Culturing of Norway lobster (*Nephrops norvegicus)*

by

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Abstract:

The objectives of this study are to investigate a methodology to improve holding conditions in Norway lobsters and to enable hatching lobster eggs. There is no significant body of research for the *Nephrops norvegicus* so that this project could be viewed as a new area of study, which may create new scientific openings as well as having an economic importance. Many marine species, such as cod, salmon, shrimps and mussels are today farmed. A means has been discovered of hatching European Lobster (*Homarus gammarus*) eggs in captivity, which is in the same family, *Nephropidae*, as the Norway lobsters. These previously collected experiences and successful farming methods will be used to develop culturing methods for *Nephrops.*

The first aim of this project was to examine and gain a better understanding of the aggressive behaviour of the animals. Aggressive behaviour, causing injury or death, is thought to be a major obstacle in keeping animals in communal tanks. It was found that the Norway lobsters are able to build a dominance hierarchy, which reduces overall aggression in communal tanks. Moreover, it was found that pheromones are necessary for establishing dominance. Follow up studies will aim to gain a better understanding of the role of aggression in natural behaviour including territoriality, resource competition and mate choice. The second aim is to develop economic holding conditions for the animals. Holding animals in communal tanks is thought to be easier to manage - in terms of feeding and maintaining good water quality - than keeping them separately. Ongoing experiments comparing holding conditions indicate that there seems to be no difference in survival rate between individual and communal holding conditions. The third aim is to decide upon the best methods of collecting eggs and to hatch them. This will be researched in the next two years. Eggs will be collected by catching berried females. Holding conditions will be tested to maximize the hatching rate of the four larval stages in the lab. The results of the study will improve the understanding of the species which could help drive better stock management. In addition, it could support the fishing industry by providing sustainability and reducing the influence of natural barriers to fishing, such as weather conditions.

The United Kingdom is the leading catcher of *Nephrops*, and as such one would not expect it to be labelled as poor quality, however, a “Food from Britain” report in Italy 2004 stated that “…in the minds of our clients, *Nephrops* are either Scottish or Danish. If they are looking for higher quality they ask for Danish products whereas if they are more interested in price they ask for the Scottish ones.” (Research on Sales and Marketing Support of *Nephrops* 2004). Countries such as Italy buy the Scottish *Nephrops* only because of the lower price, not because of the better quality. Aquaculture of Norway lobsters could help increase the value of British *Nephrops*.

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Contents

1. Introduction and Background

Aquaculture is a fast growing business aiming at 85 million tonnes of global production in 2030. In 2005, 52 million tonnes were produced from aquaculture (Utting, 2006). In 2004, the total production of shellfish in the UK was over 27,800 tonnes and valued at £22.7 million. The main shellfish species farmed are currently oysters, mussels and scallops (Defra, 2004). The United Kingdom, however, has the world's largest share of the species *Nephrops norvegicus* (Scottish Executive, 2006). In 2004 the total catch was 30,516 tonnes with a value of £70.5 million (Defra, 2004). Norway lobsters are therefore an economically important species, as they are consumed within the country (mainly as breaded scampi) as well as exporting the whole animals alive or dead, to countries such as Italy, France and Spain. However, the industry is dependent on weather and seasonal factors which decrease the volume of catch and drive the prices up. Moreover, the new rules for increasing mesh size or cutting days at sea has an influence on the total catch. A previous study by Briggs, *et al.* (1999) showed that an increase in mesh size of 10 mm decreased the catch by approximately 30%. In 2004 the catch decreased by approximately 10.000 tonnes when compared to 2003 (Defra, 2004). The Sea Fish Industry Authority (Seafish), who are interested in improving the sustainability of British seafood, have looked at improving the quality (and therefore maximising the value) of *Nephrops*. They are helping to improve the handling methods on board and they are recommending fishermen to tube the healthier prawns from the trawled catch and subsequently landing less (Linkie, 2007). Despite 50 years of Norway lobster fishing (Howard, 1989) there have been few attempts to find other ways of harvesting *Nephrops* than taking them from the natural environment. In the Kyoto Conference (1995), the importance of aquaculture in securing the demand for seafood in the future was mentioned.

