no visible differences could be observed.

Sea bass

Pipeline stunner. Sea bass killed using either the present commercial method of immersion in an ice slurry alone, or using electrical stunning by passing it through the pipeline stunner before immersion in an ice slurry, presented satisfactory sensory quality. The electric stunning in water fresh water (1000 µS/cm conductivity) did not detectably improve nor degrade the sensory properties of the fish, namely the flavour, texture or colour of the flesh. Fish slaughtered by both methods presented similar sensory quality following storage on ice for up to 10 days. The pipeline stunner met demands of partner A5 with respect to slaughter rate.

Batch stunner. The Turkish SME preferred to use a batch stunner, which is filled with seawater, on board of the harvest boat, as the required slaughter rate of 4 tonnes per hour was achieved, which is in accordance with the wishes of partner A6. The stunner is mobile, as no mains was available on board. With respect to product quality parameters analysis of pH and colour (CIE-L*a*b* values) was carried out. Differences in pH and colour between electrical stunning and live chilling were not observed
The workers at the farm assessed the outer appearance of the fish and the fillets. It appeared that loss of scales was less for the electrically stunned fish, compared to the fish that was killed by live chilling. Electrical stunning affected the eyes. It appeared that they were less convex and less dark compared to the batch that was killed by live chilling. Nevertheless, in the view of the SMEs the fish could be sold.

Turbot

Continuous stunner. Food quality aspects of farmed turbot (Psetta maxima) were compared following two methods of slaughter: the traditional method, by immersion in an ice slurry, or by first electrically stunning in water fresh water (1000 µS/cm conductivity) the fish, using a prototype continuous stunner, before immersion in an ice slurry. Quality was assessed for up to 10 days of storage on ice following slaughter. No differences were found between the slaughter methods in terms of an overall carcass quality.

Batch stunner. We compared electrical stunning of farmed to turbot to live chilling, with respect to product quality parameters. For electrical stunning we used a batch process on a 400 l scale with seawater followed by chilling, as turbot farmers in the Netherlands prefer this. A slaughter rate could be obtained that met the demands of partners A3 and A4 To measure the product quality we choose four methods. These were analysis of colour.. The obtained results revealed that the product quality of the turbot that was stunned and killed by electricity followed by chilling was similar to that of fish killed by applying live chilling.

Conclusions and recommendations

In conclusion we can state that:

- current densities needed for instantaneous stunning of tilapia, sea bass and turbot species were established, as judged from EEGs. For the sea bass and turbot these conditions were established in seawater, which is their natural environment at the farms. For eel we assumed the same condition, as established for tilapia, has to be applied. However, the EEG data are lacking for eel.

- when the fish are exposed sufficiently long to electricity recovery during killing of all selected fish species can be prevented.

- for eel the use of a saturated aqueous $Ca(OH)_2$ solution could prevent recovery. For the other fishes crushed ice was suitable for killing of the stunned species

- assessment of product quality of fish stunned by electricity showed that carcass downgrading can be prevented and the quality is similar to that obtained by current methods

- a pulsed square wave alternating current reduced power needed for instantaneous stunning by a factor of 1.9, compared to the use of a 50 Hz sinusoidal current.

- orientating the fish that enters a pipeline stunner or a continous stunner should be given attention. It should be prevented that the fish enters the electrified water with tail first, as loss of consciousness and sensibility may not be within 1 second due to exposure to the current.

- a dosing system should be developed. For industry this may be needed to minimize labour. In addition, avoidable stress in the fish should be prevented.

- Regarding food safety, the commercial stunners, which are supposed to be built and sold beyond the lifetime of the project, have to meet HACCP standards that are currently used in food industry.

- For each company that wants to use a stunner A HACCP plan has to set up.

- more extensive microbiological analysis is needed before firm conclusions about food safety regarding the reuse of water in the prototype stunners.

- slaughter rates ranging from 1 to 4 tonnes/hour, were in accordance with the wishes of the industrial partners in StunFishFirst.

- safety of the workers is addressed to in the design of the amplifiers and the pipeline stunners.

