Influence of social enrichment on transport stress in fish: a behavioural approach

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ABSTRACT

Ornamental fishes are among the most commonly owned companion animals in the world, however, the transportation process during acquisition can result in fishes being exposed to biotic and abiotic conditions which compromise welfare. While many studies have considered methods of improving welfare for food fishes through physical and social enrichment, few have considered how to improve welfare of ornamental fishes post-transport. We investigated whether (i) being introduced into an empty tank, a tank with resident conspecifics (variatus platys; *Xiphophorus variatus*), or a tank with resident heterospecifics (common mollies; *P. sphenops*) and (ii) being able to see resident fish in an adjacent tank affected stress-associated behaviour post-transport. Videos of variatus platys being introduced to their treatment tanks were taken immediately on release following transport, and at 1, 24, 72, 120 and 168 h after release. Behaviours, including biting, chasing, erratic movement and time spent immobile, were analysed across all time points. Latency to forage was analysed immediately upon release post-transport only. The social composition of the tanks that the variatus platys were placed in influenced the majority of behaviours analysed, however visual cues only had a significant effect on chasing behaviour at 168 h post release and on biting behaviours of resident fish towards transported fish. Fish placed in tanks with resident conspecifics exhibited significantly more agonistic behaviours than those introduced into empty tanks or with resident heterospecifics. Variatus platys introduced into tanks with resident conspecifics had shorter foraging latencies. It is clear that tank composition post-transport has an effect on behaviour of ornamental fishes and represents a way in which retailers can implement welfare improvements.

1. Introduction

Ornamental fishes are one of the most commonly owned companion animals (Miller-Morgan, 2009; Wood et al., 2015) with the annual global trade value estimated at around $15-$20 billion (King, 2019; Pouil et al., 2019). Until recently fish welfare research has focused on food-fishes in aquaculture (Huntingford and Kadri, 2009; Stevens et al., 2017), however, more recently the welfare of fish within the ornamental trade has received more attention (Huntingford et al., 2006; King, 2019). The ornamental fish supply chain exposes fishes to a variety of stressors, with fish transport identified as a main point where stressors can culminate in poor welfare (Jones et al., 2021). Various studies have considered welfare improvements during transport (see reviews: Stevens et al., 2017; Vanderzwalmen et al., 2019; Jones et al., 2021), but research into welfare refinements post-transport to aid recovery is lacking. Within the trade, treatment of fishes post-transport varies. In general on arrival at a retailer following national or international transport, fishes are initially floated on their destination tanks in their transport bags, with tank water gradually mixed into the bag to allow gradual acclimation to new water quality parameters (Donaldson et al., 2008). Fishes are then released into tanks which vary considerably in physical (e.g. substrate, enrichment) and social (e.g. conspecifics or heterospecifics) environment. Environmental conditions post-transport during the recovery phase are likely to have a critical effect on

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welfare and yet research into optimal conditions post-transport is lacking.

Enrichment and stocking density can influence fish behaviour (Näslund and Johnsson, 2014; Stevens et al., 2017) and increased structural complexity is a well-documented method of improving fish welfare (Näslund and Johnsson, 2014). Social enrichment for fishes has received less attention, yet the presence of conspecifics can facilitate social learning and promote adaptive social behaviours (Strand et al., 2010). The benefits of social enrichment may stem from the fact that individuals receive a broad range of sensory information during social interaction, from which they form appropriate responses (Korzan and Summers, 2007; Chen and Fernald, 2011). Most fishes rely on vision as a key source of sensory information, with the majority of species having well-developed vision and defined brain structures to support visual processing (Fernald and Wright (1985); Guthrie (1986); Sandström (1999) 'Social facilitation' has been observed in fishes, where the behaviour of one individual induces the same response in the observer; the observer then learns through expressing this behaviour and experiencing the consequences in a particular context (Brown and Laland, 2003; Ward, 2012). Social buffering, the ability for another conspecific to mitigate physiological and behavioural stress (Kiyokawa et al., 2014), has also been observed in fishes, with fishes better able to recover from aversive stimuli when conspecifics are visually present (Faustino et al., 2017). Both of these concepts may underpin the benefits of social enrichment.

Thus there is ample evidence within the literature to support the idea that the social environment of a fish, and/or the ability to see other fishes is likely to influence stress recovery. Ornamental fishes are routinely kept in mixed species compositions (Saxby et al., 2010; Sloman et al., 2011; Desjardins et al., 2012; Palagi et al., 2020) but the use of species composition as social enrichment to reduce stress post-transport to our knowledge has not been studied. Consequently, understanding whether the presence of other fishes (either physically in the same tank, or visually present in adjacent tanks) has the potential to alter recovery from transport may aid welfare refinement within the ornamental trade. The aim of the present study was to identify the effects of social environment on post-transport behaviour of ornamental fishes. We investigated whether (i) being introduced into an empty tank, a tank with resident conspecifics, or a tank with resident heterospecifics and (ii) being able to see resident fish in an adjacent tank affected stress-associated behaviour post-transport.

2. Materials and methods

Ornamental fishes used in the present study were acquired from a local wholesaler. Mixed sex juvenile variatus platys (*Xiphophorus variatus*) (~3 cm in total length) (n = 240) were used as the study species as it is one of the most popular ornamental freshwater fish species (Teletchea, 2015) and for mixed-species housing treatments another live-bearing fish species, the common molly (*Poecilia sphenops*), was used to replicate housing conditions in local retail stores. The resident fishes were initially held in stock tanks and then placed into 18 l tanks for 1 week to acclimate prior to the addition of transported fish (40 of each species). Tanks were located on a recirculation system with stock tanks and experimental tanks held at: DO_2 94 ± 3%; pH 6.9 ± 0.2; temperature 25 ± 1 °C (mean ± S.E.M.) and the light:dark period was 12:12 h. All tanks contained two identical artificial plants as enrichment (50 ×100×70 mm; taking up ~20% of the tank) which mimicked tank conditions in local retail stores; substrate was not provided in line with the conditions fish would experience in retail stores. Water quality was checked three times per week, and ammonia, nitrate and nitrite remained low throughout (<0.25 mg/l, <5 mg/l and 0 mg/l, respectively). Resident fishes were fed Mars API Tropical flakes twice daily (08.00 ± 1 h and 16.00 ± 1 h) and the same feeding regime was conducted after the addition of transported fish. Tanks were siphoned daily to remove solid waste and the recirculating system water was topped-up

with charcoal-filtered tap water. There were a few natural mortalities within the resident fish species, but no tank lost more than one resident fish during the course of the experiment.

2.1. Experimental design

The experiment investigated the behaviour of variatus platys immediately post-transport when added to tanks with differing species and visual cues. Visual cues were removed by covering the sides of the tanks with blue opaque plastic; if visual cues were present this plastic screening was removed. Six different treatments were used (Table 1) which manipulated the two factors of interest: (i) whether transported fish were introduced into tanks that were empty, contained resident conspecifics (*X. variatus*) or resident heterospecifics (*Poecilia sphenops*) and (ii) whether fish were able to see a tank of mixed species resident fish in adjacent tanks (visual cues) or not (no visual cues). The behaviours of the transported fish and also the agonistic behaviours (Table 2) of the resident fishes were analysed to identify whether any of the six treatments had an effect on transport-stress recovery or social interactions within the tank. Due to the number of tanks available, data collection occurred over five replicate time periods (five separate weeks) where the same resident fishes were used throughout but returned to stock tanks, redistributed, and allowed 1 week acclimation prior to each experimental period. Where transported fish were placed into tanks with visual cues they were able to see a mixed species assemblage (five *X. variatus* and five *P. sphenops*) in an adjacent tank.

