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Recent developments and improvements in ornamental fish packaging systems for air transport

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Abstract

Current ornamental fish packaging systems are characterized by very high fish loading densities and high metabolic wastes in the transport water after shipment. They focus mainly on management of the quality of transport water. Recent studies using the guppy as a model fish showed that post-shipment mortality could be reduced through enhancement of the stress resistance of the fish, and hence emphases should also be placed on the preparation of the fish for transport and recovery of the fish after shipment. Farmers can contribute significantly by applying nutritional prophylaxis before harvesting. Exporters may use the salinity stress test to identify fish lots of good quality for transport, apply health prophylaxis to eradicate parasites and optimize other techniques such as starvation of the fish or addition of salt to the transport water to enhance the stress resistance of the fish. Importers may adopt proper acclimation procedure and allow fish to recover in low salinity water to reduce post-shipment mortality. As the main bulk of post-shipment mortality is stress-mediated and occurs during the 1-week recovery period, the industry should consider revising the basis of the current warranty system for their customers, from death on arrival to cumulative mortality at 7 days post shipment (or death after 7 days, DA7), in order to cut down fish losses after shipment.

Keywords: air transport, fish packaging, nutritional prophylaxis, ornamental fish, post-shipment mortality, stress resistance

Introduction

In the ornamental fish business, the ability to meet the customers' needs for high-quality fish is always a critical factor. As most of the ornamental fish produced in Southeast Asia are destined for export, the fish must not only be attractive but also robust to withstand long air transportation. Since the 1950s when polyethylene bags were first used for live fish transport, the polyethylene-bag transport system has greatly reduced the shipping weight of ornamental fish consignments, and made them feasible for air transport. Nevertheless, the freight cost of fish consignments is still a major cost of the ornamental fish business. For consignments from Asia to the USA, shipping may cost more than the fish in the consignment (Lim & Chua 1993). Hence, the use of modern packaging technology for air transport to increase fish loading density and improve post-shipment survival is critical to the industry.

The key limiting factor to increase the fish loading density in a live-fish transport system is the deterioration of the quality of transport water due to accumulation of metabolic wastes. A variety of techniques have been developed to manage the quality of transport water during transport. They include starving fish before packaging (Phillips & Brockway 1954; Nemato 1957), lowering the temperature of transport water (Phillips & Brockway 1954; Norris, Brocato, Calandrino & McFarland 1960; Lim & Chua 1993; Teo & Chen 1993), addition of anaesthetics (Takashima, Wang, Kasai & Asakawa 1983; Teo, Chen & Lee 1989; Guo, Teo & Chen 1995a, b), ion exchange resin (Amend, Croy, Goven, Johnson & McCarthy 1982;

Bower & Turner 1982; Teo *et al.* 1989; Lim & Chua 1993), buffers (McFarland & Norris 1958; Amend *et al.* 1982; Teo *et al.* 1989) or drugs in the transport water (Ling, Chew, Lim, Koh & Lim 2000).

Singapore is the top ornamental fish exporting country in the world, with a 26% share in the global export market in 1998 (Anonymous 2000). The current ornamental fish packaging practice focuses mainly on the control of the quality of transport water. For ornamental fish consignments, the industry standard for warranty is 5% death on arrival (DOA); that is, exporters are expected to compensate customers for losses exceeding this standard. This paper presents an overview of ornamental fish packaging systems for air transport, with special reference to that used by Singapore exporters. It also reports recent developments in research on fish packaging technology, discusses possible improvements to the packaging system and proposes a new warranty system for the industry.

Fish packaging system

The system used for packaging ornamental fish for air transport is a closed one in which all factors to meet the requirements of the fish for survival are self-sustained. It involves packing the fish in sealed polyethylene bags filled with water that is usually pre-treated with chemicals or drugs, and over-saturated with pure oxygen. After packing, the bags with fish are placed in a styrofoam (polystyrene) box, usually four to eight bags to a box, to provide thermal insulation to prevent sudden changes in temperature of the transport water, especially when the consignments are in the cargo hold of aircraft (21–22 °C) during air transport.

The volume of transport water limits the number of fish that can be packed. The loading density of fish is expressed as biomass of fish (g) per unit volume of water (L). The loading density used by Singapore exporters generally increases with increasing body weight of the fish (Fig. 1), as large individuals consume less oxygen and produce less nitrogenous wastes per unit weight than small ones (Fry & Norris 1962). For consignments from Singapore to Europe with a 30-h transit time, the average loading density ranges from 22 g L^{-1} for neon tetra, *Paracheirodon innesi* (Myers) (average weight 0.22 g) to 272 g L^{-1} for goldfish, *Carassius auratus* (L.) (average body weight 13.8 g). They are correspondingly higher than those for foodfish juveniles, such as cyprinids and perch of

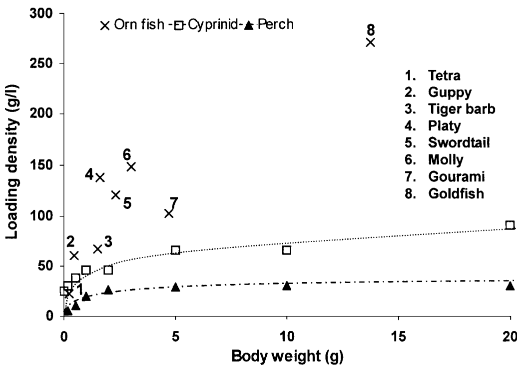

Figure 1 The average loading densities of eight common freshwater ornamental fish used by Singapore exporters. The standard densities used for foodfish juveniles (cyprinid and perch) in similar systems are shown for purposes of comparison (data derived from Berka 1986).

the same size (Fig. 1). Higher loading densities may be used if the transit time is shorter. Consignments to Asia with a transit time of 12–14 h, for instance, allow density increases of about 50%. Fish densities are decreased by about 20% for shipments to the United States (transit time 36–40 h).

