Monitoring the chain of custody to reduce delayed mortality of net-caught fish in the aquarium trade

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Abstract

Information obtained from hobby magazines and from informants in the aquarium trade during the mid-1980s indicated that marine aquarium fish had high acute mortality (50%) on the reef due to capture with cyanide, and had 30% mortality on average at each step of the chain of custody — from the village level, through export facilities, to import facilities, and to retailers in North America. More recent data show there is some reduction, although there continues to be high rates of mortality in the marine aquarium trade. The scientific literature also demonstrates that fish experience cumulative stress from being netted, bagged, crowded, and exposed to changes in pH, temperature, salinity, dissolved oxygen, light, and from the accumulation of ammonium ion in the bags (which becomes toxic un-ionised ammonia when the bags are opened). It is believed that with better capture methods (e.g. nets), as well as better handling and shipping practices, it is possible to reduce the mortality at each step of the chain of custody.

It is difficult to obtain accurate marine fish mortality information regarding cyanide-caught fish because those involved fear regulation and/or prosecution for dealing in fish captured by illegal means. Research is needed (with marine fishes held in sealed plastic bags) to determine the range of environmental conditions encountered during transport from exporting to importing countries. Research with freshwater fishes has been successful in prolonging survival in shipping bags by adding chemical additives to inhibit the proliferation of bacteria, neutralize excreted ammonia, buffer pH, and by sedating the fish to reduce their metabolism. Similar research is needed with marine aquarium fish. We hope to demonstrate that with better post-harvest care and handling it is possible to markedly reduce mortality rates of marine ornamental fish during collection and transport.

Introduction

Plastic bags were first used by tropical fish importers to package aquarium fish for transport by air and/or in motorized vehicles in the early 1950s (Miller 1956). Marine aquarium fish were exported by Earl Kennedy from the Philippines in plastic bags starting in 1958 (Robinson 1985). In 1962, Kennedy noticed greater mortalities of marine aquarium fish in his export facility associated with fish obtained from collectors on Lubang Island, south of Manila. He learned that the fish were being collected with sodium cyanide. The marine aquarium trade expanded in the 1970s, fuelled by an abundant supply of cheap fish caught with cyanide. Kennedy left the trade in disgust after he accompanied air shipments and witnessed high mortalities of the fish after their arrival in the US. Dempster and Donaldson (1974), at the Steinhart Aquarium, conducted histological studies during the mid-1960s on marine fish obtained from California waters that were experimentally exposed to sodium cyanide. They found damage to internal organs such as the liver, kidney, spleen and brain. The tissue damage matched that found in marine aquarium fish imported from the Philippines.

Rubec (1986, 1987a) summarized information from various aquarium hobby magazines and from sources in the industry concerning mortality rates for marine aquarium fish in the aquarium trade. It was estimated that 50% of the fish targeted with cyanide died from acute doses on the reef, and that there was on average 30% delayed mortality at each step of the chain of custody. It was estimated that the cumulative mortality through the four steps of the chain of custody (from villages, to export, import, and then to retail facilities) was greater than 80%, if one excluded the acute mortality on the reef (Rubec 1987b). Including the mortality on the reef, the cumulative mortality from reef to retailers was estimated to be greater than 90% (Rubec and Soundararajan 1991). The papers summarized evidence showing that delayed mortalities

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were associated with cellular damage and physiological impairments resulting from exposure to cyanide (Rubec 1986, 1987a).

However, there are those in the aquarium trade who have maintained that the problem is not cyanide, but that it is all "stress, stress, stress" (Goldstein 1997). Goldstein cited a scientific study by Hall and Bellwood (1995) in which damselfish were experimentally exposed to 10 milligrams per litre (mg L^{-1}) cyanide for 90 seconds. He asserted that high mortalities were associated with stress and that the highest mortalities were associated with fish that were both stressed and starved. He stated that there was no evidence that anaesthetic doses of cyanide caused either gut epithelial changes or more mortality than occurred with net-caught fish. Another assertion in Goldstein's (1997) magazine article was that, based on the evidence to date, net collecting did not deliver healthier fish than collecting with cyanide. No evidence was presented. Rubec et al. (2001) discussed mortalities in the aquarium trade and acknowledged that high delayed mortalities of marine fish were probably associated with a variety of factors, including cyanide, stress, ammonia, oxygen depletion, disease, and starvation. It is necessary to consider all factors influencing the fish in order to reduce mortalities occurring in the aquarium trade.

There are many factors that lead to mortalities of marine aquarium fishes, including physical damage and the use of chemicals such as sodium cyanide during collection, inferior water quality, poor handling, disease, and stress at all stages during collection and transport (Wood 2001; Wabnitz et al. 2003). Sadovy and Vincent (2002) stated that mortality levels in both the live food and live aquarium fish trades range from a few per cent to 80% or more for cyanide-caught fish and/or where poor capture, handling and maintenance practices produce stressed animals. The source of the mortality, however, is not always clear.

