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Benefits of enrichment on the behaviour of ornamental fishes during commercial transport.

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Highlights

- The effect of enrichment on fish behaviour during commercial transport was studied.
- Enrichment during transport reduced erratic swimming on arrival and post-transport.
- Enrichment during transport reduced chasing behaviours post-transport.
- Enrichment during transport reduced stress-related behaviours during recovery.
- Enrichment could reduce transport-stress for millions of ornamental fishes.

Abstract

Environmental enrichment is known to reduce stereotypical behaviours in a wide range of captive animals, providing clear welfare benefits in many species. However, one of the most significant stressors faced by captive animals is live transport but whether enrichment can alleviate transport stress is unknown. Using behavioural measures as indicators of fish welfare, we investigated whether the addition of environmental enrichment can improve the welfare of ornamental fish during commercial transport. Pairs of bags containing variatus platy (*Xiphophorus variatus*) were transported by road with or without enrichment (plastic loops) from a UK wholesaler to one of two stores, the first and the last store on a delivery route. Transport time to the first store was short (< 4 h) and longer (> 6 h) to the second. Fish behaviour and incidences of mortality, disease and injury were recorded immediately following transport and during a four-week recovery period. Immediately post-transport, significantly fewer occurrences of erratic swimming were observed in the enriched group compared to the control group, and lower levels of chasing were recorded in the enriched group during recovery. This study is the first to demonstrate the behavioural benefits of enrichment during live transport of fishes under commercial conditions.

Keywords*: environmental enrichment, ornamental fishes, stress, transport, welfare.*

1. Introduction

Enrichment is often added to the housing of captive animals with the aim of improving their welfare (Carlstead and Shepherdson, 2000; Young, 2003). Enrichment is defined as an increase in environmental complexity that aims to reduce maladaptive and aberrant traits in animals (Näslund and Johnsson, 2016). Structural complexity can be added to provide enclosures mimicking the natural environment (Young, 2003), social complexity can enrich an animal's environment through housing with conspecifics or different species (Bloomsmith and Lambeth, 2000; De Rouck et al. 2005; Sloman et al. 2011) and dietary complexity can include food flavour, texture and shape variation (Skibiel et al. 2007; Rozek and Millam, 2011). Reported benefits of enrichment include improved feeding, lowered metabolic rates, positive behavioural changes and improved cognitive performance (Millidine et al. 2006; Rozek and Millam, 2011; Mileva and Bielajew, 2015; Grimberg-Henrici et al. 2016). While diversity in the environmental requirements of animals means that the response to enrichment is often species (Costa et al. 2018), life-stage (Ullah et al. 2017) and context-specific (Wood, 1998), positive effects of enrichment have been documented across a wide range of animals (Wood, 1998; Belz et al. 2003; Baumans et al. 2005; Milgram et al. 2005; De Rouck et al. 2005; DeVries et al. 2007; Ellis, 2009; Cornale et al. 2015; Sullivan et al. 2016)

For many captive animals, one of the major challenges to their welfare is the stress they encounter during live transport (Grandin, 1997; Harmon, 2009; Nomura et al. 2009). Given the benefits of enriched housing, it is possible that enriched transport could provide similar effects. Studies on the benefits of enrichment during transport are restricted to pigs and monkeys (Peeters and Geers, 2006; Fernström et al. 2008; Roldan-Santiago et al. 2015);

while these studies have found mixed results there is some evidence to suggest benefits of enrichment during transport. For example, providing maize-filled balls to pigs during transport and lairage resulted in lower plasma lactate concentrations and reduced incidents of shoulder injuries compared to control pigs (N.B. significance was taken as $p < 0.1$) (Peeters and Geers, 2006). Addition of straw bedding during piglet transport attenuated physiological effects of transport stress (Roldan-Santiago et al. 2015). Social enrichment during simulated transport of cynomolgus monkeys (*Macaca fascicularis*) reduced stress-associated behaviours (Fernström et al. 2008).

