Trials to Assess the Effectiveness of Ionization, Chlorination and UV Irradiation for the Disinfection of Seawater

Seafish Report No.SR 473

May 1996

The Sea Fish Industry Authority

Seafish Technology

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Summary

Clean supplies of water are essential for the industry to maintain high standards of hygiene and quality. Where seawater is used, regulations demand that the water is free from microbiological contamination and that disinfection is carried out where required. In an operation such as shrimp processing where the product is cooled in seawater, cross contamination as a result of using contaminated seawater may result in food poisoning. It is thought that seawater containing a disinfectant residual may also be used to slow the microbiological spoilage of the product itself. For example using treated water in an RSW system may improve fish quality and extend shelf life.

A series of practical trials were carried out to determine the effectiveness of electrolytic ionization, UV irradiation and chlorination for killing bacteria in seawater, with a view to the development of a commercial system for use on shrimp boats. A trial was also carried out to determine the effect on white fish quality of using ionized water in an RSW system.

All treatments except chlorination using sodium hypochlorite proved to be unsuitable due to poor bacteriocidal performance and/or prohibitive costs. Sodium hypochlorite gave effective coliform bacterial kills at dose levels as low as 5 mg/l of chlorine. A marginal improvement in quality was determined over a 7 day period with treated RSW versus untreated RSW.

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1. Introduction

Supplies of clean water are essential for the industry to maintain hygiene standards for many fish handling and processing operations.

At sea, and occasionally onshore, seawater is used where a supply of fresh water is unavailable. The Food Safety (Fishery Products on Fishing Vessels) Regulations 1992 demand the seawater used for this purpose, is clean and free from microbiological contamination. This requires that disinfection is carried out where necessary.

The microbiological quality of the seawater is especially important if it comes into contact with a cooked product. Within the brown shrimp industry, seawater is usually used in the cooling and sorting of cooked shrimp. As the quality of seawater cannot be guaranteed there is a risk of contaminating the product, resulting in accelerated spoilage and more importantly the risk of food poisoning.

It is thought that seawater containing a disinfectant residual may also be used to slow the microbiological spoilage of the product itself. For example, the use of treated water in a refrigerated seawater (RSW) storage system may improve fish quality and extend shelf life.

After reviewing a wide range of disinfectant technologies, electrolytic ionization, UV irradiation; filtration and chlorination were identified as being potentially suitable for the disinfection of seawater on board small boats. This report is concerned with a series of laboratory and field trials carried out to determine the effectiveness of the above disinfectant technologies to kill bacteria in seawater. A trial was also carried out to determine the effects on white fish quality, of using electrolytically ionized water in an RSW system.

The use of ozone as a disinfectant is a further option for possible use on larger vessels but this is to be studied separately and did not form part of this study.

2. Trials Sequence

The following trials were carried out:

Trial I A laboratory investigation of the amount of copper and silver released into the treated water by an ionization cell over a range of flow rates and power settings.

Trial II A laboratory investigation of the ionization disinfection of fresh water and artificial seawater inoculated with *E.coli.*

Trial III A laboratory investigation of the ionization disinfection of seawater taken from Bridlington Harbour.

Trial IV Field investigations of the ionization disinfection of seawater taken from three areas of UK coastline.

Trial V Field investigations of the use of commercial filters to enhance ionization disinfection.

Trial VI A laboratory investigation of the extension of the shelf-life of gutted cod held in an RSW system using ionized water.

Trial VII A field investigation of the disinfection of seawater using a 6 x 30W UV chamber with and without pre-filtration.

Trial VIII Determination of the chlorine demand and the amount of chlorine required to disinfect seawater.

Trial IX A laboratory investigation of seawater disinfection at higher chlorine doses and longer contact times.

3. Equipment

3.1 Ionization Equipment

Electrolytic ionization is a relatively new disinfection technology. Small amounts of copper and silver ions are released from electrodes within the ionization cell by passing a current through the water to be treated. The metal ions are taken into the bacterial cell and interfere with DNA replication, cell wall transport, and respiratory mechanisms resulting in cell death.

The ionization equipment was supplied by Tarn Pure Ltd, High Wycombe, Bucks. Two basic types of ionization cell, flow-through and re-circulatory, were used in the work. The flow- through cell was designed to put a bactericidal concentration of metal ions into water in a single pass, whilst a re-circulatory cell was designed to be used in a closed system to maintain a consistent level of ions in the water, by topping up with a slow ion release.

The flow-through ionization cell F1 is shown in figure 1 and consisted of a 200 mm long x 100 mm diameter ABS housing which contained two 25 mm x 25 mm x 25 mm silver/copper alloy electrodes cast in a ratio of 90% silver: 10% copper. The electrodes were spaced 5 mm apart. A second similar flow–through cell F2, with 30% silver: 70% copper electrodes was used in later trials.

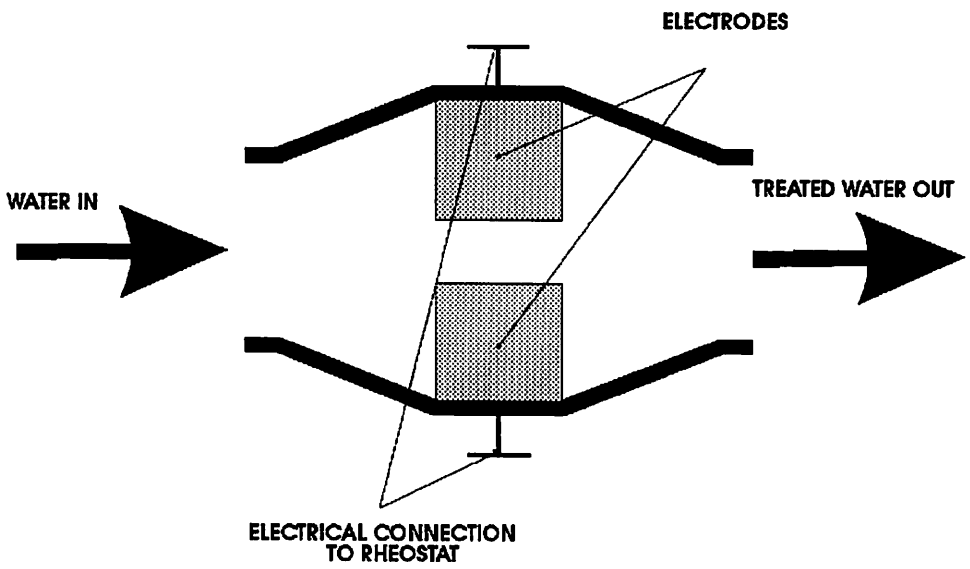

Figure 1 - Diagram of the F1/F2 flow–through electroylytic cell

The re-circulatory ionization cell F3, shown in figure 2, consisted of a 350 mm long x 50 mm diameter ABS housing which contained two 90% silver:10%copper electrodes identical to the ones in the flow-through cell but spaced 300 mm apart.

