

**Do Changes in Aeration Efficiency  
of 'Airwick' Diffusers  
explain recent High Mortalities  
during Transport of Live Fish in Bulk Bins?**

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## SUMMARY

To provide an alternative to the use of oxygen cylinders in live transport bins, Seafood Transportation Developments (STD) initiated development of an aeration unit which could be fitted to the live bins. The system provides oxygen to the live fish by air diffusion at a high flow rate through an air diffuser attached in the bottom of the bin. Initial export shipments of live fish using the STD system were very successful. The company had obtained air diffuser pipe which appeared equal to the demand of supplying sufficient oxygen into the water. Then however, high mortalities were reported for transport of coral trout.

Upon thorough investigation of all potential circumstances that may be responsible for the unsuccessful shipments, the only obvious difference seemed to be the re-ordered supply of air diffuser piping used in the bins. This research work assessed the aeration efficiencies of several air diffuser pipes with different properties and characteristics.

Airflow rates and bubble size appear to have correlation with type of diffuser piping rather than whether the specific diffuser had been previously used or not. Airflow rates were similar over short initial aeration use. The efficiency of the fine grade diffuser decreased significantly over a period equivalent to that for shipping live product to Hong Kong, for example. The coarse grade diffuser was not robust enough for the purpose of use.

The 'original' air diffuser piping supplied during development of the transport bins, even though previously used for live transport, retained acceptably high aeration rates. This efficiency decreased slightly following a continuous 96h run, however it is unlikely that this period would be demanded of a bin used in a commercial operation.

The new supply of air diffuser piping was shown to be less efficient than that of the 'original' supply. The efficiency of a previously used air diffuser made from the re-ordered supply was low, taking greater than double the time to cause 100% O<sub>2</sub> saturation of the water. This air diffuser had been used for a shipment that resulted in very high mortalities of the fish. Additionally, an uneven distribution of air bubbles was observed throughout the water column during operation of this air diffuser. Unused air diffusers that were trialed also gave variable aeration efficiencies.

The answer to the question posed as basis for this research is: YES

The specific properties of piping used for air diffusion within live transport bins are crucial with respect to aeration efficiency. Of the types of diffuser piping trialed in this work only one, that supplied from England, demonstrated appropriate characteristics for multiple re-use within live transport systems. This information remains essential to the live fish export industry as aeration system transport bins are still being manufactured.

Unfortunately, this work was carried out retrospectively in reaction to huge revenue losses by industry. If investigations, similar to this one, were conducted during development of 'new' technologies, negative Australian industry image could be prevented.

## INTRODUCTION

Selling of live fish on export Asian markets is highly lucrative, especially for species such as coral trout (*Plectropomus* spp.) which can achieve prices of \$110/kg. The distance of these markets dictates that the most efficient way to transport live fish is by airfreight.

Fish were initially transported in a 'closed' system involving fish inside a plastic bag containing oxygen saturated seawater. The bags were then packed into polystyrene boxes for shipment. This transport method was not very cost effective due to the high freight costs of shipping large quantities of water. Also, mortalities of fish were often high as the system was in no way fail-safe. Oxygen is depleted and carbon dioxide levels increase with fish respiration, hence the transport water rapidly becomes unable to sustain life. This problem is compounded if there are trans-shipment delays during airfreight.

To facilitate survival of live fish, an 'open' transport system with bulk bins was introduced. The system used compressed oxygen cylinders to supply oxygen in the water at a flow rate sufficient to maintain the fish throughout transport. In this manner, fish could be transported in high density thereby reducing freight costs. The bins were insulated and re-useable.

However, the live fish export trade using this system was short-lived due to differing interpretations of the ICAO Technical Instructions for the Transport of Dangerous Goods. The outcome was an international ban on the carriage of 'in-use' compressed oxygen cylinders by aircraft.

The ban caused live fish exporters to look for alternative methods for sustaining live fish during transport. The oxygen saturation of the seawater within the bulk bin system was sustained at very high levels at the flow rates commonly used by exporters. Investigations conducted by researchers at the Centre for Food Technology showed that the flow rate could be greatly reduced with no mortality of coral trout (Paterson and Grauf, 1999). Given this information and knowledge of the respiration rate of coral trout during airfreight, it was possible that fish could be sustained if high flow rates of air were pumped through the seawater.

The aeration module unit was developed as a transport system to replace oxygen cylinder use when transporting live fish to export markets. The aeration system was used successfully for over 6 months with mortality rates usually less than 2%. At the time of this project work there were 98 STD aeration bins in operation within the live fish export industry and the use of these generated around \$750 000 in export revenue.

Then, suddenly high numbers of fish mortalities were reported in coral trout exported in these aeration bins. There was quite a deal of variation in the number of mortalities reported, but in some cases 100 % losses were recorded while other exporters still were having no losses at all. Information gained from industry indicated that the mortalities were unlikely to be due to factors such as transport water quality, age or physical condition of fish, nor handling and pack out procedures or airfreight transport itself.

