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Long-term repeatability of behaviours in zebrafish (*Danio rerio*)

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Abstract

Animal personality is defined as individual variation in behaviour that is consistent over time and/or across contexts. Animal personality is now considered a fundamental aspect in the fields of animal behaviour and behavioural ecology, yet the majority of studies assess repeatability of behaviours over only relatively short time periods (e.g. a week) using just two measures. Understanding whether behavioural traits are repeatable over longer periods is important for the assessment of individual differences in behaviour. Using zebrafish (*Danio rerio*), we investigated the repeatability of activity and exploratory behaviours, including distance travelled, time spent in the bottom of the arena, stationary time and overall exploration of the novel arena over a 28-week period, using five intervals. All measures were repeatable over 28 weeks, but the repeatability estimates were much lower when comparing the initial week one and week two behaviours. There were clear sex differences in aspects of activity, with males more active than females. Importantly, our behavioural assays suggest that zebrafish require an initial "tank experience," prior to the main phenotyping session, to ensure that behaviours being measured are repeatable—these effects are often not considered, but have implications for the many studies that measure behaviour at a single time point only.

KEYWORDS

activity, animal personality, anxiety, exploration, novel arena

1 | INTRODUCTION

The behaviour of animals differs not just among species and populations, but also among individuals. What was originally thought to be non-adaptive variation around an adaptive population mean is now considered an important aspect of fitness (Cote, Fogarty, Weinersmith, Brodin, & Sih, 2010; Dall, Houston, & McNamara, 2004; Dingemanse, Both, Drent, van Oers, & van Noordwijk, 2002; Smith & Blumstein, 2008). In short, animal personality is defined as consistency in behaviour over time and/or across contexts (Cloninger, 1986; Dall et al., 2004). For example, some individuals tend to be bolder and more active across different situations than others (Koolhaas et al., 1999; Sih, Bell, Johnson, & Ziemba, 2004). Such behavioural strategies involve both costs and benefits for the individuals that employ them, and affect the way individuals respond to selection pressures (Dall et al., 2004; Dingemanse, Both, Drent, &

Tinbergen, 2004; Pruitt et al., 2017). Optimal behavioural plasticity might be more adaptive, but individuals tend to be constrained in their behavioural responses to stimuli and threats. That is, intra-individual behaviour tends to be consistent over time. Moreover, because variation in behaviour is maintained in populations and transmitted to subsequent generations, animal personality is a major component of fitness (Cote et al., 2010; Dingemanse et al., 2002).

To estimate repeatability, behaviours are measured more than once in the same individuals and analysed to determine what proportion of variation is owing to differences between individuals, and what portion is due to variation within individuals (Beckmann & Biro, 2013; Fangmeier et al., 2018). There are several different methods of calculating repeatability (Hayes & Jenkins, 1997; Lessells & Boag, 1987; Nakagawa & Schielzeth, 2010; Stoffel, Nakagawa, & Schielzeth, 2017), but the unifying criterion is that, for a behaviour to be repeatable, we expect relatively low within-individual variance compared to

