Animal personalities on the move

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4

Mesocosm experiments reveal the loss of migratory tendencies in a recently isolated population of three-spined sticklebacks

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Abstract

In the 1970s, water management in the Netherlands resulted in numerous isolated populations of three-spined sticklebacks, which can no longer migrate from freshwater to the sea. We tested whether ∼50 years of isolation resulted in reduced migratory tendencies in these 'resident' sticklebacks. Lab-based individual testing showed behavioural divergence between residents and migrants, but also produced counter-intuitive results, especially with regards to movement tendencies. To detect differences in migration tendencies, we set up a semi-natural mesocosm, consisting of connected ponds, where movements of numerous individuals could continually be tracked at larger spatial scales. We found that wild-caught residents and migrants exhibited no differences in movement tendencies 'within ponds', but residents moved significantly less 'between ponds' than migrants. Between-pond movements were consistent and the observed differences were robust across contexts (changes in water flow and group size). Our study reveals that larger-scale movement tendencies can diverge over short time scales in response to human-induced isolation, and highlights the importance of observing behaviour in ecologically relevant setups that bridge the gap between lab and field studies.

Introduction

Habitat fragmentation is one of the major threats for biodiversity, particularly for migratory species that depend on multiple habitats to complete their life cycle (Legrand et al. 2017). In the north of the Netherlands, pumping stations have disrupted the connectivity between marine and riverine habitats, confining some fish populations to freshwater habitats without the possibility to migrate to the sea. Such forced isolation can cause rapid phenotypic responses and life-history changes (mammals and birds: Soriano-Redondo et al. 2020; fish: Quinn and Myers 2004; Closs et al. 2013; Dodson et al. 2013; Augspurger et al. 2017). Using individual lab-based assays, we have previously shown that this is indeed true for three-spined sticklebacks (*Gasterosteus aculeatus*): 'resident' populations, isolated for ∼50 years, were found to diverge in morphology and in behaviour from their 'migrant' ancestors (Ramesh et al. 2022b), with part of the divergence having a genetic basis (Ramesh et al. 2021). Regarding movement-related behaviours, population differences uncovered in the lab were surprising at first because residents, that were expected to exhibit lower movement tendencies than migrants, were instead more active and more exploratory (Ramesh et al. 2022b). We hypothesized at that time that this may be due to stress, induced by testing in social isolation, which might have affected wild-caught migrants disproportionately more than wild-caught residents, as migrants are thought to shoal extensively as an anti-predator strategy to higher predation risk in the open sea. Alternatively, small-scale experimental settings in the lab may not be suited to study larger-scale processes like migration. More generally, for wild-caught animals, lab conditions necessarily present a novel environment and fail to mimic natural complexity in biotic and abiotic factors, including the animals' social environment (Burns et al. 2009; Calisi and Bentley 2009; Niemelä and Dingemanse 2014; Pritchard et al. 2016). However, studying dispersal or migration behaviour in the field is often logistically challenging (especially in aquatic

