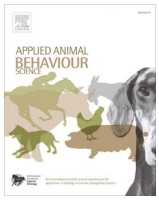
Applied Animal Behaviour Science 277 (2024) 106342

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim



Effects of simulated motion frequency related to road quality on the welfare and recovery of transported largemouth bass (*Micropterus salmoides*)

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ARTICLE INFO

Keywords:
Road quality
Motion frequency
Largemouth bass
Fish welfare
Behaviour
Physiology

ABSTRACT

Farmed fish are commonly transported between various facilities by road vehicles, resulting in inevitable exposure to uncontrolled and oscillatory movements, likely exacerbated by poor road conditions. The effect of road quality on livestock has been studied during live transport, but research into the impact of motion has been rarely examined with fish. This study investigated the effects of different motion frequencies related to road quality on the welfare and recovery of largemouth bass (*Micropterus salmoides*). Three motion frequencies were examined in this study using a non-transported control, a simulated "rough" transport treatment, and a simulated "smooth" transport treatment. Live transport was carried out for 3 h using a motion simulation platform with a movement frequency of 1.0 and 1.8 Hz for the smooth and rough treatment, respectively. Control fish were kept in static tanks for the same duration to obtain basal physiology, behaviour, and flesh quality. Water parameters were measured before and immediately after simulated transport in all groups. Behavioural, physiological, and muscle parameters were measured before simulated transport, as well as 0 h and 24 h post-transport. Total ammonia nitrogen levels increased in all treatments over time ($p < 0.001$), with significantly higher values observed in transported groups. Non-transported fish displayed increased biting ($p = 0.025$), chasing ($p = 0.010$), and threatening ($p = 0.003$) behaviour over time, suggesting potential fasting and confinement stress. During the post-transport period, a significant main effect of treatment and timepoint on freezing and thigmotaxis behaviour was found, with an increase in these behaviours over time and significantly higher levels between control and smooth transported groups. Nevertheless, aggressive behaviours were affected only by timepoint, with an increase observed between 0 h and 24 h post-transport. Neither plasma biochemical indicators nor flesh quality differed between treatments, while a significant effect of timepoint was found for plasma glucose ($p = 0.045$), plasma lactate ($p = 0.021$), and muscle pH ($p < 0.001$). Our study consequently did not find rough transport to impact fish physiology and flesh quality more than smooth transport, but behavioural results suggest there was a strong combined effect of fasting, exposure to a novel environment, and confinement over time. Future research would be valuable to study these effects on the welfare of transported bass, allowing for a longer recovery time and the use of potential mitigation options such as environmental enrichment.

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https://doi.org/10.1016/j.applanim.2024.106342
Received 9 January 2024; Received in revised form 20 May 2024; Accepted 1 July 2024
Available online 2 July 2024

1. Introduction

Animal welfare is associated with both animal and human health, product quality and safety, as well as sustainability (Broom, 2010). Farm animals are largely transported by land vehicles for economic trade and relocation purposes (Fisher et al., 2009). The Five Freedoms framework suggests that animals should not experience hunger, disease, injury, distress, and pain (Brambell, 1965; Farm Animal Welfare Council, 1993). However, farm animals are inevitably exposed to a series of events during live transport, including handling, confinement, unfamiliar environments, withdrawal of feed and water, and exposure to vibration and noise, which can individually and cumulatively have negative impacts on all aspects of the Five Freedoms (Southgate, 2008; Fisher et al., 2009). Motion characteristics including vibration level and frequency, acceleration, and amplitude and the magnitude of these influence the impact on animal welfare during live transport (Santurtun and Phillips, 2015). Vibration is determined by the direction of force (vertical, longitudinal, and horizontal), frequency, and acceleration (Aradom Messmer, 2013; Donofre et al., 2020). Frequency refers to the number of complete oscillation cycles that occur per unit of time (e.g. minute), measured in Hertz, Hz (Donofre et al., 2020). Road quality or roughness is one of the features that determines vibration levels and frequency during vehicle transport (Aradom Messmer, 2013). When a road is in poor condition or has irregularities like potholes, cracks, or uneven surfaces, vehicles will have sudden changes in height as they move along.

Excessive vibrations on vehicles lead to uncomfortable experiences, such as fear, distress, and fatigue in animals, which may compromise their welfare (Aradom Messmer, 2013). In beef cattle, it has been shown that transport under poor road conditions can increase the likelihood of traumatic bruises (Huertas et al., 2010). Lambs transported on unpaved roads have been found to have higher levels of plasma cortisol, glucose, creatine kinase, neutrophil/lymphocyte ratio, as well as poorer meat quality, compared to those transported on paved roads (Miranda-de la Lama et al., 2011). Low-frequency vertical vibrations are found to impair animal welfare more than higher frequencies (Perremans et al., 1998; Perremans et al., 2001; Van De Water et al., 2003). For example, pigs showed a significantly higher heart rate and spent 10 times less time in prostrate postures (a relaxed and quieter state indicative of better welfare) when they experienced low-frequency vibration at 2 and 4 Hz compared to 8 and 18 Hz during transport (Perremans et al., 1998; Perremans et al., 2001). One of the reasons why poor conditions affect transported animals is that unpredictable and irregular movements can induce stress responses. For example, sheep experiencing unpredictable and irregular motions exhibit increased aggression and decreased cardiac variability compared to those transported with predictable and regular movements (Navarro et al., 2018). Therefore, it might be expected that live transport on poor roads can also induce a similar negative influence on fish welfare due to excessive and unpredictable motions in transport tanks (King, 2009).

The largemouth bass (*Micropterus salmoides*) has been a commercially important food fish in China since its cultivation in the 1980s, ranking among the top eight freshwater species and contributing approximately 0.8 million tonnes in 2022 (MARA of the PRC et al., 2023). Live largemouth bass are largely transported by vehicle for selling or breeding, with a common journey duration of around three hours in the Yangtze River Delta (Bai and Li, 2018). However, largemouth bass are susceptible to physical trauma in confined tanks due to their fragile integumentary system and prominent and sharp dorsal fins (Wang, 2015). Thus, excessive vibrations may result in distress and fatal injuries to this fish species. Live transport has been found to impair fish welfare in other freshwater and seawater species, as evidenced by altered physiological and behavioural welfare indicators. For example, the count of white and red blood cells, as well as the levels of haemoglobin and haematocrit, exhibited significant increases in juvenile hybrid yellow catfish (*Tachysurus fulvidraco*♀ × *Pseudobagrus vachellii*♂) following simulated transport in bags for 4 h (Zheng et al., 2021). Plasma cortisol levels significantly increased in blood parrot cichlid (*Amphilophus citrinellus* × *Cichlasoma synspilum*) and koi (*Cyprinus carpio*) after 4-h simulated transport in plastic bags (Wu et al., 2021). Nile tilapia (*Oreochromis niloticus*) transported in tanks for 3 h showed higher opercular movements compared to non-transported fish (Félix et al., 2021). These measures are indicators of increased stress and impaired welfare in fish.

While most main roads in urban and suburban China are paved and in good condition, there are still some dirt roads in rural areas, particularly where farms are located (Wong et al., 2013; Wong et al., 2017). This potentially results in adverse welfare impacts on fish before their transport on high-quality roads, such as paved roads or highways. Unpaved or dirt roads around the fish farms may also damage transport containers and cause stress for both the fish and the truck driver (Rimmer, 1995). For example, Rebouças et al. (2019) reported that dirt roads caused higher acceleration vibration and more mechanical shocks in transport water compared to asphalt roads, resulting in elevated haematological, metabolic, and ionic responses, and more physical injuries in Nile tilapia.

This study aimed to determine the effects of motion frequencies simulating different road quality conditions on the behaviour, physiology, and flesh quality of largemouth bass, as well as water quality in transport tanks. Another objective of this study was to investigate whether largemouth bass can recover from transport stress within a 24-h period to enhance their general welfare before further transport or slaughter.

