Evaluating Re-usable Containerised Systems for Airfreighting Live Fish using Bottled Oxygen

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NON-TECHNICAL SUMMARY

Shortly after work began in this project, an international ban was applied to the carriage of 'in-use' compressed oxygen cylinders by aircraft. This effectively halted the lucrative live fish export industry and industry effort concentrated on getting the ban lifted. The ban also severely restricted the ability of the researchers to conduct the project work. The project objective of monitoring water quality changes in flight was unable to be carried out. Project work was continued, albeit under restricted operating conditions and much useful information was gained.

The project work shows how fish react under transport conditions and how this affects the demand for oxygen within the bulk bin transport system. Live fish can be shipped in large quantities, 100-300kg, and at high density (1:<2 fish/water ratio) using returnable insulated bins in which seawater is oxygenated with compressed oxygen gas.

The respiration rate of coral trout was determined. The respiration rate was high (75mgO_2/kg fish/hour) when the fish were first loaded into the bin and then reduced significantly (to 45mgO_2/kg fish/hour) once the fish settled down. The greater respiration rate at loading, with the consequent high oxygen consumption and high carbon dioxide production, results from handling and transfer stresses. Hence it is important to packout the fish as quickly and gently as possible. Additionally, it is important to have the seawater in the bulk bin well oxygenated prior to loading fish.

Research results demonstrated that the release of oxygen into the bulk bin water was found to be excessive to fish requirements. When 150kg of banded morwong were airfreighted from Sydney to Brisbane, oxygen level remained high throughout transport, >300% dissolved oxygen in the seawater. However, dissolved oxygen levels did change with pressure fluctuations in the cargo hold during takeoff and landing. Evidence from this project work indicates that current levels of oxygen supplied can be reduced.

Trials with banded morwong (70kg) packed at a fish/water ratio of 1:1 showed that oxygen flow rates from 350-700cc/min kept the dissolved oxygen level above 200% saturation. This is more than sufficient to sustain live fish. At the low gas flow rate of 350cc/min, the pH of the water dropped rapidly when the fish were first loaded indicating rapid accumulation of carbon dioxide. However, the rate of pH fall slowed with time as the fish settled, implying a slower carbon dioxide production rate and indicating that the reduced flow rate still assisted in physical removal of carbon dioxide from the water.

Work with coral trout at the same density, showed that a reduced oxygen flow rate of 350cc/min also retained high water oxygenation. These fish were quieter when packed and hence when loaded into the bin, did not cause a large drop in water pH.

These findings show that oxygen flow rates can be reduced by at least half the flow rates currently used. Further, calculation from the results obtained indicate that 250kg of fish could be transported in a bulk bin with an oxygen flow rate of as little as 1.25 litres/minute supplied. This flow rate would allow exports of fish to be made using a D-size (1.6m^3 compressed gas) cylinder rather than the currently used E-size (4.1m^3) ones. Further work is still required to confirm these findings intransit.

The project work findings provide information for assisting industry and authorities such as the Civil Aviation Safety Authority (CASA) in making descisions with respect to the carriage of 'in-use' oxygen cylinders by airlines.

BACKGROUND

Live export of fish provides a lucrative way of getting the best price for marine products. However, existing methods for exporting fish are based on systems that are closed to the atmosphere, where fish in a limited amount of seawater are put in a double plastic bag which is inflated with oxygen, sealed and then packed in a polystyrene foam box. The drawbacks of this system are widely recognised by those exporting live seafood, particularly when corrosive seawater leaks from damaged boxes into the aircraft. The quest for better packaging systems for airfreight of live fish is a major priority of the live fish industry, as discussed at industry workshops organised by the National Seafood Centre.

Disposable containers can present a waste problem at fish markets and, potentially, costly repair bills to airlines due to their lightweight construction but they are cheap to freight which is why their use has been prevalent. The use of returnable insulated plastic bins is a good alternative as fish can be transported in bulk. However, the freight cost in sending these bins is far higher as the bin may often be returned empty. The assumption that the "extra" freight cost of returnable containers is prohibitive is not necessarily true. Packing fish in many small disposable boxes with large amounts of "dead space" is more labour intensive and an inefficient and therefore more expensive use of costly aircraft cargo space than packing fish in a robust and returnable container. An additional advantage of bulk bin transport is the excellent fish to water ratios able to be achieved, hence making this transport method more efficient.

Live fish were transported in bulk bins by air, mainly to Hong Kong, between mid 1995 and mid 1996. This proved very lucrative for exporters, resulting in export earning for Australia of around $30M and predicted growth of 30% per year. However, the trade was short-lived and was stopped worldwide because of disparate interpretations of the International Civil Aviation Organisation (ICAO) Technical Instructions for the Transport of Dangerous Goods. An attempt to have the Technical Instructions amended to allow for this style of live transport, failed in October 1996.

Since this time, further developments and directed lobbying have resulted in a special technical provision for aircraft carriage of bulk bins with attached compressed oxygen cylinders. ICAO Special Provision A202 stipulates, amongst many other things, that the maximum permitted flow rate of oxygen is 1 litre per minute per $5m^3$ of cargo hold volume.

With the bulk transport system operating the way they did in the early days, this limit would rapidly be exceeded. Most of the oxygen is probably wasted, being present in excess of the fish's needs. Instead of satisfying the fish, the bubbles of oxygen are moving the seawater around the fish like an "air lift" pump and most of the gas is passing immediately into the overlying air. Water recirculation and oxygenation methods must be improved so that less oxygen is required to transport a given amount of fish. This requires knowledge about the oxygen requirements of fish crowded in bulk bins and whether the oxygen demand of the fish is constant throughout airfreight.

This preliminary study will develop methods for monitoring oxygen requirements of the fish and deterioration in water quality when fish are held in a re-useable export containers currently being used to ship live fish, then evaluate the performance of the system during commercial trials.

NEED

This project addresses the live fish export industry's need for alternative transport systems by developing methods of evaluating the performance of re-useable air-freighted fish transport containers and then using the information gained to improve the efficiency of these transport methods.

The project will also provide sound background information on respiration of fish during bulk transport shipment and whether respiration rate is affected by transport factors. This will provide knowledge of the amount of oxygen required to sustain survival of fish during airfreight of the bulk transport bin.

Such information will be useful to the CASA (Civil Aviation Safety Authority) and should assist in decisions of granting the approvals as required by ICAO (International Civil Aviation Organisation) TI Special Provision A202.

OBJECTIVES

1. To monitor changes in water quality when fish are transported in an existing fish transport system

Preliminary work carried out - initial problems with sensitivity and response time of monitoring equipment, as well as 'waterproofness' of the battery case of one of the data loggers used, were solved.
THEN an international ban on the carriage of in-use oxygen cylinders by all airlines came into force. Such ban completely prohibited further monitoring work of air-freighted fish, hence this objective remains incomplete.

2. To measure the respiration rate of fish during simulated transport

Conducted with coral trout (*Plectropomus* spp. - mostly *P.leopardus* with some *P. maculatus* present). This objective is complete.

3. To reduce the amount off bottled oxygen required to meet the respiratory needs of the fish without compromising water quality

Considered to be 90% complete prior to airline ban being enforced.
Further experimentation was planned to establish the feasibility of downsizing the oxygen cylinder used in air-freight fish transport from BOC size E (4.1m^3) to BOC size D (1.6m^3), relevant to ICAO TI Special Provision A202.

