Determining homing abilities of nesting male threespine stickleback (Gasterosteus aculeatus)

Authors: Martin, Marielle V., Van Dame, Katherine, Ryan, Sidney, and Stuart, Yoel E.

Source: BIOS, 95(4) : 246-250

Published By: Beta Beta Beta Biological Society

URL: https://doi.org/10.1893/BIOS-D-23-00016

BIOS

Research Article
https://doi.org/10.1893/BIOS-D-23-00016

Determining homing abilities of nesting male threespine stickleback (*Gasterosteus aculeatus*)

Marielle V. Martin, Katherine Van Dame, Sidney Ryan, and Yoel E. Stuart

Department of Biology, Loyola University Chicago, Chicago USA

Correspondence to: mmartin1419@outlook.com

Abstract

Homing behavior allows individuals to return to a home site when displaced, presumably increasing fitness by increasing access to known food resources, refuges from predators, and breeding opportunities. Homing has been demonstrated in *Gasterosteus aculeatus*, also known as the threespine stickleback. We hypothesized that because stickleback males guard a nest and tend to the eggs and fry, they should be particularly incentivized to home. In an experiment, we marked and displaced nesting male stickleback over a variety of distances to see how far they were able to home and whether homing success declined with distance displaced. We found that stickleback males did home, which is consistent with other studies. Additionally, we found that the return probability decreased with distance displaced, though not significantly. However, some nests were clearly occupied by new, unmarked males after displacement, meaning that even if an experimentally displaced fish returned, they might not have been able to regain their nest. Removing these cases from the data strengthened the expected negative relationship between distance and return, suggesting that stickleback use landmark cues to navigate home.

Keywords: Site fidelity; Landmark recognition; Priority effect

How to Cite: Martin MV, Van Dame K, Ryan S, Stuart YE. 2024. Determining homing abilities of nesting male threespine stickleback (*Gasterosteus aculeatus*). Bios 95:246–250. DOI:10.1893/BIOS-D-23-00016.

Received 12 July 2023; revised 7 August 2024; accepted 19 September 2024.

Introduction

Homing, when an organism returns to its home range after being displaced, is present across diverse vertebrae taxa including birds, reptiles, and fish (Taylor et al. 2016; Ward et al. 2013; Schmidt 2004). Homing may rely on "true navigation", which is characterized by long distance travel using mechanisms like magnetic fields, or it may rely on learned environmental cues such as landmarks, acoustic, or other signals (Ivanova et al. 2019; Dodson 1988). Homing ability can vary between species, populations, and even individuals (Keefer et al. 2013), making mechanisms difficult to document and understand.

Homing likely evolved due to its potential to increase individual fitness. For example, it improves survival by allowing organisms greater access to food resources and refugia from predators (Ward et al. 2013; Ivanova et al. 2019). Homing can also improve reproductive success by helping an individual maintain presence and priority on a breeding ground (Schmidt 2004; Keefer 2013).

Gasterosteus aculeatus, or the threespine stickleback, is a small bony fish with wide geographical distribution and the ability to live in both freshwater and marine environments (Bell and Foster, 1994). The morphology of the stickleback can vary, not only with location, but also during breeding season when males develop orange underbellies and blue eyes. Its wide distribution and unique and consistent behavior have led it to become a model organism for behavior. One such behavior is the ability to home (Ward et al. 2013; Ivanova et al. 2019). For example, in a study done on non-nesting river stickleback, 80% of fish returned to their capture site regardless of whether displaced upstream or downstream, with most fish orienting in the direction of their home site upon release (Ward et al. 2013). Similarly, when displaced from breeding grounds, marine stickleback were able to return up to 300 meters in a day (Ivanova et al. 2019). Ivanova et al. (2019) suggested that stickleback remember topographical information about their home range, using landmark recognition to home by swimming along shallow sections of the shore with observable landmarks instead of staying in deeper water without landmarks.