2. Materials and methods

This study was approved by the University of Queensland Animal Ethics Committee Native and Exotic Wildlife and Marine Animal Group (#2021/AE000556).

2.1. Animals

A total of 432 adult largemouth bass (mean weight of 248.7 ± 5.9 g, mean length of 22.0 ± 0.2 cm) were sourced from a commercial fish farm (Hangzhou Jianfeng Agricultural Development Co., Ltd, Hangzhou, China). The experimental fish were initially reared in outdoor recirculating water raceways located approximately 100 m from the on-farm laboratory where the experiment was carried out. The size of the laboratory was approximately 25 m^2 and the ambient temperature was controlled at 28°C during the study period. Prior to the experiment, fish were carefully captured using dipnets from the raceways and placed in several elliptical tanks with water and aerated air. The tanks were then transported by an electric tricycle to the laboratory, where fish were transferred into four 600-L tanks for one-day acclimation. Aerators were used to maintain an appropriate level of oxygen in acclimation tanks. Fish were fasted during this period to empty feed residue and subsequently reduce excrement in the transport tanks. This is common practice to maintain water quality during real-life transport (Southgate, 2008). The daily light/dark cycle was maintained at 12:12 h throughout this period. Water quality parameters were checked at 08:00 h and 17:00 h each day. Dissolved oxygen was maintained at 6.8 ± 0.5 mg/L, water temperature at 29.6 ± 0.4°C, and water pH at 7.6 ± 0.1.

2.2. Equipment and experimental protocols

The complexity of combined factors (e.g. traffic condition and driving performance) in real-life transport makes motion study on trucks challenging. To minimise confounding variables, a laboratory-based motion simulator can be used to perform reproducible “live transport” of fish (Wu et al., 2020; Félix et al., 2021; Wu et al., 2021), broilers (Zheng et al., 2020), and sheep (Santurtun et al., 2014).

In this study, a six degrees of freedom (6DOF) motion simulation

platform (Suzhou Fengda Automation Equipment Technology Co., Ltd, Suzhou, China) was used to produce vertical, longitudinal, and horizontal movements in combination. The motion platform (Fig. 1A) included a rectangular upper platform that measured 1000 × 1000 mm, positioned at a height of 661 mm from the floor, and a baseplate measuring 1083 × 1235 mm. Six electric cylinders (arms) equipped with 1-kW motors generated linear motions (surge, sway, and heave) by moving the upper platform piece up to 120 mm with an acceleration of 0.1 g. The angular motions (yaw, pitch, and roll) were able to be generated by moving the upper platform ± 20°, with an acceleration of 250°/s^2. The platform control box was connected to a laptop (Legion Y7000P, Lenovo Group Limited, Beijing, China), and pre-determined movements were simulated using MBOX Dynamic Platform Control Software (Beijing HollySys Electric Technology Co., Ltd, Beijing, China). A four-compartmented tank (100 × 100 × 80 cm; Fig. 1B) was fixed on the upper platform. Each compartment contained approximately 85 L of water. Oxygen was continuously provided during the experiment (Fig. 1C).

On the day of simulated transport, fish were randomly selected from acclimation tanks and distributed to one control and two experimental treatments: a non-transported control (C), a simulated "smooth" transport treatment (S), and a simulated "rough" transport treatment (R). For the transported groups, regular combined horizontal, lateral, and vertical movements were simulated for 3 h with a motion frequency of 1.0 Hz for the smooth transport treatment and 1.8 Hz for the rough transport treatment. Possible motion frequencies were tested before the formal study (with water only), and 1.8 Hz was determined to be the maximum value for animal and personnel safety under laboratory conditions. All other movement parameters including amplitude (10 mm) were maintained at the same value for both transported treatments. At the beginning of the study, each control and transported group consisted of 18 fish. All treatment groups commenced simultaneously each day for eight consecutive days, resulting in eight replicate groups per treatment. On each of the eight experimental days, the motion platform was executed twice (morning and afternoon), once for a "smooth" transport and once for a "rough" transport. The order was randomised with each transport time occurring four times in the morning and four times in the afternoon, as well as for the control groups. Fish groups were randomly designated to be sampled for either physiology or behaviour, to avoid physiological sampling affecting behavioural observations. Each group comprised different fish for sampling, as stressed or sampled fish could not be reused, unlike terrestrial animals. After 3 h of simulated movement, transported fish were transferred to 600-L tanks within their groups for a 24-h recovery period. Control groups were kept in static tanks for the same duration and sampled as baseline references for behaviour, physiology, and flesh quality at each sampling interval.

Fig. 1. The 6DOF motion platform (A); the four-compartmented tank (B); and an oxygen tube connected with an air generator (C).

2.3. Water quality measures

Water quality parameters were measured in control and experimental groups before (T0) and immediately after simulated transport (T1). Dissolved oxygen (DO) and water temperature were measured *in situ* in each group by a portable DO meter (JPB607A, Hangzhou Qiwei Instrument Co., Ltd, Hangzhou, China). Water pH and total ammonia nitrogen (TAN) were determined using a multiparameter water analyser (HI83200, Hanna® Instruments, Woonsocket, USA) with commercial pH reagents (HI93710–01) and ammonia reagents (HI93715–01, Hanna® Instruments, Woonsocket, USA).

2.4. Fish behavioural measures

Non-transported control fish were filmed before simulated transport (T0), and at 0 h (T1) and 24 h post-transport (T2) for 30 min using digital cameras (MJSXJ02CM, Xiaomi Corporation, Beijing, China) from a top-down view. Transported fish were only filmed in the post-transport period to minimise confounding impacts with simulated transport. One of the reasons was that we did not have underwater cameras inside the acclimated tanks to record fish behaviour as baselines. Additional capture and handling would have been required between acclimated and behavioural recoding tanks (arena) if fish were filmed before being transported. Capture and handling stress may exceed further transport stress and affect subsequent experimental results. Additionally, to eliminate any potential disturbance of human presence on the recorded behaviour, only the middle 20 minutes of each 30-minute video were analysed. BORIS© behavioural observation software (Friard and Gamba, 2016) was used to code fish behaviours. Recorded behaviours were described in advance in an ethogram (Table 1) including freezing, thigmotaxis (being in the periphery area), erratic swimming, biting, chasing, and threatening. The number of visible fish exhibiting freezing, thigmotaxis, and erratic swimming were scanned per minute and recorded as the percentage of the group engaging in each behaviour within each treatment group. Total occurrences of each aggressive

Table 1
Ethogram of recorded behaviours for largemouth bass.

Behaviour	Description
Freezing	A fish is immobile or only has minor body movements for more than 3 s (Nordgreen et al., 2014; Sánchez-Muros et al., 2017; Vanderzwalmen et al., 2021).
Thigmotaxis	The number of fish occupying the periphery area of an arena (Alfonso et al., 2020).
Erratic swimming	Occurrence of sharp, rapid, and unexpected swimming or direction change (Vanderzwalmen et al., 2020b; Vanderzwalmen et al., 2021).
Biting	A fish widely opens its mouth to bite another fish on any part of its body, and physical contact is involved (Oikonomidou et al., 2019; Oliveira et al., 2022).
Chasing	A fish rapidly follows another fish, but the action does not involve any physical contact; chasing occurs either in a circular or rectilinear motion (Oikonomidou et al., 2019).
Threatening (aggressive display)	A fish opens its opercula and rushes towards another fish without any physical contact (Oikonomidou et al., 2019); the receiver may retreat or show freezing behaviour when threatened.

behaviour (biting, chasing, and threatening) were counted for the 20-min observation period and data were converted to a rate per hour (3 × 20 mins to represent 60 mins). Approximately 10 % of videos were coded blind by a second observer to calculate the inter-rater reliability of behavioural coding (interclass correlation coefficient = 0.85, $p < 0.001$). Fish were not individually identifiable in our study, leading to visual occlusion of some fish within the group in the arena (video--recording tank area). Therefore, all behaviours were recorded at the group level, and only behaviours visible for coding were included in the formal analysis.