Three exporters reported fish losses to the value of \$42 000, \$125 000 and \$75 000 respectively over a 2 month period. These major industry players have ceased live fish export, with one industry member forced to cease operation completely due to losses sustained in this area. Other exporters have indicated that they are facing the

same future. The current trend being displayed to our overseas customers has damaged the reputation of the Australian live fish export industry.

Seafood Transportation Developments, the company responsible for the development of the aeration module unit, responded to industry concern and approached the Centre for Food Technology for assistance in determining why these large numbers of mortalities were occurring. Until the reason for the mortalities had been identified and resolved, the company indicated that it had ceased manufacture and supply of these units despite outstanding orders of: Western Australia 40; Indonesia 30; Thailand 40; and enquires re large orders for Singapore and America.

The only noticeable difference within the aeration system in the shipments where high mortality occurred compared to earlier successful shipments appeared to be in the air diffusion pipe quality. The diffusion pipe currently used is provided by the same manufacturer and is purported to be equivalent to previous diffuser supplied, however industry comments have suggested varied differences in air bubble size and the lack of "fizz" on the surface of the water.

This research work proposed to determine the aeration efficiency of the current air diffusion pipe so as to establish parameters to ensure that sufficient oxygen transfer occurs to allow fish to survive transportation.

## PROJECT OBJECTIVES

- Evaluate quality changes in aeration efficiency associated with aging of 'airwick' diffusers used in aerated live fish transport bins returned from overseas

*Completed*

- Determine the effect of conditioning, use and multiple re-use on aeration efficiency of 'airwick' diffusers

*Completed*

## **METHODS**

### ***Aeration bins:***

Trials were carried out in both 250L and 750L live transport units. The bins were fitted with a mechanical aeration unit as supplied by Seafood Transport Developments Pty Ltd, Brisbane (Plate 1). The unit consists of an air pump driven by an independent power supply which is capable of 30h continuous operation and the flow rate is >30L/min. The bin contained an 'airwick' diffuser and was filled with fresh seawater to a standard volume. The diffusers were made from black, porous, rubber piping very similar to that used for irrigation purposes in many industries.

Air diffusers with different characteristics and histories were tested:

- No.1. New – re-ordered supply
- No.2. New – No. 1 – soaked 20 hours in seawater
- No.3. Used – original supply, 40 hours use (2 return trips to Hong Kong)
- No.4. Used – No. 1 (20 hour run with no fish present)
- No.5. Fine – unused, gives finest range of bubbles
- No.6. Used – No. 1 (20 hours use, successful shipment to Singapore)
- No.7. New – imported from England, soaked 48 hour prior to use
- No.8. Used – No. 1 used in big bin, unsuccessful shipment

Diffusers for the small bin (250L) were all cut to a standard length of 880 mm where possible. Some hoses were slightly less in length than 880 mm because small cracks etc had to be cut from the ends. The length of the hose used in the big tank was not recorded.

### ***Measurement of dissolved oxygen (DO):***

An Activon Model 401 oxygen meter fitted with an AS401F oxygen probe was used to measure dissolved oxygen. This equipment was calibrated against 2 solutions. Solution 1 was spiked with sodium sulphite to reduce the oxygen content to near 0%, while Solution 2 was distilled water which was air equilibrated to a dissolved oxygen level of 100%. Monitoring of oxygen levels was achieved by connecting the Activon meter to a Datataker 600 data logger using De Logger software. The De Logger programme was set to record readings at 30 second intervals.

### ***Measurement of pH and salinity:***

Salinity and pH was measured using a TPS Ionode WP-81 salinity/pH meter.

### ***Estimation of air bubble size***

An underwater video camera was used to estimate bubble size for a number of the air hoses. To do this video footage was taken with a grid placed in the bubble stream. Still shots were taken from the video footage and the size of the bubbles was attempted to be estimated from comparison with the grid.

### ***Measurement of aeration efficiency:***

Fresh seawater was pumped into the appropriate bin to a standard volume. The water was then stripped of oxygen by flushing through with high purity nitrogen gas supplied to the bin water via 'air stones'. The dissolved oxygen level of the water was monitored until it was below 5% level.

At this point, the trial was initiated by switching on the air pump unit and the oxygen level in the water was monitored until it reached 100%. Data was then downloaded from the data taker and analysed.

Temperature, salinity and pH were measured both before and after each trial run. No differences in these parameters were recorded.

