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The Use of Feed and Water Additives for Live Fish Transport

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Abstract

The transport of live fish for aquaculture, either for food or as companion animals, presents a major issue for animal welfare. The stressors associated with live transportation are well documented with a focus on maintaining water quality during transport to reduce stress. Far less considered is our ability to enhance health and welfare during transport through the use of dietary and water additives prior to and during transport. With increasing interest in the use of plant essential oils as feed additives in food fish aquaculture and the increased availability of products claiming to alleviate stress in ornamental species, there is a need for scientific investigation into these potential welfare-promoting methods. Here we summarise current knowledge on the use of food additives, water conditioners, antibiotics, antimicrobials, and probiotics to promote fish health during transport. This review aims to highlight the gaps in our knowledge surrounding promising ways of promoting fish health during transport and to stimulate new research in this area.

Key Words: fish transport, stress, water quality, welfare management
Introduction

Fish production as a food source was valued at US$160 billion in 2014 (FAO 2016) and the estimated worth of the ornamental fish industry (including wholesale, wages, retail sales, associated materials etc.) is approximately US$15 billion (FAO 2011). In both aquaculture enterprises, fishes will be transported at some point in the production chain although the duration and the type of transport varies greatly between the two industries.

Fishes destined for food are often transported by road to various facilities in open systems where continuous monitoring of water quality and access to fish during transport is possible (Van de Sande 1974; Berka 1986; Rimmer & Franklin 1997; Lekang 2007; Espinosa-Curiel et al. 2016). In contrast, ornamental fishes tend to be transported over greater distances in closed systems (e.g. in plastic bags shipped by aeroplane) (Braker 1974; Berka 1986; Swann 1992; Rimmer & Franklin 1997; Cole et al. 1999; Marine Aquarium Council 2001; Lim et al. 2007). While fish transport practices have been reviewed extensively, exact information on mortality during transport is particularly limited for the ornamental fish trade with estimated values ranging from a few per cent to greater than 80% (Rubec & Cruz 2005). High mortality is also reported during recovery from transport (Froese 1988; Sadovy 2002; Rubec & Cruz 2005).

Despite the differences in transport practice between food fishes and fishes destined for the pet industry, welfare challenges for transportation are very similar. Stressors encountered during transport include handling prior to transport, deterioration of water quality during transport and increased susceptibility to metabolic shock, stress, infection and disease after transport (Pickering et al. 1982; Portz et al. 2006; Sampaio & Freire 2016).

There are several excellent reviews on how water quality and husbandry practices can
improve welfare (Berka 1986; Swann 1992; Cole et al. 1999; Crosby et al. 2005a, b; Lim et al. 2007; Harmon 2009), but far less scientific attention has focused on the potential to enhance welfare during transport over and above good husbandry practice. For example, a range of commercial and non-commercial products have been suggested to aid fish health during transport but there is a considerable absence of studies within the scientific literature surrounding these products. This review will firstly consider whether the stress of transport can be alleviated by feed or water treatment prior to transport, and secondly consider current knowledge surrounding water additives during transport.

Can we Prepare Fishes for Transportation Stress?

Dietary supplements are commonly used in aquaculture for a wide variety of reasons including stress reduction (Peng et al. 2013; Vallejos-Vidal et al. 2016), improving specific and non-specific immune resistance (Wang et al. 2006; Vallejos-Vidal et al. 2016), enhancing colouration (Pan & Chien 2009; Kouba et al. 2013) and increasing growth rate (Vallejos-Vidal et al. 2016). Therefore, prior to transport, there is the opportunity to use dietary supplements to enhance the immune system and improve stress tolerance (Volpatti et al. 1998; Lim et al. 2002; Rollo et al. 2006). Dietary supplements that have been tested in relation to transport in fishes include glucan, probiotics, ascorbic acid, carotenoids and herbal supplements.

Glucan is a polysaccharide which enhances non-specific immunity in fishes and reduces susceptibility to stress and the immunosuppressive effects of stress (Volpatti et al. 1998; Kim et al. 1999; Vallejos-Vidal et al. 2016). Rainbow trout (Oncorhynchus mykiss, Walbaum 1792) fed a diet with no (0%), low (0.1%), medium (0.5%) or high (1%) levels of
glucan for four weeks showed differences in non-specific immune responses to 2 h simulated transport stress (Volpatti et al. 1998). Following transport stress, lymphocytes decreased in the group fed 0% glucan, whereas in all the glucan-fed groups, lymphocytes, monocytes and neutrophils, increased demonstrating that glucan increases the non-specific immune response. Phagocytosis and respiration burst activity of cells were also higher in glucan-fed groups than in the 0% group. A sub-group of fish was fed the experimental diets for a further two months following transport to determine the long-term effect of glucan on the digestive tract. Prolonged exposure to glucan resulted in slight deterioration within the epithelial cells of the stomach and gut (Volpatti et al., 1998) suggesting that prolonged ingestion of glucan may not be beneficial.

Probiotics are either a mono or mixed culture of live micro-organisms that are ingested and multiply in the gut of host organisms in order to improve the indigenous microflora (Havenaar et al. 1992; Cross, 2002). Probiotics are increasingly being considered as a safer and more environmentally-friendly alternative to antibiotics with their use in aquaculture increasing as a response to public demand for antibiotic-free fishes (Martínez Cruz et al. 2012). The desired effects of probiotics include improved larval development, growth promotion, stimulation of the immune system, pathogen and disease control, stress resistance and improved water quality (Martínez Cruz et al. 2012). Ambas et al. (2015) simulated transport of marron (Cherax cainii, Austin 2002) reared on marron commercial feed as a control, or control feed enriched with Bacillus mycoides (Flugge 1886). After 48 h, survival of marron fed the control diet was 93.3 ± 2.8% S.E. vs. 100% in the probiotic group. Marron in the probiotic group also had a higher intestinal bacterial population and total haemocyte count than the controls, indicating an improved immune status when fed probiotics. Although studies on the direct benefits of probiotics in alleviating transport stress
are limited, the advantages of probiotics in response to stressors relevant to transport have also been demonstrated. Rollo et al. (2006) reared sea bream (*Sparus aurata*, Linnaeus 1758) larvae with a mixture of *Lactobacillus fructivorans* (Charlton et al. 1934) and *Lactobacillus plantarum* (Orla-Jensen 1919) for either 20 or 42 days using rotifers (*Brachionus plicatilis*, Müller 1786) and/or *Artemia salina* (Linnaeus 1758) as a vector. At the end of the rearing period, 600 fry were exposed to 6.3 pH for 1 h. The probiotic group had lower levels of mortality and whole body cortisol. Hsp70 gene expression increased after exposure to low pH with the greatest increase in Hsp70 found in sea bream reared with probiotics.

