Citation for final published version:

Masud, N, Ellison, A and Cable, J ORCID: https://orcid.org/0000-0002-8510-7055 2019. A neglected fish stressor: mechanical disturbance during transportation impacts susceptibility to disease in a globally important ornamental fish. Diseases of Aquatic Organisms 134 (1) , pp. 25-32. 10.3354/dao03362 file

Publishers page: http://dx.doi.org/10.3354/dao03362
<http://dx.doi.org/10.3354/dao03362>

A neglected fish stressor: mechanical disturbance during transportation impacts susceptibility to disease in a globally important ornamental fish

Running page head: Fish transport influences disease susceptibility

N. Masud, A. Ellison, J. Cable*

School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK

Corresponding author email: cablej@cardiff.ac.uk

ABSTRACT: The transport of fish in aquaculture and the ornamental trade exposes fish to multiple stressors that can cause mass mortalities and economic loss. Previous research on fish transport has largely focused on chemical stress related to deterioration in water quality. Mechanical disturbance during routine fish transport, however, is unpredictable and is a neglected potential stressor when studying fish welfare. Stress induced immunosuppression, caused by mechanical disturbance can increase the chances of contracting infections and significantly increase infection burden. Here, using the model guppy-*Gyrodactylus turnbulli* host-parasite system and a new method of bagging fish (Breathing Bags ™), which reduces mechanical disturbance during fish transport, we investigated how parasite infections contracted after simulated transport impact infection trajectories on a globally-important ornamental, freshwater species. Guppies exposed to mechanical transport disturbance suffered significantly higher parasite burden compared to fish that did not experience transport disturbance. Unfortunately, there was no significant reduction in parasite burden of fish transported in the Breathing Bags ™ compared to standard polythene carrier bags. Thus, transport induced mechanical disturbance, hitherto neglected as a stressor, can be detrimental to disease resistance and highlights the need for specific management procedures to reduce the impact of infectious diseases following routine fish transport.

KEY WORDS: Transport stress . mechanical disturbance . disease susceptibility . ornamental fish . guppy . *Gyrodactylus turnbulli*

1. INTRODUCTION

For the animal industry, transportation can lead to maladaptive traits, including reduced feeding, altered immune response and mortality (Cattle: Stockman et al*.* 2013, Swine: Zou et al. 2017, Poultry: Matur et al*.* 2016, Fish: Momoda et al*.* 2007, Castro et al. 2016). Although the impact of transport stress is a general animal welfare issue, priority of research has been placed on terrestrial livestock (Schwartzkopf-Genswein et al. 2012) over aquatic species. Furthermore, current research on transport stress in fish focusses on food fish and neglects the ornamental trade (Ashley 2007, Stevens et al. 2017) despite fish being the most abundant pet in western households (American Pet Products Association 2012). Indeed, with over 4500 freshwater fish and 1450 marine fish species traded globally as pets, the ornamental trade is a lucrative business valued at U.S. $800 million to $30 billion annually (Stevens et al. 2017) and this demand for ornamentals is increasing with expansion of the global pet trade (Saxby et al. 2010). Increased fish transport is an inevitable consequence of rising demands for exotic species and emphasis on meeting these demands includes minimising transport costs which may lead to fish being transported in sub-optimal conditions.

Stressors experienced by fish during transport can lead to immunosuppression, with the proposed mechanism linked to the release of catecholamines and glucocorticoid hormones as a stress response (Barton 2002, Ackerman et al. 2006), which may increase disease susceptibility (Caruso et al. 2002, Ramsay et al. 2009). There tends to be huge variability in immune responses to stressors (see Tort 2011 for review), with chronic or acute stressors suppressing or enhancing immunity (Dhabbar 2000). In Atlantic salmon, for example, chronic stress suppresses transcriptional immune responses to pathogenic challenge, whereas acute stress enhanced it (Webster et al. 2018). Thus, with transport stressors that remain under the radar, we are still in the dark as to how pathogens will be affected by the host's immune response. Further complications arise when variations in susceptibility to disease are linked to both host and pathogen species making the outcome of transportation on fish welfare uncertain. Chinook salmon (*Oncorhynchus tshawytscha*) and ayu (*Plecoglossus altivelis*), for example, exposed to transport conditions showed increased susceptibility to bacterial infections (Iguchi et al. 2003, Ackerman et al. 2006). Channel catfish (*Ictalurus punctatus)*, that experienced low water crowding stress as part of simulated transport conditions, only showed increased susceptibility when exposed to *Ichthyophthirius multifiliis*, but not to inoculation with the channel catfish virus (Davis et al. 2002).

