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Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics

Todd S. Harmon

Walt Disney World, Animal Programs, Lake Buena Vista, FL, USA

Correspondence
Todd S. Harmon, Walt Disney World, Animal Programs, PO Box 10 000, Lake Buena Vista, FL 32830, USA.
Email: todd.s.harmon@disney.com

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Abstract

Fish culture operations, public aquariums, fish biologists and aquatic researchers often have the need to transport live fish. These fish are frequently transported in live-haul boxes by ground transportation. Activities involved with transporting fish, such as handling, confinement and exposure to sub-optimal water quality, have the potential to create physiological changes in the fish because of increased stress. Because of the affiliation between stress and fish health, it is important to minimize the amount of potential stressors as well as to minimize the duration of exposure to stressors during these procedures. Furthermore, understanding aberrant environmental conditions and how they affect fish often leads to establishing new protocols that reduce stress. Increased survival rates and the arrival of healthy fish are dependent on transport and on the pre-handling and post-handling procedures associated with fish-hauling operations.

Key words: acclimation, fish transport, osmoregulation, stress, water quality.

Introduction

The greatest challenge with any live-fish transport is to minimize the amount of stress placed on the fish. Barton (1997) noted various definitions of stress and highlighted the difficulties of defining stress so that it suits all disciplines. As stress relates to transporting fish, the definition given by Francis-Floyd (2002) is practical and is defined as 'a condition in which an animal cannot maintain a normal physiological state because of various factors adversely affecting its well-being'. Stress in fish can be caused by biological, chemical or physical conditions. Stress can also play a major role in the susceptibility of fish to disease (Winton 2001). Transported fish are often exposed to multiple stressors within a short duration. Potential stressors associated with transporting fish include inappropriate hauling densities (Piper *et al.* 1982), tank confinement (Davis & Parker 1986), physical handling (Maule *et al.* 1988; Cech *et al.* 1996), unfavourable water quality (Weirich & Tomasso 1991; Carmichael *et al.* 1992) and conditioning fish to a new environment (Carmichael *et al.* 1984; Brick & Cech 2002). Transport-associated mortality might be the result of one severe stressor, several mild stressors or infectious disease. Moreover, the exact impact of the stress depends on the severity and duration of the stress, as well as the health of the fish (Noga 2000). Even if fish are carefully handled and transported, a group of mild stressors might act together and cause mortality (Carmichael *et al.* 2001).

Internal physiological mechanisms responsible for adapting to a stressor include nervous, immunological and hormonal mechanisms (Selye 1973). However, there is a metabolic cost associated with this adaptation, which includes diverting energy from normal metabolic functions to the functions that are used to cope with the stress (Barton & Iwama 1991). These responses are often categorized as primary, secondary and tertiary stress responses. The primary response is the release of hormones into the circulatory system, which then trigger secondary responses that can include increases in heart rate, gill blood flows and metabolic rate, as well as decreases in plasma chloride, sodium and potassium (Portz *et al.* 2006). Tertiary responses might include disease resistance, altered behaviour, reduced growth rate, and reproductive capacity, thermal tolerance and tolerance to hypoxia (Barton & Iwama 1991). Although fish have the ability to respond physiologically to stress, these response actions can be forced beyond their normal limits, thus becoming detrimental to the fish (Barton & Iwama 1991). Barton and Schreck (1987) estimated the metabolic cost of acute stress in juvenile steelhead *Oncorhynchus mykiss* (Walbaum) to be approximately one-quarter of the energy available within the scope for activity.

Stressors that affect fish can be categorized into acute (short-term) or chronic (long-term) stressors (Davis 2006). Acute stressors include handling, confinement, abrupt changes in water quality and improper acclimation, and chronic stressors include extended periods of poor water quality, improper stocking densities and improper diets. Severe stress might result in immediate mortality, presumably through ion loss (McDonald & Milligan 1997), whereas chronic stress often results in a severely compromised immune function and/or a decrease in energy stores (Portz *et al.* 2006). An immunosuppressed fish allows pathogens to initiate a disease that would otherwise normally be resisted by the fish (Wedemeyer 1997). 'Delayed mortality syndrome' and 'hauling loss' are terms used to reference fish mortality that is associated with transport and conditioning to a new environment. Delayed mortality might occur days or even weeks after transport depending on the underlying cause and severity. The direct cause of delayed mortality has not been established conclusively (Barton & Iwama 1991; Noga 2000), but is thought to be at least partially a result of blood electrolyte imbalances resulting from osmotic flux (Wedemeyer 1996). Mazic *et al.* (1991) found no immediate mortality during the transport of striped bass *Morone saxatilis* (Walbaum), but losses started 3 h after transport and lasted for 4 weeks, eventually reaching 100% mortality.