2.5. Fish physiological measures

Fish physiological samples were collected at three timepoints (T0, T1 and T2) in all treatment groups (Fig. 2). Prior to sampling, individual fish (three animals per group at each timepoint) were rapidly caught by a dipnet (< 30 s) and transferred to a plastic container containing water with 125 mg/L tricaine methane sulfonate (MS-222, South Ranch Biotech Co., Ltd, Zhengzhou, China) for humane euthanasia. Fish death was confirmed by total loss of equilibrium, and cessation of opercular movement and heartbeat (Neiffer and Stamper, 2009; Topic Popovic et al., 2012). Morphology and physiology measurements took place immediately upon confirmed euthanasia and were completed within 5 min. First, fish were weighed and measured for body length. Skin mucus was subsequently collected using a sterile cell scraper gently applied three times to both lateral sides from the head to the caudal direction of the fish, as described by Carbajal et al. (2019). Blood was collected using 1 mL heparinised syringes (26 G) from the caudal vein of the fish. Collected mucus was centrifuged at 3600 *g* for 5 min and the supernatant was transferred into 1.5 mL Eppendorf tubes. Plasma was obtained from blood supernatant after being centrifugated at 3600 *g* for 10 min. For both plasma and mucus, an equal volume of samples from three fish in the same group was pooled together at each timepoint. Pooled samples were stored at −20°C for later analysis.

Blood plasma was used to analyse the levels of cortisol, glucose, lactate, and heat shock protein 70 (HSP70). Skin mucus samples were used to measure metabolite levels of cortisol, glucose, and lactate. All biochemical assays were carried out in a commercial laboratory (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) using fish cortisol ELISA kits (H094–1–2), glucose assay kits (A154–1–1), lactate assay kits (A019–2–1), and HSP70 ELISA kits (H264–2) according to the manufacturer's instructions.

Muscle pH was measured at the dorsal muscle of the fish once the blood sampling was completed (Fig. 2), by inserting a pH probe (Testo 205, Testo SE & Co. KGaA, Titisee-Neustadt, Germany) into the front, middle, and rear position of the dorsal fillet. The muscle pH was subsequently calculated as a mean value of the three measurements. The drip loss of the fillet was determined by the retail display method described by Font-i-Furnols et al. (2015). Fillet samples (3.0 ± 0.5 g) were placed in a plastic box and then stored at 4°C for 72 h. Drip loss was calculated by the following equation (Bosworth et al., 2004):

$$\text{Drip loss (\%)} = [(W_0 - W_1)/W_0] \times 100$$

W_0 is the initial weight of the fillet sample; W_1 is the weight after storage (72 h).

2.6. Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 29 (IBM®, New York, USA). All data are expressed as mean ± standard error (SE). The critical value for detecting significant differences was considered to be less than 0.05. Figures were created using GraphPad Prism 10 (GraphPad Software Inc., San Diego, USA).

This study addressed two research questions regarding changes in fish behaviour, which required different statistical approaches. The first question was whether fish behaviour in the control groups changed over time, while the second questioned whether fish behaviour was affected by different movement frequencies.

Shapiro-Wilk tests and Q-Q plots were applied to check the assumptions of normality for residuals. If the assumptions were not met, a non-parametric Friedman's test with Bonferroni pairwise comparisons was employed to detect the differences in fish behaviour among three sampling times in the control groups, addressing the first research question on fish behaviour. Two-way repeated measures analysis of variance (ANOVAs) with Bonferroni pairwise comparisons were applied to determine the effect of motion frequency, sampling timepoint, and their interaction on fish physiology, muscle parameters, water quality, and fish post-transport behaviour. The time of sampling (timepoint) was treated as a within-subjects factor, while motion frequency (treatment) was considered as a between-subjects factor.

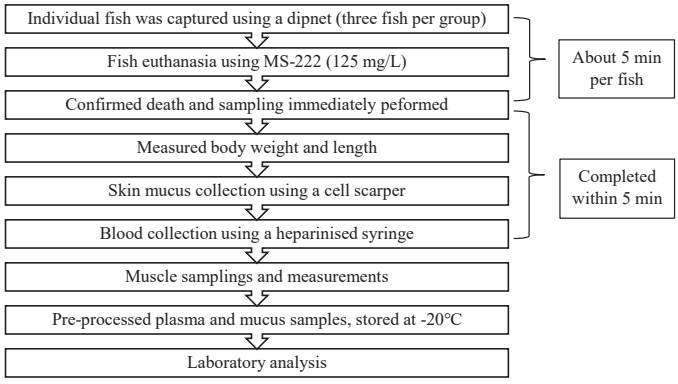

Fig. 2. A diagram of fish physiological samplings performed for each treatment group at each timepoint.

3. Results

3.1. Water quality

The mean values of each water quality parameter in three treatments between two sampling timepoints are shown in Table 2. The main effect of timepoint was significant for dissolved oxygen, with DO levels significantly decreasing between T0 (6.85 ± 0.11 mg/L) and T1 (5.86 ± 0.16 mg/L). There was a significant interaction effect for water temperature, pH, and TAN levels, although for pH the interaction was only marginally significant ($p = 0.049$). Water temperature was higher in the two transport treatments than in the control at T1, and rough-transported groups showed a significant increase between the two timepoints. The water pH significantly decreased in two transport treatments between T0 and T1, while no difference was found in the control groups. TAN levels increased in all treatments over time, with particularly elevated values observed in both transport treatments, but there was no significant difference between the two treatments.

3.2. Fish behaviours

In this study, we investigated whether fish behaviour changed among three sampling times in the control groups to understand how bass behaviour changed over time as a reference, so the effects of motion frequency on fish behaviour could be better identified.

The percentage of fish per control group showing freezing ($\chi^2_{(2)} = 4.75$, $p = 0.093$) and thigmotaxis behaviour ($\chi^2_{(2)} = 0.25$, $p = 0.882$) did not differ between timepoints (Fig. 3A); however, erratic swimming significantly decreased ($\chi^2_{(2)} = 9.87$, $p = 0.007$) over time (Fig. 3B). All types of aggressive behaviours including biting ($\chi^2_{(2)} = 7.40$ $p = 0.025$), chasing ($\chi^2_{(2)} = 9.29$, $p = 0.010$), and threatening ($\chi^2_{(2)} = 11.47$, $p = 0.003$) increased over the experimental period in control groups (Fig. 3C). Biting behaviour was seven times higher at T2 (93.00 ± 28.56 events/h) compared to T0 (13.50 ± 8.84 events/h). No chasing behaviour was observed at T0 in control fish, but the values increased to 15.75 ± 0.80 events/h at T2. Threatening behaviour in the control groups significantly increased from 2.63 ± 1.19–42.38 ± 7.66 events/h throughout the study.

To understand how fish behaviour changed in response to different transport motion frequencies during a 24-h recovery period, we examined the effects of motion frequency, sampling timepoint, and their interaction on fish behaviour by comparing data among three treatments at two post-transport timepoints (T1 and T2). However, no significant interaction effects were found for all types of fish behaviour and only the main effects were retained. The main effect of both treatment and sampling timepoint on freezing behaviour was significant (Table 3). The percentage of fish displaying freezing behaviour increased between T1 (29.75 ± 2.11 %) and T2 (36.28 ± 2.65 %; Fig. 4A). Moreover, smooth transported fish (38.90 ± 3.27 %) exhibited a significantly higher percentage of freezing behaviour compared to control groups (25.53 ± 3.27 %; Fig. 4A). Both treatment and timepoint had a significant main effect on thigmotaxis behaviour (Table 3). Similar to freezing

Table 2
Mean values and two-way repeated measures ANOVA results for water quality parameters: dissolved oxygen (DO), temperature, pH, and total ammonia nitrogen (TAN) in three treatments before simulated transport (T0) and 0 h post-transport (T1).

