# Relationship between body weight and loading densities in fish transport using the plastic bag method 

R. FROESEInstitute of Marine Science, Kiel, FRG


#### Abstract

The transportation of live fish in sealed plastic bags was examined. Water parameters and loading densities were analysed. Based on several assumptions an estimation of oxygen consumption during transport was performed indicating that metabolism during transport was about three times higher than routine metabolism. There was some evidence that small fish were more affected by transportation stress, and that large fish need a longer starvation time before transport to reduce ammonia excretion sufficiently.


## Introduction

Since the early 1950s plastic bugs have been used for transporting fishall over the world (Fry \&Norris 1962). The major problems of this method are well known: changes in temperature; depletion of dissolved oxygen; increases in acidity and carbon dioxide; and accumulation of toxic nitrogenous wastes.

The use of insulated shipping boxes and the addition of extra plastic bags filled with ice or hot water alleviated the temperature problem. The replacement of the air above the transport water with pure oxygen was a straightforward method of maintaining a sufficient concentration of dissolved oxygen. Vifferentmethods have been suggested for the control of carbon dioxide and ammonia, e.g. anaesthetics, buffers for pH , ion-exchangers, and bacteriological nitrification (Nemoto 1957; Ranade 1957; McFarland \& Norris 1958; McFarland 1960; Dick 1975; Durve 1975; Taylor \& Solomon 1979; Amend, Croy, Goven, Johnson \& McCarthy 1982; Bower \& Turner 1982; Turner \& Bower 1982; Sado 1985). While the control of temperature and oxygen is common practice, none of the latter methods has established itself so far. They are possibly too expensive or too complicated for routine use, as any overdose of anaesthetics or erroneous back titration of the pH-buffer, or the use of a buffer without ammonia control, results in high shipping mortality.

McFarland (1960) pointed out that for a given quantity of water, oxygen, and loaded fish, the time taken to reach $50 \%$ mortality depends on the body weight of the fish. Winberg (1960, 1961) demonstrated a general relationship between metabolism and body weight for all fish.

The aim of this study was to find a relationship between water parameters, metabolism, body weight, loading density, and mortality.

Materials and methods
Commercial shipments of ornamental fish from Singapore to Kiel via Hamburg, Federal Republic of Germany were examined directly after arrival in Kiel. To avoid any changes in
the gas regime, the water for the chemical analysis was taken from the closed bags by pricking a conical tube with a valve through the underwater part of the bag. After chemical analysis of transport water the bags were opened and the fish were slowly acelimatized by pouring cuptuls of new water from the tanks into the bags, until the water volume was three or four times the initial quantity. This procedure lasted about half an hour. Then the fish from each bag were transferred into a separate tank ( $60-1001$ ). Water temperature was $22^{\circ} \mathrm{C}$. The tanks were aerated and filtered with sponge filters and illuminated for 12 h per day. Half of the water was changed every second day. Mortality was monitored for 8 days. During this time the fish were fed with commercial dry feed ( $\mathrm{Tetra} \operatorname{Min}^{\mathrm{R}}$ ) and were prophylactically treated against ectoparasites and fungi (methylene blue $3 \mathrm{mg} / \mathrm{l}$ and malachite green $0.06 \mathrm{mg} / \mathrm{l}$ ).

Dissolved oxygen was measured by a modified Winkler method (Anon. 1979), accuracy $+/-1 \cdot 2 \%$. Atmosphere volume in the bag was measured by sucking the gas through a gas meter, accuracy $+/-2 \cdot 5 \%$. pH was measured with a glass pH electrode, accuracy $+/-0 \cdot 3 \%$ (Anon. 1979). Ionized ammonia was determined using the photometric test kit Spectroquant supplied by E. Merck, Darmstadt, FRG, accuracy +/- $3 \cdot 3 \%$. Un-ionized ammonia was calculated according to Emmerson, Russo, Lund \& Thurston (1975). Ammonia production was calculated by multiplying concentration with water volume, assuming that initial ammonia concentrations in the transport water used by the shippers were negligible. All values were converted to $20^{\circ} \mathrm{C}$ (Winberg 1960,1961 ) and related to total fish weight and time of transport. The estimated error of this method was $+/-12 \%$.