Once the resident fishes had acclimated to the tanks, bags containing five mixed sex *X. variatus* were transported from the ornamental fish wholesaler to the University of the West of Scotland (7 miles; 30–35 min depending on traffic and weather conditions). Fish were packed and transported as if they were being shipped from the wholesaler to local stores, and were held at the wholesaler in single species tanks. They were placed in transparent polyethylene bags (25.4 ×45.72 cm) with ~400 ml of water; the remaining space was filled with pure oxygen. Bags were securely sealed, placed in another bag, and then in a light-tight polystyrene box with heat pads and insulation/packaging to reduce any potential for mechanical disturbance and to maintain an appropriate temperature. To allow for identification, the transported *X. variatus* observed for behaviour following transport were a different colour to resident *X. variatus*.

On arrival, bags were floated on the surface of their allocated tank for 30 min. Water from the tank was gradually introduced to the bags over a 30 min period. Fish were then released into the tanks and the tanks videoed for 15 min using a Go Pro Hero 5 camera. The tanks were then videoed for 15 min again 1 h later and then 24, 72, 120 and 168 h after arrival. A technical malfunction meant that videos for one tank replicate were lost for the release and 1 h time points.

2.2. Behavioural analysis

Behavioural Observation Research Interactive Software (BORIS) (Friard and Gamba, 2016) was used to analyse behaviours exhibited by

Table 1
The six treatments used in the present study (n = 8 tanks per treatment randomly allocated over five data collection rounds). Tank stocking density was kept the same across all treatments to reduce the potential for stocking density effects influencing any behaviours observed.

Treatment	Visual cues	Resident fish present in tank	No. of transported fish added
1	No	None	10
2	No	Five *X. variatus*	5
3	No	Five *P. sphenops*	5
4	Yes	None	10
5	Yes	Five *X. variatus*	5
6	Yes	Five *P. sphenops*	5

Table 2

Ethogram of behaviours analysed. Focal fish in this instance refers to the fish being followed at a particular moment in the video, for all analyses three fish were followed in total per video (15 min).

Behaviour	Description	Interpretation
Bite	Direct contact aggression: Focal fish making direct physical contact with another fish (either a transported fish or resident fish) by biting or nipping of fins or body. The total number of individual acts of biting and nipping was recorded.	Contact aggression is a natural behaviour for dominant fish, but at elevated levels can also be an indicator of stress. The act of biting can also induce stress and injury to the attacked fish (Oldfield, 2011).
Chase	Indirect non-contact aggression: Focal fish exhibits chasing behaviour through quickly pursuing another fish (either a transported fish or resident fish) within the tank. The total number of individual acts of chasing was recorded.	Non-contact aggression is a natural behaviour for dominant fish and exhibited during social hierarchy establishment (Oldfield, 2011). However, as with biting, at elevated levels it can be an indicator for stress and in itself cause stress to resident con- and/or heterospecifics (Oldfield, 2011).
Erratic movement	Focal fish exhibiting non-stereotypical swimming behaviour through rapid alterations in direction and/or speed of swimming. The total number of individual acts of erratic movement was recorded.	An indicator of aversion to their current physical or social environment. Erratic movement has been used as a welfare indicator due to a positive correlation with stress (Egan et al., 2009; Kleinhappel et al., 2019).
Time spent immobile	Focal fish stationary within the water column or bottom of tank with no obvious fin movement. Fish were timed to identify how long they spent immobile within each data collection time period.	The time a fish spends immobile either within the water column or on the substrate can be an indicator of stress and/or anxiety and a proxy for boldness; a shorter amount of time immobile could indicate a reduction in stress (Tran and Gerlai, 2016). However, a shorter amount of time immobile combined with an increase in erratic movements is likely to be indicative of increased stress.
Foraging latency	The time it takes for the focal fish to exhibit foraging behaviour, such as nipping at the artificial vegetation, walls or bottom of tank usually at a 45° angle. Foraging latency was only recorded immediately after the focal fish were introduced into the tanks. Fish were timed from initial introduction until they exhibited foraging behaviours.	Foraging behaviours can be influenced by social environments and latency to exhibit foraging behaviours can be an indicator of how bold a fish is (Martins et al., 2012). A longer latency to feed time could indicate increased levels of stress if fish are either not motivated to feed due to physiological state, or perceive a risk to approaching food.

the transported and resident fishes. An ethogram of stress-related behaviours was developed using a range of existing literature (Table 2). Although no feed was added during the observation period, transported fishes exhibited foraging behaviours when they were first added to the tank, therefore latency to forage was included in the behavioural analysis for this time point only. For this measure, three randomly selected transported fish were followed and timed to identify how long it took for them to begin foraging.

For the transported fish, all other behaviours (Table 2) were measured at every time point, where three randomly selected fish were followed across a 15 min period for 5 min each (i.e. three transported fish were followed per 15 min video). For the behavioural analysis of the resident fishes, the same method of randomly selecting three resident fish and following one for 5 min each and repeating this three times was conducted (i.e. three resident fish were followed per 15 min video). Only aggressive behaviours were considered for the resident fishes. When

transported fish were added to empty tanks, clearly any biting or chasing behaviour recorded was a transported fish biting or chasing another transported fish. However, aggression in tanks in which transported fish were added to resident fishes had the potential to be more complex. Therefore, aggressive behaviours were explored further by identifying the targets of agonistic interactions initiated by transported fish where they were added to tanks containing either resident conspecifics or heterospecifics (i.e. were they targeting other transported fish or the resident fish?). Specifically, the incidence of aggression by (i) transported fish towards other transported fish, (ii) transported fish towards the resident fish species, (iii) resident fishes towards transported fish and (iv) resident fishes towards other resident fishes was determined.

Videos were all recorded at the same time each day (12.00 ± 1 h) to reduce the likelihood of food-anticipation related behaviours (Lall and Tibbets, 2009; Martins et al., 2012). Due to the nature of the experiment, it was not possible to analyse the videos while remaining blind to treatment. Therefore, to reduce the potential for observer bias, two independent researchers (MJ and SL) analysed all videos using BORIS and an inter-reliability score was calculated for each behaviour. SL was blind to the study aims to further reduce the potential for observer bias. Concordance was high for all behaviours (erratic movement: $r(27) = 0.859$, $p < 0.001$; biting: $r(27) = 0.930$, $p < 0.001$; chasing: $r(27) = 0.924$, $p < 0.001$ and time spent immobile: $r(27) = 0.899$, $p < 0.001$).