DO (mg/L)	Control	Smooth	Rough	Treatment	Timepoint	Interaction
T0 T1	6.66 ± 0.24 5.90 ± 0.26	6.96 ± 0.16 5.66 ± 0.33	6.93 ± 0.15 6.03 ± 0.22	$F_{2,21} = 0.30$ $p = 0.748$	$F_{1,21} = 38.76$ $p < 0.001$	$F_{2,21} = 1.03$ $p = 0.373$
Temperature (°C)	Control	Smooth	Rough	Treatment	Timepoint	Interaction
T0 T1	27.11 ± 0.16 26.73 ± 0.03^{B}	27.86 ± 0.20 28.24 ± 0.39^{A}	27.44 ± 0.08^{b} 27.94 ± 0.16^{Aa}	$F_{2,21} = 12.27$ $p < 0.001$	$F_{1,21} = 1.45$ $p = 0.243$	$F_{2,21} = 4.21$ $p = 0.029$
Water pH	Control	Smooth	Rough	Treatment	Timepoint	Interaction
T0 T1	7.78 ± 0.05 7.53 ± 0.07	7.95 ± 0.10^{a} 7.51 ± 0.10^{b}	8.11 ± 0.11^{a} 7.38 ± 0.03^{b}	$F_{2,21} = 0.88$ $p = 0.431$	$F_{1,21} = 39.25$ $p < 0.001$	$F_{2,21} = 3.51$ $p = 0.049$
TAN (mg/L)	Control	Smooth	Rough	Treatment	Timepoint	Interaction
T0 T1	0.12 ± 0.08^{b} 2.67 ± 0.22^{Ba}	0.01 ± 0.00^{b} 3.26 ± 0.18^{Aab}	0.00 ± 0.00^{b} 3.54 ± 0.12^{Aa}	$F_{2,21} = 3.21$ $p = 0.061$	$F_{1,21} = 1184.01$ $p < 0.001$	$F_{2,21} = 10.53$ $p < 0.001$

Data are expressed as mean ± SE. Superscripts are applied when a significant interaction is found ($p < 0.05$). Different lowercase letters indicate a significant difference between two timepoints within a treatment. Different uppercase letters indicate a significant difference between treatments within a timepoint.

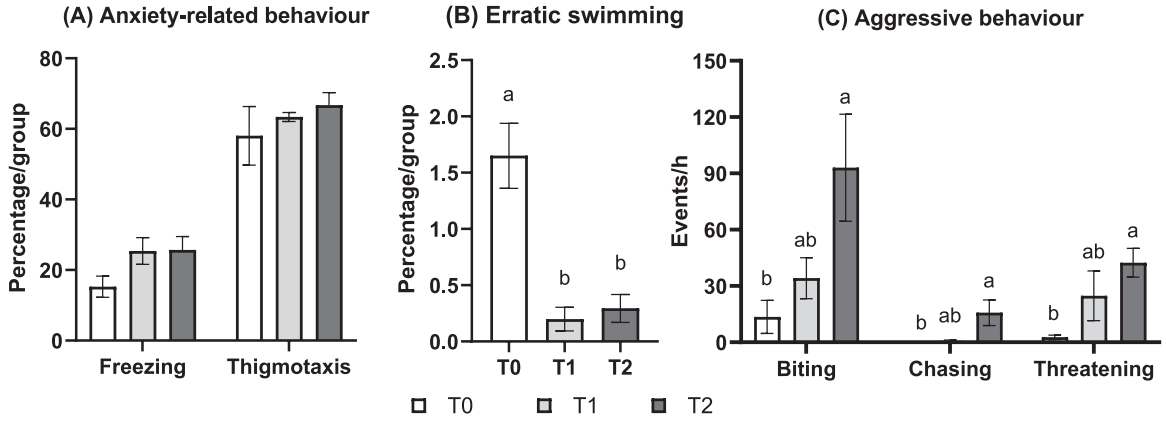

Fig. 3. Mean ± SE (error bars) for anxiety-related behaviour (A), erratic swimming (B), and aggressive behaviour (C) before simulated transport (T0), 0 h (T1) and 24 h post-transport (T2) in the control groups. Different lowercase letters indicate a significant difference ($p < 0.05$) between timepoints.

Table 3
The effects of motion frequency (treatment) and sampling time (timepoint) on the behaviour, physiology, and flesh quality of largemouth bass. The asterisk indicates a significant interaction effect.

Indicators	Treatment	Timepoint
Freezing	$F_{2, 21} = 4.35$, $p = 0.026$	$F_{1, 21} = 4.91$, $p = 0.038$
Thigmotaxis	$F_{2, 21} = 4.26$, $p = 0.028$	$F_{1, 21} = 4.75$, $p = 0.041$
Erratic swimming	$F_{2, 21} = 2.48$, $p = 0.108$	$F_{1, 21} = 0.01$, $p = 0.934$
Biting	$F_{2, 21} = 0.00$, $p = 0.999$	$F_{1, 21} = 11.91$, $p = 0.002$
Chasing	$F_{2, 21} = 1.70$, $p = 0.207$	$F_{1, 21} = 12.86$, $p = 0.002$
Threatening	$F_{2, 21} = 0.76$, $p = 0.481$	$F_{1, 21} = 13.43$, $p = 0.001$
Plasma cortisol	$F_{2, 21} = 0.73$, $p = 0.493$	$F_{2, 21} = 0.27$, $p = 0.765$
Plasma glucose	$F_{2, 21} = 0.21$, $p = 0.810$	$F_{2, 21} = 3.34$, $p = 0.045$
Plasma lactate	$F_{2, 21} = 0.16$, $p = 0.853$	$F_{2, 21} = 4.22$, $p = 0.021$
Plasma HSP70	$F_{2, 21} = 0.30$, $p = 0.741$	$F_{2, 21} = 0.08$, $p = 0.926$
Mucus cortisol*	$F_{2, 21} = 3.68$, $p = 0.043$	$F_{2, 21} = 14.47$, $p < 0.001$
Mucus glucose	$F_{2, 21} = 1.87$, $p = 0.179$	$F_{2, 21} = 24.55$, $p < 0.001$
Mucus lactate	$F_{2, 21} = 0.81$, $p = 0.458$	$F_{2, 21} = 41.80$, $p < 0.001$
Muscle pH	$F_{2, 21} = 1.21$, $p = 0.319$	$F_{2, 21} = 12.51$, $p < 0.001$
Drip loss	$F_{2, 21} = 1.44$, $p = 0.260$	$F_{2, 21} = 2.53$, $p = 0.092$

Abbreviations: HSP, heat shock protein.

behaviour, there was an increase in thigmotaxis behaviour between T1 (70.02 ± 2.05 %) and T2 (74.49 ± 2.01 %; Fig. 4B). The mean values of thigmotaxis were significantly higher in smooth transport treatment (75.99 ± 3.03 %) compared to control groups (65.03 ± 3.03 %; Fig. 4B). Neither treatment nor timepoint had a significant impact on erratic swimming among three treatments during the post-transport period.

Again, no significant interaction effect was found in aggressive behaviour between post-transport timepoints. The main effect of treatment was not significant for any aggressive behaviours, but a notable influence was observed on biting, chasing, and threatening behaviour with timepoint (Table 3). The biting rate significantly increased between T1 (40.38 ± 9.98 events/h) and T2 (85.50 ± 11.81 events/h; Fig. 5A). Similarly, a significant increase was found for chasing (from 0.75 ± 0.39–8.88 ± 2.43 events/h; Fig. 5B) and threatening behaviour (from 24.63 ± 6.78–64.00 ± 9.46 events/h; Fig. 5C) during the recovery period.