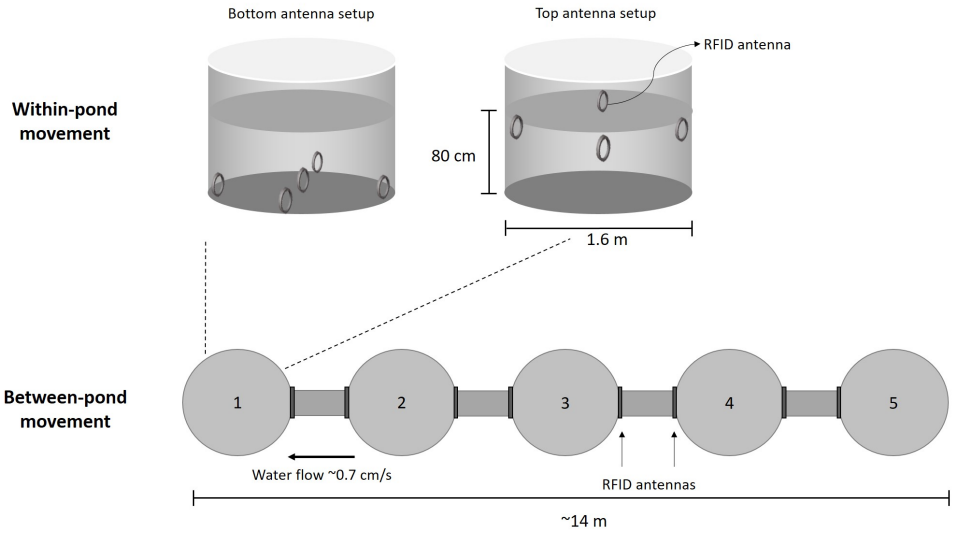

Figure 4.1 – ***Experimental setup.*** *The mesocosm consisted of two sets of five linearly connected ponds (1 to 5) equipped with circular RFID antennas that automatically detect crosses of PIT tagged individuals. Fish were released into pond 1. This pond was equipped with nine RFID antennas (five on the bottom and four on top of the water column), allowing us to quantify within-pond movements. The connections between adjacent ponds were equipped with two RFID antennas, allowing us to quantify the number and direction of movements between ponds.*

environments and for small fish) and frequently lacks data about the animals' social groups (Krause et al. 2013).

To bridge the gap between lab and field studies, we set up a semi-natural mesocosm consisting of connected ponds, in which groups of fish can be remotely tracked over extensive periods of time. We here report the first experiment that aimed to test for consistent differences in movement tendencies between wild-caught 'resident' and 'migrant' sticklebacks and to disentangle the effects of spatial scale (within and between ponds), social environment (group size), and ecological conditions (water flow) on movement patterns. The results of the second experiment, aimed at disentangling genetic and non-genetic effects, are reported in (Ramesh et al. 2021). Under these experimental conditions, we tested (a) if residents and migrants exhibit differences in their movement tendencies, (b) if the spatial scale of movement matters, and (c) how consistent these patterns are under varying conditions (group size and water flow).

Methods

Mesocosm system

The mesocosm consists of two independent systems of five ponds (each ⌀ 1.6 m, with water depth of 80 cm), connected linearly with opaque corridors (each of length ∼1.5 m and ∼ 11 cm), spanning a linear distance of ∼14 m (Fig. 4.1). The system is supplied with freshwater from a natural ditch, with the possibility of creating water flow (∼0.7 cm/s), mimicking the wild conditions, which also acts a cue for migration (Jonsson 1991). This system allowed to measure the movement of individual sticklebacks within and between ponds. The first pond (labelled 1 in Fig. 4.1), enriched with plastic plants, was used to quantify within-pond movements, while the whole system of five connected ponds was used to record between-pond movement tendencies (see details in Supp. info. 1).

We used a Radio-Frequency-Identification (RFID) system consisting of circular RFID antennas (⌀ 10 cm), data loggers and Passive Integrative Transponders (PIT tags; Trovan, Ltd., Santa Barbara, California) to record movements of tagged sticklebacks (details in Supp. info. 1). Nine circular antennas were placed in the first pond to record within-pond movements and two antennas were placed at both ends of each of the four connecting corridors to measure between-pond movement tendencies (Fig. 4.1). Each antenna records the unique PIT-tag ID of the fish along with a time stamp, stored on a USB drive in the central data logger. The sensitivity of the system was set to three reads per second per unique tag. In a pilot study, we validated the reads using video recordings and found that it corresponded well with the entry and exit times of fish.