Although many freshwater transport protocols might be similar, it is worthwhile to note differences among marine and freshwater species, including osmoregulatory differences (Moyle & Cech 1988) and sensitivity to certain water-quality parameters. It must also be noted that acceptable water-quality parameters might even differ within a species depending on life stage, health and previous holding conditions. However, with all species, maintaining proper water quality during transport is a vital component in reducing physiological stress.

Many sources of stress during transport might be unavoidable. However, stress might be minimized by following good transport procedures. Proper design and operation of the transport container can reduce many fish stressors. Moreover, proper water quality is important for maintaining fish health and reducing stress throughout transport as well as proper tempering into the receiving water. A review of hauling recommendations for various species is listed in Table 1. This paper will review water-quality parameters that should be monitored during transport as well as handling and acclimation recommendations before and after transport. This paper will focus on live transport in transport containers and will not depict the transport of fish in plastic bags, which often occurs in the ornamental industry. However, some of the stressors encountered and their effects might be similar.

Osmoregulatory stress

The immediate mortality associated with transport stress is presumably blood ion disturbances (McDonald & Milligan 1997). Marine bony fishes must drink large amounts of seawater to prevent dehydration because of the movement of water from their body into the surrounding seawater environment as a result of their hypotonic condition (Moyle & Cech 1988). Conversely, freshwater fish are hypertonic, therefore gaining water and losing electrolytes. During excitement and in stressful conditions (which typically occur in transport), epinephrine (adrenaline) is released into the bloodstream, thus affecting the permeability of water across the gill epithelia in fish (Moyle & Cech 1988). This increases the water gain and blood ion loss in freshwater fish and increases the loss of water and ion influx in marine fish, resulting in a disturbance of osmoregulatory homeostasis (Portz *et al.* 2006). Because of these conditions, a general procedure for transporting many freshwater fish is to add salts to their transport water. Many studies have documented the advantages of using salt during and after the transport of various species (Collins & Hulsey 1963; Tomasso *et al.* 1980; Johnson & Metcalf 1982; Carmichael *et al.* 1984; Mazic *et al.* 1991; Barton & Zitzow 1995; Cech *et al.* 1996; Swanson *et al.* 1996). In freshwater, salt (NaCl) has the potential to alleviate or reduce osmoregulatory dysfunction by decreasing the gradient between the water and the fish blood (Mazic *et al.* 1991). Isotonic conditions for freshwater fish are approximately one-third the salt concentration of seawater (Moyle & Cech 1988). Most of the studies listed above used 5–10‰ salt solutions in their hauling experiments (freshwater fish). Mazeaud *et al.* (1977) also stated that marine fish stop drinking when stressed as a result of a gastric muscular contraction induced by catecholamine. Catecholamine is considered to be a hormone that is released under stressful situations in an attempt to adapt to or avoid the stressor (Wedemeyer 1996).

Table 1 Hauling recommendations for various species

Species	Pre-haul	Hauling tank	Post-haul	Reference
American shad *Dorosoma penetrance*		High density to allow for schooling	13–15°C 0.5% salt water	Backman and Ross (1990)
Brook trout/lake trout *Salvelinus* spp.		Density: 69–170 g L^{-1} 0.1 g L^{-1} of $NaHCO_3$ and $CaCl_2$ 6–7°C		McDonald *et al.* (1993)
Delta smelt *Hypomesus transpacificus*		0.8% NaCl NovAqua		Swanson *et al.* (1996)
Freshwater drum *Aplodinotus grunniens*		0.5% NaCl Density: 60 g L^{-1}		Johnson and Metcalf (1982)
Hybrid striped bass *Morone chrysops* ♀ × *Morone saxatilis* ♂	MS-222 50 mg L^{-1}	25 mg L^{-1} MS-222 10 g L^{-1} NaCl		Tomasso *et al.* (1980)
Largemouth bass *Micropterus salmoides*	MS-222 50 mg L^{-1} Fasted 72 h Copper sulfate 10 mg L^{-1}*	Density: 180 g L^{-1} Temperature 16°C 25 mg L^{-1} MS-222 Salt near isotonic to fish	Acclimate in salts similar to fish plasma Copper sulfate 10 mg L^{-1}*	Carmichael *et al.* (1984)
Red drum *Sciaenops ocellatus*	MS-222 80 mg L^{-1}			Robertson *et al.* (1988)
Striped bass *M. saxatilis*		1.0% NaCl Temperature 12°C	1.0% NaCl	Mazic *et al.* (1991)
Striped bass *M. saxatilis*		0.8–1.2% NaCl Temperature <18.3°C At 12.8–18.3°C use 3–5 mg L^{-1} MS-222 on fish >76.2 cm		Yeager *et al.* (1990)
Tiger muskellunge F1 hybrid ♀ *Esox masquinongy* × ♂ *Esox lucius*		Density up to 135 g L^{-1}	<10°C abrupt temperature change	Mather *et al.* (1986)
Walleye *Stizostedion vitreum*			0.5% NaCl recovery water	Barton and Zitzow (1995)