The problems that must be overcome for the successful transportation of live fish are many and diverse (Norris et al. 1960; Fry and Norris 1962). The primary problem arises from the water's low capacity for oxygen, together with its low capability to dissipate the end products of fish metabolism. The secondary problem is that of handling. In delicate species, abrasion needs only to remove the mucus from a fraction of the area of the skin in order to rob the fish of essential protection from osmotic stress. In addition, many fish are so stimulated by handling that they readily accumulate dangerous levels of lactic acid in their blood. Excessive changes in temperature are also deleterious.

Scientific studies to determine factors causing mortality

Hanawa et al. (1989) studied the response of humbug damselfish (*Dascyllus aruanus*) to cyanide. Groups of 10 humbug damselfish were dipped into several concentrations (25 or 50 mg L^{-1}) of cyanide ion (CN^-) for either 10, 60 or 120 seconds (s); mortality was measured within 96 hours. Test damselfish exhibited no mortality after being dipped in 25 mg L^{-1} CN^- for either 10 s or 60 s. There was 60% mortality after exposure to 25 mg L^{-1} CN^- for 120 s. Likewise, there was no mortality after exposure to 50 mg L^{-1} for either 10 or 60 s; but 100% mortality occurred within 96 hours (h) after CN^- exposure for 120 s. Under stressed conditions (being bagged) previously non-lethal exposures (50 mg L^{-1} CN^- for 60 s) were 100% lethal. Hence, both stress and cyanide resulted in higher mortality after exposure to CN^- for a shorter time period. There was impairment of oxygen consumption by the liver tissue of test fish documented 2.5 weeks post-exposure. Hanawa et al. (1989) concluded that handling stress in combination with anaesthetic doses of CN^- could in part explain the delayed mortality associated with CN^- use in the tropical fish trade.

Hall and Bellwood (1995) assessed delayed mortalities of groups (16 per group) of damselfish (*Pomacentris coelestis*) exposed to cyanide, stress and starvation, alone and in various combinations, over a 13-day period. With each factor separate, the cyanide-only exposure (which also involved handling) resulted in the highest delayed mortality (37.5%), followed by stress-only (25%) and starvation-only (0%). Among the paired combinations, stress+starvation produced the highest mortality (66.7%). The stress-only condition and the handling control both had 25% mortality, indicating that those conditions were stressful to the fish. The results demonstrated that cyanide influenced the delayed mortality both alone and in combination with the other factors. While starvation-only did not produce mortality during the experimental period, the percent mortalities for cyanide+starvation and stress+starvation indicate the importance of starvation in combination with the other factors.

Concurrent conditions

During shipping, several environmental parameters (pH, dissolved oxygen, carbon dioxide, ammonia, temperature) change concurrently in sealed polyethylene plastic bags (McFarland and Norris 1958; Fry and Norris 1962). This makes it difficult to infer which environmental parameters may have killed the fish. A study by Chow et al. (1994) is the only one that has separately determined levels of each environmental parameter inducing 50% mor-

Table 1. **Per cent survival and per cent mortality of damselfish, *Pomacentrus coelestis*, exposed to cyanide, stress and starvation alone and in various combinations (Hall and Bellwood 1995).**

Treatment	Per cent survival	Per cent mortality
Cyanide-only	62.5	37.5
Stress-only	75.0	25.0
Starvation-only	100.0	0.0
Cyanide + stress	75.0	25.0
Cyanide + starvation	66.7	33.3
Stress + starvation	33.3	66.7
Cyanide + stress + starvation	58.3	41.7
Handling control 2	75.0	25.0
No handling control 1	83.3	16.7

tality over time (median tolerance limits – 48 h LD_{50}) on a marine aquarium fish species, the common clownfish (*Amphiprion ocellaris*). They also monitored changes in the levels of environmental parameters over 48 hours resulting from simulated transport of individual clownfish in sealed plastic bags. The median tolerance limits were 1.35 milli-molar (mM) total ammonia (0.079 mg L^{-1}), 57.22 micromolar (µM) un-ionised ammonia (0.003 mg L^{-1}), pH 5.5, and for temperature the upper limit was 34.46°C and the lower limit 19.49°C. It is of interest to note that environmental conditions monitored in the bags over 48 hours (temperatures near 25°C, pH from 8.45 to 6.97, total dissolved ammonia ≤0.36 mM, and un-ionised ammonia ≤1.9 µM) did not reach the median tolerance limits. Although water quality conditions were not incipiently lethal, some of the clownfish died in the bags. It was not clear how the parameters in combination influenced mortality of the clownfish (40% at 48 hours).