### ***Specific trials:***

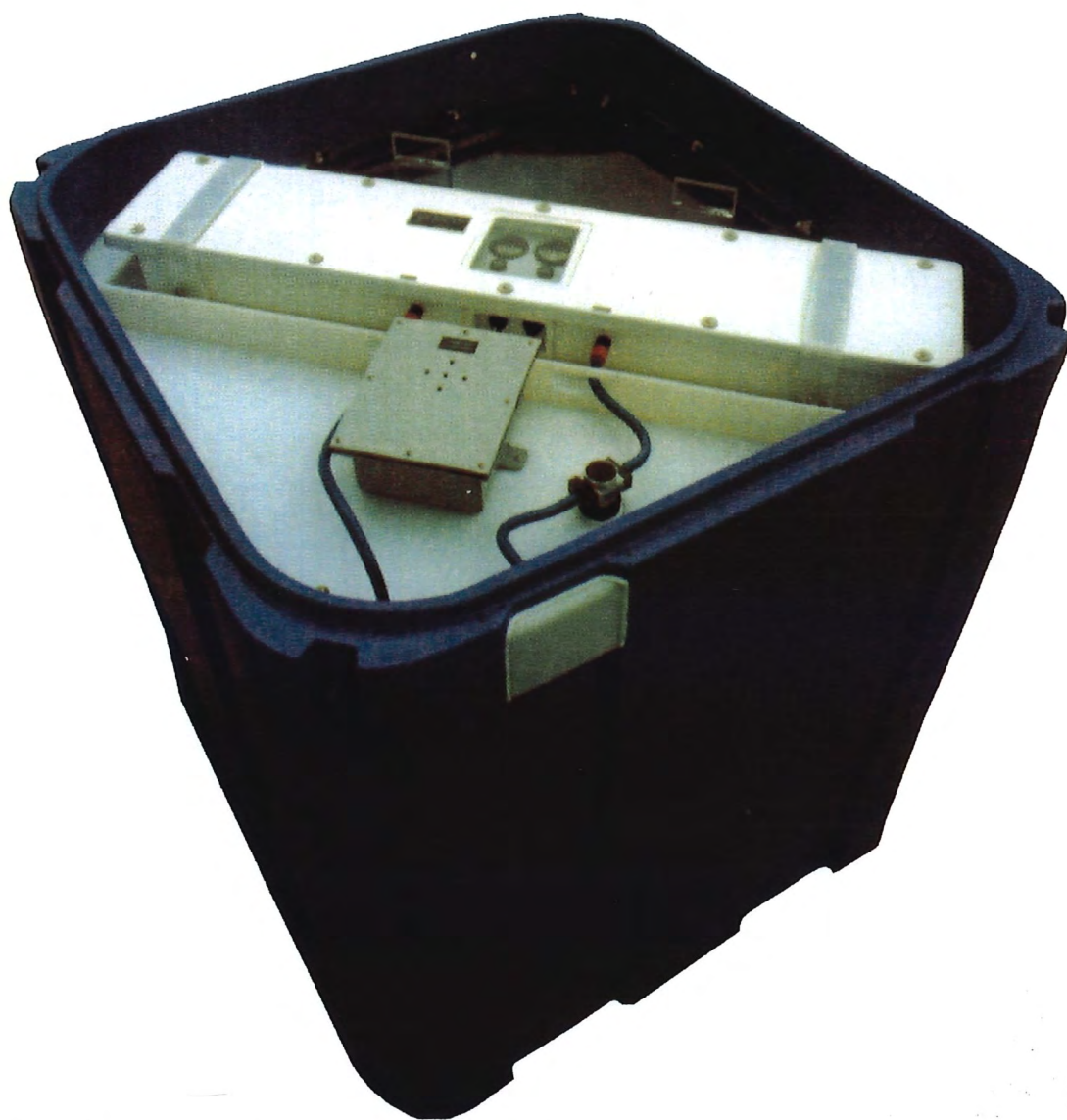
The efficiency of each of the air diffusers was gauged by measuring the time taken for each to raise the DO level in the water from 5% to 100% saturation. Each trial was replicated several times to ensure results were reproducible.

Trials were also conducted with different air diffuser hoses (no.3; no.5 and no.8) after various periods of continuous running of the aeration unit (up to 24 hours) to establish whether the aeration efficiency of the hose changes with use over time. A trial, using hose no.2, was also undertaken after 96 hours continuous running of the pump.

### ***Extra investigations:***

Information was gathered concerning other factors thought of that could possibly have an effect on the air diffuser hose functioning efficiency.

As well as 'dry' run trials, where the bin contained seawater only with no fish, trials were carried out in the small bin (250L) with the inclusion of a 9.5kg load of sea cucumbers. This species was chosen because of their high slime content. These trials were run for 72h.



**Plate 1. STD aeration unit fitted in a transport bin.**



## RESULTS and DISCUSSION

While developing the transport bin, Seafood Transport Developments (STD) obtained specific porous rubber hosing from a company for use as the air diffuser within the bins. The diffuser appeared to work effectively and consistently. Upon re-ordering more of the same product, the company was unable to supply the original piping but offered a similar product that was claimed to give exactly the same performance. In good faith, STD purchased and incorporated the new diffuser into the manufactured bins. It was shortly after this time that high mortalities occurred with coral trout transported with the STD aeration system.

STD gathered all relevant data that they could concerning both successful and unsuccessful live fish shipments and approached CFT for assistance in determining what the problem could be. As many factors as possible were investigated. From shipment histories, it appeared unlikely that mortalities were simply due to fish condition or stress at packout. No shipments had been subjected to excessive delays in transport, nor did there appear to be any unusual procedure with respect to the airlines.