*One hour bath per day for 10 days.

Dissolved oxygen

Dissolved oxygen (DO) is often the single most limiting factor in any fish-holding system. Proper DO must be maintained throughout transport. Ideally, DO should be maintained at or near 100% saturation throughout transport. The solubility of DO is dependent on water temperature, gas composition, salinity and total pressure. The solubility of oxygen decreases as the water temperature, salinity and altitude increase (Table 2). Saturation is the amount of a dissolved gas when the water and atmospheric phases are in equilibrium (Piper *et al.* 1982). Gas supersaturation can occur when the dissolved gases are greater than the equilibrium concentration (Colt 1984). When fish are exposed to supersaturated water before it equilibrates, the excess gas might cause the solution to form emboli in various tissues; this is referred to as gas bubble disease (Noga 2000). While using pure oxygen during transport it is very easy to supersaturate the water. However, because oxygen is assimilated metabolically it is less likely than other gases (such as nitrogen) to form persistent bubbles (Noga 2000). Wedemeyer (1996) noted that mortality from gas bubble disease usually does not occur if oxygen (note: not ambient air, which contains nitrogen) supersaturation is 200%. However, Wedemeyer (1996) warned that gill ventilation might be reduced because of the high levels of oxygen causing elevated carbon dioxide (CO_2) in the fish's blood.

The initial 30–60 min in the transport container is critical because of the increased activity by the fish (Piper *et al.* 1982). Fries *et al.* (1993) reported a drop from 20 to <5 mg L^{-1} DO during the initial tank loading process of channel catfish *Ictalurus punctatus* (Rafinesque). Therefore, it is very important to saturate or supersaturate the water with oxygen (O_2) prior to placing a heavy load of fish into a transport tank. It must also be noted that confinement and capturing prior to transport also pose a threat to deteriorating DO levels within the holding area.

Table 2 Point of saturation (referred to as 100% saturation) for dissolved oxygen in water with varying salinities and temperatures (barometric pressure 760 mmHg)

Temperature (°C)	Salinity (0 g L^{-1}), mg L^{-1}	Salinity (15 g L^{-1}), mg L^{-1}	Salinity (30 g L^{-1}), mg L^{-1}
0	14.602	13.180	11.896
2	13.813	12.487	11.287
4	13.094	11.853	10.730
6	12.436	11.274	10.220
8	11.832	10.742	9.752
10	11.277	10.252	9.321
12	10.766	9.801	8.923
14	10.294	9.384	8.555
16	9.858	8.998	8.214
18	9.453	8.640	7.898
20	9.077	8.307	7.603
22	8.726	7.997	7.328
24	8.400	7.707	7.072
26	8.094	7.436	6.831
28	7.808	7.182	6.606
30	7.539	6.943	6.394

Source: Colt (1984).

Additional aeration might be required, particularly if crowding the fish into a confined area is in the scope of the procedure.

