Welfare of Teleosts in Transit: Advances in Stress Minimisation Techniques in the Rainbow Trout (*Oncorhynchus mykiss*)

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A concise overview of stress biology in teleost fishes and current methodologies aimed at reducing the physiological effects of transit-induced stress.

Introduction

Transportation of commercial and ornamental fish species is a necessary undertaking for both the primary production and aquarium industry, respectively. Processes to do so are variable and dependent on the transit time and mode, size and behaviour of the species. Fish are commonly transported singly or in groups in plastic bags or tanks. Transit-induced stress is ubiquitous especially in exported species which are typically in transit for prolonged periods. Developments in improved methodologies are necessary to reduce the physiological effects of transit-induced stress. These will result in improved fish welfare and desired outcomes for both suppliers and vendors. This short review will summarise the physiological responses to chemical stressors in fish and analyse current methodologies proposed to reduce transit-induced stress in rainbow trout (*Oncorhynchus mykiss*) as a model for other commercial and ornamental species.

Chemical Stressors and Physiological Responses

Transit-induced physiological responses to stress in fish are the result of time-dependent deterioration of water quality via the accumulation of ammonia and carbon dioxide (metabolic wastes) and depletion of oxygen leading to hypoxia and anaerobic metabolism (lactate production) in cases of prolonged transit (>8hr) (Sampaio and Freire, 2016). Accumulation of carbon dioxide via aerobic metabolism results in acidification of transport water leading to acidosis (Sampaio and Freire, 2016). Acidosis/hypercapnia leads to reduced affinity of haemoglobin for oxygen and consequently a reduction in blood oxygen-carrying capacity. Ammonia accumulates as a result of protein catabolism, especially in fish that have not been fasted prior to transport (Chow, 1994; Sampaio and Freire, 2016). Carbon dioxide and ammonia production are dynamically linked by their effects on pH (Tang *et al*. 2009). Protein catabolism results in ammonia production and subsequent increase in pH, whilst aerobic metabolism of glucose stores results in carbon dioxide production and a consequential drop in pH (Mommsen *et al*. 1999; Tang *et al*. 2009).

Therefore, the primary water chemical parameters which should be monitored to reduce stress responses in transit are both ammonia and carbon dioxide concentrations which are difficult to regulate in closed systems (Sampaio and Freire, 2016). Conversely, variations in pH and oxygen saturation in closed transport systems are easier to regulate through the addition of buffers and supply of excess pure atmospheric oxygen, respectively (Treasurer, 2010; Shabani *et al*. 2016). All of these water chemistry parameters will fluctuate depending on the volume of transit water, duration of transit, stocking density and environmental temperature (Noga, 2000; Sampaio and Freire, 2016).

Physiological stress responses in fish occur in three distinct phases; primary, secondary and tertiary (Iwama et al. 2006), of which primary and secondary are most important in the context of transit-induced stress. Primary neuroendocrine responses involve the release of catecholamines from the chromaffin tissue of the anterior kidney and activation of the hypothalamic-pituitary-interrenal cellular system resulting in the secretion of cortisol from interrenal tissue of the anterior kidney (Iwama *et al*. 2006; Sampaio and Freire, 2016). Cortisol mobilises glucose to facilitate increased respiration (George et al. 2013). Secondary stress responses are the physiological and biochemical effects of the hormones released during the primary response. They include variations in activation of heat-shock proteins, haematological parameters, metabolites and ions in the blood as well as increases in increases in blood glucose, commonly detected and analysed as a marker of the secondary stress response (Barton, 2002; Iwama *et al*. 2006).

Stress Reduction in Transport

Tacchi, *et al.* (2015) assessed the effectiveness of the addition of salt (NaCl 5g/L) to water in reducing transit stress in the rainbow trout (*Oncorhynchus mykiss*) and the mechanism by which salt may acts as a stress moderator. Salt addition was found to shape the bacterial community of the skin of fish by selecting for salt-tolerant species and reducing the overall bacterial load as seen by scanning electron microscopy (SEM). However, this pattern was not reflected in skin-associated bacterial quantification by *in vitro* culture (colony-forming units) which showed relatively similar increases in bacterial numbers in both test groups (salt treated and non-salt treated). Thus, salt addition did not appear to reduce the potential for stress-induced infection. Stress in transit resulted in increased mucus production in both test groups, trapping abundant bacteria, as seen by SEM. However, mucus production in the salt treated group was patchy, suggesting a reduced stress response in these fish. Salt addition did not affect cortisol responses directly, but inhibited the release of glucose in the blood of the salt-treated fish compared to the untreated group. This study, among others, highlights that the addition of salt to transport water may reduce non-specific stress responses in fish resulting in improved welfare outcomes (Harmon, 2009).