A recent study by Johansson *et al.*, (2007) demonstrated that the understanding of physiology and behaviour is important in order to farm animals successfully. Therefore, in this project the behaviour of the Norway lobster were and will be further studied, as well as the factors affecting their physiological welfare. This decapod species lives at a depth of 200 to 500 metres (Aguzzi *et al.*, 2004), where the visibility is limited. Species from the same family, such as American lobsters, use chemicals in their urine to communicate, especially during fighting (Karavanich and Atema, 1998a). It is hypothesized that chemical signals are also used by *Nephrops*. Adult Norway lobster were paired for initial “boxing matches”, to examine their behaviour as well as their urine release. Aggressive interactions can cause injuries or death. Animals kept in communal tanks may therefore have a high death rate than animals kept individually. However, Atema and Engstrom (1971) showed the important aspect of the pheromone system, which plays an important role in the animal’s behaviour. It was shown that in American lobsters (*Homarus americanus*) pheromones present in the urine are crucial for the maintenance of dominance hierarchies (Karavanich & Atema, 1998a) Therefore, in American lobsters aggression is reduced by urinary signals. My study addresses the mechanisms of dominance in Norway lobsters. Do urinary pheromones play a similarly important role in the regulation of dominance in *Nephrops* as they do in *Homarus*? A better understanding of *Nephrops’* aggressive behaviour has been achieved and the animals kept for a significant time in the tanks in a healthy condition. However, more research on behaviour is necessary such as the role of aggression in territoriality and mate choice in order to have a better control on the animals in the tank.

The next step in developing a Norway lobster culture will be to develop a technique to hatch the eggs. The adult *Nephrops* are distributed on the bottom of the seabed; the larvae on the other hand have three pelagic larval stages (I, II and III). They then metamorphose to the post-larval stage (IV) and settle on the sea bed (White *et al.,* 1988). A study by Nates and McKenney Jr (2000) showed that the quantity and the composition of crustacean larvae changes during development and varies depending on biotic and abiotic factors. Therefore methods for raising eggs and holding larvae in captivity have to be tested. Previous studies, for example on shrimp aquaculture (Boopathy, *et al.,* 2006), on the European lobster hatchery (Jørstad, *et al.* 2005) and on fin-fish farming (Conceição, *et al.*, 2007), will help to develop a methodology for farming Norway lobster. The first steps on culturing Norway lobster were researched by Figueiredo and Vilela (1972), who looked at rearing Norway lobster eggs at different temperatures. Another study investigated biochemical changes during embryonic stages (Rosa et.al. 2003).

2. Material and Method

2.1 Holding conditions:

In this experiment two types of holding conditions are tested. For the acclimatising of the *Nephrops* after transport they are washed by exposing them for a few seconds to sea water in order to collect the ammonium waste accumulated during transport. After washing, they are introduced into a different tank (holding tank). Fifty animals are divided into two groups kept in different conditions. One population is separated individually, whilst the other twenty five animals are not separated and can therefore interact with each other. The water quality and temperature is the same. At the end of this experiment it will be clear which methodology is an efficient way of transporting and holding *Nephrops*.

2.2 Agonistic Behaviour:

Twenty-six *Nephrops* were matched into pairs of similar size. The animals were isolated in individual tanks for seven days before the fight. Each individual was labelled. The fights were recorded with a camera and the tank floor was fitted with a velvet sheet to prevent the animals from slipping. The animals were introduced to the fighting tank and were given thirty minutes to acclimatise. The fighting time was thirty minutes. In these experiments, the hypothesis that *Nephrops* establish a dominance hierarchy was tested. The second part of this experiment will be to look at the importance of pheromone cues in fighting. I compared the fight dynamics of unrestrained combatants with that of animals whose chemical communication is blocked. The methods of Breithaupt and Atema (2000) were used.

