Supplementary material

Species interactions and environmental context affect intraspecific behavioural trait variation and ecosystem function

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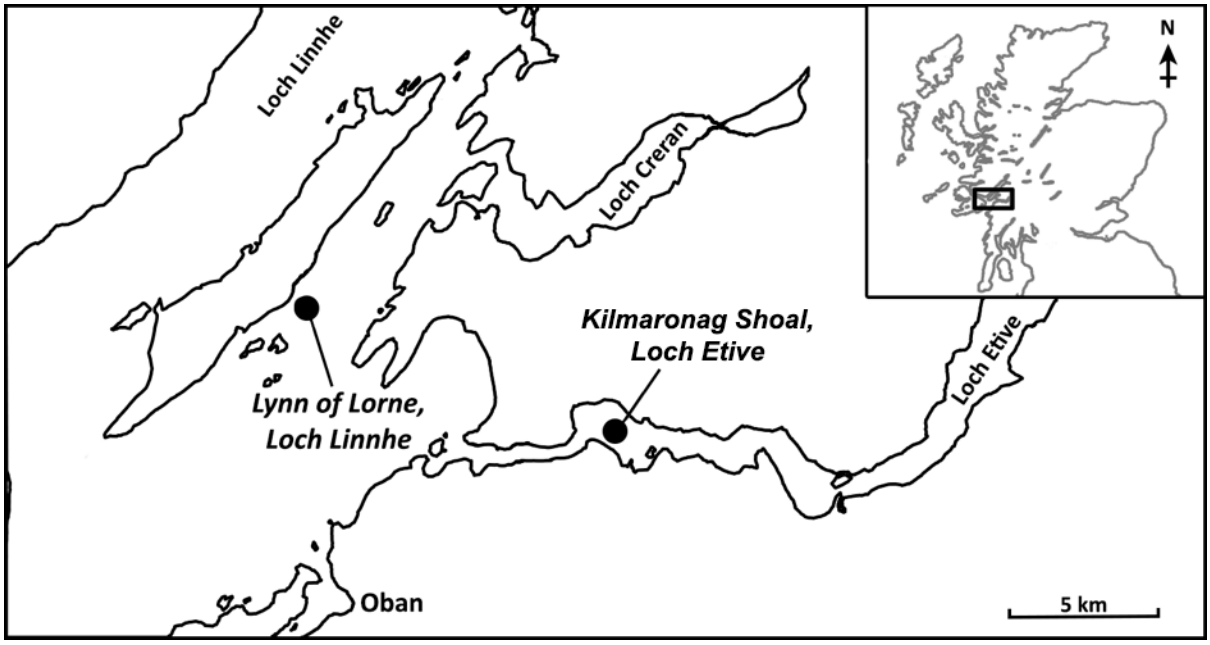
Fig. S1 Sampling locations at Kilmaronag Shoal, Loch Etive (56°27'34.20"N, 5°20'29.28"W), and the Lynn of Lorne, Loch Linnhe (56°29'49.6"N, 5°29'56.2"W), Scotland, UK.



Sediment parameters

Sediment parameters were measured by laser diffraction (Malvern Mastersizer 2000) at the Department of Geography, University of Cambridge following standard protocols (http://www.geog.cam.ac.uk/facilities/laboratories/techniques/). Particle size parameters were calculated using logarithmic graphical measures (Blott & Pye, 2001, doi: 10.1002/esp.261).

Table S1:

Sediment parameters (mean ± SD) for sediment samples drawn from sampling sites (n = 5).

	Kilmaronag Shoal, Loch Etive	**Lynn of Lorne, Loch Linnhe**	**Hamble Estuary**
Mz (µm)	233.4 ± 44.9	253.5 ± 35.5	26.3 ± 1.6
Mz (Phi)	3.6 ± 1.1	2.9 ± 0.4	6.7 ± 0.1
Sorting (µm)	298.0 ± 66.7	209.6 ± 63.6	31.9 ± 2.2
Sorting (Phi)	2.4 ± 0.2	2.1 ± 0.4	1.7 ± 0.02
Kurtosis (µm)	14.4 ± 8.9	6.0 ± 2.7	8.8 ± 1.0
Kurtosis (Phi)	3.3 ± 1.1	4.5 ± 1.6	2.3 ± 0.03
Skewness (µm)	3.7 ± 0.7	1.3 ± 0.6	2.3 ± 0.2
Skewness (Phi)	0.6 ± 0.7	1.4 ± 0.5	0.01 ± 0.07
Results below 63 µm (%)	44.7 ± 22.8	23.8 ± 9.4	94.4 ± 1.0
TOC (%)	9.9 ± 5.4	2.9 ± 0.8	7.6 ± 0.6

Fig. S2: Total organic carbon (TOC) content (mean ± SE) (%) at Loch Etive (56°27'34.20"N, 5°20'29.28"W, n = 4), Loch Linnhe (56°29'49.6"N, 5°29'56.2"W, n = 5), and Hamble (50°52'23.1"N 1°18'49.3"W, n = 5), showing a significant difference between sites (ANOVA: $F_{2,10}$= 30.78, P < 0.001).