Various methods have been used to achieve and maintain proper DO levels throughout fish transport, including compressed gaseous oxygen, agitators, aerators and liquid oxygen. In a given volume, liquid oxygen holds more oxygen than in the gaseous form. However, a liquid oxygen dewar will lose approximately 2% daily; thus, if long-term storage is necessary it could become an issue (Timmons *et al.* 2002). Using the correct diffuser is also important for efficiency. Smaller bubbles from fine-pore diffusers have a greater air to water surface area compared with the same volume of gas with larger bubbles. This is important with regard to the amount of oxygen needed for transport. Agitators are inefficient compared with pure O_2 injection through diffusers, but are important in removing CO_2. However, agitators can cause excessive foaming in salt water (Carmichael *et al.* 2001). Carmichael *et al.* (1992) recommends a combination of agitators and pure oxygen diffusers for high-density transports. A secondary source of oxygenation is always recommended in the event of failure of the primary source. Stocking density and travel distance often play important roles in the method/s used to maintain proper DO levels. When using compressed or liquid oxygen caution must be taken to keep away from flammable materials and to make sure that the cylinders are securely fastened. Additional care must be taken when using liquid oxygen because any contact with epithelial tissue will result in severe burns.

Suspended solids and ammonia

Suspended solids from fish faeces can pollute the water as well as physically damage the gills of fish. Mechanical-type cartridge filters connected to a submersible pump have excellent filtration capabilities and can easily be mounted onto a transport box (Fig. 1). Ammonia toxicity is also a concern in fish transport, especially long hauls. Ammonia is produced as a by-product from fish metabolism and is primarily excreted through the gills by diffusion (Colt & Armstrong 1981). The accumulation of ammonia can be minimized by fasting fish prior to transport and/or adding ammonia-reducing agents to the transport water. Fasting fish for at least 24 h is recommended to reduce the accumulation of faeces and ammonia in the tank (Carmichael *et al.* 2001). Wedemeyer (1996) found that when transporting salmonids a typical protocol is to fast the fish for 48–72 h prior to transport. Phillips and Brockway (1954) reported that trout fasted for 63 h produced half as much ammonia as recently fed fish. Filtration is often used during long-distance transport, whereas just fasting the fish prior to transport can often be successful for short trips.

Temperature of the transport tank

Because fish are poikilotherms, the surrounding water is critical to their physiological reaction rates. As their body temperature increases biochemical reaction rates increase.

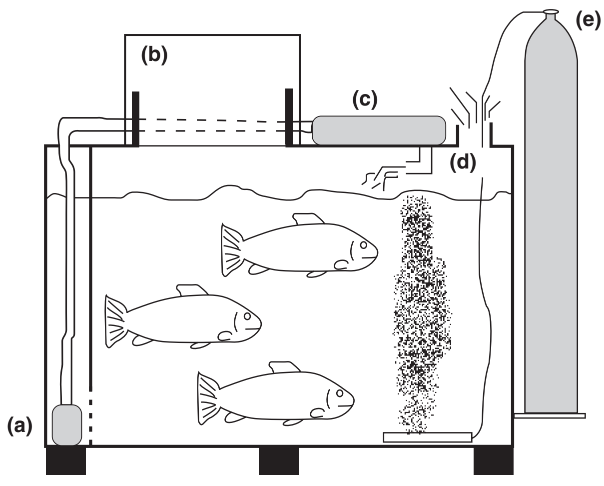

Figure 1 Schematic of a self-contained fish transport box. (a) Submersible pump, (b) large hatch, (c) mechanical filter, (d) air vent and (e) compressed oxygen cylinder.

Conversely, as their body temperature decreases, metabolic processes decrease. Thus, cooling the transport water has advantages. Cooling the water will slow the metabolism of the fish, which in turn reduces ammonia production, oxygen consumption and ammonia toxicity and increases oxygen solubility. Wedemeyer (1997) found that by reducing the hauling water by 10°C, most warm-water species will reduce oxygen consumption and ammonia production by 50% and, therefore, recommends lowering the hauling water temperature by 5–10°C. Cooling the water by 5–7°C is a widely used protocol in many salmonid transports (Wedemeyer 1996). However, caution must be used in the cooling process to ensure that there is not too much of a gradient difference between the holding water temperatures and the hauling temperatures as an abrupt change in temperature itself could be a stressor. Most experienced haulers should know their limits of each particular species and cool the hauling water accordingly.