2.3. Statistical methods

Statistical analyses were conducted using R statistical analysis software version 3.6.2 (R Core Team, 2018). Data in figures are shown as means ± SE, with significance at the 5% level ($p < 0.05$). Data from each tank at each time period were found to be over-dispersed (using the *check_overdispersion* function from the 'glmmTMB' package), therefore family distributions were changed from Poisson to negative binomial. The aggressive behaviours of the transported and resident fishes were analysed to identify the incidence of aggression by (i) transported fish towards other transported fish, (ii) transported fish towards the resident fish species, (iii) resident fishes towards transported fish and (iv) resident fishes towards other resident fishes. Variances in biting, chasing and erratic movement occurrence as well as time spent immobile and foraging latency were analysed using generalised linear models with template model builders using the '*glmmTMB*' package. The presence/absence of visual cues, tank composition and sampling time were considered as fixed effects, with data collection round nested within tank number included as a random variable to account for any differences in conditions during the five different data collection rounds. Interactions between fixed effects (visual cues presence/ absence and tank composition) were also analysed. Each model was tested for zero-inflation and residuals visually assessed via Q-Q plots of theoretical quantiles and standardised residuals vs model prediction plots, both using the '*DHARMa*' package. Significance of main effects and their interaction were investigated using the *anova.glmmTMB* function; if interactions and/or random effects were found to be non-significant they were removed for model simplification. If significance was identified, *post-hoc* analyses using the *emmeans* package were used to conduct pairwise comparisons for main effects and any potential interactions using the Tukey method (Lenth, 2016). Between- and within- group variations were also analysed for main effects and post hoc analyses conducted using the *TukeyHSD* function.

2.4. Ethical approval

This research was approved by the Animal Welfare and Ethics Review Boards for the University of the West of Scotland (Submission Number 14836) and Waltham Petcare Science Institute.

3. Results

3.1. Biting

The number of bites performed by the transported fish was affected by tank composition but not by visual cue presence/ absence, with biting also increasing over time (Table 3; Fig. 1). No significant interactions between the tank composition and visual cues were found. Fish placed into empty tanks post-transport, and those placed into tanks containing heterospecifics, performed significantly fewer bites than those placed into tanks containing resident conspecifics (Fig. 1). This was particularly evident immediately after introduction, 1 h and 24 h after addition to the tanks where levels of biting were much higher in the tanks where fish were added to resident conspecific groups (Fig. 1). However, 1 h after introduction, fish placed in an empty tank also exhibited more biting behaviour than fish that were added to resident heterospecifics.

There was no significant difference in the number of bites performed among the transported fish regardless of tank composition, visual cue presence/absence or sampling time (Table 3). However, more bites were
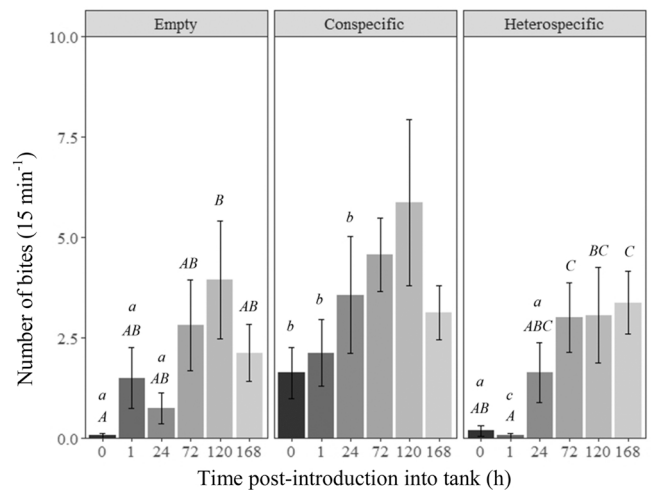
Table 3
Summary of main statistical findings, with significant results ($p < 0.05$) highlighted in bold.

Behaviour	Tank Composition	Visual Cue Presence/ Absence	Time	Tank Composition* Visual Cue
Total bites performed by transported fish	**$\chi^2 = 14.0$, df= 2, $p<$ 0.001**	$\chi^2 = 0.04$, df= 1, $p = 0.85$	**$\chi^2 = 46.99$, df= 5, $p< 0.001$**	$\chi^2 = 0.79$, df= 2, $p = 0.67$
Bites among transported fish	$\chi^2 = 0.04$, df= 1, $p = 0.85$	$\chi^2 = 1.95$, df= 1, $p = 0.16$	$\chi^2 = 8.72$, df= 5, $p = 0.12$	$\chi^2 = 0.34$, df= 1, $p = 0.53$
Bites by transported fish towards resident fish	**$\chi^2 = 4.05$, df= 1, $p= 0.04$**	$\chi^2 = 0.02$, df= 1, $p = 0.89$	$\chi^2 = 10.34$, df= 5, $p = 0.07$	$\chi^2 = 0.07$, df= 1, $p = 0.8$
Bites among resident fish	**$\chi^2 = 9.97$, df= 1, $p= 0.002$**	$\chi^2 = 0.81$, df= 1, $p = 0.38$	$\chi^2 = 1.77$, df= 5, $p = 0.88$	$\chi^2 = 0.48$, df= 1, $p = 0.49$
Bites by residents to transported fish	**$\chi^2 = 5.28$, df= 1, $p= 0.02$**	**$\chi^2 = 5.25$, df= 1, $p= 0.02$**	**$\chi^2 = 11.94$, df= 5, $p= 0.04$**	$\chi^2 = 0.89$, df= 1, $p = 0.35$
Total chases performed by transported fish	**$\chi^2 = 7.04$, df= 2, $p= 0.03$**	$\chi^2 = 1.43$, df= 1, $p = 0.23$	**$\chi^2 = 82.26$, df= 5, $p< 0.001$**	$\chi^2 = 0.61$, df= 2, $p = 0.77$
Chasing among transported fish	$\chi^2 = 0.42$, df= 1, $p = 0.52$	$\chi^2 = 0.74$, df= 1, $p = 0.39$	**$\chi^2 = 68.96$, df= 5, $p< 0.001$**	$\chi^2 = 0.03$, df= 1, $p = 0.86$
Chases by transported fish towards resident fish	$\chi^2 = 0$, df= 1, $p = 0.99$	$\chi^2 = 0.53$, df= 1, $p = 0.47$	**$\chi^2 = 14.19$, df= 5, $p= 0.01$**	$\chi^2 = 0$, df= 1, $p = 0.99$
Chases among resident fish	$\chi^2 = 0.46$, df= 1, $p = 0.5$	$\chi^2 = 0.48$, df= 1, $p = 0.49$	$\chi^2 = 2.71$, df= 5, $p = 0.74$	$\chi^2 = 3.41$, df= 1, $p = 0.06$
Chases by residents to transported fish	$\chi^2 = 0.66$, df= 1, $p = 0.42$	$\chi^2 = 1.33$, df= 1, $p = 0.25$	$\chi^2 = 4.92$, df= 5, $p = 0.43$	$\chi^2 = 0.33$, df= 1, $p = 0.56$
Erratic Movements	**$\chi^2 = 8.17$, df= 2, $p= 0.02$**	$\chi^2 = 0.89$, df= 1, $p = 0.35$	**$\chi^2 = 31.08$, df= 5, $p< 0.001$**	$\chi^2 = 3.25$, df= 2, $p = 0.2$
Foraging Latency	**$\chi^2 = 14.62$, df= 2, $p< 0.001$**	$\chi^2 = 0.02$, df= 1, $p = 0.9$	n/a	$\chi^2 = 2.97$, df= 2, $p = 0.23$
Time Immobile	$\chi^2 = 1.64$, df= 2, $p = 0.44$	$\chi^2 = 0.94$, df= 1, $p = 0.33$	$\chi^2 = 10.5$, df= 5, $p = 0.06$	$\chi^2 = 0.60$, df= 2, $p = 0.74$

Fig. 1. Number of bites (mean $\pm$ SE) performed by the focal transported fish that were either introduced into empty tanks, with resident conspecifics or with resident heterospecifics, recorded upon arrival, 1, 24, 72, 120 and 168 h after arrival. Lower case letters indicate significant post-hoc differences between treatments within the same sampling time (Tukey; $p < 0.05$) and capital letters indicate significant post-hoc differences between sampling times within treatment (Tukey; $p < 0.05$), where bars sharing the same letter are not significantly different. Absence of letters on a set of bars indicates that there are no significant differences.

performed by the transported fish towards resident fish of the same species (*X. variatus*) than towards resident heterospecifics (*P. sphenops*) (Fig. 2 A); sampling time and visual cue presence/absence had no significant effect on the number of bites performed by the transported fish towards the resident fish. Resident *X. variatus* were also more aggressive towards each other than resident *P. sphenops* (Fig. 2B); sampling time and visual cue presence/absence had no significant effect on the number of bites performed within resident fish groups.