Mortalities through chain of custody

Indonesian exporter

Schmidt and Kunzmann (in this issue) monitored mortalities in shipments of marine aquarium fish obtained from middlemen and collectors at a field station in Goris and transported to a central export facility in Denpasar, on Bali in Indonesia. Large variations in mortality were found between shipments and between species. Total losses per shipment — from the point of delivery at the Goris station to the point of packing for export at the Denpasar facility — ranged from 24–51%. The losses were partitioned into: losses due to injury (wounds on the fish), total dead-on-arrival (DOA), and total dead-after-arrival (DAA). The total DAA was defined to include all of the fish dying during acclimation in the field and central stations (not including living fish with wounds screened out at Goris), including fish dying during transport between the stations and in the holding system in Denpasar. The total mortality rate per shipment ranged from 10–40% (Schmidt and Kunzmann in this issue). Most of the mortalities (50–80% of the DAA) occurred in the stock system in Denpasar. Possible reasons suggested for the post-harvest mortality were: a) physical damage to the fish and/or the use of cyanide (during collection); b) poor handling, diseases and stress (in the facilities); c) inferior water quality during transport and in the tanks; and d) the collection of unsuitable species and/or species life stages (e.g. obligate coral feeders).

It seems likely that most of the DOA and DAA reported by Schmidt and Kunzmann (in this issue) can be attributed to the collection and transport methods used by the collectors and middlemen rather than to the handling practices and water quality at the exporter's field and central facilities. The mortalities attributed by the authors to stress and disease in the exporter's facilities appear to be secondary effects induced by cyanide collection, bad handling, and poor water quality during holding and transport in the field and at the village level. Schmidt and Kunzmann noted that the export company could not control the collection and handling methods used by the collectors and stressed the urgent need to switch the collection method from cyanide to the use of nets.

One of the largest aquarium fish exporters in the Philippines admitted to the senior author that fish mortalities in its facility ranged from 30–40%, resulting in a loss of 250,000 US dollars (USD) in dead fish per year. Vallejo (1997) likewise reported that mortality rates at Philippine export facilities ranged from 30–40%, which is greater than the 10% rate estimated for Sri Lanka by Wood (1985) and the 10–20% rate reported for Puerto Rico by Sadovy (1992). These per cent mortality ranges represent the most frequent rates that occur, rather than the overall range.

Mortalities at import and retail levels

Importers were estimated to experience, on average, 30% mortality of fish imported from the Philippines during the mid-1980s (Rubec 1986; Rubec and Sundararajan 1991). In addition, there were reports during that period of fish being left out on the tarmac by the airlines in mid-winter in the importing countries, which resulted in 100% DOA (Rubec et al. 2001).

Robert Fenner, who worked for a large US pet marketing retail chain in the early 1990s, noted that marine aquarium fish mortalities in the stores varied widely from week to week and between species. The stores had an average cumulative mortality of about 20%. The experience of staff and their ability to provide sufficient care was found to be the critical factor at some stores in terms of lowering losses. He noted that most stores did not keep good records of fish losses, other than of what the trade calls "disputable losses" that occur within a day of arrival (DOA).

Chris Whitelaw, the livestock manager for one of Canada's largest aquarium retail chains (20 stores), has been involved with the purchase and sale of marine aquarium fish for over 15 years (C. Whitelaw, pers. comm. 2004). In the early 1990s, he purchased Philippine marine aquarium fishes from a Toronto-based importer. The former owner of this import facility recently informed Whitelaw that the total DOA plus DAA on shipments of fish he obtained ranged from 30–60%. During the same time period, the stores associated with Whitelaw experienced 20–25% mortality on fishes purchased from this wholesaler. The fish exhibited symptoms believed typical of cyanide poisoning, either wasting away and dying despite feeding ravenously, or refusing to eat and dying suddenly despite decent water quality and no apparent signs of disease. Whitelaw later purchased fish from both local and US-based transshippers, which were believed to be cyanide-caught, and experienced mortalities of 20–30%.

In 1996, Whitelaw (pers. comm. 2004) started to import net-caught fish directly from Philippine exporters who had obtained their fish from collectors trained by either the Haribon Foundation/ Ocean Voice International (OVI) or the International Marinelife Alliance (IMA). The fish rarely, if ever, showed signs of cyanide, but they frequently showed signs of starvation and poor handling. After working with these suppliers to determine proper densities of fish per unit volume of water in bags placed in shipping boxes for the 36–48 hour trip, the rate of DOA experienced was less than 5% and the rate of DAA an additional 5–10% of the total number of fish received per shipment. Hence, the total DOA plus DAA with net-caught fish presently ranges from 10–15%.

Heidel and Miller-Morgan (2004) recently conducted veterinary studies at wholesale/import facilities on the west coast of the US on fish imported from Indonesia and the Philippines. The studies involved more than 300 individual fish representing 79 species, and they were conducted within hours of the fishes' arrival. The condition of dead, moribund and healthy fish was assessed through water analyses, necropsies and histological and microbiological analyses. Overall mortality ranged from 0–16%, but in some species mortality rates were as high as 100% (J. Heidel pers. comm. 2004). Aside from mortality, other external signs included disequilibrium, gaping, flared opercula, skin ulcerations, haemorrhages, increased mucus production, gill damage and external protozoa and bacteria. Water quality was not always optimal, and the data indicated that many fish arrived with pre-existing health problems and/or infections. The presumption was that the stress of capture, holding and transport, together with deteriorating water quality and opportunistic or epizootic infections, led to the observed fish losses (Heidel and Miller-Morgan 2004). Although the study is not complete, preliminary results indicate significant water chemistry imbalances, while infectious and parasitic diseases were found to be minimal (J. Heidel pers. comm. 2004).

