Consistent behavioural syndromes across seasons in an invasive freshwater fish

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Abstract

The linkage between behavioural types and dispersal tendency has been suggested to be a widespread phenomenon and understanding its mechanisms has become a pressing issue in light of global change and biological invasions. Here, we investigate whether individuals who colonize new habitats exhibit a certain set of behavioural types that differs from those remaining in the source population. We focussed on a feral population of guppies (*Poecilia reticulata*) that is assumed to undergo a yearly (re)colonization process. Guppies are among the most widespread invasive species in the world, but in temperate regions these fish can only survive in thermally altered freshwaters due to their tropical origin. The investigated population has sustained in a thermally-altered stream in Germany for over 50 years, where they find year-round suitable water temperatures around a warm-water influx. However, in warm seasons, peripheral parts of the warm-water flume become thermally accessible and may be colonized. We sampled fish from the source population and from a winter-abandoned downstream site in March, June and August. Fish were tested for boldness, sociability and activity involving open field tests as well as interactions with a biomimetic robot as social partner. Guppies differed consistently among each other in all three traits. Behavioural trait expression in the source population differed across seasons, however, we could not detect differences between source and downstream populations. Instead, all sampled populations showed a remarkably stable behavioural syndrome between boldness and activity despite strong changes in water temperatures across seasons. We conclude that random drift (as opposed to a personality-biased dispersal) is a more likely dispersal mode for guppies, at least in the investigated stream system. In the face of highly fluctuating environments, guppies seem to be extremely effective in keeping their behavioural expressions constant, which could help explain their successful invasion and adaptation to disturbed habitats.

Keywords: animal personality, dispersal, range expansion, invasive species, thermally altered freshwaters, guppy

Introduction

Consistent behavioural differences among individuals (e.g. behavioural types or animal personality; Réale et al. 2007) seem to be a ubiquitous biological feature (Bell et al. 2009, Bierbach et al. 2017) that was proposed to have substantial ecological and evolutionary importance (Sih et al. 2012, Wolf and Weissing 2012). For one, behavioural types are thought to be relevant in determining success (Chapple et al. 2012, Carere and Gherardi 2013, Canestrelli et al. 2015) and impact of biological invasions (Cote et al. 2010a, Fogarty et al. 2011, Juette et al. 2014), which is becoming an ever more pressing issue in the light of global change and ongoing translocations of species among habitats (Seebens et al. 2017, 2018).

It is assumed that individuals, which invade novel habitats, are a non-random subset of their source populations (Shine et al. 2011, Sih et al. 2012, Juette et al. 2014, Canestrelli et al. 2015, Spiegel et al. 2017). These invaders may share certain phenotypic and life-history characteristics that are associated with their behavioural types (reviewed in Juette et al. 2014). Behavioural types of invading individuals may determine whether they are transported (by humans) or move (by themselves) into new environments (first invasion stage; after Lockwood et al. 2011), whether they can survive and establish a non-native population (second stage), and whether they may spread to new habitats thereafter (third stage). Behavioural types shown to be relevant in this regard are boldness (Cote et al. 2011, Myles-Gonzalez et al. 2015), aggression (Duckworth and Badyaev 2007, Hudina et al. 2015), activity (Brown and Irving 2014, Thorlacius et al. 2015) as well as sociability (Cote et al. 2010b, 2011, Rasmussen and Belk 2012). Further, it was predicted that individuals carrying correlated suits (i.e., behavioral syndromes, Sih et al. 2012) of bold, aggressive, active and asocial types may predominantly be found at the invasion front (Duckworth and Badyaev 2007, Cote et al. 2010b, 2011, Juette et al. 2014). However, such spatially and/or temporally assorted compositions of behavioural types among populations along invasion gradients have only rarely been investigated under natural conditions in the wild.

The difficulty to study invasion phenomena in the wild is their random and unpredictable occurrence (Lockwood et al. 2011), which makes it nearly impossible to follow active invasion

fronts. In the rare cases where range expansions could be tracked in real time (e.g., western bluebirds: Duckworth and Badyaev 2007; Afican jewelfish: Lopez et al. 2012; round goby: Thorlacius et al. 2015; signal crayfish: Hudina et al. 2015; cane toad: Gruber et al. 2017), the invasion origin is often unknown or unclear due to multiple introduction events. Thus, we need to identify study systems that allow us to investigate dispersal across sensible temporal and spatial scales. Thermally altered freshwater systems (TAFs) in the temperate zones provide fruitful conditions to study this phenomenon. Here, natural and/or anthropogenic warm water influxes create unique temperature refuges that allow non-native species (especially those of tropical origin) to establish populations (Langford 1990, Jourdan et al. 2014). While ambient water temperatures drop in winter (e.g., less than +1°C in the river Rhine; Jourdan et al. 2014), areas close to constant warm water influx will remain suitable for non-native tropical species to survive. Once peripheral areas become thermally suitable again in warmer seasons, the winter-abandoned areas are assumed to be (re)colonized. Such a scenario enables us to investigate the role of behavioural types during *in situ* dispersal of non-native species from a single, well-determined source into new (i.e. winter-abandoned) habitats.