Optimal hauling temperatures can vary considerably between species, so historical information can be helpful with this decision process, particularly with regard to how well a species tolerates a change in temperature and how quickly a temperature change should take place. It might also be beneficial to use the transport time as an acclimation process between the arrival water and the pre-transport water. Piper *et al.* (1982) recommends channel catfish hauling temperatures of 7.7–10°C (45–50°F) in winter and 15.6–21.1°C (60–70°F) in summer. However, Piper *et al.* (1982) warn that for channel catfish fry it is not recommended to cool the hauling water. Studies have recommended that water for the transport of hybrid bass should be cooled to <18.3°C (65°F) (Yeager *et al.* 1990). Piper *et al.* (1982) reported that the optimal temperature for hauling striped bass is 12.8–18.3°C (55–65°F). Transporting the largemouth bass *Micropterus salmoides* (Lacepède) was most successful in 16°C water (Carmichael *et al.* 1984).

Maintaining the temperature of the tank

Temperature control involves maintaining the water temperature during transport within a desired range. Insulated boxes, temperature-controlled box trucks, chillers and/or ice can all assist in controlling the temperature. The tank material can also have an influence on maintaining the temperature, particularly if the water temperature and air temperature are very different. Many tanks today are made of fibreglass or aluminium, with an insulating material such as polyurethane sandwiched in the middle. This type of tank compared with an aluminium tank containing no insulation or a fibreglass tank with no insulation has very different thermal conductivity properties. A few of the more popular tank materials are listed in Table 3. The lower the *k* value the better the insulating properties of the material. Therefore, tank construction can play a vital role in maintaining temperature and in determining whether or not an elaborate heating or cooling system is needed. However, during long transports, where the ambient temperature is much different than the tank temperature, a chiller or heater might be necessary to maintain the temperature of the tank within the desired range.

Table 3 Thermal properties of typical hauling tank materials

Material	Thermal conductivity (*k*)
Plywood (Douglas Fir)*	0.8
Aluminium (1100 alloy)*	1532
Fibreglass†	0.25
Polyurethane*	0.16
Urethane†	0.18
Expanded polystyrene (extruded)†	0.26

*Taken from the American Society of Heating, Refrigerating and Air-Conditioning Engineers (1981).
†Taken from Piper *et al.* (1982).
Thermal conductivity (*k*) is the amount of heat in British Thermal Units (BTU) that is transmitted in 1 h through 1 ft^2 material 1 inch thick for each degree °F difference between the two surfaces of the materials.

Carmichael and Tomasso (1988) and Johnson (2000) reported that ice is commonly used to cool transport water, whereas chillers are less common. One pound of ice (0.45 kg) will lower two gallons (7.56 L) of water 5.5°C (Timmons *et al.* 2002). If ice is used and it is made from a chlorinated water source, sodium thiosulphate ($Na_2S_2O_3$) or sodium sulphite (Na_2SO_3) should be added to remove the residual chlorine. Wedemeyer (1996) recommends 7.4 mg L^{-1} $Na_2S_2O_3$ to 1 mg L^{-1} chlorine and 2 mg L^{-1} Na_2SO_3 to 1 mg L^{-1} chlorine for the neutralization of chlorine. There are also numerous products that are sold as 'water conditioners' that claim to work for chlorine removal.

Carbon dioxide

Carbon dioxide is produced as a by-product of fish metabolism. Wedemeyer (1996) points out that a high concentration of CO_2 can be a greater risk than elevated ammonia levels during transport. Elevated CO_2 levels in the holding tank can reduce the concentration gradient between the blood and the water where diffusion occurs through the gills. Excess levels of CO_2 could result in hypercapnia (high CO_2 levels in the blood) and acidosis and quite possibly narcosis and death (Wedemeyer 1997). Clinical signs of toxicity include slowed respiration

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(although respiration might temporarily increase just prior to toxicity) and fish lying on the bottom of the tank (Stoskopf 1993). Wedemeyer (1996) recommends keeping CO_2 concentrations below 30–40 mg L^{-1} during transport. However, he warns that if the DO is not saturated this level might be reduced.

Any type of water disturbance, such as agitators or heavy aeration, can help to remove CO_2 from the water. However, many transport containers have a lid and are 'closed'. These tanks can pose a secondary problem. Carbon dioxide that has been stripped from the water can cause an increase in the partial pressure of CO_2 in the airspace above the water, which might eventually prevent CO_2 from off gassing from the water. In this case, any type of opening in the top of a transport container can provide a pathway for atmospheric air exchange (Fig. 1). Furthermore, Forsberg and Summerfelt (1999) reported superior water quality (i.e. no drop in pH and lower CO_2) in transport tanks fitted with ram-air ventilators compared with tanks with a single vent containing 40.2 g L^{-1} of walleye *Stizostedian vitreum* (Mitchill) fingerlings.

