The eco-evo-devo of stickleback personalities

Gismann, Jakob R.L.

DOI:
10.33612/diss.1165822955

2

Mesocosm experiment reveals scale dependence of movement tendencies in sticklebacks

J. Gismann*
A. Ramesh*
A.G.G. Groothuis
F.J. Weissing
& M. Nicolaus

Biology Letters (2023), 19(4), 20220602
* Joint first authors

2

Abstract

Habitat fragmentation can have negative impacts on migratory organisms that rely on the functional connectivity between growing and breeding grounds. Quantifying the population-level phenotypic consequences of such fragmentation requires fine-scaled tracking of individual behaviour and movements across relevant scales. Here we make use of a natural experiment where some populations of 'migrant' three-spined sticklebacks (*Gasterosteus aculeatus*) became 'residents', following habitat fragmentation five decades ago. To test whether residents have a lower movement tendency than migrants, we developed a novel experimental platform that allows the automated tracking of individual movements via RFID technology in a semi-natural mesocosm where spatio-temporal scales and environmental conditions can be manipulated. We found that residents moved significantly less than migrants at large but not at small spatial scale. This pattern was consistent across time and contexts (water flow and group size). Our study substantiates prior literature on rapid phenotypic divergence in sticklebacks in response to human-induced isolation and highlights the importance of observing behaviour in ecologically relevant set-ups that bridge the gap between laboratory and field studies.

2.1 Introduction

Habitat fragmentation is a major threat for many animals, particularly for migratory species that depend on multiple habitats to complete their life cycle (Legrand et al., 2017). Water management efforts worldwide have disrupted the connectivity between marine and freshwater habitats, confining some fish populations to only freshwater habitats without the possibility of migrating to the sea. Such forced isolation can cause rapid phenotypic responses and life-history changes (mammals and birds: Soriano-Redondo et al. (2020); fish: Augspurger et al. (2017); Closs et al. (2013); Dodson et al. (2013); Quinn and Myers (2004)). Species' responses to reduced connectivity, forced isolation, decreased densities and smaller population sizes will inform us whether and how animal populations can cope with these human- induced changes. In this context, behavioural responses are crucial in determining success in persisting in fast-changing environments, especially in the initial stages (Sih et al., 2016). However, studying such responses typically requires quantification of behaviour and movement at the individual level, which is often challenging in small-bodied species and especially so in the aquatic environment. For example, in the wild, individual tracking of small organisms' movements is often either impossible or gives rise to uncertainty in the observations, as a large proportion of data is often missed (mark–recapture experiments in the wild often have low recapture rates or biased recapture due to animal behaviour or weather conditions Lee et al. (2014); Mazerolle (2015). While laboratory studies can be highly controlled and allow for experimental manipulation, they regularly suffer from spatio-temporal limitations and most importantly lack ecological relevance due to limited environmental complexity compared to natural environments (Calisi and Bentley, 2009; Krause et al., 2013; Niemelä and Dingemanse, 2014; Pritchard et al., 2016).

Using individual laboratory-based assays, we have previously shown that, in the Netherlands, 'resident' populations of three-spined sticklebacks (*Gasterosteus aculeatus*), isolated in freshwater ditches due to man-made barriers for approximately 50 years, diverged in behaviour (and morphology) from their 'migrant' ancestors (Ramesh et al., 2022). Yet, contrary to our expectations (and other findings for example on higher swimming performance and endurance of migrants in comparison to residents (Dalziel et al., 2012; Dalziel and Schulte, 2012)), migrants did not exhibit higher movement tendencies than residents. Moreover, residents were more active and exploratory than migrants (Ramesh et al., 2022). We speculated that the counterintuitive nature of the results could be due to a freezing or stress response of migrants in the absence of a social group (Ramesh et al., 2022; Huntingford and Wright,

1993). Further, the small- scale experimental settings in the laboratory may not be suited to study larger scale movement, which we explicitly aim to test here.