One interesting feature of stickleback life history that might incentivize homing is male nesting behavior. In a reversal of sex roles, males build and guard a nest to raise offspring (Bell and Foster 1994). Nesting male stickleback defend against predators and other stickleback with aggressive actions such as biting and dorsal pricking, which is

Figure 1. A marked stickleback, with a blue bead superglued to its left pelvic spine.

accompanied by other non-defensive nesting behavior such as nest fanning and fry retrieval (Bell and Foster 1994). As such, they likely have a smaller home range than non-nesting stickleback and a greater incentive to return.

While non-breeding stickleback have been shown to home back to their areas of capture, it is currently unknown whether nesting male stickleback are able to home to their specific nests when displaced. The aim of this study was to test whether nesting male stickleback return to their nests after displacement, and whether their tendency to return diminishes when the distance they are displaced increases. If stickleback use landmark recognition to home, as hypothesized by Ivanova et al. (2019), then an increase in distance would decrease the likelihood of having familiar landmarks the stickleback could use to home, reducing return rates.

Materials and Methods

In June 2022 we worked in Lower Stella Lake, Vancouver Island, British Columbia. This was a clear, fully freshwater lake where breeding males had been observed (T. Sasser *personal communication*). The water clarity was good enough to see around 10m in any direction and the nests were always less than 2m deep. The stickleback population in this lake is permanently lake locked (i.e., it is not a migratory anadromous population). We worked from a boat launch on the northeast side of the lake (50°18′44.5″N 125°32′18.9″W). The habitat in this area was a bed of grassy reeds on a flat shelf approximately ten meters wide spanning approximately sixty meters of shoreline at 1-2 meters depth. We dry-suit snorkeled to find nesting males: divers slowly swam the habitat, pausing when individual or schools of stickleback were spotted. Nesting males tended to have orange underbellies and vibrant blue eyes and were less likely to flee from divers than non-nesting stickleback.

Upon sighting a potential male, we paused for several minutes until we saw nesting behavior, including egg fanning, aggression towards other males, leading females away from their suspected nest, and repeatedly returning to this nest after being scared off by the diver (Bell and Foster 1994). Then, we used dip nets to collect the nesting male, and placed a marker with a numbered flag near the nest. A partner in a canoe took a GPS coordinate at the nest. For some nests, we also left a GoPro camera overnight set to take a photo every 60 seconds. Captured males were brought to shore by canoe, where they were marked (Fig. 1).

To mark stickleback, we anesthetized individuals one at a time using a dilute tricaine solution (MS222) until they were no longer responsive. We then used Super Glue to adhere one size-6 glass “seed bead” to each pelvic spine, noting bead color for each side. This marking method was adapted from Ivanova et al 2019, where they deemed it a reliable marking method. Bead colors included black, pink, red, orange, yellow, light green, dark green, light blue, dark blue, and white for a total of ten colors and 100 color combinations. We allowed stickleback to recover from anesthesia and, after observing healthy swimming behavior, we released them at a fixed point near the center of the work area. We used the GPS coordinates of the release point and the nests to calculate the distance between the nesting site and the point of release. A total of 41 fish were displaced with a minimum distance of 3 meters and a maximum distance of 58 meters. Animal use was approved by Loyola University Chicago IACUC (Project 2853) and a permit to collect fish was issued from the Ministry of Forests, Lands, Natural Resource Operations and Rural Development of British Columbia between May 12th and July 15th of 2022. (Permit number NA22-685471).

We used several strategies to quantify nest returns. First, we analyzed GoPro images for marked stickleback. Unfortunately, there was a low confirmation rate with GoPro cameras due to technical issues with cameras and difficulty identifying marked fish observed in photos. Because of this, we also snorkeled to observe nests directly, finding flagged nests and recording bead color and nearest nest number for marked fish in a dive notebook. When diving, we moved slowly and remained at marked nests until other, unmarked stickleback were present to help ensure that our presence had not scared away any marked stickleback that may have returned.