Ornamental fishes within the aquarium trade experience long transports (from a few hours to >48 h) by road and aeroplanes. Stressors associated with transport include handling, crowding and degrading water quality. The aim of the present study was to assess potential benefits of adding structural enrichment during commercial transport of ornamental fishes. We hypothesised that structural enrichment would provide shelter and disrupt physical contact between fish during transport, leading to reduced stress-related behaviours post-transport. The behaviour of variatus platy (*Xiphophorus variatus*), transported by road with or without enrichment (loops of floating plastic), was examined immediately post-transport and during four weeks of recovery at a retailer.

2. Methods

2.1 Study animals and data collection

Variatus platy (random mix of males and females) were transported from a UK wholesaler to two retail stores (journey range: 8.5-10.7 km). Transportation time to the first store was ~2 h 30 min (short transport) and 7 h to the second store (long transport). At the wholesaler, platys (~3.5 cm in length) were netted and placed into polythene bags containing tank water (5-10 fish in 1 l, 25.5 x 35 cm bags, bags were ~25% filled). The air at the top of the bags was replaced with pure oxygen and the bags air-tight sealed and placed into an insulated packing box along with a variable number of other bags. The number of fishes in the bags varied based on the orders that were placed by the stores. Twelve bags containing environmental enrichment (five plastic loops per bag, 31.3 cm in diameter and 1.8 ± 0.32 cm in width; mean ± SD, Fig. 1) and 12 control bags without enrichment were tracked, with six bags of each treatment going to each store. On each journey, bags were tracked in pairs (one control, one enriched), and each pair was assigned a number for statistical analyses accounting for different journey times dependent on traffic delays. Pairs of bags tracked together always contained the same number and colour strain of fish. However, different pairs of bags contained different colour strains to ensure that the fish from different shipments could be identified. At store 1 (short transport) all the fish from one bag were sold prior to the end of the data collection, so this pair was excluded from statistical analysis.

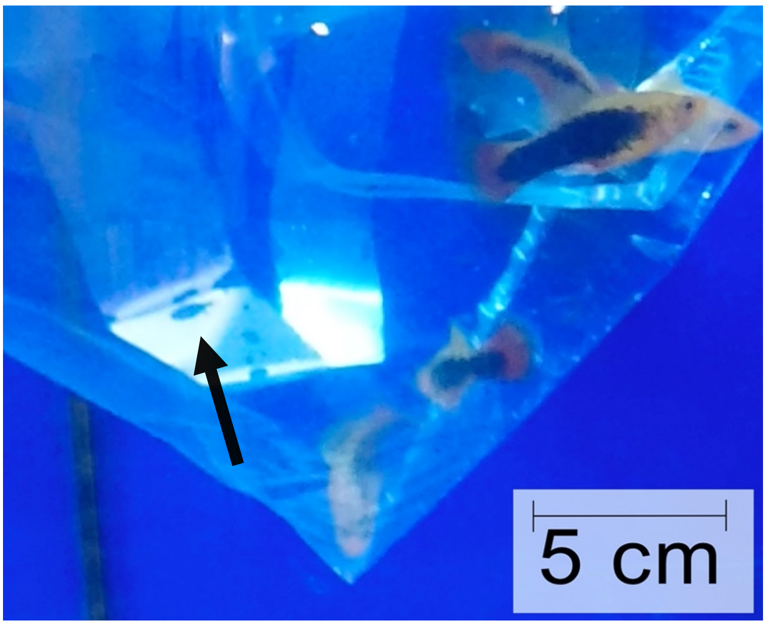

Figure 1: Enrichment. Example photograph of plastic loop used as enrichment during transport (indicated by the arrow).