Under a personality-biased dispersal hypothesis, where individuals with a certain set of personality traits are assumed to leave their source population to colonize new habitats, we predicted that *(i)* the source population's average personality traits should change over the course of the year due to the drain of the emigrating individuals, *(ii)* newly founded populations should differ in their average personality traits compared to their source populations, and *(iii)* behavioural variation should decrease both in the source as well as in the newly founded populations due to the non-random migration patterns. In addition, *(iv)* population-wide correlations among personality traits (behavioural syndromes) may emerge or disappear because of assumed non-random migration patterns.

In addition to these predictions arising from non-random migration patterns, behavioural types may change due to the influence of seasonal fluctuations in abiotic (e.g., temperature; Spiegel et al. 2015) and/or biotic (e.g., resource availability and predator pressure; Barbosa et al. 2018) factors common in temperate climates. If so, one would predict that population averages,

behavioural variation and syndromes should change over the course of the year (similar to *i* and *iii* and *iv*) but in both established and newly founded populations to a similar extent (opposing prediction *ii*), especially when those populations face similar external conditions.

In Germany, tropical fishes have established populations in several artificially heated stream systems (Lukas et al. 2017a), the most investigated being the Gillbach near Cologne (see Fig. 1a; Jourdan et al. 2014, Lukas et al. 2017b, Kempkes et al. 2018). To estimate the seasonal fluctuations within the sampled range of the stream, we continuously recorded water temperatures with submerged data-loggers. We sampled feral guppies (*Poecilia reticulata*) from the Gillbach during different seasons (temporal scale: spring, early summer, late summer) and, if possible, at different sites (spatial scale: core area of warm water influx versus more peripheral downstream area; see Fig. 1a) and repeatedly assessed behavioural types in terms of boldness, sociability and activity in a laboratory setting.

Material& Methods

Study site & Sampling

The Gillbach (Rhine catchment, Germany) has been under the influence of thermal pollution and anthropogenic modifications for over six decades. Its headwaters are exclusively fed by the warm water discharge of the brown coal power plant Niederaußem (Fig. 1a). This creates a temperature regime, which is highly dependent on the volume and temperature of the plant's water discharge. Generally exhibiting above ambient water temperatures (Fig. 1b), the Gillbach harbours several established populations of non-native fish, invertebrate and plant species, many of which are of tropical or subtropical origin (see Supplementary material Appendix 1, Table A2 for a list of native and non-native fish species found in the Gillbach).

Here, feral guppies (*Poecilia reticulata*) are highly abundant and as successful global invaders (Deacon et al. 2011), they are well suited to investigate the expression of behavioural traits across time and space. Samplings were conducted at two sites (~300m transects, see Fig. 1a) in March, June and August 2016. To target guppies, we collected specimen by seine and dip netting (2.5 mm mesh width) until we reached sufficient numbers for behavioural testing (i.e., 35-45 adult individuals). Captured fish were transferred into well-aerated coolers and kept separately by sampling site.

Water temperatures at sites 1 and 2 were monitored using HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA) for a period of 16 months (Fig. 1b). For the period of sampling and behavioural testing (March – August 2016), the Gillbach experienced a thermal gradient of ~2°C/km (mean ± SD, site 1: 25.53°C ± 3.21°C; site 2: 23.49°C ± 3.4°C; Fig. 1b). We expect this trend to be even more pronounced in colder months, thus decreasing the likelihood of survival for guppies outside the core area during winter (see also Jourdan et al. 2014). Interestingly, the temperature range at both sites was similar (min – max, site 1: 8.38°C – 43.11°C; site 2: 10.46°C – 39.5°C), with two cases of temperature drops below the guppies' lower critical minimum temperature (approx. 12°C according to Jourdan et al. 2014).