However, it was apparent that mortality (sometimes > 80%) occurred only in bins fitted with the new-supplied air diffuser and then only after the second use of the bin. This led to enquiries concerning events that occurred with the bin after the fish had been removed in the destination country. As best as could be ascertained, adequate procedures were followed and only rarely was the diffuser allowed to dry out. As well, AQIS was approached to confirm quarantine procedures that occurred when the bin re-entered Australia. It was assured that AQIS used methyl bromide gas to remove potential insect infestation but that no chlorine, at any strength, was used for sanitation. This therefore negated the possibility that a concentrated solution of chlorine was causing degradation of the diffuser.

It was also suggested that slime from the coral trout and other fish could be accumulating on the piping and, if allowed to dry out, hardening to an extent that changed the porosity of the diffuser. Microscopic examination of extremely thin sections of the diffuser did not reveal any obvious difference between 'good functioning' diffuser and 'malfunctioning' diffuser.

It was concluded that the currently supplied diffuser used for the air diffusers was changing somehow in its physical capabilities after first use and was simply not adequate to the requirement.

### ***Flow rates of air diffusers***

The flow rate through various air diffuser pipes, both used and unused, was assessed at different continuous run times (Table 1).

**Table 1. Air flow rates of various diffusers.**

Diffuser No.	Type	History	Run Time	Flow Rate (l/min)	Back Pressure (kpa)
1	Current supply	Unused	10 min	34	12.5
			20 min	33	12.5
			30 min	33	12.5
2	Current supply	Unused. soaked for 20 hours in seawater	10 min	35	13
3	Original supply	Used - 2 trips (40 hours)	10 min	37	12.5
			20 min	37	12.5
4	Current supply	Unused	10 min	35	12.5
			20 hour	35	12.5
5	Fine grade	Unused	10 min	37	13
			20 min	37	13
			16 hour	30	13
			20 hour	28	12.5

No unused 'original' supply of air diffuser was available for testing. However results showed that 'original' diffuser (diffuser No. 3) after 2 x 20h successful transport trips to Hong Kong (<2% mortalities for both trips) had a flow rate greater than other types of unused diffuser and hence would be deemed to be still functioning efficiently to maintain survival of fish. The success of return trips with this diffuser also adds evidence that AQIS quarantine procedures were not adversely affecting the performance of the diffuser.

The re-ordered piping then supplied (diffuser No. 1) had a slightly lower flow rate than the 'original' supplied diffuser even when 'conditioned' by 20h soaking in seawater prior to first use.

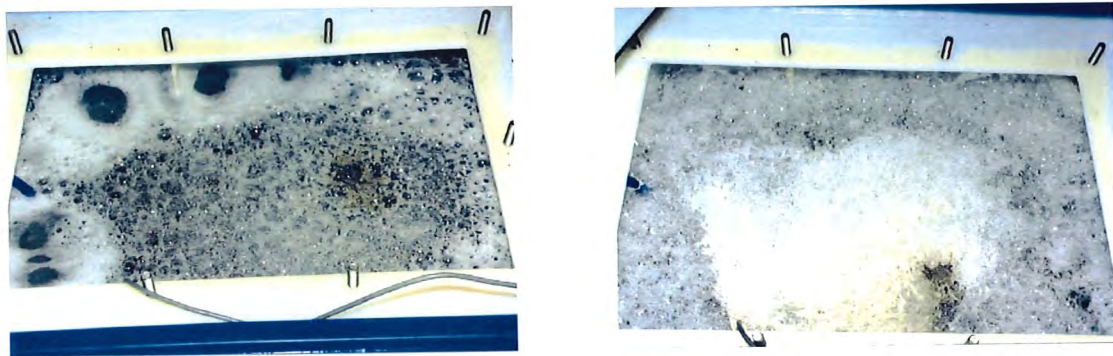
Additionally, a very fine grade piping (diffuser No. 5) was tested (Table 1). After only minutes of running, the diffuser performed very effectively with a flow rate as high as the 'original' diffuser. However, with continuous running of the system, the flow rate dropped significantly for this diffuser type. It is possible that the porosity of the diffuser changed after the piping material expanded during continuous use and hence the air holes constricted to some extent.

An alternative coarse grade diffuser was acquired and it was intended to trial this for comparison purposes. However, although coiled loosely and handled gently prior to the trial, when it was to be tested there were gaping tears along the diffuser. Hence, no further use of this type of diffuser was considered.

### **Bubble size from diffusers**

During flow rate trials, it was observed that actual bubble size produced from the various diffusers was somewhat different (Plate 2 and 3). Visually, diffuser No. 3 had consistent and uniform bubbles arising in the water column. The diffuser supplied for the re-order appeared to have bubble size uniformity and consistency very similar to

that of the 'original' diffuser supply. During initial trials the bubbles produced from the fine grade diffuser were ideal and uniformly minute, but became quite inconsistent over time.



**Plate 2. Aeration through different air diffusers.**



**Plate 3. Bubble size from different diffusers in the water column.**

Semi-quantitative measurement of air bubble size was attempted but proved too difficult without very expensive instrumentation. There was too much activity in the water to permit the photographic measurement of the bubbles against a standard grid. Attempts were made to "hold the bubbles still" by 'fixing' on a clean glass plate smeared with vaseline, but again bubble movement in the water column was just too great to allow definitive measurement. From many attempts, best estimate of actual bubble size ranged from 0.5mm to 5mm. This is too wide a range to establish differences between bubble size produced from different diffusers.