3.3. Fish physiology and flesh quality

We compared plasma physiological-biochemical parameters among treatments at three sampling times. Treatment did not show a statistical impact on plasma cortisol, glucose, lactate, and HSP70, while a significant main effect of sampling timepoint was observed for plasma glucose and lactate (Table 3). Plasma glucose showed an overall increase from T0 (9.23 ± 0.44 mmol/L) to T2 (10.59 ± 0.52 mmol/L), but no significant differences were found among three timepoints (Fig. 6A). Plasma lactate levels significantly decreased from 4.60 ± 0.19 mmol/L to 3.98 ± 0.16 mmol/L throughout the study (Fig. 6B). No differences were found in plasma cortisol (Fig. 6C) among the three treatments throughout the experimental period.

For mucus parameters, there was a significant interaction effect for mucus cortisol ($F_{4,21} = 2.64$, $p = 0.047$; Table 3). The levels of mucus cortisol were significantly higher in smooth transported groups compared with control groups between T0 and T1 (Fig. 7A). Mucus

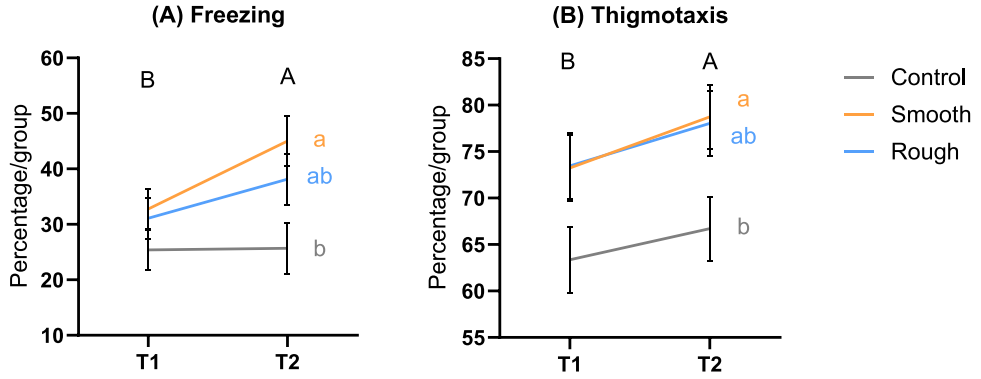

Fig. 4. Mean ± SE (error bars) for freezing (A) and thigmotaxis (B) between 0 h (T1) and 24 h post-transport (T2) in control, smooth and rough transport treatment. Different lowercase letters indicate a significant difference between treatments. Different uppercase letters indicate a significant difference between timepoints.

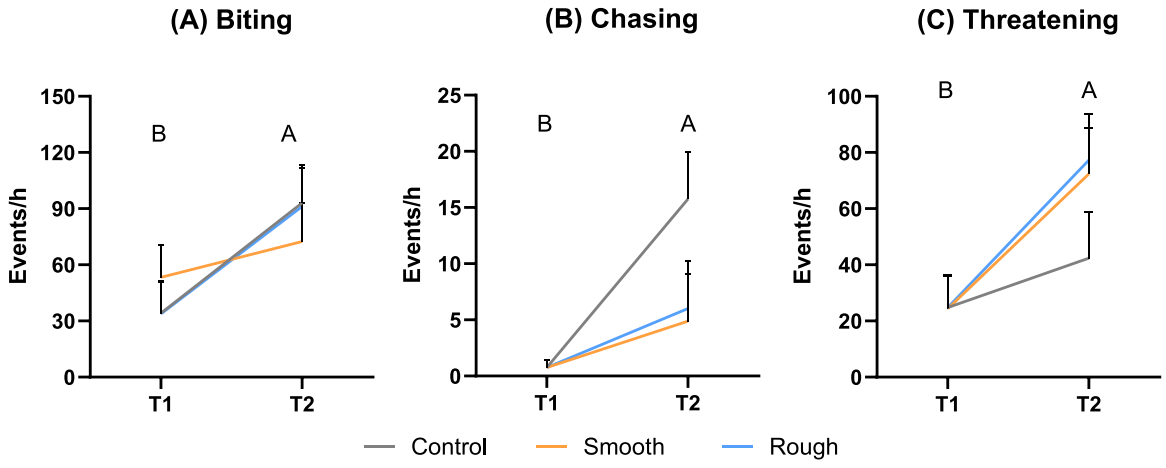

Fig. 5. Mean ± SE (error bars) for biting (A), chasing (B), and threatening (C) between 0 h (T1) and 24 h post-transport (T2) in control, smooth and rough transport treatments. Different uppercase letters indicate a significant difference between timepoints.

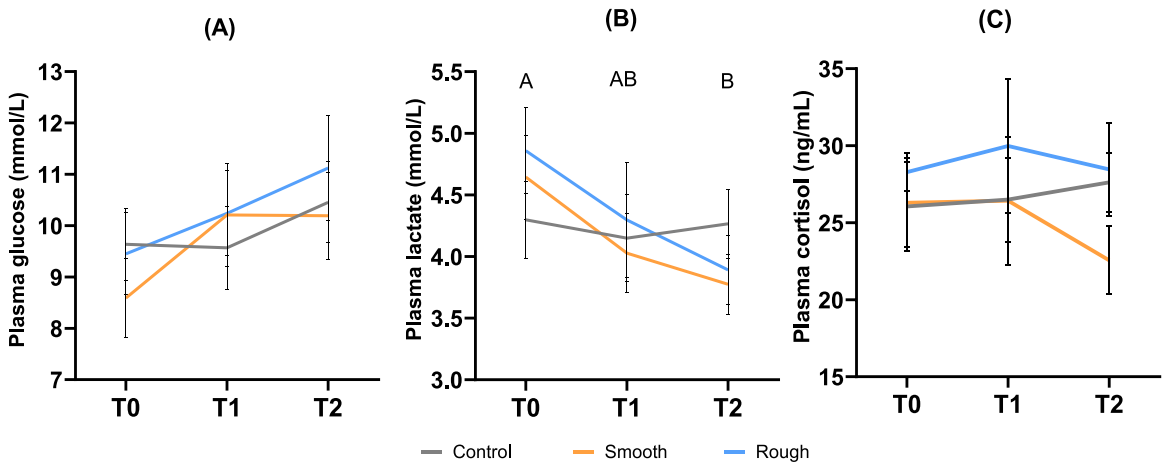

Fig. 6. Mean ± SE (error bars) for plasma glucose (A), plasma lactate (B), and plasma cortisol (C) of largemouth bass before simulated transport (T0), 0 h (T1) and 24 h (T2) post-transport in control, smooth and rough transport treatment. Different uppercase letters indicate a significant difference between timepoints.