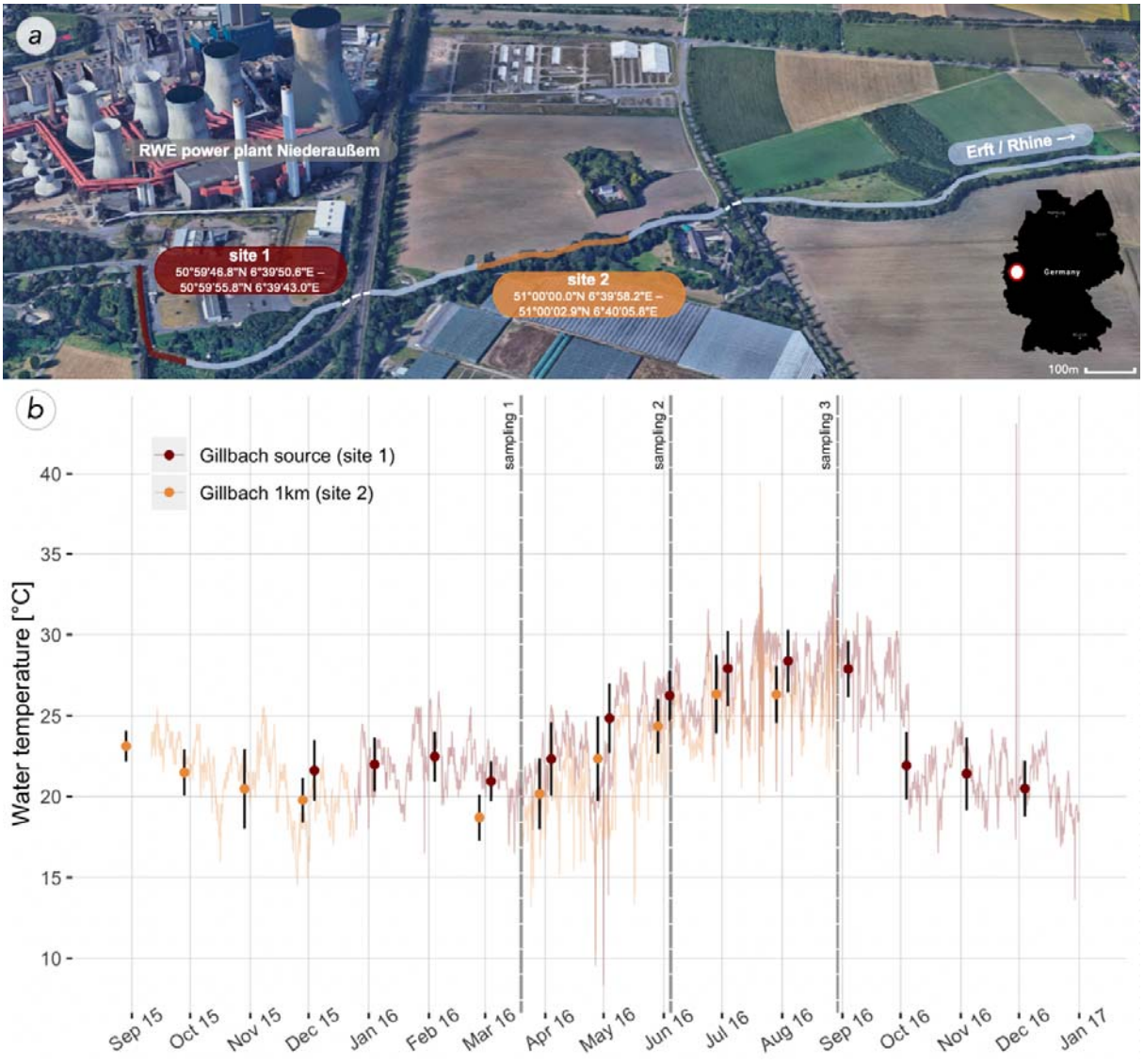

Figure 1: The Gillbach stream and its altered temperature regime. a) Warm water discharge from a power plant feeds into the Gillbach (map created using © 2019 Google Earth with data from 2009 GeoBasis-DE/BKG) and generates a thermal gradient above ambient water temperatures that is preserved for several kilometres (site 1 – site 2). **b)** 4-hourly water temperature* with error bars indicating monthly means ± SD for each site (site 1 – Gillbach source: red; site 2 – one kilometre downstream: orange). Dotted lines correspond to the three sampling occasions. * Note that due to a loss of loggers, continuous monitoring was interrupted for certain periods (site 1: no data from 11.09.2015 – 24.12.2015; site 2: no data from 24.12.2015 – 19.03.2016 nor from 29.08.2016 – 31.12.2016).

Behavioural assessment

Fish collected for behavioural testing were transferred to the laboratory at Humboldt University of Berlin, Germany. Fish were kept in one holding tank (270 litres), but were separated into mesh-divided compartments by collection time and site (‘populations’ hereafter). Each compartment (24 cm × 58 cm × 40 cm L×W×H) contained artificial java moss and natural gravel. Females and males were housed communally. Water was maintained at 26°C and kept under continuous aeration and filtration. Fish were held under diurnal lighting (12:12h light:dark cycle) and fed commercial flake food (TetraMin® Tetra GmbH) twice a day. Feeding stopped 12 hours before experiments and recommenced afterwards. To acclimate the fish to laboratory conditions and reduce potential short-term effects of the different sampling habitats on behavioural trait expression, no behavioural testing was conducted for at least 30 days (see Fig. 2a for detailed experimental design). One week prior to testing, adults of suitable size (SL>15 mm) were marked using visible implant elastomer tags (VIE; Northwest Marine Technology Ltd, Shaw Island, WA, USA) to allow for individual identification throughout the experiment. To be mindful of capture biases towards a specific behavioural type, we used all fish within a compartment for testing.

We tested a total of 159 guppies (95 females, 64 males; for population-specific sample sizes see Fig. 2a). Test fish were introduced to an open field test arena (88 x 88 cm, 75 mm water depth) filled with aged tap water kept at 26°C. To reduce disturbing stimuli, the arena was enclosed in black, opaque plastic and illuminated with artificial light reproducing the daylight spectrum. Behavioural observations were recorded via a camera (Ximea 4K USB 3.1 camera) mounted above the arena. Position information was extracted using EthoVision 10 XT software (Noldus Information Technology, Wageningen, Netherlands).