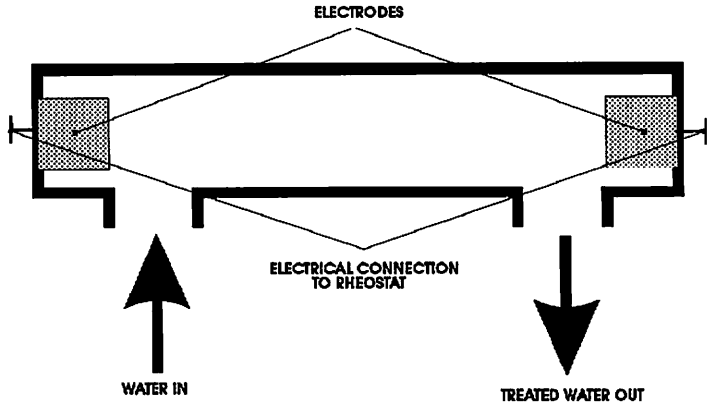

Figure 2 - Diagram of the recirculating ionization cell

The ionizers were powered by either a 240V AC or 24V DC supply. Current was supplied to the electrodes through a transformer/control box (coarse adjustment) and a separate in–line rheostat (fine adjustment). Periodically the polarity automatically switched to prevent uneven electrode wear.

The amount of copper and silver ions released into the water during ionization was controlled by adjusting the current to the electrodes(indicated by a 0-10A ammeter on the control box). At full power (10A reading on the ammeter) the measured current and voltage at the electrodes of the flow–through cells in 34 parts per thousand (ppt) seawater was 8.5A and 10V respectively, giving the equipment an overall power consumption of approximately 90W.

3.2 Filters

The following filters were used in trials V, VII and VIII to filter the seawater prior to disinfection.

Sand Filter - The sand filter used in the work was a 24 inch diameter Lacron™ filter with a manual back flush valve. The filter contained a medium grade sand to give 10μm filtration.

Cartridge Filter - The cartridge filter used in the work was a 360 mm x 170 mm diameter "2 inch" Amiad™ ABS cartridge filter. The filter used a 100μm element which consisted of grooved plastic disks which were compressed during filtration but separated for easy cleaning when the element was removed. The filter could be back flushed to allow cleaning without removal of the element.

4. Common Experimental Method

The trials detailed in this report were carried out between June 1994 and March 1995, in the Seafish Technology laboratory at Hull or in the field.

4.1 Disinfection Procedure

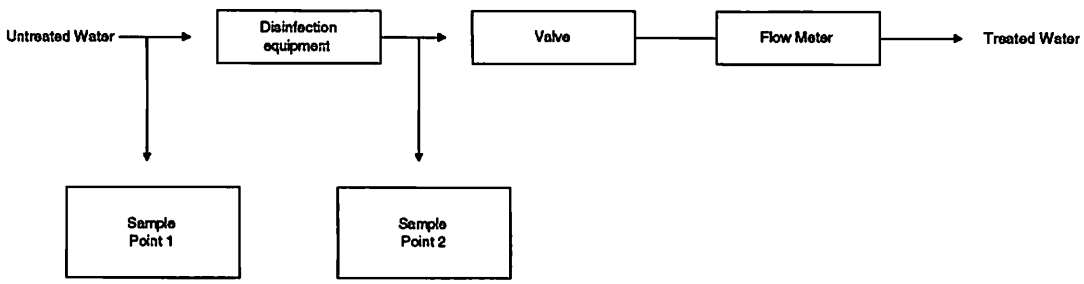

Figure 3 - Diagram to show the common experimental procedure

Figure 3 shows the common experimental arrangement used to disinfect water. In laboratory trials I to III, 20 litre samples of treated water were collected directly after the flow meter, then thoroughly mixed. Mixing was carried out because preliminary work with the ionizer had shown that the amount of copper put into the sample could vary by up to 0.08 mg/l depending on the stage of switching cycle. Sample points 1 and 2 were used for simultaneous sampling of pre and post treated water in trials IV, V and VII.

The flow-through disinfection equipment was adjusted by a gate valve. The flow rate was measured using an inline turbine type electronic flow meter. All pipework was in ABS or flexible PVC reinforced tube.

All samples were collected in sterile Sterilin™ or Nalgeine™ bottles.

In trials V and VII where pre-filtration of the water was required, the filter was placed immediately after sample point 1.

4.2 Microbiological Analysis

Water samples were analysed by the PHLS department of Hull Royal Infirmary. Samples were analysed for total viable count (TVC) total coliform (TC) and *E.coli*. Organisms were enumerated by either a spiral plate count or membrane filtration technique according to the standard methods detailed in HMSO document No.71. Results were reported as colony forming units (cfu) per ml or per 100 ml.

4.3 Metal Ion Analysis

Water samples were analysed for copper content using a calibrated Hach DR2000 spectrophotometer at 450mm. Fish flesh samples were analysed for copper by the University of Hull, Department of Analytical Chemistry. Samples (including fish skin) were washed, digested in nitric acid and analysed using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS).

Numerous attempts were made to find an accurate and reliable method for the determination of silver in seawater but all methods failed due to complexing of the silver and interference caused by the salts present in seawater.

4.4 Chlorine Dosing

In trials VIII and IV water was dosed with sodium hypochlorite solution to kill bacteria. The amount of free chlorine and total chlorine in a stock solution of sodium hypochlorite (nominal 5% available chlorine) was determined using a calibrated Hach DR2000 spectrophotometer and DPD reagents,

This stock solution was then used to dose the water to be treated with the specified amount of chlorine.

5. Trial I - Determination of the amount of copper and silver released into freshwater and seawater using the Tarn Pure F1 ionization cell

5.1 Introduction

The aim of the trial was to determine the effect of salinity, flow rate and power setting on the level of copper and silver ions deposited into the treated water by the F1 ionization cell. Fresh water and artificial seawater (ASW) at 24 ppt and 34 ppt was ionized over a range of flow rates and power settings.

5.2 Method

A 500 1 Alibert container (No. 21624) was filled with fresh (tap) water. The F1 ionization cell was set up and the trial was carried out according to the common experimental method detailed in Section 4.1. The water was treated at 1A (the maximum current achieved with fresh water) at a flow rate of 20 l/min, 40 l/min and 70 l/min. At each flow rate, 20 l of treated water was collected in a stainless steel container, mixed, and two samples were taken for immediate copper analysis.