Three importers of marine aquarium fish near Tampa, Florida revealed in 2004 that the pH of the water in plastic bags received from the Philippines generally ranges from 6.1–6.5 after flight durations exceeding 35 hours. The drop in pH to levels below 6.5 in sealed plastic bags associated with air shipments seems to be a significant source of stress.

It generally takes about five to seven days after the removal of fish from sealed containers for corticosteroid and glucose stress hormone levels to decline to baseline levels (Carmichael et al. 1984a,b). Most importers do not hold the fish in their facilities long enough for this to happen before they ship the fish on to wholesalers or retailers. Most retailers have no experience in acclimating marine fish received from transshippers. Mortality rates of fish received directly from overseas to retail stores are generally higher than mortality rates for fish received from domestic importers or wholesalers (Rubec et al. 2001). Fish shipped for longer time periods over greater distances experience more stress, which may contribute to higher rates of DAA.

Acclimation procedures

Because of the accumulation of ammonium ion (NH_4^+) and the drop in pH in the bags resulting from carbon dioxide excretion by the fish, most exporters and importers use acclimation procedures when removing fish from plastic bags. Prior to 1996, most importers receiving fish from overseas dumped the fish, along with the water from the bags, into plastic bins or other containers. They then dripped clean seawater into the bins to gradually raise the pH and acclimatize the fish to the seawater in their holding systems. The Canadian store manager (Whitelaw) noted that his staff also added Ammolock® to the water in the bins to neutralize the ammonia. Starting in 1996 they transferred the fish more quickly to clean seawater with the pH lowered using hydrochloric acid. Three marine fish importers near Tampa, Florida, acknowledged using carbon dioxide to lower the pH of the seawater. One claimed that he had markedly reduced his mortality rates using this method, compared to earlier methods such as using monobasic sodium phosphate (NaH_2PO_4) or various acids to reduce the pH of the acclimation water. Another stated that his mortalities were reduced by 5% since adopting the new method in 1999. A third importer noted that her DOA+DAA using the carbon dioxide method was, on average, 8%. However, she also acknowledged that with some shipments, all of the damselfish died after arrival. She speculated that this might be because they were collected with too high a concentration of cyanide.

The carbon dioxide method is now in use by some exporters in the Philippines and Indonesia. According to Schmidt (2003), carbon dioxide gas was infused into seawater to lower the pH from 8.3 down to 6.75 to 6.85 at the Goris field station. Once the fish were placed in acclimation tanks containing the low-pH water, the carbon dioxide infusion was stopped. The pH of the water was then allowed to slowly increase (via dissipation of the carbon dioxide to the atmosphere) to a range of 8.1–8.4 over a period of at least three hours. Similar methods were used in the central export facility situated in Denpasar. The pH in the bags on arrival at the Denpasar facility ranged from 6.90–7.55. The pH was slowly raised in the acclimation system to a pH range of 8.0 to 8.2. The fish were then transferred to the holding system where the pH of the seawater ranged from 8.1–8.5.

Reducing mortality with net-caught fish

The mortalities reported for marine aquarium fish shipped in sealed plastic bags through the chain of custody from the villages to export facilities, to overseas import/wholesale facilities, and then to retail stores are too high (Rubec 1986; Rubec and Soundararajan 1991). Rubec et al. (2001) stated that mortalities could be reduced if the fish were captured using nets rather than cyanide, and if better shipping and handling methods were adopted. A feasibility study for the Marine Aquarium Council found that the mortality rates for aquarium fish caught using barrier nets and subject to better handling were reduced from 30% to less than 5% on fish transported from the villages to Manila-based exporters (Rubec and Cruz 2002).

Rubec et al. (2001) noted that a small importer situated in New Jersey, US experienced more than 30% mortality with cyanide-caught fish compared to less than 10% mortality with shipments of net-caught fish imported from the Philippines, when both were properly acclimated. The Canadian importer in Toronto had mortalities of 30–60% associated with cyanide-caught fish, while the livestock manager (Whitelaw) importing net-caught fish from the Philippines experienced 10–15% DOA+DAA. Rubec et al. (2001) suggested that if the cumulative mortality through the chain of custody could be reduced from more than 90% to less than 10%, everyone would make more money. It seems likely that large reductions in mortality rates can be achieved by stopping cyanide fishing and stabilizing environmental conditions by using chemical additives in shipping bags. At present, most exporters do not add any chemicals to the shipping water to stabilize the water quality.