Since no measurements were made when the fish were packed in Singapore, the following assumptions had to be made to evaluate oxygen consumption and ammonia production: the bags were inflated with pure oxygen; the used transport water was air-saturated and free of ammonia; water temperature was $25^{\circ} \mathrm{C}$ at the beginning and changed linearly during transit; transit starts 4 hours before take-off in Singapore.

The oxygen (in mg) available during transit is the sum of dissolved oxygen $\left(\left(\mathrm{O}_{2}\right)_{\mathrm{W}}\right)$, equation 1) and atmosphere oxygen in the bag $\left(\left(\mathrm{O}_{2}\right)_{A}\right)$, equation 2$)$ at the beginning of transport.

```
\(\left(\mathrm{O}_{2}\right)_{w}=V_{w} \times C_{25}\)
(Equation 1)
\(V_{W} \quad=\) water volume \(\left(\mathrm{dm}^{3}\right)\)
\(C_{25}=\) oxygen concentration in the water (ppm)
\(\left(O_{2}\right)_{A}=V_{G} \times F_{l} \times P_{L} \times P_{N}^{-1} \times T_{N} \times\left(T_{N+t}\right)^{-1}\)
\(V_{G} \quad=\) volume of the atmosphere \(\left(\mathrm{dm}^{3}\right)\)
\(F_{1} \quad=\) coefficient transforming oxygen from \(\mathrm{dm}^{3}\) into \(\mathrm{mg}=>1429\)
\(P_{L} \quad=\) air pressure at the end of transport
\(P_{N} \quad=\) normal air pressure \(=>1013.25\) (hectopascal)
\(T_{N}=\) normal temperature \(=>273 \cdot 15\) ( \({ }^{\circ} \mathrm{Kelvin}\) )
\(t \quad=\) temperature \(\left({ }^{\circ} \mathrm{C}\right)\)
```

Upon arrival, dissolved oxygen and temperature were measured. The amount of oxygen in the atmosphere $\left(\left(\mathrm{O}_{2}\right)_{A}\right)$ was calculated using the Bunsen coefficient (equation 3).
$\left(O_{2}\right)_{A}=C_{25} \times V_{G} \times P_{L} \times\left(P_{L}-P_{W}\right)^{-1} \times T_{N} \times\left(T_{N}+t\right)^{-1} \times \mathrm{a}$
$P_{W} \quad=$ vapour pressure of water (hectopascal)

```
P}w=6.1244+0.42056\timest+0.1736\times\mp@subsup{t}{}{2}+0.00012\times\mp@subsup{t}{}{3}+0.00001\times\mp@subsup{t}{}{4
    (fitted to tabulated values given in Anon. (1975)).
a = Bunsen coefficient
a}=1.7119-0.0628\timest+0.00125\times\mp@subsup{t}{}{2}-0.00001\times\mp@subsup{t}{}{3
    (fitted to tabulated values given in Anon. (1975)).
```

Total oxygen consumption in a bag $\left(\mathrm{O}_{2}\right)_{c}$ is evaluated by equation 4 .
$\left.\left(\mathrm{O}_{2}\right)_{C}=\left(\left(\mathrm{O}_{2}\right)_{W}+\left(\mathrm{O}_{2}\right)_{A}\right)\right)_{\text {starl }^{-}}\left(\left(\mathrm{O}_{2}\right)_{W}+\left(\mathrm{O}_{2}\right)_{A}^{i}\right)_{\mathrm{cnd}}$
All values for oxygen consumption were converted to $20^{\circ} \mathrm{C}$ (Winberg 1960), and related to loading densities and transit time. The estimated error of this method was $+/-14 \%$.

Results
A total of 100 bags containing about 3000 fish of 33 species were examined. These data are summarized in Table 1.

