Viking Fish Farms Ltd

Ardtoe Marine Laboratory

A second progress report on Seafish Project no. 6320701

Setting welfare standards of cod during transportation to improve safety and efficiency

Prepared for the

Seafish Industry Authority

by

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1. General Objectives

The investigation of the relationship between fish size, stocking density, and water quality changes during live fish transportation is expected to give information that could form the basis of an industry code of practice for marine finfish transport which would improve the safety and welfare of fish under such conditions.

This report covers a second series of trials testing the effects of a range of variables on water quality during simulated transport conditions, using the cod, *Gadus morhua*, as the test species.

2. Testing the effects of different buffering agents

2.1. Trial objectives

To examine the effects of three different buffers on water quality changes, fish condition and fish welfare in a simulated 48 hour transit of 28.6 g cod at density of 20 kg m^{-3}. The buffers tested were:

(1) Sodium bicarbonate (Howard Dryden)
(2) TRIS =Tris (hydroxymethyl) methylamine (BDH Laboratory Supplies, Poole Prod 271193G)
(3) Magnospheres (Howard Dryden)

The changes in pH and other water quality parameters in these treatments were also compared with tanks where no buffer was provided.

2.2. Trial methods

A sample of 50 fish from the main stock holding tank was examined prior to the trials commencing to check the body and fin condition, and for deformity to the jaws and gill covers.

Black polyethylene tanks of 70 litre capacity were used as simulated transport tanks and were filled to a measured 50 litre mark. The tanks were supplied with filtered and UV treated seawater of 33.3 ppt and a starting temperature of 8^{o}C. No aeration was provided but oxygen was supplied through small wooden block diffusers to give a saturation value around 120%. The mean recorded initial value was 9.8 mg l^{-1} but after 20 minutes the mean oxygen concentration was 12.75 mg l^{-1}. The tanks were maintained in the dark with an open cover to allow filming. A video camera with an inbuilt infra red lighting unit was positioned 50 cm from the side of the tank on a tripod and angled at ca. 10 degrees to prevent reflection from the infra red light. The camera was linked by cable to a video unit in an adjoining room and recordings were made on high quality VHS tape. Each tank was labelled and filmed for 2 minutes before transfer to the next tank until all six tanks had been filmed. This procedure was repeated every hour for the first 12 hours post loading time and thereafter every 3 h until the trial was completed. The trial was initially scheduled to last for 24 h as this was taken to be a suitable transfer time representing an average loading, transit and discharge time from the Scottish mainland to an ongrowing site. However, as juvenile cod may have to be transported from Scotland to Norway, Denmark or Ireland the trial was extended to 48 h. The fish were bulk weighed on transfer to the trial tanks and then the fish counted on completion of the trial. Transport handling was carried out and fish were transferred to the test transfer tanks, to give equal

stocking densities in each tank of 20 kg cod juveniles m^{-3}. On commencing the trial and at 1 hour intervals thereafter temperature, salinity, oxygen concentration and pH were recorded. Alkalinity, and total ammonia were measured each 6 hours and suspended solids and turbidity were recorded at the end of the trial.

Fish condition was checked in 25 fish from each tank (=50 each treatment) when the fish were transferred to the holding tanks. The fins, body, snout and operculae were examined closely for any damage. Fish behaviour was analysed from the video material and fish behaviour categorised as:

(a) Swimming speed : 0 = no movement; 1=slow; 2= moderate; 3=very active
(b) Location in the tank = numbers of fish at the surface
(c) Gulping/non gulping
(d) 1, Aggression; 2, non aggression
(e) Opercula movement: 1=slow; 2=moderate, 3=rapid respiration
An average score for a 1 minute observation period was calculated for each tank.