In recent decades, the use of passive integrated transponders (PIT tags) has become very prominent in the studies of movement patterns in wild populations Gibbons and Andrews (2004), including small organisms such as passerine birds (Nicolaus et al., 2008; Schroeder et al., 2011), insects (Niemelä et al., 2021) and fish (Cousin et al., 2012). With this technology, individual behaviour and social associations can be measured using remote detections at fixed locations. In this study, we describe a novel experimental mesocosm set-up that uses PIT tags and a radio-frequency identification (RFID) system to quantify individual behaviour and movement of small fish in semi-natural conditions, thus providing relevant environmental complexity and temporal scales while allowing the investigation of movement across different spatial scales. The mesocosm consists of several connected semi-natural ponds equipped with RFID antennas to monitor individual movement tendencies. Here we particularly address if (i) spatial scale matters for uncovering population divergence in movement tendencies and (ii) movement tendencies of migrant and resident fish are consistent across ecological conditions such as water flow and group size.

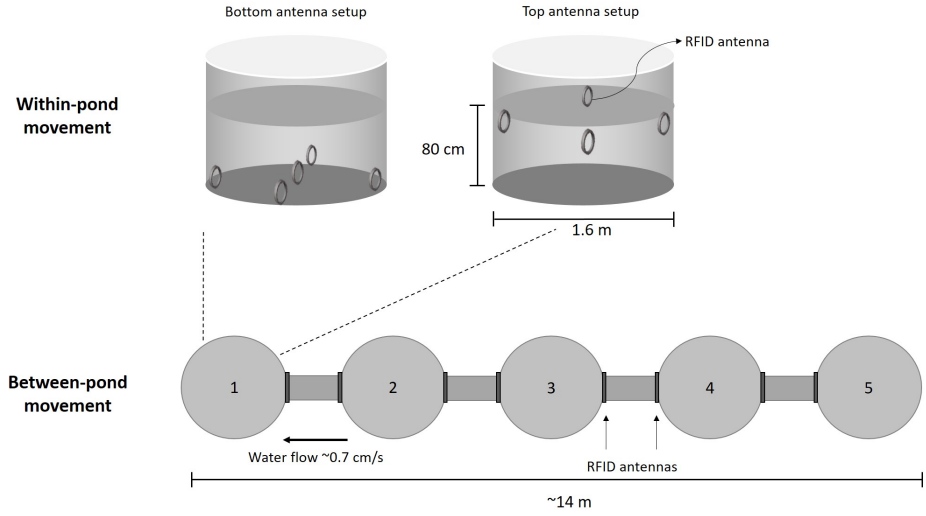

Figure 2.1 | Experimental set-up. Each mesocosm consisted of five linearly connected above-ground ponds (1–5) equipped with circular RFID antennas that automatically detect crosses of PIT-tagged individuals. Fish were released into pond 1. This pond was equipped with nine RFID antennas (five on the bottom 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.

2.2 METHODS

2.2.1 MESOCOSM SYSTEM

The experiments were conducted in two independent mesocosms of five above-ground ponds (each Ø 1.6 m, with a water depth of 80cm), connected linearly with opaque corridors (each of length approx. 1.5 m and Ø 11 cm), spanning a linear distance of approximately 14 m (Figure2.1). The system is supplied with freshwater from a natural ditch with the possibility of creating water flow (approx. 0.7 cm/s) that mimics conditions in Dutch canals and represents a directional migration cue together with seasonal changes of temperature and photoperiod (Jonsson, 1991). This system enabled measurement of the movements of individual sticklebacks within- and between-ponds. The first pond (labelled 1 in Figure 1), enriched with plastic plants, was used to quantify within-pond movement, while the whole system of five connected ponds was used to record between-pond movement tendencies (see Supplementary Information 1, Fig A1 for details).

Our tracking system consisted of circular RFID antennas (Ø 10 cm), data loggers and PIT tags (Trovan, Ltd, Santa Barbara, California) to record movements of PIT-tagged sticklebacks (details in Supplementary Information 2). 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 (Figure2.1). 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 no detections were missed and that reads corresponded well with the entry and exit times of fish.

2.2.2 ETHICS

Wild animals were sampled using a fishing permit from Rijksdienst voor Ondernemend Nederland (the Netherlands) and an angling permit from the Hengelsportfederatie Groningen-Drenthe. Housing and testing of behaviours were in adherence to the project permit from the Central Committee on Animal Experiments (CCD, the Nether-lands) under the licence number AVD1050020174084.