Fishes cannot synthesise ascorbic acid (vitamin C) and rely on absorption through their food (Sales & Janssens 2003; Peng et al. 2013). Supplementing feed with ascorbic acid has been found to reduce mortality following a stressor (Lim et al. 2002). Peng et al. (2013) fed silver pomfret (*Pampus argenteus*, Euphrasén, 1788) a diet supplemented with ascorbic acid (L-ascorbyl- 2-polyphosphate, 35% ascorbic acid equivalent at a concentration of 100, 450 or 800 mg ascorbic acid kg\(^{-1}\) diet) for 9 weeks. Silver pomfret were then transported in darkened plastic bags for 4 h. Diet supplementation with ascorbic acid significantly reduced serum cortisol and glucose levels as well as mortality, indicating that high ascorbic acid successfully reduced stress associated with transport.

Abreu et al. (2014) fed wild-caught pencilfish (*Nannostomus trifasciatus*) one of four experimental diets with 0%, 0.01%, 0.1% or 0.5% beta 1,3 glucan added for 7 days. The fish were then fasted and transported for 24 h. The addition of beta 1,3 glucan reduced the net loss of K\(^+\) during the first 3 h of transport. The addition of 0.5% beta 1,3 glucan to the feed increased the net influx of Na\(^+\) between 3 and 12 hours of transport.
Combinations of ascorbic acid and glucan as supplements have been trialled in relation to transport stress. Barros et al. (2014) supplemented the diet of Nile tilapia (Oreochromis niloticus, Linnaeus 1758) with a basal diet of 125 mg kg\(^{-1}\) of ascorbic acid (BD) followed by a diet containing 0.1% β-glucan and 600 mg kg\(^{-1}\) ascorbic acid (GD). The fish were reared on BD for 20 days, after this period the fish were fed GD for variable durations (7, 15, 30, or 45 days) prior to undergoing transport stress. Fish from all four treatments were transported for 4 h in 100 l net cages within a 600 l fish transport tank. During recovery from the transport, fish fed 0.1% β-glucan and 600 mg kg\(^{-1}\) ascorbic acid for 7 days had the highest cortisol concentrations and greatest increase in red blood cells and haemoglobin. However, longer exposure to β-glucan and ascorbic acid reduced plasma cortisol and after 72 h, cortisol had returned to baseline in Nile tilapia fed this diet for longer than 7 days. As β-glucan and high levels of ascorbic acid were not tested separately, it is unclear which supplement provided the observed improvements or whether it was the two combined.

Organisms undergoing stress can experience a shortage of oxygen at the cellular level causing abnormal oxidative reactions in the aerobic metabolic pathway and the generation of reactive oxygen species (ROS) (Rånby & Rabek 1978; Pan et al. 2010) which if not inactivated can cause oxidative damage to lipids, proteins, carbohydrates and nucleotides (Yu 1994; Chew 1995; Halliwell & Gutteridge 2015). Carotenoids are pigment molecules with known antioxidant properties that are important to animal health by inactivating free radicals. Fishes cannot synthesise carotenoids de novo and must acquire them through their diet. In food fish aquaculture, diets are often supplemented with carotenoids to improve fillet colouration and to reduce oxidative stress. The use of carotenoids, in particular astaxanthin, as a dietary supplement in aquaculture has been extensively reviewed and found to improve...

Dietary carotenoids such as astaxanthin and β-carotene have been shown to help fish cope with stressors that may act as components of transport stress (e.g. hypoxia, Pan et al. 2010; high ammonia, Pan et al. 2011). Therefore, the potential benefits of carotenoids as dietary supplements to prepare fish for transport stress warrants future study.

In aquaculture, plant extracts are mostly used as feed supplements, although some may be added directly to the water (see Additives During Transport below). In 2011 the total global herbal drug market was estimated at US $62 billion and is predicted to grow to US $5 trillion by 2050 with an annual growth rate of 5-15% (Harikrishnan et al. 2011). Plant-derived products can be effective as antioxidants, growth promoters, appetite stimulators, immune stimulants and stress reducers along with having anti-inflammatory and anti-carcinogenic properties (Citarasu 2010; Harikrishnan et al. 2011; Merlini et al. 2014; Bulfon et al. 2015). The use of plant essential oils as dietary supplements in aquaculture was recently reviewed (Sutili et al. 2017) but to our knowledge only two studies have considered the effects of plant extracts on stress tolerance during transportation through administration as a dietary supplement. Turmeric (Curcuma longa, Linnaeus 1758) has a variety of documented medicinal properties (anti-inflammatory, Araújo & Leon 2001; immunostimulant, Chattopadhyay et al. 2004; antioxidant, Luthra et al. 2001, Saccol et al. 2016; anaesthetic, Saccol et al. 2016; anti-microbial, Luthra et al. 2001; anti-parasitic, Araújo & Leon 2001).