Typically, fingerlings, juveniles and small fish are transported in plastic bags, filled with 25-30% water and 70-75% air or pure oxygen (Carneiro & Urbinati 2001, Conte 2004). Presence of air pockets in polythene bags for fish increases the chances of mechanical stress due to water movement. In mechanical terms, stress is defined as a force applied across a surface per unit area for all orientations of that surface (Chen & Han 2007). In addition, accumulation of carbon dioxide from respiring fish can lead to displaced available oxygen, especially if stocking densities are high (Conte 2004). Thus, traditional transport carriers can expose fish to multiple stressors, including capture, handling, overcrowding, abrupt changes in temperature and physical trauma (Robertson et al. 1988, Portz et al. 2006). A decline in water quality caused by the accumulation of ammonia (Ackerman et al. 2006), fluctuations in dissolved oxygen and pH (Moran et al. 2008, Sampaio & Freire 2016) which are known fish stressors, is another consequence of transportation (Patterson et al. 2003,). Micro-porous transport bags (Breathing Bags ™, Kordon ®) unlike traditional polythene bags allow exchange of respired carbon dioxide with atmospheric oxygen. Being porous means the bags can be completely filled with water (without the need to add air or oxygen) and since water is incompressible relative to air, this should provide natural cushioning against mechanical stress for fish being transported (Thiagarajan et al. 2011). The impact of other stressors associated with fish transport has been previously investigated (water quality: Ackerman et al. 2006; Dhanasiri et al. 2011, capture and handling: Caruso et al. 2002, Thompson et al. 2016, stocking densities: Ramsay et al. 2009) whereas mechanical stress has thus far remained neglected.

The transport procedure for fish varies globally depending on local animal trading laws and whether fish are transported locally or internationally. The latter routinely involves fish quarantine procedures before transport and border inspections post-arrival (Portz et al. 2006). In addition, such fish will experience extended transport disturbance including multiple handlings due to inspections. Fish transportation procedures typically lack routine screening procedures for parasites and therefore represent a wide-scale welfare issue (Ashley 2007, Stevens et al. 2017). Ornamentals transported from the wild or local pet shops may be reservoirs of undiagnosed infections that become more pernicious due to stress-imposed immunosuppression following transport (Bonga 1997). Due to mixing of species from different geographic regions, disease dynamics in wholesalers, retailers and hobbyist aquaria may result in parasite host switching and increased virulence (Kelly et al. 2009). Accidental or intentional introduction of exotic species into local fish populations can cause transmission of highly virulent parasites to which native fish species may be especially susceptibile (Smit et al. 2017).

Ornamental fish trade practices routinely involve the addition of antiparasitic chemicals into water and removal of weak or diseased fish which reduces disease outbreaks and keep parasite numbers to a minimum (Stevens et al. 2017). Diseases with distinctive symptoms, such as those caused by *Ichthyophthirius multifiliius* or *Saprolegnia parasitica*, are relatively easy to detect through visual inspection of fish, leading to either quarantine or euthanizing infected individuals to halt spread of infections (FAO 2012, Stentiford et al. 2017). Such standard practice for fish farmers and hobbyists does reduce maintenance cost and for legal reasons many countries only sell or display fish that appear healthy (Washington & Ababouch 2011). However, many parasites at low levels of infection do not affect fish phenotype, making them undetectable to non-specialists. Ectoparasites, such as *Gyrodactylus* species, typically require thorough microscopic examination to determine parasite burden (Maceda-Veiga & Cable 2018), which is not a routine procedure for fish at any point in the aquaculture or ornamental trade. For gyrodactylosis, there is no 100% effective treatment and parasites can remain at low frequencies in fish populations that are being transported and then in favourable conditions they can increase exponentially until stock survival is severely affected (Cable 2011). Thus, even if species harbour low-level infections due to the presence of anti-parasitic chemicals, stressful transport conditions can sufficiently weaken the immune system allowing large infection sources to be established in healthy stocks.

Amongst the most popular tropical fish species is the guppy (*Poecilia reticulata*, see Maceda-Veiga 2016), which has been transported worldwide as an ornamental and biological control agent, with 41 recorded introductions outside its native habitat (Magurran 2005). The most common parasites of wild and ornamental guppies are viviparous monogenean *Gyrodactylus* spp. known for their ‘Russian doll’ reproduction and direct transmission (Cable 2011). This makes them capable of rapidly colonising a fish population, affecting their behaviour, including courtship, feeding and shoaling (Kennedy et al. 1987, Kolluru et al. 2009, Hockley et al. 2014) and survival (Cable & van Oosterhout 2007, Yamin et al. 2017).

Here we investigated the impact of simulated transportation on fish infection dynamics. Specifically, we assessed how mechanical disturbance associated with traditional polythene carrier material impacts susceptibility to disease in fish exposed to parasites after simulated transport. In addition, we tested the efficacy of Breathing Bags ™ in helping alleviate mechanical disturbance-induced elevated disease susceptibility and mortality.

2. MATERIALS AND METHODS

2.1. Host and parasite species maintenance

Male guppies (standard length: 12.1-17.4 mm) bred from a stock originating in the Lower Aripo River in Trinidad, were initially housed at Exeter University before being transferred to Cardiff University in October 2014. Guppies were kept in 70 L breeding tanks, containing artificial plants and refugia. They were maintained under a 12 h light: 12 h dark photoperiod (lights on 07:00-19:00) at 24 ± 1°C and fed daily on dry food flake (Aquarium ®) and every alternate day on live freshly hatched *Artemia* nauplii. Experimental infections utilized the Gt3 strain of *Gyrodactylus turnbulli*, isolated from a Nottingham aquarium shop in October 1997 and subsequently maintained at Cardiff University since 1999 on inbred guppies prior to this study. All work was approved by the Cardiff University Animal Ethics Committee and conducted under UK Home Office licence PPL 303424.

