

Lionfish (*Pterois volitans*) do not show directional preferences for ambient underwater soundscapes during diurnal hours

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Supplementary Material

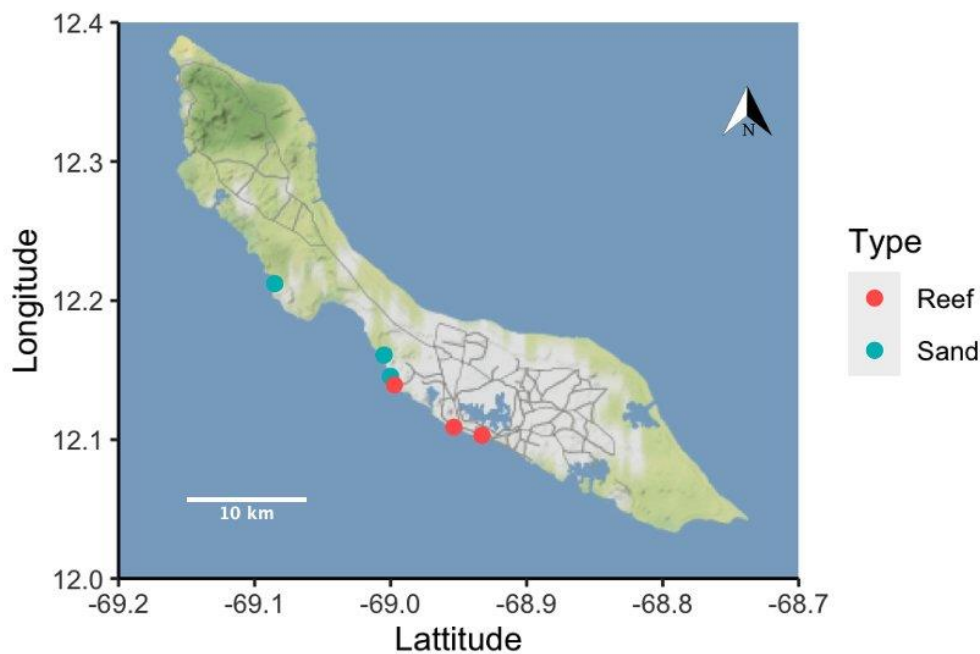


Fig. S1: Sound recordings were taken at 4–6 m depth in three locations (each > 200 m apart) for reef habitat (Red dots: Reef 1: Punda (12°06'10.9"N 68°55'58.8"W), Reef 2: Water Factory (12°06'34.1"N 68°57'16.5"W) and Reef 3: Snake Bay (12°08'22.3"N 68°59'51.0"W)) and sandy habitat (Blue dots: Sand 1: Daaibooi (12°12'43.7"N 69°05'06.9"W), Sand 2: Fort St Michael (12°08'43.9"N 68°59'59.9"W) and Sand 3: Kokomo (12°09'38.7"N 69°00'17.9"W)) sites in Curacao, Caribbean Netherlands.