When analysing the number of bites performed by the resident fish (either resident *X. variatus* or *P. sphenops*) towards the transported fish added to their tank it was found that tank composition, visual cue presence/absence and sampling time all had a significant effect on the number of bites performed by the resident fish (Table 3, Fig. 2 C). Resident *X. variatus* performed significantly more bites towards the transported fish than the resident *P. sphenops* did and resident fish with the ability to see fish in adjacent tanks performed significantly fewer bites towards the transported fish than those without visual access to fish in adjacent tanks. Sampling time also had a significant effect, with a greater number of bites performed overall towards the transported fish 1 h after release compared to 120 h after release.

3.2. Chasing

Chasing frequency was affected by tank composition, with time also having a significant effect (Table 3, Fig. 3). Visual cue presence/ absence had no effect on chasing frequency overall and there was no significant interaction between tank composition and visual cues. Transported fish placed in tanks with resident conspecifics performed significantly more chases than fish placed in empty tanks or tanks with heterospecifics; post-hoc analyses showed a significant difference 24 h after arrival (Fig. 3). Chasing frequency significantly increased over time, which was evident when fish were added to tanks containing resident conspecifics with significantly more chases performed at sampling times 72, 120 and 168 h after arrival, than immediately after release (Fig. 3).

Within the treatments where transported fish were added to tanks containing either resident conspecifics or heterospecifics, time had a significant effect on the incidence of transported fish chasing other transported fish, with the number of chases increasing with time
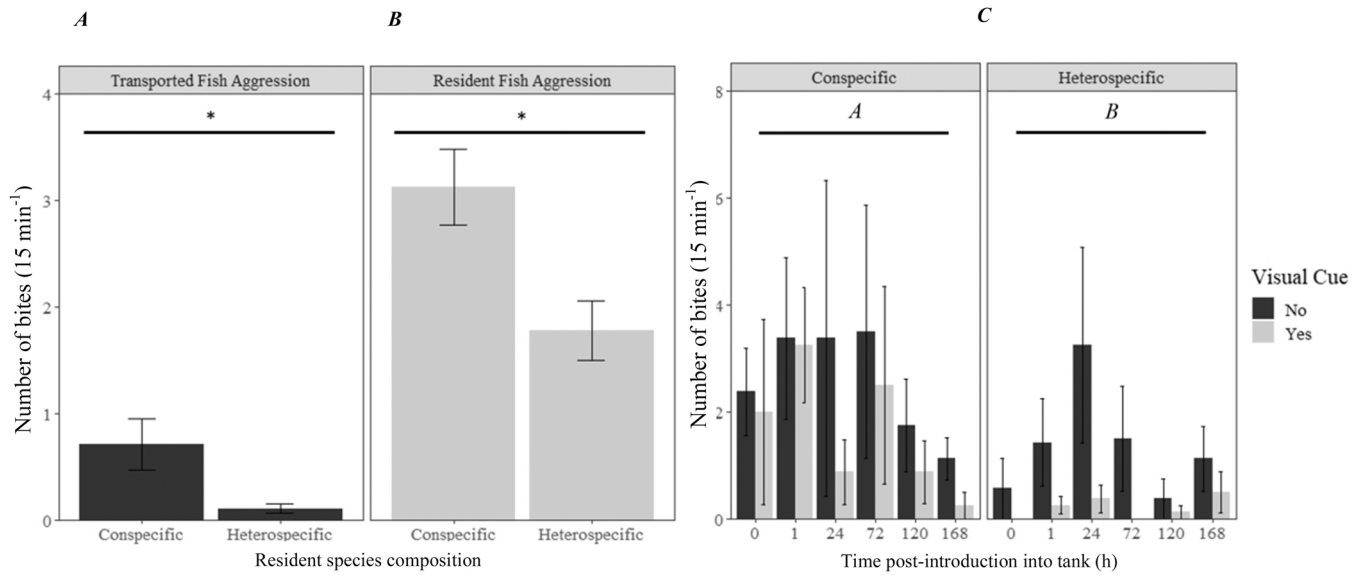
Fig. 2. A: Number of bites (mean ± SE) performed by the focal transported fish towards resident fish (conspecifics or heterospecifics). Asterisk indicates a significant post hoc difference (Tukey; $p < 0.05$). **B**: Number of bites (mean ± SE) performed by focal resident fish towards other resident fish (conspecifics or heterospecifics). Asterisk indicates a significant post hoc difference (Tukey; $p < 0.05$). **C**: Number of bites (mean ± SE) conducted by focal residents (conspecifics or heterospecifics) towards transported fish. Capital letters at the top of each panel indicate a significant post-hoc difference between tank composition (conspecifics and heterospecifics) (Tukey; $p < 0.05$). An overall effect of sampling time and visual cue presence/absence was also identified (see text for further analyses). As significant effects of sampling time and visual cues were found, data are shown for each sampling time (upon arrival, 1, 24, 72, 120 and 168 h after arrival) and with visual cues present or absent.
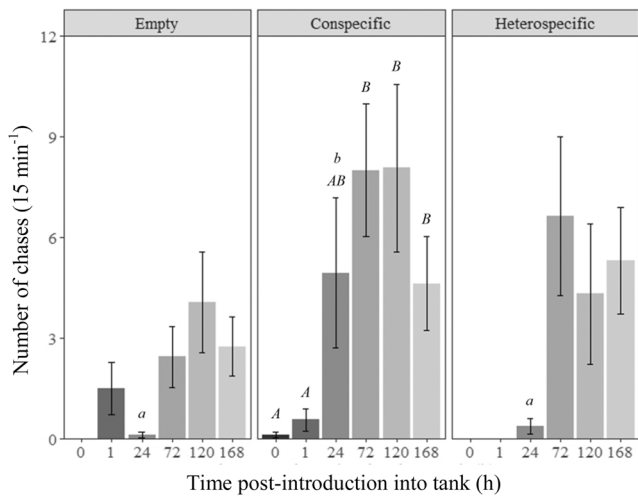