The following body of the report is separated into sections based on the work conducted for each project objective.

SECTION 1. Changes in water quality during high density transport of fish.

The performance of a system being used to transport live fish was assessed by measuring changes in seawater quality within the bulk transport bin.

Methods

The existing transport system

Initially, S.E.A. Food International transported fish in plastic barrels (c.20L capacity) fitted inside a large insulated plastic fish bin (750L capacity). Oxygen (medical EP grade) was supplied from a gas cylinder via a regulator and distribution manifold to carbon diffusers - 'air stones' - in the bottom of each barrel. This system allowed reasonable survival rate of fish at the point of destination and up to 100kg of fish could be shipped this way in each fish bin. The system was further modified immediately prior to commencement of investigations for this project, by eliminating the barrels and simply filling the fish bin directly with seawater.

The fish transport bins used by S.E.A. Foods International and other companies are straightforward in principle. Rugged insulated plastic bins are modified by plastic welding panels and partitioned inside to create an inner storage compartment for water/fish and another for containment of the oxygen cylinders. The water/fish compartment is lidded and sealed with rubber gaskets and stainless steel wing nuts or clips. A small hole in the compartment lid and another in the lid of the bin allows waste gas to escape but at the same time prevents water leaking outside the transport bin.

Plates 1 & 2. **Insulated bulk fish transport bin**

The bins in use by S.E.A. Foods International used two E-size oxygen cylinders. Gas lines from the regulator on each cylinder were plumbed to hose connectors on the partition walls. On the other side of the partition, hoses connected to air-stones were attached to the connectors bringing the oxygen into the compartment.

Fish species

Initial experiments were carried out with mainly banded morwong (*Cheilodactylus spectabilis*) and some parrotfish (*Choerodon* spp.) included. Further work was conducted with coral trout (*Plectropomus* spp. - a combination of *P. leopardus* and *P. maculatus*).

Water quality monitoring

The quality of the seawater within the transport bin was measured by the parameters of dissolved oxygen; temperature; pH (correlates to carbon dioxide level) and ammonia.

Two submersible data loggers were used for monitoring: a DO300 which records oxygen and temperature; and a CTDP300 which measures conductivity, temperature, depth and pH (Greenspan Technology, Qld). The use of data loggers allowed automatic and continuous data to be obtained throughout all transport stages.

First trials employed the oxygen sensor as supplied with the instrument, however membranes were changed to more sensitive ones after the first data collections to provide greater and more rapid response to oxygen tension changes. The data loggers were factory set to read up to 500^{+} % saturation dissolved oxygen. This was to allow for the commercial practice of saturating the seawater with oxygen just prior to loading fish into the bin. Normal atmosphere is about 20% oxygen by volume, so saturating seawater with pure oxygen will give about 500% dissolved oxygen saturation reading at 1 atmosphere.

The ammonia concentration in the water was measured using the Palintest photometer 5000 prior to loading fish into the bin and at the time of unloading the bin at the end of transport.

Results and Discussion

Water will foul when fish are crowded together in oxygenated water. The oxygen level of the water may be high but toxic wastes such as carbon dioxide and ammonia will still accumulate, potentially reaching levels critical to the fish. The pH of seawater falls as carbon dioxide increases, so this parameter provides a useful index of carbon dioxide accumulation (Figure 1).

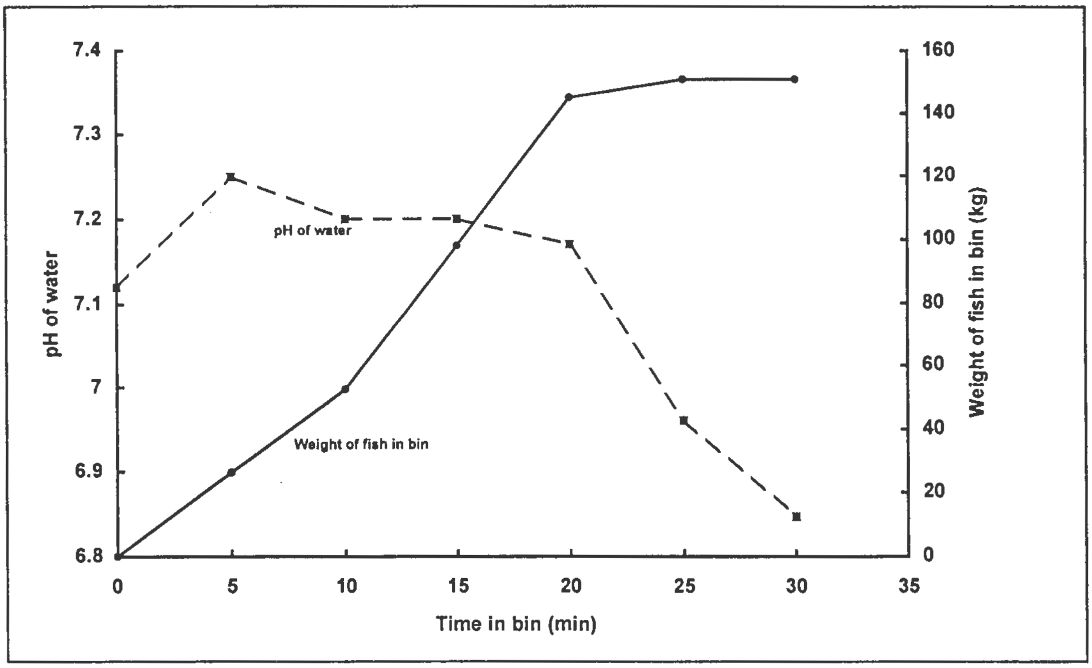

Figure 1. **Water pH changes as fish are loaded into bin.**

We proposed to monitor the changes in these variables when fish were air-freighted in the system used by S.E.A. Foods International to provide information on that system and to obtain data by which to compare that of modified systems

Packouts for these trials were conducted very rapidly and efficiently. Fish were hand netted from the holding tanks, placed in fish crates and immediately transferred to the transport bin, 7-10kg at a time.

The seawater in the bin was saturated with oxygen prior to loading the fish and data obtained in various trials does not suggest any rapid drop in oxygen while the bin was being loaded. Oxygen levels remianed at 400-500% saturation throughout loading. No downward trend was observed at the end of loading, implying that oxygen is entering the seawater faster than the fish can consume it. However, these comments are restricted to data covering the loading peroid only and it may be that the fish take some time to reach maximum oxygen consumption rate.

Data obtained from a shipment of 150 kg of fish (mostly banded morwong with some parrotfish, 13kg) sent from Sydney to Brisbane is given in Figure 2. The fish were loaded in a ratio of 1:1.7 seawater. Total transport time, floor to floor, was close to 7 hours. The ammonia level at destination was measured to be 13.2mg NH_3-N/L which is relatively high but to be expected after the period of transport.