Studies with chemical additives

In the case of ornamental fish produced by means of aquaculture it may be possible to improve the condition of the fish by improving water quality in the ponds through the use of antibiotics and other prophylactic treatments and/or by the addition of food additives such as vitamin C (Lim et al. 2002; Lim et al. 2003). This paper is more concerned about measures that can be taken with marine aquarium fish collected from the wild, such as the use of chemicals that can be added to sealed plastic bags at various points through the chain of custody from the field to retailers.

Buffers

McFarland and Norris (1958) studied changes in the pH of seawater in sealed containers containing California killifish (*Fundulus parvinnus*) to mimic conditions in plastic bags. They found that the pH of the seawater declined rather quickly (most of the decline occurred within the first 8 hours). The low pH and/or the accumulation of dissolved carbon dioxide at low pH values were believed to contribute to 50% mortality as the pH declined to 6.0 over a 50-hour period. They also reported that even though the water contained adequate dissolved oxygen, the fish could not use the oxygen at high carbon dioxide levels because of Bohr and Root effects, which inhibit the ability of the blood to transport oxygen at low pH. The accumulation of carbon dioxide acidified the water, explaining the drop in pH.

McFarland and Norris (1958) found that they could markedly improve water quality conditions and the survival time of the fish kept in sealed containers by adding tris buffer. Tris buffer was found not to be harmful to 25 species of marine fish and four species of freshwater fish. In the control tests lacking buffer mentioned in the previous paragraph, 50% mortality occurred with California killifish (65 g of fish per gallon) in sealed containers as the pH dropped from 7.8 to 6.0. Tests with similar densities of killifish were conducted by adding 10 g per gallon of tris buffer. In the first treatment, the water was buffered to a starting pH of 8.25. The killifish experienced 50% cumulative mortality by the sixth day as the pH fell to 6.7. In the second treatment, the starting pH was 7.8 and the fish experienced 50% mortality after 4.9 days, at a pH of 6.5. The killifish survived much longer with tris buffer than in the control case where the seawater was not buffered. The buffer slowed the rate of pH decline and increased the time to reach 50% mortality.

McFarland and Norris (1958) and Amend et al. (1982) found that tris buffer reduced the rate of decrease in pH in studies involving California killifish and southern platyfish (*Xiphophorus maculatus*) held in sealed containers in comparison to controls lacking buffer. McFarland and Norris (1958) found that adding tris buffer reduced the rate of accumulation of carbon dioxide in comparison to two control groups lacking buffer. The accumulation of carbon dioxide followed the trend to be expected in the presence of a substance capable of buffering the carbon dioxide (by converting it to less toxic bicarbonate ion).

Elimination of ammonia

Two methods are commonly used to control the accumulation of ammonia in transport water: preventing ammonia formation by slowing the metabolism of the fish and removing ammonia after it has been excreted (Bower and Turner 1982). The first method uses sedatives or lowers water temperatures; the second method uses compounds that bind the ammonia, such as ion-exchange resins or zeolites. Clinoptilolite is a naturally occurring mineral that acts as a zeolite (hydrated silicates of aluminium with alkali metals and/or alkaline earth metals) capable of binding with ionised ammonium (NH_4^+) and un-ionised ammonia (NH_3) to remove ammonia from solution in water.

Bower and Turner (1982) demonstrated that clinoptilolite was effective in significantly reducing ammonium ion ($p<0.01$) and un-ionised ammonia ($p<0.05$) concentrations in freshwater during simulated transport of goldfish (*Carassius auratus*) held in sealed polyethylene bags. Although small clinoptilolite particles remove ammonia more effectively than large particles because of their greater surface area, large 2–5 mm diameter particles were recommended because they did not cause the water to become turbid.

Problems have been identified with respect to the removal of ammonia from freshwater with increasing weights of clinoptilolite (Teo et al. 1989, 1994). Higher percent mortalities of guppies (*Poecilia reticulata*) and tiger barbs (*Barbus tetrazona*) occurred at higher densities of clinoptilolite, despite the fact that total ammonia ($NH_3 + NH_4^+$) concentrations in the packing water declined with increasing weights of clinoptilolite. This may have been related to the turbidity caused by fine clinoptilolite particles in the water, which may have clogged the gills of the fish, causing asphyxiation. Currently, the aquarium trade uses larger particles of clinoptilolite placed in plastic mesh bags inside sealed plastic bags during shipping of freshwater fishes to overcome this problem.

Clinoptilolite is not effective in seawater (Turner and Bower 1982). Liquid formulations (e.g. Amquel®/Cloram-X®) need to be evaluated for their ability to bind unionised ammonia in seawater during shipping. However, Amquel® cannot be used in conjunction with tris buffer (Robertson et al. 1987). Apparently, this is because the tris buffer molecule has an amine grouping, which binds to the Amquel. This problem can be overcome by using buffers lacking amines in conjunction with liquid agents that bind ammonia.