high among-individual variance (Bell, Hankison, & Laskowski, 2009). Repeatability of behaviour has been demonstrated in a wide range of taxa and for different behaviours (Bell et al., 2009; Brommer & Class, 2017; Dingemanse et al., 2002; Dziewczynski & Crovo, 2011; Hedrick & Kortet, 2012), but estimates of longer-term repeatability are generally lacking (but see Carlson & Tetzlaff, 2020; David, Auclair, & Cézilly, 2012; Petelle, McCoy, Alejandro, Martin, & Blumstein, 2013; Wuerz & Krüger, 2015) and the time between interval testing has been shown to affect repeatability estimates (Bell et al., 2009). Most studies which do estimate repeatability of behaviours only do so over short time periods of days or weeks (Bell et al., 2009), often only by using two measures (Bell et al., 2009; Biro & Stamps, 2015), and only occasionally over months (Niemelä & Dingemanse, 2018). Both the interval and the number of repeated measures have been shown to influence repeatability estimates (Beckmann & Biro, 2013; Bell et al., 2009; Holtmann, Lagisz, & Nakagawa, 2017). For example, only 9% of repeatability estimates from a 2009 meta-analysis were classified as long intervals (>1 year) and all but three of these were estimated using just two measures of behaviour (Bell et al., 2009). Using the fish (*Pisces*) papers only, just three used intervals longer than 30 days (17.6%). This issue has not improved over time, as a search for repeatability in fish from 2018 to 2019 (TOPIC: behavio*r AND repeatab* AND fish; Web of Science and Scopus) reveals that just 21% of the 33 relevant papers use intervals longer than 30 days, though 22 of these studies do use more than two measures, and only one study has an interval longer than 100 days. The situation is somewhat improved in birds, with ~ 58% of repeatability estimates measured using intervals greater than 30 days (~42% >100 days), with ~ 58% of these using more than two measures (Holtmann et al., 2017). Behaviour has been shown to exhibit significantly lower repeatability estimates when the interval between repeated measures increases and with increased repeated measures (Bell et al., 2009; Holtmann et al., 2017). The precision of estimates is also expected to increase with increased repeated measures (Wolak, Fairbairn, & Paulsen, 2012). Thus, more longer-term studies with multiple repeated measures are needed to fully understand the repeatability of behaviour.

Selection often acts differently on certain behaviours of males than it does on females. Sex differences in behaviour are commonly observed in animal populations (Lehmann & Boesch, 2008; Schuett & Dall, 2009). Males are often bolder and more aggressive than females; this has been shown in fish and birds, but less extensively in other taxa (Carazo, Noble, Chandrasoma, & Whiting, 2014; Dahlbom, Lagman, Lundstedt-Enkel, Sundström, & Winberg, 2011; Harris, Ramnarine, Smith, & Pettersson, 2010; Schuett & Dall, 2009). Furthermore, males and females have been shown to differ in repeatability for behavioural traits—behaviours have been found to be more repeatable in females than males for a wide range of taxa (Bell et al., 2009).

Here, we test whether behaviours related to activity, anxiety and exploration (as described by Réale, Reader, Sol, McDougall, & Dingemanse, 2007) are repeatable in zebrafish over a 28-week period using five repeated measures. Zebrafish live for ~3.5 years in captivity (Gerhard et al., 2002), so 28 weeks represents ~ 14% of their laboratory life span, though they may only live for

1–2 years in the wild (Spence et al., 2006). Individual variation in behaviour is under strong selective pressure (Ariyomo, Carter, & Watt, 2013; Tran & Gerlai, 2013) and linked to reproductive success (Ariyomo & Watt, 2012). While several zebrafish behaviours have been shown to be repeatable, these studies assessed repeatability over just one- or five-week periods (Baker, Goodman, Santo, & Wong, 2018; Toms & Echevarria, 2014; Tran & Gerlai, 2013). Both age and sex have been shown to affect zebrafish behaviour—under 10 months of age, males show higher activity levels, while after age 22 months, females are more active (Philpott, Donack, Cousin, & Pierret, 2012). Our fish were aged between 6 and 13 months; thus, we predict that males will tend to be more active than females. However, female zebrafish have also been shown to be more consistent in their activity levels than males, at least over a 1-week interval (Tran & Gerlai, 2013); hence, we predict that females will be more repeatable in their behaviours. Our behavioural measures (see methods below) represent typical and routine behavioural responses of zebrafish (and of other fishes) used in a variety of pharmacological and eco-toxicological studies, which often measure behaviour only once (Egan et al., 2009; Lamb, Chia, & Johnson, 2020; Maximino et al., 2013; Volkova et al., 2015); hence, a better understanding of how consistent behaviours are over time is warranted.