The above procedure was repeated using Peacock Seamix™ pre-mixed salt and tap water to make two 5001 batches of ASW with salinity of 24 ppt and 34 ppt.

With seawater, a higher maximum current of 10A was attainable. Each batch of seawater was treated at 3A, 6A and 10A at a flow rate of 20 l/min, 40 l/min and 70 l/min.

5.3 Results

The amount of copper in each sample is shown in Table 1 overleaf.

Table 1 - Amount of Copper (mg/l) deposited into fresh water and artificial seawater after treatment using the F1 cell

Ionizer Power Setting Current (A)	Average Total Copper Level (mg/l)								
	Fresh Water			24ppt ASW			34ppt ASW		
	Flow rate (l/ min)			Flow rate (l/ min)			Flow rate (l/ min)		
	20	40	70	20	40	70	20	40	70
Untreated control	0.1	0.05	0.05	0.06	0.06	0.06	0.08	0.08	0.08
1A(fresh water only)	0.22	0.15	0.14	-	-	-	-	-	-
3A	-	-	-	0.28	0.16	0.10	0.19	0.15	0.12
6A	-	-	-	0.32	0.20	0.14	0.22	0.16	0.12
10A	-	-	-	0.34	0.25	0.19	0.39	0.33	0.33

Fine black particles of silver chloride were observed in the artificial seawater samples immediately after treatment. Treated fresh water developed a rose/grey discolouration after 2-3 hours standing.

5.4 Conclusions

At a given flow rate doubling the current to the electrodes did not double the amount of copper deposited into the water. Likewise at a given power level, halving the flow rate did not double the amount of copper in the treated water as would be expected.

The black particles of silver chloride formed in treated artificial seawater could cause problems if the water is used for product washing purposes (i.e. black particles sticking to the product).

The amount of silver in each water sample could be estimated using the following formula.

$$\text{Estimated amount of silver in sample due to ionization (mg/l)} = \left[\text{Amount of copper in treated water (mg/l)} - \text{Amount of copper in control (mg/l)} \right] \times 9$$

6. Trial II - Disinfection of fresh water and artificial seawater inoculated with *E. coli* using the Tarn Pure F1 ionization cell

6.1 Introduction

It is well documented that copper and silver ions are toxic to bacteria, algae and viruses in fresh water. However, no papers are available which are concerned with the fate and biocidal performance of copper and silver in seawater. This trial was carried out using the F1 ionization cell to disinfect fresh water and 34ppt artificial seawater (ASW) inoculated with *E.coli.* Microbiological sampling of the treated water was carried out at intervals after ionization to determine the rate of disinfection. Treated samples were held at 5 °C and 20 °C to see if the bacteriological effect was temperature dependent.

It has been reported that silver ions may only have a bacteriostatic effect. The bacteria appear to have been killed, but in time the metal ions are pumped out of the cell which then recovers and continues to grow. After 48 hours samples held at 5 °C were moved to 20 °C storage, a temperature shock which should revive cells in stasis.

6.2 Method

Fresh water (300 litre) was inoculated with *E.coli* NTC No.10418 to give approximately 1 x 10^5cfu/100 ml. The F1 ionization cell was set up and the trial was carried out according to the common method detailed in section 4.1. The water was treated at 1A (the maximum current achievable with fresh water) at a flow rate of 40 l/min and a 20 litre sample of the treated water was collected in a stainless steel container then mixed. A second 20 litre sample of water was collected with the ionizer switched off to give an untreated control. Samples of one litre were taken from the initial inoculum, treated water and the control and stored separately at 5 °C and at 20 °C.

The amount of copper in each sample was measured immediately after collection. Microbiological analysis of each 1 litre sample was carried out 6 hours, 24 hours and 48 hours after collection.

The procedure was repeated with 34 ppt ASW treated at 40 l/min and 10A.

This was then repeated with water inoculated to 10^8 cfu/100 ml. After 48 hours samples stored at 5 °C were moved into storage at 20 °C, and a final microbiological analysis was carried out after 72 hours.

6.3 The Results

The results of the 10^5 cfu/100 ml inoculum disinfection trial are shown in Table 2. Repeating the trial with 10^8 cfu/100 ml inoculum gave the same pattern of disinfection (results not shown). No growth was observed in the samples held at 5 °C which were incubated at 20 °C after 48 hours.

Table 2 - Percentage kill of *E.coli* in treated fresh water and artificial seawater inoculated at 10^5 cfu/ml and stored at 5°C and 20°C

	Storage Temperature	Treatment	Time to analysis after treatment (h)	*E.coli* (cfu/100 ml)	% kill
Fresh Water	5 °C	Ionized 40 l/min 1A	6	30000	94.5
			24	0	100.0
			48	0	100.0
		Control	0	550000	0.0
			6	750000	(–36.4)
			24	520000	5.4
			48	930000	(–69.0)
	20°C	Ionized 40 l/min 1A	6	-	-
			24	20000	96.3
			48	5000	99.0
		Control	0	550000	0.0
			6	630000	(–14.5)
			24	490000	10.9
			48	530000	3.6
Artificial Seawater 34ppt	5°C	Ionized 40 l/min 10A	6	280000	6.6
			24	10000	96.6
			48	5000	98.3
		Control	0	300000	0.0
			6	1400000	(–366.6)
			24	670000	(–123.3)
			48	610000	(–103.3)
	20°C	Ionized 40 l/min 10A	6	300000	0.0
			24	40000	86.6
			48	100000	66.6
		Control	0	300000	0.0
			6	290000	3.3
			24	180000	40.0
			48	180000	40.0

Results showed that in treated fresh water samples held at 5 °C all the bacteria were killed within 24 hours (i.e. a 3 log kill). In fresh water samples held at 20 °C the rate of disinfection was considerably slower with only a 99.0% kill (2 log) and a 98.8% reduction in *E.coli* observed after 48 hours.

ASW samples held at 5 °C showed a slower rate of disinfection which resulted in a less than 2 log reduction in *E.coli* after 48 hours. No significant reduction in *E.coli* was observed when seawater samples were held at 20 °C.

Salinity of the ASW was measured at 33 ppt. Copper levels in the un–treated seawater and fresh water samples were 0.08 mg/l and 0.04 mg/l respectively. The treated ASW gained 0.25 mg/l of copper which indicated 2.25 mg/l of silver was also added to the sample. Treated freshwater gained 0.10 mg/l which indicated an estimated 0.9 mg/l of silver was also added to the sample.

6.4 Conclusion

The effectiveness of disinfection appeared to be reduced in ASW. This may be due to the complexing of copper and silver ions by salts present in the seawater. Disinfection was enhanced at lower temperatures in both freshwater and ASW.