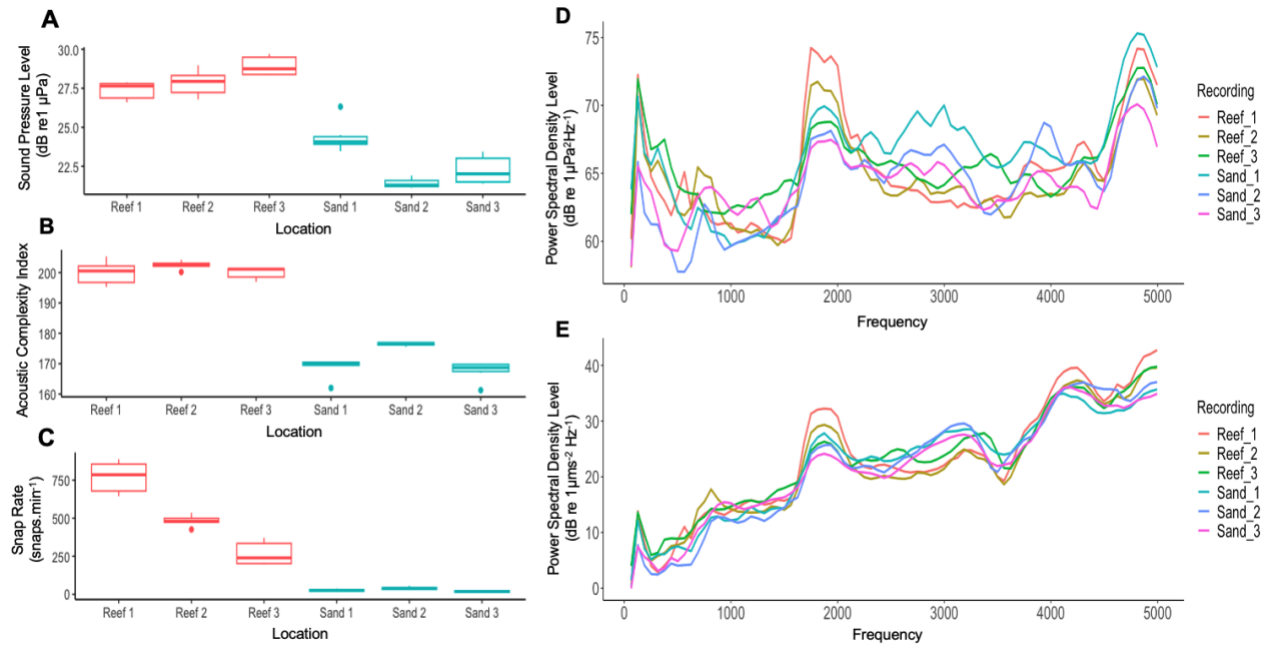


Fig. S2: Average eco-acoustic indexes of the original recordings showing **A)** Sound Pressure Level (dB re 1 μ Pa) Butterworth filter 50–2000Hz, **B)** Acoustic Complexity Index (ACI) and **C)** Shrimp snap rate (snaps min^{-1}) and **D)** Power spectral density plots (Butterworth filter 50–5000 Hz) of the sound pressure and **E)** particle acceleration of one representative example of each playbacks at 1 m from the speaker of each habitat treatment type (Sand 1: Daibooui, Sand 1: Fort St Michael and Sand 3: Kokomo, Reef 1: Punda, Reef 2: Waterfactory and Reef 3: Snake Bay; see Figure S1). Boxplots show median and 1st-3rd interquartile ranges and whiskers represent 95% confidence interval; points represent outliers.