The ratio of water and gas volume in the bags was $2 \cdot 4$ units gas per unit water $(n=99$, $\mathrm{sd}=$ 0.57 ).

Total fish load (FL), oxygen consumption $\left(\mathrm{Q}_{\mathbf{c}}\right)$, and ammonia production (AP) in relation to body weight $(\mathrm{W})$ could be fitted by a $\log _{10}-\log _{10}$ transformed linear regression (equations 5, 6, 7; Figs 1 and 2).
$\mathrm{FL}=38.1 \times \mathrm{W}^{0.49} \quad \mathrm{~g} \mathrm{x} \mathrm{dm}^{-1} \quad(n=100 ; r=0.873) \quad$ (Equation 5)
$Q_{c}=1.29 \mathrm{x} \mathrm{W}^{0.51}$
$\mathrm{mg} \mathrm{x} \mathrm{h}{ }^{-1} \quad(n=95 ; r=0.885)$. (Equation 6)
$A P=0.20 \mathrm{X} \mathrm{W}^{0.64}$
$\operatorname{mgx~h} \quad{ }^{-1} \quad(n=50 ; r=0.851)$
(Equation 7)

Table 1. Values measured aftertransit: shipments from Singapore in scaled plastic hags

| Variable | L'nil | 11 | Median | Minimum | Maximum |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Transit time | h | 100 | 3.4 | 31 | 37 |
| Mortality | \% | 83 | 4 | 0 | 100 |
| Fish per bag | n | 100 | 50 | 1 | 250 |
| Fish per bag | 8 | 100 | 135 | 1.6 | 460 |
| Body weight | g | 100 | 2.7 | 0.07 | 11.4 |
| Fish load | $\mathrm{g}^{*} \mathrm{dm}^{-3}$ | 100 | $55 \cdot 5$ | 7.4 | $155 \cdot 4$ |
| Water volume | $\mathrm{dm}^{3}$ | 100 | $2 \cdot 3$ | $0 \cdot 1$ | 4.4 |
| Gas volume | $\mathrm{dm}^{3}$ | 99 | $5 \cdot 5$ | $0 \cdot 2$ | 12.9 |
| Temperature | ${ }^{\circ} \mathrm{C}$ | 100 | 23.9 | 19.5 | $27 \cdot 3$ |
| pH |  | 100 | 6.0 | $5 \cdot 4$ | $6 \cdot 5$ |
| Oxygen | ppm | 95 | $10 \cdot 6$ | $0 \cdot 3$ | 21.3 |
| Oxygen saturation | \% | 95 | 128.2 | $3 \cdot 5$ | 246.9 |
| Oxygen consumption | rngVh-' | 95 | 0.87 | $0 \cdot 21$ | $5 \cdot 46$ |
| NHI | $\mathrm{p}^{\mathrm{pm}}$ | 50 | $37 \cdot 1$ | $12 \cdot 1$ | 98.3 |
| $\mathrm{NH}_{3}$ | ${ }_{\text {ppm }}$ | 50 | 0.017 | 0.003 | $0 \cdot 121$ |
| Ammonia production | $m g^{*} g^{-1 *} h^{-1}$ | 50 | 0.013 | 0.003 | 0.102 |



Figure 1. Relationship between $\log _{10}$ fish load and $\log _{10}$ body weight in different fish species. Shipments from Singapore in sealed plastic bags.


Figure 2. $\log _{10}$ oxygen consumption and $\log _{10}$ ammonia production as $n$ function of $\log _{10}$ body weight in different Ash species. Shipments from Singapore in sealed plasticbags.

Overall mortality was analysed by the multiple regression method taking under consideration all measured parameters as independent variables. No satisfactory model was obtained. Mortality over all examined shipments was $12 \cdot 8 \%$ after 8 days. $5 \%$ arrived dead in the bags. The highest mortality in the tanks occurred on the day after arrival. (The raw data are available in Collected Reprints 1988, Institutfur Meereskunde).