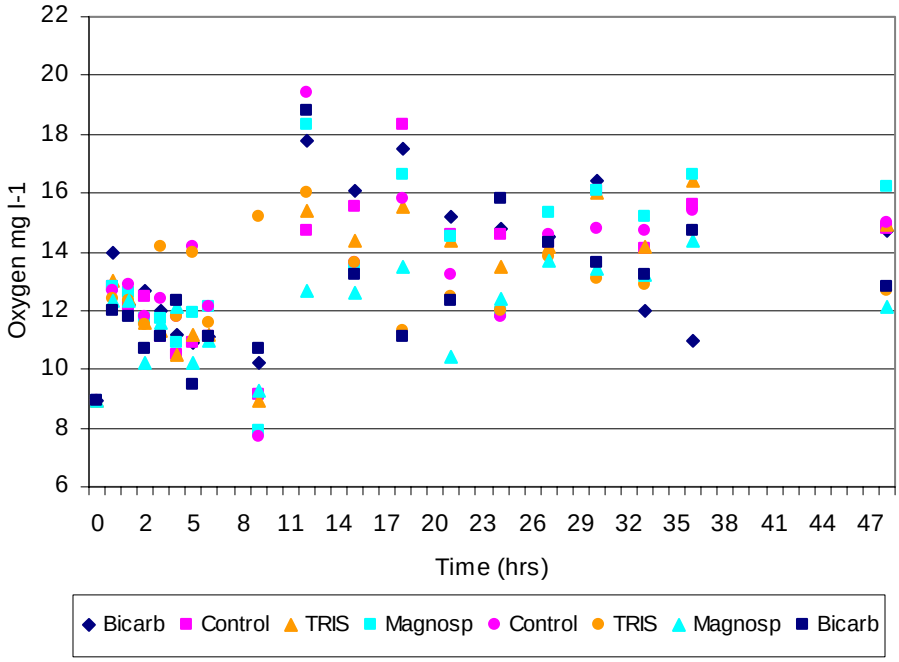
2.3. Trial results

The oxygen levels varied in the initial 3 hours of the experiment and oxygen flows into each tank thus had to be adjusted individually until levels stabilised at ca. 120%.

2.3.1. Salinity, temperature and oxygen.

The salinity during the trial was 33.3 ppt and, as no water exchange was used, this did not vary between tanks or during the trial. Oxygen levels remained in the range 7.9 to 16.6 mg l^{-1} (Fig. 1). The temperature was 7.9°C at the start of the trial and 8.6°C after 48 h.

Fig 1. Trial 3 : Change in Oxygen Concentration with Time



2.3.2. pH

Initially the buffers were added a rate of:

0.5 g l^{-1} of Tris to each of two tanks,
0.5 g l^{-1} of bicarbonate to each of two tanks
1 g l^{-1} of magnospheres to each of two tanks, held in a mesh bag suspended in the tanks.

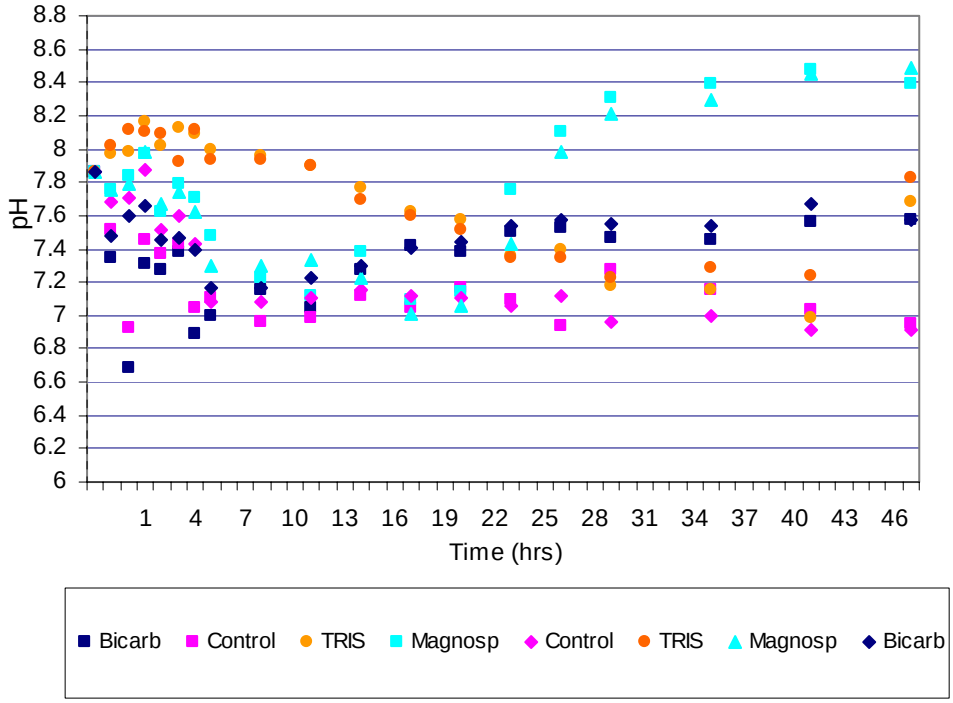
The pattern of further additions of buffer is shown in Table 1. Briefly, further additions of bicarbonate and magnospheres were made after 12 and 21 hours, whilst Tris was only augumented after 42 hours.