Experiment 1

We created five groups of migrants and six groups of residents, each consisting of 10 randomly selected individuals (total: $N_{mig} = 49$ and $N_{res} = 60$). While we always tried to maintain the group size to 10 fish, tag-loss and other technical difficulties led to one group of migrants having nine fish and another with 11 fish. Groups were housed in separate small holding ponds for 24 hours before the start of the experiment. On the experimental day, one resident and one migrant group were released simultaneously (to avoid temperature or seasonal biases) into separate mesocosms. The individuals in each group were first monitored for within-pond movement by confining the fish to the starting pond for the first five hours (Fig. 4.1) and then for between-pond movement for ∼16.5 hours, after opening the connection to the other ponds (Fig. 4.1; Supp. info. 2).

Experiment 2

In a next step (after ∼one month), we combined all migrants and, separately, all residents (after excluding 12 fish which either had died or lost tags) into two large groups ($N_{mig} = 45$, $N_{res} = 52$) and quantified between-pond movements in these two groups in the same separate mesocosm setups over four days. In addition, we alternated

flow and no-flow conditions on consecutive days (see Supp. info. 1).

Analyses

For each individual, we quantified within-pond movement as the number of times a fish crossed different bottom and surface antennas separately (Fig. 4.1). We deemed the number of separate visits to a particular antenna unreliable for measuring movement patterns because fish that stayed longer near an antenna were recorded as multiple disconnected set of reads, as if they visited the antenna multiple times. Between-pond movement was quantified as the number of crosses a fish made through the corridors connecting two ponds (Fig. 4.1). Fish that did not get detected by any antenna were given a score of zero crosses.

We then analysed if residents and migrants differed in the number of crosses for within- and between-pond movements (Experiment-1) and whether they were consistent across contexts (group size and flow; Experiment-2). Briefly, we considered the number of crosses within or between ponds as response variable separately in univariate generalized linear mixed models with Poisson errors. In all models, we included origin (resident vs. migrant) as a fixed factor and group-ID and an observation-level 'Obs' (Observation-level random effects to control for overdispersion (Harrison 2014), as random effects. For Experiment-2, treatment (flow vs. no flow) and its interaction effect with origin were added as fixed effects and individual-ID as a random effect to account for individual repeats. Additionally, we analysed whether the fraction of fish that did not exit the first pond differed between migrants and residents using Fisher's exact test. Repeatability and correlation of number of crosses across contexts were also calculated (Supp. info. 3). All analyses were carried out in R (R Core Team 2021). For complete description of the analyses see Supp. info. 3.

Results

In Experiment-1, residents and migrants showed a broad distribution of number of crosses at both bottom and top antennas (Fig. 4.2 a, b) and the differences between the groups were in both cases not statistically significant (Table 4.1; Median bottom-antenna crosses: Residents=23, Migrants=14; Median top-antenna crosses: Residents=3.5, Migrants=8). In contrast, residents exhibited much lower numbers of crosses between ponds than migrants (Fig. 4.2 c; significant effect of Origin in Table 4.1; Median pond crosses: Residents=0, Migrants=16). Furthermore, the proportion of 'non-leavers', i.e., individuals that did not exit the first pond, was significantly higher in residents than in migrants (55% in residents vs. 28.6% in migrants, odds ratio=3.02, p=0.007). In Experiment-2, as in Experiment-1, residents moved consistently less between ponds than migrants (Fig. 4.2d). Furthermore, fish moved more between ponds in the presence of flow and the trend was slightly stronger for residents than migrants (Fig. 4.2d; significant Origin × Treatment effect in Table 4.1). Individual movement tendency between ponds was moderately repeatable across ecological contexts but very weakly correlated over social contexts (Supp. info. 3). However, we clearly see from