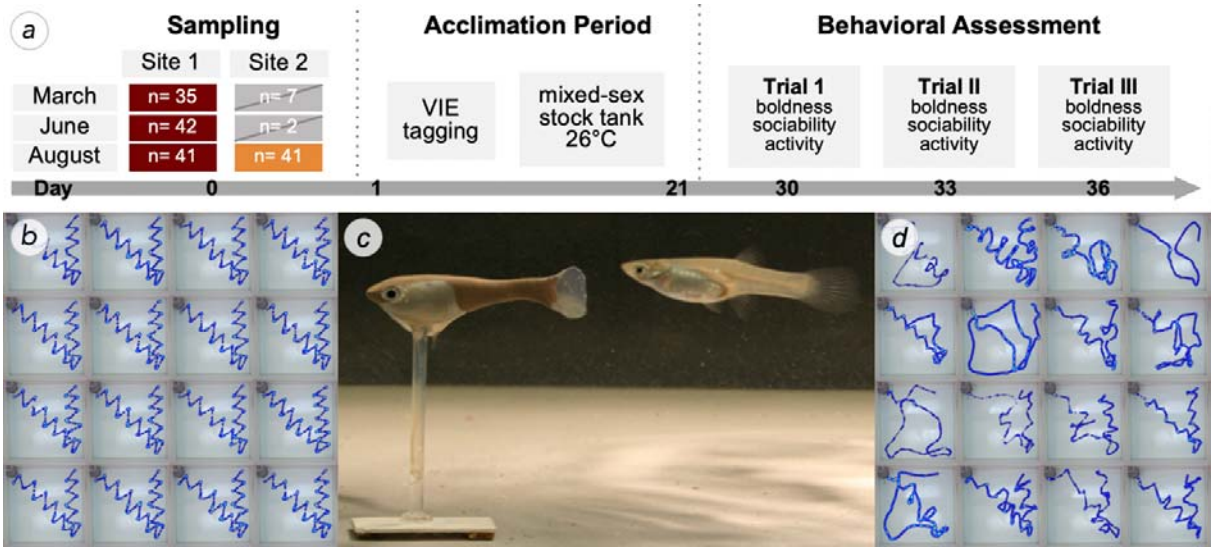

Figure 2: Behavioural testing with Robofish setup. a) Test fish were collected three times and, when possible, at two sites (site 1 and 2, see Fig. 1a). After an initial 30-day acclimation period, fish were tested a total of three times (trial I – III, sample sizes indicate tested individuals, for total catch numbers see Supplementary material Appendix 1). **b-d)** During trials, the Robofish replica was moved remotely through the test tank (c). Once the focal individual had emerged from its shelter, the biomimetic Robofish initiated its predefined path (b) and the fish's swimming trajectory was recorded (d).

(1) Boldness: Boldness was measured as latency to emerge from a shelter with a low latency time indicating high levels of boldness. Fish were introduced into a grey opaque shelter box (Ø 100 mm; top left of each image in Fig. 2b and d) and allowed to acclimate. After one minute, a sponge was removed from the entrance (40mm x 25mm), allowing the fish to emerge and explore the test arena. A fish was scored as having emerged when its full body was visible outside of the shelter. If fish did not emerge after five minutes, the lid of the shelter box was removed. Fish that failed to emerge within eight minutes were given the maximum score of 480 seconds and the box was removed entirely.

(2) Sociability: A three-dimensional fish replica was stationed 1 cm away from the shelter entrance. The replica, which resembled a large-sized guppy female (SL=30mm; left fish in Fig. 2c), could be moved by a robot via a magnetic base (see Landgraf et al. 2016, Bierbach et al. 2018 for details). Upon the fish's emergence (or the removal of the shelter box), the biomimetic robot started to move through the arena in a standardized sequence (Fig. 2b) imitating a conspecific.

We calculated the mean distance the fish kept to the biomimetic robot as a proxy of sociability with lower values indicating higher levels of sociability. Consequently, fish that followed the robot closer were assumed to be more sociable (see Bierbach et al. 2018).

(3) Activity: After completion of the Robofish's predefined path, the replica was removed from the arena by hand and once the fish resumed normal swimming behaviour, it was allowed to explore the arena. As a measure of activity, we calculated the mean velocity with which the fish explored the open arena during 3 minutes.

After the first behavioural assay, we recorded the body length of each individual. Fish were transferred back to their respective compartment and allowed 72 hours resting before being retested following the same protocol. Fish were tested a total of three times in randomized order. All reported experiments comply with the current German law approved by LaGeSo (G0117/16 to Dr. D. Bierbach).

Statistical analysis

Datasets with sample sizes less than 10 were excluded from all further analyses (i.e. site 2 in March and June; see Fig. 2a).

To test for differences in average trait expression over a temporal and/or spatial scale, we used linear mixed models (LMM, *GENLINMIXED* procedure; SPSS v. 25.0, IBM Corp., Armonk, NY, USA) with each behavioural trait (boldness, sociability, activity) as a separate response variable. 'Boldness' and 'sociability' were log10-transformed to fulfil normality assumptions. All models included sample population as the fixed effect of interest (four levels: March – site 1, June – site 1, August – site 1, August – site 2). Behaviour in poeciliids is often size- and/or sex-dependent (Brown and Braithwaite 2004, Harris et al. 2010), thus we included standard length (SL) as a covariate and 'sex' as an additional fixed factor. To account for the repeated testing of individuals, we further included 'trial' as a fixed factor and 'fish ID' as a random factor. Post-hoc LSD tests were employed in case fixed factors were found to have a significant effect. For the covariate 'body size', we also performed Pearson's correlations to better evaluate the relationship.