Table 1. Quantities of buffer used in trial

Time	Tris	Magno	Bicarb
start	0.5g/l	1 g/l	0.5 g/l
12 h	-	+2 g/l	+1 g/l
21 h	-	+10 g/l	+1 g/l
36 h	-	-	-
42 h	+0.5 g/l	-	-

The pH in tanks with no buffer had fallen to 7.1 within 6 hours (Fig. 2), and declined to minimum values of 7.0 after 12 h from the start of the transport trial. The pH values of tanks with TRIS added at 0.5 g l^{-1} were 7.9 after 12 hours but had fallen to

Fig 2. Trial 3 : Effect of Various Buffers on pH Change



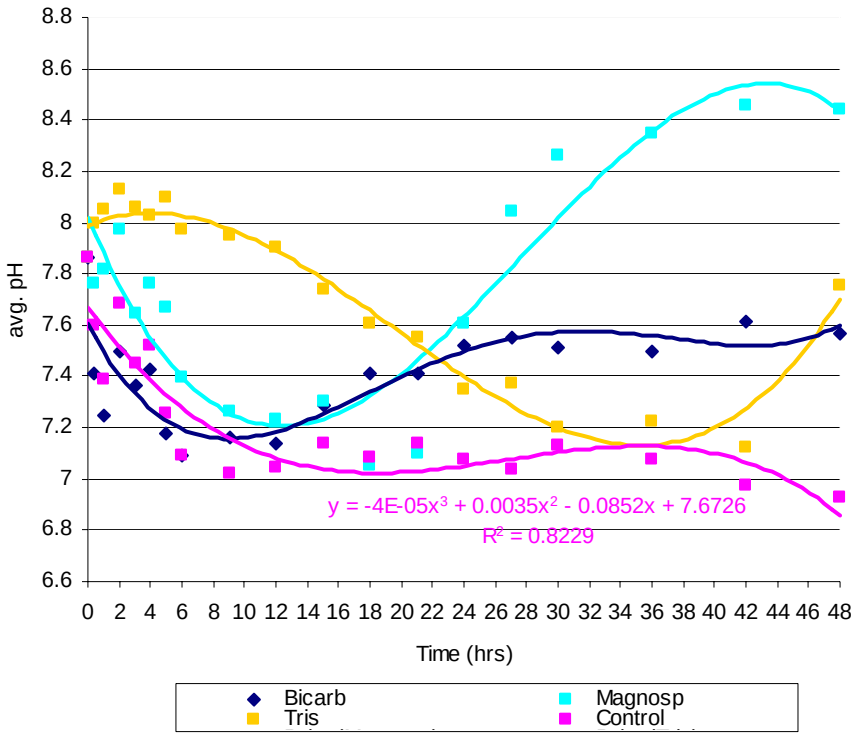
7.34 after 24 hours and continued to fall gradually to a minimum of 6.99 and 7.24 after 42 hours. To determine whether the pH in the tanks could be recovered a further 0.5 g l^{-1} of Tris was added to the tanks after 42 hours, and by the trial end 48 hours post transfer, the pH had increased to 7.63 and 7.87 respectively.

The pH in the tanks with bicarbonate declined at similar rates to the control tanks with values of 7.0 and 7.17 after 6 hours (Fig. 2). A further 1 g l^{-1} of bicarbonate was added to each tank after 12 hours and the pH in the two tanks rose to 7.38 and 7.44 respectively after 21 hours. A further 1 g l^{-1} of bicarbonate was added and the pH rose to 7.5 and 7.54 respectively at 24 hours and remained stable in the tanks through to the end of the transport at 48 hours when pH was 7.57 in both tanks.

The pH in the tanks with Magnospheres declined following transport, but more slowly than in the control tanks and tanks with bicarbonate, to 7.48 and 7.3 respectively after 6 hours but, after 12 hours, the pH was 7.12 and 7.33 (Fig. 2). A further 2 g l^{-1} (100 g) of magnospheres was then added but pH continued to decline and was 7.14 and 7.04 after 21 hours. At this point 10g l^{-1} (500 g) of magnospheres was added to these tanks and within 4 hours the pH was rising and attained highest values of 8.39 and 8.3 by 36 hours after addition, and was 8.39 and 8.49 at the end of the trial.