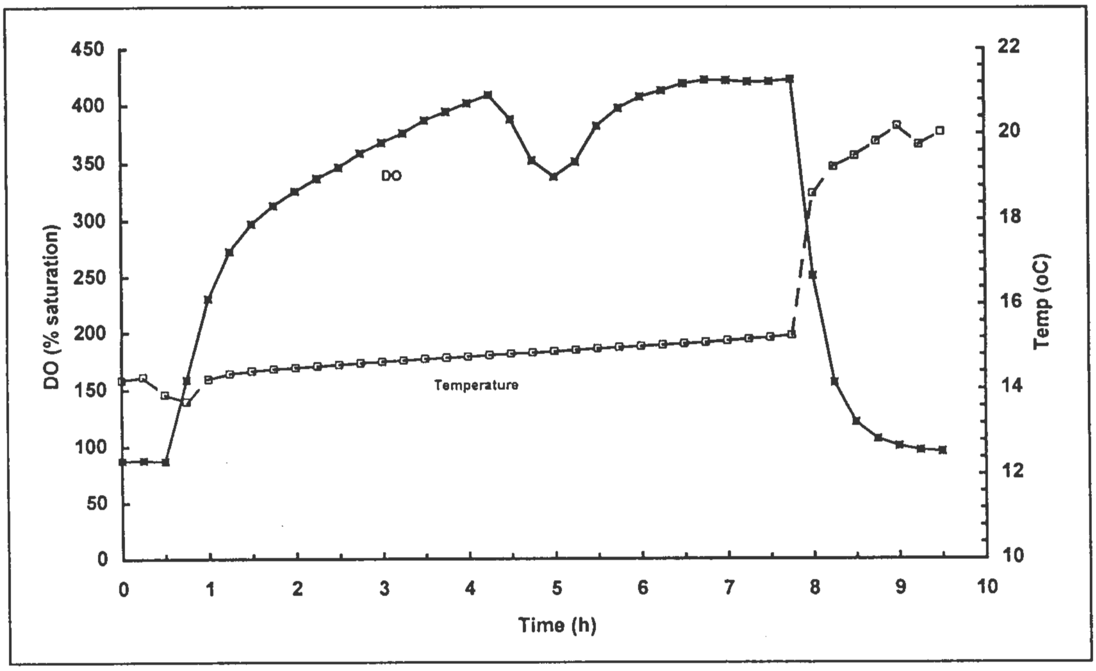

Figure 2. **Dissolved oxygen and temperature changes during shipment of fish from Sydney to Brisbane.**

Oxygen level throughout transport is consistently high, only falling when the datalogger was removed at destination. The dip in oxygen level (observed at 5h) coincides with the time the plane landed in Brisbane, but that maybe because of a lag between pressure changes in the cargo hold during the flight and subsequent changes in the pressure of oxygen in the water. Consistent with this, it was found that if the datalogger is carried dry as baggage it reports a proportionally similar fall in atmospheric oxygen level during a flight, apparently because the atmospheric pressure falls below 1 atm in the cargo hold. Transient changes in atmospheric pressure in the cargo hold as it is pressurised and depressurised, change the partial pressure of oxygen in the air and hence the amount of dissolved oxygen it is possible to achieve in the oxygenated seawater.

Figure 2 depicts data from initial trials with the membrane as supplied with the instrument and the smoothness of the line indicates that the datalogging oxygen sensor is too insensitive to rapid fluctuations in oxygen level ('noise') of the kind observed with other sensors. The DO300 sensor is primarily designed for long term use in zero flow environments and it is clear that some information is being lost due to the slow response time of the sensor membrane.

The datalogger insensitivity was resolved by modifying the sensor membrane to a much thinner one. This effectively sped up response to minor changes in oxygen levels and made the logger more sensitive to flow, however, placement location of he instrument within the transport bin chamber needs to be carefully considered to maximise response.

It was at this time of the trials that the airline ban came into force and hence no further work of this nature was able to be undertaken.

SECTION 2. Respiration rate of fish during simulated transport

Information from the respiration rate of fish during simulated air transport will assist fish exporters to tailor oxygen supply to the fish, rather than relying upon consumption rates averaged over an entire journey.

Precise measurements of respiration rate are required to determine whether the demand for oxygen is constant throughout transport. Is the demand higher initially when fish are first loaded into the transport bin (due to handling stress) and then does it decrease to a steady level? If this occurs, over what period of time and how high is the initial demand? Do atmospheric pressurisation changes during air transport change the oxygen demand of the fish? All these questions have ramifications for managing oxygen delivery during air transport of fish and for the total volume of compressed oxygen needed to be carried.

Information in this area became even more critical when the Special Provision A202 amending the ICAO Technical Instructions for the Transport of Dangerous Goods came into being. This Provision stipulates a maximum for gas flow rate during airfreight and therefore the specific oxygen demand of fish during transport is needed. Knowing the respiration rates of fish allows amounts of oxygen to be supplied to the live transport bin sufficient to the need and not in excess.

Methods

The model respirometer

A model transport container system was designed so that it could operate as a flow-through respirometer, allowing measurements of how much oxygen the fish consumed. The respirometer was constructed to operate automatically and record respiration rate of fish packed under conditions simulating high-density live transport.

The system consists of two chambers, one which contains fish packed at high density and the other containing inert objects to provide a similar volume to water ratio as that in the fish chamber (Figure 3). Oxygenated seawater is pumped past an oxygen sensor mounted in-line in a pipe and then the flow is split into each chamber. The seawater then flows out of the individual chambers and is piped through a solenoid valve array that alternatively directs water from each chamber past a second oxygen sensor for several minutes. A bench-top datalogger with two oxygen meters and sensors was used for convenience rather than the submersible dataloggers. The system must be adequately earthed otherwise earth-loops cause problems with meter/logger performance and it is essential that the oxygenated seawater flow rate to both chambers is exactly the same.

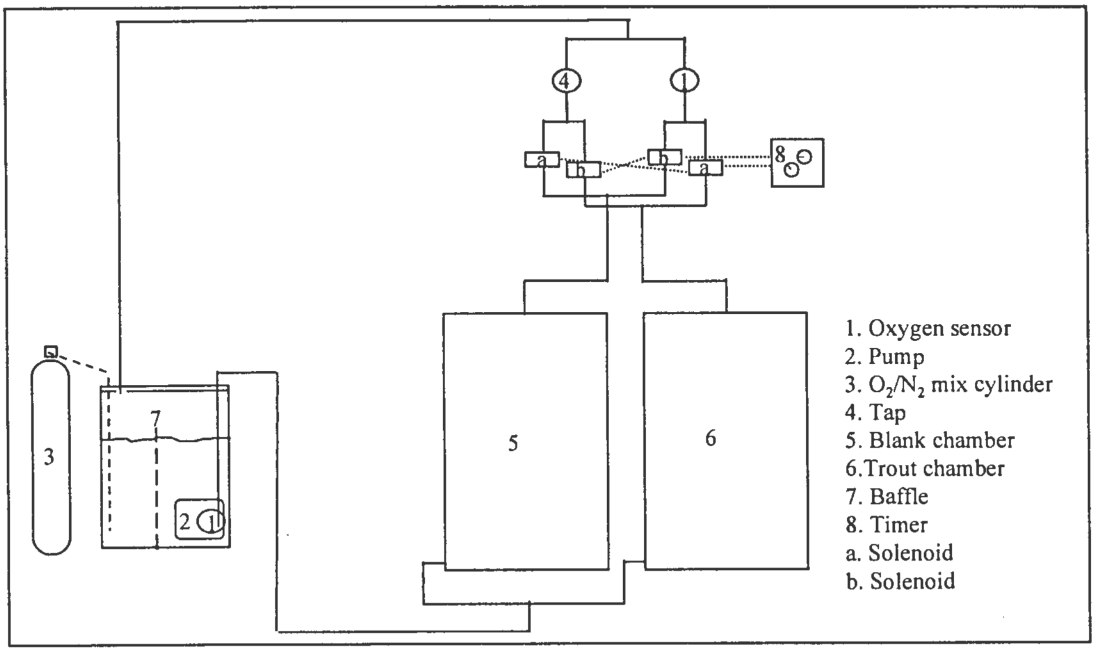

Figure 3. **Respirometer diagram**

Water flow control, sampling of the water and measurement of oxygen tension is performed automatically so that the system can run efficiently with minimal supervision and records respiration rate periodically for several hours. Oxygen, and also nitrogen, gas can be used to regulate the oxygen level in the system at around 180% saturation.