Supplementing the diet of juvenile yellow tail tetra (Astyanax aff. bimaculatu, Linnaeus 1758) with turmeric for 60 days before a simulated 24 h transport reduced mortality, plasma lactate and plasma glucose concentrations compared to controls (Ferreira et al., 2017). Aloe vera (Burman 1768) is commonly used in humans for a wide range of medicinal properties.
Choi & Chung 2003; Mahor & Ali 2016) but little is known about the benefits of using A. *vera* as a dietary supplement in aquaculture. Zanuzzo *et al.* (2017) administered one of four diets (0%, 0.5%, 1% or 2% A. *vera*) for 10 days prior to a 4 h transport of juvenile pacu (*Piaractus mesopotamicus*, Holmberg 1887). Immediately following transport, the pacu were divided into three sub-groups: a non-injected control group; a buffer injected group and a group injected with inactivated *Aeromonas hydrophila* (Chester 1901) to stimulate their immune system. This is particularly relevant to transport stress studies as fishes can become highly susceptible to bacterial and viral infections during transport (Yanong 2003; Crosby *et al.* 2005b). On arrival and after 24 h recovery, non-injected A. *vera* fed groups had significantly higher cortisol concentrations than the non-injected control fish. However, in the *A. hydrophila* injected fish, the cortisol levels of the fish fed 1% A. *vera* were significantly lower than in the other groups. Immediately after transport, leukocyte respiratory burst was higher in all (injected and non-injected) A. *vera* fed fish than in the control fish but no significant difference was found between the treatments after 24 h of recovery. The haemolytic activity of the complement system was significantly higher in the A. *vera* fed fish on arrival and after recovery than in the control fish. The results indicate that A. *vera* improved the immune system of juvenile pacu after transport and improved the stress recovery of infected individuals. No explanation for why A. *vera* slowed recovery rate of the non-injected juvenile pacu yet improved the recovery rate of the injected group was given. Further research into the use of herbal dietary supplements in relation to transport of fishes is clearly needed.
The Use of Additives During Transport

There are many reviews that have considered the importance of good water quality during live fish transport (Berka 1986; Swann 1992; Cole et al. 1999; Crosby et al. 2005a, 2005b; Lim et al. 2007; Harmon 2009) and a variety of products can be added to the water to maintain water quality or alleviate the problems of waste products (e.g. pH buffers, zeolites, AmQuel®, nitrifying bacteria). Even when water quality is optimal for the duration of transport, there are many other stressful factors (e.g. handling, high loading density and crowding) (Pickering et al. 1982; Portz et al. 2006; Sampaio & Freire 2016); far less research has focused on whether it is possible to add compounds to the water to alleviate the physiological effects of stress. As demonstrated above, the benefits of dietary supplements for improving health and welfare during transport may require many weeks of preparation; the addition of compounds to the water for the period of transportation requires far less advance planning. Some products such as water conditioners and salt are added directly to the transport water along with fishes while others may require exposure for a short time immediately prior to transport (e.g. anaesthetics). The promotion of physiological well-being through addition of compounds to the water can occur via different physiological mechanisms such as sedation, protection of mucus integrity and disease prevention. While the use of traditional water additives such as salt and synthetic anaesthetics have been well explored, the use of plant extracts as water conditioners and antimicrobial agents has received less attention. Here we will briefly discuss the more traditional water additives but will focus primarily on the use of novel and emerging water additives.

Managing Stress by Sedation
Anaesthetics are one of the most commonly used additives in the transport of fish (Lim et al. 2003; Harmon 2009; Cupp et al. 2017). Anaesthesia is defined as “a state caused by an applied external agent resulting in a loss of sensation through depression of the nervous system” (Akerman et al. 2005). Sedation can lower a fish’s metabolic rate (Ross & Ross 2008) resulting in improved water quality (Pattanasiri et al. 2016), lower levels of stress (Ims 2011) and often allows transportation at higher loading densities (Cupp et al. 2017).

Frequently used synthetic anaesthetics, such as MS-222 (ethyl 3-aminobenzoate methanesulfate) and benzocaine, have been widely reviewed elsewhere (Ross et al. 2008; Carter et al. 2011; Javahery et al. 2012; Readman et al. 2013; Husen & Sharma 2014) and will not be discussed in detail here. Generally, synthetic anaesthetics initially induce stress before having a delayed stress-reduction effect (Ims 2011; Readman et al. 2013). An alternative to sedation through synthetic compounds is to use natural compounds such as essential oils.

The most commonly used essential oil is clove oil (Syzygium aromaticum, Linnaeus 1758). Clove oil has been extensively reviewed for its use as an anaesthetic (e.g. Javahery et al. 2012) but many other less researched essential oils have been considered for use during fish transport. Most studies have considered the use of essential oils during transport by adding them to the water at set concentrations. Condalia buxifolia (Reisseck 1861) methanolic extract induced sedation in silver catfish (Rhamdia quelen) for 6 h and during transport improved survival, water quality and reduced ion loss (Becker et al. 2013). Salbego et al. (2015) also found similar results when they sedated non-starved silver catfish with methanolic extract of C. buxifolia prior to transport and added C. buxifolia solution to the transport water. Addition of C. buxifolia improved water quality, reduced total ammonia
nitrogen (TAN) levels, slowed metabolism and reduced net ion efflux. Lipoperoxidation and carbonylation of proteins decreased in silver catfish transported in C. buxifolia, demonstrating less ROS production. Low concentrations (0.5-10 µl l⁻¹) of C. buxifolia induced fast sedation and higher doses did not cause harmful effects suggesting it is safe to use to induce sedation (Becker et al. 2013).

The use of essential oils in the water is summarised in Table 1. Interestingly, Pattanasiri et al. (2016) tested the release rate of low-density polyethylene (LDPE) bags coated with clove oil over a 48 h period. After the initial 2 h, the release rate of clove oil was almost constant at 12 mg l⁻¹ when the bag contained 75 ml water and 14 mg l⁻¹ when the bag contained 150 ml water. These levels induced sedation in Siamese fighting fish (Betta splendens, Regan 1910) but not anaesthesia. Survival of Siamese fighting fish transported in the clove oil-coated bags was significantly higher, with lower ammonia concentrations and higher dissolved oxygen than in control un-coated bags. Siamese fighting fish did not appear to experience any detrimental effects of prolonged clove oil exposure.