2 | MATERIALS AND METHODS

2.1 | Subjects and husbandry

Zebrafish (*Danio rerio*) were bred in the Otago Zebrafish Facility on 22 March 2016 and reared in a temperature-controlled facility (25–27°C) with a 08:00 to 22:00 light cycle. The conductivity, temperature and pH were maintained between 390–458 μS, 25.2–26.1°C and 7–7.8 pH, respectively. At ~4 months of age, 48 fish (24 males, 24 females) were randomly selected from the cohort and each fish was tagged dorsally with visible implant elastomer (VIE) tags (using combinations of red, yellow, green, orange and pink) to allow identification of individuals. At ~5 months of age, the 48 fish were moved to the Department of Zoology for phenotyping and held at 25°C with a 07:00 to 20:30 light cycle. Fish were kept on a closed 27-tank recirculating system in 4 tanks containing groups of 12, six males and six females per tank, with the sexes separated by a divider (Tecniplast tanks; 284 mm × 169 mm × 114 mm). Fish were fed 2–3 times a day, dry feed (Zebrafish Management Ltd.) in the mornings and afternoons and a live feed of brine shrimp (*Artemia* sp.) at midday. Fish were allowed ~3.5 weeks of acclimation to their new housing conditions before behavioural assays started.

2.2 | Behavioural assays

Each fish was tested individually in a test tank (300 mm H × 270 mm W × 154 mm D), with three tanks filmed simultaneously. The

tanks had two sheets of translucent white acrylic on the back outside of the tank and were lined on the inside with white film on two sides and the bottom to eliminate glare and reflection. Tanks were lit from behind and above. The tanks were filmed front-on using a Basler acA1300-60/gc GigE camera with a 4.4–11 mm lens that was positioned 112 cm away from the test tanks.

Fish were netted and added to the test tanks randomly. We assessed each individual's activity and anxiety levels and exploratory behaviour in the novel arena for 10 min, with no acclimation period. Locomotor activity was measured as the total distance travelled and mean velocity during the test. An active fish was defined as one that travelled far and fast. To measure exploration, we estimated stationary time, as well as an exploration index, where a 3 × 3 grid of equal-sized rectangles (70 mm H × 90 mm W) was superimposed onto the tank during video analysis (within the behavioural software EthoVision XT; Noldus), and the cumulative duration spent in each square was recorded. An exploration index was calculated by using the standard deviation of the amount of time each fish spent in each of the nine zones. A low standard deviation meant that a fish was exploratory and spent time in all of the zones equally (Thomson, 2017). The amount of time spent in the bottom part of the tank (within ~8 cm of the bottom) is a measure of anxiety, where a fish that spends more time in the bottom part of a tank is considered more anxious, as zebrafish tend to dive to the bottom in an unfamiliar environment and then begin to explore the other areas of the tank after a period of time (Collymore, Tolwani, & Rasmussen, 2015; Levin, Bencan, & Cerutti, 2007; Parker, Millington, Combe, & Brennan, 2012).

To control for variation in circadian rhythms, testing was carried out between 9:00 and 15:00, and always within the same 1-hr window for each individual fish (we tested for time of a day effect in our models). On the day of testing, fish were not fed until after behaviours were assayed. Care was taken to net fish as quickly as possible to minimise disturbance. Behavioural tests were repeated at 2, 6, 15 and 28 weeks after the initial test, corresponding to ~29, 30, 35, 42 and 55 weeks of age.

2.3 | Sample sizes

Four females and three males died during the 28-week period; thus, they were removed for all analyses. The final sample size for distance travelled, bottom time and stationary time was 41 (20 females and 21 males). The exploration index was obtained by reanalysing all the videos after testing, but a few videos were corrupt, so the final exploration sample size across the five trials was 36 (15 females and 21 males).

2.4 | Statistical analysis

All data were analysed using R version 3.6.1 (R Core Development Team 2019).