Physical handling

The stress associated with the physical capture and handling of fish is often overlooked because of the preparations for the actual transport of the fish. Maule *et al.* (1988) reported that the most stressful event in their salmonid study was loading the fish into the tanks and not the actual transport. Robertson *et al.* (1988) also suggested that capturing and handling prior to transport proved most traumatic to red drum *Sciaenops ocellatus* (Linnaeus). Johnson and Metcalf (1982) also found that capturing and handling was a major cause of mortality in the transport of freshwater drum *Aplodinotus grunniens* (Rafinesque). If at all possible, fish should be moved without removing them from the water. McDonald and Milligan (1997) highlighted several references that reported exposure to air after exercise for even short periods of time can have a significant impact on mortality rates.

However, handling fish in nets is almost inevitable during transport procedures. When nets are necessary, it is advisable to use nets that are less abrasive. Nets made from polypropylene or polyethylene should be avoided; these nets tend to be stiff and can cause scale loss (Yeager *et al.* 1990). Scale loss can result in an opening to the epidermis that provides a pathway for bacterial, fungal and viral pathogens. Any practice to minimize the removal of fish mucus and scale loss is preferred. Fish mucus acts as an antibacterial agent as well as a physical barrier between pathogenic organisms in the water and the fish (Francis-Floyd 2002). Any loss of the mucus will increase the chance of infection, especially secondary fungal infections (Wedemeyer 1996). It is also advisable to use coated nets when moving fish with spines. This will help prevent the spines from getting tangled in the netting material.

Carrying capacity

The number (or weight) of fish that can be successfully transported depends on water quality, the duration of the transport, water temperature, fish size and the species. Piper *et al.* (1982) pointed out that with trout the maximum permissible weight is directly proportional to their length. Thus, if a tank can hold 20 kg of 5 cm trout then it can hold 40 kg of 10 cm trout. In a survey of fish culturists in the USA, Carmichael and Tomasso (1988) found great variability in transport of the same species. For example, brown trout 0.05–0.29 kg L^{-1}, grass carp 0.05–0.27 kg L^{-1}, striped bass 0.02–0.29 kg L^{-1}, hybrid striped bass 0.04–0.23 kg L^{-1}, channel catfish 0.01–0.48 kg L^{-1} and rainbow trout 0.02–0.30 kg L^{-1}. This variability within a single species results from fish size, transport time, water temperature and personnel preferences. Various species and their suggested transport densities are listed in Table 4. As a general rule, as the transport time increases (particularly >8 h) the carrying capacity should decrease.

Table 4 Suggested hauling densities for various species of fish as reported by Piper *et al.* (1982)

Species	Size	Carrying capacity (g L^{-1})	Notes
Chinook salmon *Oncorhynchus tshawytscha*	3.81 cm	60–120	–
Chinook salmon	6.35 cm	120–240	–
Coho salmon *Oncorhynchus kisutch*	10.1–12.7 cm	240–360	–
Rainbow trout *Oncorhynchus mykiss*	20.3–27.9 cm	300–420	–
Largemouth bass *Micropterus salmoides*	15.2–25.4 cm	240	Up to 10 h transport
Striped bass *Morone saxatilis*	91 g	180	10 h transport
Striped bass	10 g	60	19–24 h
Striped bass	91 g	90	15 h
Channel catfish *Ictalurus punctatus*	227 g	708	8 h, 18.2°C
Channel catfish	9.1 g	414	8 h, 18.2°C
Channel catfish	3.6 g	354	8 h, 18.2°C

Water conditioners

Many transport protocols include the addition of water conditioners to the tanks. There are products available that act as anti-foaming agents, pH stabilizers, ammonia removers, electrolytes and 'slime coat' agents. However, limited research has been conducted on these agents, mainly because most are not used with food fish in the USA. The US Food and Drug Administration regulates the drugs used on these animals and, depending on whether or not the compound is considered a drug, will determine if the product can be used on food fish legally. A study by Swanson *et al.* (1996) using delta smelt *Hypomesus transpacificus* (McAllister) found that the addition of NovAqua (Kordon LLC, Hayward, CA, USA) increased survival over 72 h by 26.9%. Wedemeyer (1996) reported a significant reduction in mortalities when using Polyaqua (Kordon LLC) when trucking chinook salmon *Oncorhynchus tshawytscha* (Walbaum) and steelhead *O. mykiss* (Walbaum). Both of these products are polymer formulations that form a temporary coating on exposed tissue that has lost mucus. These chemical agents, particularly pH stabilizers and ammonia removers, are widely used in the transport and shipping procedures of non-food fish.