The most important factor in live-fish transport is an adequate supply of dissolved oxygen. In Singapore, the volume of pure oxygen supplied to the transport bag used to be up to six times the volume of transport water, and it has now been reduced to three to four times the volume of water. Even at these reduced volumes, dissolved oxygen content is never a limiting factor. Several fish packaging experiments have recorded over-saturated oxygen content of above 10 mg L^{-1} (at 25 °C, 100% saturation = 8.26 mg L^{-1}) after 24–48-h shipment, even when there was high fish mortality of up to 20% (Froese 1988; Teo *et al.* 1989). Because of the high loading densities used, the volume of oxygen used for packaging ornamental fish is very high compared with the volume of water used for foodfish, such as cyprinid and perch juveniles (Berka 1986). The oxygen-to-fish ratio, expressed in volume of oxygen (mL) to the total weight of fish (g), is a good measure of the actual amount of oxygen used in relation to the fish biomass. For freshwater ornamental fish shipments from Singapore to Europe, the ratios range from 14 in large-sized fish such as goldfish to 120 in small-sized fish such as neon tetra (Fig. 2). All the ratios for fish from Singapore are similar to those used for cyprinid and perch juveniles, indicating that in terms of fish biomass, the amounts of oxygen used by the two groups of fish are similar.

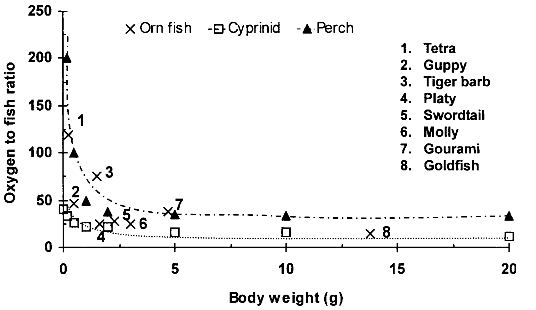

Figure 2 The average oxygen to fish ratios of eight common freshwater ornamental fish used in the Singapore packaging system. The ratios used for cyprinid and perch juveniles in similar systems are shown for purposes of comparison (data derived from Berka 1986).

Overview of the current industry practice

Conditioning of fish for packaging

Ornamental fish are conditioned before packaging in three stages, namely, prophylactic treatment, starvation and pre-packaging. Prophylactic treatment is usually performed on fish that are possibly infected with parasites or other pathogens, especially those reared in earthen ponds enriched with organic manure. The period of treatment varies from 1 day to a week or more, depending on the species and condition of the fish. Fish after prophylactic treatment are healthier, and hence more likely to withstand transport stress during the journey to their destination. Before packaging, fish are starved to prevent regurgitation of partially digested food material and to reduce the amount of excreta during transport. Singapore exporters starve their fish for 1 or 2 days for small fish such as guppy and neon tetra and 2 days or more for larger fish such as goldfish and koi, *Cyprinus carpio* L. Counting of fish is usually performed before packaging. After counting, the fish are pre-packed in polyethylene bags that are then placed in an air-conditioned room at 22–23 °C to enable them to acclimate to the packaging conditions, such as confinement, crowding, high pressure and low water temperature.

Quality control

The packaging process initiates severe stress in fish (Nikinmaa, Soivio, Nakari & Lindgren 1983; Maule, Schreck, Bradford & Barton 1988; Schreck, Solazzi, Johnson & Nickelson 1989; Barton & Iwama 1991), and when fish are unable to recover homeostasis during and after the process, mortality may ensue. It is therefore imperative for exporters to examine and evaluate the quality of their fish before packaging, and ship only healthy and good-quality fish with high stress resistance to increase survival after transport.

The current industry practice is to screen fish, starting from the time of counting fish to the actual packaging, based on visual inspection for pathological signs, such as dark body colour, closing of finnage, cloudy eyes, lack of appetite and lethargy or even the presence of external parasites. Pre-packaging is an important means of quality control, and fish that fail to adapt to the packaging conditions and show signs of sickness are eliminated from the shipment to reduce the risk of mass mortality.

Control of temperature of transport water

The objective of live fish transport is to maximize loading density and concurrently to maintain the fish in good condition on arrival. As the metabolism during transport is about three times higher than the routine metabolism (Froese 1988), the existing packaging system focuses on lowering the metabolic rate of the fish, to reduce oxygen consumption and accumulation of acidity, carbon dioxide and ammonia in the transport water. High water temperature reduces the loading density of fish due to the increased metabolic rate of fish. It results in faster bacteria growth and lower dissolved oxygen levels, leading to increased waste production and decreased availability of oxygen to the fish. Hence, the temperature of transport water is usually reduced to the level that the fish can tolerate, that is, 22 °C for tropical fish and 15–18 °C for temperate species. After pre-packaging, fish are allowed to acclimate to the packaging temperature in an air-conditioned room for 4–6 h. Thereafter, they are packed in water that has been cooled to the desired temperature at packaging.