(Co)Variance structure was nested within ‘population’, which enabled us to calculate the total behavioural variance as well as within- and among-individual variance components in each population. Based on this, we calculated behavioural repeatability for each of the four populations. We assumed that repeatability and/or variance estimates differed among populations when 95% confidence intervals (CIs) were not overlapping (see Nakagawa and Schielzeth 2010).

To evaluate the existence of behavioural syndromes (i.e. correlation among behavioural traits) within our populations, we used linear regression models to test for significant correlation between each trait pair. In order to minimize the effects of mean differences in behavioural traits on trait correlation, we mean-centred each behavioural trait within ‘trial’ and ‘population’ (z-transformation). Each model included one trait as the dependent variable and another as covariate. We further compared whether trait correlation differed among populations (i.e., difference in slopes termed ‘covariate nested within population’). Visualizations were done with GraphPad Prism (version 8.01, GraphPad Software Inc., La Jolla, CA, USA).

Results

Differences in average trait expression among populations

Expression of sociability and activity differed between populations, while boldness did not ('population' effect in Table 1; Fig. 3a).

On a temporal scale, fish sampled at site 1 in August showed higher levels of sociability (EMMs ± SD, August – site 1: 1.18 ± 0.03) compared to March and June populations (March – site 1: 1.3 ± 0.03, June – site 1: 1.27 ± 0.03; post-hoc, August site 1 vs. March – site 1: $p = 0.003$, August site 1 vs. June – site 1: $p = 0.025$). Similarly, fish sampled at site 1 in August were more active (August – site 1: 7.81 ± 0.33) than populations sampled in March and June (March – site 1: 6.38 ± 0.33, June – site 1: 6.03 ± 0.27; post-hoc, August site 1 vs. March – site 1: $p = 0.002$, August site 1 vs. June – site 1: $p < 0.001$).

On a spatial scale, we found no evidence that fish sampled in August at site 1 and site 2 differed in boldness (1.4 ± 0.08; post-hoc, August site 1 vs. August site 2: $p = 0.76$), sociability (1.18 ± 0.04; post-hoc, August site 1 vs. August site 2: $p = 0.92$), nor activity (7.74 ± 0.31; post-hoc, August site 1 vs. August site 2: $p = 0.89$).

Boldness and activity showed a size-dependency (covariate 'body size'; Table 1, Fig. 3b) with smaller individuals being bolder (Pearson's $r = 0.32$; $p < 0.001$) and more active (Pearson's $r = -0.35$; $p < 0.001$) than larger ones. We found no such relation between body size and sociability (Pearson's $r = 0.002$; $p = 0.98$). While sex is correlated with size in the sexually dimorphic guppy, sex did not affect any of the measured behavioural traits (non-sig. 'sex' effect; Table 1). We further observed a habituation to the repeated testing during sociability and activity assays (sig. 'trial' effect; Table 1). Fish were considerably less social (trial 1: 1.03 ± 0.21, trial 2: 1.27 ± 0.21, trial 3: 1.4 ± 0.21) and less active (trial 1: 8.09 ± 0.21, trial 2: 6.93 ± 0.21, trial 3: 5.96 ± 0.21) with each consecutive measurement.

Table 1: Results from linear mixed models. For each behavioural trait, the LMM included ‘population’, ‘trial’ and ‘sex’ as fixed effects and ‘body size’ (SL in mm) as covariate. Fish ID was used as random factor. Significant effects ($p < 0.05$) are indicated in bold.

	Boldness (log_{10})		**Sociability (log_{10})**		**Activity**	
Effect	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p
population	$F_{3,469} = 0.57$	0.634	$F_{3,469} = 3.8$	**0.01**	$F_{3,469} = 8.44$	**< 0.001**
trial	$F_{2,469} = 1.72$	0.18	$F_{2,469} = 135.9$	**< 0.001**	$F_{2,469} = 42.32$	**< 0.001**
sex	$F_{1,469} = 0.06$	0.81	$F_{1,469} = 2.28$	0.132	$F_{1,469} = 0.24$	0.622
body size (SL)	$F_{1,469} = 8.43$	**0.004**	$F_{1,469} = 0.02$	0.884	$F_{1,469} = 3.58$	0.059