2.2.3 Experiment 1: movement across spatial scales in the mesocosm

We created five groups of migrants and six groups of residents, each consisting of 10 randomly selected individuals (total: Nmig = 49 and Nres = 60). While we always tried to maintain the group size to 10 fish by making up the group to 10 with untagged individuals, tag loss and other technical difficulties (such as retagging and waiting for recovery) led to one group of migrants having nine fish and another with 11 fish, in order to test all the tagged fish. Groups were housed in separate small holding ponds for 24 h before the start of the experiment. On the experimental day, one resident and one migrant group were released simultaneously (to avoid temperature or temporal 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 5 h (Figure 2.1) and then for between-pond movement for approximately 16.5 h, after opening the connection to the other ponds (Figure 2.1; Supplementary Information 1 and 2).

2.2.4 Experiment 2: effect of group size and water flow on movement

In a next step (after about one month), we combined all migrants and, separately, all residents (after excluding 12 fish that had either died or lost tags) into two large groups, which reflect natural conditions more closely, as stickleback prefer larger over smaller groups (Thünken et al., 2014) (Nmig = 45, Nres = 52; one group of migrants and one group of residents) and quantified between-pond movements in these two groups in the two mesocosm set-ups over 4 days. In addition, we alternated flow and no-flow conditions on consecutive days (see Supplementary Information 1).

2.2.5 Analyses

We first cleaned the data from small read errors which could be easily corrected given the high sensitivity of the RFID system (3– 5 reads/second). Then, for each individual, we quantified within-pond movement as the number of times each fish moved between different bottom antennas or different surface antennas, respectively (Figure2.1). We deemed the number of consecutive visits to a particular antenna unreliable for measuring movement patterns because of the possibility that a prolonged visit to a given antenna might be recorded as multiple disconnected set of reads, appearing as if the fish visited the antenna multiple times. Between-pond movement was quantified as the

number of crosses a fish made through the corridors connecting two ponds (Figure2.1). Fish that were not 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 and whether they were consistent across contexts (group size and flow). Briefly, we considered the number of crosses within- and between-ponds as response variables separately in univariate generalized linear mixed models with Poisson errors. In all models, we included origin (resident versus migrant) as a fixed factor and group-ID (to account for pseudo replication) and an observation level 'Obs' (to control for overdispersion, Harrison (2014)) as random effects. Additionally, we analysed whether the fraction of fish that did not exit the first pond differed between migrants and residents in Experiment 1, using Fisher's exact test. Repeatability and correlation of number of crosses across contexts were also calculated (electronic supplementary material, S3). For Experiment 2 (effect of group size and water flow on movement), our effective sample size was one per origin, hence we refrained from conducting statistical analysis and interpreted results from the plot. We estimated individual consistency of between-pond movements using repeatability and correlation coefficients, which are provided in the supplementary materials (Supplementary Information 3). All analyses were carried out in R v. 4.1.0, (R Core Team, 2021). Complete description of the analyses and code are given in the supplementary materials.

2.3 RESULTS

2.3.1 MOVEMENT ACROSS SPATIAL SCALES IN THE MESOCOSM

Within the first pond, residents and migrants showed a broad distribution of number of crosses at both bottom and top antennas (Figure 2.2a,b) and the number of crosses were not different between the two groups in both cases (table 2.1; median bottom-antenna crosses: residents=23, migrants= 14; median top-antenna crosses: residents=3.5, migrants= 8). By contrast, the number of movements between ponds was smaller in residents than in migrants (Figure 2.2c; effect of Origin in table 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 higher in residents than in migrants (55% in residents versus 28.6% in migrants, odds ratio = 3.02, p = 0.007).