2.2. Experimental design

To test the impact of traditional polythene bags versus Breathing Bags ™ on fish susceptibility to disease, guppies (20 per experimental treatment) were experimentally infected after experiencing simulated transport. All guppies were netted carefully from breeding tanks to minimise handling stress and transferred to separate tanks for a 24 h holding period. Fish were not fed for the holding period to ensure a post-absorptive stage and to minimise build-up of nitrogenous waste, as per standard aquacultural practice (Berka 1986). To simulate transport stress, fish were randomly allocated into either 48 x 21 cm polythene bag treatments (provided by Aquatic World, Cardiff) or

36 x 19 cm Breathing Bags™ treatments. The polythene bags were filled with one-third dechlorinated water to two-thirds air which is the most common method of transporting small fish in aquaculture (Conte, 2004). Air was not added to the Breathing Bags ™ as per supplier instructions to reduce mechanical disturbance due to sloshing (Thiagarajan et al. 2011). Fish stocking density was 4 fish/l for both bag treatments, which falls within approved guidelines for tropical freshwater species stocking densities (OATA 2008) and each bag contained water volumes of up to 1.5l. To prevent handling fish with nets, they were placed into bags while fully submerged. Bagged fish were then contained in an insulated sealed thermal box (dimensions: 30 x 24 x 19 cm, 24±1°C) and placed onto an orbital shaker (Stuart®) for 24 h at 50 rpm to simulate transport motion. The rotator allowed for orbital movement on a horizontal platform, similar to any flat surface fish would be placed on in a transport vehicle or aircraft (Portz et al. 2006). Control fish (n=20) were kept in bags without turning on the orbital rotator, adjacent to an operating rotator to ensure fish were exposed to the same noise levels.

2.3. Experimental infections

To perform controlled infections, guppies were lightly anaesthetised with 0.02% MS222 and each fish was infected with two gyrodactylid worms. Parasite transfer was conducted using a dissection microscope with fibre optic illumination following standard methods of King and Cable (2007). Briefly, two worms from heavily infected donor fish were transferred to the caudal fin of recipient hosts by placing the anaesthetised donor fish in close proximity to an anaesthetised naïve host with the transfer monitored continuously using the dissecting microscope. Parasite infections were then monitored every 48 h by anesthetising fish and the total number of gyrodactylids counted over the first 17 days of infection. At Day 17, all fish that survived were treated with Levamisole

(Norbrook ®, UK) according to Schelkle et al. (2009) and their post-treatment recovery and any further mortalities monitored for 3 weeks.

2.4. Water quality

As water quality can impact disease susceptibility (Ackerman et al. 2006), we measured water ammonia (freshwater master test kit, API ®), pH (battery powered checker HANNA ®) and oxygen saturation (dissolved oxygen meter, Lutron Electric Enterprise CO., LTD.) to ensure this did not vary between treatment and control groups (n=5 bags per experiment). All water quality levels within the polythene bags and Breathing Bags ™ post-transport were within normal ranges (ammonia levels undetectable for both bag treatments), pH (pH 7.1-7.8) and oxygen saturation (20.4-21.4 %) and consistent between treatments (Fisher's Exact test: oxygen, p= 0.958, pH, p=0.909).

2.5. Statistical analysis

All statistical analyses were conducted using RStudio v2.1 (R Development Core Team, 2015). *G. turnbulli* mean intensity for all experiments, was defined as the average number of worms on infected hosts (Bush et al. 1997). A generalized linear mixed model (GLMM) with a negative binomial error family in the MASS R package was used to analyse the relationship between transport treatments (polythene bags and Breathing Bags ™) and mean parasite intensity. Host standard length, bag type (polythene bags and Breathing Bags ™) and treatment (transport and no-transport) were treated as fixed factors. As parasite intensity was recorded for each individual fish at different days, 'Fish ID' and 'days since initial infection' was included as a random effect in the GLMM to avoid pseudoreplication by incorporating repeated-measures. Fish length was included in the initial model but was removed because the size range did not explain significant variation (Thomas et al. 2013). Area under the curve (AUC) is a statistical parameter that provides a measure for analyzing infection trajectories over time using the trapezoid rule (White 2011). Area under the curve was analysed using a second GLMM with a negative binomial error family. Finally, we used a Generalised Linear Model (GLM) to analyse how peak parasite day and maximum parasite count varied with treatment. For analysing maximum parasite count we used a negative binomial error family with a log link function and a gaussian error family with an identity link function for peak parasite day. All error families were determined based on the lowest Akaike Information Criterion (AIC) value. A logistic regression was used to analyse motality between transported and control fish and between bag types.