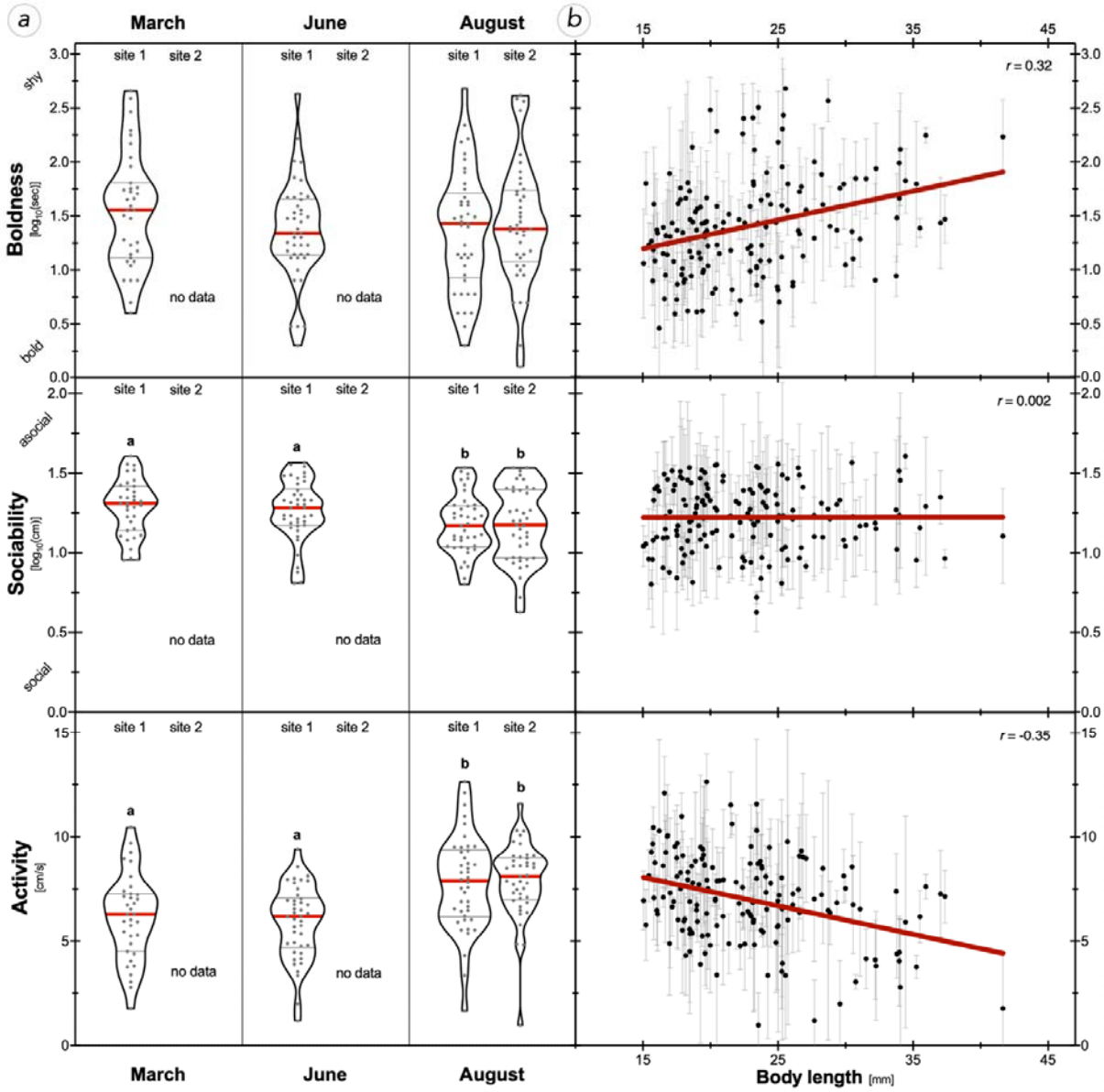

Figure 3: Average personality traits across temporal and spatial scale. *(a)* Median trait scores (and IQRs) for boldness* (i.e., time to emerge from shelter), activity (i.e., velocity during open field exploration) and sociability* (i.e., distance to biomimetic Robofish). Different letters depict significant differences based on post-hoc LSD tests after global linear mixed models ($p < 0.05$). ***(b)*** Mean traits scores (± SD) in relation to body size (standard length; n=159). To visualize the relationships, we show linear regressions lines (red) calculated from individual means (see main text for significance levels and Pearson's r). * Note that low scores of boldness and sociability indicate high trait expression.

Among- and within-individual variation in behavioural traits

Fish within each sampled population differed consistently in their expression of boldness, sociability and activity across trials (sig. repeatability estimates; Table 2). Only fish sampled in August at site 2 had a significantly higher repeatability estimate for sociability than fish from the March sampling at site 1 (non-overlapping CIs; Table 2). There was no significant difference among populations in estimated overall behavioural variances in any of the examined traits (see 'total variation' in Table 2; for within- and among-individual variance estimates see Supplementary material Appendix 1, Table A2).

Table 2: Repeatability estimates and total behavioural variance estimates. *p*-values are derived from Wald's *z* statistics (see Supplementary material Appendix 2, Table A3 for within- and among-individual variation estimates). Significant differences among populations can be assumed when 95%CI do not overlap.

	Boldness (log_{10})			**Sociability (log_{10})**			**Activity**		
Population	Repeat-ability [95% CI]	*p*	Total Variation [95% CI]	Repeat-ability [95% CI]	*p*	Total Variation [95% CI]	Repeat-ability [95% CI]	*p*	Total Variation [95% CI]
March site 1	0.49 [0.40–0.57]	**0.004**	0.24 [0.15–0.4]	0.26 [0.15–0.4]	0.05	0.06 [0.04–0.1]	0.27 [0.17–0.41]	**0.042**	6.86 [4.3–11.87]
June site 1	0.40 [0.31–0.5]	**0.005**	0.31 [0.2–0.5]	0.32 [0.23–0.43]	**0.011**	0.07 [0.05–0.11]	0.30 [0.21–0.42]	**0.015**	5.75 [3.74-9.33]
August site 1	0.54 [0.47–0.6]	**0.001**	0.34 [0.22–0.54]	0.34 [0.25–0.45]	**0.009**	0.06 [0.04–0.09]	0.38 [0.29–0.47]	**0.005**	7.15 [4.61-11.48]
August site 2	0.53 [0.46–0.6]	**0.001**	0.3 [0.19–0.48]	0.49 [0.42–0.57]	**0.001**	0.08 [0.05–0.13]	0.36 [0.27–0.46]	**0.007**	5.92 [3.81-9.57]

Behavioural syndrome structure

Boldness and activity were negatively correlated in all four sampling populations (sig. correlation between boldness and activity: $F_{1,157}$ = 52.23; p < 0.001; no sig. difference in slopes among populations: $F_{3,151}$ = 0.45; p = 0.715). Fish with high average activity levels also left the shelter quicker, confirming that activity and boldness are part of a larger behavioural syndrome (Fig. 4).