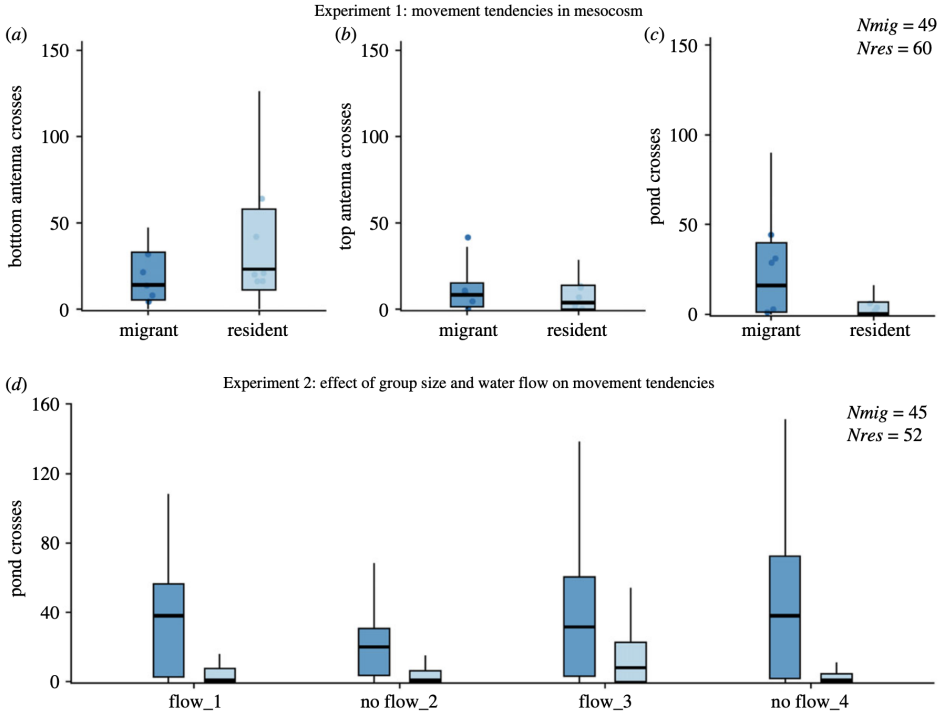

Figure 2.2 | Within-pond and between-pond movement of resident and migrant sticklebacks. **(a,b)** Within-pond movement between bottom and top antennas, respectively (Experiment 1); **(c)** between-pond movement in Experiment 1; **(d)** between-pond crosses in relation to the daily flow treatment in Experiment 2. In all graphs, boxplots with median are shown for migrant (dark blue) and resident (light blue) sticklebacks. For (a–c), we have also represented the medians of each test group as shaded dots (migrant = five groups, resident = six groups).

Table 2.1 | Results of the statistical analysis of movement within- and between-ponds using generalized linear mixed models. Estimates of fixed effects (β) on a log-scale are given with their 95% confidence intervals (CI), Z-values (Wald statistic), p-values and variance components are given with their s.d. Sample sizes Experiment 1: Nmig = five groups (49 individuals), Nres = six groups (60 individuals).

	Experiment-1		
	Bottom crosses	Top crosses	Pond crosses
Fixed effects	β	β	β
	(95% CI)	(95% CI)	(95% CI)
Intercept	**2.61**	**1.98**	**1.90**
	(2.13, 3.08)	**(0.30, 3.63)**	**(0.63, 3.13)**
Origin[1]	0.51	-0.68	**-2.26**
	(-0.12, 1.15)	(-3.03, 1.53)	**(-4.04, -0.58)**
Random effects	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)

2.3.2 Effect of group size and water flow on movement

In large social groups, residents again moved consistently less between ponds than migrants (median pond crosses over the 4 experimental days range between 1 and 6 for residents and between 20 and 38 for migrants, Figure 2.2d). Therefore, we conclude that differences between residents and migrants were maintained regardless of changing group size or differing ecological (flow) conditions.

2.4 Discussion

Previous studies in sticklebacks that have quantified population movement tendencies under laboratory conditions showed mixed or counterintuitive patterns: residents showed either higher (Ramesh et al., 2022) or inconsistent patterns (Di-Poi et al., 2014) in activity/exploration levels compared to migrants. In this study, we show that migrants and residents differ in their movement tendency only on a larger spatial scale (between- ponds), while no differences could be detected on a smaller scale (within-ponds). It is thus conceivable that previous inconsistencies stem from the fact that the experimental set-ups did not offer biologically relevant testing conditions. The use of semi-natural mesocosms, as described here, may thus be a more appropriate way to characterize individual and population movement related

to migration.

It is biologically plausible that movement tendencies are scale dependent. Movement measured at very different spatial scales (from 30 cm in the laboratory to 1.5 m within- ponds to 14 m across ponds) may reflect functionally different behaviours. For example, individual measurements on smaller scales, in the laboratory, may be an indication of a stress response to social isolation (Huntingford and Wright, 1993). By contrast, 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 tens of kilometres inland within a few days (pers. comm. from water authorities) and thus require sufficient space to express their natural behaviour.