The metal ions appeared to kill *E.coli* and did not just give a bacteriostatic effect, as raising the temperature from 5 °C to 20 °C for 24 hours failed to revive any bacterial cells.

The higher initial bacterial loading of 10^8 cfu/100 ml had no effect on the rate of disinfection.

7. Trial III - Disinfection of Bridlington seawater using the Tarn Pure F1 ionization cell

7.1 Introduction

The aim of this laboratory trial was to investigate the level of treatment required to disinfect bacteria naturally present in seawater, and to confirm the results of the previous trial which suggested the bacteriocidal effect was more pronounced at lower temperatures.

7.2 Method

A 300 litre sample of seawater was collected from inside Bridlington Harbour which is known to be a contaminated area. The F1 ionization cell was set up and the trial was carried out according to the common experimental method detailed in section 4.1. The seawater was treated at 10A with a flow rate of 40 l/min and a 20 litre sample of treated seawater was collected in a stainless steel container and mixed. A second 20 litre sample of seawater was collected with the ionizer switched off to give a treated control. 1 litre samples were taken from the original seawater, the ionized seawater and the control and stored at 5 °C and 20 °C.

The amount of copper in each sample was measured immediately after collection. Microbiological analysis of each sample was carried out at 3 hours, 24 hours and 48 hours after collection.

To provide a more extreme test the trial was repeated with the seawater treated at 3A with a flow rate of 80 l/min.

7.3 Results

In seawater samples which received a low level of treatment (80 l/min, 3A), no reduction of TCVs or total coliform organisms at either temperature was observed (results not shown).

The results of the higher level of treatment are shown in table 3 overleaf.

Table 3 - Percentage kill of bacteria in Bridlington Harbour seawater samples ionized with the F1 ionization cell and stored at 5°C and 20°C

Sample storage temperature (°C)	Treatment	Time to Analysis (h)	Bacterial Count		% kill	
			TVC (cfu/ml)	TC (cfu/100ml)	TVC	TC
5	40 l/min 10A	3	120	0	93.3	100
		24	30	0	98.3	100
		48	180	0	90	100
	Control	0	1800	600	0.0	0.0
		3	1700	1400	5.5	(–133.3)
		24	1900	1400	(–5.5)	(–133.3)
		48	2800	600	(–55.5)	0
20	40 l/min 10A	3	2800	0	(–55.5)	100
		24	3100	0	(–72.2)	100
		48	500	0	72.2	100
	Control	0	1800	600	0.0	0.0
		3	1700	1000	5.5	(–66.7)
		24	1900	400	(–5.5)	33.3
		48	1200	-	33.3	-

TVC = Total Viable Count, TC = Total Coliforms

In treated samples, all total coliform bacteria were killed within 3 hours at both temperatures. No significant reduction in TVC bacteria held at 20 °C was observed.

Salinity of the seawater was measured at 32 ppt. The copper level in untreated seawater samples was 0.12 mg/l. Seawater treated at 40 l/min, 10A gained 0.33 mg/l of copper which indicated 2.97 mg/l of silver was also added to the sample. Seawater treated at 80 l/min, 3A gained 0.09 mg/l of copper which indicated 0.81 mg/l of silver was also added to the sample.

7.4 Conclusions

The lower level of treatment was not sufficient to kill any TVC or TC bacteria within the sample.

The results suggest that TVC bacteria in natural seawater are more resistant to disinfection by copper and silver ions than coliform bacteria.

In natural seawater the disinfection of coliform bacteria was not temperature dependent as suggested by the previous trial with artificial seawater. However, TVC bacteria were more readily killed at 5 °C than at 20 °C.

These findings are consistent with the TVCs including robust bacteria naturally adapted to the marine environment whilst the coliforms are likely to be contaminants and be already stressed in that environment.

8. Trial IV - Disinfection of seawater using the Tarn Pure F1 and F2 ionization cells

8.1 Introduction

To confirm the results of the previous trial, three comprehensive field tests were carried out onboard small shrimp boats in the Wash and Morecambe Bay, to determine the optimum conditions for the flow-through ionization of seawater.

Both the F1 and F2 ionization cells were used. The F2 cell with a lower silver content has the advantage of lower production costs and it was thought that it may be more effective in seawater.

In the third test a 5μm laboratory-scale cartridge filter was used to pre-filter the seawater before ionization. It was thought that filtration may enhance the bactericidal action of ionization treatment by removing organic particles from the water which can bind metal ions. It may also directly remove some bacteria which are bound to larger particles.

8.2 Method

The F1 ionization cell was set up on board a small shrimp boat according to the common experimental method detailed in section 4.1. Seawater was drawn directly from the sea, approximately 1 km from the mouth of the river at Kings Lynn and treated at 10A with a flow rate of 40 l/min (test A).

Pre and post treated seawater samples (150 ml) were taken simultaneously; this was repeated up to ten times and samples were stored at 0°C. The sampling procedure was repeated with samples stored at 20°C. Microbiological and copper analysis was carried out 3 hours after sampling.

The procedure was repeated with the F2 cell.

The test was repeated twice (tests B and C) onboard a small shrimp vessel approximately 500 m off shore in Morecambe Bay.

In test C, additional seawater samples were pre-filtered before treatment. Filtration only samples were taken as a control.

8.3 Results

The seawater samples from Kings Lynn, (test A) contained a very low level of bacteria which made meaningful interpretation of the microbiological results impossible (results not shown).

The results of tests B and C are shown in Table 4 below.

Table 4 - Average percentage bacterial kill in seawater ionized using F1 and F2 ionization cells, with and without filtration and held at 0 °C and 20 °C

Seawater Treatment	Sample storage temp° C	Test	Average Bacterial Count						Average % Kill		
			Pre-Treatment			Post-Treatment					
			TVC (cfu/ml)	TC (cfu/100ml)	*E.Coli* (cfu/100ml)	TVC (cfu/ml)	TVC (cfu /100ml)	*E.Coli* (cfu /100ml)	TVC	TC	*E.Coli*
F1 Ionizer	5°C	B	335	8115	7065	200	5750	5057	40.3	29.1	28.5
	5°C	C	188	2300	1800	96	630	230	47.8	72.6	87.2
	20°C	B	453	9833	7667	113	4167	3867	75.0	57.6	52.1
	20°C	C	134	220	1200	42	288	50	68.6	86.9	95.8
F2 Ionizer	5°C	B	919	20,000	17,950	171	3400	3820	81.3	83.0	78.7
	5°C	C	160	3800	2700	82	720	520	48.7	81.0	80.7
	20°C	B	540	8555	7511	27	906	698	95.0	89.4	90.7
	20°C	C	124	4400	3100	56	2260	910	54.8	48.6	70.6
5μm Filtration Only	20°C	C	116	2400	1300	122	1010	520	(-5.2)	57.9	60.0
F2 Ionizer with 5μm pre-filtration	20°C	C	156	2000	1010	40	50	50	74.3	97.5	95.0

TVC = Total Viable Count, TC = Total Coliforms

The salinity of the seawater in both trials was measured at 34 ppt. The amount of copper in the untreated seawater samples was 0.07 mg/l. Seawater treated with the F1 cell gained 0.33 mg/l of copper which indicated 2.97 mg/l of silver was also added. Seawater treated with the F2 cell gained 1.33 mg/l of copper which indicated 0.56 mg/l of silver was also added.