During the winter months in importing countries, exporters usually attach a heat pack to the underside of the cover of the packaging box to prevent cold-shock to the fish. The heat generated from the heat pack induced by rocking movement will increase the temperature in the packaging system. In this case, the loading density of fish is reduced accordingly, due to the anticipated increase in temperature.

Control of metabolic wastes

As ornamental fish are packed in a small volume of water at a high loading density, the metabolic waste accumulates rapidly in the transport water. Ammonia and carbon dioxide are the two major metabolic wastes produced in transport water. Ammonia accumulates in transport water due to the excretion from fish and the bacterial action on fish excreta and dead fish. There are two forms of ammonia: the ionized (NH_4^+) ammonia and the unionized or free ammonia (NH_3). Only the unionized ammonia is able to pass tissue barriers and is toxic to fish. Fish may succumb if the toxic unionized ammonia in the tissue is above tolerable levels. Cation exchange resins such as clinoptilolite, a natural zeolite, is commonly used to remove ammonia from the transport water. The resins have the ability to absorb ammonia by selective ion exchange. They are either wrapped in a net bag or added directly into the transport water at 15–20 g L^{-1} of water. They do not control the accumulation of carbon dioxide in the transport water.

Bacterial growth is another major source of metabolic wastes. Bacteria not only increase the ammonia load and compete with fish for oxygen in the transport water, but also weaken or cause diseases. Drugs such as neomycin sulphate, methylene blue and acriflavine are added to the transport water to control bacterial growth.

Addition of salts to transport water

Osmoregulatory dysfunction is common in fish that are exposed to transport stress, and the addition of salt to transport water is effective in reducing the osmoregulatory dysfunction and other physiological responses to stress (Johnson & Metcalf 1982; McDonald & Milligan 1997), resulting in reduced fish mortality (Tomasso, Davis & Parker 1980; McDonald & Milligan 1997). In Singapore, coarse salt containing 95–98% sodium chloride is commonly used to reduce effects of stress of the fish. It is added directly to transport water at 0.5–3.0‰, and the concentration used varies with species and exporters.

Recent developments and possible improvements

Current ornamental fish packaging practices focus on minimizing stress imposed on the fish through the control of metabolic rate and the removal of metabolic waste from the transport water. There is insufficient attention to enhancement of the stress resistance of the fish, which would enable the fish to overcome the stressful conditions during transportation better. To improve the post-shipment performance of the fish, efforts from different sectors of the ornamental fish industry should be made to reduce the stress imposed on the fish and enhance their stress resistance.

Characteristics of transport water and fish mortality pattern after shipment

A survey on the packaging of guppy was conducted in Singapore to determine the quality of transport water and the fish mortality pattern after transport. A total of 104 bags were checked for the quality of transport water after a 40-h simulated shipment. Fish from 51 bags were stocked in aquaria to monitor their post-shipment mortality for 1 week. The water quality of the transport water after the 40-h shipment was characterized by relatively high temperature, super-saturated dissolved oxygen (at 27.5 °C, 100% saturation = 7.90 mg L^{-1}), low pH and high total ammonia and unionized ammonia (Table 1).

After 40-h simulated shipments, the mean fish mortality at unpacking was only 2.6% (Table 2). The median mortality indicated that in half of the bags, the mortality was 0.5% or less. The mean cumulative mortality at 7 days post shipment varied from 0% to

Table 1 Quality parameters of the transport water of guppy after 40-h simulated shipments

Parameter	*N*	Mean ± SD	Median	Minimum	Maximum
Temperature (°C)	104	27.34 ± 0.22	27.40	26.90	27.70
Dissolved oxygen (mg L^{-1})	104	18.50 ± 1.80	19.27	12.61	20.00
pH	104	6.17 ± 0.16	6.20	5.90	6.40
Total NH_3 (mg L^{-1})	88	28.26 ± 8.56	25.68	13.60	53.05
Unionized NH_3 (mg L^{-1})	88	0.031 ± 0.006	0.031	0.018	0.047

Table 2 Cumulative post-shipment mortality (%) of guppy during the 1-week recovery period after 40-h simulated shipment (fish density 100 fish L^{-1} or 35 g L^{-1}; number of bags = 51)

	Day after unpacking							
Parameters	0	1	2	3	4	5	6	7
Mean ± SD	2.63 ± 3.52	4.02 ± 5.96	4.87 ± 6.36	6.39 ± 6.83	7.74 ± 6.95	8.76 ± 7.14	10.14 ± 6.85	10.84 ± 7.19
Median	0.5	1.25	2.5	4.5	6.25	7.00	9.00	9.50
Minimum	0	0	0	0	0	0	0	0
Maximum	9.90	22.77	24.75	24.75	24.75	24.75	24.75	26.25

26%, with a mean mortality of 10.8% and a median mortality of 9.5%. The high variation is attributable to the varying quality of fish acquired from different farms (Table 2). Transportation of freshwater drum, *Aplodinotus grunniens* Rafinesque or largemouth bass, *Micropterus salmoides* Lacepde resulted in fish mortality that occurred either immediately or secondarily due to osmoregulatory dysfunction or infectious diseases (Johnson & Metcalf 1982; Carmichael, Tomasso, Simco & Davis 1984). At present, industry practice is for exporters to provide a warranty of 5% DOA to their customers. Exporters are expected to compensate customers for losses exceeding the standard. The mortality pattern of ornamental fish reflects the fact that only a small portion of the fish is killed directly by the reductions in the quality of transport water. Most post-shipment mortality was due to osmoregulatory dysfunction or stress-mediated diseases and occurred during the first week after transport. It is therefore suggested that the industry revises the basis of the warranty system to their customers from DOA to cumulative mortality at 7 days post shipment (or death after 7 days, DA7) to cut down mortality after arrival to customers.