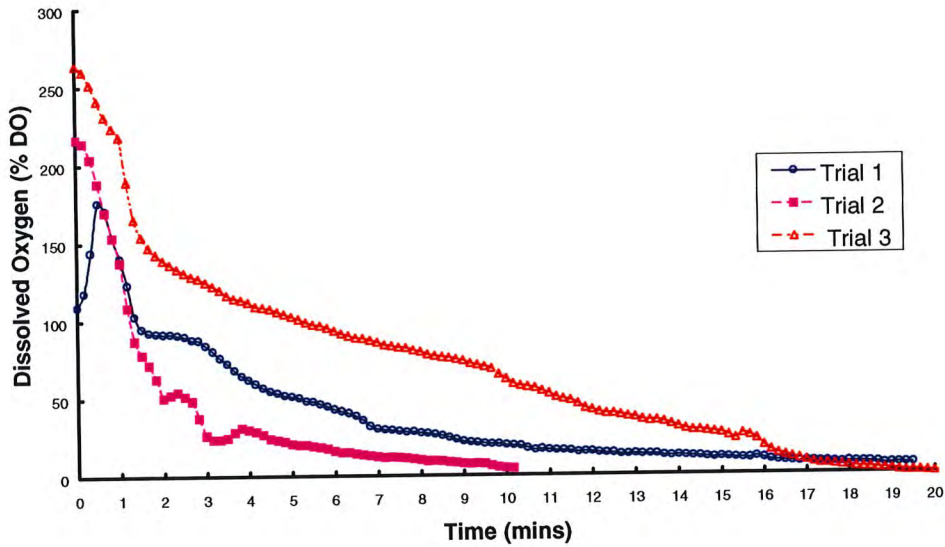
Queries submitted to international seafood technology and marine fisheries discussion lists on the internet returned answers that we were trying to do the impossible with simple technology. There are extremely expensive instruments (c. \$800 000) that could achieve our aim but none were available in Australia.

### ***Aeration efficiencies***

The technique of removing oxygen from the seawater in the transport bin by purging with pure nitrogen, worked well and efficiently (Figure 1). Oxygen was removed very rapidly over the first two minutes to a level dependent on the initial oxygen saturation

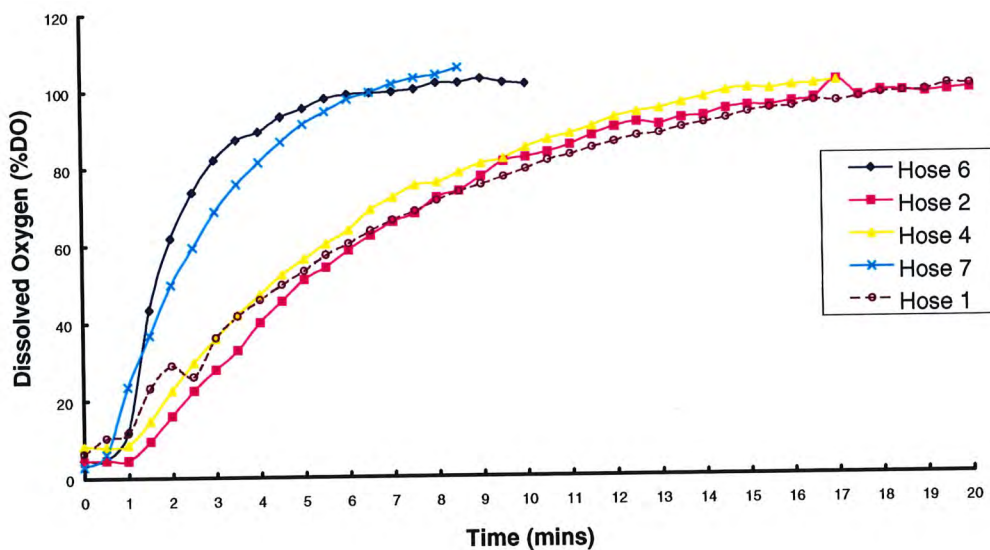
level of the water. Levels were reduced to less than 10% dissolved oxygen within 20 minutes even when initial saturation levels were >250% DO.

**Figure 1. Oxygen removal from seawater by use of nitrogen.**



The aeration efficiency of the diffusers, as measured by the time required to re-oxygenate the water by the aeration system, was hugely different between the different air diffusers trialed (Figure 2).