8.4 Conclusions

A consistently high level of disinfection was not achieved.

Summation of the average % kills at both temperatures showed little difference between the overall disinfection performance of the two ionizers, the F2 cell being very slightly more effective.

There is no evidence to suggest that the bactericidal effect of the F2 ionization cell was temperature dependent. However, the F1 cell was more effective at 20 °C.

The percentage kill values for the ionizers varied considerably between trials.

Filtration alone did not remove TVC organisms but did remove approximately 60% of coliform bacteria, which would be expected to be associated with larger particles. Bacterial kills were significantly enhanced when the water was pre-filtered prior to treatment. Effective filtration may be the key to improving the bactericidal performance of ionization. However, a filter for commercial use would have to be easily cleanable and capable of being used for extended periods without blocking.

At the rate of copper deposition observed in this trial, it was calculated that the two 100 g F1 electrodes would only last 25 hours in continual use before being used up, whilst F2 electrodes would last 44 hours. The rapid wear and cost of replacement of electrodes (approximately £250–£350 depending on silver content) would make this technology uneconomical, unless the same level of disinfection could be achieved with a lower dose of metal ions.

9. Trial V - Disinfection of seawater using the Tarn Pure F2 ionization cell in conjunction with commercial filters

9.1 Introduction

To make ionization a commercial proposition for the flow-through disinfection of seawater, pre-filtration may enhance the bactericidal kill and thus reduce the rate at which the electrodes wear by allowing a lower dose of ions to achieve an effective bactericidal kill.

In this trial, seawater from Bridlington Harbour was pre-filtered using either a 100 μm agricultural cartridge filter or a 10 μm sand filter before being treated using a F2 ionization cell. Both filters were relatively inexpensive, rugged and compact and capable of handling flow rates of up to 10 m^3/hr. Both had back flush valves for ease of cleaning. As the previous trials showed that the storage temperature of the samples had little effect on bacterial kill, samples were stored at ambient temperature until analysis.

9.2 Method

The F2 ionization cell was set up according to the common experimental method detailed in section 4.1. Seawater drawn from inside Bridlington Harbour was treated at 10A at flow rates of 20 l/min, 80 l/min and 130 l/min. Pre and post treatment seawater samples (150 ml) were taken simultaneously; this was repeated three times at each flow rate. Samples were stored for 3 hours at approximately 10 °C before microbiological and copper analysis was carried out.

The above procedure was repeated with the sand filter, and again with the agricultural cartridge filter to pre-filter the seawater before treatment. Pre-filtration and post-filtration samples were taken at 80 L/min for each filter as a control.

9.3 Results

The results of the trial are shown in table 5 overleaf.

Table 5 - Average percentage bacterial kill in natural seawater samples, treated with the F2 cell with and without pre-filtration

Seawater Treatment	Flow Rate (L/min)	Average Bacterial Count						Average % Kill		
		Pre-Treatment			Post-Treatment					
		TVC (cfu/ml)	TC (cfu/100 ml)	*E.coli* (cfu/100 ml)	TVC (cfu/ml)	TC (cfu/100 ml)	*E.coli* (cfu/100 ml)	TVC	Total Coliform	*E.coli*
10μ Sand Filtered only	80	108	1200	1000	141	963	738	(–30.5)	(–19.8)	26.2
100μ Cartridge Filtered only	80	102	250	50	80	525	188	21.5	(–110)	(–276.0)
Ionization only	20	+98	417	1	60	37	47	38.7	91.2	73.5
	80			7	97	117	53	0.6	72.0	69.8
	130			7	105	100	83	(–8.2)	76.0	53.19
10μ Sand Filtered and Ionization	20	+30	200		235	50	36	23.7	75.0	60.8
	80			9	62	143	127	79.8	28.3	(–38.0)
	130	8		2	300	50	18	2.8	(–75.0)	5.5
100μ Cartridge Filtered and Ionization	20	+10	500	2	34	367	0	66.6	26.6	100
	80			3	143	500	300	(–40.1)	0.0	(–25.5)
	130	2		9	125	500	417	(–22.5)	0.0	(–74.4)

TVC = Total Viable Count, TC = Total Coliforms
+ Some pre-treatment samples were lost due to damaged containers. Each pre-treatment average bacterial count is an average of three surviving seawater samples, collected during each treatment

The salinity of the seawater was measured at 34 ppt. The amount of copper in the untreated seawater samples was 0.08 mg/l. Seawater samples treated at 20 l/min, 80 l/min and 130 l/min, gained 0.63 mg/l, 0.26 mg/l and 0.01 mg/l of copper respectively. This indicated 0.27 mg/l, 0.11 mg/l and 0.004 mg/l of silver was also added to each sample respectively.

9.4 Conclusions

Ionization was not sufficiently effective to be commercially viable with or without pre-filtration.

Even at the highest treatment level (lowest flow rate) relatively low kill rates were observed. For a disinfective treatment to be considered effective it should be able to consistently kill at least 3 log or 99.9% of the coliform bacteria present.

The running costs of ionization (electrode wear) and its unreliable disinfective performance make it an unsuitable treatment for the flow-through disinfection of seawater.

10. Trial VI - Determination of the effect of using ionized seawater in an RSW system

10.1 Introduction

As the previous trials have shown ionization can kill some of the bacteria in seawater, it was thought that keeping fish in an ionized refrigerated seawater system (RSW) may reduce the rate at which the fish spoiled.

A laboratory trial at Hull was carried out to compare the quality of fish stored in an ionizer treated RSW system with fish stored in a normal RSW system and with a boxed and iced control.

10.2 Method

Two 1000 mm x 600 mm x 800 mm deep polypropylene tanks were each fitted with a pump, valves and ABS/PVC pipework to allow water to be drawn out of one end of the tank, recirculated outside the tank and pumped in at the other end. Tank 1 had the F3 recirculatory ionizer plumbed into the pipework. Tank 2 was not fitted with an ionizer and was used as a control. Each tank was filled with 20 litres of seawater (34 ppt) taken from inside Bridlington Harbour. The tanks were placed in a chill store to maintain the seawater between 0 °C to –1 °C. A 70 kg sample of head on gutted cod was obtained from a single haul of a local day boat. Each tank was filled with 20 kg of fish. The water in both tanks was recirculated at 0.35 l/min. Copper levels in the ionizer treated RSW tank were monitored daily and the ionizer current adjusted to keep a level of 0.25 mg/l.