3. Results

3.1 Holding conditions:

Introduction: In this experiment it is expected that the animals need to be transported with a temperature controlled vehicle in water with an oxygen supply to avoid high mortality rates and to reduce stress. Moreover, the animals should be caught in creels (pots) and not trawled, as it has a significant effect on the mortality rate (Ridgway, *et al.,* 2006). Norway lobsters are solitary animals and therefore it could be expected that they prefer to be kept separately. The study of Karavanich and Atema (1998b) showed, however, that other crustacean species, such as the American lobster (*Homarus americanus*) are able to recognise individuals, which allows them to form a stable dominance relationship. It can therefore be expected that there is no difference between keeping them separately or together.

Results: The experiment is going as planned with some difficulties at the beginning, which were resolved. So far, five groups were tested under communal holding condition and individual holding condition. In each group there are fifty animals and they are divided in two populations of twenty five animals. One population is kept individually while the other population is kept in a communal tank. The issue of reliable supply had, and will continue to have, an influence on the speed of development of the project. This was predictable as it continues to be a major issue to this industry and it is the aim of the project to improve this situation. The results so far (see figure 3.1.1.) showed that there are no survival rate differences between the communal holding and individual holding condition. However, as the numbers of repeats are too low to have a significant statement, further experiments are necessary to assess a result. The additional two years will be used for more repeats and significant analysis.

Experiment 3.1.: Holding

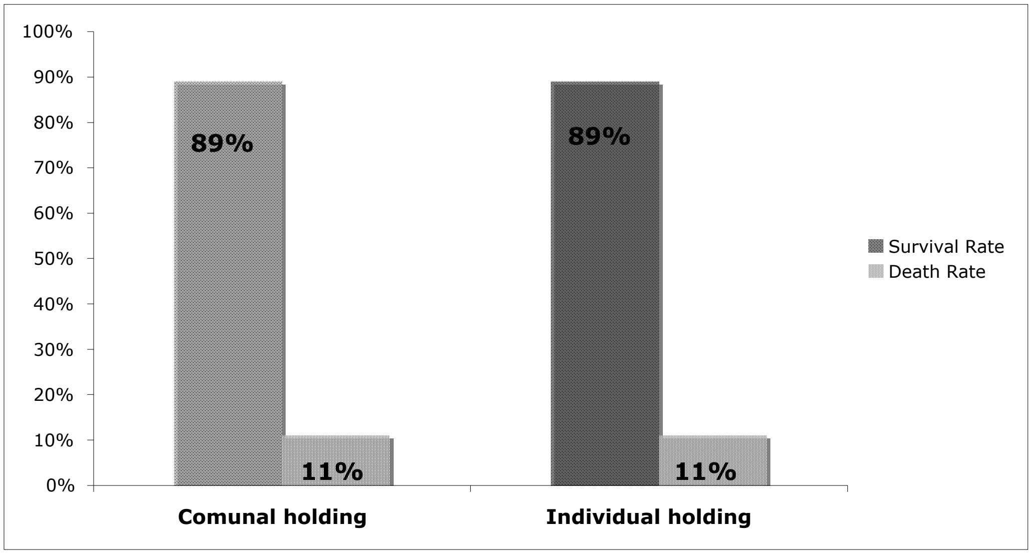

Figure 3.1.1. Survival and death rates of five experiments. Comparison of the survival and death rates of *Nephrops norvegicus* between two different holding conditions. There was no significant difference between communal holding and individual holding condition.