Plan for using and disseminating the knowledge

The consortium intends to exploit the results to improve competitiveness through introduction and training of the fish farming SMEs to control application of stunning. All partners have a focus on improving quality (product as well as quality perceived by consumers) by stunning of farmed fish. The project will also promote the utilisation of the findings with respect to the model to predict conditions for stunning of species not selected in the project. In addition to the SME partners involved in the project, numerous private companies will be kept updated on the progress made with respect to stunning of the selected species, via the end-users.
In the first half of the project, dissemination of information about the project will remain limited to the consortium partners. During the second half of the project, articles will be published in scientific journals as well as in more industrially oriented magazines. These publications will include all scientific results in all areas of the projects. The project coordinator will coordinate the publication effort of the consortium. The SMEs, Research Institutes and University will allow the free use of scientific results obtained through the proposed project after their presentation through publications, international conferences and open workshops. These results will be available to all EU aquaculture industry.
In addition, the expected results of the proposed project offer great possibilities of exploitation by the participating SMEs. It is clear from their profiles that all of them are willing to take part in research and development in this field.
In the plan a strategy to disseminate the knowledge at mid-term, at the end of the project, as well as beyond the consortium during the lifetime of the project and afterwards will be given. The strategy is as follows. If necessary, an update of this plan will be included as an extra deliverable at the end of month 12 of the interim report.

Strategy

When deemed necessary, knowledge will be protected before results are disseminated beyond the consortium. The coordinator (B1) and partners C1, B2, B3 and B4 are experienced in protection of knowledge. In general, protection of knowledge can be achieved within 2-3 months. This period is needed to write a patent application, submit it and have it registered at an Office for Industrial Property in e.g. the Netherlands.
Once this procedure has been finished, the consortium has freedom to disseminate the protected results as widely as possible, especially to the European aquaculture industries. In order to achieve this, the results will be disseminated widely through all appropriate media (e.g. trade, technical and scientific journals, workshops and internet).
The following steps are foreseen.

- the manufacturers show prototype equipment for humane slaughter at trade shows (the exhibition in Glasgow, Trondheim and the Seafood Exhibition in Brussels)
- the RTD performers submit papers for publication in trade and scientific journals and to present the project results at national and international conferences and national workshops
- the preparation of guidelines as procedures of humane slaughter of the selected species and other farmed fish species by the SMEs and RTD performers

- dissemination the results beyond the lifetime of the project comprise: brochures, video once the commercial equipment has been developed (this will be done beyond the life time of the project), published papers in trade and scientific journals
- exploitation of results

We would like to emphasize that dissemination of knowledge beyond the consortium and lifetime is included. In addition, one manufacturer A7 will update its website to present the commercial stunners (photographs and video images), results obtained by using the prototype, experiences from companies using the equipment for humane slaughter and results obtained from scientific assessment. Moreover, the manufacturers have the intention to advertise in trade and technical journals.

The guidelines for humane slaughter are also disseminated beyond the lifetime of the project, as they will be available as information on which EU future recommendations or legislation can be based.

Exploitation of the results

In order to ensure that the SMEs are able to assimilate and exploit the results of the project, a number of steps are foreseen. The consortium clearly sees the importance of active research participation by the SMEs in co-operation with the RTD performers at their own facilities. All of the SMEs have adequate scientific expertise for such participation. During the project, RTDs and SMEs will work together, be in close touch and communicate extensively. Outside the set meetings, communication between the partners will be intensive and will involve practical experience exchange at the various sites of the project. In order to promote and facilitate this, the project has been designed in such a way that SMEs and RTDs work together on the same tasks. In addition, several involved SMEs and RTDs have bilaterally co-operated in the past. As a result, the ‘doorsteps’ are low and informal contacts already exist.

We would like to emphasize our intent to protect, knowledge, when deemed necessary by one or more SMEs, before results are disseminated beyond the consortium.

Raising public participation and awareness: Socio-economic benefit for the general public

The socio-economic benefits of humane slaughter of fish in aquaculture, resulting in further development of European aquaculture, also provide economic justification for the proposed research project. Currently, European aquaculture in general is in need of methods to improve welfare of fish during production to ensure further growth of the industry and to ensure maintenance of the level reached today. When the European marine aquaculture industry is provided with equipment for humane slaughter, it could very well enlarge the prospects and secure the future of the whole sector.

Further growth enhances economic activities and generates employment opportunities within the whole production chain. In addition, it leads to a more diverse supply of good quality aquaculture products throughout the year, promoting fish consumption among European consumers, and thus contributing health of consumers, which contributes to an increased demand for fish products.