## Discussion

Mortality could not be successfully related to any other parameters, probably because the shippers empirically eliminated all that could have helped to identify such relationships. The fact that $61 \%$ of the monitored mortality occurred after arrival indicates the importance of a careful acclimatization to the tank water (see also Eddy, Lomholt, Weber \& Johansen [1977] for the effects of sudden changes of pH ).

The method to estimate oxygen consumption from commercial shipments without measurements on departure required several assumptions which give the results the status of a hypothesis. Compared with general values given by Winberg $(1960,1961)$ and Pauly ( 1981 , 1982a, b) metabolism during transit was about three times higher than routine metabolism, and small fish seemed to be more affected by stress than large fish. Stress responses of fish to transportation were also described by McFarland (1960) and Specker \& Schreck (1980).

Ammonia production depends on the metabolic rate and the time and quantity of the last intake of food. The power linking ammonia production to body weight should be about the same as for oxygen consumption. This was signific antly not the case ( $t$-test, 0.05 level). Large fish produced more ammonia than suggested by their different metabolic rate. This might be explained by the practice of the shippers to keep all fish without food for $24-48 \mathrm{~h}$ before transport. As the time to empty the stomach is related to body weight (Faenge \& Grove 1979), 24 h might be too short for large fish. The difference between 24 and 48 h of starvation could explain the higher variance in the data for ammonia production compared with oxygen consumption.

Although the shippers of the examined fish used a ratio of about 3 parts oxygen to 1 part water (D. Seet, Coral Scene, Singapore, personal communication), the ratio on arrival was 2.4:1 only. This could be explained by a slight permeability of the polyethylene bags for gas. Although the bags were filled until they were taut on departure, they appeared deflated on arrival. The ratio on arrival was assumed to represent the actual available oxygen for the fish.

The shippers used empirical values for fish load, depending on the species, on the size of the fish, and on the expected transit time, including a safety period for delays (D. Seet, Coral Scene, Singapore, personal communication). An equation to estimate loading densities can be derived as follows:

At a volume of $2 \cdot 4 \mathrm{dm}^{3}$ oxygen per $1 \mathrm{dm}^{3}$ water and at a temperature of $25^{\circ} \mathrm{C}$, the quantity of available oxygen is about 3140 mg oxygen per $\mathrm{dm}^{3}$ water (equation 2). For a maximum transit time of 48 h , the fish in $1 \mathrm{dm}^{3}$ can consume about 65 mg oxygen per hour. Dividing this amount by oxygen consumption ( $Q_{o}$ )results in the number of fish which can be transported in $1 \mathrm{dm}^{3}$ water. When this number is multiplied with the average body weight (W) the maximum fish load (ML) results:
$M L=65 * Q_{c}{ }^{-1 *} W$
(Equation 8)

If the estimated oxygen consumption during transit (equation 6) is inserted in equation 8 then equation 9 results.

$$
\begin{equation*}
M L=50 * W^{0.49} \tag{Equation9}
\end{equation*}
$$

The exponent of this equation exactly matches the exponent of the fish load in the analysed shipments (equation 5). The multiplicative factor is about $30 \%$ higher. These $30 \%$ could be the above mentioned empirically determined safety factor.

## Conclusion

Transport conditions for fish in sealed plastic bags have been examined. Oxygen consumption under real transport conditions was determined. It was found to be about three times higher than routine metabolism.

Based on metabolic considerations, an equation for fish weight per unit water was developed. If a safety factor of $30 \%$ is included, this equation corresponds to the fish load empirically used by the exporters of ornamental fish.

Ammonia production of large fish was higher than suggested by their metabolic rate and there was some evidence that starvation time before transport should also be related to body weight.

## Acknowledgments

I thank Dr W. Nellen for his supervision of the study. Thanks are also due to all who helped me gain the data. Special thanks to Dr D. Pauly for many fruitful discussions.