Nutritional prophylaxis and harvesting at farm

Ornamental fish are often reared under intensive conditions and are subjected to physiological challenges from changes in water chemistry and culture procedures to behavioural interactions among fish (Wedemeyer 1997). Such chronic stress may manifest as health problems and physiological disturbance. Control of the stress response of ornamental fish to prepare them for transport should therefore start at the farming stage. Assuming that the primary cause of post-shipment mortality is partly associated with the low stress resistance of fish, use of stress resistance enhancement techniques before the transportation process may reduce post-shipment mortality of the fish.

Vitamin C, ascorbic acid, is an essential dietary nutrient for aquatic organisms. It assists in maintaining normal growth and collagen formation, improving disease resistance and reducing stress (Menasveta 1994). Nutritional prophylaxis using vitamin C supplementation worked well in enhancing the stress resistance of the guppy (Lim, Dhert, Chew, Dermaux, Nelis & Sorgeloos 2002b; Lim, Wong, Koh, Dhert & Sorgeloos 2000). Supplementing the diet of guppies with vitamin C at 2000 mg kg^{-1} for 10 days significantly improved the cumulative mortality of the fish at 7 days post shipment, from 23% in the control fish to 8% in the vitamin C-supplemented fish (Fig. 3). As fish were stressed during transport, the lower cumulative mortality in the vitamin C-supplemented fish could be attributed to the enhanced stress resistance of the fish. Guppies fed the vitamin C supplement were also more resistant to disease. When the fish were exposed to *Tetrahymena*, vitamin C supplementation reduced cumulative mortality at 7 days post shipment from 90% in the control fish to 14% in the vitamin C-supplemented fish (Fig. 3). Similarly, Li & Lovell (1985) reported that feeding mega-doses of vitamin C at 3000 mg kg^{-1} completely protected channel catfish, *Ictalurus punctatus* (Rafinesque) against experimental *Edwardsiella ictaluri* Hawke (enteric septicaemia) infections. The beneficial effects of vitamin C in enhancing the disease resistance were also recorded in other aquaculture species such as turbot larvae, *Scophtalmus maximus* (L.) (Merchie, Lavens, Dhert, Gomez, Nelis, De Leenheer & Sorgeloos 1996), postlarvae of *Penaeus monodon* (Fabricius) (Kontara, Merchie, Lavens, Nelis, De Leenheer & Sorgeloos 1997) and Atlantic salmon, *Salmo salar* L. (Waagbø, Glette, Raa-Nilsen & Sandnes 1993). Hence, fish farmers can contribute to the success of ornamental fish transport by use of nutritional prophylaxis using

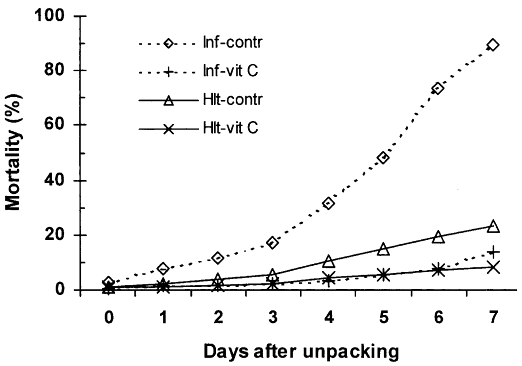

Figure 3 Cumulative mortality of vitamin C-supplemented guppies and the control fish during the 1-week recovery period after 40-h simulated shipment. Two batches of fish were tested, with one batch infected with *Tetrahymena*. Values represent the mean of three replicates (Inf-contr: infected fish fed control diet; Inf-vit C: infected fish fed vitamin C supplement; Hlt-contr: healthy fish fed control diet; Hlt-vit C: healthy fish fed vitamin C supplement).

dietary vitamin C supplementation to enhance stress resistance of their fish before capture for shipment.

During fish collection for shipment, farmers should take precautions to avoid injury to the fish, exposure of the fish to air for a prolonged period or overcrowding. These stress factors may be super-imposed by capture and, if not controlled, could be more severe than the effects of transport (Iversen, Finstad & Nilssen 1998).