Fig. 3. Number of chases (mean ± SE) performed by the focal transported fish that were either introduced into empty tanks, with resident conspecifics or with resident heterospecifics, recorded upon arrival, 1, 24, 72, 120 and 168 h after arrival. Lower case letters indicate significant post-hoc differences between treatments within the same sampling time (Tukey; $p < 0.05$), where bars sharing a lower-case letter are not significantly different (Tukey; $p > 0.05$). Capital letters indicate significant post-hoc differences within each treatment across sampling times (Tukey; $p < 0.05$), where bars sharing the same capital letter are not significantly different (Tukey; $p > 0.05$). Absence of letters on a set of bars indicates that there are no significant differences.
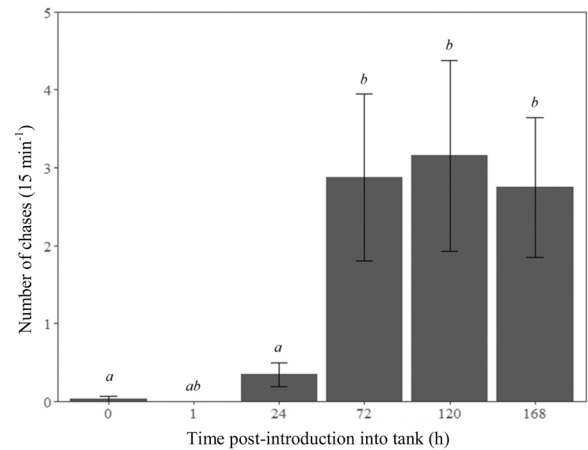
(Table 3, Fig. 4). Tank composition and visual cue presence/absence had no significant effect on the incidence of transported fish chasing other transported fish. There was no significant difference in the number of chases performed by transported fish towards either resident conspecifics or heterospecifics with visual cue presence/absence also having no effect. Sampling time was found to have an effect but *post-hoc* analyses found no significant differences between time points.

Fig. 4. Number of chases (mean ± SE) performed by the focal transported fish towards other transported *X. variatus*. As there were no significant effects of tank composition, data for these treatments have been combined. Lower case letters indicate a significant post hoc difference (Tukey; $p < 0.05$) between sampling times.

No significant difference in the number of chases performed by the resident fish species towards other resident fish was found, regardless of tank composition, visual cue presence/absence or sampling time. There was no significant difference in the number of chases performed by either of the resident fish species towards the transported fish with visual cue presence/absence and sampling time also having no significant effect.

3.3. Erratic movement

The remaining behaviours were considered for transported fish only. Erratic movement of transported fish was affected by tank composition, with time also having a significant effect (Table 3, Fig. 5). Visual cue
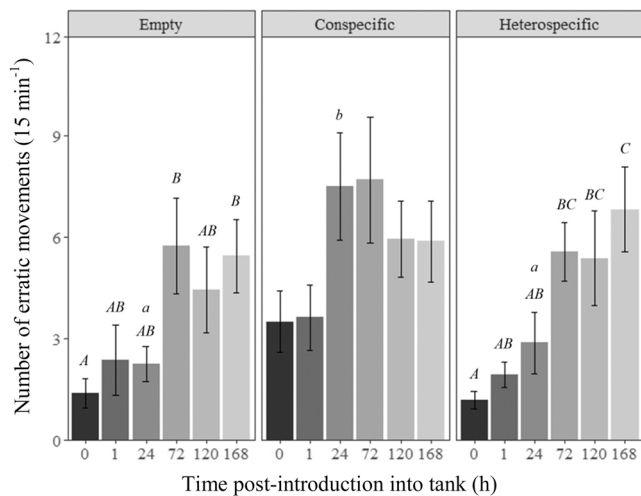
Fig. 5. Erratic movement frequency (mean ± SE) performed by the focal transported fish that were either introduced into empty tanks, with resident conspecifics or with resident heterospecifics, recorded upon arrival, 1, 24, 72, 120 and 168 h after arrival. Lower case letters indicate significant post-hoc differences between the different tank composition groups within the same sampling time (Tukey; $p < 0.05$), where bars sharing a lower case letter are not significantly different (Tukey; $p > 0.05$). Absence of letters on a set of bars indicates that there are no significant differences. Capital letters indicate significant post-hoc differences within each tank composition group across sampling times (Tukey; $p < 0.05$), where bars sharing the same capital letter are not significantly different (Tukey; $p > 0.05$).

presence/ absence had no significant effect on erratic movement frequency overall. No significant interaction between tank composition and visual cues was found. Fish placed into empty tanks post-transport exhibited significantly fewer erratic movements than those placed into tanks with resident conspecifics at 24 h post transport (Fig. 5). The number of erratic movements increased over time in both the empty tanks and those housing mixed compositions, with a significant increase appearing between 0 and 72 h post-transport. Higher levels of erratic movement in the tanks housing resident conspecifics immediately post-transport meant that no significant effects of time were seen for this treatment.

3.4. Foraging latency

Tank composition had a significant effect on foraging latency, with transported fish introduced into empty tanks exhibiting foraging behaviours significantly faster than fish introduced into tanks with resident conspecifics or heterospecifics (Table 3, Fig. 6). Visual cue presence/ absence had no significant effect on foraging latency. No significant interaction between tank composition and visual cues was found. Foraging latency was recorded immediately after introduction into the tanks only, hence there are no repeated time points.

3.5. Time immobile

There was no significant effect of tank composition, visual cue presence/absence or time on the time that transported fish spent immobile, and no significant interaction between tank composition and visual cues was found (Table 3).

4. Discussion

The aim of this study was to identify whether tank composition or the presence/absence of visual cues had an effect on stress-related behaviours (i.e. biting, chasing, erratic movements, foraging latency and time immobile) induced by short-term transportation. Although it was
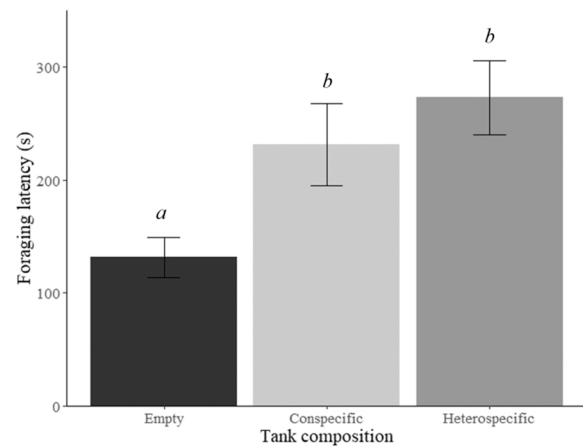

Fig. 6. Latency to exhibit foraging behaviours (mean ± SE) by focal transported fish immediately after introduction into tanks that were either empty, with resident conspecifics or with resident heterospecifics. Foraging latency was recorded upon arrival immediately after introduction into the tank. Lower case letters indicate a significant post hoc difference (Tukey; $p < 0.05$) between treatments where bars sharing a letter are not significantly different.

hypothesised that having visual or physical access to conspecifics would reduce the amount of time transported fish took to recover from transport stress, the contrary was found. While the presence of visual cues had minimal effects on fish behaviour, tank composition had a significant effect on all behaviours, apart from time spent immobile. Fish introduced into tanks containing resident conspecifics exhibited significantly more stress-related behaviours compared with fish placed into empty tanks or into tanks containing resident heterospecifics post-transport. In particular, increased aggression was seen which may have occurred through disruption of existing resident social hierarchies when transported fish were introduced and subsequent hierarchy re-establishment. Although biting behaviours were significantly higher in tanks containing conspecifics, the overall number of bites was quite low, so it is possible that this difference in biting did not have major effects on the levels of stress in each treatment. However, chasing behaviours within the tanks containing conspecifics were around double that of the other treatment groups, which coupled with the increased biting would likely result in elevated stress and impacts on welfare. Aggression appeared to stabilise over time, with a notable reduction in aggression from resident conspecifics towards transported fish. Identifying optimum tank compositions and understanding how they influence post-transport behaviour can facilitate faster recovery post-transport, with economic benefits as improved welfare can reduce disease, injury and potential mortalities (Jones et al., 2021). Compared with fish added to tanks containing residents, transported fish introduced into empty tanks resumed foraging faster, potentially indicating lower stress levels through the absence of competition posed by resident fishes (Ward et al., 2008; Martins et al., 2012).