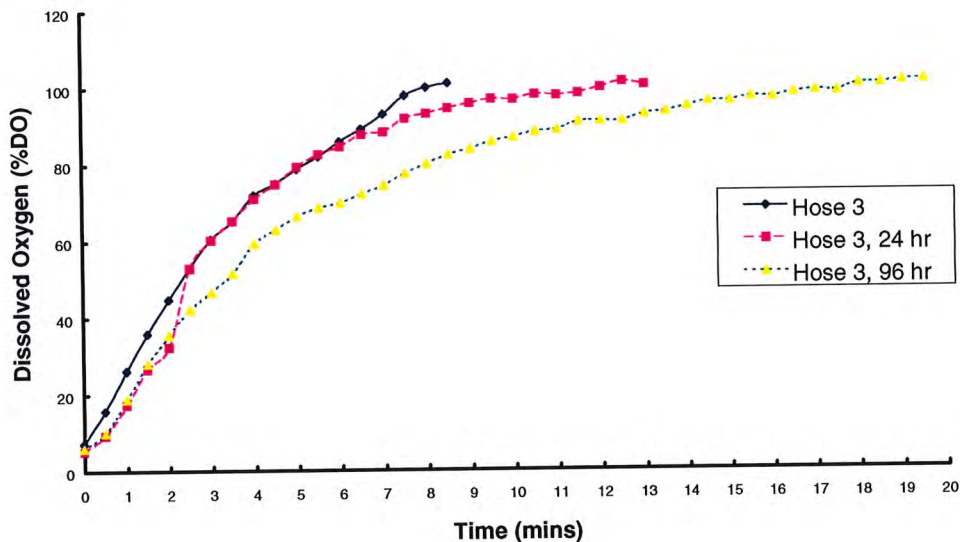
**Figure 2. The aeration efficiency of various diffusers.**



Diffusers No. 1, 2 & 4 have poor aeration efficiency while diffuser No. 7 ( the new type imported from England, presoaked according to manufacturer instruction) had an acceptably efficient aeration rate. Similar efficiency was exhibited by diffuser No.6 which was that used in a successful transport shipment of fish to Singapore.

The only available supply of 'original' air diffuser, used in the transport bins, had been used for two shipments of live fish to Hong Kong (2 x 20hours). So it had a run time use of 40 hours and had been handled, stored and sent through AQIS quarantine twice. Both shipments were successful with <2% mortalities occurring. The flow rate of this air diffuser was acceptably high and Figure 3 shows that 100% DO was achieved in about 9 minutes with the first trial run, post shipment.

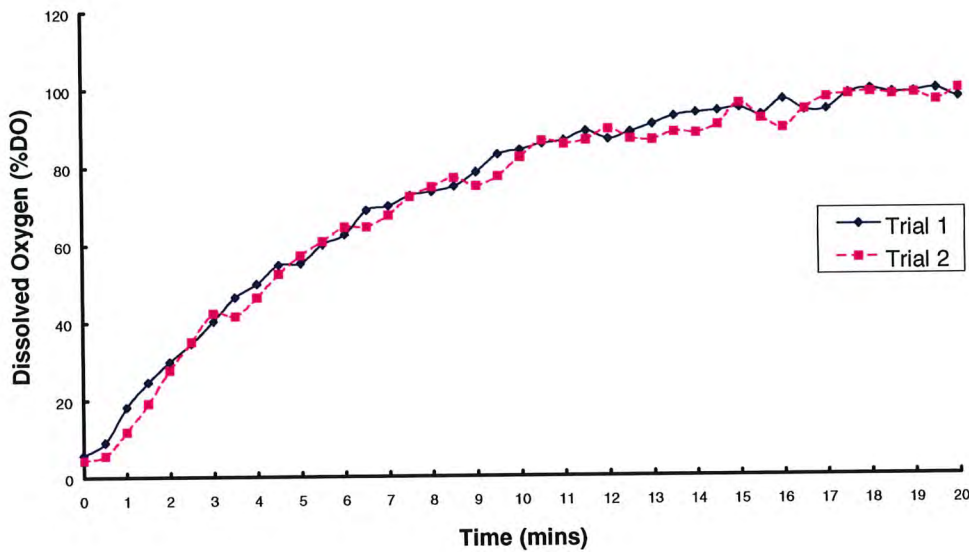
**Figure 3. Aeration efficiency of used 'original' supply diffuser (No 3).**



However, with continuous running of the aeration unit, the aeration efficiency reduced markedly, requiring almost double the time to achieve oxygen saturation after 96h running. The reduction in aeration efficiency of the diffuser with use indicates that some sort of changes are occurring which are detrimental to air flow through the water column. However, this was an extreme trial and it is unlikely that the transport bin would ever be demanded to run continuously for this extended time. Over this period, salinity of the water changed from 3.23% to 3.35%.

The efficiency of a previously used air diffuser of the type from the re-ordered supply (Diffuser No.8) was low, taking 18 - 20min for 100% DO to be reached (Figure 4). This air diffuser had been used for a shipment that resulted in very high mortality of live fish.