As a further control, a fish box was filled with 20 kg of the fish and layered with ice in a 3:1 fish to ice ratio and placed in the chill store.

Prior to storage, samples of three fish were sent for microbiological and copper analysis and a further three fish were assessed for raw appearance and odour and cooked odour and flavour by an expert sensory assessment panel using the Torry scoring scheme (Appendix I).

The analysis and assessments were repeated after 7 days storage as appropriate.

10.3 Results

The results of the trial are shown in Table 6 overleaf.

Table 6 - Cooked flavour and bacterial counts of gutted cod in normal RSW, ionizer treated RSW and boxed and iced storage after 7 days

Storage Period	Storage Conditions	Average Cooked Torry Freshness Score	Bacterial Level in Fish Samples			Copper Levels in Fish (mg/kg)
			TVC (cfu/g)	TC (cfu/100g)	*Pseudamonas sp* (cfu/g)	
Pre-storage	—	9.5	43,000	1.6	1000	0.15
After 7 days	Normal RSW	7.00	170,000	3	13,000	0.16
	Ionized RSW	7.66	16,000	<0.1	3,000	0.26
	Boxed and Iced	7.33	220,000	2	6,400	0.22

TVC = Total Viable Count, TC = Total Coliforms

Fish kept in treated RSW had the lowest bacterial counts and the highest sensory assessment scores. Fish in both types of RSW storage developed a salty flavour and a firmer texture than fish stored in ice. Copper levels in the fish stored in ionizer treated RSW were not significantly raised.

10.4 Conclusions

The results indicate that using ionizer treated water in an RSW system may marginally reduce the rate of spoilage over a 7 day period. It is recommended that further trials are carried out with mackerel or herring which are more commonly stored in RSW, although the metal ions may catalyse and accelerate oxidative rancidity in these oily fish.

11. Trial VII - Determination the effectiveness of a 6 x 30W UV system for disinfection of filtered and unfiltered seawater

11.1 Introduction

With ionization proving unreliable, costly and insufficiently effective for seawater treatment, an alternative method of disinfection was sought.

UV irradiation is a well tried, effective and relatively inexpensive method already in use for the disinfection of seawater but the efficiency of UV disinfection is known to be reduced for seawater in comparison to fresh water and reduces further with water turbidity. This trial was carried out to assess the effectiveness of a 6 x 30 W UV unit for the disinfection of filtered and unfiltered seawater drawn from inside Bridlington Harbour. This unit has a nominal capacity for treating 150 l/min of fresh water or 120 l/min of clean seawater.

Seawater can be highly turbid especially in the areas where brown shrimp are caught. Water from the Humber Estuary is likely to be typical of the highest level of turbidity which can be encountered. As part of this trial the absorption coefficients of filtered and unfiltered Bridlington Harbour and Humber water were determined. This absorption coefficient enabled the power of UV equipment required to treat the water to be estimated.

11.2 Method

The equipment was set up according to the common experimental method detailed in section 4.1. The ionizer was replaced by a 1000 mm x 300 mm diameter UVAQ™ 630/8 UV unit containing six 30 W UVA tubes powered by a 240 V 2.5 KW petrol generator. Seawater from inside Bridlington harbour was pumped through the unit at 80 l/min. Pre and post treated samples were collected simultaneously. This was repeated with the seawater pre-filtered using the 10 μm sand filter and again using the 100 μm cartridge filter. Pre-filtered samples were also taken with the UV switched off. Microbiological analysis was carried out within three hours.

Samples were also sent to UV Systems Limited for determination of the absorbance coefficient using a UV spectrophotometer. From this they calculated the UV dose actually received by the water flowing through the sterilisation unit. A dose of 25–30 mJ/cm^2 is usually considered necessary to produce potable water.

11.3 Results

A summary of the results is shown in Tables 7 and 8 below.

Table 7 - Average percentage bacterial kill in Bridlington seawater with UV disinfection and with 10μm and 100μm pre-filtration

Treatment	Absorption coefficient of the treated seawater	Calculated UV dose received (mJ/cm3)	Average Bacterial Count						Average % Kill		
			Pre-Treatment			Post-Treatment					
			TVC (cfu/ml)	TC (cfu/100 ml)	E.coli (cfu/100 ml)	TVC (cfu/ml)	TC (cfu/100 ml)	E.coli (cfu/100 ml)	TVC	TC	E.coli
10μ Sand Filtration alone	0.16	—	108	1200	1000	141	963	738	(-30.5)	19.7	26.2
100μ Cartridge filter alone	0.24	—	102	250	50	80	525	188	21.5	(-110.0)	(-276.0)
UV alone	0.24	82	100	225	225	83	250	18.3	17.0	(-11.1)	18.7
10μ Sand Filtration & UV	0.16	108	82	275	105	100	50	5	(-21.9)	81.8	95.2
100μ Cartridge Filter & UV	0.24	82	62	450	60	27	41	23	56.4	90.8	61.7

TVC = Total Viable Count, TC - Total Coliforms

Table 8 - Turbidity

Seawater	Absorption Coefficient
Bridlington Unfiltered	0.24
Bridlington Sand Filtered 10μm	0.16
Bridlington Cartridge Filtered 100μm	0.24
Humber Water Unfiltered	3.64
Humber Water Sand Filtered 10μm	filter blocked
Humber Water Cartridge Filtered 100μm	filter blocked

A steady flow rate could not be achieved when passing River Humber water through both types of filter as blocking occurred within minutes.

Salinity of the Bridlington water was 33 ppt. Salinity of the River Humber water was 15 ppt.

11.4 Conclusions

The power of the UV unit was insufficient to give a significant bacterial kill. Even after filtration, the UV absorption coefficient of the seawater was sufficiently high to result in the water receiving a low UV dose.

Both filters enhanced the kill by UV irradiation even though the cartridge filter did not appear to reduce the absorbance coefficient of the seawater. However, turbid seawater rapidly blocked both types of filters and so they are not suitable for use as part of a commercial treatment system.

The absorption coefficient of the water taken from the River Humber was very high. It was calculated that to treat this water to potable standards (minimum dose of 30 mJ/cm^3) at a flow rate of 80 l/min an 11 kW UV system at a cost of approximately £35,000 or an expensive automatic filtration unit and a smaller UV would be required.