## References

Amend D. F., CroyT. R., Goven B. A., Johnson K. A. \& McCarthy D. H. (1982) Transportation of fish in closed systems: methods to control ammonia, carbon dioxide. pH and bacterial growth. Transactionsofthe American Fisheries Sociery5, 603-11.
Anon. (1975) Tabellenbuch Chemie. VEB Deutscher Verlag fuer Grundstoffindustrie, Leipzig, ODR.
Anon. (1979) Deutsche Einheitsverfahrenzur Wasseruntersuchung. Verlag Chemie GmbH, Weinheim, FRG.
Bower C. E. \& Turner D. T. (1982) Ammonia removal by clinoptilolite in the transport of ornamental freshwater fishes. Progressive Fish-Cullurist 44, 19-23.
Dick G. L. (1975) Some observations on the use of MS 222 Sandoz with grey mullet. JournalofFish Biology 7, 263-8.
Durve V. S. (1975) Anaesthetics in the transport of mullet seed. Aquaculure 5, 53-63.
Eddy F. B., Lomholt J. P., Weber R. E. \& Johansen K. (1977) Blood respiratory properties of rainbow trout (Salmo gairdneri) kept in water of high $\mathrm{CO}_{2}$ tension. Journal of Experimenal Biology 67, 37-47.
Emmerson K., Russo R. C., Lund R. E. \& Thurston R. V. (1975) Aqueous ammonia equilibrium calculations: Effect of pH and temperature. Journal of the Fisheries Research Board of Canada 32, 2379-83.
Faenge R. \& Grove D. (1979) Digestion. In: Fish Physiology, Vol. 8 (ed. by W. S. Hoar, D. J. Randall \& J. R. Brett), pp. 208-11. Academic Press, New York.
Fry F. E. ]. \& Norris K. S. (1962) The transportation of live fish. In: Fish As Food (ed. by G. Borgstrom). Academic Press, New York.
McFarland W. N. (1960) The use of anaesthetics for the handling and the transport of fishes. California Fish and Game 46, 407-31.
McFarland W. N. \& Norris U. S. (1958) The control of pH by buffers in fish transport. California Fish and Game 44, 291-310.
Nemoto C. M. (1957) Experiments with methods for air transport of live fish. Progressive Fish-Culurist 19, 147-57.

Pauly D. (1981) The relationships between gill surface area and growth performance in fish: a generalization of von Bertalantfy's theory of growth. Mecresforschung8, 251-82.
Pauly.D. (1982a) Studying single-species dynamics in a multispecies context. In: Theory and management oftropical fisheries (ed. by D. Pauly \& G. I. Murphy). ICLARM Conference Proceedings 9.
Pauly D. (1982b) Further evidence for a limiting effect of gill size on the growth offish: the case of the Philippine goby (Mistichthy'sluisnensis). Philippine Journal ofBiology 11, 379-83.
Ranade N. R. (1957) Experimental air-shipment of carp fry in plastic bags. Indian Journal of Fisheries 4, 290-3.
Sado E. K. (1985) Influence of the anaesthetic quinaldine on some lilapias. Aguaculuure 46, 55-62.
Specker J. L. \& Schreek C. B. (1980) Slress responses to transportation and fitness for marine survival in coho salmon (Oncorhynchuskisuch) smolıs. Canudian Journal of Fisheries and Aquatic Sciences 37, 765-9.
Taylor A. L. \& Solomon D. J. (1979) Critical factors in the transport of live freshwater fish. III. The use of anaesthetics as tranquillizers. Fisheries Management 10, 153-7.
Turner D. T. \& Bower C. E. (1982) Removal of ammonia by bacteriological nitrification during the simulated transport of marine fishes. Aquaculture 29, 347-57.
Winberg G. G. (1960) Rate of metabolism and food requirements of fishes. Fisheries Research Board of Canada Translation Series No. 194.
Winberg G G. (1961) New in formation on metabolic rate in fishes. Fisheries Research Board of Canada, Translation Series No. 362.