3.2 Agonistic behaviour:

Introduction: Numerous agonistic experiments have been done, demonstrating that aquatic crustacean establish and maintain dominance hierarchies, for example with snapping shrimp (*Alpheus heterochaelis*) (Obermeier and Schmitz, 2003), crayfish (Delgado-Morales, *et al.,* 2004, Breithaupt and Eger, 2002), American lobsters (Atema and Voigt, 1995) and hermit crabs (*Pagurus bernhadus*) (Briffa and Williams, 2006). Pheromone communication in general appears to play an important role in the regulation of dominance in crustaceans (Atema and Steinbach, 2007). Pheromones are conveyed in the urine (Karavanich and Atema, 1998a, Breithaupt and Atema, 1999) Therefore in this section of experiments it will be expected that *Nephrops* do establish dominance hierarchies and need urinary signals for the regulation of dominance.

Results: The agonistic behaviour of male *N. norvegicus* followed a common pattern and therefore it was possible to categorise different behaviour (see Table 1). It was observed that Norway lobsters have a species specific display, which is called meral spread with contact. This display was not found in other similar species such as in European lobsters or Crayfish. Meral spread is mainly used for threatening the opponent while meral spread with contact is used to fight. In this display the animals are face to face with spread claws and push each other. In this experiment it was found that the dominant male display meral spread without physical contact more than comparing to subordinate males (see Fig. 1 in the attached paper). The first experiment shows that Norway lobsters have the ability to recognise dominant opponents after separation for twenty four hours (see Figure 3.2.3.). Subordinates generally showed more defensive behaviour and backed away immediately, while the dominate male showed more aggressive behaviour (see Figure 3.2.1. and Figure 3.2.2). When paired for the second fight with urine release blocked, subordinate Norway lobsters from their first fight fought and were aggressive (see figure 3.2.4.). The results demonstrate that Norway lobsters are able to recognize dominant conspecifics and that they use urinary chemical signals to communicate social status. In addition, the frequency of antennule flicking showed that it is more important for the subordinate male to recognise the dominant male (see figure3.2.5.).

Table 1. Definition of agonistic levels for fighting N. norvegicus (adapted from Atema & Voigt, 1995)

Level	Behaviour	Definition
-2	Fleeing	Walking backwards, Walking away or turning away, tailflipping
-1	Avoidance	Walking around but avoid opponent, Body pressed to the ground
0	Separate	No activity
L	Separate	Locomotion, cleaning
1	No physical contact	Facing, Approaching, Turning towards, Following
2	Physical contact	Contact without aggression
3	No physical contact (Threat display)	Claw up, Meral spread (horizontally spread claws without physical contact), High on legs
4	Physical contact (Threat display with physical contact)	Meral spread (horizontally spread claws with physical contact), Claw touching, Claw smacking, Claw pushing
5	Physical contact (Claws not used to grasp)	Smacking, Pushing, Antennal touching
6	Physical contact (Claws used to grasp)	Claw lock, Claw grabbing, Punching