Difficulty was encountered in finding suitable small square or rectangular plastic bins that could be made watertight. The walls are prone to bow under pressure and this caused the difficulties in making the bins watertight. This problem was overcome by employing small drums that have a screw-top lid and gasket (of the kind used for home-brewing).

Measuring respiration rate

The respirometer operates by pumping water through either of two chambers, one with fish (test) and one without fish (blank). Knowing the flow rate of the water (L/h), the difference in oxygen tension between the first and second oxygen sensors gives the amount of oxygen removed in each chamber (mgO_2/L) and it is possible to calculate the rate of oxygen removal in the chamber (mgO_2/h). The difference in oxygen removal from the seawater between the 'test' chamber and the 'blank' chamber gives the oxygen consumption rate of the fish. The fish can be weighed before the experiment, allowing weight specific respiration rate (mgO_2/kg fish/h).

Trials

The chambers of the respirometer were filled with seawater and oxygenated to around 180% dissolved oxygen. The system was then sealed and checks made that there were no 'changes' in oxygen level recorded by the sensors caused by artefacts of the system.

Fish were netted from the holding tank, placed in tared fish crates and weighed. Fish were then transferred to the model respirometer chamber as soon as possible. Water was topped up in the chamber, the top sealed and the solenoid device timing manipulated to ensure that water flow from the test chamber was measured for the first part of the experiment so that initial oxygen consumption by the fish was recorded. Temperature of the seawater in the chambers was recorded at frequent intervals throughout the trial.

Testing the respirometer system

Coral trout were too valuable to use while fine-tuning the respirometer prior to beginning trials. Instead, barramundi (*Lates calcarifer*) were used as they were readily available in our holding tanks. Observations were made at relatively high oxygen levels, with DO*in* of about 200% saturation, because of transporting live fish with pure oxygen. This partial pressure was achieved by balancing flow of gas from cylinders of both oxygen and nitrogen gases. Later work used a commercial mixture of oxygen/nitrogen which, under the conditions of the experiment, gave DO readings much closer to the normal range of oxygen saturation.

Results

Respirometer system testing

With 5-6 kg of barramundi (approx. 20 fish) in the test chamber and running the system for several hours at flow rates of about 5L/min, produced a DO*out* of approximately 40% saturation below that of DO*in*. The 'blank' chamber respiration was negligible by comparison. When plotted versus time, the DO*out* was usually a stable square-wave indicating good mixing within the chamber (Figure 4).

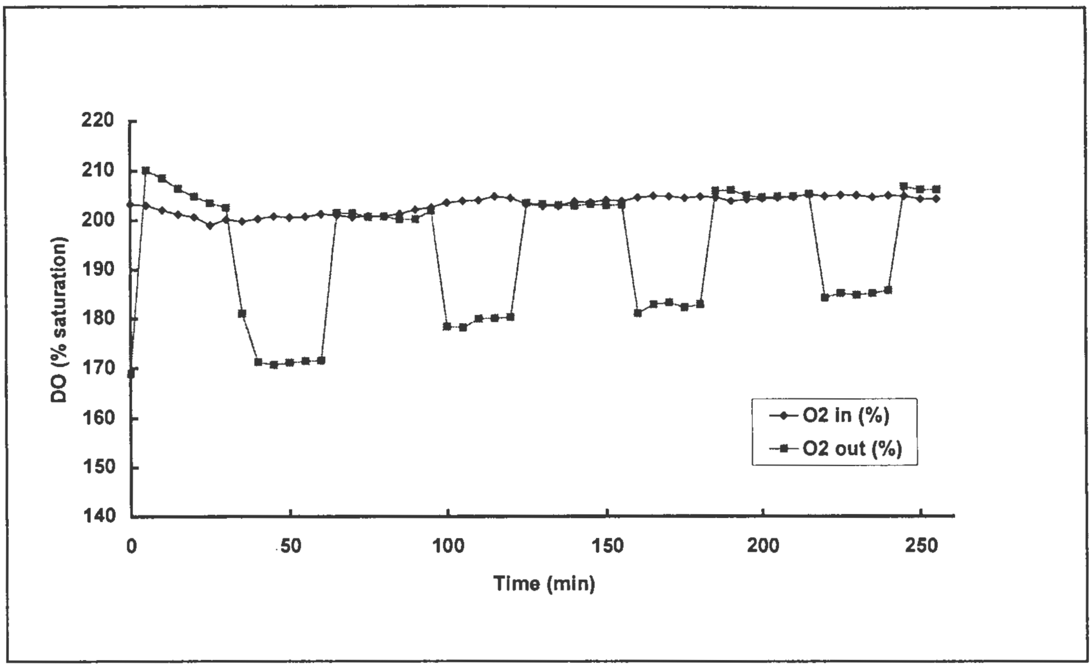

Figure 4. **Respirometer data of oxygen consumption by barramundi**

As the system was run repeatedly, an artefact was seen where a 'drift' pattern appeared in the plot of pO_2out (Figure 5).

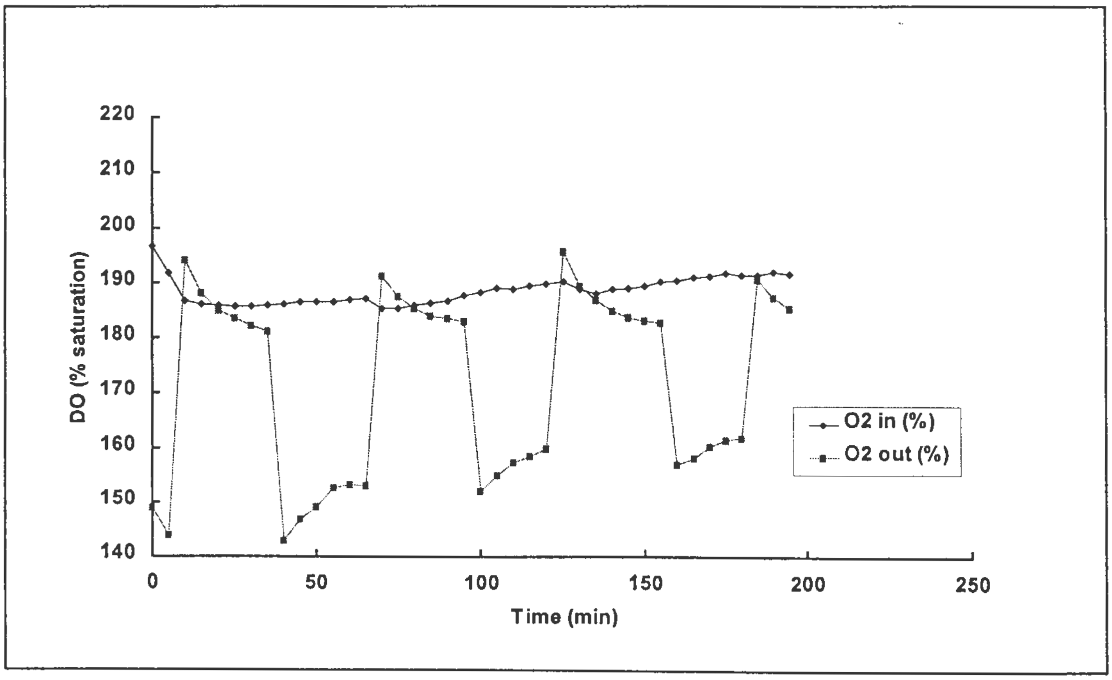

Figure 5. **Respiration data for barramundi after multiple use of the respirometer.**