In summary, several essential oils are effective in inducing sedation in fish, however, most essential oils have only been tested at one or a few concentrations and in a single species; few have been tested for use in fish transport. The mechanism of action can vary between essential oils and in the most part is poorly understood. It is known that different species have different aversive reactions to anaesthetics, so there is a need to further explore the use of essential oils on individual species of interest (Javahery et al. 2012; Husen & Sharma 2014; Chambel et al. 2015). With the increased commercialisation of essential oils, or components of, for use in fish husbandry (e.g. AQUI-S; http://aquatactics.com/aqui-s-
the potential for better, more refined chemicals for improving fish welfare during transport compared to more traditional synthetic chemicals seems likely.

Maintaining Mucus Integrity

The epidermal layer of fishes excretes protective mucus that serves as a barrier to the external environment (Harnish et al. 2011; Ottesen & Olafsen 1997; Shephard 1994). During transport and handling, fishes can lose their protective mucus, resulting in an increased risk of injuries (Harnish et al. 2011). Mucus loss can result in disturbed osmoregulation, loss of scales, skin damage, and bacterial, fungal and parasitic diseases (Wedemeyer 1996). When fishes are stressed or transported in large numbers within a single bag, the risks of losing mucus increases as fish are more likely to come into contact with the bag or other individuals. Protecting epidermal mucus can result in a higher quality of fish at arrival in terms of physical health, reduced risk of infection and improved aesthetic appearance.

Water conditioners usually refer to compounds added directly to the transport water of fish to reduce stress by means other than sedation. Water conditions may be either pure herbal extracts or combined with other products into a commercially available product. A review on the use of polymer-based water conditioners to reduce handling-related injuries (Harnish et al. 2011) identified three studies (summarised in Table 2). Wedemeyer (1996) presented findings of user surveys and husbandry procedures finding that polymer-based water conditioners reduce mortality.

Stress Coat® (API Aquarium Pharmaceuticals Inc., n.d.) is a commercially available water conditioner which is recommended for addition to water during transport and to tank water during other potentially stressful procedures. Several studies have used Stress Coat® in...
husbandry procedures (Earley et al. 2006; Colburn et al. 2008; Harnish et al. 2011; Wong et al. 2015), however, much of the information on Stress Coat® is not in the peer-reviewed literature. Snellgrove et al. (2007) found that water cortisol levels excreted by goldfish exposed to Stress Coat® were lower following netting compared to goldfish netted with no Stress Coat® exposure. Edmonds (2016) found that Stress Coat® exposure during transport did not reduce excretion of cortisol but reduced conspecific aggression in guppies post-transport.

The main components of Stress Coat® (manufactured by Mars Fishcare) are Aloe barbadensis Mill (also known as A. vera) (1-10%), water (>80%), polyvinylpyrrolidone (PVP) and other non-hazardous ingredients (trade protected) (1-10%) (Mars Fishcare Inc. 2014). Most of the limited peer-reviewed research carried out directly on A. vera focus on it as a dietary supplement used to alleviate stressors not relevant to the transport of fish (e.g. Dotta et al. 2014; Gabriel et al. 2015a,b; Kim et al. 1999; Taiwo et al. 2005; Zanuzzo et al. 2015a,b). One study looked at A. vera as a dietary supplement prior to transport (Zanuzzo et al. 2017; see above) and an additional study by the same group used A. vera in the water during transport of fish. Zanuzzo et al. (2012) dissolved A. vera powder (concentrations: 0, 0.02, 0.2 and 2 mg l⁻¹) in the transport water of matrinxã (Brycon amazonicus, Spix and Agassiz 1829). Aloe vera increased the activity of the immune system, by enhancing the respiratory activity of leukocytes, in matrinxã following handling but the effects of A. vera were no longer apparent at the end of the 4 h transport. Aloe vera has potential to improve the condition of fish during transport procedures but more research is needed to gain a better understanding of the possible benefits and the best method of administration.

Sung et al. (2012) tested another patented product Pro-Tex® (Bradan Limited, a soluble variant of TEX-OE®) to determine whether it improved the resistance of juvenile carp.
to high ammonia levels. Pro-Tex® contains an extract of the prickly pear cactus (Opuntia ficus indica, (L.) Miller 1925) which increases heat-shock protein expression in humans and fishes (Wiese et al. 2004; Roberts et al. 2010; Sandilands et al. 2010). Exposure of carp to Pro-Tex® (2 μl 50 l⁻¹ water) for 2 h increased survival from 50 to 95% and 0 to 20% when exposed to 5.92 mg l⁻¹ and 14.21 mg L⁻¹ of NH₃ respectively for 1 h. Sung et al. (2012) also found that Pro-Tex® increased expression of heat shock protein (Hsp70) in gill and muscle tissue of carp which may suggest potential benefits of Pro-TEX® for fish transport. Hales et al. (1990) evaluated the injury-preventing capacity of a water-soluble gel-coating (composed of antibiotics and non-disclosed pharmaceutical components) applied to the hands of fish handlers. The unexpected results showed that spot croaker (Leiostomus xanthurus, Lacepède 1802) handled with gel-coating had a higher mortality than fish handled by collectors with non-coated hands. The authors hypothesised that the composition of the gel was different from that of the mucus of the fish as mucus mostly consists of protein along with lipids, carbohydrates and nucleic acids (Al-Hassan et al. 1982). Austin et al. (2009), published a non-peer reviewed report about the use of ULTIMATE® (AquaScience Technologies) in the transport water of koi carp. ULTIMATE® has two main components, the ingredients for ClorAm-X® (the original AmQuel®) (composed of sodium hydroxymethanesulfonate, AquaScience Technologies) and the ingredients for Stress-X® (the original NovAqua®) (water, sodium thiosulfate, buffers, electrolytes, proprietary synthetic polymer formulation and preservatives, Aquarium Solutions). In addition, ULTIMATE® contains a dechloraminating agent, electrolytes (including calcium, sodium and chloride ions), a polymer system and product stabilizers (Austin et al. 2009). After 8 h of simulated sealed transport, the koi transported in ULTIMATE® had shorter recovery times than the control fish indicated by accelerated reduction in mucosal levels of haemoglobin. Unfortunately, sample size was not sufficient for
definitive results, and in addition, the methods used to detect levels of haemoglobin in the mucus were not precise enough. No published peer-reviewed paper was found that used ULTIMATE® in fish transport, however it is an area worth investigating based on the observations made by Austin et al. (2009). Despite many studies using substances such as Stress Coat®, Novaqua® and Polyaqua®, little peer-reviewed information is available on the efficacy of these substances, particularly in maintaining mucus integrity.