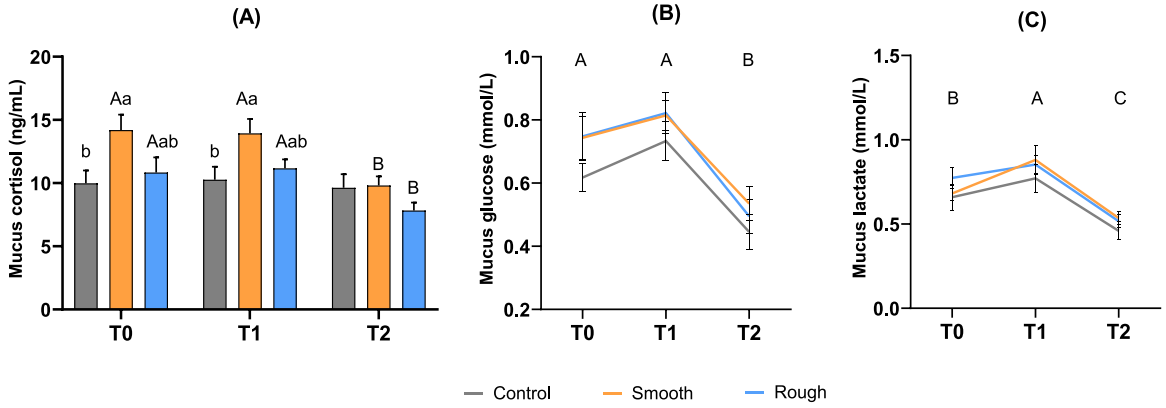

Fig. 7. Mean ± SE (error bars) for in mucus cortisol (A), mucus glucose (B), and mucus lactate (C) of largemouth bass before the simulated study (T0), 0 h (T1) and 24 h post-transport (T2) in control, smooth and rough transport treatment. Different uppercase letters indicate a significant difference between timepoints. Different lowercase letters indicate a significant difference between treatments.

cortisol did not change over time in the control groups, but the values significantly decreased in two transport treatments after 24 h of recovery (Fig. 7A). Additionally, the main effect of sampling time was significant for mucus glucose and lactate (Table 3). Mucus glucose was found to peak at T1 (0.79 ± 0.03 mmol/L) and then decreased to 0.49 ± 0.03 mmol/L at T2 (Fig. 7B). Similarly, mucus lactate increased between T0 (0.70 ± 0.04 mmol/L) and T1 (0.83 ± 0.04 mmol/L), and the values significantly decreased to 0.50 ± 0.03 mmol/L after 24 h of recovery (Fig. 7C).

For flesh quality, there was a significant main effect of sampling time on muscle pH (Table 3). Muscle pH showed an increase from T0 (6.94 ± 0.04) to T1(7.05 ± 0.03) and continued to rise to 7.12 ± 0.02 at T2 (Fig. 8A). No significant difference was observed for drip loss in all treatments throughout the experimental period (Fig. 8B).

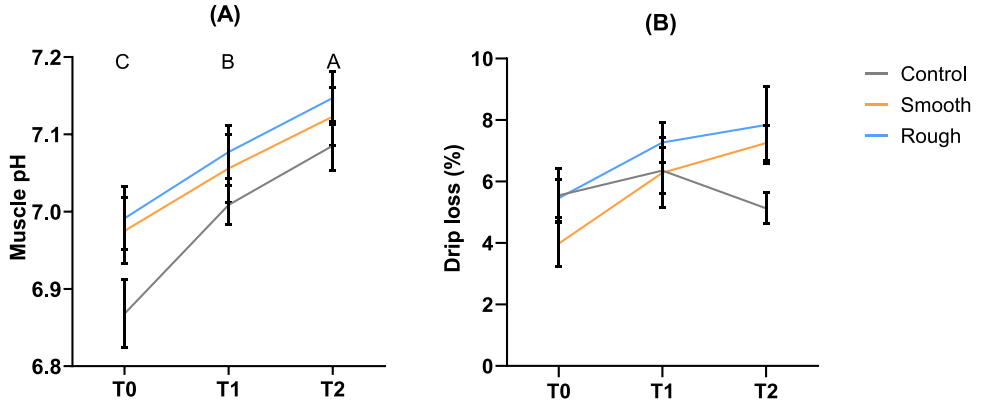

Fig. 8. Mean ± SE (error bars) in muscle pH (A) and drip loss (B) of largemouth bass before simulated transport (T0), 0 h (T1) and 24 h post-transport (T2) in control, smooth and rough transport treatment. Different uppercase letters indicate a significant difference between timepoints.

4. Discussion

In this study, we determined the effects of different motion frequencies on the welfare and recovery of largemouth bass, and water quality in a transport environment. To our knowledge, this is the first study that used a combination of physiological and behavioural welfare indicators, as well as flesh quality parameters, to assess the welfare of transported largemouth bass through both pre-transport and post-transport recovery stages. Overall, we found that water quality parameters changed throughout the study, with notable alterations in water temperature, pH, and total ammonia nitrogen in the transported groups. Non-transported fish showed an increase in aggressive behaviours over time. During the post-transport period, freezing and thigmotaxis behaviour was found to be affected by both treatment and timepoint, while aggressive behaviours significantly increased over time. Neither motion frequency nor sampling time had a significant impact on plasma cortisol, but a significant main effect of timepoint was found for plasma glucose and lactate. The interaction between treatment and timepoint had a significant impact on mucus cortisol, while only a significant timepoint effect was found for mucus glucose and lactate. Although there was a significant effect of timepoint on muscle pH, the flesh quality of bass did not appear to be reduced due to simulated transport in our study.

4.1. Water quality

Dissolved oxygen is one of the essential factors to maintain during live transport (Southgate, 2008; Harmon, 2009). In this study, DO levels decreased significantly between two timepoints. Ideally, DO should be kept at 100 % of saturation during fish transport (Harmon, 2009). However, the levels are expected to decline after transport based on the previous studies (Qiang et al., 2018; Wang et al., 2023). Although oxygen is generally provided from compressed tanks or aerators throughout the journey in real-life transport, it is suggested to include a secondary oxygen source to prevent any failure of the primary supply and to ensure fish welfare (Harmon, 2009; Sampaio and Freire, 2016).

Fish are poikilothermic animals and their metabolism is highly associated with environmental temperature (Harmon, 2009). In our study, water temperatures were significantly higher in two transport treatments at T1, indicating fish had higher metabolic rates and might be more stressed while being transported. This finding is in agreement with the previous research (Pakhira et al., 2015). A significant decrease was observed in water pH in two transport treatments at T1, likely because of oxygen consumption and carbon dioxide accumulation in water (Sampaio and Freire, 2016). After 3 h of simulated transport, the pH values in our study remained within the required range of 6.5–8.5 for farmed freshwater fish in China (MEE of the PRC, 1989), causing little impairment to bass.

High ammonia nitrogen levels especially unionised ammonia (NH_3) are toxic to fish by impairing their physiological functions (e.g. anti-oxidative enzyme activities), causing immunosuppression and hypoxia (Sun et al., 2014; Zhang et al., 2016; Zheng et al., 2021; Wang et al., 2023). In the present study, TAN levels significantly increased in all treatments from T0 to T1, and higher values were observed in both transport treatments, reaching 3.54 mg/L in rough transported groups. This indicated that transport can be a significant factor in elevating TAN levels due to increased metabolic rate and stimulated nitrogenous excretion in fish. Although no significant difference was found between the two transport treatments, there was a tendency for TAN to increase as the motion frequency increased. Similar increases were shown in a previous study using the same species (Wang, 2015). Currently, there are no standards or guidelines for the range of water ammonia during transport of freshwater fish in China. The existing Chinse national standard (GB 11607–1989) requires that the maximum level of un-ionised ammonia in farming water should be within 0.02 mg/L (MEE of the PRC, 1989). Exposure to 0.13 mg/L NH_3 for 24 h was found to induce anaerobic glycolysis and cell apoptosis in largemouth bass, but this stressor did not result in a prominent effect on fish health or growth during this period (Zhao et al., 2020). Another study indicates that largemouth bass has a high tolerance for 28 days of exposure to ambient ammonia of 8.31 mg/L (or 0.29 mg/L NH_3) (Egnew et al., 2019). Both studies suggest that largemouth bass are tolerant to a period of mild ammonia stress, but prolonged exposure to high ammonia can potentially have deleterious effects on bass welfare and health. Therefore, it is important to monitor ammonia during transport to safeguard fish welfare and health, particularly during trips with high stocking density and when water exchange is not possible.

4.2. Fish behavioural indicators

4.2.1. Control fish behaviours

Behavioural responses of fish are an important indicator of welfare status (Johansen et al., 2020). In the present study, freezing and thigmotaxis behaviour did not show a significant change in the control groups among three timepoints. However, we observed increased levels of biting, chasing, and threatening behaviour over time.