There was no evidence for correlations between sociability and boldness ($F_{1,157}$ = 0.026; p = 0.872; no sig. difference in slopes among populations: $F_{3,151}$ = 1.8; p = 0.149), nor between sociability and activity ($F_{1,157}$ = 0.062; p = 0.803; no sig. difference in slopes among populations:

$F_{3,151} = 0.816$; $p = 0.487$). Thus, sociability measured as distance kept to the moving Robofish does not seem to be part of a behavioural syndrome with neither boldness nor activity.

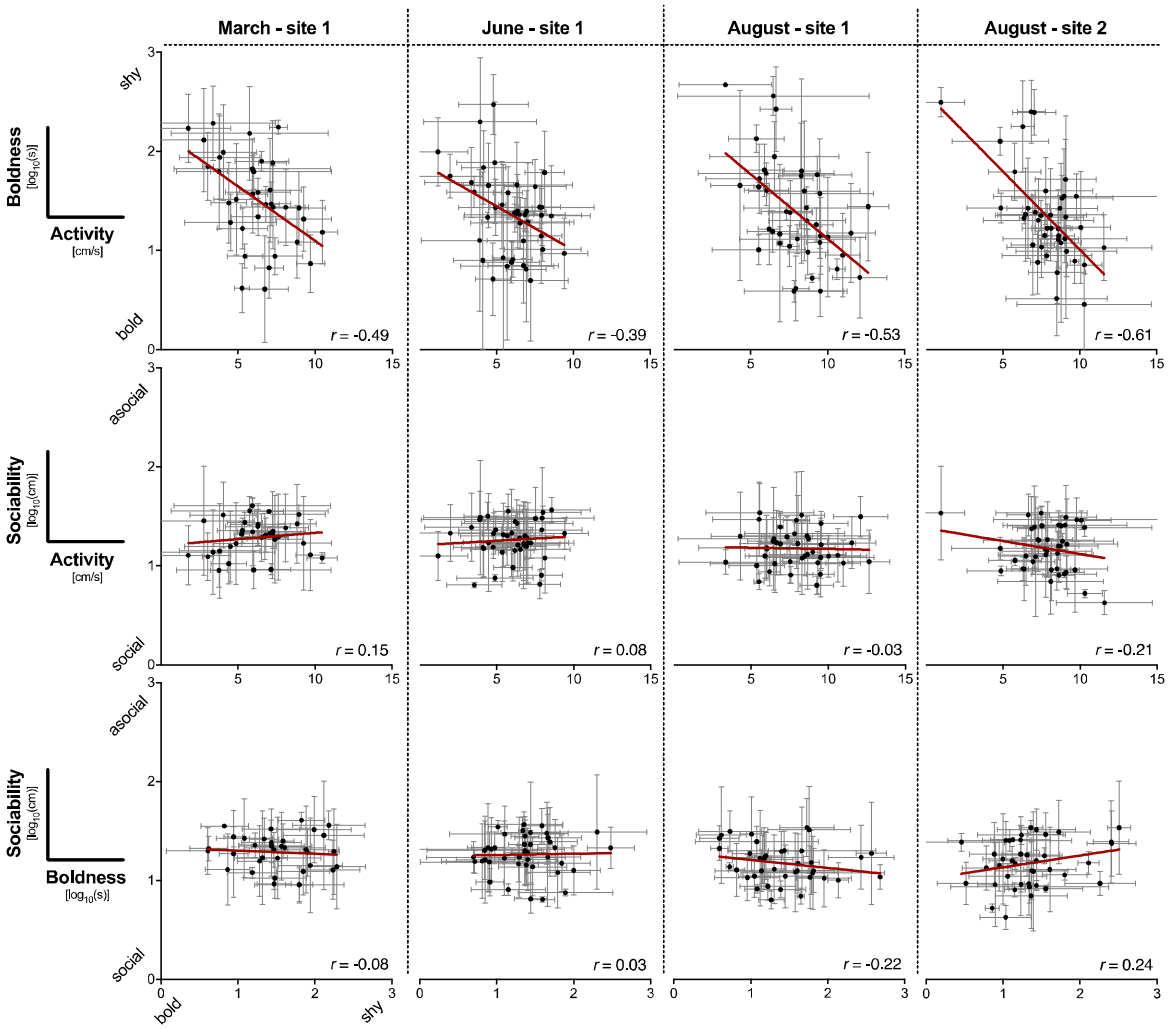

Figure 4: Correlations between behavioural traits across temporal and spatial scale. Mean trait scores* (±SD) of individual test fish are shown for each population. Linear regressions (red line and Pearson's *r*) visualize the relationships between traits (see main text for detailed analysis of trait correlation; $n = 159$). * Note that for boldness and sociability lower values indicate a high trait score.