Five behavioural response variables were modelled: total distance travelled, mean swimming speed, total bottom time, total stationary time (log-transformed to meet normality assumptions) and the exploration index. Sex differences in behaviour and changes in behaviour over time were analysed with linear mixed effects models in R using the package "lme4" (Bates, Maechler, Bolker, & Walker, 2015), with sex, week (mean-centred) and the interaction between sex and week treated as fixed effects and individual identity included as a random effect. Time of day was included as a covariate in the models, but was non-significant for all behaviours except stationary time (and only marginally significant, see below), so was removed from the other models. Confidence intervals were calculated using the *confint* function, and model effects with confidence intervals that do not include 0 are considered significant. We note that we attempted to digitally extract fish size from our videos (to include as a covariate in our analyses), but repeated size measurements of the same fish were too variable to have any confidence in the measures. We do note that individual differences in activity levels were independent of the size of zebrafish in previous studies though (Baker et al., 2018; Tran & Gerlai, 2013).

Repeatability estimates and variance components were obtained using the package "rptR" (Stoffel et al., 2017), all with a Gaussian distribution, using 10,000 bootstrapping runs and 10,000 permutations. Sex was included as fixed effect and week and fish ID as random effects in the repeatability models. The statistical significance of repeatability estimates was inferred from whether or not confidence intervals included zero. Repeatability estimates and variance components of males and females were calculated using two separate models, using data across the entire 28-week period, with fish ID and week included as random effects. We recognise that our sample sizes (ranging from 15 to 21) may be too small to estimate sex-specific repeatability with any precision (Wolak et al., 2012), but we feel that the sex-specific repeatabilities are still worth reporting given the large differences in within variance estimates observed for males and females.

We note that we also tested whether individuals differed in patterns of behaviour over time, by specifying week as a random slope effect (Biro, O'Conner, Pedini, & Gribben, 2013; Biro & Stamps, 2015), but this was non-significant for all behaviours (likelihood ratio tests, *p*-values ranging from 0.38 to 0.67), suggesting that trend lines for individuals were parallel and did not diverge over time; hence, we used the repeatability models described above.