This was corrected by adjusting slight inconsistencies in flow rate that had developed between the blank and test circuits of the respirometer. Subsequently, careful notice was taken to ensure that flow rate was balanced through both sides of the system.

Respiration of coral trout at high density

Records of respiration rate taken at five minute intervals while the test chamber was being monitored show that the oxygen uptake rate of the fish tended to be high shortly after they entered the chamber and that the level fell subsequently (Figure 6).

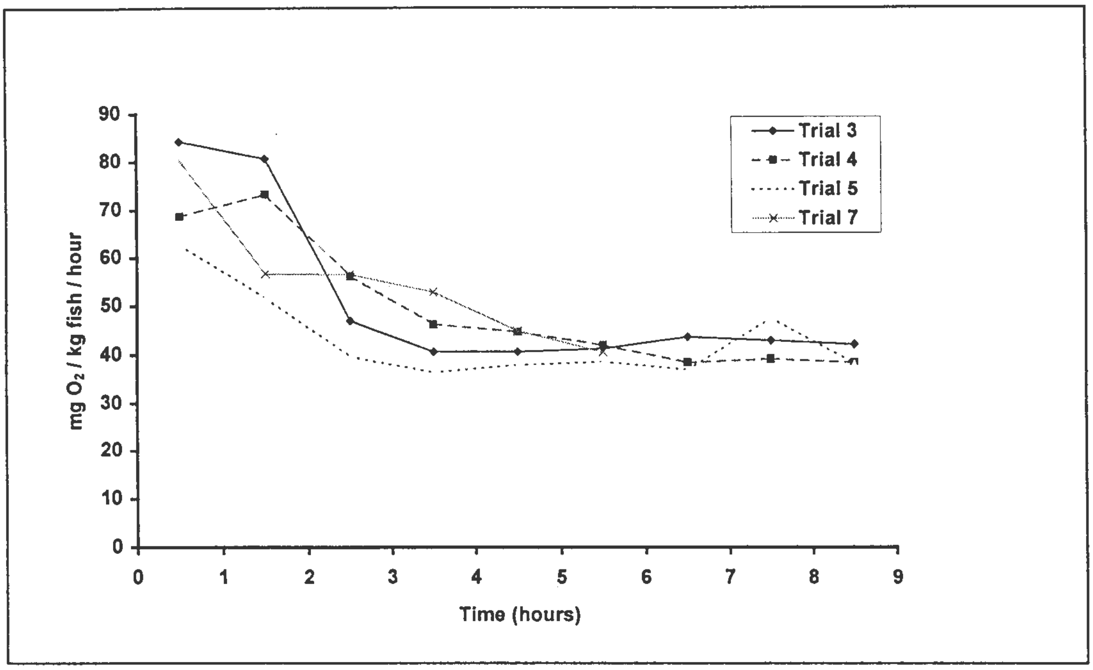

Figure 6. **Respiration rate of coral trout from replicate trials**

The average respiration rate during each monitoring period was calculated to integrate the oxygen consumed by the fish over the full 30 minutes and the results overall summarised by calculating the mean oxygen uptake rate per monitoring period for all runs (Figure 7).

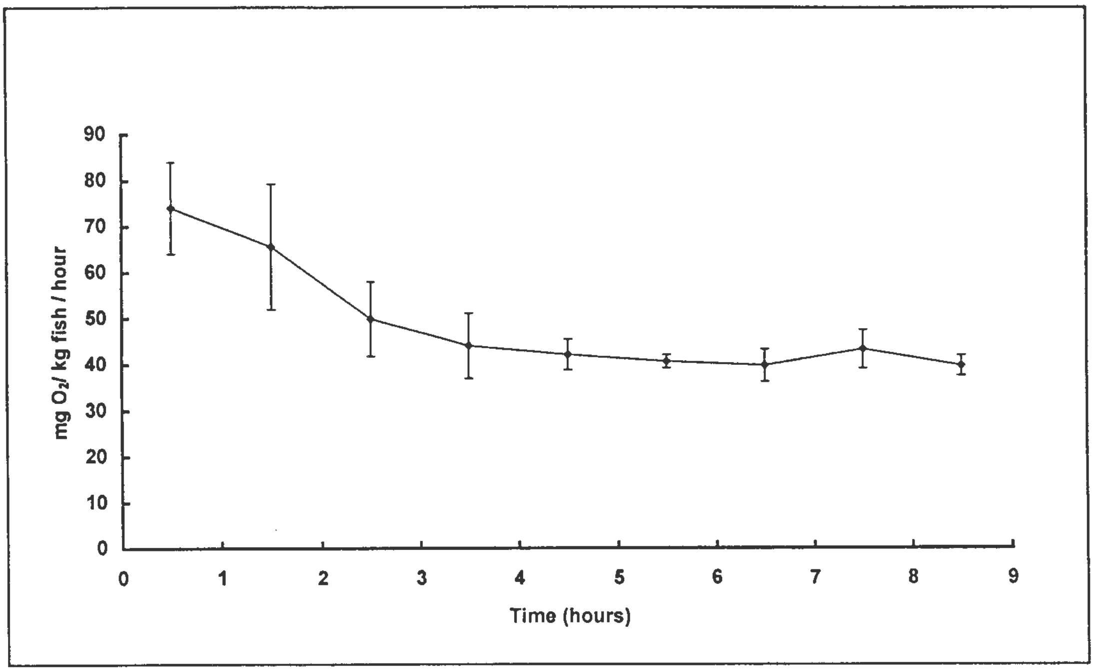

Figure 7. **Respiration rate of coral trout averaged across trials**

Discussion

When coral trout are netted from a tank, weighed and placed at high density in a simulated transport tank, their collective respiration rate of around 75mgO_2/kg fish/h was nearly halved within the first three hours and remained relatively stable at around 40mgO_2/kg fish/h for the duration of the experiment. Handling, exercise and brief periods in air all cause stress in fish and will consequently increase the respiration rate. Thus it isn't surprising that it takes several hours for the respiration rate of the fish to fall as the animals 'settle' in the respirometer. The fish are so crowded in the chamber that they are unlikely to swim, so the effect noted is probably entirely caused by previous handling rather than activity within the 30-litre respirometer chamber. For example, the fish may be repaying an oxygen debt caused by lactic acid accumulation. Another reason why the respiration rate of the confined fish would fall and then stabilise at a low level, is that the crowded fish may be anaesthetising themselves in their own respiratory carbon dioxide.