Control of bacteria during shipping

Amend et al. (1982) tested various chemicals to assess their ability to retard bacterial growth in plastic bags compared to untreated controls and to test the tolerance of fish to each chemical. There was no mortality associated with neomycin sulfate. It also was the least expensive of the chemicals tested. Neomycin sulfate has also been used in other experiments with freshwater aquarium fish to control bacterial proliferations in shipping bags (Teo et al. 1989, 1994).

Invasive bacteria are responsible for skin and fin infections on marine fish, which frequently occur after handling operations (Colorini and Paperna 1983). Experiments with a marine fish (*Sparus aurata*) and a freshwater fish (*Oreochromis mossambicus*) demonstrated that immersing the fish in 100 mg L^{-1} of nitrofurazone for 6 hours effectively reduced the proliferation of bacteria in the water, prevented bacterial colonization of skin lesions and minimized the possibility of developing a systemic infection. Post-mortem analyses of the fish found that the nitrofurazone was not absorbed internally. Hence, nitrofurazone was found to be suitable for use associated with netting and/or transfers that cause scale loss and minor injuries. It has the advantage that it can be used with biological filters because it does not interfere with gram-positive nitrifying bacteria to any significant extent. Because nitrofurazone is not taken up and retained by the fish it is less likely to induce antibiotic-resistant strains of bacteria than other chemicals. Interviews with several US importers and several exporters in the Philippines indicated that nitrofurazone has been used in the transport and holding of marine aquarium fish.

Anaesthetics/sedation

Anaesthetics have been shown to be effective in lowering metabolic rates by reducing the motor activities of fish, and they may be used to further reduce metabolic waste production (McFarland 1960). The use of anaesthetics in the transport of ornamental fish has not been fully explored (Lim et al. 2003). Anaesthetics may be used to limit the stress responses of fish, but there are conflicting reports on this subject. The sedatives most commonly used by the industry to ship aquarium fish are quinaldine or quinaldine sulfate, and tricaine methane sulfonate (Cole et al. 2001).

A number of experiments using the anaesthetic 2-phenoxyethanol have been conducted with guppies, tiger barbs, and mollies (*Mollenesia sphenops*) — all freshwater fish, either alone or in combination with other factors (Teo et al. 1989, 1994; Kwan et al. 1994). The experiments indicate that 2-phenoxyethanol alone was capable of lowering mortality rates. Associated water quality parameters such as total ammonia and total carbon dioxide were generally not directly lethal. There is some indication that low dissolved oxygen concentrations were lethal to tiger barbs (Teo et al. 1994). The data indicate that the 2-phenoxyethanol reduced the mortality associated with sublethal levels of total ammonia and/or the drop in pH measured during simulated transport experiments with fish in sealed plastic bags (Teo et al. 1989, 1994; Kwan et al. 1994).

Chemical combinations

Tests with 2-phenoxyethanol plus tris buffer resulted in lower percent mortalities than the other paired combinations (clinoptilolite+tris buffer, clinoptilolite+2-phenoxyethanol), both with guppies and with tiger barbs (Teo et al. 1989, 1994). The low percent mortalities with the combination of sedative and buffer were not associated with either lower carbon dioxide or lower total ammonia concentrations compared to the water quality obtained for the two other paired combinations. Highest percent mortalities were associated with the clinoptilolite plus tris buffer combination for both guppies and tiger barbs. In tests using 20 g L^{-1} clinoptilolite and 0.02 M tris buffer with various densities of tiger barbs (40, 60, 80 fish per 3 L of water and 3 L of oxygen gas per bag), the mortality jumped up from 0% at 60 fish per bag to 83.3% at 80 fish per bag (Teo et al. 1994). This was attributed to a low concentration of dissolved oxygen (1 mg L^{-1}) for the test using 80 fish per bag. However, no depletion of dissolved oxygen (20.6 mg L^{-1}) occurred with clinoptilolite plus tris buffer that could explain the associated mortality (11.7%) of guppies at a density of 20 fish per 400 ml of water per bag (Teo et al. 1989).

An analysis of variance (ANOVA) was conducted by Teo et al. (1989) to assess the influences of clinoptilolite, tris buffer and 2-phenoxyethanol alone and in various combinations on water-quality parameters and the mortality of guppies. All three chemical additives added separately or together in paired combinations had significant effects on ammonia concentrations ($p \leq 0.01$). Both 2-phenoxyethanol alone and the interaction term for clinoptilolite*2-phenoxyethanol had significant effects ($p \leq 0.01$) on the dissolved oxygen concentrations in the bags containing guppies (the fish consumed less dissolved oxygen when they were anaesthetised). All three chemical additives together exhibited a significant ($p \leq 0.05$) three-way interaction effect in relation to dissolved oxygen. Both tris buffer alone and 2-phenoxyethanol alone had significant effects ($p \leq 0.01$) on reducing mortality rates of guppies in sealed plastic bags. Clinoptilolite also contributed to a significant ($p \leq 0.05$) reduction in mortality.

