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

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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. Different methods 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

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275

# 276 R. Froese

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 acclimatized by pouring cupfuls 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°C. The tanks were aerated and filtered with sponge filters and illuminated for 12h 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 (Tetra  $Min^{R}$ ) and were prophylactically treated against ectoparasites and fungi (methylene blue 3mg/l and malachite green 0.06mg/l).

Dissolved oxygen was measured by a modified Winkler method (Anon. 1979), accuracy +/- 1.2%. Atmosphere volume in the bag was measured by sucking the gas through a gas meter, accuracy +/- 2.5%. pH was measured with a glass pH electrode, accuracy +/- 0.3% (Anon. 1979). Ionized ammonia was determined using the photometric test kit Spectroquant supplied by E. Merck, Darmstadt, FRG, accuracy +/- 3.3%. 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°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°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  $((O_2)_W)$ , equation 1) and atmosphere oxygen in the bag  $((O_2)_A)$ , equation 2) at the beginning of transport.

$$(O_2)_w = V_w \times C_{25}$$
(Equation 1)  

$$V_W = \text{water volume (dm^3)}$$

$$C_{25} = \text{oxygen concentration in the water (ppm)}$$

$$(O_2)_A = V_G \times F_I \times P_L \times P_N^{-1} \times T_N \times (T_N + t)^{-1}$$
(Equation 2)  

$$V_G = \text{volume of the atmosphere (dm^3)}$$

$$F_I = \text{coefficient transforming oxygen from dm^3 into mg = >1429}$$

$$P_L = \text{air pressure at the end of transport}$$

$$P_N = \text{normal air pressure = >1013.25 (hectopascal)}$$

Upon arrival, dissolved oxygen and temperature were measured. The amount of oxygen in the atmosphere  $((O_2)_A)$  was calculated using the Bunsen coefficient (equation 3).

$$(O_2)_A = C_{25} \times V_G \times P_L \times (P_L - P_W)^{-1} \times T_N \times (T_N + t)^{-1} \times a$$
 (Equation 3)

= vapour pressure of water (hectopascal)  $P_W$ 

= temperature (°C)

= normal temperature = >273.15 (°Kelvin)

 $T_N$ 

t

1)

- $P_{W} = 6.1244 + 0.42056 \text{ x } t + 0.1736 \text{ X } t^{2} + 0.00012 \text{ x } t^{3} + 0.00001 \text{ x } t^{4}$ (fitted to tabulated values given in Anon. (1975)).
- *a* = Bunsen coefficient
- $a = 1.7119 0.0628 \text{ x } t + 0.00125 \text{ x } t^2 0.00001 \text{ x } t^3$

(fitted to tabulated values given in Anon. (1975)).

Total oxygen consumption in a bag  $(O_2)_c$  is evaluated by equation 4.

$$(O_2)_C = ((O_2)_W + (O_2)_A))_{\text{start}} ((O_2)_W + (O_2)_A)_{\text{end}}$$
(Equation 4)

All values for oxygen consumption were converted to 20°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 2.4 units gas per unit water (n = 99, sd = 0.57).

Total fish load (FL), oxygen consumption  $(Q_c)$ , and ammonia production (AP) in relation to body weight (W) could be fitted by a  $\log_{10} - \log_{10}$  transformed linear regression (equations 5, 6, 7; Figs 1 and 2).

$FL = 38.1 \text{ x W}^{0.49}$	$g \ge dm^{-1}$	(n = 100; r = 0.873)	(Equation 5)
$Q_c = 1.29 \text{ x } W^{0.51}$	mg x $h^{-1}$	(n = 95; r = 0.885).	(Equation 6)
$AP = 0.20 \text{ X W}^{0.64}$	mg x $h^{-1}$	(n = 50 ; r = 0.851)	(Equation 7)

Variable	Unit	п	Median	Minimum	Maximum
Transit time	h	100	34	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		100	2.7	0.07	11.4
Fish load	g*dm <sup>-3</sup>	100	55.5	7.4	155-4
Water volume	dm <sup>3</sup>	100	2.3	0.1	4.4
Gas volume	dm <sup>3</sup>	99	5.5	0.2	12.9
Temperature	°C	100	23.9	19.5	27.3
pH		100	6.0	5.4	6.5
Oxygen	ppm	95	10.6	0.3	21.3
Oxygen saturation	%	95	128-2	3.5	246.9
Oxygen consumption	rngVh-'	95	0.87	0.21	5-46
NHI	ppm	50	37.1	12.1	98.3
NH <sub>3</sub>	ppm	50	0.017	0.003	0.121
Ammonia production	mg*g-1*h-1	50	0.013	0.003	0.102

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

278 R. Froese

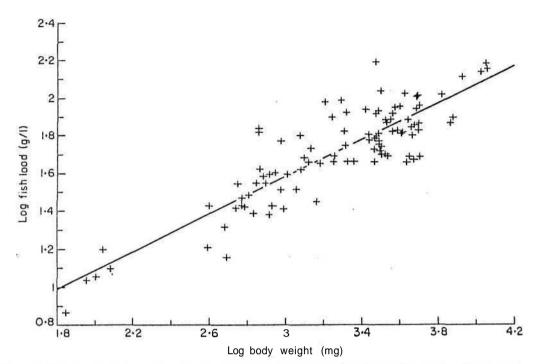


Figure 1. Relationship between  $log_{10}$  fish load and  $log_{10}$  body weight in different fish species. Shipments from Singapore in sealed plastic bags.

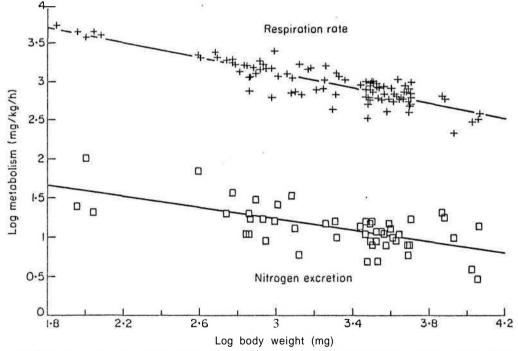


Figure 2.  $Log_{10}$  oxygen consumption and  $log_{10}$  ammonia production as n function of  $log_{10}$  body weight in different Ash species, Shipments from Singapore in sealed plastic bags.

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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, Institut fur 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 significantly 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 24–48h before transport. As the time to empty the stomach is related to body weight (Faenge & Grove 1979), 24h might be too short for large fish. The difference between 24 and 48h 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  $2.4 \text{ dm}^3$  oxygen per  $1 \text{ dm}^3$  water and at a temperature of  $25^{\circ}$ C, the quantity of available oxygen is about 3140mg oxygen per  $\text{ dm}^3$  water (equation 2). For a maximum transit time of 48 h, the fish in  $1 \text{ dm}^3$  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  $1 \text{ dm}^3$  water. When this number is multiplied with the average body weight (W) the maximum fish load (ML) results:

$$ML = 65 * Q_c^{-1} * W$$

(Equation 8)

## 280 R. Froese

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

 $ML = 50 * W^{0.49}$ 

(Equation 9)

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.

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