Shabani *et al.* (2016) assessed current transport conditions affecting water quality and stress in rainbow trout to develop suggestions for improved handling practices. Heavy oxygen saturation and elevation of carbon dioxide during transport, despite suboptimal water quality and excessive respiration rates, proved to be the primary factors contributing to stress. The research suggested that minimising air exposure time and preventing exposure to either supersaturated or suboptimal concentrations of dissolved oxygen would result in reduced effects of stress and improved welfare of fish.

Mohammadi and Khara (2015) examined the effects of clove oil, an anaesthetic agent, on stress responses in rainbow trout compared to those induced by three other anaesthetic agents; MS-222, ketamine and tobacco extract. Clove oil induced the smallest increase in blood cortisol and lactate concentrations, suggesting it has potential in minimising stress responses when utilised as a transport water additive. However, these observed effects were based on a spectrum of anaesthesia-inducing concentrations (20-60mg/L) of clove oil, rather than appropriate transit doses.

Conclusion

These studies highlight advances in methodologies which may be used to reduce stress responses in commercial and ornamental fish species during transport. Whether methodologies involve the addition of agents (anaesthetics and salts) to water or avoidance of specific water quality conditions/states, reduced stress responses are best ameliorated via an integrated approach. Furthermore, an understanding of the physiology of fish stress and the inciting stressors involved, coupled with improved maintenance of water quality in transit will better equip exporters/suppliers with the necessary skills to optimise fish welfare during transportation.

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References

Barton, BA 2002, 'Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids', Integrative and Comparative Biology, vol. 42, no. 3, pp. 517-525.

Chow, PS, Chen, TW & Teo, LH 1994, 'Physiological responses of the common clownfish, amphiprion ocellaris (cuvier), to factors related to packaging and long-distance transport by air', Aquaculture, vol. 127, no. 4, pp. 347-361.

George, N, Peter, VS & Peter, MCS 2013, 'Physiologic implications of inter-hormonal interference in fish: Lessons from the interaction of adrenaline with cortisol and thyroid hormones in climbing perch (anabas testudineus bloch)', General and Comparative Endocrinology, vol. 181, no., pp. 122-129.

Harmon, TS 2009, 'Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: A review of the basics', Reviews in Aquaculture, vol. 1, no. 1, pp. 58-66.

Iwama, GK, Afonso, LO & Vijayan, MM 2006, 'Stress in fish', The physiology of fishes, vol. 3, no., pp. 319-342.

Mohammadi, M & Khara, H 2015, 'Effect of different anesthetic agents (clove oil, tricaine methanesulfonate, ketamine, tobacco) on hematological parameters and stress indicators of rainbow trout oncorhynchus mykiss, walbaum, 1792', Comparative Clinical Pathology, vol. 24, no. 5, pp. 1039-1044.

Mommsen, TP, Vijayan, MM & Moon, TW 1999, 'Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation', Reviews in Fish Biology and Fisheries, vol. 9, no. 3, pp. 211-268.

Noga, EJ 2011, Fish disease: Diagnosis and treatment, John Wiley & Sons.

Sampaio, FDF & Freire, CA 2016, 'An overview of stress physiology of fish transport: Changes in water quality as a function of transport duration', Fish and Fisheries, vol. no., pp. n/a-n/a.

Shabani, F, Erikson, U, Beli, E & Rexhepi, A 2016, 'Live transport of rainbow trout (onchorhynchus mykiss) and subsequent live storage in market: Water quality, stress and welfare considerations', Aquaculture, vol. 453, no., pp. 110-115.

Tacchi, L, Lowrey, L, Musharrafieh, R, Crossey, K, Larragoite, ET & Salinas, I 2015, 'Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (oncorhynchus mykiss)', Aquaculture, vol. 435, no., pp. 120-127.

Tang, S, Thorarensen, H, Brauner, CJ, Wood, CM & Farrell, AP 2009, 'Modeling the accumulation of co2 during high density, re-circulating transport of adult atlantic salmon, salmo salar, from observations aboard a sea-going commercial live-haul vessel', Aquaculture, vol. 296, no. 1–2, pp. 102-109.

Treasurer, JW 2010, 'Remediation of ammonia accumulation during live transport of juvenile cod, gadus morhua l., and the effects of fast period on ammonia levels and water quality', Aquaculture, vol. 308, no. 3–4, pp. 190-195.