Across all tank compositions, both biting and chasing behaviours increased over the first 72 h post-transport. It is likely that initial low frequencies of aggressive behaviours were due to the immediate stress of transport and introduction into a novel environment (Lucon-Xiccato et al., 2022). Once this behavioural inhibition ceased, time and energy were invested into exploring the novel environment, establishing social hierarchies and agonistic behaviours associated with their formation (Carbonara et al., 2019). The process of combining two social groups of equal size into one larger group is known as group fusion (Couzin, 2006), and can result in social instability with increased aggression until a new hierarchy is formed. As a result of the standard "picking" procedure, fish within the same transport bag are unlikely to originate from the same hierarchy and the inherent stress associated with transport is likely to preclude any short-term aggression within bags. In the present

study, the transportation process replicated the normal transport from wholesaler to retailer with a stocking density of five fish *per* bag, added to a tank containing an established hierarchy of five resident fish. Where there were no resident fish, two bags of transported fish were added to the empty tanks. Therefore, all transported fish were added to a separate group of fish, necessitating the formation of new social hierarchies.

The number of erratic movements significantly increased with time, and was seen most prominently between 0 and 72 h in tanks containing only transported fish or where transported fish were added to resident heterospecifics. The highest level of erratic movements was observed in tanks where transport-stressed fish were added into tanks containing acclimated conspecifics at 72 h. Increased levels of erratic movements may be an indication that an environment is unfavourable (Brandão et al., 2021). In the present study, water quality levels remained within acceptable levels so the increased erratic movements may be related to unfavourable social conditions. In all tanks, a new social hierarchy needed to establish which can take time and requires energetic investment. Consequently, the increased erratic movements are likely to be related to coping with the initial stress of transport combined with hierarchy establishment.

Individuals that have prior knowledge of an environment (residents) are more likely to invest time and energy into agonistic behaviours when new individuals (intruders) arrive, as residents already have knowledge of available resources (Harwood et al., 2003). This prior-residency effect stems from the pay-off asymmetry between the two parties; the intruder is unaware of what the available resources are, therefore, the resident has more to lose. Although often referred to in the context of territoriality (value asymmetry hypothesis: Nijman and Heuts, 2011), prior residency can also result in a dominance advantage when forming social hierarchies (Braddock, 1949; Figler and Einhorn, 1983). This effect is seen both intraspecifically and interspecifically (Skoglund et al., 2012). In the present study, the high levels of agonistic behaviours (biting, chasing) seen in tanks where transported fish were placed with resident conspecifics is likely to be related to prior residency with resident conspecifics defending existing territories. Although the prior residency effect has been seen between heterospecifics (Skoglund et al., 2012), in the present study, significantly increased levels of agonistic behaviours by residents towards transported fish were only seen intraspecifically, and not interspecifically.

Competition within intraspecific and interspecific groups may be associated with different resources (Ward et al., 2006; Eurich et al., 2018). Although less biting occurred between transported fish and resident heterospecifics compared with resident conspecifics, similar amounts of chasing behaviour occurred in both treatments. Any existing hierarchies among resident conspecifics were likely disrupted through group fusion when new conspecifics were added (Flood and Wong, 2017). However, when transported fish were added to heterospecific residents, it is possible that although there was increased competition, the existing hierarchy was not disrupted to the same extent. Avoiding the escalation of aggressive acts from chasing to physically damaging behaviours such as biting (Oldfield, 2011) appears possible where competition occurs between heterospecifics, but resident conspecifics have more to defend in terms of hierarchy formation. Differences in aggression by residents towards transported fish could also relate to species differences as aggression between residents was higher for *X. variatus* than for *P. sphenops*. *P. sphenops* are a schooling fish so should routinely be kept in groups (Rogers et al., 2013) and although they are not technically classified as a schooling fish, *X. variatus* are a social species and fare better both physically and behaviourally when kept in groups (Beaugrand et al., 1984).

Agonistic behaviours are a natural component of a fish's behavioural repertoire and some level of aggression should be expected, with this likely to increase when new fish are added to existing social groups. However, for fish welfare, it is important that aggression is kept below levels which would compromise welfare. Following a stressor such as transport, fish may engage in displaced aggressive behaviours as a way

of coping with their own stress (Sneddon et al., 2016) and this continued aggression once hierarchies are established can cause chronic stress (Sloman and Armstrong, 2002) with an increased likelihood of physical damage. Environmental enrichment was present in each tank and is often added as a form of shelter to allow fish to escape aggressive individuals (Kochhann and Val, 2016). Indeed, the presence of environmental enrichment (plastic loops) during transportation has been found to lessen the effects of transport-induced stress in *X. variatus* (Vanderzwalmen et al., 2020). However, the presence of physical enrichment may also represent a defendable resource resulting in increased rather than decreased aggression (Woodward et al., 2019). To our knowledge, no studies have considered the effects of environmental enrichment on recovery post-transport; interactions between social and environmental enrichment is an avenue for further research.

Contrary to existing studies (Oliveira et al., 2017; Silva et al., 2019), the presence of fish in adjacent tanks (i.e. with visual cues) had a minimal effect on behaviours measured. When resident fish were able to see fish in adjacent tanks they reduced bites towards transported fish, compared to those that did not have visual cues. Fish can infer social rank purely by observation (Grosenick et al., 2007), therefore, in the treatments that had visual cues it may have provided the fish with additional information that was then used to consider where they were placed in the hierarchy of both the tank they were in but also the adjacent tank. Another potential explanation is that the resident fish with visual cues were exposed to other "novel" fish (i.e. other resident fish) in adjacent tanks (although not able to interact with them), thus resulting in a more diluted response when the transported fish were added into the tank. Why this was seen with heterospecific residents but not conspecific residents remains unclear.

Currently, the main focus on reducing stress of ornamental fish post-transport is on abiotic factors however, we have shown that additional consideration of social factors in the housing of fish within wholesalers and retailers could be used to refine fish welfare. Stress-associated behaviours including contact aggression were reduced when fish were placed into either empty tanks or tanks with resident heterospecifics post-transport. Ornamental fishes are often housed in mixed-species compositions where species that share similar water quality requirements and life-histories, such as the variatus platy and the common molly (both poecilid live-bearers) are housed together. Poecilid live-bearers represent a large proportion of freshwater tropical fishes sold in the UK (OATA, 2020); refining and standardising procedures concerning the welfare of this group will therefore have significant benefits for the trade. Although a large number of different species are sold within the ornamental trade, based on UK data, over 75% of fish sold are freshwater and within tropical freshwater fish sales, 70% of species sold fall within five main groups (OATA, 2020). Use of simple, easily quantifiable, behavioural measures such as those in the present study could effectively refine welfare post-transport for many fish sold. This could be complemented by further research into the tank composition that fish choose post-transport; for example, through visual choice tests or social preference tests in which transported fish can choose between empty tanks or different social compositions (Nagel et al., 2018; Ward et al., 2020). Further understanding of the behaviour of ornamental fishes would also contribute to the potential development of operational welfare indicators (OWIs) for the trade (Jones et al., 2021).