Disruption of epidermal mucus can cause many detrimental effects including disruption to osmoregulation. The stress of transport itself can also cause changes in osmoregulation (Barton & Iwama 1991; Baldisserotto et al. 2007) and so during transport of freshwater species, salt (NaCl) can be added to the water to reduce the difference between the internal osmolality of the fish and that of its environment thereby reducing physiological workload required to maintain homeostasis (Nikinmaa et al. 1983). Table 3 gives examples of studies that have investigated the effects of NaCl addition during transport. It is clear that for some species, the addition of NaCl during transport may be beneficial but given the range of salinities that different species inhabit and differences in osmoregulatory capacity from stenohaline to euryhaline, the use of NaCl as an additive will always be very species and life-stage specific. Additionally, Tacchi et al. (2015) compared the skin morphology of non-transported rainbow trout to that of rainbow trout transported in fresh or salt water (5 g NaCl l⁻¹) using electron microscopy. Fish transported in salt water had a thin layer of mucus whereas fish transported in fresh water had a thick deposit of mucus. It was suggested that the addition of NaCl slowed down the release of mucus from goblet cells. A ~50-fold increase in skin-associated bacteria in fish transported in fresh water was seen compared to a ~10-fold increase in the salt water group (Tacchi et al. 2015). While salt water transported fish had a thinner mucus layer, the mucus layer of these fish appeared to be in better condition showing
that NaCl also has the potential to reduce subsequent stress caused by bacteria and improve skin mucus condition.

Prevention of Stress-Related Diseases

When fishes experience high levels of stress they become more susceptible to bacterial diseases, which can result in higher mortality (Yanong 2003, Crosby et al. 2005b). To prevent proliferation of bacteria in the transport water while the fish’s immune system is weakened, antibiotics are sometimes used. Amend et al. (1982) tested the efficacy of several antibiotics (kanamycin, gentamicin, chloramphenical, streptomycin, neomycin and furazolidone, each at 20 mg l⁻¹), the antisepic acriflavine (10, 20, and 100 mg l⁻¹), the disinfectant chlorine dioxide at 20 mg l⁻¹, and the antimicrobial methylene blue (10 and 100 mg l⁻¹). The tests were done by simulating 48 h transport with southern platy (Xiphophorus maculatus, Günther 1866) and checking the efficacy of each substance to control bacteria levels. Kanamycin and gentamicin were found to be toxic to the fish at these concentrations. Methylene blue, chlorine dioxide, furazolidone, and acriflavine did not prevent bacteria growth. Acrafiavine at 100 mg l⁻¹, chloramphicol and streptomycin controlled bacteria levels, but caused mortality in the fish. Only neomycin was effective against bacteria and safe for the fish. Antibiotic resistance in ornamental fishes has been studied since the late 1970s and the literature shows that resistance emerges when new antibiotics become widely available (del Rio-Rodriguez & Turnbull 2002; Rose et al. 2013; Trust & Whitby 1976; Verner-Jeffreys et al. 2009). A study on ornamental fishes by Rose et al. (2013) found that the most effective antibiotics (cefotaxime and kanamycin) were effective against only 45% and 44% of their target bacteria respectively, and of these, 16% and 35% had developed resistance. In this study, nine bacteria were resistant to all the tested antibiotics, only one showed no
resistance at all. Dixon et al. (1999) found similar results in a study looking at bacterial resistance in fish imported from Singapore. Over 50% of the bacteria isolated were resistant to 7 out of 12 tested antibiotics. In addition to increased resistance, antibiotics can cause increased levels of stress in fishes. Cururu stingray (Potamotrygon cf histrix, Müller and Henle 1841) transported in water containing tetracycline (200 mg l\(^{-1}\)) for 24 h had elevated corticosterone levels after 12 h compared to controls (Brinn et al. 2012). The administration of antibiotics during transport is also problematic because the use of antibiotics is strictly monitored and regulated in many countries (Cole et al. 1999; Crosby et al. 2005b; Brinn et al. 2012).