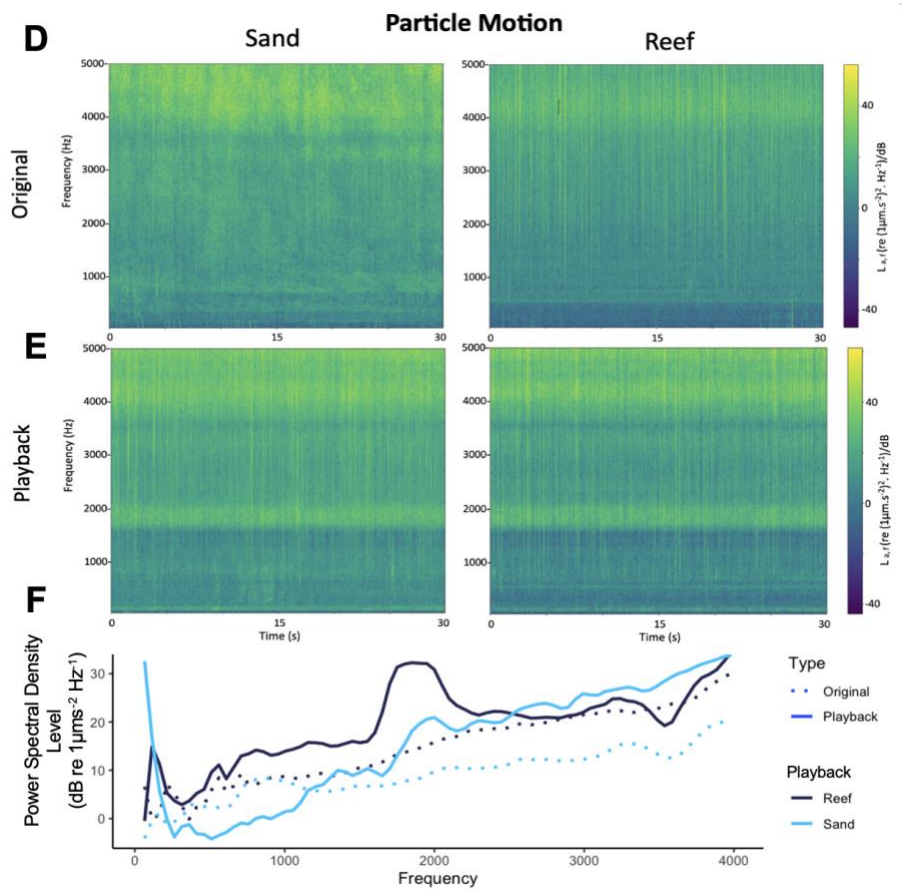
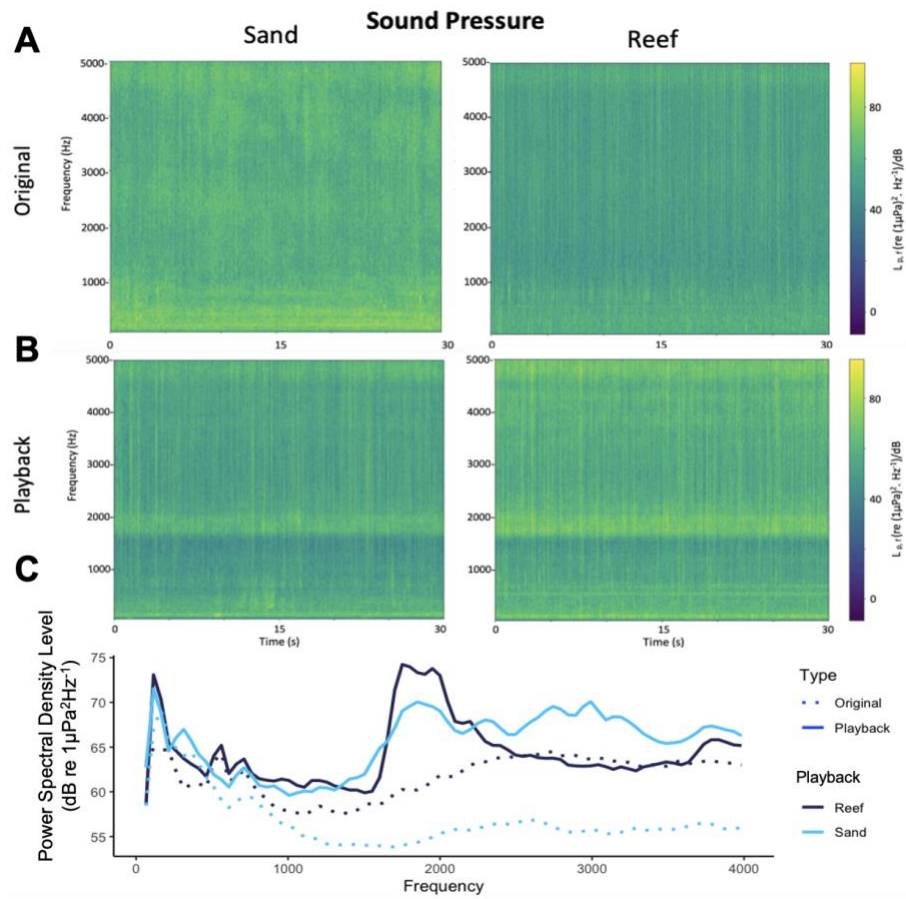


Fig. S3: Spectrograms of sound pressure (**A-C**) and the particle acceleration (**D-F**) of 30 second recordings of the original recording (**A** and **D**) and playback sounds (**B** and **E**; Butterworth filter 50–5000 Hz), and power spectral density plots of **C**) the sound pressure and **F**) particle acceleration of a recording of a representative example of sand (Sand 3; light blue) and reef (Reef 2; dark blue) habitat respectively at 1 m from the speaker with dotted lines representing the original recording and solid lines representing the playback (Butterworth filter 50–4000 Hz).