3. RESULTS

Parasite dynamics were influenced by fish simulated transport, with guppies being transported suffering significantly higher mean parasite intensity than untransported control fish (GLMM: Z= 2.51, SE=0.16, p=0.009; Fig. 1). The carrier type used to simulate transport (polythene bags or Breathing Bags ™), however, did not affect the mean intensity between transported and untransported fish (GLMM: Z= 2.51, SE= 0.15, p=0.19). Total infection trajectory over 17-days, as measured through Area Under Curve (AUC) was significantly greater in fish that experienced simulated transport versus controls (GLMM: Z=2.42, SE=0.17, p=0.01). Similarly, peak parasite day (t=2.24, SE=0.25, p=0.02) and the associated maximum parasite count (Z=6.73, SE=0.06, p<0.001) were significantly different between transported and control fish: with maximum parasite count on peak days having approximately 51% greater parasite load compared to controls in both carrier types (Breathing bags™= 50.9% greater, polythene bags= 51.2% greater). There was no significant difference in mortality between simulated transport and control fish within the same bags or between Breathing Bags™ and polythene bags (between bags, GLM: Z =0.18 ,SE=0.35, p= 0.85; within same bags, GLM: Z = 0.89, SE= 0.36, p= 0.371).

4. DISCUSSION

Simulated transport significantly affected guppy susceptibility to infections with *Gyrodactylus turnbulli* showing for the first time the impact of mechanical disturbance on disease dynamics. Unfortunately, increased parasite burdens were not ameliorated following use of specialized Breathing Bags ™ even though these bags did reduce the level of water sloshing during mechanical disturbance; although it should be noted that there could be additional benefits of these bags, not tested here, for example in terms of maintaining higher water quality. Mechanical stress is a broad term that encompasses aspects of physical forces such as pressure and impulse (Thiagarajan et al. 2011) and reduced slosh does not rule out the possibility of such forces acting on fish within Breathing Bags™ during the transport simulation. Thus, fish transported in Breathing Bags™ may indeed have experienced a form of mechanical stress despite reduced water sloshing leading to increased susceptibility to disease.

The link between stressors and susceptibility to disease in fish is influenced by the production of cortisol, which is an immunosuppressant (Tort et al. 2003, Tort 2011). While the relationship between a stress event and immunosuppression is far from clear (reviewed by Tort 2011), the transport process for fish is associated with multiple stressors including handling and netting, with water quality deterioration considered the major stressor linked to high stocking density (see Braun & Nuñer 2014), which has been implicated in elevated cortisol levels, increased disease susceptibility and significant mortality levels (Caruso et al. 2002, Iguchi et al. 2003, Cho et al. 2009, Robertson et al. 2017). However, for the current study fluctuating water quality, temperature, lighting, noise, netting and stocking densities were controlled, leaving mechanical disturbance as the major stressor. While we are unaware of how long a stress response would last in guppies post-transport, as there is likely a species level difference in cortisol production (Honryo et al. 2018), mechanical disturbance in our transported guppies could have caused elevated cortisol production during a stress response leading to immunosuppression. Surprisingly, guppies exposed to gyrodactylid infection immediately prior to experiencing mechanical transport disturbance did not show a significant effect of transportation on total infection infection trajectories or mean parasite intensity compared to untransported fish (Appendix), which indicates immune status at the time of initial infection is the most important factor determining disease outcome.

Undiagnosed infections on imported fish are a major biosecurity risk in the ornamental trade (Maceda-Veiga & Cable 2018), particularly as they may introduce novel parasite species to which local hosts have no immunity (Paterson et al. 2012). The current study emphasises the need for stricter screening procedures after transport, as diseases such as gyrodactylosis are difficult to diagnose without thorough microscopic screening and can cause an explosion in parasite burden due to transport stress. Application of anesthetic agents, like clove oil and MS-222, into water prior to transport has shown limited efficacy in reducing stress and mortality in transported fish (Rubec et al. 2000) and actually is associated with the risk of respiratory failure (Wagner et al. 2003, Pramod et al. 2010). In contrast, addition of compounds, such as salt, prior to fish transportation, can reduce transport-related mortality (Oyoo-Okoth et al. 2011); however, they have variable efficacy on diseases such as gyrodactylosis, as treatment is often time, concentration and species dependant (Schelkle et al. 2011). Studies of parasite diversity in the ornamental trade (pet shops, retailers and home aquaria) highlight *Gyrodactylus* spp. as one of the most common group of parasites detected during screening procedures (Trujillo-González et al. 2018, Maceda-Veiga & Cable 2018). Thus, the impact of this monogenean infection remains a serious welfare issue for global ornamental trade. For the first time our investigation highlights that even when water quality, stocking density and temperature are stable, mechanical disturbance during transport, hitherto neglected as a potential stressor, significantly impacts susceptibility to infections in fish. With disease remaining the major factor limiting the expansion of global fish trade (FAO 2016), investigating stressors that have remained under the radar thus far may prove crucial in a growing trend emphasizing the need for improved fish welfare.

Acknowledgements. This study was partially supported by the Welsh Government and Higher Education Funding Council for Wales through the Sêr Cymru National Research Network for Low Carbon, Energy and the Environment (NRN-LCEE) AquaWales project.

5. LITERATURE CITED

Ackerman PA, Wicks BJ, Iwama GK, Randall DJ (2006) Low levels of environmental ammonia increase susceptibility to disease in chinook salmon smolts. Physiol Biochem Zool 79:695-707 DOI:10.1086/504615

American Pet Products Association (2012). APPA National Pet Ownership Survey 2011-2012. American Pet Productions Association, Available at: http://www.americanpetproducts.org/press industrytrends.asp.