Probiotics can be added to transport water to improve water quality and reduce stress arising from low quality water. Efinol\(^\circledR\)L is a commercial probiotic product containing Bacillus subtilis (Ehrenberg 1835), Bacillus licheniformis (Weigmann 1898), Lactobacillus acidophilus (Moro 1900) and Saccharomyces cerevisiae (Hansen 1883); it also comprises amino acids, vitamins, minerals, free-flow and anti-caking agents (calcium carbonate and silica). Marbled hatchetfish (Carnegiella strigata, Günther 1864) were transported in a water solution containing 10 mg l\(^{-1}\) probiotic Efinol\(^\circledR\)L. After 24 h of transport, water containing the probiotic solution had higher dissolved oxygen levels and lower ammonia concentrations (Gomes et al. 2008). Fish in the control group had higher body cortisol levels and higher efflux of Na\(^+\) and K\(^+\). The reduced stress levels seen in marbled hatchetfish treated with probiotics could be attributed to either a direct effect of Efinol\(^\circledR\)L on physiology or an indirect effect of improved water quality. Using a similar protocol, Gomes et al. (2009) transported cardinal tetra (Paracheirodon axelrodi, Schultz 1956) in water containing Efinol\(^\circledR\)L. The addition of Efinol\(^\circledR\)L resulted in a higher survival, higher water alkalinity and lower total ammonia in the water. The cortisol levels of the cardinals in the Efinol\(^\circledR\)L group were
significantly lower after transport compared to control fish. Efinol® L is not the only probiotic to have been added directly to the water. Zink et al. (2011) transported yellowfin tuna (Thunnus albacares, Bonnaterre 1788) yolk sac larvae one day post-hatching for 24 h. In the probiotic treatment, 300 ml of EcoAqua® (108 colony-forming units ml⁻¹ in a mix of B. subtilis, B. licheniformis, B. megaterium (Bary 1884), and B. laterosporous (Laubach 1916) EcoMicrobials LLC, Miami, Florida) was added to the water. No difference in survival was recorded between the treatments although the water quality of the bags containing probiotics was greater than in the control bags (lower pH, lower TAN and higher dissolved oxygen). Although the mechanisms of effect of these probiotic solutions are not yet fully understood, and it is unclear whether there are benefits related to reduced disease susceptibility, the positive effects seen in this limited number of studies suggest that more widespread investigation into the addition of probiotics during transport is warranted.

Concluding Remarks

There is a growing market for novel compounds which can be administered either via the diet or water to alleviate stress in fishes with the aim of increasing welfare. Perhaps driven in part by an increasing desire of consumers (either of food fishes or pet fishes) to purchase products which have not been exposed to synthetic chemicals, many of the emerging products are based on natural compounds or enhancing natural processes. While there is growing evidence that some of these compounds can improve welfare, much of the evidence remains anecdotal and the mechanisms of effect have been overlooked. Dietary supplements such as glucan, ascorbic acid, carotenoids, herbal supplements and probiotics may have the potential to reduce stress and mortality during transport but far more research is required to understand the capabilities of these supplements. Several commercially available water
conditioners have been considered in relation to transport stress, but there is a significant lack of peer-reviewed publications and publically available data on the testing of these products. Although, the process is not fully understood, probiotics can also be effective in reducing stress and mortality when added directly into transport water but as yet susceptibility to disease agents following such treatment remains unexplored. In order to enhance the welfare of fishes transported within aquaculture there is an urgent need to explore these emerging areas.

References


Ackerman PA, Morgan JD, Iwama GK (2005) Anesthetics. Supplement to the CCAC guidelines on: the care and use of fish in research, teaching and testing. Available at: https://www.ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Fish_Anesthetics.pdf


Lim KC, Yusoff FM, Shariff M, Kamarudin MS (2017) Astaxanthin as feed supplement in aquatic animals. Reviews in Aquaculture.


Swann L (1992) Transport of Fish in Bags. Extention publications (MU), Illinois-Indiana Sea Grant Program.


Table 1. Research into the use of essential oils during the transport of fishes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life stage/Size</th>
<th>Essential oil</th>
<th>Concentration</th>
<th>Stressor</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver catfish</td>
<td>Juvenile (mean:</td>
<td><em>Lippia alba</em></td>
<td>0, 10 μl l⁻¹</td>
<td>5, 6 or 7 h transport</td>
<td>Reduction of lipoperoxidation (LPO), catalase, superoxide dismutase and glutathione-S-transferase in the liver in fish transported for 5 h.</td>
<td>Azambuja <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>(<em>Rhamdia quelen</em>)</td>
<td>64.5 ± 6.1 g and 18.85 ± 0.57 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver catfish</td>
<td>mean: 262.0 ± 73.5 g, 38.5 ± 1.1 cm</td>
<td><em>Aloysia triphylla</em></td>
<td>0, 30, 40 μl l⁻¹</td>
<td>6 h transport</td>
<td>Lower plasma cortisol and ion loss compared to control, and higher plasma Na⁺ and Cl⁻ concentrations.</td>
<td>Zeppenfeld <em>et al.</em>, 2014</td>
</tr>
<tr>
<td>(<em>Rhamdia quelen</em>)</td>
<td>38.5 ± 1.1 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
concentrations in the liver.

Lower muscle lactate and higher muscle glucose levels.

<table>
<thead>
<tr>
<th>Fat snook</th>
<th>Juvenile</th>
<th>Menthol (5-Methyl-2-(propan-2-yl)cyclohexan-1-ol)</th>
<th>0, 3.7 or 7.4 mg l⁻¹</th>
<th>10 h transport</th>
<th>Effective anaesthetic for short term handling.</th>
<th>Separčho et al., 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Centropomus</td>
<td>(mean ±</td>
<td>SEM: 1.6 ± 0.4 g and 4.8 ± 0.4 cm)</td>
<td>10 h transport</td>
<td></td>
<td>No effects on mortality, ammonia, dissolved oxygen, nitrite levels after transport.</td>
<td>Sepurčho et al., 2016</td>
</tr>
<tr>
<td>parallelus)</td>
<td>SEM: 1.6</td>
<td>0.6 g and 4.8 ± 0.4 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fat snook</th>
<th>Juvenile</th>
<th>Nectandra megapotamica</th>
<th>0, 15, 30 µl l⁻¹</th>
<th>10 h transport</th>
<th>Higher post-transport mortality in the 30 µl l⁻¹ than the 15 µl l⁻¹ and the control group.</th>
<th>Tondolo et al., 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Centropomus</td>
<td>(Centropomus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parallelus)</td>
<td>parallelus</td>
<td>megapotamica</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Silver catfish</th>
<th>mean ±</th>
<th>Condalia buxifolia</th>
<th>0, 5, 10 µl l⁻¹</th>
<th>6 h transport +</th>
<th>Lower water TAN in both groups</th>
<th>Salbego et al. 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rhamdia quelen) SEM:</td>
<td>pre-sedation transported in C. buxifolia.</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>420.1 ± 8.8 g and 21.2 ± 2.3 cm</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Lower net efflux of Na⁺, Cl⁻ and K⁺ in both groups transported in C. buxifolia.