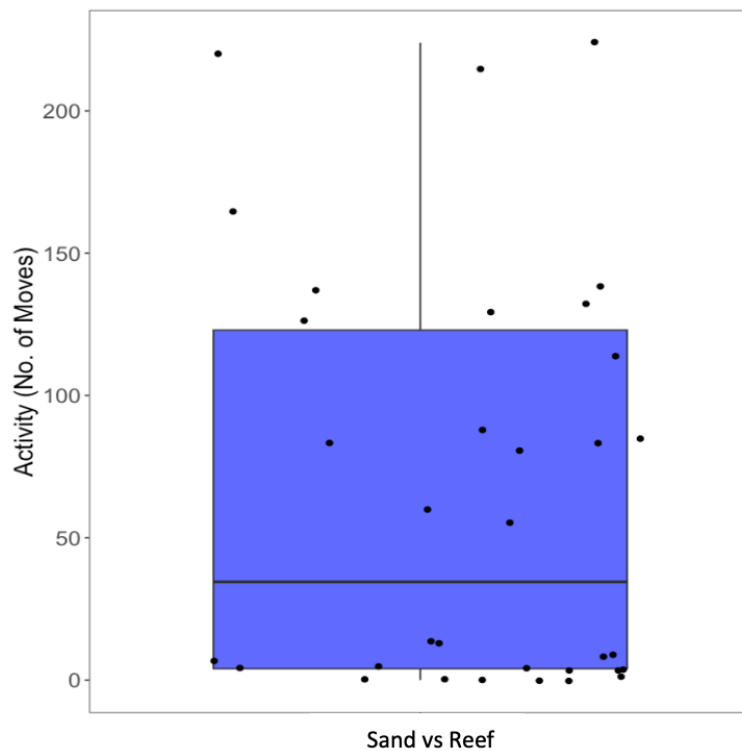


Fig. S4: The activity (number of moves) of lionfish during the choice experiment with simultaneous and reef habitat playbacks. Boxplots show the median and 1st and 3rd interquartile ranges and whiskers show the 95% confidence interval.

Table S1: Results of statistical analyses as reported in the text. Significant p -values are shown in bold, and number of asterisks denotes level of significance.

<i>Analysis</i>	<i>Model</i>	<i>Model term</i>	<i>AIC</i>	<i>Test statistic</i>	<i>Estimate</i>	<i>p</i>
<i>First choice ~ Treatment * Fish size + (1 Fish ID)</i>	Binomial GLMM	Treatment: Fish size	76.15	$X^2_1 = 0.11$	1.10	0.74
<i>First choice ~ Treatment + Fish size + (1 Fish ID)</i>	Binomial GLMM	Treatment	74.25	$X^2_1 = 1.18$	1.44	0.28
		Fish size		$X^2_1 = 3.18$		0.075
<i>First choice (any sound) ~ 1 + (1 Fish ID)</i>	Binomial GLMM	-	74.48	$Z = 0.14$	0.04	0.89
<i>First choice (Sand vs Reef) ~ Fish size</i>	Binomial GLM	Fish size	37.09	$F_1 = 0.012$	-0.002	0.92
<i>Overall preference ~ Treatment*Fish Size + (1 Fish ID)</i>	Binomial GLMM	Treatment: Fish size	63.53	$X^2_1 = 0.27$	0.01	0.60
<i>Overall preference ~ Treatment+ Fish Size + (1 Fish_ID)</i>	Binomial GLMM	Treatment	61.80	$X^2_1 = 0.0041$	0.04	0.95
		Fish size		$X^2_1 = 0.046$	-0.0023	0.83
<i>Overall preference (any sound) ~ 1 + (1 Fish_ID)</i>	Binomial GLMM	-	57.85	$Z = -1.70$	-0.55	0.09
<i>Choice~ Fish Size</i>	Binomial GLM	Fish size	36.42	$F_1 = 0.58$	0.013	0.45
<i>Activity ~ Treatment* fish size + time</i>	LMM	Treatment: Fish size	616.43	$X^2_1 = 2.19$	0.35	0.14
		Time		$X^2_1 = 0.38$	-0.07	0.54
<i>Activity ~ Treatment+ fish size + time</i>	LMM	Treatment	615.49	$X^2_1 = 0.047$	-1.77	0.83
		Fish size		$X^2_1 = 38.33$	1.61	<0.001
		Time		$X^2_1 = 0.17$	-0.05	0.68