Discussion

Our results document the annual (re)colonization process of an artificially heated stream by a non-native fish in temperate Germany. As guppies dispersed into winter-abandoned areas in warmer months, we expected this process to be driven by dispersal-enhancing behavioural traits. Contrary to expectations, we found no evidence that the source and the newly-founded population differed in terms of boldness, sociability and activity. Likewise, we did not detect a decrease in behavioural variation (except sociability in March and August) indicative of a “thinning out” of disperser’s behavioural types. Instead, we found a behavioural syndrome among activity and boldness that was consistently detectable throughout the year and across sampled populations. However, average personality traits in the source population changed over the course of 6 months with August-sampled individuals being more active and more sociable than those sampled earlier in the year, while boldness scores remained unchanged. To sum up, we found no strong evidence for a personality-biased dispersal in this feral population of *Poecilia reticulata*. Instead, the detected seasonal differences in average trait expression (sociability, activity) are likely due to changed environmental factors such as temperature, predator abundance and/or resource availability.

The observed changes in behavioural traits across seasons but not between sites were likely caused by changes in environmental conditions experienced by both populations rather than by a drain of individuals carrying certain behavioural types from source to downstream populations. This assumption is congruent with the Gillbach’s temperature profile that clearly shows strong seasonal variations within but not across sites. The observed seasonality in temperature in combination with a variation in day length, food quality/quantity and predation are likely to be important modifiers of population-wide behaviours (Biro et al. 2010, Eccard and Herde 2013, Spiegel et al. 2015, Uchida et al. 2016, Dhellemmes et al. 2020). There is further evidence that prior acclimation could, in fact, alter offspring phenotypes (Beitinger et al. 2000, Seebacher et al. 2014). This might explain the increased activity exhibited by fish in August, as higher developmental temperatures lead to more active phenotypes (Seebacher et al. 2014). Out of the studies finding personality-biased compositions along a dispersal front, only two noted context-

dependency: spatial sorting of behavioural types did not emerge under predation (Cote et al. 2013) and was lost as the population aged (Thorlacius et al. 2015). Our findings now highlight heterogeneity across seasons, however, teasing apart the effects of abiotic and biotic fluctuations in resources and predation will require an experimental approach.

Besides seasonal differences in average behavioural traits, we found stable behavioural syndromes among sampled populations that involved a strong positive correlation between boldness and activity. This is consistent with several studies, where this boldness-activity syndrome has been documented in various taxa (van Oers et al. 2004, Pintor et al. 2008, Wilson and Godin 2009, Cote et al. 2010b, Eccard and Herde 2013, Muraco et al. 2014), including guppies (Brown and Irving 2014). Natural selection through predation favours the evolution of behavioural correlations (Dingemanse et al. 2007, Harris et al. 2010, Dhellemmes et al. 2020). A positive boldness-activity correlation might thus represent an optimal trait combination in the fact of predation pressure experienced by both populations. However, the lack of differences in the level of (individual) behavioural variation among populations might be a result of a random downstream drift and a low overall behavioural plasticity in this population. This can be a consequence of the assumed bottleneck origin of this feral population that most likely stems from an unknown number of released domesticated guppies (see Gertzen et al. 2008 for estimates of propagule pressure for popular aquarium species). Investigations involving other populations of the guppy and more distantly related species are highly recommended, especially as several environmental factors are predicted to impact behavioural variation, especially in fish (e.g., Harris et al. 2010, Laskowski et al. 2016, Barbosa et al. 2018). Alternatively, guppies and especially those from the Gillbach that experience highly fluctuating temperature regimes may have the ability to adjust their behavioural expressions within very short times, e.g., within the 30-days acclimatisation period in the laboratory used here. It is known that guppies have a remarkable capacity for thermal adaptation (Chung 2001, Jourdan et al. 2014) and have successfully established populations in environments with very different thermal regimes compared to their native habitats (Deacon et al. 2011). Testing of individuals after different acclimation periods could be a possibility to investigate this issue further.

Theoretical models (Cote et al. 2010a, Spiegel et al. 2017) as well as several studies across taxa (Duckworth and Badyaev 2007, Pintor et al. 2009, Cote et al. 2010b, Myles-Gonzalez et al. 2015, Coates et al. 2019) provide evidence for the prominent role of individual differences in behaviour during dispersal and invasion processes (Bowler and Benton 2005, Chapple et al. 2012, Canestrelli et al. 2015). Nevertheless, the picture might be more complicated: our current study exemplifies that population-wide behavioural analysis can fail to provide evidence for behaviourally-mediated migration patterns and future analysis may focus on tracking individuals along their travel routes across seasons and locations. Furthermore, analysis may incorporate the invasion history, environmental factors or the species-specific genetic backgrounds which all can affect behavioural variation in wild populations (Sih and Bell 2008, Bergmüller and Taborsky 2010, Stamps and Groothuis 2010).

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Authors' contributions:

JL and DB designed the study; JL, FWM, DB and GK jointly collected the fish; DB, TL and JK developed the Robofish system; JL and DB conducted the behavioural experiments, analysed the data and wrote the manuscript; all authors approved the final draft.