Anaesthetics

Anaesthetics are widely used prior to and during transport to slow the metabolism of the fish, thus reducing oxygen uptake and decreasing CO_2 and ammonia production. Anaesthetics also lessen the stress response caused by increased activity and handling (Wedemeyer 1996). The proper dosage is critical and will vary with species and fish size. Only a light sedation should be used if anaesthetics are used during transport (Wedemeyer 1997). It is important that the physiological functions of the fish and its orientation in the water column are not hindered by the anaesthetic, which they would be if a dose for anaesthesia rather than sedation was administered. Carmichael *et al.* (1984) found that the use of tricaine methanesulphonate (MS-222) both before (50 mg L^{-1}) and during (15 mg L^{-1}) transport reduced stress in the largemouth bass *M. salmoides* (Lacepède). Yeager *et al.* (1990) found that the use of MS-222 at 3–8 mg L^{-1} during the transport of striped bass *M. saxatilis* (Walbaum) brood fish was successful. Robertson *et al.* (1988) recommended rapid anesthetization with MS-222 (80 mg L^{-1}) prior to capture and that no anaesthetic was used during shipment for red drum *S. ocellatus* (Linnaeus). As expected, these dosages and techniques vary among species. Currently, MS-222 is the only anaesthetic approved by the US Food and Drug Administration's Center for Veterinary Medicine for use on food fish, but MS-222 has a 21 day withdrawal time (Schnick 2006).

Acclimation

Acclimation can often be overlooked in fish hauling operations because of the time spent on the actual transport as well as knowing that the fish have arrived safely. However, fish can become stressed if not acclimated properly and can become immunosuppressed, possibly leading to delayed mortality. The preferred method to reduce the stress associated with the acclimation process is to mimic the water from which the fish was taken. When hauling fish this involves the transport container water and the receiving water after transport. Abrupt changes in water parameters, such as temperature, pH, hardness and salinity, should be avoided (Noga 2000). Fish should be acclimated to receiving water if it is much different from the transport water. However, as mentioned previously, adding salts as well as reducing the hauling temperature of freshwater species can be beneficial during the hauling procedure. Therefore, depending on the gradient difference an acclimation procedure might need to be part of the transport procedure.

Noga (2000) recommends a 1°C change per hour for most fish, but acknowledges that certain species will tolerate a more rapid change. However, Wedemeyer (1996) noted that healthy salmonids could tolerate up to a 10°C change in water temperature with only mild stress and that it is a common practice for culturists to gradually acclimate over a few hours if the temperature difference is >10°C. Timmons *et al.* (2002) recommended that a temperature change should not exceed 5.5°C in 20 min and if the pH differs by more than one unit to exchange 10% of the tank water every 10–20 min with the receiving water until it is similar. Most fish seem to tolerate a rapid drop in temperature better than the equivalent rise in temperature (Noga 2000). Because of the uncertainty of variables, such as cumulative stressors and the overall condition of the fish, past experience in similar situations is certainly beneficial, but might vary among shipments. Moreover, the extreme variation in the acclimation procedures often reported probably results from differences in the aforementioned variables.

Final considerations

Prior knowledge of transport techniques and familiarity with a species' tolerances and the history of the fish being transported is invaluable to biologists. A list of recommendations for hauling conditions and acclimation of various species is presented in Table 1. Carmichael and Tomasso (1988) evaluated survey results on transport methods for 13 species of fish and found great variability among haulers. Each species along with life stage and genetics might differ in water-quality tolerances as well as susceptibility to transport-induced stressors (Barton & Iwama 1991). Furthermore, the health of the fish plays an important role in survivability and health after transport. It is not only the transport procedures that are important – the holding conditions before transport and the receiving waters after transport can also play an important role in the success of live-fish transports. A fish in poor condition before any transport activities is more likely to be overcome by the physiological challenges associated with transport-induced stress than a healthy fish.

During transport some sources of potential stress might be unavoidable; however, there might be practices that will reduce the amount and duration of stress placed on the fish. With these practices and procedures in place the biologist/culturist will be more successful during transport and will also increase overall fish health after transport.

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