The trend in pH change under each treatment can perhaps be seen a little more easily in Fig. 3 where the average pH for the two replicates of each treatment has been plotted against time. Trend lines have also been fitted to the data though it has to be borne in mind that, other than for the control, these are not true regressions as additional buffers were added during the course of the trial.

Fig 3. Trial 3 : Average pH vs Treatment



As can be seen, only the TRIS prevented an initial sharp decline in pH at the start of the trial and thereafter allowed a much slower decline in pH as its buffering capacity was used up. The other two buffers did hold the pH up by 0.2-0.4 units compared to the control during the first few hours of the trial but the rate of decline was broadly similar in the latter three treatments. However, when additional bicarbonate and manospheres were added at around 12 and 24 hours, the pH quickly rose away from that of the control. The pH of the tanks with bicarbonate remained at around 7.6

thereafter whilst the pH of the tanks with magnospheres gradually rose to the natural buffer pH of this material at around 8.4. This indicates that both these buffers need to be added at a much higher concentration when the fish are first stocked in the transport tanks and that doing so would probably prevent the initial crash in pH seen in this trial.

2.3.3. Ammonia

The ammonia recorded at the start of the trial was 0.006 mg l^{-1} and increased through the 48 h simulated transport to 0.3-0.6 mg l^{-1} NH_3-H. However, the pattern of ammonia readings showed considerable variation during the trial.

2.3.4. Fish behaviour

The initial fish activity was high as seen in the video material but after 2 hours the fish descended in the water column and were more sedate. There was no difference in activity levels between tanks with different buffers. Towards the end of the 24 hour transport period the behaviour was still sedate.

2.3.5. Fish condition

No difference was noted in fish condition scores from the beginning to the end of the 24 h trials.

2.3.6. Mortality

There were to 2 mortalities in each of the tanks at the termination of the trial. These were smaller fish and showed evidence of fin nipping due to the starvation period.

2.4. Trial 3 Discussion and conclusions

As in previous trials pH dropped from 7.8 to minimum values of 7 within 6 hours of commencing the transport trial. In the present study there was no previous experience to indicate the amount of buffer that would have to be added per litre per kg of fish density, so trial quantities of 0.5 g l^{-1} Tris, 0.5 g l^{-1} bicarbonate and 1 g l^{-1} Magnospheres were added to the tanks before the fish were transferred. After 6 hours the pH was constant only in the group with TRIS buffer and, when bicarbonate and magnospheres were used, the pH was not significantly different from the control tanks. This suggested that the amount of buffer added in these cases had been under-estimated and further bicarbonate and Magnospheres were added. While the addition of a further 1 g bicarbonate per litre increased the pH to 7.3 a further addition of 1 g l^{-1} was required to achieve pH values of 7.5 at the end of the trial. An addition of 2 g magnospheres l^{-1} had little effect on pH and an addition of 10 g l^{-1} was required to give an elevated level of 8.4 and 8.5. During this addition it was noticed that the effectiveness of the Magnospheres may have been diminished as they were held in a small mesh bag. The aerator was therefore inserted in the bag to aid diffusion. The weight of magnospheres was clearly too high and an intermediate figure would be more appropriate. It was noted that, although the pH levels remained constant for 6 hours and 12 hours with TRIS buffer that the pH did drop gradually to 7.3 after 24 hours. Further addition of TRIS should therefore be made 21 hours into the transport.

Based on these results the following levels of buffer are recommended for cod at a stocking density of 20 kg m^{-3}:

- *TRIS 0.5 g l^{-1} transport water; addition of another 0.5 g l^{-1} after 21 hours*
- *Bicarbonate 2 g l^{-1}*
- *Magnospheres 10g l^{-1} is too high, so a figure of 5 g l^{-1} is suggested with aeration to assist mixing.*

These results highlight the need to replicate this trial with a higher stocking density of cod, closer to 30 kg m^{-3}. The recommended additions of buffer should also be checked to determine if adequate levels of pH can be maintained, and to see if the buffer has to be supplemented after a certain period in to the transport.

However, the physical condition of the fish was not different between treatments and there was no indication of damage to the body, fins, gill cover or snout. Ammonia readings did increase from 0.3 to 0.6 mg l^{-1} NH_4-N during the transport and work should be carried out to determine whether these levels are a problem and whether they can be controlled.