3 | RESULTS

The total distance travelled ranged from 1,017.2 to 5,604.9 cm across all five time points, while mean swimming speed ranged from 1.7 cm/s to 9.3 cm/s. Time spent at the bottom ranged from 6.8 to 600 s and stationary time ranged from 17.2 to 372.7 s. Across all time points, the exploration index ranged from 18 s (high exploration) to 140 s (low exploration). Swimming speed and distance were strongly correlated (adjusted r = 0.99, *df* = 203, *p* < .0001), so speed
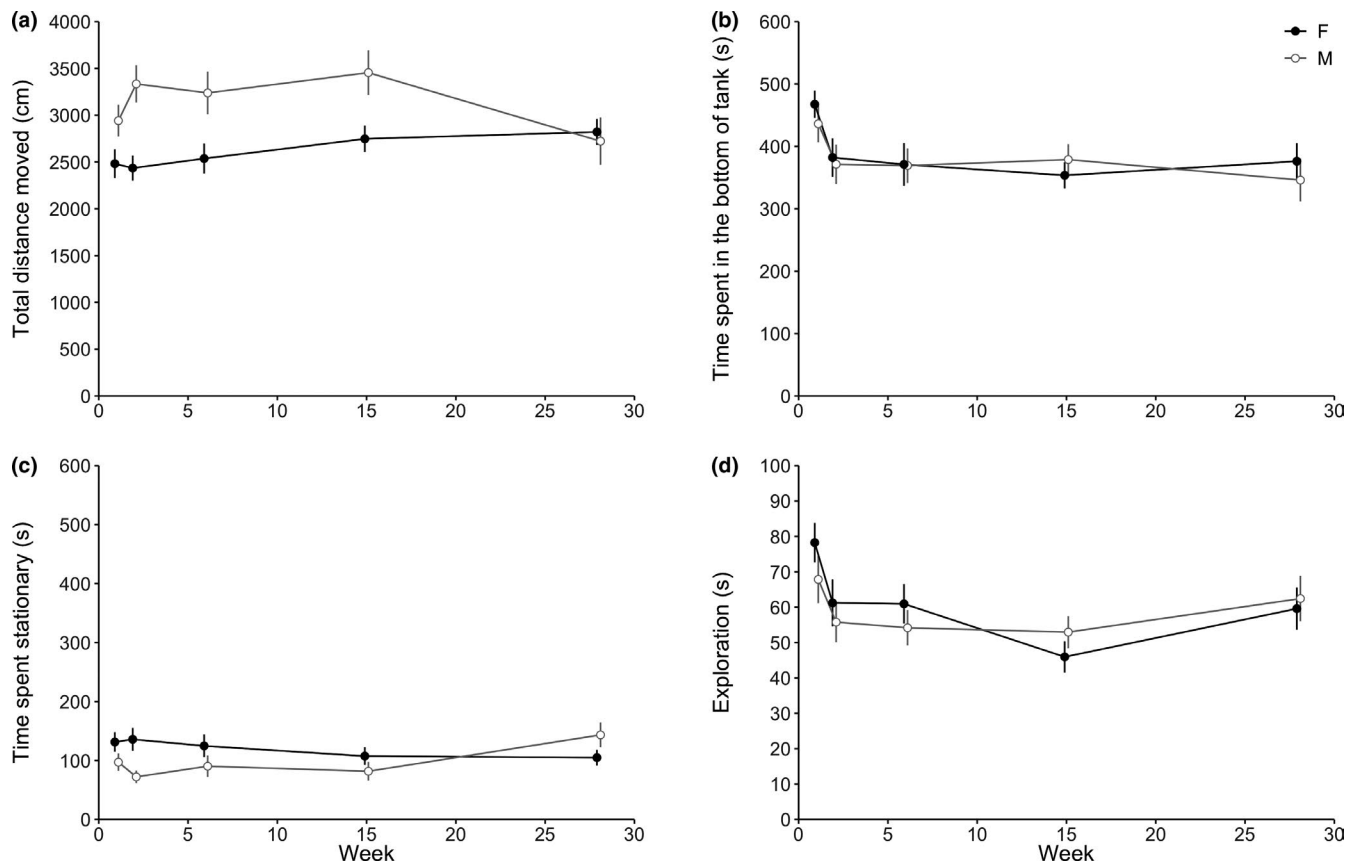
FIGURE 1 Mean (± standard error) total distance moved (a), time spent in the bottom of the tank (b), time spent stationary (c) and exploration (d) across 28 weeks of testing (assayed at 1-, 2-, 6-, 15- and 28-week time points) for male (open circle) and female (filled circle) AB wild-type zebrafish. Weeks correspond to 29, 30, 35, 42 and 55 weeks of age post-fertilisation

data are not presented. Distance travelled was negatively correlated with bottom time ($r = -0.39$, $df = 203$, $p < .0001$), stationary time ($r = -0.89$, $df = 203$, $p < .0001$) and exploration ($r = -0.61$, $df = 178$; $p < .0001$). In contrast, bottom time was positively correlated with stationary time ($r = 0.46$, $df = 203$, $p < .0001$) and exploration ($r = 0.66$, $df = 178$, $p < .0001$), and stationary time was positively correlated with exploration ($r = 0.65$, $df = 178$, $p < .0001$). Despite these correlations, we present data for individual behavioural measures, rather than take a principal component approach, as behaviours measured in studies tend to be highly variable across research groups.

3.1 | Distance travelled

Males travelled significantly larger distances than females, on average 534 cm farther (Est = 533.73 [95% confidence interval = 135.22, 932.25]; Figure 1a). While distance travelled significantly increased over time (Est = 145.96 [21.47, 270.46]), the males decreased their activity over time (Est = −267.18 [−441.13, −93.23]), which was driven by a decline in distanced travelled by males at the 28-week point (Figure 1a). Despite these effects over time, the distance travelled during a trial was significantly repeatable over the 28 weeks

($R = 0.46$; Table 1). Interestingly, distance travelled was highly repeatable in males ($R = 0.57$), but less repeatable in females ($R = 0.24$; Table 1). This difference in repeatability is due to females displaying greater within-individual variance than between-individual variance, compared to males, and due to females displaying less variability compared to males (Table 1; Figures S1a and S2). Importantly, we found that mean behavioural responses across the study were unequal, in such that the behaviours exhibited by the zebrafish (of both males and females) tended to be more consistent from the second testing onwards in comparison with the initial time point. Because of this, we additionally assessed the repeatability of behavioural traits with and without week 1 included. Repeatability was much lower for the week 1 and week 2 comparison ($R = 0.33$; Table 2), though excluding week 1 from the overall analysis did not alter the overall repeatability estimate (Table 2).