Ashley PJ (2007) Fish welfare: current issues in aquaculture. Appl Anim Behav Sci 104:199-235 DOI: https://doi.org/10.1016/j.applanim.2006.09.001

Bakke TA, Cable J, Harris PD (2007) The biology of gyrodactylid monogeneans: the ‘Russian-doll killers’. Adv Parasit 64:161-460 DOI: 10.1016/S0065-308X(06)64003-7

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticostreroids. Integr and Comp Biol 42:517-525 DOI:10.1093/icb/42.3.517

Bonga SEW (1997) The stress response in fish. Physiol Rev 77:592-616 DOI:10.1152/physrev.1997.77.3.591

Bourne NK (2016) On the Shock Response of Polymers to Extreme Loading. Journal of Dynamic Behavior of Materials 2:33-42 DOI:10.1007/s40870-016-0055-5

Brydges NM, Boulcott P, Ellis T, Braithwaite VA (2009) Quantifying stress responses induced by different handling methods in three species of fish. Appl Anim Behav Sci 116:295-301 DOI:10.1016/j.applanim.2008.09.003

Braun N, Nuñer AO (2014) Stress in *Pimelodus maculatus* (Siluriformes: Pimelodidae) at different densities and times in a simulated transport. Zoologia (Curitiba) 31:101-104 DOI: 10.1590/S1984-46702014000100012

Bush AO, Lafferty KD, Lotz J, Stostakj A (1997) Parasitology meets ecology on its own terms: Margolis et al. re- visited. J Parasitol 83:575-583. DOI:10.2307/3284227.

Cable J (2011) Poeciliid parasites. In: Evans, J.P., Pilastro, A., Schlupp, I. (Eds.), Ecology & Evolution of Poeciliid Fishes. Chicago University Press, p. 82-89

Cable J, van Oosterhout C (2007) The impact of parasites on the life history evolution of guppies (*Poecilia reticulata*): The effects of host size on parasite virulence. Int J Parasitol 37:1449-1458 DOI:10.1016/j.ijpara.2007.04.013

Cable J, Harris PD, Bakke TA (2000) Population growth of *Gyrodactylus salaris* (Monogenea) on Norwegian and Baltic Atlantic salmon (*Salmo salar*) stocks. Parasitology 121:621-629 DOI:10.1017/S0031182000006971

Carneiro PCF, Urbinati EC (2001) Salt as a stress response mitigator of *matrinxã*, *Brycon cephalus* (Günther), during transport. Aquac Res 32:297-304 DOI:10.1046/j.1365-2109.2001.00558.x

Caruso D, Schlumberger O, Dahm C, Proteau JP (2002) Plasma lysozyme levels in sheatfish *Siluris glanis* (L.) subjected to stress and experimental infection with *Edwardsiella tarda.* Aquac Res 33:999-1008 DOI:10.1046/j.1365-2109.2002.00716.x

Castro PL de, Lewandowski V, Souza MLR de, Coradini MF, Alexandre AA da C, Sary C, Ribeiro RP (2016) Effect of different periods of pre-slaughter stress on the quality of the Nile tilapia meat. Food Science and Technology 37:52-58 DOI:10.1590/1678-457x.05616

Chen W-F, Han D-J (2007) Plasticity for Structural Engineers. J. Ross Publishing. pp. 606

Cho SJ, Caldwell CA, Gould WR (2009) Physiological stress responses of Rio Grande silvery minnow: effects of Individual and multiple physical stressors of handling, confinement, and transport. N Am J Fish Manage 29:1698-1706 DOI:10.1577/M09-043.1

Conte FS (2004) Stress and the welfare of cultured fish. Appl Anim Behav Sci 86:205-223 DOI: 10.1016/j.applanim.2004.02.003

Davis KB, Griffin BR, Gray WL (2002) Effect of handling stress on susceptibility of channel catfish Ictalurus punctatus to Ichthyophthirius multifiliis and channel catfish virus infection. Aquaculture 214:55-66 DOI: 10.1016/S0044-8486(02)00362-9

Dhabhar FS (2000) Acute stress enhances while chronic stress suppresses skin immunity. The role of stress hormones and leukocyte trafficking. Ann N Y Acad Sci, 917876-917893. DOI:10.1111/j.1749-6632.2000.tb05454.x

Dhanasiri AKS, Kiron V, Fernandes JMO, Bergh O, Powell MD (2011) Novel application of nitrifying bacterial consortia to ease ammonia toxicity in ornamental fish transport units: trials with zebrafish. J Appl Microbiol 111:278-292 DOI:10.1111/j.1365-2672.2011.05050.x

FAO. The State of World Fisheries and Aquaculture (2016) Contributing to food security and nutrition for all. Rome. p 200. Available at: http://www.fao.org/3/a-i5555e.pdf

Hockley FA, Wilson CAME, Graham N, Cable J (2014) Combined effects of flow condition and parasitism on shoaling behaviour of female guppies *Poecilia reticulata.* Behav Ecol Sociobiol 68: 1513-1520 DOI: 10.1007/s00265-014-1760-5

Honryo T, Oakada T, Kawahara M, Kurata M, Agawa Y, Sawada Y, Miyashita S, Takii K, Ishibashi Y (2018) Estimated time for recovery from transportation stress and starvation in juvenile Pacific bluefin tuna *Thunnus orientalis*. Aquaculture 484:175-183 DOI: 10.1016/j.aquaculture.2017.11.023