On arrival at the retail stores, bags were floated in separate recovery tanks for 30 min, during which time fish were recorded using a GoPro hero 5 camera set to 1080P (progressive scan) 120 fps (frames per seconds) with a wide angle. Cameras were mounted on a monopod attached to the outside of the tank. The bags were then opened to allow for slow mixing of the water and after a further 30 min the fish were released into the tank. Staff at the retail stores were unaware of the treatments or study aims to avoid any bias in daily husbandry practices. Preliminary trials found that water parameters in the bags on arrival at the stores were pH: 6.3 ± 0.34; oxygen: 188.65% ± 23.41; and temperature: 23.5 °C ± 1.22 (mean ± SD), and were not significantly different between transport times (P>0.1 all parameters). Ammonia and nitrate remained at safe levels (i.e. total ammonia nitrogen <0.93 ppm, unionised ammonia < 0.004 ppm, nitrate <21.2 ppm) during transport and recovery.

During recovery, fish behaviour was recorded once every 7 days for 4 weeks at random times of the day, but not within 1 h of feeding. The duration of recordings and the camera set up were the same as on arrival. Videos were subsequently used to analyse fish behaviour (Table 1) using Behavioural Observation Research Interactive Software (BORIS) (Friard and Gamba, 2016). Randomly selected focal fish were tracked for 3 min, repeated consecutively from the start to the end of the video such that 10 individuals were tracked. Behaviours for each focal fish were then added to obtain a bag score. Due to the nature of the enrichment analysing the videos blind was not possible as the enrichment was visible in the videos recorded on arrival. Therefore, a subsample (~10%) of videos were analysed blind by an additional observer (inter-observer reliability: Kendall's W coefficient: 0.99). From the videos, any visible body and fin injuries were recorded for focal fish and given a severity score from 0 – 3 where 0 = no injuries and 3 = major injury (Vanderzwalmen et al. 2019). Scores for fin and body injuries were summed to obtain a total injury score recorded for each fish which were then summed to obtain a total injury score per bag. This score was then adjusted for the number of fish that remained at each data collection point to account for fish that had been sold or died. From arrival of the fish until the end of the 4-week recovery period, mortality and occurrence of disease was recorded daily by the retail store staff. All fish were fed a commercial diet *ad libitum* twice daily and kept on a 12 h light/dark regime. Preliminary trials found that water parameters in the tanks at the stores were pH: 6.96 ± 0.51; oxygen: 101% ± 1.93; temperature: 22.42°C ± 1.6 (mean ± SD), and were not significantly different between stores ($p>0.1$ all parameters).

Table 1. Ethogram of the behaviours recorded.

Behaviour	Description	Relation to welfare
Biting	Fish biting or nipping a conspecific.	Biting can cause injuries and may lead to mortality (Pitcher, 1986; Noble et al. 2012).
Chases	Occurrence of a fish chasing a conspecific.	Aggressive interaction has been associated with elevated stress (Vera Cruz and Brown, 2007; Kadry and Barreto, 2010; Gronquist and Berges, 2013).
Erratic swimming	Occurrence of rapid swimming and direction change in the absence of being chased.	Erratic swimming is an indicator of elevated stress, distress or pathogenic condition and may be used as a sign of poor welfare (Conte, 2004).
Ventilation rate	Frequency per minute (Alvarenga and Volpato, 1995).	Ventilation rate can increase as a result of stressors and is a highly sensitive mechanism involved in stress coping strategies (Barreto and Volpato, 2004; Martins et al. 2012).