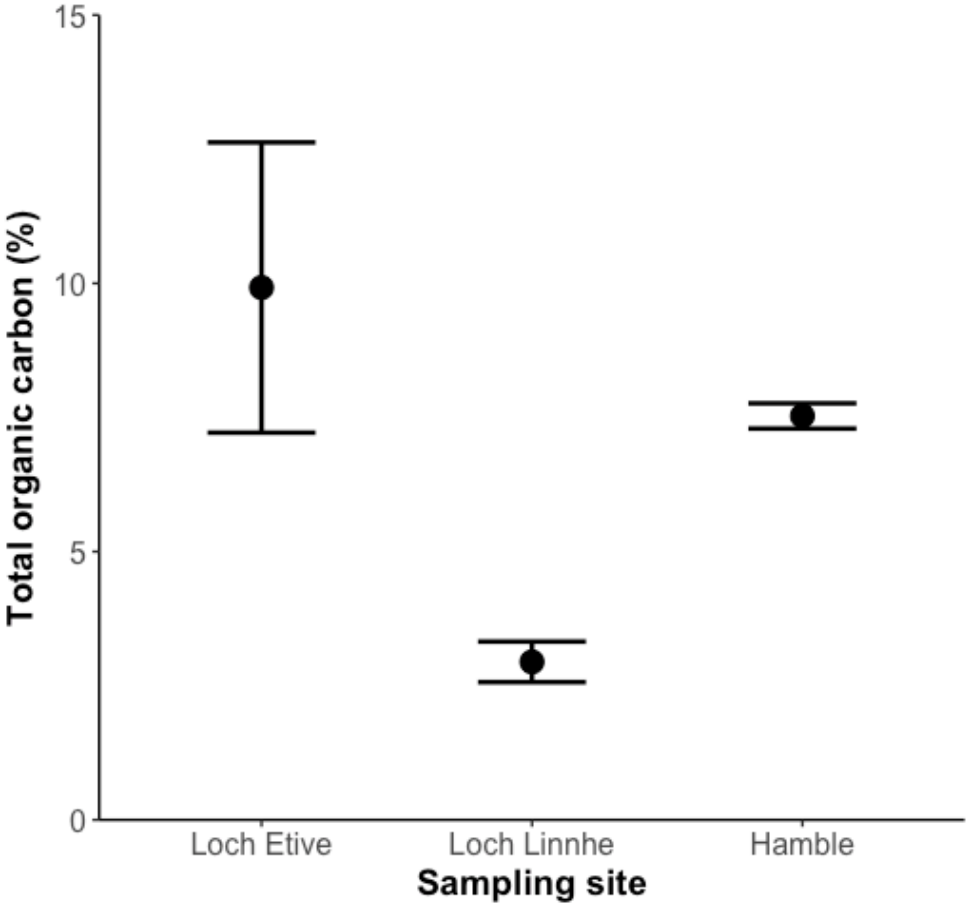


Fig. S3: Cumulative sediment particle size distributions for sampling sites at Lynn of Lorne, Loch Linnhe and Kilmaronag Shoal, Loch Etive, and for sediment used during mesocosm incubations from Hamble-le-Rice, Hampshire.