Experiment 3.2.1.: Behaviour comparison between loser and winner

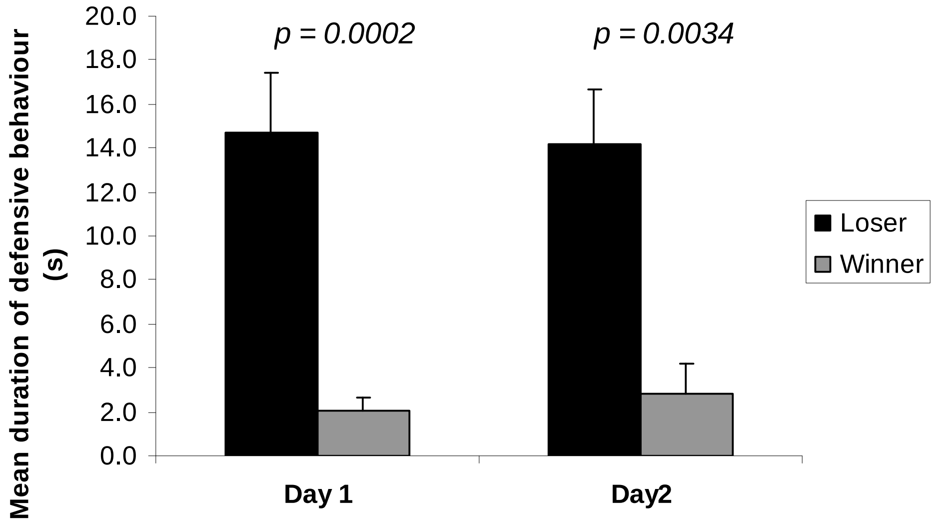

Figure 3.2.1. Defensive behaviours (levels -2 and -1, see table 1). Comparison of mean duration spent with defensive behaviour (seconds) between loser and winner in *Nephrops norvegicus* on two consecutive days (SEM, N = 13). The p-value indicates significant differences between loser and winner using Wilcoxon signed-rank test.

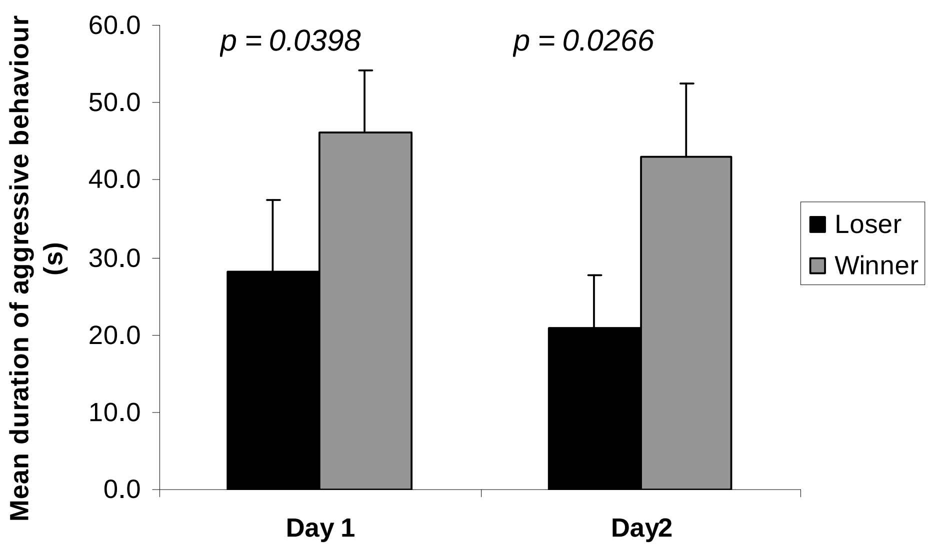

Figure 3.2.2. Aggressive behaviours (levels 4-6, see table 1). Comparison of mean duration spent with aggressive behaviour (seconds) between loser and winner in *Nephrops norvegicus* on two consecutive days. (SEM, N = 13). The p-value indicates significant differences between loser and winner using Wilcoxon signed-rank test.

Experiment 3.2.2.: Dominance hierarchy

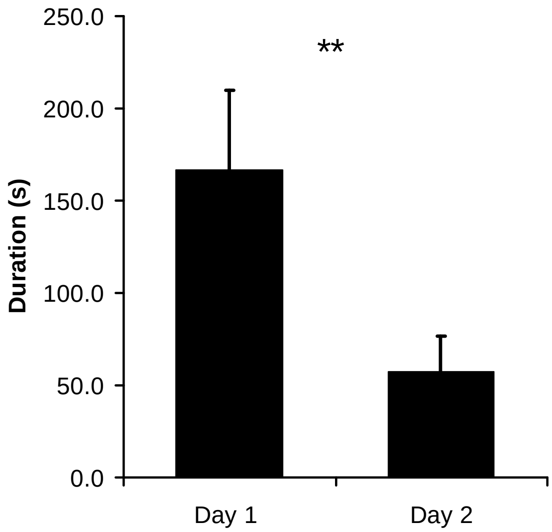

Figure 3.2.2. Unrestrained fights. Fight duration (seconds) in paired fights of sized-matched *Nephrops norvegicus* on two consecutive days (means + SEM, N = 13). Asterisks indicate significant difference between the two days (Wilcoxon signed-rank test: 2-tailed p = 0.0005);