Health prophylaxis

Parasitic infection is common in ornamental fish. In many cases, the infection is mild and asymptomatic, and the infection alone may not kill the fish. However, elevation of corticosteroid (mainly cortisol) levels in plasma as a primary response to stress can be immuno-suppressive, resulting in increased vulnerability of the fish to pathogens (Barton & Iwama 1991). Hence, an apparently harmless infection may turn lethal when ornamental fish are stressed and when immune function is suppressed during transport. Parasitic infection may also pre-dispose fish to secondary bacterial infections that further compromise the immune system and aggravate the problem of post-shipment mortality. A recent challenge test using guppies that were conditioned in the hatchery for 1 month demonstrated that the fish, when challenged with *Tetrahymena*, exhibited a significant decrease in its stress resistance without displaying visible signs of infection (Lim *et al.* 2000). However, when the fish were stressed during a 40-h simulated air transport, the challenged groups suffered heavy mortality of 24% at the time of unpacking, and 75% at 7 days post shipment. This is in contrast to control fish that had almost 100% survival at 7 days post shipment. Wise, Schwedler & Otis (1993) showed that handling, transport and confinement stress increased the mortality rate of channel catfish that were challenged with *Edwardsiella ictaluri*. A similar experiment on guppies demonstrated that treatment with chlorine dioxide at 20 mg L^{-1} in the transport water reduced post-shipment mortality significantly, both at the time of unpacking (4%) and at 7 days post shipment (31%). It is therefore critical for exporters to treat their fish for pathogenic infections before packaging.

Several protocols have been developed in Singapore to treat common bacterial pathogens such as *Aeromonas* and parasites such as *Tetrahymena*, *Gyrodactylus* spp. and *Cestodes* in guppy and *Hexamita, Costia* and *Trichodina* in angelfish. Most treatments involve bathing or feeding the fish with appropriate chemicals or drugs before packaging and treating fish with chemicals or drugs in the transport water (Table 3). In all cases, post-shipment survival rates of the treated fish are improved. In the latest development, the treatment protocols for *Tetrahymena* infection in guppy and *Hexamita* infection in angelfish have been simplified (Ling *et al.* 2000). Fish are treated directly in the pre-packaging and transport water with appropriate drugs (Table 3), and hence the need for tank-space and manpower to maintain the fish is reduced.

Starvation

Starving fish before live-fish transport has been used to improve the survival of fish during transport since the beginning of the 19th century (McCraren 1978). Its primary objectives are to reduce metabolic rate and improve the quality of transport water. The metabolism of blue gourami was found to decrease with increasing duration of starvation (Chow, Chen & Teo 1994a), but in guppy, starvation for 2–5 days before packaging did not affect the oxygen consumption of the fish (Teo & Chen, 1993). A recent study showed that starvation enhanced the stress resistance of the guppy. The stress resistance of guppies starved for 1 day was significantly higher than that of fish starved for 2 days, which in turn was higher than that of non-starved fish or fish starved for half a day or 3 days

Table 3 Improvements of post-shipment survival of guppy and angelfish through health prophylaxis

Fish species	Targeted pathogens	Treatment protocols	Survival rate at 7 days post shipment	Sources
Guppy	*Gyrodactylus* and *Cestode*	Bathe fish in 20 mg L $^{-1}$ formalin, 2 mg L $^{-1}$ acriflavine and 2‰ salt solution for 1 h before packaging and add 2 mg L $^{-1}$ acriflavine and 2‰ salt solution in transport water during transportation (36 h)	Increases from 78% in control fish to 99% in treated fish	Ling, Lim & Cheong (1996)
	Tetrahymena and *Aeromonas* sp.	One-day bath in 10 mg L $^{-1}$ chloramphenicol and 2‰ salt solution, followed by 1-h bath in 0.1 mg L $^{-1}$ malachite green, 50 mg L $^{-1}$ formalin and 2‰ salt solution, and then add 0.1 mg L $^{-1}$ malachite green and 2‰ salt to transport water during transportation (36 h)	Increases from 67% in the control group to 91% in the treated group	Loo, Ling & Lim (1998)
	Tetrahymena	Add 20 mg L $^{-1}$ chlorine dioxide to pre-packaging water (6 h) and transport water (40 h)	Improves from 61% in control fish to 89% in treated fish.	Ling *et al.* (2000)
Angelfish	*Hexamita, Costia* and *Trichodina*	Bathe fish in 2 mg L $^{-1}$ acriflavine and 2‰ salt solution for 3 days, and during the first 2 days, feed them with dried pellet incorporated with 2% metronidazole, twice a day (36 h)	Increases from 3% in control group, to 42% in group with bath treatment, and to 96% in group given full treatment	Ling & Khoo (1997)
	Hexamita	Add 3.5 mg L $^{-1}$ metronidazole (soluble form) in pre-packaging water (6 h) and transport water (40 h)	Improves from 58% in the control to 95% in treated group	Ling *et al.* (2000)

(Lim *et al.* 2000). Subsequent fish packaging experiments revealed that while starvation had no effect on mortality at unpacking, guppies starved for a day before shipment had significantly higher survival than control fish at 7 days post shipment (Fig. 4). Starvation for half a day or for 2 or 3 days did not improve post-shipment survival. The same study found that there were no significant differences in all parameters in the transport water between the control group and the group starved for 1 day. These results indicated that reduced fish mortality was attributable to the increased stress resistance of the fish. Hence for ornamental fish transport, the optimal duration for starving small fish such as guppy should be limited to 1 day only, as prolonged starvation may have adverse effects on the stress resistance of the fish, and hence increase post-shipment mortality.

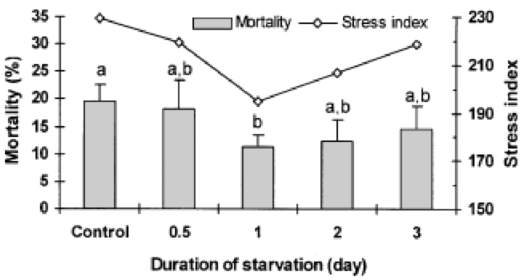

Figure 4 Effects of the duration of starvation on stress resistance of guppy and cumulative mortality of the fish at 7 days post shipment. Values represent the mean of three replicates. For fish mortality, the vertical bar represents standard deviation. Different alphabet letters indicate significant differences between means ($P < 0.05$).