We used a logistic regression to test for a relationship between the binary response variable of being re-spotted at the nest of capture, and our continuous explanatory variable, distance displaced from that nest. For the data set containing all the marked and released fish, we used the *glm* function in the base R package *stats* (R Core Team 2022; *glm(recapture ~ distance*, *family = binomial (link = logit)*). Then, we re-ran the model on a data subset as follows: in some cases when divers returned to marked nests to check for beaded males, the nests of those marked stickleback appeared taken over by other male stickleback. This was evidenced by an un-beaded male at the marked nest location that was displaying nesting behaviors and aggressive tendencies to other males. If we interpreted the situation correctly, and these new males were indeed holding the nest site, then marked males may have returned to their nest only to be dissuaded by new males, as a type of priority effect. Our data would therefore have recorded these "evicted" males as non-homing, even though they may have returned. Thus, we subset the data just to nests without a possible eviction event and re-ran the logistic regression as above.

Results

We caught and marked 42 nesting stickleback males in Lower Stella Lake. Forty-one survived marking and were released (Table 1). Displacement distance averaged 28.8m (s.d. = 17.0m; range 3m–58m). We re-spotted 20 beaded stickleback after release with 14 spotted by diver alone, four observed by both GoPro and diver, and two fish observed by GoPro alone (Table 1). The largest return distance was 51 meters. All the re-spotted stickleback were observed at the nests from which they were taken, indicating that the males can and do return to their nests following displacement. We found that the likelihood of return was negatively but not significantly related to distance displaced ($\beta = -0.02$, $p = 0.233$; Table 2) for the complete data set. When the seven "evicted males" were removed from the data set, the strength of the relationship increased ($\beta = -0.04$), nearly significantly so ($p = 0.057$), despite the smaller sample size.

Discussion

We found that nesting males could return to their nests following displacement. The frequency of return tended to decrease with distance displaced, though not with statistical significance. Previous studies have found that up to 80% of stickleback are able to return within a day (Ward et al. 2013; Ivanova et al. 2019), which is much higher than our return rate of 49%. Notably, when males that were classified as "evicted" were removed from the analysis, the negative correlation between distance and homing was stronger and marginally more significant, despite a reduced sample size. If this is indicative of a true signal, then our result supports Ivanova et al.'s (2019) hypothesis that stickleback are using local environmental cues to home because increasing displacement distance should make it less likely for a stickleback to recognize environmental landmarks. A future experiment to determine whether stickleback do indeed use landmark recognition to home could be to introduce different objects into an artificial environment and remove or alter them when the fish are trying to home.

This negative correlation between distance and homing ability could also be explained by something like a reliance on olfactory cues to home. As with landmark recognition, olfactory cues could become more unfamiliar with increases distance from the home site, though the distance displaced may not have resulted in a change.

While we are not aware of any literature on male stickleback taking over nests, there are some similar behaviors that have been observed. Male stickleback have been shown to eat, steal, and fertilize eggs present in the nests of other male stickleback (FitzGerald 1993). Since it is the nest itself that draws the female, perhaps a male that had fertilized some of the eggs of the previous owner decided to move in in his absence. It is also possible that a male moved his eggs from a less favorable nest to an empty one.

While it's not known for certain why homing by landmark recognition would evolve in this population, being able to return to the nest if displaced when looking for food or evading predators would be a beneficial trait to have. In other environments, such as rivers or marine environments, currents or tides could also displace stickleback, so it is possible that this behavior evolved in a marine population and was inherited by freshwater populations.

We did recover the predicted negative rate of return with distance displaced, though the relationship was not significant. We propose several reasons why, some technical and some biological. First, we likely lacked statistical power. Our experiment only used 41 fish due to space and time constraints. Our analysis suggests that there was a nearly significant negative relationship between distance and return and increasing the sample size might push the results to a significant level. Second, we may have mis-identified some of our individuals at the start of the nesting males when they were not, which would make them less incentivized to home and would weaken any relationship between distance and return. Third, we did not confirm whether nests had eggs in them, as we did not want to destroy the nests. Males begin displaying homing behaviors prior to having eggs in their nests; if some of our experimental males did not have eggs in their nest at capture, the fitness consequences of not returning may have been lower, weakening incentive to home.

Table 1. Data used for analysis including the fish being displaced, bead combination (with acronyms indicating bead color and the ":" separating left color from right color), ID of the marker placed at the nest, displacement distance, whether the fish was re-spotted (by diver or by GoPro), whether an evicted male was present, and the nest ID where the fish was re-spotted.