5. Conclusion

Overall, this study highlights the importance of understanding species-specific social dynamics when attempting to alleviate the effects of stress post-transport. Introducing fish post-transport into empty tanks without other resident fishes aids behavioural recovery, evidenced by decreased foraging latency and decreased levels of aggression. Ideally within retail stores it is recommended that fish are introduced into empty tanks post-transport at an appropriate density for the species; densities that are too high or too low can exacerbate aggression (Sloman

and Armstrong, 2002). However, the feasibility of placing new arrivals into empty tanks may be limited by space or be impractical if insufficient numbers of fish are being transported. In such instances, the most suitable tank composition for variatus platys would be to place them with resident (compatible) heterospecifics, such as the common molly, rather than into a tank with resident conspecifics. Through relatively minor refinements to current procedures, the welfare of one of the most popular ornamental fish, the variatus platy, can be improved and this is likely to hold true for similar species which form strong intraspecific hierarchies. Continued research into the benefits of optimum social compositions for transported ornamental fishes is necessary to improve fish welfare following transport.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This research was conducted as part of the first author's doctoral research. D.S. is an employee of the WALTHAM Petcare Science Institute, a subsidiary of Mars Petcare UK., and P.S. with Aquasense, which both provided some funding towards the Ph.D. which this study forms part of. Neither D.S. nor P.S. were involved in the data collection or analysis aspects of this study, but were involved in its conceptualization and provided feedback on the final manuscript prior to submission. The interpretation of the results and the content of the manuscript remained the final decision of the authors.

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References

Beaugrand, J.P., Caron, J., Comeau, L., 1984. Social organisation of small heterosexual groups of green swordtails (*Xiphophorus helleri*, Pisces, Poeciliidae) under conditions of captivity. Behaviour 91, 24–59.

Braddock, J.C., 1949. The effect of prior residence upon dominance in the fish *Platypoecilus maculatus*. Physiol. Zool. 22, 161–169.

Brandão, M.L., Dorigão-Guimarães, F., Bolognesi, M.C., Dos Santos Gauy, A.C., Pereira, A.V.S., Vian, L., Carvalho, T.B., Gonçalves-de-Freitas, E., 2021. Understanding behaviour to improve the welfare of an ornamental fish. J. Fish. Biol. 99, 726–739.

Brown, C., Laland, K.N., 2003. Social learning in fishes: a review. Fish Fish 4 (3), 280–288.

Carbonara, P., Dioguardi, M., Cammarata, M., Zupa, W., Vazzana, M., Spedicato, M.T., Lembo, G., 2019.) Basic knowledge of social hierarchies and physiological profile of reared sea bas Dicentrarchus labrax (L.). PLoS One 14 (1), E0208688.

Chen, C.-C., Fernald, R.D., 2011. Visual information alone changes behaviour and physiology during social interactions in a cichlid fish (*Astatotilapia burtoni*). PLoS ONE 6 (5), e20313.

Couzin, I.D., 2006. Behavioural ecology: Social organisation in fission-fusion societies. Curr. Biol. 16 (5), 169–171. https://doi.org/10.1016/j.cub.2006.02.042.

Desjardins, J.K., Hofmann, H.A., Fernald, R.D., 2012. Social context influences aggressive and courtship behaviour in a cichlid fish. PLoS ONE 7 (7), E32781.

Donaldson, M.R., Cooke, S.J., Patterson, D.A., MacDonald, J.S., 2008. Cold shock and fish. J. Fish. Biol. 73, 1491–1530.

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kaleuff, A.V., 2009. Understanding behavioural and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res. 205 (1), 38–44.

Eurich, J.G., McCormick, M.I., Jones, G.P., 2018. Direct and indirect effects of interspecific competition in a highly partitioned guild of reef fishes. Ecosphere 9 (8), e02389. https://doi.org/10.1002/ecs2.2389.

Faustino, A.I., Tacão-Monteiro, A., Oliveira, R.F., 2017. Mechanisms of social buffering of fear in zebrafish. Sci. Rep. 7, 44329.

Fernald, R.D., Wright, S.E., 1985. Growth of the visual system in the African cichlid fish, *Haplochromis burtoni*: Accommodation. Vis. Res. 25 (2), 163–170.

Figler, M.H., Einhorn, D.M., 1983. The territorial prior residence effect in convict cichlids (*Cichlasoma nigrofasciatum* Günther): Temporal aspects of establishment and retention, and proximate mechanism. Behaviour 85 (1/2), 157–183.

Flood, C.E., Wong, M.Y.L., 2017. Social stability in times of change: effects of group fusion and water depth on sociality in a globally invasive fish. Anim. Behav. 129, 71–79. https://doi.org/10.1016/j.anbehav.2017.05.003.

Friard, O., Gamba, M., 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol. Evol. 7 (11).

Grosenick, L., Clement, T.S., Fernald, R.D., 2007. Fish can infer social rank by observation alone. Nature 445 (25), 429–432. https://doi.org/10.1038/nature05511.

Guthrie, D.M., 1986. Role of vision in fish behaviour. In: Pitcher, T.J. (Ed.), The behaviour of teleost fishes. Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-8261-4_4.

Harwood, A.J., Griffiths, S.W., Metcalfe, N.B., Armstrong, J.D., 2003. The relative influence of prior residency and dominance on the early feeding behaviour of juvenile Atlantic salmon. Anim. Behav. 65, 1141–1149.

Huntingford, F.A., Kadri, S., 2009. Taking account of fish welfare: lessons from aquaculture. J. Fish. Biol. 75, 2862–2867.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P., Turnbull, J.F., 2006. Current issues in fish welfare. J. Fish. Biol. 68 (2), 332–372.

Jones, M., Alexander, M.E., Snellgrove, D., Smith, P., Bramhall, S., Carey, P., Henriquez, F.L., McLellan, I., Sloman, K.A., 2021. How should we monitor welfare in the ornamental fish trade? Rev. Aquac. 14, 770–790. https://doi.org/10.1111/raq.12624.

King, T.A., 2019. Wild caught ornamental fish: a perspective from the UK ornamental aquatic industry on the sustainability of aquatic organisms and livelihoods. J. Fish. Biol. 94 (6), 925–936.

Kiyokawa, Y., Honda, A., Takeuchi, Y., Mori, Y., 2014. A familiar conspecific is more effective than an unfamiliar conspecific for social buffering of conditioned fear responses in male rats. Behav. Brain Res. 267, 189–193.

Kleinhappel, T.K., Pike, T.W., Burman, O.H., 2019. Stress-induced changes in group behaviour. Sci. Rep. 9, 17200.

Kochhann, D., Val, A.L., 2016. Social hierarchy and resting metabolic rate in the dwarf cichlid *Apistogramma agassizii*: the role of habitat enrichment. Hydrobiologica 789 (1). https://doi.org/10.1007/s10750-016-2806-7.