Hur JW, Park IS, Chang YJ (2006) Physiological responses of the olive flounder, *Paralichthys olivaceus*, to a series stress during the transportation process. Ichthyol Res 54:32-37 DOI:10.1007/s10228-006-0370-2

Iguchi K, Ogawab K, Nagaec M, Itod F (2003) The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). Aquaculture 220:515-523 DOI:10.1016/S0044-8486(02)00626-9

Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasite spillback: a neglected concept in invasion ecology? Ecology 90:2047-2056 DOI:10.1890/08-1085.1

Kennedy CEJ, Endler JA, Poynton SL, McMinn H (1987) Parasite load predicts mate choice in guppies. Behav Ecol Sociobiol 21:291-295 DOI:10.1007/BF00299966

Kolluru GR, Grether GF, Dunlop E, South SH (2009) Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). Behav Ecol 20:1045-2249 DOI:10.1093/beheco/arn124

Maceda-Veiga A, Cable J (2018) Diseased fish in the freshwater trade: from retailers to private aquarists. Dis Aquat Org. DOI: 10.3354/dao03310

Maceda-Veiga A, Domínguez-Domínguez O, Escribano-Alacid J, Lyons J (2016) The aquarium hobby: can sinners become saints in freshwater fish conservation? Fish Fish 17:860-874 DOI:10.1111/faf.12097

Magurran AE (2005) Evolutionary Ecology: *The Trinidadian Guppy.* Oxford University Press. pp 206

Magurran AE, Phillip DAT (2001) Implications of species loss in freshwater fish assemblages. Ecography 24:645-650 DOI:10.1111/j.1600-0587.2001.tb00526.x

Matur E, Akyazi I, Ersalan, E, Ekiz EE, Eseceli HE, Keten M, Metiner K, Bala DA (2016) The effects of environmental enrichment and transport stress on the weights of lymphoid organs, cell-mediated immune response, heterophil functions and antibody production in laying hens. Anim Sci J 87:284-292 DOI:10.1111/asj.12411

Momoda TS, Schwindt AR, Feist GW, Gerwick L, Bayne CJ (2007) Gene expression in the liver of rainbow trout, *Oncorhynchus mykiss*, during the stress response. Genomics and Proteomics 2:303-315 DOI:10.1016/j.cbd.2007.06.002

Moran D, Wells RMG, Pether SJ (2008) Low stress response exhibited by juvenile yellowtail kingfish (*Seriola lalandi* Valenciennes) exposed to hypercapnic conditions associated with transportation. Aquaculture Research 39:1399-1407 DOI:10.1111/j.1365-2109.2008.02009.x

Ornamental Aquatic Trade Association (OATA 2008) Water Quality Criteria. www.ornamentalfish.org (accessed January 2019).

Oyoo-Okoth E, Cherop L, Ngugi CC, Chepkirui-Boit V, Manguya-Lusega D, Ani-Sabwa J, Charo-Karisa H (2011) Survival and physiological response of *Labeo victorianus* (Pisces: Cyprinidae, Boulenger 1901) juveniles to transport stress under a salinity gradient. Aquaculture 319:226-231 DOI:10.1016/j.aquaculture.2011.06.052

Paterson RA, Townsend CR, Tompkins DM, Poulin R (2012) Ecological determinants of parasite acquisition by exotic fish species. Oikos 121:1889-1895 DOI: 10.1111/j.1600-0706.2012.20143.x

Paterson BD, Rimmer MA, Meikle GM, Semmens GL (2003) Physiological responses of the Asian sea bass, *Lates calcarifer* to water quality deterioration during simulated live transport: acidosis, red-cell swelling, and levels of ions and ammonia in the plasma. Aquaculture 218:717-728 DOI:10.1016/S0044-8486(02)00564-1

Portz DE, Woodley CM, Cech Jr. JJ (2006) Stress-associated impacts of short-term holding on fishes. Rev Fish Biol Fisher 16*:*125-170 DOI:10.1007/s11160-006-9012-z

Pramod PK, Sajeevan TP, Ramachandran A, Thampy S, Somnath PS (2010) Effects of Two Anesthetics on Water Quality during Simulated Transport of a Tropical Ornamental Fish, the Indian tiger barb *Puntius filamentosus*. N Am J Aquacult 72:290-297 DOI:10.1577/A09-063.1

R Development Core Team (2010) R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. URL. http://www.R-project.org

Ramsay JM, Watral V, Schreck CB, Kent ML (2009) Husbandry stress exacerbates mycobacterial infections in adult zebrafish, *Danio rerio* (Hamilton). J Fish Dis 32:931-941 DOI:10.1111/j.1365-2761.2009.01074.x

Robertson L, Thomas P, Arnold CR (1988) Plasma cortisol and secondary stress responses of cultured red drum (*Sciaenops ocellatus*) to several transportation procedures. Aquaculture 68:115-130 DOI:10.1016/0044-8486(88)90235-9

Rubec PJ, Cruz F, Pratt V, Oellers R, Lallo F (2000) Cyanide-free, net-caught fish for the marine aquarium trade. SPC Live Reef Fish Information Bulletin 7:28-34