Table S2: Results of models when fish which did not visit both sides of the experimental arena during trials were included. The only results that was qualitatively different from the models excluding fish that visited both sides was the test for overall preference for any playback (sand or reef) versus a silent control (see discussion below).

<i>Analysis</i>	<i>Model</i>	<i>Model term</i>	<i>AIC</i>	<i>Test statistic</i>	<i>Estimate</i>	<i>p</i>
<i>First choice ~ Treatment * Fish size + (1 Fish ID)</i>	Binomial GLMM	Treatment: Fish size	76.15	$X^2_1 = 0.11$	1.10	0.74
<i>First choice ~ Treatment + Fish size + (1 Fish ID)</i>	Binomial GLMM	Treatment	74.25	$X^2_1 = 1.18$	0.66	0.28
		Fish size		$X^2_1 = 3.18$	-0.018	0.075
<i>First choice (any sound) ~ 1 + (1 Fish ID)</i>	Binomial GLMM	-	74.48	$Z = 0.14$	0.04	0.89
<i>First choice (Sand vs Reef) ~ Fish size</i>	Binomial GLM	Fish size	42.68	$F_1 = 0.46$	-0.00	0.50
<i>Overall preference ~ Treatment*Fish Size + (1 Fish ID)</i>	Binomial GLMM	Treatment: Fish size	74.68	$X^2_1 = 0.10$	0.32	0.75
<i>Overall preference ~ Treatment+ Fish Size + (1 Fish_ID)</i>	Binomial GLMM	Treatment	72.78	$X^2_1 = 0.04$	0.11	0.85
		Fish size		$X^2_1 = 0.11$	0.003	0.74
<i>Overall preference (any sound) ~ 1 + (1 Fish_ID)</i>	Binomial GLM	-	68.92	$Z = -2.21$	-0.69	0.027*
<i>Choice~ Fish Size</i>	Binomial GLM	Fish size	41.25	$F_1 = 1.91$	0.019	0.17
<i>Activity ~ Treatment* fish size+ time</i>	LMM	Treatment: Fish size	616.43	$X^2_1 = 2.19$	0.35	0.14
		Time		$X^2_1 = 0.38$	-0.074	0.54
<i>Activity ~ Treatment+ fish size+ time</i>	LMM	Treatment	615.49	$X^2_1 = 0.047$	-1.77	0.82
		Fish size		$X^2_1 = 38.32$	1.61	<0.001
		Time		$X^2_1 = 0.17$	-0.050	0.68

Interpretation of inconsistent findings

When fish that did not visit both sides of the arena were included in analyses, one model on the preference tests suggested that fish overall avoided sounds of the playbacks versus the silent control. This is because in these excluded trials (total excluded trials from Preference Tests: 19 out of 60) which were removed due to not leaving the acclimation area (9 out of 19 excluded cases) or fish not visiting both sides (10 out of 19 excluded cases), individuals spent

the vast majority of their time with the silent control, versus the playbacks (sand or reef). However, in the cases where fish left the acclimation area but did not visit both sides, the majority of these fish first moved to the side of the arena with the playback of sand or reef and remained there until the end of the first half of the trial (3 out of 10 chose control, 7 out of 10 chose sand or reef). At the end of the first half of the trial, the playbacks on the speakers switched sides, resulting in the fish subsequently spending the majority of the time on the side with the silent control, despite having initially decided to move towards the side with the playbacks of reef or sand. Therefore, we do not interpret this result as a genuine avoidance response to the playbacks of sand or reef.