2.2 Statistical analysis

R 3.6.1 was used for statistical analyses (Bolker et al. 2009; R Core Team, 2019). One outlier from the bite data was removed as it was 22.5 SD away from the mean (Miller, 1991). General linear mixed models (GLMM) were carried out on ventilation rate using the lme function from the lme4 package (Bates et al. 2015). Treatment, recovery stage and transport duration were set as explanatory variables; strain had no significant effect on behaviour and was included as a random variable, as was pair number. Number of fish per bag was five in all cases except in one pair where 10 fish per bag were ordered that week. Therefore, number of fish was not included as a random variable due to the low variation causing singularity when included in models. The interactions between the explanatory variables were also analysed. The distribution of residuals was determined visually (R Core Team, 2019). For response variables recorded as counts, a generalised linear mixed model (GLMM) using poisson family from the lme4 package was used with the same variables as above (Bates et al. 2015). As necessary, data were log or square root transformed to obtain normally distributed residuals. Percentage data were expressed as proportions and a binomial GLMM was carried out with the fixed and random variables as described above. The best fit model was selected by disregarding explanatory variables that did not significantly improve the fit of the model (Bates et al. 2015). The significance of the explanatory variables was tested using ANOVA at $P<0.05$ (Fox and Weisberg, 2011; Pinheiro et al. 2018) and pair-wise post-hoc Tukey tests were carried out (Lenth, 2017). Figures were created using ggplot2 (Wickham et al. 2016).

2.3. Ethics

This study was approved by the University of the West of Scotland Ethics Committee as well as the Animal Welfare Ethics Review Board at the Waltham Petcare Science Institute.

3. Results

Erratic swimming was significantly affected by treatment, recovery stage and transport duration (Table 2). Erratic swimming was consistently lower in fish transported with enrichment than controls (Fig. 2). In fish that experienced the short transport, erratic swimming decreased during week 1 of recovery but then rose within the following weeks to similar levels seen on arrival (Fig. 2). In fish from the long transport, however, erratic swimming was lower during recovery than on arrival and remained low throughout recovery (Fig. 2).

Table 2 ANOVA result of best fit model for erratic swimming and chasing with *F* values, degrees of freedom (df), and *P* values. Significant p-values are indicated in bold.

Behaviour	Fixed Effect	numDF	denDF	*F*-value	*P*
Erratic swimming	Treatment	1	78	125.8	**<0.0001**
	Recovery Stage	4	78	108.31	**<0.0001**
	Transport Duration	1	9	36.92	**<0.0001**
	Treatment * Recovery Stage	4	78	2.413	0.075
	Recovery Stage * Transport Duration	4	78	48.062	**<0.0001**
	Treatment * Transport Duration	1	78	0.391	0.563
	Treatment * Recovery Stage * Transport Duration	4	78	2.404	**0.046**
Chasing	Treatment	1	88	173.614	**<0.0001**
	Recovery Stage	4	88	35.09	**<0.0001**
	Transport Duration	1	5	0.008	0.842
	Treatment * Recovery Stage	4	88	16.184	**<0.0001**
	Treatment * Transport Duration	1	95	0.196	0.669