3.2 | Bottom time

Time spent on the bottom of the arena did not differ between females and males (Est = −9.80 [−66.73, 47.14]), and male and female bottom-dwelling behaviour did not change over time [−0.42 [−29.37, 28.53]]. While time was marginally non-significant in the full model

TABLE 1 Variance components, repeatability values and associated 95% confidence intervals of each behaviour for all fish and for each sex, estimated using the *rptR* package. k = 5 repeat measures over 28 weeks; n = 20 females and 21 males for distance, bottom time and stationary time; and n = 15 females and 21 males for exploration. Note that stationary time is log-transformed

	Distance (cm)	Bottom time (s)	Stationary time (s)	Exploration (s)
All				
Var$_{Among}$	343,471 [175067, 555192]	6,603 [3192, 11079]	0.03 [0.02, 0.05]	234 [107, 405]
Var$_{Within}$	406,096 [320611, 496885]	10,324 [8161, 12721]	0.05 [0.04, 0.06]	372 [288, 462]
Repeatability	0.47 [0.32, 0.62]	0.36 [0.21, 0.51]	0.37 [0.21, 0.51]	0.35 [0.18, 0.51]
Males				
Var$_{Among}$	582,561 [239,358, 1,059,563]	10,262 [3,975, 18,673]	0.05 [0.02, 0.09]	338 [126, 627]
Var$_{Within}$	428,668 [305,288, 570,783]	8,267 [5,906, 10,992]	0.05 [0.03, 0.06]	349 [249, 463]
Repeatability	0.58 [0.35, 0.74]	0.53 [0.30, 0.70]	0.45 [0.22, 0.64]	0.48 [0.24, 0.65]
Females				
Var$_{Among}$	103,549 [8,336, 235,601]	2,702 [0, 6,595]	0.02 [0.002, 0.04]	87 [0, 235]
Var$_{Within}$	323,196 [226,666, 429,117]	12,738 [8,984, 16,902]	0.05 [0.04, 0.07]	397 [26, 551]
Repeatability	0.24 [0.04, 0.46]	0.16 [0, 0.35]	0.24 [0.03, 0.45]	0.15 [0, 0.36]

TABLE 2 Repeatability values and 95% confidence intervals of each behaviour across weeks 1–2 and weeks 2–28. Repeatability was calculated using the *rptR* package, accounting for sex in the models. k = the number of repeat measures; n = 41 fish for distance, bottom time and stationary time; and n = 36 for exploration

	k	Distance (cm)	Bottom time (s)	Stationary time (s)	Exploration (s)
Weeks 1–2	2	0.33 (0.03–0.58)	0.25 (0–0.52)	0.21 (0–0.49)	0.35 (0.04–0.63)
Weeks 2–28	4	0.45 (0.29–0.59)	0.37 (0.21–0.51)	0.36 (0.21–0.51)	0.38 (0.21–0.53)

(−19.68 [−40.40, 1.04]), there was an obvious decline in bottom-dwelling behaviour after the first phenotyping, and removal of the non-significant interaction confirmed this time effect (Est = −19.89 [−34.36, −5.42]; Figure 1b). Time spent in the bottom part of the tank was significantly repeatable over the 28 weeks (R = 0.39; Table 1) and did not change when excluding the week 1 data (R = 0.39; Table 2), but repeatability was lower, and non-significant, when comparing just week 1 and week 2 (R = 0.25; Table 1). Time spent in the bottom of the arena was highly repeatable in males (R = 0.55), but not in females, where it was much lower (R = 0.18; Table 1; Figures S1b and S3).