There are three main reactions to the physiological stress response in fish: primary, secondary, and tertiary responses (Schreck and Tort, 2016). Erratic swimming, such as darting and flashing, is thought of as a tertiary stress response due to the release of hormones at the whole--animal level (Nomura et al., 2009; Schreck and Tort, 2016). In the present study, control fish showed declined erratic swimming behaviour over time, which may indicate a reduction of handling stress after being transferred from the acclimated tanks to the arenas. However, they did display more biting, chasing, and threatening behaviour, which was likely due to the establishment of dominance hierarchies in the arena (Noble et al., 2012; Vanderzwalmen et al., 2021; Jones et al., 2023). Aggressive behaviours are part of the natural behaviours of most fish species and they do display some aggression, especially when social structure is being re-established (Magurran, 1986; Jones et al., 2023). Limited food or territory are possible reasons for increased competitive aggression among fish individuals, which may lead to the establishment of dominant rank-based hierarchies (Martins et al., 2012). Continued aggressive behaviours due to the established hierarchy may induce chronic stress and lead to physical injury, thereby further compromising the welfare of fish (Noble et al., 2012). Additionally, feed withdrawal in the video-recording tanks may lead to more aggressive behaviours in control fish over time, even though they were not exposed to transport stress. Short-term feed restriction can result in permanent changes in the aggressive behaviour of Atlantic salmon (*Salmo salar*), with a significantly higher occurrence of attacks in fasted fish (Canon Jones et al., 2017). When the food source is limited in an arena, the application of environmental enrichment may benefit in reducing aggression. Previous research suggests that environmental enrichment can improve the well-being of farmed fish in aquaculture, helping meet their welfare needs and reducing aggression in some species, such as Nile tilapia (Arechavala-Lopez et al., 2022). However, the effect of different environmental enrichments on fish aggressive behaviour has not been fully understood and has had contradictory results between species (Zhang et al., 2022). For example, Zhang et al. (2020) found that the medium- and high-level physical structure enrichment reduced aggressive behaviour in black rockfish (*Sebastes schlegelii*). Variatus platy (*Xiphophorus variatus*) also displayed less chasing behaviour in the enriched environment during live transport and recovery period compared to the non-enriched groups (Vanderzwalmen et al., 2020a). In contrast, the presence of rod structure enrichment had no effect on aggression in the zebrafish (*Danio rerio*) (Wilkes et al., 2012). No studies have been carried out yet to explore the effects of different types of environmental enrichment on the agonistic behaviours of largemouth bass, either during the farming period or live transport.

Overall, the behaviour results of control fish suggest a strong cumulative and negative combined effect of confinement and fasting over

time, evidenced by an increase in aggressive behaviours. Future studies should be considered to understand the effects of feed withdrawal and environmental enrichment on the behaviour of largemouth bass when kept in tanks (e.g. post-transport recovery aquarium) for more than 24 h.

4.2.2. Post-transport behaviours

To examine whether fish behaviour was influenced by motion frequencies, we compared post-transport behaviours between the control and two transport treatments. Freezing behaviour is a known response to acute stress by fish from activation of the inhibitory behavioural system (Galhardo and Oliveira, 2009). In the present study, smooth--transported fish displayed a higher level of freezing behaviour compared to the control groups. It is possible that largemouth bass were still experiencing transport-related stress and they were avoiding aggression from conspecifics (Vanderzwalmen et al., 2021). No difference in freezing behaviour was found between the two transport treatments, indicating a negligible impact from motion frequency. However, increased thigmotaxis behaviour was found in smooth-transported fish, possibly due to an anxiety state after transport and being transferred to a novel environment (Alfonso et al., 2020; Jones et al., 2023).

It is important to avoid excessive aggressive behaviours that have a negative effect on fish welfare (Jones et al., 2023). In our study, all types of aggressive behaviours significantly increased over time. This suggests that adult bass experienced stress during the 24 h of the recovery period, either due to simulated transport or environmental change, leading to an increase in aggression to displace their conspecifics. These behaviours are likely a result of hierarchy re-establishment in a novel tank (Noble et al., 2012; Vanderzwalmen et al., 2021; Jones et al., 2023). No significant main effect of motion frequency was found for aggressive behaviours during the recovery. Therefore, we did not find that 3-h rough transport affected the aggression of largemouth bass more than smooth or no transport. However, increased threatening behaviours during the recovery indicated that the novel environment can be considered a more acute stressor for adult bass, rather than motion frequency. All fish were kept within the same group for behavioural observation during the recovery and were not introduced to new conspecifics in each tank. In real-life transport, fish are unlikely to be kept in the same tanks to maintain their social hierarchies, as they will be all transferred into larger recovery tanks upon arrival at the destination (Yang et al., 2021). Aggressive behaviour and anxiety-like behaviours may increase in the post-transport recovery tanks due to adding new fish and consequently compromise fish welfare. Our results therefore provide implications to improve fish welfare during the recovery post-transport, particularly for the species that required multiple transport events. The minimum period during which largemouth bass adapt to the novel environment and social group after live transport would be valuable to measure in future studies, especially with the addition of new conspecifics. Notably, these topics would be challenging to achieve in the real-life transport of fish for selling purposes because the events are generally consecutive and cumulative. It is recommended to conduct studies on such topic under laboratory conditions, which typically involves minimising transport-related stress and suffering to fish. The outcomes may subsequently be applicable to the commercial aquaculture context.

4.3. Fish physiological indicators

Transport operations can induce physiological stress in fish with the activation of the hypothalamus-pituitary-interrenal (HPI) axis to promote a cascade of hormone release (Schreck and Tort, 2016).

Changes in cortisol levels are one of the most commonly used physiological indicators reflecting acute stress status in fish (Martínez-Porchas et al., 2009; Ellis et al., 2012; Sadoul and Geffroy, 2019). In the present study, 3 h of simulated transport did not result in any change in the plasma cortisol of bass, which is contradictory to the results of previous transport studies using polyethene bags on rohu (*Labeo rohita*) (Pakhira et al., 2015), channel catfish (*Ictalurus punctatus*) (Refaey and Li, 2018), and golden pompano (*Trachinotus ovatus*) (Hong et al., 2019). A possible reason could be the application of different transport systems. In our study, oxygen was continuously aerated throughout the experiment, following an open system approach (Berka, 1986). Conversely, in closed systems using sealed bags, no oxygen is provided during transport (Berka, 1986), leading to significant decreases in oxygen levels and increases in ammonia, as observed in studies by Pakhira et al. (2015) and Refaey and Li (2018). These conditions potentially induce more stress on fish. However, another simulated transport study indicates that largemouth bass exposed to a motion frequency of 1.0 and 1.7 Hz showed a non-significant elevation in serum cortisol compared to the non--transported groups, while a significant increase was observed for frequencies exceeding 2.1 Hz (Wang, 2015). This suggests that a motion frequency below 2.0 Hz may not strongly elevate blood cortisol levels among largemouth bass. Moreover, in this study, we only introduced regular and predictable motions to fish up to 1.8 Hz, whereas in real-life transport by vehicles, irregular and unpredictable movements, along with higher vibration frequencies, are common. This limitation may explain the non-significant outcomes for blood cortisol.

Blood glucose and lactate are commonly measured indicators of secondary stress response in fish (Barton, 2002; Martínez-Porchas et al., 2009). The levels of blood glucose are generally higher after transport because of cortisol-induced glucogenesis and glycogenolysis, as well as increased energy demand (Martínez-Porchas et al., 2009; Pakhira et al., 2015; Refaey and Li, 2018). In our study, plasma glucose did not increase in any treatments, despite observing an increase over time. Given the stable levels of plasma cortisol in both control and transported fish, we expected non-significant results for glucose. Similarly, no significant change in blood glucose was found in cururu stingray (*Potamotrygon* cf. *histrix*) and African catfish (*Clarias gariepinus*) after 3 h of transport and 24 h post-transport (Brinn et al., 2012; Manuel et al., 2014). The endocrine system for glucose control in fish has not been fully understood (Sampaio and Freire, 2016). Prolonged pre-transport fasting in adult bass likely contributed to maintaining relatively stable plasma glucose levels.