3. The influence of fish weight on water quality and stress during transport

3.1. Objectives

To examine pH and other water quality changes and buffer requirements in fish of different size at transport, in fish of 9.8 g and 18.4 g mean weight respectively. Fish were maintained at a stocking density of 1.33 kg in 50 litre trial tanks, equating to a stocking density of 26.6 kg m^{-3}. Fish condition and fish welfare were examined in a simulated transport of 24 hour duration. The buffer used was TRIS (BDH Chemical Supplies) and two concentrations were used, at 0.5 g l^{-1} and 1 g l^{-1} transport water. In addition two tanks were allocated with no buffer, acting as controls. Tanks were allocated on a random number basis to each treatment. The treatments were therefore:

Tank number	Fish size	Treatment
1	Large	TRIS buffer 0.5 g l^{-1}
2	Small	TRIS buffer 0.5 g l^{-1}
3	Large	TRIS buffer 1.0 g l^{-1}
5	Small	TRIS buffer 1.0 g l^{-1}
4	Large	No buffer
6	Small	No buffer

3.2. Methods

As in Trial 3 a sample of 50 fish from the holding tank was examined prior to the trials commencing to check the body and fin condition, jaw condition and gill covers.

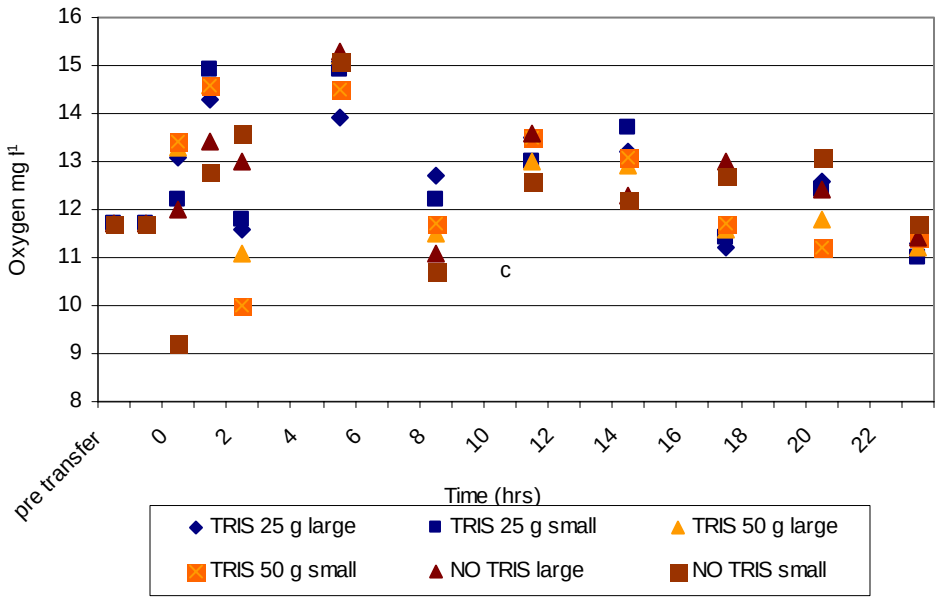
The tanks used for the simulated transport, the fish handling, sampling procedures and the videoing and observational procedures were the same as in Trial 3. The trial was scheduled to last for 24 h as this was taken to be a suitable transfer time representing an average loading, transit and discharge time from the Scottish mainland to a receiving site. The fish were starved for 24 h prior to transport and maintained in the dark before transfer to the test transfer tanks. The stocking densities in each tank were identical, at 26.6 kg cod juveniles m^{-3}.

3.3. Results

3.3.1. Salinity, temperature and oxygen

The oxygen levels varied in the initial 3 hours of the experiment and each tank had to be adjusted individually until levels stabilised at ca. 120%. Oxygen levels were 12 to 13.5 mg l^{-1} on commencement of the trial and the range thereafter was from 11.0 to 14.9 with minor adjustments made to oxygenation as required (Fig. 4). The temperature during the trial increased from 9.0 to 9.6^{o}C. The salinity was 31.5 ppt and did not vary during the trial.