3.3 | Stationary time

Males spent significantly less time stationary than females (Est = −0.13 [−0.25, −0.01]), and this behaviour did not change over time (Est = −0.04 [−0.08, 0.005]; Figure 1c) until week 28, when males spent significantly more time stationary (Est = 0.10 [0.04, 0.16]). Time of day was a significant covariate, but only marginally so (Est = 0.12 [0.003, 0.24]). Overall, stationary time was significantly repeatable (R = 0.36; Table 1), but was lower and non-significant when comparing week 1 and week 2 (R = 0.21; Table 2). Excluding week 1 did not alter the overall repeatability (R = 0.37; Table 2). As with distance and bottom time, stationary time was highly repeatable in males

(R = 0.50), but less repeatable in females (R = 0.24; Table 1; Figures S1c and S4).

3.4 | Exploration index

There was no significant sex difference in exploration (Est = −2.55, [−14.17, 9.07]; Figure 1d), but exploration did marginally decline over time (Est = −5.12, [−9.76, −0.48]; Figure 1d). Overall, the standard deviation of exploration was significantly repeatable over the 28 weeks (R = 0.36; Table 1), with a similar repeatability between weeks 1 and 2 (R = 0.35; Table 2). Exploration was significantly repeatable in males (R = 0.50), but not in females (R = 0.18; Table 1; Figures S1d and S5).

4 | DISCUSSION

The aim of this study was to investigate the repeatability of personality-related behaviours from five repeated measures over 28 weeks, a significantly longer period and larger number of repeated measures that are commonly tested, using zebrafish (*Danio rerio*) as a model. All behaviours assessed were fairly repeatable over 28 weeks, ranging from 0.36 to 0.46 (Table 1). There were also clear sex differences in distance travelled and stationary time,

and the repeatability of behaviours exhibited by females was generally lower and not always repeatable. Importantly, behaviours tended to be more repeatable from the week 2 time point than from the initial assay, which is consistent with shorter-term repeatability studies on zebrafish (Tran & Gerlai, 2013) and which has important implications as researchers often only use two repeated measures over a week.

Distance travelled, our proxy measure for activity, was significantly repeatable over the entire study period. There is a similar overall pattern for repeatability of bottom time, our proxy for anxiety, over time. For both variables, we found little repeatability between the week 1 and week 2 time points, suggesting that the fish needed an experience with the test tanks first before exhibiting their natural behaviour (Tran & Gerlai, 2013). This is especially evident in the distance travelled and time spent stationary by males. Other researchers have suggested that just one or two measurements are insufficient to estimate an individual's behaviour (Beckmann & Biro, 2013; Fangmeier et al., 2018). Some researchers have also recommended that activity should be measured in a non-novel environment to get an accurate measure of an individual's normal activity levels (Réale et al., 2007; Renner, 1990). However, anxiety is always tested in a novel arena (Egan et al., 2009; Parker et al., 2012).

Exploration is often measured as stationary time, but we also generated an exploration index, where we calculated the standard deviation of the cumulative duration spent in each segment of the superimposed grid during the novel arena test. Both variables were repeatable overall, though the repeatability of stationary time dropped when we compared week 1 and week 2 only (Table 2). There is abundant existing research into exploratory behaviour (Carlson & Langkilde, 2013; Dingemanse et al., 2002; Jones & Godin, 2010; Renner, 1990; Thomas, Herrel, Hardy, Aujard, & Pouydebat, 2016), and it is generally found to be repeatable. Toms and Echevarria (2014) found exploratory behaviour in zebrafish (when tested every day) to be repeatable over seven days; their given repeatability value of 0.4 is similar to that presented in the current study. Likewise, Baker et al. (2018) reported repeatabilities of 0.21–0.40 for explorative behaviours measured over five weeks in wild-caught zebrafish and Fangmeier et al. (2018) reported repeatabilities of 0.2–0.3 for explorative behaviours in wild-type laboratory zebrafish.