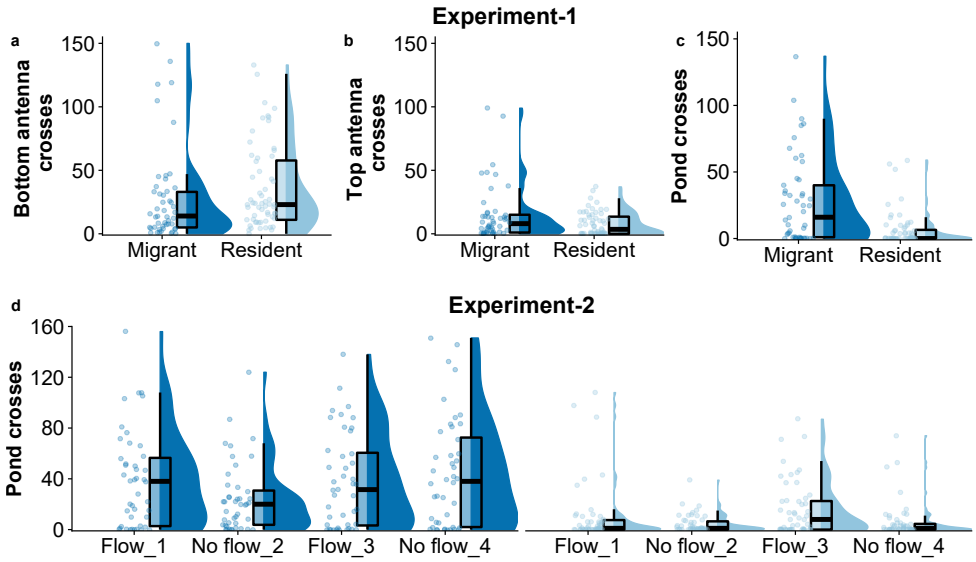

Figure 4.2 – ***Within-pond and between-pond movement of resident and migrant sticklebacks.*** *a,b) within-ponds crosses at the bottom and top antennas respectively (Experiment-1); c) between-pond crosses in Experiment-1; Sample size: Nmig=49, Nres=60; d) between-pond crosses in relation to the daily flow treatment in Experiment-2. Sample size: Nmig=45, Nres=52. In all graphs, individual crosses (dots), boxplots and density kernels are shown for migrant (dark blue) and resident (light blue) sticklebacks.*

Fig. 4.2 and Table 4.1 that the difference between residents and migrants was maintained across different contexts.

Discussion

We have previously shown that ∼50 years of isolation potentially led to rapid behavioural and morphological divergence of residents from migrants (Ramesh et al. 2022b), which mimics the divergence observed in another long-isolated population of sticklebacks (Di-Poi et al. 2014). Both studies assayed individual movement tendencies under artificial housing conditions in the lab and showed counter-intuitive patterns: residents showed either higher (Ramesh et al. 2022b) or inconsistent patterns (Di-Poi et al. 2014) in activity/exploration levels compared to migrants. Here, we show that the same populations as in (Ramesh et al. 2022b) exhibited movement tendencies as predicted previously, when they were tested in a semi-natural setting (relevant social/ecological context and spatial scale): Resident populations exhibited lower movement tendencies than their migrant counterparts. These differences, detected only at large spatial scale, remained consistent across ecological and social contexts. Together with the previous results on F1 lab-born juveniles (Ramesh et al. 2021), this study suggests

Table 4.1 – *Results of the statistical analysis of movement within and between ponds using generalised linear mixed models. Estimates of fixed effects (β) in log-scale are given with their 95% confidence intervals (CI) and variance components are given with their standard deviation. Fixed effects that significantly differ from zero are denoted in bold. Sample sizes experiment-1: N_{mig}=5 groups (49 individuals), N_{res}=6 groups (60 individuals); experiment-2: N_{mig}=1 group (45 individuals), N_{res}=1 group (52 individuals). 1:'migrant' is used as reference category; 2:'flow' is used as reference category*