Experiment 3.2.3.: Blocked nephropore

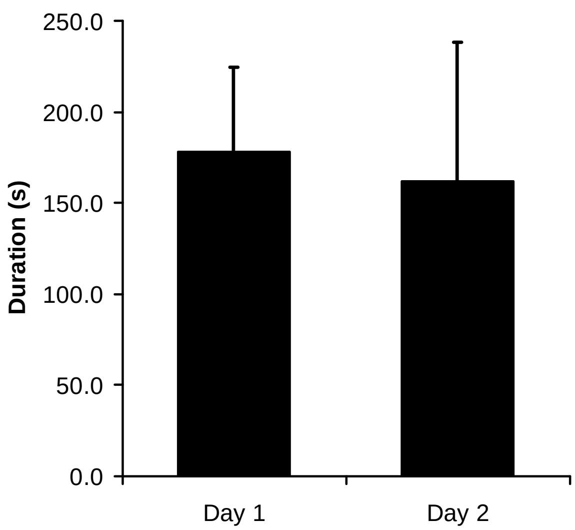

Figure 3.2.3. Urine-blocked fights. Fight duration (seconds) in paired fights of sized-matched *Nephrops norvegicus* on two consecutive days (means + SEM, N=13). Animals had catheters fitted on both days. Urine release was blocked only on day 2. There was no significant difference in fight duration between day 1 and day 2 (Wilcoxon signed-rank test: 2-tailed p = 0.64).

Experiment 3.2.4: Antennule flicking

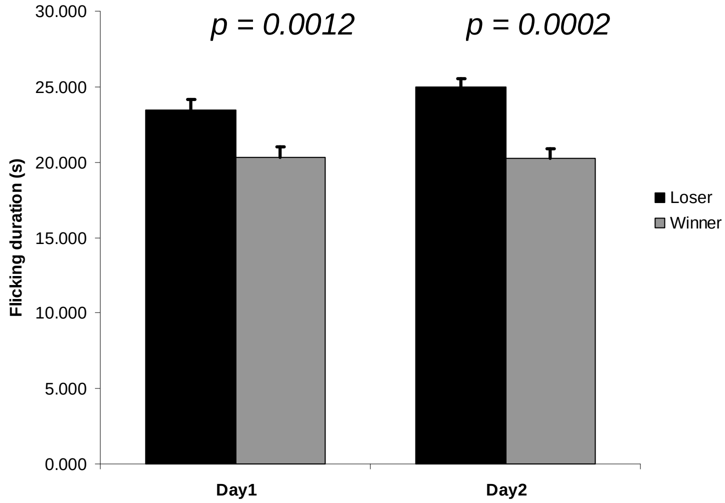

Figure 3.2.4. Antennule flicking frequency. The frequency of antennule flicking (seconds) in paired fights of sized-matched *Nephrops norvegicus* on two consecutive days (means + SEM, N13). The p-values indicate significant difference between loser and winner antennule flicking frequency using the Wilcoxon signed-rank test.

Please see attached paper for further details on this study.

3.3 Conclusion:

The conclusion of this study so far is that Norway lobsters are able to establish a dominance hierarchy and reduce their aggression over time. This may explain the preliminary results of section one, where there is no difference in survival rate between communal holding condition and individual holding condition. Urine plays a crucial role in maintenance of hierarchies, since it is responsible for the recognition of the dominance animal by the subordinate animal (Atema and Steinbach, 2007). The importance of pheromone recognition is further demonstrated by the loser displaying a greater flick (sniff) rate than the winner.

Further studies are predicted to gain a better understanding of the natural behaviour including territoriality, the role of dominate males and mating choice.