Higher \( \text{PvO}_2 \), \( \text{PvCO}_2 \) and \( \text{HCO}_3^- \) in 5 µl l⁻¹ group.

Lower hepatic lactate in the 10 µl l⁻¹ group.

Lower muscle lactate in both 5 and 10 µl l⁻¹ vs. control group.

Improved antioxidant status.

<p>| Silver catfish mean ± | L. alba 0, 30, 40 µl l⁻¹ 6 h transport + Lower water TAN. Becker et al. 2015 | 48 |</p>
<table>
<thead>
<tr>
<th>(Rhamdia quelen)</th>
<th>SEM:</th>
<th>pre-sedation</th>
</tr>
</thead>
<tbody>
<tr>
<td>420.1 ±</td>
<td></td>
<td>Lower net efflux of Na(^+), Cl(^-) and K(^+).</td>
</tr>
<tr>
<td>8.8 g and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.2 ± 2.3 cm</td>
<td></td>
<td>Higher P(_v)O(_2), P(_v)CO(_2) and HCO(_3)(^-) in the 40 µl l(^{-1}) group.</td>
</tr>
</tbody>
</table>

Higher plasma cortisol in the 30 µl l\(^{-1}\) compared to the control group.

<table>
<thead>
<tr>
<th>Silver catfish</th>
<th>mean ±</th>
<th>L. alba 0, 30, 40 µl l(^{-1}) 6 h transport + pre-sedation</th>
<th>Lower net efflux of Na(^+), Cl(^-) and K(^+).</th>
<th>Salbego et al. 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rhamdia quelen)</td>
<td>SEM:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>420.1 ±</td>
<td></td>
<td>An exposure of 30-40 µl l(^{-1}) induced oxidative stress and elevated cortisol.</td>
<td></td>
<td></td>
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<tr>
<td>8.8 g and</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>21.2 ± 2.3 cm</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Silver catfish</th>
<th>mean ±</th>
<th>C. buxifolia 0, 25, 50 µl l(^{-1}) 12 h transport</th>
<th>Lower non-ionized ammonia levels.</th>
<th>Becker et al. 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rhamdia quelen)</td>
<td>SEM:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish Species</td>
<td>Stage</td>
<td>Treatment</td>
<td>Duration</td>
<td>Outcome</td>
</tr>
<tr>
<td>--------------</td>
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<td>---------</td>
</tr>
<tr>
<td>Swordtail fish (Xiphophorus hellerii)</td>
<td>mean: 1.50 ± 0.02 g and 165.7 ± 22.5 g</td>
<td>Valerian root (Valeriana officinalis)</td>
<td>1 g l⁻¹</td>
<td>Lower net efflux of Na⁺, Cl⁻ and K⁺.</td>
</tr>
<tr>
<td>Nile tilapia (Oreochromis niloticus)</td>
<td>Juvenile, mean: 1.34 ± 0.07 g and 4.25 ± 0.22 cm</td>
<td>Alpinia galanga</td>
<td>150 mg l⁻¹</td>
<td>Lower mortality and whole body cortisol.</td>
</tr>
<tr>
<td>Silver catfish (Rhamdia quelen)</td>
<td>Juveniles, mean: 25, 35 µl l⁻¹</td>
<td>Myrcia sylvatica</td>
<td>6 h transport</td>
<td>Lower plasma cortisol and lactate levels, increased Na⁺/K⁺-ATPase gill activity</td>
</tr>
<tr>
<td>Value</td>
<td>Description</td>
<td></td>
<td></td>
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<td>---------</td>
<td>----------------------------------------------------------------------------</td>
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<tr>
<td>8.9 ± 2.7</td>
<td>Lower gene expression of corticotropin-releasing hormone,</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>proopiomelanocortins, prolactin and somatolactin indicating lower stress</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>12.4 ± 1.3</td>
<td>pathways activation</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. Studies investigating the effects of polymer-based water conditioners (adapted from Harnish et al. 2011).