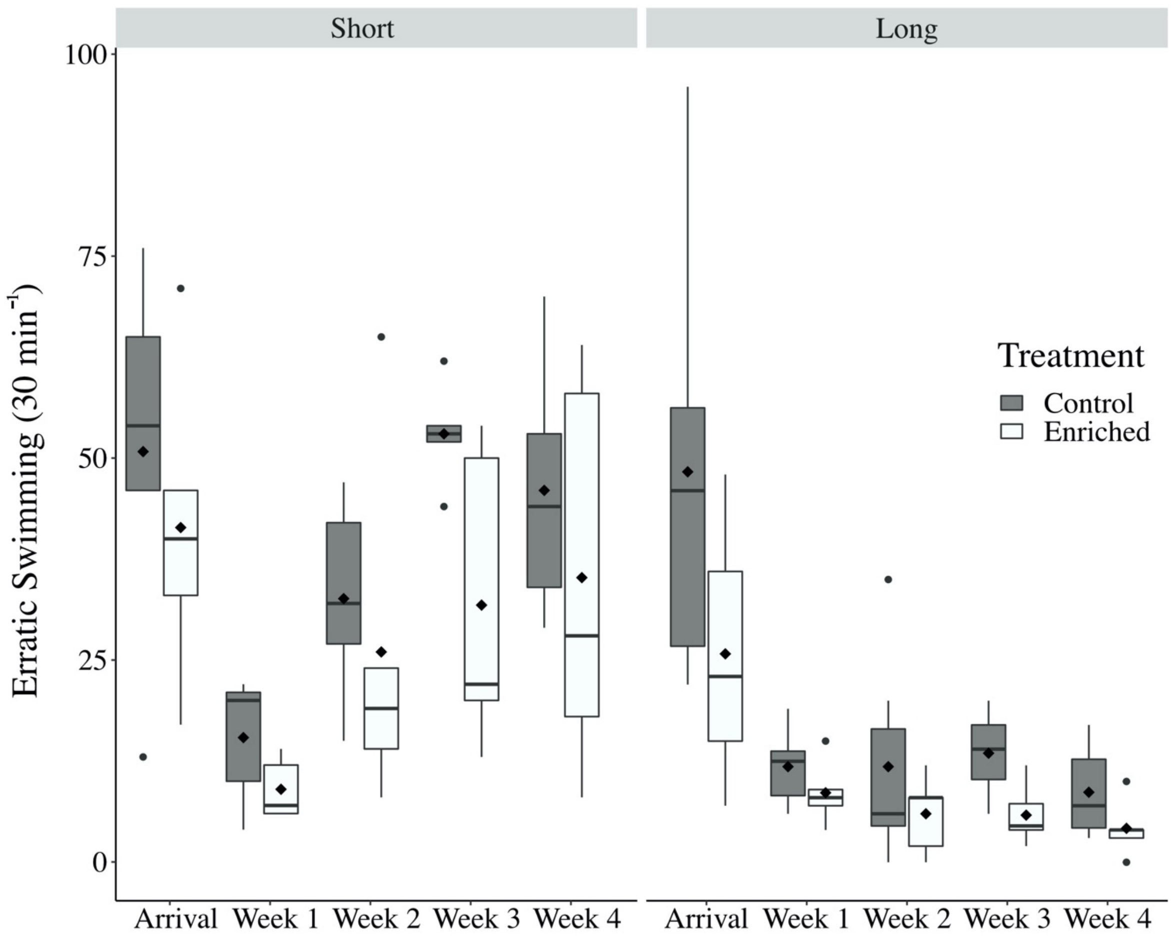

Figure 2: Occurrence of erratic swimming in fish transported with or without environmental enrichment for a short and long transport. Data are mean (diamond), median, upper and lower 25th percentile, and outliers, n=5 bags per treatment for short and n=6 for long transport. Asterisks indicate a significant difference between treatments within a time point (post- hoc Tukey, P<0.05). Lowercase letters indicate significance between stage of transport within control (a-f) and enriched (v-z) treatments, with separate post-hoc analyses used for short and long transport duration. Boxes sharing a letter are not significantly different (post-hoc Tukey, P <0.05).

Chasing was significantly affected by treatment and recovery stage with a significant interaction between the two factors (Table 2). However, unlike for erratic swimming, there was no effect of transport duration on chasing. Chasing behaviour was low on arrival at the stores for both treatments but increased during the recovery period, remaining lower in the fish transported with enrichment (Fig. 3). In fish from the control treatment, chasing was significantly higher than on arrival by week 1 of recovery and remained high, while the number of chases in the enriched treatment only began to increase slowly from week 3 of recovery (Fig. 3). Biting behaviour, injury score, mortality and ventilation rate were not significantly affected by treatment, recovery stage or duration (P >0.05 for all, Table S1 supplementary material) and remained low throughout (Table S2 supplementary material). Occurrence of disease was too low to carry out statistical analyses.