Cost makes this form of treatment prohibitive for this application.

12. Trial VIII - Determination of the amount of chlorine required to disinfect seawater

12.1 Introduction

The previous trials showed that ionization, filtration and UV were not suitable treatments for this application. A simple inexpensive solution was sought. Sodium hypochlorite is a common chemical disinfectant often used for potable water treatment. It can be purchased in a concentrated but relatively safe form and is easily dosed into water to make its use on board small boats possible.

In this laboratory trial, water from Bridlington Harbour and the Humber Estuary was treated with increasing amounts of sodium hypochlorite to determine the amount of chlorine required to carry out effective disinfection. It was thought that when chlorinated water is used to cool hot shrimp, some of the available chlorine may react with the shrimp thus reducing the amount available to kill bacteria in the water. To simulate this, the available chlorine was neutralised with sodium thiosulphate, after a short contact time with the water.

Available and total chlorine concentration were measured to determine the chlorine demand of the seawater.

12.2 Method

A three litre sample of seawater drawn from Bridlington Harbour was thoroughly shaken to re-suspend any small particulates. Sodium hypochlorite solution was added to 250 ml samples of the seawater to give a chlorine dose of 0, 0.2 mg/l, 0.5 mg/l , 1.0 mg/l, 5.0 mg/l and 20 mg/l (see Section 4.4).

After one minute 100 ml of each chlorinated seawater sample was removed and neutralized with 4 ml of 2 M sodium thiosulphate solution. The free and total chlorine concentration in the remainder of each sample was analysed using a Hach DR2000 spectrophotometer. Each neutralised sample along with two unchlorinated seawater samples and 2 control samples (containing only 4 ml of 2 M sodium thiosulphate in 100 ml of seawater) were sent for microbiological analysis.

The trial was repeated with water drawn from the River Humber (approximately 20 miles up river from the mouth of the estuary), using 1 minute and 2 minute contact times.

12.3 Results

The results of the trial are shown in Table 9 below and Table 10 overleaf.

Microbiological comparison of the thiosulphate control with untreated seawater showed that the addition of sodium thiosulphate had no biocidal effect (results not shown).

The salinity of the Bridlington and Humber seawater was 33 ppt and 15 ppt respectively. The DPD reagent method of analysing chlorine in seawater proved to be inaccurate. However, the chlorine demand of the Bridlington seawater could be tentatively estimated at 0.5-1 mg/l due to the jump in free chlorine values when dosed at higher levels.

Table 9 - Percentage bacterial kill in seawater chlorinated at 0.2 mg/l, 0.5 mg/l, 1 mg/l, 5 mg/l, and 20 mg/l

	Chlorine Dose (mg/l)	Bacterial Count			Bacterial % kill		
		TVC (cfu/ml)	TC (cfu/100 ml)	*E.coli* (cfu/100 ml)	TVC	TC	*E. coli*
Bridlington seawater (+ 4% sodium thiosulphate, one minute contact time)	0	20	400	150	-	-	-
	0.2	42	350	150	(–110.0)	12.5	0.0
	0.5	26	100	50	(–30.0)	75.0	66.7
	1	17	50	12	15.0	87.5	92.0
	5	31	8	2	(–55.0)	98.0	98.7
	20	23	6	4	(–15.0)	98.5	97.3
Humber seawater (+ 4% sodium thiosulphate, one minute contact time)	0	2000	6750	4500	-	-	-
	0.2	1500	6500	4000	25.0	3.7	11.0
	0.5	2600	5000	2500	(–30.0)	25.9	44.4
	1	3600	1750	1500	(–80.0)	74.0	66.7
	5	1200	500	25	40.0	92.6	99.4
	20	1300	50	17	35	99.3	99.6
Humber seawater (+ 4% sodium thiosulphate, two minutes contact time)	0	2000	6750	4500	-	-	-
	0.2	800	6000	6000	60.0	11.1	(–33.3)
	0.5	2300	6500	6000	(–15.0)	3.7	(–33.3)
	1	1000	1000	200	50.0	85.2	95.6
	5	1100	32	100	45.0	99.5	97.8
	20	2000	500	50	0.0	92.6	98.9

TVC = Total Viable Count, TC = Total Coliforms

Table 10 - The actual and measured total and free chlorine concentration (mg/l) in chlorinated seawater

Added Chlorine (mg/l)	Measured Chlorine			
	Bridlington Seawater		Humber Water	
	Total chlorine (mg/l)	Free chlorine (mg/l)	Total chlorine (mg/l)	Free chlorine (mg/l)
0.0	0.07	0.02	0.2	0.18
0.2	0.10	0.05	0.59	0.53
0.5	0.17	0.05	0.67	0.67
1.0	0.51	0.27	0.51	0.45
5.0	4.20	4.70	1.3	2.2
20.0	13.50	13.50	2.5	4.0

12.4 Conclusions

Chlorination gave a total coliform and *E.coli* kill consistently close to or greater than 99% with a dose of 5 mg/l or higher in both types of seawater. Doubling the contact time had no effect on the kill rate. As with the other forms of disinfection tested, TVC organisms were more resistant to chlorination than *E. coli.* A longer contact time and/or chlorine dose would be required to give a greater reduction of TVC. A further trial with higher dose levels and longer contact times is required to explore the limits of seawater chlorination.

The total and free chlorine concentrations measured for each sample were lower than expected throughout the range. It is thought that the salinity and turbidity of the seawater interfered with the analysis, as standards made up with deionized water could be measured accurately. Other methods for measuring chlorine in seawater should be assessed.

13. Trial IX - Determination of the degree of seawater disinfection achieved using higher levels of chlorine dosing and longer contact times

13.1 Introduction

The previous trial showed that chlorination could be consistently effective at coliform reduction at low dose levels and given a short contact time, but not at TVC reduction. This trial was carried out to see if disinfection could be enhanced at higher concentrations of up to 100 mg/l and longer contact times of up to 10 minutes.

13.2 Method

The trial was carried out on River Humber water using the method detailed in trial VIII (section 12.2). Seawater was dosed with sodium hypochlorite to give a chlorine does of 0 mg/l, 60 mg/l and 100 mg/l with contact times of 1 minute, 5 minutes and 10 minutes.

13.3 Results

The results of the trial are shown in Table 11 overleaf.