Tests in the laboratory, though invaluable for studies on animal behaviour owing to controlled settings, are not without drawbacks. Laboratory tests are usually performed in highly controlled and novel environments. 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 mesocosms or other semi-natural set-ups (e.g. Coates et al. (2019); Dhellemmes et al. (2020); Hirsch et al. (2017); Niemelä et al. (2021); Schirmer et al. (2019); Sudo and Tsukamoto (2015); Thorlacius et al. (2015); Thorlacius and Brodin (2018)), to bridge laboratory and field studies. They circumvent the mentioned drawbacks and may provide valuable insights undetectable in classical behavioural set-ups, especially for wild populations. In addition, the modular nature of the described mesocosm offers flexibility in the spatial organization of the individual ponds and antennas. This allows for classical tests, such as choice tests, to be conducted in a more sophisticated manner. We are confident that such systems, enabling remote tracking and yielding high-resolution data over longer periods of time will become common in behavioural studies.

Our results show that freshwater-induced phenotypic changes in sticklebacks can occur on contemporary timescales (see also Dalziel et al. (2012); Garcia-Elfring et al. (2021); Hosoki et al. (2019)) and a follow-up study showed that some of these have a genetic component (Ramesh et al., 2021). The direction of these phenotypic changes is similar to the behavioural and morphological adaptions reported in stickleback populations that have colonized freshwater habitats after the last glacial retreat (Chan et al., 2010; Colosimo et al., 2005;

Kitano et al., 2012; Lescak et al., 2015; Tudorache et al., 2007). Residents in our study populations are thus likely on a trajectory to lose their migration tendencies and already (partially) adapted to complete residency.

2.5 Acknowledgements

We thank Dennis de Worst and Willem Diderich for help with fish care and advice on experimental design and other animal caretakers for looking after the sticklebacks. We thank Peter Paul Schollema from the Water Authorities Hunze en Aa's and Jeroen Huisman from van Hall Larenstein, University of Applied Sciences for help with catching sticklebacks. We also thank the two anonymous reviewers and the editor for their valuable feedback on the manuscript.

References

Augspurger, J. M., Warburton, M., and Closs, G. P. (2017). Life-history plasticity in amphidromous and catadromous fishes: a continuum of strategies. *Reviews in Fish Biology and Fisheries*, 27(1):177–192.

Bolnick, D. I., Snowberg, L. K., Patenia, C., Stutz, W. E., Ingram, T., and Lau, O. L. (2009). Phenotype-dependent native habitat preference facilitates divergence between parapatric lake and stream stickleback. *Evolution*, 63(8):2004–2016.

Calisi, R. M. and Bentley, G. E. (2009). Lab and field experiments: Are they the same animal? *Hormones and Behavior*, 56(1):1–10.

Chan, Y. F., Marks, M. E., Jones, F. C., Villarreal, G., Shapiro, M. D., Brady, S. D., Southwick, A. M., Absher, D. M., Grimwood, J., Schmutz, J., Myers, R. M., Petrov, D., Jónsson, B., Schluter, D., Bell, M. A., and Kingsley, D. M. (2010). Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx*1 enhancer. *Science*, 327(5963):302–305.

Closs, G. P., Hicks, A. S., and Jellyman, P. G. (2013). Life histories of closely related amphidromous and non-migratory fish species: a trade-off between egg size and fecundity. *Freshwater Biology*, 58(6):1162–1177.

Coates, W. D., Hale, R., and Morrongiello, J. R. (2019). Dispersal decisions and personality in a freshwater fish. *Animal Behaviour*, 157:209–218.

Colosimo, P. F., Hosemann, K. E., Balabhadra, S., Villarreal, G., Dickson, H., Grimwood, J., Schmutz, J., Myers, R. M., Schluter, D., and Kingsley, D. M. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*, 307(5717):1928–1933.

Cousin, X., Daouk, T., Péan, S., Lyphout, L., Schwartz, M. E., and Bégout, M. L. (2012). Electronic individual identification of zebrafish using radio frequency identification (rfid) microtags. *Journal of Experimental Biology*, 215:2729–2734.

Dalziel, A. C. and Schulte, P. M. (2012). Correlates of prolonged swimming performance in F2 hybrids of migratory and non-migratory threespine stickleback | Journal of Experimental Biology. *Journal of Experimental Biology*, 215(20):3587–3596.