	Experiment-1			**Experiment-2**
	Bottom crosses	Top crosses	Pond crosses	Pond crosses
Fixed effects	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Intercept	**2.61 (2.13, 3.08)**	**1.98 (0.30, 3.63)**	**1.90 (0.63, 3.13)**	**2.53 (1.87, 3.17)**
Origin[1]	0.51 (-0.12, 1.15)	-0.68 (-3.03, 1.53)	**-2.26 (-4.04, -0.58)**	**-1.77 (-2.68, -0.87)**
Treatment[2]	-	-	-	-0.14 (-0.44, 0.16)
Origin[1] × Treatment[2]	-	-	-	**-0.72 (-1.18, -0.27)**
Random effects	Var (sd)	Var (sd)	Var (sd)	Var (sd)
Group-ID	0.11 (0.33)	2.94 (1.72)	0.95 (0.98)	-
Obs	1.21 (1.10)	1.14 (1.07)	5.02 (2.24)	0.81 (0.90)
Individual-ID	-	-	-	4.11 (2.02)

that our mesocosm setup, by allowing water flow, testing in groups and larger spatial scale (14 m length), is much better suited to characterize individual movement patterns related to migratory behaviour than lab-based assays in social isolation in small tanks.

Our study reveals that the detection of population differences in stickleback behaviour was scale-dependent (only detectable between, but not within ponds). This is probably because in the wild, sticklebacks exhibit considerable foraging movements over days (median of 40 m upstream, (Bolnick et al. 2009) and hence their within-pond movements, representing foraging movements, may not differ between populations. However, wild migrants in our field system travel 10s of kilometres inland within a few days (pers. comm. from water authorities) and thus require sufficient space and navigation cues (e.g. flow velocity; Sommer-Trembo et al. 2017) to express their natural behaviour.

Tests in the lab, though invaluable for studies on animal behaviour owing to controlled settings, are not without drawbacks. Firstly, they cannot offer the more natural conditions mentioned above (e.g. spatial scale, appropriate social or ecological contexts), which may be particularly important for wild-caught animals. They may constrain the level of behavioural expression to some extent, such as the 'freezing' behaviour of wild-caught migrants in our previous studies (Ramesh et al. 2022b). Reassuringly, we observed that this was much less of an issue for lab-bred animals: lab-born F1 juveniles did not freeze in lab tests and their movement-related behaviours measured in the lab and in the mesocosm positively correlated (Fig. 4.A1). Secondly, lab-tests

are performed in highly-controlled or novel setups. This can lead to homogenization of behavioural expression (e.g. decreased variance over time; Sommer-Trembo et al. 2017) or uncovering 'cryptic' behavioural variation (with novel behaviours and increased variance in behavioural expression (Schlichting 2008). We thus advocate using mesocosm or other semi-natural setups (e.g. Thorlacius et al. 2015; Sudo and Tsukamoto 2015; Hirsch et al. 2017; Thorlacius and Brodin 2018; Coates et al. 2019; Schirmer et al. 2019; Dhellemmes et al. 2020; Niemelä et al. 2021), to bridge lab and field studies. They circumvent the mentioned drawbacks and provide valuable insights undetectable in classical behavioural setups, especially for wild populations.

Our results further support the idea that forced isolation in freshwater is followed by phenotypic changes as reported for sticklebacks isolated after the last glacial retreat (e.g. reduction in lateral plates and reduced swimming abilities; Tudorache et al. 2007; Dalziel et al. 2012; Kitano et al. 2012). Many of these morphological and behavioural changes are underlined by genetic differentiation and are true adaptations to a resident lifestyle (Colosimo 2005; Chan et al. 2010). Additionally, we show that freshwater-induced phenotypic changes in sticklebacks can occur even on contemporary timescales (see also Lescak et al. 2015; Hosoki et al. 2020; Garcia-Elfring et al. 2021) and can have a genetic component (Ramesh et al. 2021). Residents in our study populations are thus likely on a trajectory to losing their migration tendencies and already (partially) adapted to complete residency. Current conservation management includes building fishways to reconnect land-locked and migratory populations. In this context, it is important to consider that residents may be less likely to use fishways due to lowered migration tendencies. This may require a revision in the evaluation criteria for the success of these conservation efforts. An exciting future avenue will be to study to what extent and how quickly individual migration tendencies will be affected when the two populations reconnect.