Given that the fish are largely inactive and probably to some extent anaesthetised, the respiration rate which the fish settle to may well be close to their standard metabolic rate. However, this is difficult to say for certain because elementary information about the respiratory physiology of *Plectropomus* spp and other relatively large tropical reef species is unavailable.

The minimum respiration rate of a fish is termed its 'standard' respiration rate. Under more 'natural' circumstances, measuring this quantity often presents practical difficulties because exercise and activity raise the respiration rate of a fish and animals are often spontaneously active during experiments. The oxygen consumption required to satisfy this spontaneous activity is the 'routine' respiration rate. While interest in fish respiration has a long history, much of the work focuses on relatively small species from temperate climates (Brett and Groves, 1979). It is well known that temperature and body size influence the respiration rate of fish and other organisms so, at first glance, measurements obtained on temperate species are unlikely to be relevant to the situation of live reef fish transport.

The routine respiration rate of groups of 1kg starved-Atlantic cod (*Gadus morhua*) at 10°C is 70-80 mgO_2/kg/h (Saunders, 1963). Standard respiration rate of individual starved-Atlantic cod of similar size was 58 and 39 mgO_2/kg/h at 10° and 5°C respectively (Saunders, 1963). The routine respiration rate of *G. morhua* at 10°C (Saunders, 1963) is similar to the routine respiration rate at 25°C of *Colossoma macropomum,* a tropical fish from the Amazon (Saint-Paul, 1983). This suggests specifically that the measurements of respiration rate found here for *Plectropomus* spp.confined at 20°C are similar to that we might expect from a finfish at the low edge of its thermal range. Also, and perhaps more generally, this is a re-affirmation that, regardless of *prima-facie* differences in temperature, the respiration rates of fish species from different latitudes are subject to compensatory adaptations (Brett and Groves, 1979).

Recently, a study of cod (Staurnes *et al* , 1994) during simulated live transport showed that respiration rate of the crowded fish (1:1 fish to water ratio, 2kg average wet weight) at 8 °C was about 110 mgO_2/kg/h, rather higher than that expected based on the earlier study (Saunders, 1963).

While measuring the respiration of several fish crowded in a respirometer is more meaningful in terms of the conditions applying to live transport, what we haven't attempted to simulate here is whether disturbance of the fish after packing, (for example when the bin is moved to and from aircraft and during take-offs and landings) will raise the demand for oxygen within the container. Perhaps one solution to this is to use an anaesthetic such as Aqui-S in the transport water to ensure that the fish don't become agitated in transit. However, perhaps another anaesthetic is superfluous if the fish are already largely immobilised by confinement and high carbon dioxide levels. Clearly further work in this area is warranted. Food-grade anaesthetics such as Aqui-S, could well be useful when handling fish prior to loading the transport bin to minimise the peak oxygen demand at the beginning of the journey. However, no anaesthetics are currently approved for use when transporting live fish within or from Australia.

This study shows that the respiration rate of coral trout is not constant during simulated live transport, but rather there is a peak in respiration rate soon after packing, which disappears after a few hours.

There has been recent proposals from some that an alternative to using compressed pure oxygen in cylinders would be high pressure high flow rate air. This would avert concerns of the airlines with respect to the Technical Instructions as air cannot be considered a 'Dangerous Goods' item. However, an air pump cannot oxygenate water as efficiently as compressed oxygen because of the physical limit on the amount of oxygen that will dissolve in seawater from normal atmosphere. Thus it is even more imperative when using this method that a satisfactorily wide safety margin is maintained between fish respiration and water aeration. Clearly, handling methods that minimise the initial peak demand for oxygen, making the respiration of the fish more predictable, may be beneficial both for the survival of the fish and for the economic viability of the industry that depends upon them.

SECTION 3. Effect of oxygen gas flow rate on water quality

When shipping fish, oxygen must flow through the water to supply that required for the fish to respire and stay alive. However, the amount of oxygen used within the existing transport system is far in excess of the amount of oxygen the fish need. This has extreme relevance with respect to the Special Provision A202 of the ICAO Technical Instructions which specify a maximum flow rate of 1 L/min/5m^3 of cargo hold volme.

Possible scope for improvement in this area comes from some brief calculations: 100kg fish consuming oxygen at the rate of 70 mLO_2/kg/h would, in the course of a 9h flight, require about 63L of oxygen. The current system of live transport goes onto the aircraft with 4000L (BOC size E cylinder). If gas diffusion into the water was total, then this amount of oxygen could satisfy the needs of about 6.5 tonnes of fish.

Bubbling oxygen through seawater not only helps dissolve the gas into the water, it also helps to blow out the carbon dioxide. Therefore, lowering the flow rate of the gas may change the rate of removal of carbon dioxide which could have consequent detrimental effects on water quality.

Obviously, if oxygen does not enter the seawater fast enough to match the consumption by the fish, then the oxygen level will decrease. In order to drive the diffusion of oxygen into the seawater, a certain amount of oxygen gas must still pass in bubbles through the water column and out into the air. The atmosphere within the bubbles of oxygen begins to change as they rise through the water column. Oxygen diffuses out into the water and also carbon dioxide diffuses into the bubble. The rising bubble therefore "scrubs" some carbon dioxide from the water limiting stagnation of the water. Thus, in reducing the flow of oxygen into the system, it is essential to demonstrate that the flow is still sufficient to remove the unwanted carbon dioxide before there is a measurable fall in oxygen level.

One obstacle to reducing the amount of oxygen required while transporting live fish is the reliability of the regulators employed. Some fail in transit, leading to massive mortality of the fish. Due to this uncertainty, it has been necessary to freight live fish with redundant oxygen supplies. For example, to transport 150kg of fish currently requires two D-size cylinders with independent control. There is twice as much oxygen present than is needed - so that, if one regulator fails the fish will survive on the remaining cylinder. This redundancy is expensive and not in line with current CASA requirements.

Methods

Controlling gas flow rate

When initiating experiments for this part of the research, it rapidly became clear that one of the major issues facing the industry in transporting live fish was the method of controlling gas flow from the cylinder into the transport bin.

Currently, transport of live fish is supported by standard BOC regulators and flow meters. These meters are intended for measuring bulk flow and when sending live fish, the needle valve is turned right down until nearly closed. So low is the flow that it does not even suspend the indicator bead. The flow rate must be below 1L/min and, with no accurate way of measuring the flow when setting it nor of ensuring the needle valve remains in the fixed position, it is likely that each flow meter currently in use is different and possibly can change from the setting during transit.

The common way most exporters of live fish use to gauge flow rate is by a feel for the amount of effervescence coming from the submerged air-stones. Such calibration by eye can be reasonably effective when done by very experienced personnel. The gas flow rate from a couple of randomly chosen operating cylinders was measured and they had been set to 750cc and 700cc/min. However, a simple gentle tap on the knob of the needle valve was enough to change the flow rate by up to 100cc/min. Clearly, this is not an ideal situation if fine gas flow control is required.

Flow meter

A flow meter was purchased and attached to fittings that allowed it to be placed in line between the air-stone and the cylinder regulator. Using this meter, it is possible to measure the flow rate of oxygen from each cylinder in a bin fairly accurately.