Buffer + clinoptilolite + temperature + sedative

In another series of tests by Teo et al. (1989) the density of guppies was varied (40, 50, or 60 fish per bag containing 600 ml of water) at two temperatures (20 and 25°C), with 0.02 molar (M) tris buffer, with or without the presence of 0.22 grams per litre (g L^{-1}) 2-phenoxyethanol. Mortalities were lower in the presence of 2-phenoxyethanol. At higher densities of guppies (50 or 60 fish per bag) the mortality was lower at 25°C than at 20°C with the two factors used together. At a density of 50 fish per bag, the mortality was 2% at 25°C, and 6% at 20°C. At a density of 60 guppies per 600 ml of water, the mortality was 5% at 25°C, and 5.6% at 20°C. The results of these trials showed that with the application of suitable concentrations of tris buffer, clinoptilolite, and 2-phenoxyethanol, it was possible to increase the packing density of the guppies.

The environmental conditions recorded by Teo et al. (1989) at various densities of guppies are summarized in Table 2 (the data in the authors' original table are reorganized here to separate the tests at 20°C from those at 25°C). The data presented are averages for the environmental conditions associated with three bags of fish for each test and control condition.

It is apparent from Table 2 that total dissolved carbon dioxide levels measured and the associated percent mortalities were higher in those tests where 2-phenoxyethanol was not added (Teo et al. 1989). At 20°C and densities of 40 and 60 fish per bag the dissolved oxygen (DO) levels were lower where 2-phenoxyethanol was absent than in those tests where the anaesthetic was present. At 25°C the DO level was higher in the test lacking the anaesthetic (40 fish per bag) in comparison to DO levels where the anaesthetic was present. There also were higher total ammonia concentrations in bags held at 25°C than at 20°C. Total ammonia was higher in two out of three cases where the anaesthetic was absent compared to where the anaesthetic was present along with the buffer and clinoptilolite. At a density of 40 fish per bag, the presence of the anaesthetic lowered the accumulation of ammonia at 20°C ($p<0.05$). At 25°C and a density of 40 guppies per bag the total ammonia concentration was lower with the anaesthetic present than without but the difference was not significant ($p>0.05$). The authors concluded that 2-phenoxyethanol appeared to be needed for packing guppies at 20°C. In preliminary tests, lowering the water temperature below 20°C increased the mortality of guppies (all died at 15°C). Froese (1998) summarized data that indicated tropical fish survive best when water temperatures during shipping match those found in their natural environments (22–30°C).

Discussion

The data obtained from persons involved with importing marine aquarium fish from the Philippines indicated there was a lower mortality associated with net-caught fish than with cyanide-caught fish. Several importers of fish originating from the Philippines have stated that their mortalities declined during the latter part of the 1990s. This may be related to the decline in the prevalence of cyanide in marine aquarium fish monitored by six cyanide detection test (CDT) laboratories run by the IMA (Rubec et al. 2003). The presence of cyanide declined from 43% in 1996 to 8% in 1999, and then increased to 29% in 2000, based on 7703 aquarium fish specimens tested. Other factors such as improvements in filtration systems and changes in acclimation procedures discussed in the present paper may also have influenced mortality rates over this time period.

The water quality associated with holding systems and during transport in plastic bags appears to be very important. Several exporters have informed the senior author that they experienced higher mortality when the seawater used in their facilities was obtained from Manila Bay. Lower mortalities occurred when they obtained their system water at distances further away from Manila. One exporter of net-caught fish obtains seawater transported by tank truck from Subic Bay. This exporter believes that it is important to obtain unpolluted seawater and to maintain good water quality in the facilities.

The scientific literature reviewed herein indicated that fish died in plastic bags, even when water quality conditions were not incipiently lethal. Studies have demonstrated that chemical additives can be used to increase the density of guppies, tiger barbs and mollies shipped in sealed plastic bags

Table 2. **Water quality parameters and percent mortalities monitored with guppies held in sealed plastic bags in 600 ml of water, with 20 g clinoptilolite, 0.02 M tris buffer, and 40 to 60 fish per bag, held for 48 hours at either 20°C or 25°C, with or without 2-phenoxyethanol (Teo et al. 1989).**

Treatment				After exposure				
Fish density (no. bag^{-1})	Total fish weight (g)	Temperature (°C)	2-phenoxyethanol (g L^{-1})	Carbon dioxide (mg L^{-1})	Total ammonia (mg L^{-1})	pH	Dissolved oxygen (mg L^{-1})	Mortality (%)
40	32.1	20	0.22	542	3.03	7.43	18.0	3.3
40	28.3	20	0.00	748	5.44	7.50	11.0	6.7
50	32.8	20	0.22	579	7.04	7.57	18.0	6.0
60	37.4	20	0.22	234	5.80	7.10	20.3	5.6
60	34.8	20	0.00	841	26.90	7.05	6.3	13.0
40	31.4	25	0.22	497	13.99	7.15	5.9	5.8
40	28.7	25	0.00	681	16.56	7.35	7.1	10.0
50	34.4	25	0.22	588	14.25	7.26	6.6	2.0
60	36.3	25	0.22	494	14.70	7.11	10.3	5.0