Quality control: evaluation of stress resistance

The existing industry practice for quality control based on visual inspection is unreliable, as fish with low stress resistance are detectable only when they begin to exhibit disease symptoms after shipment. To overcome this problem, a salinity stress test was developed to evaluate the stress resistance of the guppy (Lim *et al.* 2000). It entails exposure of 10 guppies to osmotic shock in 500 mL saline water made up of pre-aerated water and salt (sodium chloride). The mortality of the fish is monitored and cumulative mortality is recorded at 3-min intervals over a 2-h period. Stress resistance is expressed as the stress index, which is obtained as the sum of 40 cumulative mortality readings recorded over the test period. A higher stress index is a result of either earlier or higher mortality or both, and indicates a lower stress resistance of the fish. The optimal salinities for use in the stress test are 35‰ for market-sized guppy and 30‰ for guppy fry (Lim *et al.* 2000). Optimal salinity levels for testing juveniles of other species are 35‰ for molly, 25‰ for platy and swordtail and 15‰ for black neon tetra, *Hyphessobrycon herbertaxelrodi* Géry (Lim, Cho, Dhert, Wong, Nelis & Sorgeloos 2002a). Salinity was chosen for the stress test because it is an important factor determining the osmoregulatory pattern of aquatic organisms (Lim *et al.* 2000). The use of saline water as a test medium has the advantages of being easy and convenient to prepare, and it is more stable than other parameters such as temperature, pH and ammonia. Compared with visual inspection, the stress test provides a more objective, reliable and quantifiable measure of the physiological conditions of the fish. A similar test using sodium nitrite as the test medium was also developed for comparing the stress resistance between fish cultured in freshwater and those in brackish water (Lim *et al.* 2000).

Temperature insulation using styrofoam box

Maintaining transport water temperature throughout transit is a major problem of the fish packaging system. Recent water quality data showed that the temperature of transport water increased from 22–23 °C at packaging to 26.9–27.7 °C after 40-h simulated shipments (Table 1), indicating poor thermal insulation of the styrofoam boxes used by the industry. The temperature may drop below 20 °C or 15 °C when the consignments are placed in unheated cargo holds (Chen & Teo 1990; Froese 1998). As the majority of the ornamental fish in the export trade are of tropical origin, and are relatively stenothermic, they are readily stressed by any drastic change in the environmental temperature and mortality will occur at sub-optimal temperature (Chow, Chen & Teo 1994b; Froese 1998). Hence, it is imperative that styrofoam boxes used for ornamental fish packaging should provide good insulation during the transit time. In Singapore, two sizes of styrofoam boxes, which measure 48.5 cm × 36.5 cm × 36.5 cm (thickness: 2.0 cm) and 60.5 cm × 45.5 cm × 30.5 cm (thickness: 2.0 or 1.30 cm), respectively, are most commonly used. Froese (1998) recommended that cube-shaped styrofoam boxes with at least 2.5-cm wall thickness should be used for ornamental fish packaging to reduce heat loss during transport. Further research work on improving the insulating property of styrofoam boxes to cut down fish loss due to thermal stress should be conducted.

Management of quality of transport water

Table 1 shows that the pH of transport water after 40-h simulated shipment is below the acceptable level of 7.0–8.5 for guppy and many other tropical ornamental fish (Sandford 1995). The low pH readings reflect a high level of carbon dioxide in the transport water due to the low carbon dioxide stripping efficiency in the closed packaging system (Colt & Orwicz 1991). At high carbon dioxide and low pH levels, the oxygen-carrying capacity of blood is markedly reduced through the Bohr effect, and fish mortality may occur even if oxygen levels are within acceptable ranges (Hoar 1984). A sudden drop in pH may also cause gill damage that may lead to osmoregulatory dysfunction (Tomasso *et al.* 1980). In addition, high concentration of carbon dioxide may anaesthetize fish in the transport water (Bell 1964; Gebhards 1965). Although buffers such as tris-buffer were effective in stabilizing the pH of transport water (Teo *et al.* 1989), the resulting higher pH may increase the amount of free ammonia. The application of buffers in fish transport is therefore limited.

Ammonia toxicity to fish depends upon the concentration of unionized ammonia, and its relative proportion increases with increasing pH and temperature and decreasing salinity (Emerson, Russo, Lund & Thurston 1975). Despite the low pH of the transport water, the unionized ammonia (mean 0.029 mg L^{-1}; median 0.030 mg L^{-1}) exceeded the acceptable level of 0.02 mg L^{-1} for intensive fish culture (Wedemeyer 1997). The high ammonia level in transport water may cause reduced excretion of ammonia by the fish and consequently a build-up of ammonia in the fish tissues (Chen & Teo 1990). Brockway (1950) suggested that ammonia may inhibit the ability of haemoglobin to combine with oxygen, altering the oxygen-carrying capacity of the blood.