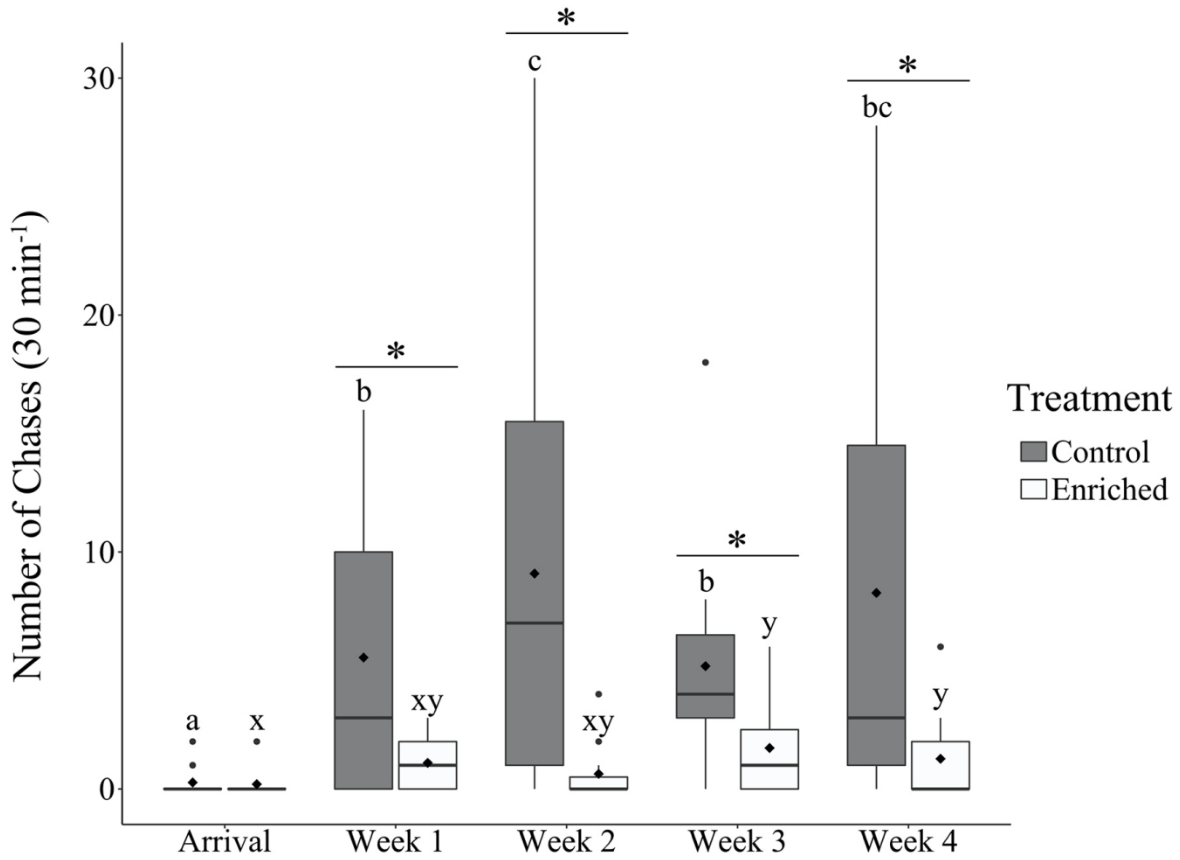

Figure 3: Occurrence of chasing in fish transported with or without environmental enrichment. Data are mean (diamond), median, upper and lower 25th percentile, and outliers, n=11. Asterisks indicate a significant difference between treatments within a specific time point (post-hoc Tukey, P <0.05). Letters indicate a significant difference between stages of transport within control (a-c) and enriched (x,y) treatments where boxes sharing a letter are not significantly different (post-hoc Tukey, P <0.05).

4. Discussion

Fish that were transported with enrichment showed a reduction in stress-associated behaviours including erratic swimming and chasing. Erratic swimming in the enriched group was lower on arrival at stores than in the control group, and during recovery fish shipped with enrichment showed lower levels of chasing.