This flow meter (Fischer & Porter 10A6100 Purgemaster, FP-1/8-16-G-5/81; Elgas Bailey, Victoria) has the usual bead-and-tube reading design but the scale can register to 2.5L/min compared to the other regulators with a 12-16 L/min scale. This low scale is far more functional and within the operating range for live fish transport.

Fish species

Trials were undertaken with banded morwong (*Plectorhynchus* spp.) as they were available on-site at the S.E.A. Foods Sydney factory and then subsequently concentrated on coral trout (*Plectropomus* spp.).

Trials

An existing transport bin from S.E.A. Foods International was modified by installing a temporary partition in the fish compartment which allowed replicate experiments to be carried out at the same time. Gas cylinders supplied each compartment separately.

Fish were loaded into the divided fish compartment at a fish to seawater ratio of 1:1. Each side was then oxygenated for 1h at the original settings of the regulators (near 12 on the scale of the Fischer-Porter flowmeter, 600-700cc/min). Temperature, dissolved oxygen and pH were measured periodically.

After recordings were taken, fish were removed and rested. Further trials used fresh seawater and lower flow rates each time.

Results and Discussion

When the banded morwong were put into the bin, there was a period of high activity prior to the fish settling down. At a stocking density of 1:1 the fish had little room to move and were seen gulping amidst the proteinaceous froth on the water surface whipped up by the effervescing oxygen bubbles. Not surprisingly, it was observed that lower flow rates produced significantly less froth after 1h.

Oxygen flow rates of 700cc, 500cc and 350cc/min kept the dissolved oxygen level above 200% saturation though it is apparent in Figure 8 that as oxygen flow rate fell, the pH of the shipment water fell as well indicating increasing levels of carbon dioxide.

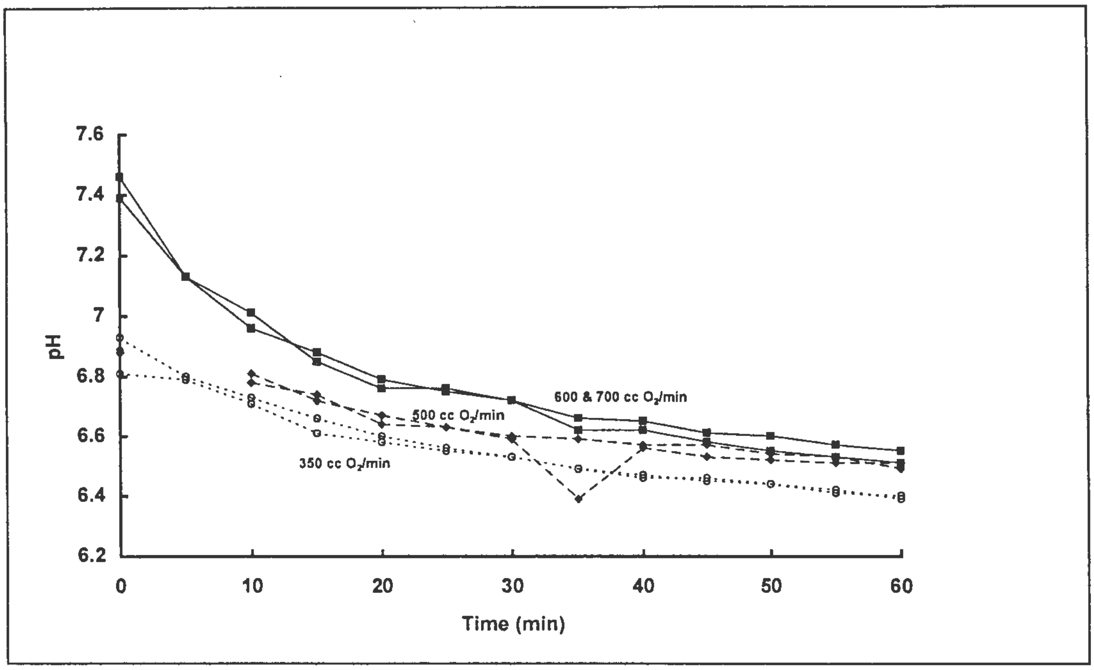

Figure 8. **Effect of reducing gas flow rate on the pH of seawater containing banded morwong (70kg) at a fish/water ratio of 1:1.**

From this data it appears that the lower gas flow rates are unable to remove carbon dioxide accumulation and thereby limit water stagnation. However, noting the large differences in the initial pH of the fresh seawater, recorded when the fish were first loaded, it seems that this is only relevant for a short period of high activity when the fish were first packed. Given the differences in initial pH starting point, the rate of fall in pH thereafter occurs at a similar rate for all gas flow rates.

A likely explanation for this response is that the fish are anaesthetising themselves by their own carbon dioxide. After an initial flurry of activity, they settle and produce carbon dioxide at a fairly steady rate.

It is of note that the data was obtained using the same fish after spelling for 1 to 2 hours between trials. However, if the fish were exhausted by re-use it would be expected that they would produce less carbon dioxide in the subsequent trials, not more.

In practice, if the conclusion of why the pH effect occurs is correct, one way to ensure that the carbon dioxide level is kept low while loading the fish would be to load the fish while using a factory gas cylinder with a high flow rate. Once the bin is fully loaded and ready to leave the factory, change to the low flow supply from the cylinder(s) in the bin.

Coral trout were much quieter when handled than the banded morwong and did not splash about when loaded into the bin. This lethargy, likely to be due to the 18°C water temperature of the holding tank (they originated from Cairns) is probably the primary reason that the pH of the water fell more slowly than it did for banded morwong at a flow rate of 350cc/min (Figure 9). Temperature is obviously going to be an important management tool when transporting live fish by this method.

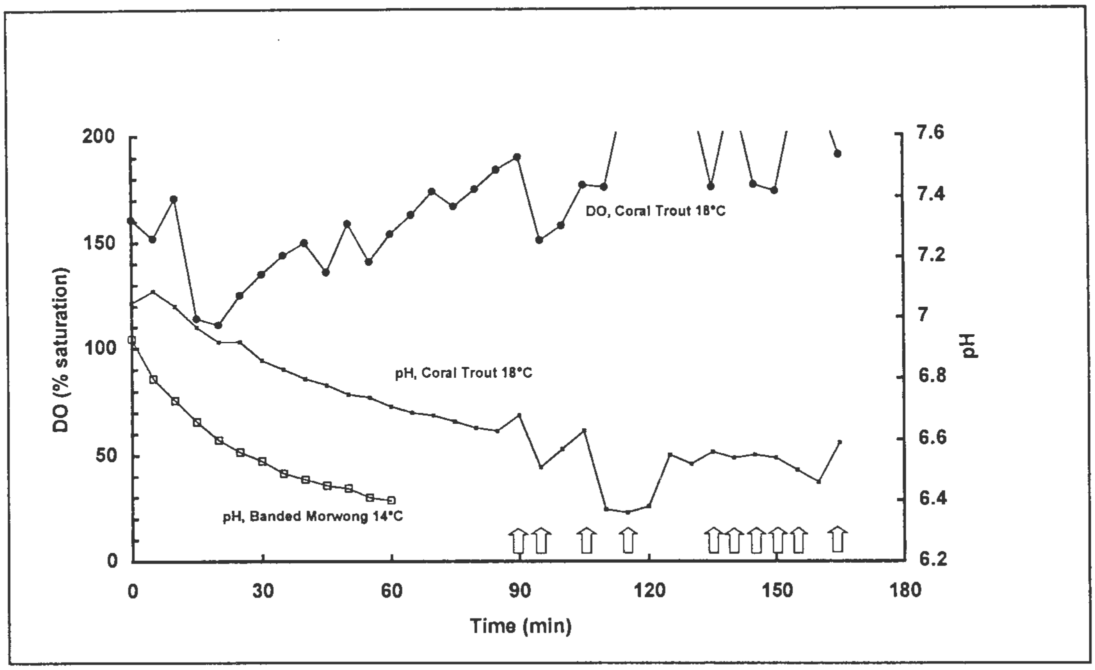

Figure 9. **Changes in dissolved oxygen and pH in seawater with 70kg of coral trout or banded morwong. Arrows represent periods of struggling by the coral trout.**