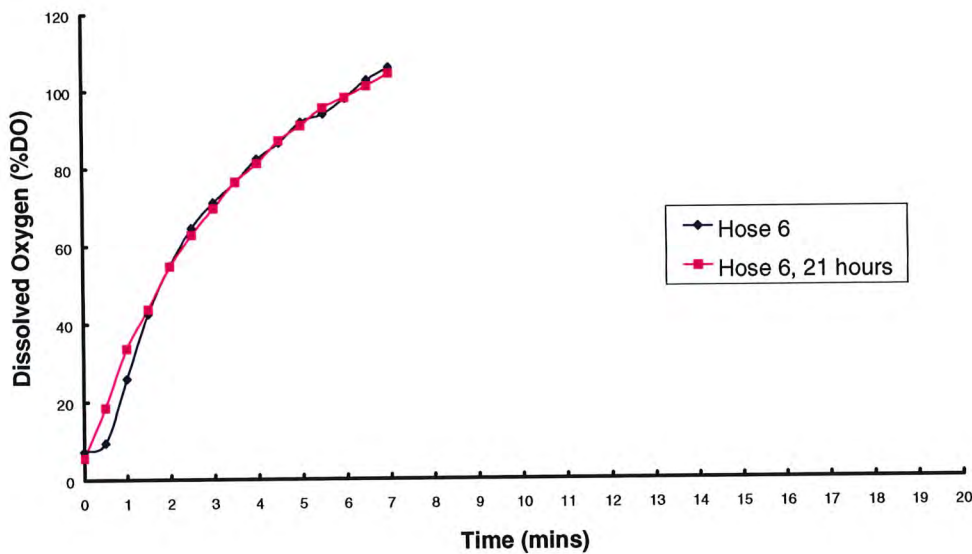
**Figure 4. Aeration efficiency of air diffuser used in an unsuccessful shipment of live fish.**



Very uneven distribution of air bubbles throughout the water column in the bin was observed when using this air diffuser.

However, diffuser of the same type was used to transport live whiting to Singapore (20h trip tank to tank) with no mortalities. Aeration efficiency for this diffuser is shown in Figure 5 and the system achieves 100% DO within just 5 minutes. This efficiency was retained after a further run of 21 hours.

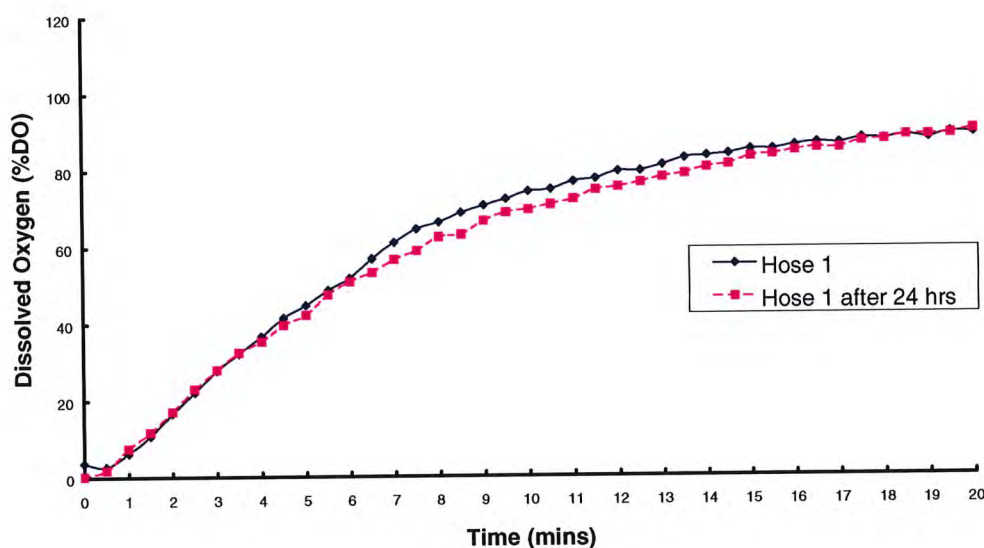
**Figure 5. Aeration efficiency of diffuser No. 6.**



All the unused air diffusers trialed gave variable aeration efficiencies, diffuser to diffuser.

The new, unused re-order supply air diffuser (diffuser No.1) had a low aeration efficiency similar to that of the 'original' diffuser after 96h run time. However as seen in Figure 6, the efficiency did not change with running time.

**Figure 6. Aeration efficiency of new diffuser No.1.**



### ***Effect of slime presence***

Diffuser No.7 was used for a trial in which 9.5 kg of sea cucumbers were held in the bin for 72 hours. The air pump was run for the duration of this trial and the dissolved oxygen content of the water remained at near 100 % for the full 72 hours, despite the water becoming very contaminated, slimy and smelly after the first few hours of the trial (Figure 7). This indicated that the diffuser was not fouling and becoming clogged with all the extraneous matter in the water. It should be noted that most of the animals died (they were in very poor condition to start with) during the trial so oxygen consumption would have been minimal.