Dalziel, A. C., Vines, T. H., and Schulte, P. M. (2012). Reductions in prolonged swimming capacity following freshwater colonization in multiple threespine stickleback populations. *Evolution*, 66(4):1226–1239.

Dhellemmes, F., Finger, J. S., Laskowski, K. L., Guttridge, T. L., and Krause, J. (2020). Comparing behavioural syndromes across time and ecological conditions in a free-ranging predator. *Animal Behaviour*, 162:23–33.

Di-Poi, C., Lacasse, J., Rogers, S. M., and Aubin-Horth, N. (2014). Extensive Behavioural Divergence following Colonisation of the Freshwater Environment in Threespine Sticklebacks. *PLoS ONE*, 9(6):e98980.

Dodson, J. J., Aubin-Horth, N., Thériault, V., and Páez, D. J. (2013). The evolutionary ecology of alternative migratory tactics in salmonid fishes. *Biological Reviews*, 88(3):602–625.

Garcia-Elfring, A., Paccard, A., Thurman, T. J., Wasserman, B. A., Palkovacs, E. P., Hendry, A. P., and Barrett, R. D. H. (2021). Using seasonal genomic changes to understand historical adaptation to new environments: Parallel selection on stickleback in highly-variable estuaries. *Molecular Ecology*, 30:2054–2064.

Gibbons, W. J. and Andrews, K. M. (2004). Pit tagging: simple technology at its best. *BioScience*, 54:447–454.

Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, 2:e616.

Hirsch, P. E., Thorlacius, M., Brodin, T., and Burkhardt-Holm, P. (2017). An approach to incorporate individual personality in modeling fish dispersal across in-stream barriers. *Ecology and Evolution*, 7(2):720–732.

Hosoki, T., Mori, S., Nishida, S., . . . , M. K. E., and undefined 2019 (2019). Diversity of gill raker number and diets among stickleback populations in novel habitats created by the 2011 tōhoku earthquake and tsunami. *Evol. Ecol. Res.*, 20:213–230.

Huntingford, F. A. and Wright, P. J. (1993). The development of adaptive variation in predator avoidance in freshwater fishes. *Marine Behaviour and Physiology*, 23(1-4):45–61.

Jonsson, N. (1991). Influence of water flow, water temperature and light on fish migration in rivers. *Nordic Journal of Freshwater Research*, 66:20–35.

Kitano, J., Ishikawa, A., Kume, M., and Mori, S. (2012). Physiological and genetic basis for variation in migratory behavior in the three-spined stickleback, *Gasterosteus aculeatus*. *Ichthyological Research*, 59(4):293–303.

Krause, J., Krause, S., Arlinghaus, R., Psorakis, I., Roberts, S., and Rutz, C. (2013). Reality mining of animal social systems. *Trends in Ecology and Evolution*, 28(9):541–551.

Lee, K. A., Huveneers, C., Gimenez, O., Peddemors, V., and Harcourt, R. G. (2014). To catch or to sight? a comparison of demographic parameter estimates obtained from mark-recapture and mark-resight models. *Biodiversity and Conservation*, 23:2781–2800.

Legrand, D., Cote, J., Fronhofer, E. A., Holt, R. D., Ronce, O., Schtickzelle, N., Travis, J. M., and Clobert, J. (2017). Eco-evolutionary dynamics in fragmented landscapes. *Ecography*, 40:9–25.

Lescak, E. A., Bassham, S. L., Catchen, J., Gelmond, O., Sherbick, M. L., Van Hippel, F. A., Cresko, W. A., Von Hippel, F. A., and Cresko, W. A. (2015). Evolution of stickleback in 50 years on earthquake-uplifted islands. *Proceedings of the National Academy of Sciences of the United States of America*, 112(52):E7204–E7212.

Mazerolle, M. J. (2015). Estimating detectability and biological parameters of interest with the use of the r environment. *Journal of Herpetology*, 49:541.

Nicolaus, M., Bouwman, K. M., and Dingemanse, N. J. (2008). Effect of pit tags on the survival and recruitment of great tits parus major. *Ardea*, 96:286–292.