Some chasing and biting are expected during hierarchy establishment, but prolonged chasing can lead to chronic stress for the fish subjected to the aggressive behaviour (Braddock, 1945; Scott and Currie, 1980). Following stress, fish can display displaced aggression behaviours, such as chasing, as a coping mechanism to manage their own stress (Sneddon et al., 2016). Reduced aggression could therefore indicate improved stress-coping capabilities in the enriched group (Sneddon et al., 2016). Lower levels of chasing in the enriched group may also suggest a less aggressive establishment of hierarchy. Biting behaviours are a greater welfare concern than chasing due to risks of injury and infection but were low throughout the present study.

Erratic swimming was higher following the short transport compared to the long transport, potentially as the longer time that elapsed between packing and unpacking allowed greater recovery from handling on route for the long transport fish. This hypothesis is supported by previous studies on fish transport which have found that handling is the most stressful part of transport and recovery begins within the first 2-4 h of transport duration (Pickering et al. 1982; Acerete et al. 2004; Nomura et al. 2009). The majority of studies that have considered the welfare of ornamental fishes during transport have used simulated transport and focussed on stress recovery for 24-96 h post-transport (Zanuzzo et al. 2012; Ramírez-duarte et al. 2013; Salaro et al. 2015; Pattanasiri et al. 2017). However, as animals can experience stress for extended periods of time following transport (Knowles et al. 1993; Dembiec et al. 2004; Fernström et al. 2008), in the present study indicators of stress were recorded for four weeks post-transport. While differences in behaviour between treatments were seen over this time frame, mortality, disease and injury were not affected by enrichment most likely as levels of all three remained low throughout the study.

Although a wealth of literature exists on the use of environmental enrichment for fish holding conditions (Näslund and Johnsson, 2016), to the authors' knowledge, no previous studies have looked at the potential benefits of enrichment for fishes during commercial transport. However, there are a few studies that have considered benefits of enrichment during periods of acute stress. For example, zebrafish (*Danio rerio*) from enriched tanks released less cortisol than zebrafish from bare tanks following a pursuit stress (Giacomini et al. 2016) and a similar result was seen in jundiá (*Rhamdia quelen*) (Barcellos et al. 2009). Exposure to blue subtrate prior, during and after a confinement stress also improved the recovery rate of serotonin and plasma glucose in gilthead seabream (*Sparus aurata*) (Batzina et al. 2014). The effects of enrichment during transport have been considered in other animals showing positive effects of social enrichment in cynomolgus monkeys (Fernström et al. 2008) and the addition of toys (Peeters and Geers, 2006) and substrate (Roldan-Santiago et al. 2015) for pigs.

Plastic loops were chosen as the enrichment as they are already used within the ornamental trade to reduce the stress experienced by some species, meaning it is an enrichment which reflects current practice and could be implemented for other species. Prior to the present study, there had been no research on the use of plastic loops as enrichment during ornamental fish transport. Plastic loops are likely to disturb visual contact between fishes within the transport bag and might provide hiding places, however, further research is needed to better understand the functionality of the plastic loops as enrichment. More research is also needed to identify other potential forms of enrichment during ornamental fish transport across different species and life-stages. Consideration must be given to the fact that the type of environmental enrichment provided is likely to affect both the response of the animal and any beneficial effects which has been shown in non-transport related studies. For example, goldfish (*Carassius auratus*) displayed increased vigilance in the presence of a predator when a solid block was used as enrichment but not when artificial plants were available (Ingrum et al. 2010). Black bucks (*Antilope cervicapra*) were found to prefer a tree trunk/branch combination as enrichment compared to vertical brushes (Bono et al. 2016) and cattle (Charolais cross heifers) prefer scratching enrichments over olfactory enrichments (Wilson et al. 2002). Goldfish also prefer enriched areas over non-enriched areas with no preference between artificial or real plants indicating that the functionality of enrichment can be more important than the material it is made from (Sullivan et al. 2016).