Fish ID	Bead ID	Nest site ID	Distance displaced (m)	Re-spotted …	… via diver	… via Go Pro	Nest where Re-spotted	Evection event?
021	DB:DB	24	3	no	no	no	NA	yes
034	O:DB	38	3	no	no	no	NA	yes
028	B:LB	34	7	yes	yes	no	34	no
022	B:DG	25	8	no	no	no	NA	no
037	O:LB	40	8	no	no	no	NA	no
038	O:P	39	8	yes	yes	no	39	no
040	LG:R	48	8	yes	yes	no	48	no
027	B:DB	33	10	yes	yes	no	33	no
029	B:LG	43	10	yes	yes	yes	43	no
032	O:B	45	12	no	no	no	NA	no
039	LG:B	49	13	no	no	no	NA	yes
033	O:W	29	16	yes	yes	no	29	no
024	B:B	11	17	no	no	no	NA	yes
026	B:O	36	18	yes	yes	no	36	no
036	O:DG	44	19	yes	yes	no	44	no
012	LB:DB	31	20	no	no	no	NA	yes
015	LB:P	26	20	yes	yes	no	26	no
041	LG:O	46	20	no	no	no	NA	no
014	LB:DG	30	22	yes	yes	no	30	no
013	LB:LG	10	28	yes	yes	yes	10	no
030	B:P	41	30	yes	yes	no	41	no
001	R:B	05	31	yes	yes	yes	05	no
035	O:LG	37	31	yes	yes	no	37	no
025	B:W	35	32	yes	no	yes	35	no
020	DB:DB	28	34	no	no	no	NA	no
031	O:R	42	38	yes	yes	no	42	no
042	LG:DB	47	38	no	no	no	NA	no
016	DB:R	21	41	no	no	no	NA	no
008	LB:B	02	42	yes	no	yes	02	no
002	R:R	08	44	no	no	no	NA	no
003	R:O	01	44	no	no	no	NA	no
004	R:DB	01	44	yes	yes	no	01	no
005	R:LB	07	46	no	no	no	NA	no
010	LB:O	20	46	yes	yes	no	20	no
011	LB:LB	20	46	no	no	no	NA	no
006	R:DG	09	50	no	no	no	NA	yes
023	B:R	12	50	no	no	no	NA	yes
009	LB:W	06	51	yes	yes	yes	06	no
017	DB:B	04	57	no	no	no	NA	no
019	DB:O	22	57	no	no	no	NA	no
018	DB:W	27	58	no	no	no	NA	no
007*	LB:R	23	NA	NA	NA	NA	NA	NA

* Died during marking. Not included in the statistical models.

Fourth, it may be that we didn't displace fish far enough. Ivanova et al (2019) found homing distances as great as 300m so it is possible that the cruising range of stickleback in this population exceeds the maximum distance that the stickleback were experimentally displaced. We were limited by the habitat due to transition from a grassy reed bed to a flat sandy shelf, in which nesting stickleback were not found. The grassy shelf extended only 60 meters from the release point, making this the maximum distance displaced. If this experiment were to be repeated, a larger distribution of distances could be used to better test the impact of distance on homing ability. It is also possible that nests were raided for eggs and destroyed while males were gone for marking and travel home. Thus, any motivation the fish had to remain at their nest site once returned was taken away. Finally, the beads used to mark the stickleback could have been dislodged, and the nesting males we marked may have been returning to nests but counted as unmarked fish. The presence of two beads, one on each pectoral fin, hopefully reduced this possibility.

Table 2. Results of the binary logistic regression for (A) the complete data set and (B) data subset to only males whose nests had not been taken over by unmarked males.

	Beta	Standard error	Z value	P value
(A) Complete data				
Intercept	0.0608	0.6345	0.969	0.338
Distance	−0.02291	0.01922	−1.192	0.233
(B) Without evictions				
Intercept	1.7978	0.8632	2.083	0.0373
Distance	−0.0463	0.02431	−1.905	0.0568

Possible reasons for the low return rate seen here could also be due to predators such as trout eating marked stickleback. Other possibilities include the stickleback losing their territory once moved, or an imperfect homing system.