Niemelä, P. T. and Dingemanse, N. J. (2014). Artificial environments and the study of 'adaptive' personalities. *Trends in Ecology & Evolution*, 29:245–247.

Niemelä, P. T., Tiso, S., and Dingemanse, N. J. (2021). Density-dependent individual variation in male attractiveness in a wild field cricket. *Behavioral Ecology*, 32:707–716.

Pritchard, D. J., Hurly, T. A., Tello-Ramos, M. C., and Healy, S. D. (2016). Why study cognition in the wild (and how to test it)? *Journal of the Experimental Analysis of Behavior*, 105(1):41–55.

Quinn, T. P. and Myers, K. W. (2004). Anadromy and the marine migrations of Pacific salmon and trout: Rounsefell revisited. *Reviews in Fish Biology and Fisheries*, 14(4):421–442.

R Core Team (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ramesh, A., Domingues, M. M., Stamhuis, E. J., Groothuis, T. G. G., Weissing, F. J., and Nicolaus, M. (2021). Does genetic differentiation underlie behavioral divergence in response to migration barriers in sticklebacks? a common garden experiment. *Behavioral Ecology and Sociobiology*, 75:161.

Ramesh, A., Groothuis, T. G. G., Weissing, F. J., and Nicolaus, M. (2022). Habitat fragmentation induces rapid divergence of migratory and isolated sticklebacks. *Behavioral Ecology*, 33:167–177.

Schirmer, A., Herde, A., Eccard, J. A., and Dammhahn, M. (2019). Individuals in space: personality-dependent space use, movement and microhabitat use facilitate individual spatial niche specialization. *Oecologia*, 189(3).

Schlichting, C. D. (2008). Hidden reaction norms, cryptic genetic variation, and evolvability. *Annals of the New York Academy of Sciences*, 1133(1942):187–203.

Schroeder, J., Cleasby, I. R., Nakagawa, S., Ockendon, N., and Burke, T. (2011). No evidence for adverse effects on fitness of fitting passive integrated transponders (pits) in wild house sparrows passer domesticus. *Journal of Avian Biology*, 42:271–275.

Sih, A., Trimmer, P. C., and Ehlman, S. M. (2016). A conceptual framework for understanding behavioral responses to hirec. *Current Opinion in Behavioral Sciences*, 12:109–114.

Sommer-Trembo, C., Petry, A. C., Gomes Silva, G., Vurusic, S. M., Gismann, J., Baier, J., Krause, S., Iorio, J. d. A. C., Riesch, R., and Plath, M. (2017). Predation risk and abiotic habitat parameters affect personality traits in extremophile populations of a neotropical fish (*Poecilia vivipara*). *Ecology and Evolution*, 7(16):6570–6581.

Soriano-Redondo, A., Gutiérrez, J. S., Hodgson, D., and Bearhop, S. (2020). Migrant birds and mammals live faster than residents. *Nature Communications*, 11:5719.

Sudo, R. and Tsukamoto, K. (2015). Migratory restlessness and the role of androgen for increasing behavioral drive in the spawning migration of the japanese eel. *Scientific Reports*, 5(1):1–7.

Thorlacius, M. and Brodin, T. (2018). Investigating large-scale invasion patterns using-small scale invasion successions—phenotypic differentiation of the invasive round goby (*Neogobius melanostomus*) at invasion fronts. *Limnology and Oceanography*, 63(2):702–713.

Thorlacius, M., Hellström, G., and Brodin, T. (2015). Behavioral dependent dispersal in the invasive round goby, *Neogobius melanostomus* depends on population age. *Current Zoology*, 61(3):529–542.

Thünken, T., Eigster, M., and Frommen, J. G. (2014). Context-dependent group size preferences in large shoals of three-spined sticklebacks. *Animal Behaviour*, 90:205–210.

Tudorache, C., Blust, R., and Boeck, G. D. (2007). Swimming capacity and energetics of migrating and non-migrating morphs of three-spined stickleback Gasterosteus aculeatus L. and their ecological implications. *Journal of Fish Biology*, 71(5):1448–1456.