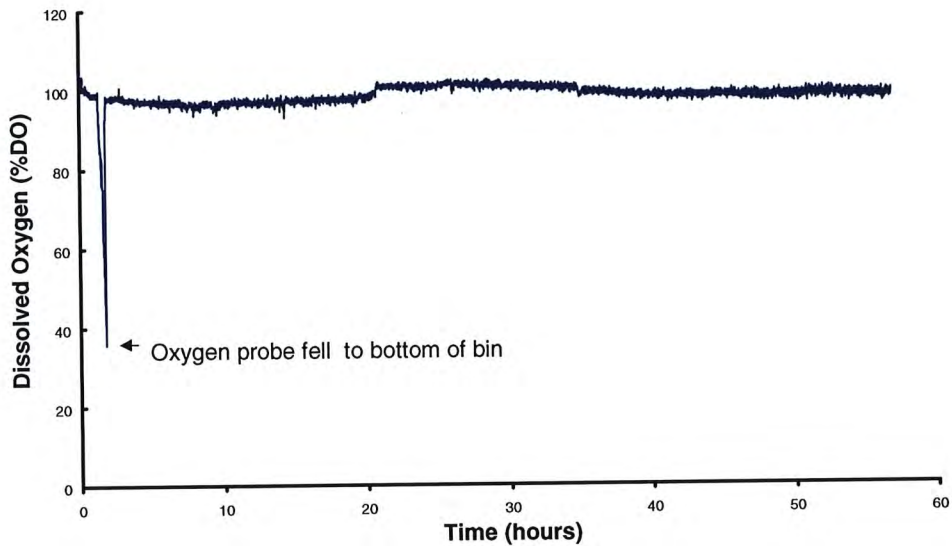
The pH, temperature and salinity measurements during the 72 hours ranged from:

PH	8.10	to	8.07
Temp	20.4°C	to	22.1°C
Salinity	3.22%	to	3.35%

The diffuser was then washed off and left to dry out for approximately 7 days before putting it back into the bin and refilling with water. Oxygen was stripped from the

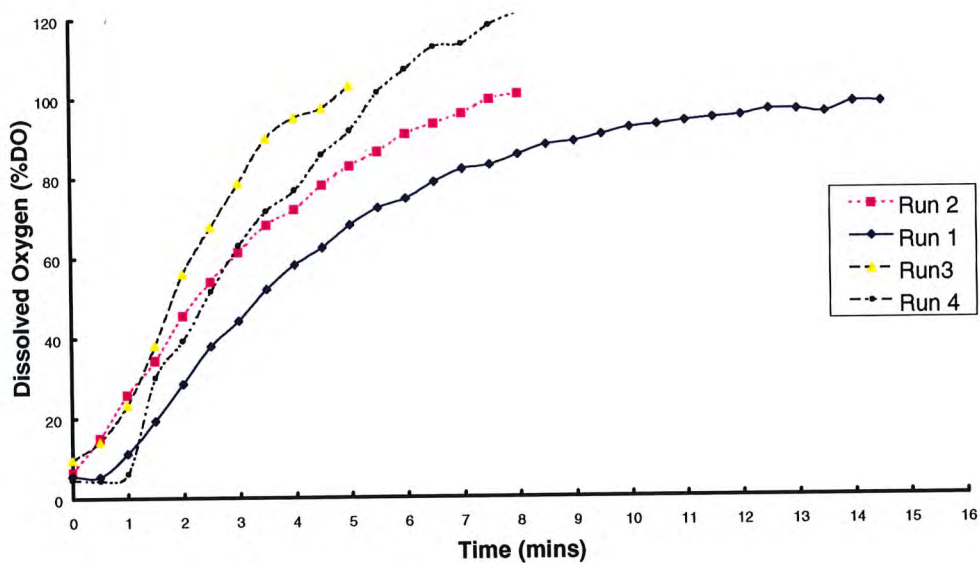
water, after which the air pump was started and oxygen logging commenced. The aeration efficiency is depicted in Figure 8.

**Figure 7. Dissolved O<sub>2</sub> levels in the presence of sea cucumbers.**



Even though the diffuser was badly abused in the presence of large quantities of slime which was allowed to encrust on and most likely in the air diffuser during the dry-out time, rate of oxygenation of the water is still reasonably fast. As best as can be ascertained from overseas, diffusers in the bins are not permitted to dry out completely. Despite having subjected the diffuser to extreme abuse conditions, results indicate that slime encrustation in the diffuser pores is an unlikely cause of deficiency in aeration rate.

**Figure 8. Aeration efficiency of diffuser after abuse treatment.**





## CONCLUSION

The answer to the question posed as basis for this research is: YES

Airflow rates and bubble size appear to have correlation with diffuser type rather than whether the specific diffuser had been previously used or not. Airflow rates were similar over short initial aeration use.

The efficiency of the fine grade diffuser decreased significantly over a period equivalent to that for shipping live product to Hong Kong, for example. The coarse grade diffuser was not robust enough for the purpose of use.

The method of stripping the oxygen out of the water with nitrogen was very effective and permitted trials to occur in rapid succession.

The 'original' air diffuser supplied during development of the transport bins, even though previously used for live transport, retained acceptably high aeration rates. This efficiency decreased slightly following a continuous 96h run, however it is unlikely that this period would be demanded of a bin used in a commercial operation.

The new supply of air diffuser was shown to be less efficient than that of the 'original' supply. The efficiency of a previously used air diffuser from the re-ordered supply was low, taking greater than double the time to cause 100% O<sub>2</sub> saturation of the water. This air diffuser had been used for a shipment that resulted in very high mortalities of the fish. Additionally, an uneven distribution of air bubbles was observed throughout the water column during operation of this air diffuser.

Unused air diffusers that were trialed also gave variable aeration efficiencies.

Having put the most efficient air diffuser to the extreme test of excessive quantities of slime contact and a complete dry-out phase, results indicate that it is not slime hardening in the diffuser pores that decreases the aeration rate.