Acknowledgements

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Appendix

Supplementary information 1: Description of the mesocosm and the tracking setup

Experiment-1

Within-pond movements:
On the morning of testing (∼10 a.m.), one test group of each origin was released into the first pond that was temporary disconnected from the other ponds by a cap blocking the entrance to the corridor. There was no water flow when recording within-pond movement tendencies. Five circular antennas were placed upright on the bottom of the pond ('bottom antennas'), and four antennas were placed just below the water surface ('surface antennas'; Fig. 4.1). To assess within-pond movements, we computed crosses that an individual made between bottom antennas or the surface antennas separately. Crosses that were made between a bottom and surface antenna were excluded as these hardly occurred. The experiment lasted for five hours.

Between-pond movements:
After five hours, we gently removed all antennas from the first pond. At this point we also turned on the flow in the system to create a cue for migration (Fig. 4.2c). Fish were given 30 minutes to recover from the disturbance caused by removing the antennas after which the connection from pond 1 to the other ponds was gently opened. We then recorded the movement of fish between the five connected ponds ('crosses') for the next 16.5 h (∼3.30 p.m. – 8 a.m.). At the end of the experiment, fish were returned to their original smaller housing ponds. Testing all 5 migrant and 6 resident groups took place over a week (temperature ranged between 12°C and 15°C). All fish were checked at the end of the experiment to see if they still carried the tags and if the tags functioned correctly.

Experiment-2

Between-pond movements Two weeks after we finished recording each individual for movement tendencies as above, we created one large group each of migrant and resident by combining all the fish ($N_{mig} = 1$ groups, 45 individuals; $N_{res} = 1$ group, 52 individuals) and monitored only the movement tendencies between-ponds simultaneously for the two groups and continuously for four consecutive days. During the study period, we furthermore alternated days with and without water flow (flow turned on / off at 10:00 a.m each day and hence kept in that condition for ∼24 hours). The flow treatment allowed testing whether the populations react differently to the presence of a migration cue.

Supplementary information 2: Study populations and housing of fish

We caught incoming migrants at a sea lock at the mouth of a river in Nieuwe Statenzijl ('NSTZ'; 53°13′54.49″, 7∘12′30.99″), and resident sticklebacks in an adjacent landlocked polder ('LL-A'; 53°17′56.14″, 7°2′1.28″) in the province of Groningen, The Netherlands (1). Fish were caught at the onset of inland migration, over a period of four weeks in March and April 2020. Fish of ≥ 4 cm in total length (from the tip of the snout to the tip of the tail) were transported to the lab in aerated plastic bags within two hours of capture. After acclimatization, fish were housed in groups of 25, separated by their origin (migrant or resident), for a week prior to experimentation in small holding ponds (∼100 L tanks filled with freshwater from a nearby ditch) under natural temperature and light conditions. Fish were fed a mixture of brine shrimps and blood worms (3F Frozen Fish Food b.), once a day, ad libitum. Fish were tagged with 8 mm Passive Integrated Transponders (PIT tag; Trovan, Ltd., Santa Barbara, California) for individual identification, under anaesthetization in buffered MS-222 solution (0.25 – 0.30 g/L ; pH = 7.5 - 8.0). PIT tags were injected in the abdominal cavity (following (2)). Before experiments, all fish were allowed at least five days of recovery in the housing pond with the same group. Mortality rate after PIT tagging was very low (<1% in the first week).