Plasma lactate levels are highly indicative of fish metabolism and are particularly influenced by short-term transport stress, often resulting in a decrease (Sampaio and Freire, 2016). Consistent with this observation, our study also demonstrates a significant decline in lactate levels between T0 and T2. Similar decreases in blood lactate over time have been reported in other studies involving the transport of common carp (*Cyprinus carpio* L.) for 7 h (Dobšíková et al., 2006) and European eel (*Anguilla anguilla*) for 3 h (Boerrigter et al., 2015). The initial handling and loading of fish at T0 may lead to higher lactate levels, as fish unavoidably experienced air exposure and crowding, resulting in relatively anaerobic conditions. However, once fish acclimated to the transport environment or became calm in recovery tanks with sufficient oxygen, plasma lactate levels decreased to lower levels. This phenomenon provides a possible explanation for the observed decline in plasma lactate in our study, which coincides with increased glucose levels over time.

In addition to fasting and transport-related stress, water temperatures were relatively high throughout the study period. The optimal temperature range for farming adult bass (size between 10 and 40 cm) is 26–29°C during the daytime (Coutant, 1977). Our study was conducted during the summer with water temperatures of approximately 28°C, nearly reaching the suggested limit. Therefore, the subsequent transport stress at different motion frequencies might not be a significant factor in affecting plasma stress indicators when compared with high water temperatures. This limitation was noted in the study. Yang et al. (2021) found that live transport of farmed fish occurs less frequently in China during the summer due to higher transport losses and fish mortality at high air and water temperatures. The findings of physiological parameters consequently suggest that unnecessary transport should be minimised during periods of high temperature that exceed the optimal range

for largemouth bass.

Skin mucus has been demonstrated to detect physiological stress in fish from a non-invasive perspective (Fernández-Alacid et al., 2018; Ouyang et al., 2020). In the present study, skin mucus cortisol did not change between simulated motion frequencies, while the values decreased after 24 h of recovery in two transport treatments. In contrast, Bertotto et al. (2010) found that mucus cortisol levels significantly increased in rainbow trout and European sea bass (*Dicentrarchus labrax*) after 1.5 h of transport, and in common carp after 3 h of transport (Bertotto et al., 2010). A positive correlation between blood and mucus cortisol has been identified in largemouth bass (Bertotto et al., 2010), gilthead seabream (*Sparus aurata* L.) (Guardiola et al., 2016), meagre (*Argyrosomus regius*) (Fernández-Alacid et al., 2018), and rainbow trout (Carbajal et al., 2019). In our study, since plasma cortisol did not show a significant increase over time in two transport treatments, similar changes were expected to be observed for mucus cortisol.

To our knowledge, no data exist investigating the effect of road quality on mucus cortisol and metabolites of largemouth bass during recovery post-transport. The time lag may account for the varying changes observed in mucus and plasma cortisol levels when fish are exposed to stressors. Previous studies suggest that the minimum time for cortisol to be released into water and faeces is 0.5–1 h in sea bream, respectively (Guardiola et al., 2016; Herrera et al., 2016). The delivery time of cortisol from blood to the skin mucus is still unclear in most farmed species, including largemouth bass. Therefore, understanding the time lag of cortisol between blood and mucus could be crucial to determine whether mucus cortisol can be used as a reliable stress biomarker in largemouth bass to assess transport stress.

4.4. Flesh quality

For product quality and safety concerns, it is necessary to maintain good welfare and reduce stress in fish while they are being kept alive for human consumption (Hardy-Smith, 2015).

Muscle pH significantly increased over time in our study. A similar increase in muscle pH was found in a previous study involving the transport of rainbow trout for 3 h 20 min, with higher muscle pH observed after 48 h of transport (Shabani et al., 2016). In our study, the depletion of plasma lactate in transported bass may explain the increase in muscle pH over time. Simulated transport could present an extended effect on lactate, particularly under food withdrawal. Lactate may be converted into pyruvate and used in the citric acid (Krebs) cycle (Boerrigter et al., 2015). In contrast, muscle pH is reported to significantly decrease in channel catfish (*Ictalurus punctatus*) exposed to transport stress for 3.5 h (Refaey et al., 2017). High levels of blood stress hormones such as cortisol can accelerate glycogenesis in muscle due to increased glucose levels (Daskalova, 2019). However, plasma cortisol did not increase over time in our study, leading to contradicting results compared to the literature mentioned earlier.

Water-holding capacity is one of the crucial attributes of fresh meat, impacting both the yield and quality of the product (Daskalova, 2019). Loss of moisture in meat leads to economic losses due to lower saleable weight, reduced product quality, and nutritional components like proteins and vitamins (Abraha et al., 2018). Neither treatment nor time-point was found to have a significant effect on the drip loss of bass fillets in the present study. Live transport was reported to significantly increase drip loss in channel catfish fillets, but the values dropped to the baseline after 168 h of recovery (Refaey et al., 2017). Although a 3-h simulated transport did not result in a statistical difference in drip loss of bass fillets in our study, an increasing trend was still observed in transported fish after 24 h of recovery. Future studies could consider exploring whether water loss can be reduced in transported bass with a longer period of recovery before being slaughtered.

5. Conclusions

This study was the first to determine the effect of motion frequencies on the behaviour, physiology, and flesh quality of largemouth bass using a motion platform. Three hours of simulated transport was found to reduce water quality, with higher total ammonia nitrogen and lower pH observed in transported groups. Although no statistical difference was detected between the two motion frequencies, there was a trend of ammonia increase accompanied by higher motion frequencies, posing a potential risk of impairing fish welfare during transport on poor road conditions. An increase in aggressive behaviours among non-transported fish indicates a strong cumulative negative effect of fasting and confinement over time. Altered anxiety-like and aggressive behaviours during the post-transport period demonstrate that adult bass were still under stress due to the combined transport and environment change. This is possibly because of the establishment of social hierarchies in a barren recovery tank. Therefore, the effects of environmental enrichment and the addition of new fish on post-transport aggressive behaviour of bass could be explored in future. Our study did not find rough transport to impact fish physiological indicators more than smooth transport. The results for muscle pH and drip loss suggest that 3 h of simulated transport within 1.8 Hz was not a primary stressor to reducing flesh quality in adult bass.

The results also suggest that a 24-h period was insufficient for largemouth bass to recover from transport-related stress. Future research on unpredictable motion and longer recovery times is encouraged to ensure the welfare of farmed fish during transport, particularly for species that require multiple consecutive journeys.

Funding sources

This study was funded as part of the Animal Welfare Standards Project by Good Ventures Foundation and Open Philanthropy.

CRediT authorship contribution statement

Yifei Yang: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Edward Narayan:** Writing – review & editing, Supervision, Methodology. **Clive J.C. Phillips:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Sonia Rey Planellas:** Writing – review & editing, Methodology. **Lu Zheng:** Resources, Investigation. **Xiaofang Ruan:** Investigation. **Arnaud Fabrice Tegomo:** Investigation. **Hao-Yu Shih:** Formal analysis. **Qingjun Shao:** Supervision, Resources, Methodology, Funding acquisition. **Kris Descovich:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

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.

Acknowledgement

The authors sincerely thank Hangzhou Jianfeng Agricultural Development Co., Ltd for providing on-site laboratory and experimental fish. The authors are also grateful for the assistance provided by Yanfeng Zhang, Shengyu Hang, Yisong Lv, and other anonymous farmers during the experimental period on the farm.

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