The idea to tune prototype equipment to the needs of the companies in the consortium, originates from the SMEs themselves. In addition, the technical files will be established according to the needs and the wishes of the SMEs involved, ensuring accessibility to the results. The technical files will be practical manuals rather than scientific papers. In addition, as a result of the short lines and close cooperation between RTDs and SMEs during the whole project, transfer of information is natural and direct.

Update

Partner B4 discussed the prototypes with the Humane Slaughter Association, which is based in the UK. The Humane Slaughter Associaton agreed on the approach which wass chosen in the project. In the Netherlands the project was presented to four major Animola protectionists organisations (Rechten Voor Al Wat Leeft, De Vissenbescherming, Stichting Wakker Dier en de Nederlandse Vereniging tot Bescherming van Dieren) on 2 March 2007. A presentation of the project was also held at the Dutch Ministry of Agriculture and Food Quality. The animal protectionists were pleased about the outcome of the project.
Partners A7 and B4 presented the pipeline stunner at the Aquaculture exhibition in Glasgow May 2006.
Partner B4 received a letter of support from the Humane slaughter Association.
In June 2006 the project partners C1, B1 and B2 presented the project to the EU-FVO-MISSION DG(SANCO)/8041/2006 EVALUATION ON ANIMAL WELFARE.
The RTD performers intend to submit four manuscripts for publication in scientific journals, provided that this does not conflict with the interests of the SMEs. RTD performers intend to submit four manuscripts for publication in scientific journals, provided that this does not conflict with the interests of the SMEs.
Preliminary results obtained with the prototypes were presented to a Dutch animal protectionists organisation (Vissenbescherming) in March 2007.
On June 6th progress made in StunFishFirst was presented to DG Sanco.
A general overview of StunFishFirst was presented a workshop of Cost Action 867, Arcachon, France. The project was also presented at the Dutch Ministry of Agriculture, Nature and Food Quality.
The research partners have planned interviews and submission of papers, as shown beneath.

Planned/ actual Date(s)	**Type**	**Type of audience**	**Countries addressed**	**Size of audience**	**Partner responsible /involved**
5/10/2005	Dutch television	General public	Unrestricted	wide	B1 and B2
17-19 May 2006	Glasgow fisheries exhibition	Companies, researchers	EU and non-EU member states	~ 200	A7, A8 and B4
March 2006	Project presented to a Dutch group of animal protectionists (Vissenbescherming)	Animal protectionists	The Netherlands	3	B1
6 May	Visit DG Sanco: Presentation progress made in StunFishFirst	Commission	all	6	C1, B1 and B2
October 2006	Project presented at Cost Action 867 in Arcachon, France	researchers	European and non European	60	B1
February 2007	Project presented at the Dutch Ministry of Agriculture, Nature and Food Quality	Policy advisors, assisting staff	Netherlands	20	B1 and B2
2 March 2006	Project Presented to the 4 major Dutch Animal Protectionists Organistations (Rechten Voor Al Wat Leeft, Vissenbescherming, De Dierenbescherming, Stichting Wakker Dier)	Animal Protectionists	Netherlands	10	B1 and B2
March-May 2007	Interview in Fish Farming International	Fish farmers in UK and in Europe	Europe	Major farms and processor s in Europe	B4
March-June 2007	Interview in Dutch trade journal Aquacultuur	Fish farmers in the Netherlands and Flemish	Netherlands and Flemish part of Belgium	Farmers and	B1

Planned/ actual Date(s)	**Type**	**Type of audience**	**Countries addressed**	**Size of audience**	**Partner responsible /involved**
		part of Belgium		processors in Nl en Flemish part of Be	
2006/2007	Publications in technical and scientific journals The effect of electrical stunning at slaughter on the carcass, flesh and eating quality of farmed sea bass (Dicentrarchus labrax) by Toby Knowles and others. The effect of electrical stunning at slaughter on the quality of farmed turbot (Psetta maxima) by Toby Knowles and others. ELECTRICAL STUNNING OF SEA BASS (Dicentrarchus labrax) IN SEAWATER FOLLOWED BY CHILLING: neural and behavioural assessment and product quality by Bert Lambooij and others A humane protocol for electro-stunning and killing of Nile tilapia in fresh water by Bert Lambooij and	Companies, government, veterinary inspectors, science	unrestricted	wide	All research partners

Planned/ actual Date(s)	**Type**	**Type of audience**	**Countries addressed**	**Size of audience**	**Partner responsible /involved**
	others				

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