Korzan, W.J., Summers, C.H., 2007. Behavioural diversity and neurochemical plasticity: selection of stress coping strategies that define social status. *Brain*. Behav. Evol. 70, 257–266.

Lall, S., Tibbets, S.M., 2009. Nutrition, feeding, and behaviour of fish. Vet. Clin. North Am. Exot. Anim. Pract. 12 (2), 361–372.

Lucon-Xiccato, T., Montalbano, G., Reddon, A.R., Bertolucci, C., 2022. Social environment affects inhibitory control via developmental plasticity in fish. Anim. Behav. 183, 69–76. https://doi.org/10.1016/j.anbehav.2021.11.001.

Martins, C.I.M., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M.T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J.-C., Carter, T., Planellas, S.R., Kristiansen, T., 2012. Behavioural indicators of welfare in farmed fish. Fish. Physiol. Biochem. 38 (1), 17–41. https://doi.org/10.1007/s10695-011-9518-8.

Miller-Morgan, T., 2009. A brief overview of the ornamental fish trade and hobby. In: Roberts, H.E. (Ed.), Fundamentals of Ornamental Fish Health. Wiley-Blackwell, Chichester, pp. 25–32.

Nagel, R., Kirchbaum, F., Engelmann, J., Hofmann, V., Pawelzik, F., Tiedemann, R., 2018. Male-mediated species recognition among African weakly electric fishes. R. Soc. Open Sci. 5, 170443 https://doi.org/10.1098/rsos.170443.

Näslund, J., Johnsson, J.I., 2014. Environmental enrichment for fish in captive environments: effects of physical structures and substrates. Fish Fish 17 (1), 1–30.

Nijman, V., Heuts, B.A., 2011. Aggression and dominance in cichlids in resident-intruder tests: the role of environmental enrichment. Neotrop. Ichthyol. 9 (3), 543–545. https://doi.org/10.1590/S1679-62252011005000031.

OATA (2020) Looking to the future: Annual review 2020/21. www.ornamentalfish.org/wp-content/uploads/OATA-Annual-Report-2020–21-online.pdf. (Accessed on 1 September 2022).

Oldfield, R.G., 2011. Aggression and welfare in a common aquarium fish, the Midas cichlid. J. Appl. Anim. Welf. Sci. 14 (4), 340–360.

Oliveira, T.A., Idalencio, R., Kalichak, F., dos Santos Rosa, J.G., Koakoski, G., de Abreu, M.S., Giacomini, A.C.V., Gusso, D., Rosemberg, D.B., Barreto, R.E., Barcellos, L.J.G., 2017. Stress responses to conspecific visual cues of predation risk in zebrafish. PeerJ 5, E3739. https://doi.org/10.7717/peerj.3739.

Palagi, E., Barbuti, R., Norscia, I., 2020. Aquarium cichlid *Tropheus moori* flexibly adjust hierarchy when tank fish species composition changes: A pilot study. *Journal of Zoo and Aquarium*. Research 8 (2), 86–93.

Pouil, S., Tlusty, M.F., Rhyne, A.L., Metian, M., 2019. Aquaculture of marine ornamental fish: overview of the production trends and the role of academic in research progress. Rev. Aquac. 12 (2), 1217–1230. https://doi.org/10.1111/raq.12381.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rogers, G.M., Kimbell, H., Morrell, L.J., 2013. Mixed-phenotype grouping: the interaction between oddity and crypsis. Oecologia 172, 59–63. https://doi.org/10.1007/s00442-012-2473-y.

Sandström, A., 1999. Visual ecology of fish – a review with special reference to percids. Fisk. Rapp. 2, 45–80.

Saxby, A., Adams, L., Snellgrove, D., Wilson, R.W., Sloman, K.A., 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.

Silva, P.F., Garcia de Leaniz, C., Luchirari, A.C., 2019. Fear contagion in zebrafish: a behaviour affected by familiarity. Anim. Behav. 153, 95–103.

Skoglund, H., Einum, S., Forseth, T., Barlaup, B.T., 2012. The penalty for arriving late in emerging salmonid juveniles: differences between species correspond to their interspecific competitive ability. Funct. Ecol. 26, 104–111. https://doi.org/10.1111/j.1365-2435.2011.01901.x.

Sloman, K.A., Armstrong, J.D., 2002. Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena? J. Fish. Biol. 61, 1–23. https://doi.org/10.1006/jfbi.2002.203.

Sloman, K.A., Baldwin, L., McMahon, S., Snellgrove, D., 2011. The effects of mixed-species assemblage on the behaviour and welfare of fish held in home aquaria. Appl. Anim. Behav. Sci. 135 (1–2), 160–168.

Sneddon, L.U., Wolfenden, D.C.C., Thomson, J.S., 2016. Stress management and welfare. Fish. Physiol. 35, 463–539. https://doi.org/10.1016/B978-0-12-802728-8.00012-6.

Stevens, C.H., Croft, D.P., Paull, G.C., Tyler, C.R., 2017. Stress and welfare in ornamental fishes: what can be learned from aquaculture? J. Fish. Biol. 91 (2), 409–428.

Strand, D.A., Utne-Palm, A.C., Jakobsen, P.J., Braithwaite, V.A., Jensen, K.H., Salvanes, A.G.V., 2010. Enrichment promotes learning in fish. Mar. Ecol. Prog. Ser. 412, 273–282.

Teletchea, F., 2015. Domestication level of the most popular aquarium fish species: is the aquarium trade dependent on wild populations? Cybium: Int. J. Ichthyol. 40 (1), 21–29.

Tran, S., Gerlai, R.T., 2016. The novel tank test: Handling stress and the context specific psychopharmacology of anxiety. Curr. Psychopharmacol. 5, 169–179.

Vanderzwalmen, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D., Sloman, K.A., 2019. The use of feed and water additives for live fish transport. Rev. Aquac. 11 (1), 263–278.

Vanderzwalmen, M., Carey, P., Snellgrove, D., Sloman, K.A., 2020. Benefits of enrichment on the behaviour of ornamental fishes during commercial transport. Aquaculture 526. https://doi.org/10.1016/j.aquaculture.2020.735360.

Ward, A.J.W., Webster, M.M., Hart, P.J., 2006. Intraspecific food competition in fishes. Fish Fish 7 (4), 231–261. https://doi.org/10.1111/j.1467-2979.2006.00224.x.

Ward, A.J.W., Sumpter, D.J.T., Couzin, L.D., Hart, P.J.B., Krause, J., 2008. Quorum decision-making facilitates information transfer in fish shoals. Proc. Natl. Acad. Sci. 105, 6948–6953.

Ward, A.J.W., Kent, M.I.A., Webster, M.M., 2020. Social recognition and social attraction in group-living fishes. Front. Ecol. Evol. 8 (15) https://doi.org/10.3389/fevo.2020.00015.

Wood, L., Martin, K., Christian, H., Nathan, A., Lauritsen, C., Houghton, S., Kawachi, I., McCune, S., 2015. The pet factor – companion animals as a conduit for getting to know people, friendship formation and social support. PLoS ONE 10 (4), e0122085.

Woodward, M.A., Winder, L.A., Watt, P.J., 2019. Enrichment increases aggression in zebrafish. Fishes 4 (22). https://doi.org/10.3390/fishes4010022.