Males were more repeatable than females in the total distance travelled and in the amount of time they spent stationary, with males travelling farther on average and spending less time stationary than females. Similarly, males displayed highly repeatable bottom time behaviour and exploration behaviour, with females displaying low and non-significant repeatability in both bottom time and explorative behaviour. There was, however, no significant difference in the actual bottom time or exploration index values between males and females; thus, while the females were less consistent in their bottom time and exploratory behaviour, there was no mean sex difference in the expression of these behaviours. It is important to note that the confidence intervals of the male and female repeatabilities overlap (Table 1), suggesting that these observed differences

between males and females are not significantly different. Our estimates of sex-specific repeatability may not be very precise due to smaller sample sizes when splitting the data set into sexes (Wolak et al., 2012), but we think it is important to highlight these differences, so that other researchers are aware that males and females may differ in the consistency of their behaviours. The findings that males were more repeatable than females are in contrast with a previous studies on zebrafish, which either found females to be more consistent in activity-related behaviour over a week than males (Tran & Gerlai, 2013) or found no differences between females and males in their exploratory behaviours over a five-week period (Baker et al., 2018). As noted by Tran and Gerlai (2013), there may be numerous reasons why males and females respond differently to certain tasks, including exploratory drive or behaviours associated with reproduction. Our fish were also tested over a much longer period of time, but the observed differences in repeatability between males and females were present even between the week 1 and week 2 time points (e.g. repeatability for activity between week 1 and 2 time points, male $R = 0.54$ [0.18, 0.79], female $R = 0.01$ [0, 0.47]; data for other behaviours not reported). Alternatively, the age of the fish may have played a role—our fish were between 6 and 13 months of age during testing, whereas the Baker et al. (2018) fish were much older (21 months), so perhaps males and females exhibit behavioural strategies that are context-dependent earlier in life. Unfortunately, sex differences are not often reported in zebrafish, in comparison with other taxa (Seltmann, Helle, Htut, & Lahdenperä, 2019). We encourage researchers to report sex and account for its effects on behaviour.

There is a suggestion that early life measures of behaviour may not reflect behaviours later in life (David et al., 2012; Herde & Eccard, 2013). Up to the age of ten months, male zebrafish are more active than females, but, after 22 months of age, females are more active (Philpott et al., 2012). The zebrafish used in this experiment were tested from the age of ~ six months over a fairly large portion of their lifespan, until the age of ~13 months; zebrafish in captivity typically live for ~3.5 years (Gerhard et al., 2002). Thus, while age-related effects could have been present in this experiment, the zebrafish were already of reproductive age at the start and did not reach near their maximum lifespan or the age at which Philpott et al. (2012) observed altered effects of sex on behaviour, so any age-related effects were probably small. Assessment of personality traits over ontogeny/biological milestones is rarely studied in any species (Wuerz & Krüger, 2015), and many questions remain about how animal personalities form (Bierbach, Laskowski, & Wolf, 2017).

The overall aims of this study were to determine whether personality-related behaviours in zebrafish are repeatable over time, and whether there are sex differences in these traits or in the repeatability estimates. We found that the behaviours studied were all significantly repeatable when tested at five time points over a 28-week period. There were convincing sex differences in behaviours in distance travelled (i.e. activity) during the novel arena assay, but not in exploration. Future studies are needed to tease out why sex differences occur, whether they exist earlier and whether they are

related to life-history trade-offs. There was also evidence that an initial experience in the testing arena may be necessary to ensure better representation of an individual's usual behaviour. It is important to study the repeatability of behaviours because it may provide insight into why and how personality is maintained in wild populations. Even studies carried out on captive populations and model organisms provide a base of knowledge which can be applied both to wild animals and to further behavioural testing in the laboratory. The findings from this study contribute to furthering our understanding of behavioural repeatability over time and support the idea that a single time point should not be relied upon for information about an individual's behaviour.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

All procedures were carried out under the standard operating procedures for zebrafish husbandry from the Otago Zebrafish Facility, and with the permission of the University of Otago Animal Ethics Committee (protocol 65/14).

DATA AVAILABILITY STATEMENT

The data associated with this manuscript are available in the Supplementary Material.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.