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 17:1055-1072 DOI: 10.1111/faf.12158

Saxby A, Adams L, Snellgrove D, Wilson RW, Sloman KA (2010) The effect of group size on the behaviour and welfare of four fish species commonly kept in home aquaria. Appl Anim Behav Sci 125: 195-205 DOI:10.1016/j.applanim.2010.04.008

Schelkle B, Shinn AP, Peeler E, Cable J (2009) Treatment of gyrodactylid infections in fish. Dis Aquat Organ 86:65-75 DOI:10.3354/dao02087

Schelkle B, Doetjes R, Cable J (2011) The salt myth revealed: Treatment of gyrodactylid infections on ornamental guppies, *Poecilia reticulata*. Aquaculture 311:74-79 DOI:10.1016/j.aquaculture.2010.11.036

Schelkle B, Faria PJ, Johnson MB, van Oosterhout C, Cable J (2012) Mixed Infections and Hybridisation in Monogenean Parasites. PLoS ONE 7(7): e39506. DOI:10.1371/journal.pone.0039506

Schwartzkopf-Genswein KS, Faucitano L, Dadgar S, Shand P, González LA, Crowe TG (2012) Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. Meat Science 92:227-243 DOI:10.1016/j.meatsci.2012.04.010

Smit NJ, Malherbe W, Hadfield KA (2017) Alien freshwater fish parasites from South Africa: Diversity, distribution, status and the way forward. Int J Parasitol 6:386-401 DOI:10.1016/j.ijppaw.2017.06.001

Stentiford GD, Sritunyalucksana K, Flegel TW, Williams BAP, Withyachumnarnkul B, Itsathitphaisarn O (2017) New Paradigms to Help Solve the Global Aquaculture Disease Crisis. PLoS Pathog 13:2 DOI:10.1371/journal.ppat.1006160

Stevens CH, Croft DP, Paull GC, Tyler CR (2017) Stress and welfare in ornamental fishes: what can be learned from aquaculture? J Fish Biol 91:409-428 DOI:10.1111/jfb.13377

Stockman CA, Collins T, Barnesa AL, Millera D, Wickhama SL, Beatty DT, Blchec D, Wemelsfelderd F, Fleminga PA (2013) Flooring and driving conditions during road transport influence the behavioural expression of cattle. Appl Anim Behav Sci 143:18-30 DOI:10.1016/j.applanim.2012.11.003

Thomas R, Vaughan I, Lello J (2013) Data Analysis with R Statistical Software: A Guidebook for Scientists. Newport, U.K. Eco-explore.

Thiagarajan KP, Rakshit D, Repalle N (2011) The air–water sloshing problem: Fundamental analysis and parametric studies on excitation and fill levels. Ocean Engineering 38:498-508 DOI:10.1016/j.oceaneng.2010.11.019

Thompson RRJ, Paul ES, Radford AN, Purser J, Mendl M (2016) Routine handling methods affect behaviour of three-spined sticklebacks in a novel test of anxiety. Behavioural Brain Research 306:26-35 DOI:10.1016/j.bbr.2016.03.015

Tort L, Balasch JC, Mackenzie S (2003) Fish immune system. A crossroads between innate and adaptive responses. Immunology 22:277-286 http://www.inmunologia.org/Upload/Articles/6/0/602.pdf

Tort L (2011) Stress and immune modulation in fish. Dev Comp Immunol 35:1366-1375 DOI: 10.1016/j.dci.2011.07.002

Trujillo-González A, Becker JA, Vaughan DB, Hutson KS (2018) Monogenean parasites infect ornamental fish imported to Australia. Parasitology Research 117:995-1011 DOI:10.1007/s00436-018-5776-z

Wagner GN, Singer TD, McKinley RS (2003) The ability of clove oil and MS-222 to minimise handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac Res 34:1139-1146 DOI:10.1046/j.1365-2109.2003.00916.x

Washington S, Ababouch L (2011) Private standards and certification in fisheries and aquaculture: current practice and emerging issues. *FAO Fisheries and Aquaculture Technical Paper.* No. 553. Rome, FAO. p 181 http://www.fao.org/docrep/013/i1948e/i1948e00.htm