(Teo et al. 1989, 1994; Kwan et al. 1994). However, there appeared to be an upper limit to the density of fish that could survive together in the shipping bags. In the case previously discussed involving paired combinations of clinoptilolite and tris buffer (Teo et al. 1994) one might attribute the tiger barb mortality (83.3%) to the clinoptilolite becoming saturated with ammonia, since the total ammonia concentration was high (27.1 mg L^{-1}) at the highest fish density (80 fish per bag). However, in the case of guppies using the same combination of chemical additives, the total ammonia concentration was low (3.7 mg L^{-1}) and the dissolved oxygen concentration was high (20.6 mg L^{-1}), yet the mortality (11.7%) was relatively high (Teo et al. 1989). Other factors such as physiological stress due to crowding may account for the observed mortalities. This may explain why mortalities increased markedly at the highest fish densities in the bags.

Fish experience cumulative stress from being netted, bagged, crowded, and exposed to changes in pH, temperature, salinity, dissolved oxygen, light, and from the accumulation of ammonium ion in the bags, which becomes toxic un-ionised ammonia when the bags are opened (Rubec et al. 2001). The main finding of experiments with freshwater fish using chemical additives in various combinations was that 2-phenoxyethanol in combination with tris buffer had a marked influence in reducing mortality rates of guppies, tiger barbs, and mollies (Teo et al. 1989, 1994; Kwan et al. 1994). No explanation for how 2-phenoxyethanol reduced the mortality rates was provided. Experiments with rainbow trout (*Oncoryhynchus mykiss*) found that 2-phenoxyethanol reduced the excretion of cortisol in the blood, which is commonly associated with physiological stress responses (Iwama et al. 1989).

Study of chemical additives for shipping marine fish

No experiments have been published involving the use of more than one chemical additive in plastic bags used to ship tropical marine aquarium fishes. There is a need to conduct studies on marine aquarium fish similar to those conducted on freshwater fish.

Fish collectors travel long distances by boat to find reefs that have not been devastated by the use of cyanide and other types of destructive fishing such as explosives (Rubec 1986, Rubec 1988; Rubec et al. 2003, Cervino et al. 2003). We plan to study post-harvest handling and transport practices from the moment the fish are captured using nets and during their transport to the villages, as well as how they are held prior to shipment to export facilities in Manila. We plan to study this closely because much of the mortality through the chain of custody appears to be related to the collection and transport methods used by collectors and middlemen. Where it is possible, we will compare mortalities for net-caught fish with those for cyanide-caught fish handled and transported in a similar manner.

Many fish are held in plastic bags at the village level, adding to post-harvest stress (Baquero 1995). Alternatives to this practice include the use of floating cages, submerged net-bags, and regional holding facilities (Rubec and Cruz 2002). We intend to test these alternatives and to document fish mortality rates under each of them.

After determining mortality rates, by species, associated with conventional shipping methods lacking chemical additives, experiments will be conducted to test the effects of various chemicals added to the shipping bags. The chemical additives will be evaluated by making shipments from the villages through the chain of custody to retailers in the US. Plastic bags with one-quarter seawater and three-quarters oxygen gas (by volume), along with chemical additives, will be used for export of the fish. The numbers of DOA and DAA will be monitored at each step of the chain.

We plan to first bag fish and place them in shipping boxes and hold them for 48 hours with and without chemical additives at a Philippine export facility. We plan to evaluate the chemicals alone and in various combinations in a manner similar to the research by Teo et al. (1989, 1994). The concentrations of needed additives will first be determined from the published literature. After that, we plan to vary the concentrations of some chemicals to determine optimal concentrations that are safe for use with the fish in the bags. Chemical additives will be used to stabilize the pH, to neutralize un-ionised ammonia as it is excreted and to stop the proliferation of bacteria in the shipping bags. This will allow us to choose the best chemical combinations and optimal concentrations of each chemical additive.

By stabilizing water quality conditions in the plastic bags during transport, we hope to eliminate or markedly reduce shipping stress and secondary effects (like disease) on the fish caused by stress-inducing factors such as rough handling, ammonia, and other adverse water quality conditions. The scientific literature indicates that this is possible. While there may be some companies using some of these techniques, they are not commonly applied to the shipping of marine aquarium fish. We believe that mortality rates of less than 1% at each step of the chain may be achieved. This may allow the exporters to pay the collectors more for net-caught fish and increase the profitability of marine aquarium fish enterprises through the chain of custody from reefs to retailers. This does not imply that the prices for net-caught fish being exported need to be higher. We believe that net-caught fish can be exported at prices competitive with those of exporters selling cyanide-caught fish.

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