Fig.4. Trial 4 : Change in Oxygen Concentration with Time



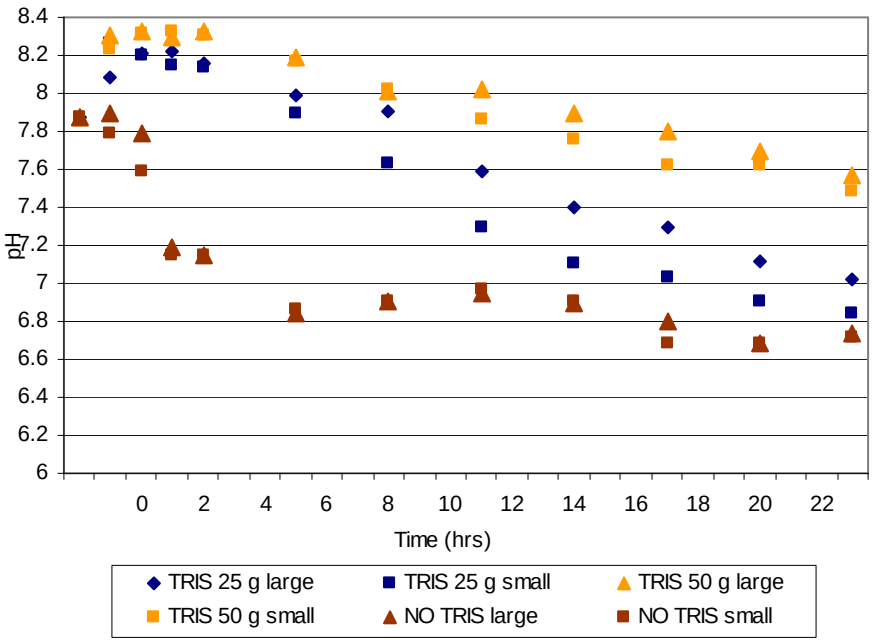
3.3.2. pH

Initially 0.5 g l^{-1} of Tris was added to two tanks holding fish of each size. This was repeated in a further two tanks but with 1 g l^{-1} Tris, and there were also two tanks with no buffer (control). No further additions of buffer were made during the 24 hour trial period.

The pH in tanks with no buffer had fallen to 6.85 within 6 hours (Fig. 5). The pH values in tanks with TRIS added at 0.5 g l^{-1} were 7.99 and 7.89 with large and small fish respectively and 8.19 and 8.17 when 1 g l^{-1} was used. There was a marked

decline in pH when 0.5 g l^{-1} TRIS was used falling to 7.59 and 7.3 after 12 hours

Fig 5. Trial 4 : pH Change with Time



compared with 8.02 and 7.86 with 1 g l^{-1} TRIS and the differences were more pronounced 24 hours after the start of the trial.

There were also differences in pH related to the size of fish. Although the biomass of fish in the tanks was equal the pH in tanks with larger fish was consistently higher than with small fish, when both 0.5 g l^{-1} and 1 g l^{-1} TRIS were used.

3.3.3. Ammonia

The ammonia level was zero at the start of the trial and increased to 0.04 mg l-1 NH_3-H after 12 hours and 0.10 after 24 hours.

3.3.4. Fish behaviour

Fish swimming speed was initially high but this declined after 2 hours. There were no differences in activity patterns between large and small fish and the fish behaviour was calm after 24 hours.

3.3.5. Fish condition

There was no deterioration in fish condition through the trial and no difference was detected between fish of different weights assessed by physical appearance including fin condition.

3.3.6. Mortality

Mortalities (n=3) were only recorded from tank 2 (small fish 0.5 g l^{-1} TRIS).

3.4. Trial 4 Discussion and conclusions

The trial demonstrated clear differences in the patterns of pH changes with large and small cod during transport. The pattern was similar when both 0.5 g and 1 g l^{-1} TRIS was used, with tanks with smaller fish having consistently and significantly lower pH compared with tanks with larger cod, although total biomass in each case was identical. However, the trial also confirmed findings from trial 3 that pH was maintained more effectively when 1 g l^{-1} TRIS was used rather than 0.5 g l^{-1}. The pH remained above 7.5 for 24 hours and no top up was required. However, it appeared that the TRIS would have to be supplemented after 12 hours when using 0.5 g l^{-1}.

Based on these results the following levels of buffer are recommended for cod at a stocking density of 27 kg m^{-3}:

TRIS 0.5 g l^{-1} transport water, with an addition of 0.5 g l^{-1} after 12 hours, or
TRIS 1 g l^{-1}, with no additional buffer required for 24 hours.

4. Future transport trials

Further trial work is required to refine the amount of other buffers such as bicarbonate and magnospheres that should be used with cod stocking densities up to 30 kg m^{-3}, in assessing the effects of ammonia, and in examining the effect of length of starve period on safety of transports.