The current practices aimed at reducing the amount of metabolic wastes in transport water, such as fasting, lowering the temperature of transport water and use of clinoptilolite, are insufficient to lower the ammonia levels and stabilize the pH in transport water to acceptable levels. Anaesthetics have been shown to be effective in lowering metabolic rates by reducing the motor activity of fish (McFarland 1960), and may be used to reduce metabolic waste production further. Teo *et al.* (1989) reported that 2-phenoxyethanol was effective in reducing the ammonia excretion of the guppy. Guo *et al.* (1995a) found that 2-phenoxyethanol and quinaldine sulphate significantly reduced ammonia and carbon dioxide excretion by platy. Anaesthetics may be used to limit the stress responses of fish, but conflicting reports exist on this subject. Some researchers reported that rapid anaesthesia before handling was effective in reducing or even eliminating the stress responses in many fish species (Strange & Schreck 1978; Tomasso *et al.* 1980, Davis, Parker & Suttle 1982, Limsuwan, Limsuwan, Grizzle & Plumb 1983; Robertson, Thomas & Arnold 1988). Guest & Prentice (1982) found that in blueback herring, *Alosa aestivalis*, the anaesthetized fish had higher survival than non-dosed fish after transportation. Tomasso *et al.* (1980) and Carmichael *et al.* (1984) reported decreases in stress response in hybrid striped bass and largemouth bass when the fish were anaesthetized before capture and kept sedated during transport. Robertson *et al.* (1988), on the contrary, found that this method was ineffective in red drum, *Sciaenops ocellatus* (Linnaeus). Other researchers have also reported that fish treated with sedating doses did not exhibit lower stress responses (Strange & Schreck 1978; Davis *et al.* 1982).

The use of anaesthetics in the transport of ornamental fish has not been fully explored. Teo *et al.* (1989) reported zero mortality in a guppy packaging experiment using 2-phenoxyethanol at 0.11 and 0.22 $g L^{-1}$ after a 20-h simulated shipment. Although the fish loading density used in the experiment (50 L^{-1}) was only half the standard density (100 L^{-1}) used by the industry for a 40-h shipment, the results indicated that the potential application of anaesthetics in ornamental fish packaging is promising, and that it warrants further research.

Enhancement of stress resistance of fish using salts

During the air transportation process, fish are constantly subjected to a series of stressors starting from capture at farms to packaging for air transport, the adverse water quality and crowded and over-pressurized conditions during transport. It is well known that stress leads to osmoregulatory dysfunction (Harrell & Moline 1992; Weirich, Tomasso & Smith 1992) and causes fish mortality (Tomasso *et al.* 1980). The addition of salt to transport water has been found to be effective in reducing osmoregulatory disturbances and fish mortality in several foodfish species (Carneiro & Urbinati 2001). Similarly, when guppies were held in saline water for 40 h, their stress resistance increased with increasing salinity to 9‰, which is close to isotonic conditions (Lim *et al.* 2000, Fig. 5). Subsequent experiments showed that when guppies were packed in saline water of 1‰, 3‰ or 9‰ and stocked in their respective salinities for recovery (with 30% daily dilution with freshwater) after shipment, all three treatments displayed a significantly lower cumulative mortality than the control group at 7 days post shipment (Fig. 6).

While there is clear evidence on the beneficial effects of salts in live-fish transport, there were considerable variations in the recommended amounts of salt used for different species. Johnson (1979) recommended a low concentration of 2‰ salt as a guideline for live-fish transport, while Carneiro & Urbinati (2001) found that 6‰ salt was most effective for minimizing the physiological response of matrinxã, *Brycon cephalus* (Günther), to transport stress. Weirich & Tomasso (1991) recommended that red drum juvenile should be transported in water that is nearly isosmotic to plasma (11‰). Similarly, a concentration of 10‰ salt was found to be effective for hauling and transport of striped bass, *Morone saxatilis* (Walbaum) (Powell 1970; Mazik, Simco & Parker 1991; Harrell 1992).

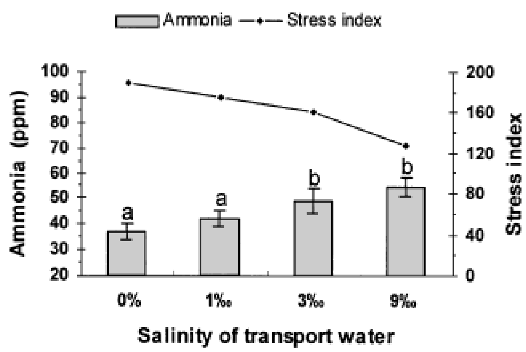

Figure 5 Effects of salt content on the stress resistance of guppy and the total ammonia content in transport water. The value represents mean of four replicates. For total ammonia content, the vertical bar represents standard deviation. Different alphabet letters indicate significant differences between means ($P < 0.05$).

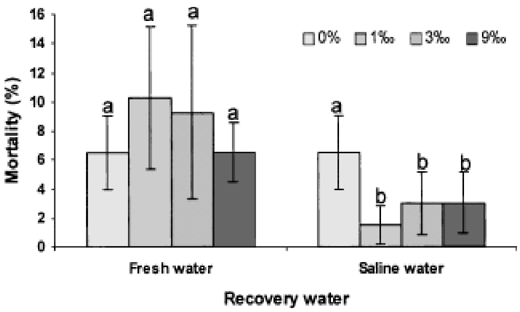

Figure 6 Effects of salt content in the transport water on cumulative mortality of guppy at 7 days post shipment. Two batches of fish were tested and they were stocked in freshwater or in water with initial salinity similar to their transport water (with daily 30% dilution with freshwater) during the 1-week recovery period after shipment. The value represents the mean of four replicates and its standard deviation. Different alphabet letters within the same group of recovery water indicate significant differences between means ($P < 0.05$).