4. Developing hatchery techniques

Background information: The experiments for this part are planned for the second and third year. However, further literature reviews were carried out and more information was collected to gain a better understanding of what would be required in implementing this study. The eggs hatch in late spring to early summer, which can vary from season to season dependent on factors such as temperature and weather conditions. The larvae progress through three pelagic zoeal stages before metamorphosing into the postlarval stage (Eriksson and Baden, 1997). Figueiredo and Vilela (1972) recommend collecting berried females in an advanced stage of development. They found that the best temperature level for incubation and survival rate is 11-14 degrees. A study by Rosa, et.al. (2003) investigated the total and free amino acid profiles and lipid dynamics during embryogenesis of this species. Moreover, Eriksson (2000) looked at the fluctuation of manganese in the eggs, which influence the egg development. The understanding of biochemical changes during ontogeny is necessary for completing the reproduction of the Norway lobster. Studies are also needed on different factors such as, differing temperatures, food and the issue on bacterial growth. In this project the information will be used for improving the survival rate of larva in an artificial environment.

Methods: Eggs in an advanced stage will be collected from the females. The water might need to be exchanged very regularly in order to avoid bacterial growth. Research done by Marte (2003), showed moreover the importance of the diet, which can influence growth and development. So far, the most satisfactory seems to be the living eggs of *Crangon Crangon*. There was no evidence found in previous studies that this experiment will not work. Which eggs are more successful in reaching the larvae stage shall be examined. The water temperature will also be changed to see if it is a factor which increases the speed of the egg development. If the animals do reach the larvae stage some will be held together and some separated to see if there is a difference in growth and mortality.

Expected results: The best time to catch berried females is between August and October, as discovered in a study by Tuck, *et al.*, (2000), which also found that the egg losses from the pleopods are extremely high during the long egg-development period. Therefore it is expected to catch the berried females in mid or late spring before the hatching starts and the eggs are in an advanced stage. Moreover, it will be expected that after a certain larval stage separation is necessary.

5. Summary

The first year of the project started with many issues, such as trying to keep the animals alive and obtaining a reliable supply of animals, which depended on the fishing conditions that were influenced greatly by the weather. However, the holding conditions improved and the survival rates increased making it possible to use the animals for experiments. Moreover, experiments were designed for the agonistic behaviour and catheterizing of the Norway lobster. All animals survived after the treatment. The results were presented in a poster at a conference (Bioactive water borne chemicals: Pheromones and welfare indicator in Fish (2007)) in Faro, Portugal. Useful feedback was given and many delegates were interested in the results, such as Dr. R. Earley from California State University. Therefore, the first year was a successful with many experiences and results to build on.

In the second and third year, the research will focus mainly on egg development and in culturing them through some larval stages successfully. Further studies will be made in progressing an understanding of natural behaviour including territoriality, the role of dominant males and mating choice. For example, the aggressive behaviour over territory reduces during the mating season, when soft-shell males and females approach for reproductive purposes on the seabed, after moulting (Aguzzi, 2004). Pheromones might be released during the mating season, which reduces the aggressive behaviour of the animals. Overall pheromone signals might be a stress-reducing factor. The celebrity chef, Heston Blumenthal, and Prof. N. Douglas (2007) showed that stress has an affect on the flavour of the dead animal. Therefore stress reduction will improve the taste of live transported Norway lobsters. Moreover, companies from the *Nephrops* industry, such as Whitby Seafoods (a scampi processor) or D.R. Collins, Angelbond Ltd and Moir Seafoods (live specialists), have shown strong interest in furthering their knowledge.

For the industry itself, it is important to have good handling and holding methods to be able to supply or receive good quality product. Poor holding methods wastes money and product, as it is often no longer saleable (e.g. animals with melanosis or animals that are delivered dead when they should be alive). Microbial growth can be easily detected as the animals have black spots on their carapace and occur through poor handling and poor holding methods. This project will give a better understanding of the species and by that be able to improve holding conditions and develop hatching techniques of Norway lobster.

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