5. Conclusion

Millions of animals are transported daily across the world. Transport is stressful for these animals, yet surprisingly few studies have assessed the effect of enrichment during animal transport. This study is the first to use enrichment during the commercial transport of ornamental fishes, finding a clear benefit in the reduction of stress-associated behaviours. With growing pressure on industries to improve the welfare of the animals they care for, further research into environmental enrichment to manage stress during commercial transport is warranted.

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End Section Statements

Competing Interests

The authors declare no competing interests.

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Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Myriam Vanderzwalmen: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft

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Supplementary Material

Table S1 ANOVA result of best fit model for biting, injury score, mortality and ventilation rate with *F* values, degrees of freedom (df), and *P* values.

	Fixed Effect	numDF	denDF	*F*-value	*P*
Biting	Treatment	1	91	3.634	0.55
	Recovery Stage	4	91	0.366	0.83
	Transport Duration	1	9	0.098	0.75
Injury score	Treatment	1	72	0.044	0.833
	Recovery Stage	4	72	0.271	0.846
	Transport Duration	1	5	2.225	0.195
Mortality	Treatment	1	94	2.434	0.122
	Recovery Stage	4	94	0.764	0.551
	Transport Duration	1	5	0.054	0.825
Ventilation Rate	Treatment	1	92	0.003	0.955
	Recovery Stage	4	92	2.16	0.079
	Transport Duration	1	4	0.215	0.661

Table S2: Mean (± SD) for non-significant welfare indicators. Actual mortality data are not presented due to commercial sensitivity.

Stage	Treatment	Transport duration	Biting (30 min^{-1})	Ventilation rate min^{-1}	Injury score (average per fish, see text)
Arrival	Control	Short	0 (±0)	105.05 (±9.11)	0 (±0)
Arrival	Enriched	Short	0 (±0)	99.70 (±4.71)	0 (±0)
Arrival	Control	Long	2.6 (±5.81)	106.80 (±10.20)	0.04 (±0.09)
Arrival	Enriched	Long	0 (±0)	116.49 (±28.45)	0.08 (±0.18)
Week 1	Control	Short	0 (±0)	105.74 (±5.71)	0 (±0)
Week 1	Enriched	Short	0.80 (±1.30)	98.66 (±7.79)	0.08 (±0.18)
Week 1	Control	Long	0.4 (±0.89)	102.27 (±12.75)	0.267 (±0.60)
Week 1	Enriched	Long	0 (±0)	106.41 (±13.81)	0.32 (±0.46)
Week 2	Control	Short	0.4 (±0.89)	107.54 (±14.60)	0.08 (±0.18)
Week 2	Enriched	Short	0 (±0)	110.57 (±13.93)	0.2 (±0.45)
Week 2	Control	Long	0.5 (±0.55)	106.04 (±13.62)	0.07(±0.10)
Week 2	Enriched	Long	0.17 (±0.41)	105.49 (±13.23)	0.8 (±0.167)
Week 3	Control	Short	0.2 (±0.45)	111.89 (±8.05)	0.28 (±0.39)
Week 3	Enriched	Short	0 (±0)	106.19 (±7.88)	0.04 (±0.09)
Week 3	Control	Long	0.33 (±0.82)	112.83 (±12.63)	0.12 (±0.31)
Week 3	Enriched	Long	0 (±0)	114.65 (±20.74)	0.32 (±0.49)
Week 4	Control	Short	0.2 (±0.45)	106.73 (±10.03)	0 (±0)
Week 4	Enriched	Short	0 (±0)	103.99 (±10.03)	0.1 (±0.22)
Week 4	Control	Long	0.5 (±0.84)	102.41 (±13.44)	0.5 (±0.78)
Week 4	Enriched	Long	0 (±0)	103.58 (±17.01)	0.19 (±0.30)