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 temporarily 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. 2.1). 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. 2.1). 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 16.5h (∼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 land-locked 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 $\geq$ 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 ($\sim$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: Consistency of between-pond movements across ecological and social context

To quantify individual consistency in between-pond movements across ecological contexts (i.e., individuals' movement under flow vs no-flow conditions), we ran univariate generalised linear mixed models (GLMMs) with Poisson errors using the dataset from Experiment-2 and the lme4 package (3) and calculated repeatabilities using the 'rptR' package (4). For repeatability across social context (i.e., individuals' movement in small vs large groups), we combined the data on the number of pond crosses from Experiment-1 (small group) with days 1 and 3 (which had water flow, thus excluding differences in ecological context) of Experiment-2 (large group).

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, (5)). 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_{ind}) to total variance ($V_{total} = V_{ind} + V_{residual}$l). We calculated repeatabilities in their original scale of measurement (not log scale), along with their confidence intervals using the 'rpt' function with 1000 bootstraps using the 'rptR' package (4). We were not able to calculate repeatabilities for different social contexts due to lack of model convergence. Hence, we resorted to using Spearman correlation to quantify consistency of pond crosses in the small group vs first day in the large group. All analyses were carried out in R v. 4.1.0, (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 the timescale and the sample size were not balanced between Experiment-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.

Additionally, in order to investigate the relationship between measurements in the laboratory and in the mesocosm, we calculated the Spearman rank correlation coefficient between activity as measured in a classical lab test and across-pond movement in the mesocosm (Fig. A1) on a separate set of fish (F1 sticklebacks raised in the lab from (1)).

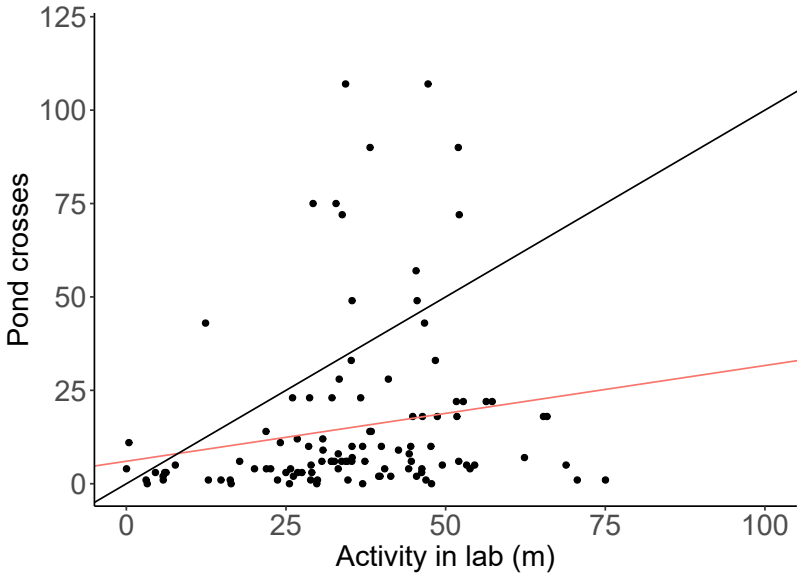

Figure A1 | Correlation of movement tendencies measured of lab-raised F1 sticklebacks tested in the lab and in the mesocosm. In a separate experiment and on a separate set of fish, (F1 sticklebacks of migrant, resident and hybrid backgrounds raised in the lab from (1)), we performed both, an activity 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. 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 correlated. Since the data were not normally distributed, we calculated the Spearman rank correlation coefficient; $\rho = 0.33$, $p < 0.01$.

References Appendix

1. Ramesh A, Groothuis TGG, Weissing FJ, Nicolaus M. Habitat fragmentation induces rapid divergence of migratory and isolated sticklebacks. *Behav Ecol.* 2022;33(1):167-77.

2. Cousin X, Daouk T, Pean S, Lyphout L, M S, M B. Electronic individual identification of zebrafish using radio frequency identification (RFID) microtags. *J Exp Biol.* 2012;215:2729–34.

3. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Soft.* 2015;67(1):1-48.

4. Stoffel MA, Nakagawa S, Schielzeth H. rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution.* 2017;8(11):1639-44.

5. Harrison XA. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ.* 2014;2:e616.

6. Ramesh A, Domingues MM, Stamhuis EJ, Groothuis TGG, Weissing FJ, Nicolaus M. Does genetic differentiation underlie behavioral divergence in response to migration barriers in sticklebacks? A common garden experiment. *Behav Ecol Sociobiol.* 2021;75(12):1-2.