Table 11 - Percentage bacterial kills in Humber water chlorinated at 20 mg/l, 60 mg/l and 100 mg/l over a range of contact times

Chlorine Dose (mg/l)	Contct time (min)	Bacterial Count			% Kill		
		TVC (cfu/ml)	TC (cfu/100ml)	*E. coli* (cfu/100ml)	TVC	TC	*E. coli*
0	-	2000	2500	2000	-	-	-
20	1	800	22	12	60	99.1	99.4
	5	600	-	45	70	99.2	97.8
	10	300	0	0	85	99.8	100
60	1	1400	20	18	30	99.2	99.1
	5	500	26	10	75	98.9	99.5
	10	300	10	0	85	99.6	100
100	1	30	30	30	75	98.8	98.5
	5	500	20	0	75	99.2	100
	10	300	8	0	85	99.6	100

TVC = Total Viable Count, TC = Total Coliforms

The salinity of the Humber water was 15 ppt.

13.4 Conclusions

As expected, the results showed that a higher level of disinfection could be obtained by increasing the chlorine dose or increasing the contact time. However, the results indicate that in a practical application on board boat, there would be little advantage in using a dose of over 20 mg/l.

14. Conclusions and Discussions

This series of simple trials highlighted the unsuitability of ionization and UV irradiation as practicable means of disinfecting seawater. Sodium hypochlorite dosing appeared to be the most suitable technology for further development to a commercial scale. Ozone is yet to be investigated.

Trials with all three disinfectants showed TVC organisms were more resistant to disinfection than coliforms. This is to be expected as coliforms which thrive in the conditions found in the human or animal gut will be injured or weakened by the hostile condition encountered in seawater.

The presence of E. coli and/or other faecal coliform bacteria in seawater indicate human or animal sewage pollution. These easily detected and enumerated enteric bacteria are used as indicator organisms. If a high level of faecal coliforms are present it is likely that a high level of harmful bacteria and viruses are also present. If a disinfectant achieves a high coliform kill it is likely that pathogens with a similar physiology such as Yersinia, Vibrio, Shigella, Salmonella, Listeria, Staphylococcus and Verotoxic *E.coli* are also being killed.

Ionization was not considered a suitable treatment for the flow through bacterial disinfection of seawater. Bacterial kills of both TVC and coliform bacteria were inconsistant and often low. Rapid electrode wear would make ionization an expensive method of water treatment for this application. Calculations indicate that it would cost approximately £2.50 to treat 1 tonne of water using the F2 cell. However, ionization may have a beneficial effect when incorporated into enclosed RSW systems as fewer metal ions may be required which would make the treatment more cost effective. However, if pelagic fish are stored in this way the metal ions may catalyse and accelerate oxidative rancidity. Further research would be required. There is also increasing public awareness concerning the toxicity of heavy metals in food. This method of prolonging freshness may add a small amount of metal ions to the food product which may result in consumer concern.

The high turbidity of seawater which can be encountered in areas where shrimp are caught makes the use of UV disinfection equipment impractical. Although highly turbid seawater is not always encountered a disinfection system must be able to handle worst case conditions. To treat turbid seawater to an acceptable level, a high powered UV system would be required which is unacceptable from both a cost and power consumption point of view. Alternatively a self cleaning filtration system could be used in conjunction with a smaller UV source, but cost would still be a prohibitive factor for the shrimp industry.

Chlorination appeared to be the most effective method for the disinfection of seawater. Sodium hypochlorite gave a consistently high kill of coliform bacteria at a low concentration. As sodium hypochlorite is dosed into water the chemical reacts to form hypochlorous acid and sodium hydroxide. The hypochlorous acid molecules enter the cell and although the exact mechanism by which chlorine kills microorganisms it not fully understood, it is known that the chlorine interferes with the function of vital enzymes which results in cell death (ref. 1).

It is strongly suspected that even when the treated seawater comes into contact with hot shrimp during cooling, that disinfection will continue and the rate may be accelerated due to the increased temperature. The contact time will be far longer than for the trials detailed in this report where disinfection was halted with a chemical agent after a few minutes. It is expected that shrimp cooled in treated seawater would have a lower bacterial count than shrimp cooled in untreated seawater.

For further seawater disinfection trials it would be sensible to use a chlorine dose of 20 mg/l. At this level pathogenic enteroviruses such as hepatitis A and Norwalk like viruses present in the water as a result of sewage contamination should begin to be inactivated. This dose level is well below the threshold of approximately 500 mg/l at which shrimp washed in chlorinated seawater pick up hypochlorite taints. (ref. 2). However, the effective minimum dose should be determined in pilot scale trials on board boat.

Hypochlorite treatment would be relatively cost effective. Calculations indicate that it would cost 4p (bulk buy) to treat 1 tonne of seawater at 20 mg/l. Sodium hypochlorite can be purchased in a sufficiently concentrated form (5%–15%) to ensure a large volume of chemical does not have to be stored on board. Equipment to dose the chemical into the seawater is rugged and relatively inexpensive.

The disadvantages of chlorination include the formation of trihalomethanes (THMs) when chlorine reacts with humic acid compounds. At high levels in drinking water THMs are considered to be harmful to health. It is worth noting that wash waters with a significantly higher level of chlorination of 150 mg/l and above are already used commercially to wash salad, raw chickens and fish fillets. For this application at a dose of 20 mg/l it is likely that the amount of THMs produced and passed to the food product would be negligible. It is thought that incorporating water treatment into an onboard shrimp cooking operation in conjunction with hygienic handling and storage would improve product quality and safety.

As a result of these laboratory trials, chlorination dosing equipment has been installed on board shrimp boats and trials are currently underway to compare the microbial and sensory quality of brown shrimp cooled in chlorinated water against shrimp cooled in untreated water. (ref. 2).

15. References

1. CLIFFORD WHITE, G. (1992). Handbook of Chlorination and Alternative Disinfectants (3rd Edition). Chapman and Hall. ISBN 0-442-006 93-4, pp 230-231.

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Appendix I
Torry Score Sheet

Freshness Score Sheet for Iced Cod
Cooked Fish

Score	Odour	Flavour	Texture, Mouth Feel and Appearance	Score
10	Initially weak odour of sweet, boiled milk, starchy followed by strengthening of these odours	Watery, metallic, starchy. Initially no sweetness but meaty flavours with slight sweetness may develop	Dry, crumbly with short tough fibres	10
9	Shellfish, seaweed, boiled meat, raw green plant	Sweet, meaty, creamy, green plant, characteristic	Succulent, fibrous. Initially firm going softer with storage. Appearance originally white and opaque going yellowish and waxy on storage	9
8	Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity		8
7	Woodshavings, woodsap, vanillin	Neutral		7
6	Condensed milk, caramel, toffee-like	Insipid		6
5	Milk jug odours, boiled potato, boiled clothes-like	Slight sourness, trace of 'off' flavours		5
4	Lactic acid, sour milk, 'byre-like'	Slight bitterness, sour, 'off' flavours		4
3	Lower fatty acids (e.g. acetic or butyric acids), composted grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide		3