<table>
<thead>
<tr>
<th>Species</th>
<th>Life stage/Size</th>
<th>Water conditioner</th>
<th>Concentration</th>
<th>Stressor</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallmouth bass (Micropterus dolomieu)</td>
<td>Adult</td>
<td>Catch’n’Release Formula®</td>
<td>5 g l⁻¹</td>
<td>Live release angling tournaments</td>
<td>Cardiac disturbances recovered within ~60min for control fish. Cardiac disturbances in fish exposed to Catch’n’Release lasted for ~180min</td>
<td>Cooke et al. (2002)</td>
</tr>
<tr>
<td>Delta smelt (Hypomesus transpacificus)</td>
<td>mean: 4.7 (August)</td>
<td>NovAqua® in 8 g l⁻¹ NaCl</td>
<td>0.5 ml l⁻¹</td>
<td>Holding and transport post-capture</td>
<td>NovAqua in 8‰ NaCl increased 72 h survival (54.8%) when compared to the control with 8‰ NaCl (27.9%).</td>
<td>Swanson et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>5.1 cm (November)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Largemouth bass (Micropterus salmoides)</td>
<td>Unreported</td>
<td>Unspecified commercial product§</td>
<td>1 mg 75 l⁻¹</td>
<td>Live release angling tournaments</td>
<td>Survival for fish held in water with conditioner for 3-9 h was higher (96.5%) than fish held in unconditioned water (90.8%).</td>
<td>Plumb et al. (1988)</td>
</tr>
</tbody>
</table>
The water conditioner contained unspecified quantities of sodium chloride, potassium chloride, sodium thiosulfate, pyrogenic silica, dimethylketone, alpha-methylquinoline, methylene blue, nitromersol, ethylenediaminetetraacetate, triethylene glycol, and acriflavine (Plumb et al. 1988).
Research into the effects of adding NaCl to the water of fishes during transport.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life stage/Size</th>
<th>Concentration</th>
<th>Stocking density</th>
<th>Stressor</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown trout (Salmo trutta)</td>
<td>mean: 76.2 ± 1.7 g</td>
<td>0.6 g l⁻¹ NaCl</td>
<td>100 g l⁻¹</td>
<td>14 h transport</td>
<td>Smaller increase of blood oxygen carrying capacity than fish in the control group.</td>
<td>Nikinmaa et al. 1983</td>
</tr>
<tr>
<td></td>
<td>1.7 g, 20.4 ± 0.1 cm</td>
<td></td>
<td></td>
<td></td>
<td>No reduction of plasma osmolality and higher plasma levels of Cl⁻ and Na⁺.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Higher liver glycogen and muscle lipid contents when compared to control fish.</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Mean ± SE</td>
<td>Fresh Water (5, 10, 20, 40, 80 mg l⁻¹ Ca²⁺)</td>
<td>Salt Water (1, 36.4 g l⁻¹ NaCl)</td>
<td>Confinement</td>
<td>Highest Survival for Fish in Fresh Water</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Freshwater drum (Aplodinotus grunniens)</td>
<td>36.5 cm 60 g l⁻¹ NaCl 6 h transport</td>
<td>Reduction in immediate and delayed mortality after transport.</td>
<td>Johnson &amp; Metcalf 1982</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Striped bass (Morone saxatilis)</td>
<td>72 ± 2.5 g 180 g l⁻¹ NaCl 5 h transport</td>
<td>Reduction in delayed mortality over a 4-week period following transport.</td>
<td>Mazik et al. 1991</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White bass (M. chrysops) x striped bass hybrids</td>
<td>38.7 ± 1.1 g 10, 20, 40, 80 mg l⁻¹ Ca²⁺</td>
<td>Highest survival for fish in fresh water was at 80 mg l⁻¹ Ca²⁺ at both stocking densities</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>58.7 ± 1.1 g 55.2 g l⁻¹ NaCl 6.5 h confinement</td>
<td>Ca²⁺ at both stocking densities (with higher survival at higher</td>
<td>Weirich et al. 1992</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
8, 16, 24 g l$^{-1}$

NaCl stocking).

Highest survival in salt water was at 8 g l$^{-1}$ NaCl, with similar survival at both densities.

Plasma osmolality decreased during confinement in fresh water.

In sea water, fish in 8 g l$^{-1}$ maintained plasma osmolality.

Plasma osmolality at 16 and 24 g l$^{-1}$ increased.
mean group weights = 3.7 ± 0.3 to 55.8 ± 1.7 g

Fresh water (5, 10, 20, 40, 80 mg l⁻¹ Ca²⁺) 60 g l⁻¹ 12 h simulated transport Fish in all treatments had <5% mortality with no variation in mortality levels between the treatments.

Salt water (1, 8, 16, 24 g l⁻¹ NaCl) 61.75 g l⁻¹ 48 h transport Lower blood glucose levels after transport in both 0.5 and 1 g l⁻¹ NaCl groups compared to levels in fish transported in fresh water or with zeolites.

Xenocara (Ancistrus triradiatus) mean: 10.4 ± 0.5, 1 g l⁻¹ 61.75 g l⁻¹ 48 h transport

No significant difference between 0.5 and 1 g l⁻¹ NaCl

Ramírez-Duarte et al. 2011
groups.

Lower mortality immediately post transport and 7 days post transport in both 0.5 and 1 g l\(^{-1}\) NaCl groups compared to levels in fresh water or with zeolites.

No significant difference between 0.5 and 1 g l\(^{-1}\) NaCl.

<table>
<thead>
<tr>
<th>Xenocara (Ancistrus triradiatus)</th>
<th>mean: 9.0 ± 6.4 g and 7.0 g l(^{-1}) NaCl ± 1.4 cm</th>
<th>1 or 2 g l(^{-1}) NaCl</th>
<th>137.5 g l(^{-1})</th>
<th>12 h simulated transport</th>
<th>Lower blood glucose concentrations.</th>
<th>Ramírez-Duarte et al. 2013</th>
</tr>
</thead>
</table>

Reduction in mortality following transport.
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Duration</th>
<th>Treatment</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or 2 g l(^{-1})</td>
<td>48 h</td>
<td>NaCl</td>
<td>Lower blood glucose</td>
</tr>
<tr>
<td>82.3 g l(^{-1})</td>
<td>simulated transport</td>
<td>48 h</td>
<td>concentrations but higher mortality during the 7 day post transport recovery period in the 2 g l(^{-1}) NaCl treatment.</td>
</tr>
</tbody>
</table>

**Astyanax altiparanae Fingerlings** (mean: 0.37 ± 0.05 g)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Duration</th>
<th>Treatment</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, 3, 6, 9, 12, and 15 g l(^{-1})</td>
<td>96 h salinity exposure with no food.</td>
<td>NaCl</td>
<td>All fish survived for up to 6 h in 0, 3, 6, and 9 g L(^{-1}) NaCl. After 96 h, morality was 75% on the 9 g L(^{-1}) NaCl and 100% in the 12, and 15 g L(^{-1}) NaCl.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Duration</th>
<th>Treatment</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, 3, 6, and 9 g 22, 30 and 37 g l(^{-1}) NaCl</td>
<td>8 h simulated transport</td>
<td>1(^{-1})</td>
<td>Blood glucose levels were significantly lower in the 30 and 37 g l(^{-1}) fish transported in the 3, 6, 9 g l(^{-1}) NaCl compared to the 0 g l(^{-1}) NaCl.</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>mean: 200 g</td>
<td>0.5 g l(^{-1}) NaCl</td>
<td>Unknown</td>
</tr>
<tr>
<td>---------------</td>
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<td>---------</td>
</tr>
<tr>
<td><em>(Oncorhynchus mykiss)</em></td>
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<td></td>
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</tbody>
</table>