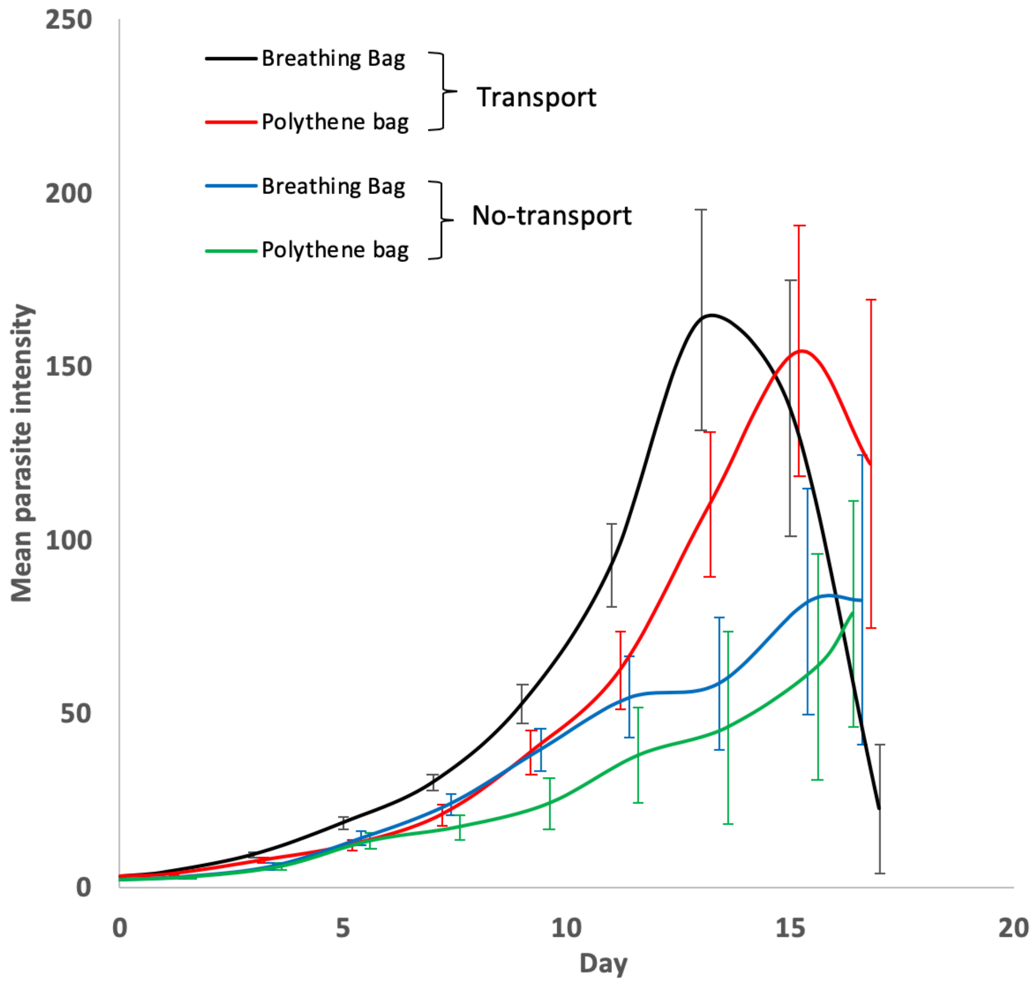
Webster TMU, Rodriguez-Barreto D, Martin SAM, Oosterhout CV, Orozco-terWengel P, Cable J, Hamilton A , Leaniz CGD, Consuegra S (2018) Contrasting effects of acute and chronic stress on the transcriptome, epigenome, and immune response of Atlantic salmon. Epigenetics 13:12, 1191-1207 DOI: 10.1080/15592294.2018.1554520

White N (2011) The parasite clearance curve. Malaria J 10:278 DOI:10.1186/1475-2875-10-278

Yamin G, Zilberg D, Levy G, Rijn JV (2017) The protective effect of humic-rich substances from monogenean parasites infecting the guppy (*Poecilia reticulata*). Aquaculture 479:487-489 DOI:10.1016/j.aquaculture.2017.06.022

Zou Y, Hu XM, Zhang T, Wei HK, Zhou YF, Zhou ZX, Peng J (2017) Effects of dietary oregano essential oil and vitamin E supplementation on meat quality, stress response and intestinal morphology in pigs following transport stress. J Vet Med Sci 79:328-335 DOI:10.1292/jvms.16-0576

Fig. 1. Mechanical disturbance significantly impacted susceptibility to disease in *Poecilia reticulata* exposed to *Gyrodactylus turnbulli* infections after experiencing 24h simulated transport in both types of transport bags (Breathing Bags^{TM} and polythene bags). Standard Error bars slightly transposed to one side to prevent overlap.



Appendix: Transport induced mechanical stress impact on infection trajectories of guppies with pre-existing infections

Here, we investigated how mechanical disturbance during simulated transport impacted pre-existing high burden infections in guppies (*Poecilia reticulata*).

Materials and Methods

Each guppy (n=20 per treatment) was experimentally infected with 30 *G. turnbulli* worms, packaged in standard polythene bags and then exposed to simulated transport (as described in the Main Text) or left as untransported controls. After 24h of simulated transport, all fish (transport and control) were individually isolated in 1l pots and screened every 48h over 17 days to monitor their infection trajectories and the data was analysed as described in the Main Text.

Results

Mean parasite intensity and total infection trajectories over 17 days as measured by AUC were not significantly different between transported guppies and controls (mean parasite intensity: GLMM: Z=1.64, SE= 0.1, p= 0.1; AUC: GLM: Z= 0.6, SE= 0.38, p=0.54, A.1.). Peak parasite burden, however, was significantly higher in guppies that experienced simulated transport (GLM: Z=2.72, SE= 0.05, p=0.006) and timing of peak parasite burden was also earlier in these guppies compared to controls (GLM: t=-3.83, SE= 0.03, p=0.0001).

A.1. Infection trajectory with standard error bars showing *Poecilia reticulata* exposed to a starting point *Gyrodactylus turnbulli* infection of 30 worms prior to transport did not suffer significantly elevated mean parasite intensity compared to controls.