Supplementary information 3: Estimating consistency of between-pond movements

To quantify individual consistency in between-pond movements across ecological contexts (flow/no flow), we ran univariate generalised linear mixed model (GLMMs) with Poisson errors using the dataset from Experiment-2 and the lme4 package (3). For repeatability across social environments (small vs large group size), we combined the crosses data from Experiment-1 and day 1 and 3 of Experiment-2 with flow. We used the number of crosses between ponds as the response variable, with origin (resident vs. migrant), treatment (social context: small vs large group size or ecological context:

flow vs no-flow in two separate models) and their interaction (*origin* × *treatment*) as a fixed factors and individual-ID as a random effect. In addition, we added Obs as observation-level random effects to control for over-dispersion (OLRE, (4)). We used these 'full' and 'simplified' models (omitting all the fixed effects) to calculate 'adjusted' and 'raw' repeatabilities respectively. Repeatabilities are defined as the ratio of among-individual variance (V_{ind}) to total variance ($V_{total} = V_{ind} + V_{residual}$). We calculated repeatabilities in their original scale, along with their confidence intervals using the 'rpt' function with 1000 bootstraps using the 'rptR' package (5). We were not able to calculate repeatabilities for different social contexts due to lack of model convergence. Hence we resorted to using Spearman correlation as the data is not normally distributed. All analyses were carried out in R (R Core Team 2021).

Between-pond movement was moderately repeatable across ecological contexts (Adjusted R(95% CI) = 0.42 (0.34, 0.51) and Raw R(95% CI) = 0.38 (0.30, 048)). Across social context, individuals were not very consistent with low correlation coefficients (Spearman $\rho = 0.35$, $p < 0.001$). This could be because timescale and sample size were not balanced between Experiments 1 and 2. While repeated data were collected over consecutive days in Experiment 2, single data points were collected a month apart in Experiment-1. However we see that the residents were consistently moving less than migrants in all contexts.

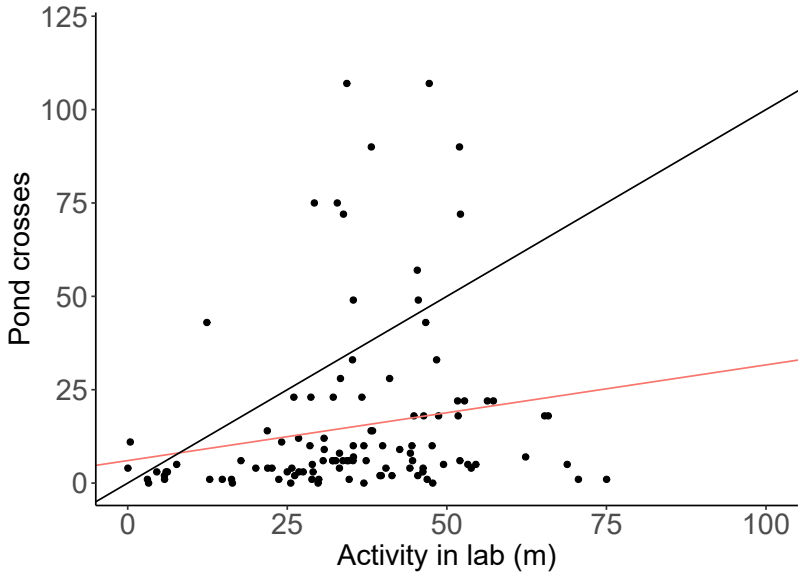

Figure 4.A1 – *Correlation of movement tendencies of lab-raised F1 migrants, residents and hybrids tested in the lab and in the mesocosm. In a separate experiment and set of animals, (F1 sticklebacks raised in the lab from (1)), we performed both, an activity assay in the lab, where individual fish were assessed for general movement tendencies for 20 minutes in their home tank (30 × 16 × 18 cm (L × W × H)) (according to methods in (6)) and movement tendencies across-ponds in the mesocosm as in Experiment-1. Black line represents the identity lines, $x = y$. The red line is the ordinary least squares regression line. Lab-based activity (total distance covered in meters in 20 mins) and number of pond crosses in the mesocosm were positively and significantly correlated (Spearman $\rho = 0.33$, $p < 0.01$).*