A future experiment to determine whether stickleback do indeed use landmark recognition to home could be to introduce different objects into an artificial environment and remove or alter them when the fish are trying to home. Overall, this experiment indicates a negative correlation between distance and ability to home indicating that nesting male stickleback are able home back to their specific nests. Additionally, we found evidence that when a nesting male is displaced, a new male may take advantage and move into the empty nest.

Acknowledgements

We thank D. Bolnick, T. Neely-Streit, Z. Pitsenberger, D. Rennison, T. Sasser, T Veen, and J Weber for help in study design and field work. We thank the British Columbia Ministry of Environment for research permits. Funding was provided by Loyola University Chicago, consisting of a Provost Fellowship (SR), a College of Arts and Sciences Biology Department Summer Research Fellowship (MVM), and CAS Mulcahy Fellowships (MVM and SR).

References

Taylor MD, Payne NC, Becker A, Lowry MB. 2017. Feels like home: homing of mature large-bodied fish following translocation from a power--station canal. ICES Journal of Marine Science. 74(1):301–310. https://doi.org/10.1093/icesjms/fsw168

Ward AJW, James R, Wilson ADM, Webster MM. 2013. Site fidelity and localised homing behaviour in three-spined sticklebacks (*Gasterosteus aculeatus*). Behaviour. 150(14):1689–1708. https://doi.org/10.1163/1568539x-00003115

Schmidt KA. 2004. Site fidelity in temporally correlated environments enhances population persistence. Ecology Letters. 7(3):176–184. https://doi.org/10.1111/j.1461-0248.2003.00565.x

Ivanova TS, Ivanov MV, Bakhvalova AE, Polyakova NV, Golovin PV, Kucheryavyy AV, Yurtseva AO, Smirnova KA, Lajus DL. 2019. Homing ability and site fidelity of marine threespine stickleback on spawning grounds. 20(3):297–315.

Dodson JJ. 1988. The nature and role of learning in the orientation and migratory behavior of fishes. Environmental Biology of Fishes. 23(3):161–182. https://doi.org/10.1007/bf00004908

Keefer ML, Caudill CC. 2013. Homing and straying by anadromous salmonids: a review of mechanisms and rates. Reviews in Fish Biology and Fisheries. 24(1):333–368. https://doi.org/10.1007/s11160-013-9334-6

Bell MA, Foster SA. 1994. The evolutionary biology of the threespine stickleback. Oxford; New York: Oxford University Press.

R Core Team. 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.r-project.org/

FitzGerald GJ. 1993. The Reproductive Behavior of the Stickleback. Scientific American. 268(4):80–85. https://doi.org/10.1038/scientificamerican0493-80

Bolnick DI, Snowberg LK, Patenia C, Stutz WE, Ingram T, Lau OL. 2009. Phenotype-dependent native habitat preference facilitates divergence between parapatric lake and stream stickleback. Evolution. 63(8):2004–2016. https://doi.org/10.1111/j.1558-5646.2009.00699.x

Gonzalez-Voyer A, Winberg S, Kolm N. 2009. Distinct evolutionary patterns of brain and body size during adaptive radiation. Evolution. 63(9):2266–2274. https://doi.org/10.1111/j.1558-5646.2009.00705.x

Hiromichi Mitamura, Uchida K, Miyamoto Y, Toshiharu Kakihara, Miyagi A, Kawabata Y, Ichikawa K, Arai N. 2012. Short-range homing in a site-specific fish: search and directed movements. Journal of experimental biology. 215(16):2751–2759. https://doi.org/10.1242/jeb.065052

Park PJ, Bell MA. 2010. Variation of telencephalon morphology of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology. Journal of Evolutionary Biology. 23(6):1261–1277. https://doi.org/10.1111/j.1420-9101.2010.01987.x

Author Contributions

This project was designed and run by MM and YS. Funding for this project was secured by MVM, SR and YS. Data was collected by MVM, KVD, SR and YS. Results were analyzed by MVM. All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interest.