While coral trout were generally quiet, only gently ventilating their gills with slow movements of their opercula, they did sometimes become agitated particularly later during the 3-hour trial. These periods of struggling (indicated by arrows in Figure 9) caused the oxygen and pH readings to fluctuate but with the overall trend for both to respectively increase and decrease. In these trials, the fish compartment was so confined that just a few struggling fish have no room to move and end up flapping on top of the mass. When considering fish to water ratios, physical damage is obviously a factor under high density conditions.

The results of these trials indicate that 350cc/min is sufficient to maintain survival of 70kg of both banded morwong and coral trout. This rate may not be the lowest flow rate possible for sustaining the life of these species at this loading density.

SUMMARY OF FINDINGS

General considerations:

- the importance of consistent oxygen flow during transport necessitates involved personnel be fully conversant with the care for, assembly and operation of the system's equipment
- packout of fish needs to be as rapid and gentle as possible to ensure minimum stress to the fish
- demand for oxygen is high when fish are first loaded into the bin due to the handling stresses of packout
- degree of handling stress varies with fish species; banded morwong were extremely active when first loaded in the bin, whereas coral trout were calmer. Hence the initial oxygen demand in the system varies with species
- loading densities of 1:1, seawater to fish ratio, were achieved successfully
- high density loading of fish in the bulk transport bin means there is little room for fish to move; this has two effects - fish remain calmer but there is greater possibility of physical damage to fish if they do struggle

Water quality:

- initial oxygen consumption when fish are first loaded affects seawater quality by depletion of oxygen and accumulation of carbon dioxide (indicated by fall in pH)
- saturating the water with pure oxygen prior to loading results in sufficient dissolved oxygen present to cope with the initial high oxygen demand
- the pH falls rapidly during loading of fish in the bin due to the increased level of carbon dioxide
- once the fish calm down, the pH fall slows indicating that the rate of carbon dioxide accumulation is slowing
- the amount of dissolved oxygen in the seawater changed with changes in pressurisation of the cargo hold during flight

Respiration rate:

- project work has resulted in the development of a simple, functional respirometer which monitors oxygen consumption automatically and continuously
- respiration rate was high initially when fish were loaded, then slowed as the fish settled
- under the trial conditions used, the respiration rate for coral trout initially was around 75 mgO_2/kg fish/h then dropped to about 45mgO_2/kg fish/h after the fish had settled

Oxygen flowrate:

- transport of fish at high densities in insulated bins is only possible when the seawater is oxygenated at a flow rate sufficient to sustain respiration
- at that flow rate, the amount of oxygen available in the compressed gas cylinders needs to last considerably longer than the airfreight transport time to ensure a safety margin is allowed for trans-shipment mishaps

- the arbitrary flow rates used currently may be higher than necessary; given respiration rates determined in this work, simple calculations indicate that the flow rate is providing over four times that required
- the flow rate can be reduced significantly (to 350cc/min for 70kg fish) and still sustain coral trout survival
- the low flow rate was still sufficient to assist in the physical removal of accumulating carbon dioxide
- reducing the flow rate will allow smaller size gas cylinders to be used

BENEFITS

As identified in the original application, the major beneficiaries of this work are all live fish exporters. Additionally though, the CASA has been provided with data on which to base sound decisions with respect to granting approval for transport of live fish in bulk bins with compressed gas cylinders. Such approval is required from the 'appropriate authorities' in the country of export, the operator and the destination country.

Results from this project provide the live fish export industry with a sound basis by which to optimise transport of coral trout. The information gained allows exporters to make informed decisions about the most appropriate way to maximise survival while minimising freight costs for bulk bin shipments of live fish to distant markets.

Successful shipment of live fish can be highly lucrative. During the period of the project work undertaken, live coral trout landed in Hong Kong frequently achieved $60-$80/kg and in some Chinese markets, were commanding up to $120/kg. This compares to the price of around $10/kg obtained for chilled fillets. Hence, the option to land coral trout live in overseas markets is of obvious advantage for increasing export revenue and obtain maximum return from a resource.

The results from this project have assisted the live exporting industry to transport live coral trout to such markets using systems which minimise mortalities of fish.

Coral trout is a prized species in Asian markets but the demand is for premium quality. Landing fish live is the epitome of optimal quality and hence fulfils the market need. With reliable transport systems for shipping live product there, Australian suppliers will develop a reputation for excellent quality product, consistency of supply and therefore perpetuate demand for our product.

A spin-off benefit of the work in this project is the data obtained on respiration rates for the fish species tested could well be useful as base information for research work in other areas. To date, this information has not been available.

The completion of this work was unfortunately interrupted by the total ban on carriage of oxygen cylinders by airlines.

However, the existence of the ban resulted in links being established with Ansett Australia and others who were initiating related work to this project in an effort to provide the required information to CASA for deliberation on granting the appropriate approvals for allowing transport of live fish at high density.

FURTHER DEVELOPMENT

Further work needs to be done to see whether flow rates below 350cc/min are practical for sustaining 70kg of fish during transportation at a stocking density close to 1:1. This is important as this is the area where there could be a quantum step down from carriage of size E cylinders to size D.

It would be of benefit to conduct some 'real' airfreight trials with full monitoring of events during shipment to confirm the simulated findings of this project.

Now that a functional and easily operated respirometer has been developed, it would be very useful to obtain further information on the respiration rates of other frequently transported species.

CONCLUSIONS

The results of this project provide some answers to the live fish export industry on the requirements of coral trout during airfreight transport. Thereby allowing decisions to made for optimising transport of live fish to export markets.

The information gained through this work is also appropriate to the CASA now has data on which to base sound decisions with respect to granting approval for transport of live fish in bulk bins with compressed gas cylinders.

The outcomes of the research with respect to the project objectives are:

1. Water quality during transport

Quantified information on how fish react under transport conditions and the changes in demand for oxygen from loading and transport. This provides a basis for making recommendations for the appropriate handling and packout of fish for live export markets, although the findings here need to be confirmed with inflight monitoring.

2. Respiration rates

Information is now available on the respiration rate of coral trout within simulated bulk bin transport. Again, this provides the basis for recommending appropriate packout procedures. However, as above, the findings need to be confirmed inflight.

3. Oxygen flow rates

Data now available to show that flow rates as supplied to bulk transport systems currently are in excess of that required to keep the fish alive. Research results demonstrated that the flow rate can be reduced significantly, potentially allowing the use of a smaller size compressed gas cylinder.

These outcomes provide a good basis of information for the CASA to consider faced with granting approvals for transporting live fish on aircraft.

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