In Singapore, the salt concentration used for packaging ornamental fish is 0.5–3.0‰, which is much lower than 9‰ recommended by Cole, Tamaru, Bailey, Brown & Ako (1999). The experiment with guppy showed a drastic increase in the total ammonia content when the salinity of transport water was increased to 3‰ or 9‰, due to a decrease in the efficiency of clinoptilolite (Fig. 6). These results suggest that among the three effective salinities ranging from 1‰ to 9‰, guppies should be packed at 1‰. Higher salinity should be avoided as it may result in undesirable quality of the transport water.

Acclimation and recovery of fish after shipment

On arrival, ornamental fish should be allowed to recover from transport stress in tanks and acclimate to local water conditions before being distributed to retailers. The acclimation operation includes floating sealed bags in recovery tanks until the temperature difference between the transport water and the recovery water is less than 2 °C. This may take about 30 min for a temperature difference of 4–5 °C. The pH and hardness of the recovery water should also be close to the optimal values for the respective species.

The addition of salt to the recovery water to enhance the stress resistance of the fish is effective in reducing post-shipment mortality. Guppies stocked in freshwater for recovery after transport exhibited no significant differences in cumulative mortality at 7 days post shipment among the three groups of fish packed in saline water (1‰, 3‰ and 9‰) and the control group (Fig. 6). However, when the fish were stocked in their respective salinity of transport water for recovery (with 30% daily dilution with freshwater), all three salinity treatments displayed a significantly lower cumulative mortality than the control group. Fish packed in 1‰ also had significantly higher survival in water of the same salinity than when placed in freshwater during recovery. These results suggest that the addition of salt, even only 1‰, is critical to the recovery of the guppy after transport. Similar beneficial effects of salt for recovering and maintaining osmotic homeostasis during the recovery stage from transport stress were reported for other fish species. They include 5‰ for hybrid striped bass fingerlings (female white bass, *Morone chrysops* (Rafinesque) × male striped bass, *M. saxatilis*) raised in freshwater (Reubush & Heath 1997) and 10‰ for striped bass (Mazik *et al.* 1991).

Conclusion

The current state of the art in the packaging of ornamental fish focuses mainly on the control of metabolic waste products to reduce the stress imposed on the fish during transport. The existing packaging system is characterized by a very high fish loading density, which helps to reduce the freight cost of the fish consignment, but at the same time, leads to very high ammonia and carbon dioxide, and low pH in the transport water at unpacking. Deterioration of water quality may cause severe stress to the fish and would result in about 10% cumulative mortality at 7 days post shipment.

Stress resistance is an important factor determining the post-shipment performance of the fish. However, insufficient attention has been given to enhancing the stress resistance of the fish to increase their chances of survival after transport. To increase the loading density and reduce post-shipment mortality of ornamental fish, efforts should be made to lower the stress responses of the fish and enhance their stress resistance. As the effects of transport of ornamental fish extend beyond the actual transportation period, emphasis should also be placed on the preparation of the fish for the transport and recovery of the fish after shipment. Control of the stress response of ornamental fish should start on the farm. Farmers can contribute by applying nutritional prophylaxis before capture and minimize stress to the fish during the capture operation. Feeding the guppy with diets supplemented with vitamin C at 2000 mg kg $^{-1}$ for 10 days enhances both disease resistance to *Tetrahymena* and the stress resistance of the fish, leading to reduced post-shipment mortality.

For exporters, the visual inspection for pathological problems is not satisfactory, as parasitic infection could be mild and asymptomatic, and the fish may show symptoms only after shipment. Exporters may use the salinity stress test to identify fish lots of good quality for transport to reduce fish loss after shipment. Quality enhancement through health prophylaxis to eradicate parasites would significantly improve the cumulative mortality of ornamental fish at 7 days post shipment. A new treatment protocol involving treatment in pre-packaging and actual transport water has been developed to eliminate the need of tank maintenance, thus leading to a saving in manpower and space. Recent experiments using guppy suggested that starvation for 1 day is effective in enhancing their stress resistance. The fish should be starved for an optimal duration only, as prolonged starvation leads to a decrease in the stress resistance of the fish. A low concentration of salt should be used to enhance the stress resistance of guppy, both in the transport water and in the recovery water after shipment. High salinity should be avoided as the salt content reduces the efficiency of clinoptilolite and lowers the quality of transport water.

Data on the water quality of transport water suggest that the current practice in reducing the metabolic wastes in transport water is insufficient to lower ammonia and stabilize pH to acceptable levels. Use of anaesthetics to lower the metabolic rate and enhance the stress resistance of the fish could be a promising solution, but it warrants further research. To lower fish loss due to thermal shock or heat loss, further research on the design of styrofoam boxes with better insulation properties is needed.

Finally, as the mortality is stress-mediated and occurs during the 1-week recovery period, the industry should consider revising the basis of the warranty system to their customers, from DOA to cumulative mortality at 7 days post shipment (or death after 7 days, DA7) to reduce fish losses after shipment.

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