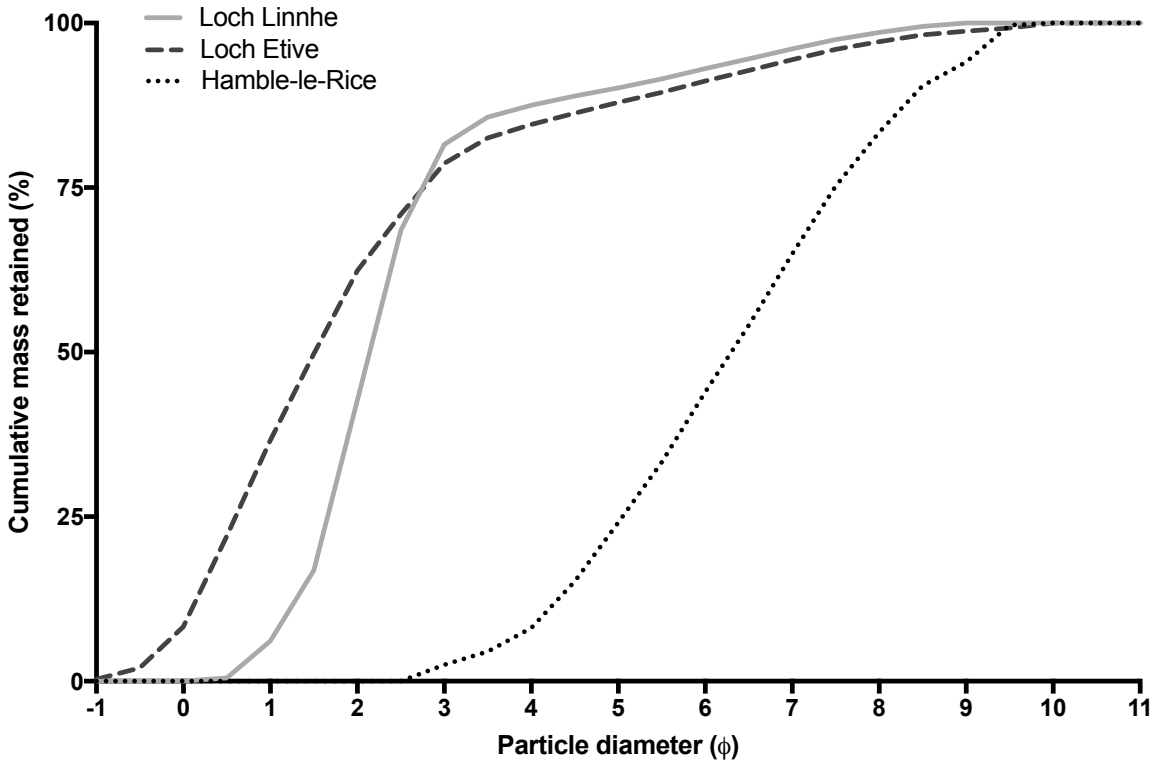


Table S2: Aspects of ecological context manipulated in fully cross-factored design, showing the number and nature of treatment levels, and identity of individuals in the respective mesocosms.

	Population (Sampling site)	**Species treatment**	**Density**
Number of treatment levels	2	3	3
Treatment levels	1) Kilmaronag Shoal, **Loch Etive** 2) Lynn of Lorne, **Loch Linnhe**	1) ***Amphiura filiformis* monoculture** *(Species identity of component individuals: A. filiformis* in monoculture) 2) ***Ampihura chiajei* monoculture** *(Species identity of component individuals: A. chiajei* in monoculture) 3) ***A. filiformis-A. chiajei* mixed community** *(Species identity of component individuals: A. filiformis* in mixed community, or *A. chiajei* in mixed community)	1) **Low** (250 ind. m^{-2} *A. filiformis*, 175 ind. m^{-2} *A. chiajei*) 2) **Medium** (500 ind. m^{-2} *A. filiformis*, 350 ind. m^{-2} *A. chiajei*) 3) **High** High (1000 ind. m^{-2} *A. filiformis*, 700 ind. m^{-2} *A. chiajei*)

Table S3: Number of replicate mesocosms (n= 102), and *A. filiformis* (n = 370) and *A. chiajei* (n = 242), for all combinations of context. Replicates were constrained by abundance of *A. chiajei* from the Loch Etive site. Six cores were lost to mortality. Two individuals representing the species mixture were randomly selected from each core (n = 192).

		***Ampihura filiformis* monoculture**	***Amphiura chiajei* monoculture**	**Mixed species treatment**
Loch Linnhe	Low density	n = 6 250 ind. m^{-2} *A. filiformis* 3 ind. $core^{-1}$	n = 6 175 ind. m^{-2} *A. chiajei* 2 ind. $core^{-1}$	n = 6 250 ind. m^{-2} *A. filiformis* 2 ind. $core^{-1}$ *A. chiajei* 1 ind. $core^{-1}$
	Medium density	n = 6 500 ind. m^{-2} *A. filiformis* 6 ind. $core^{-1}$	n = 6 350 ind. m^{-2} *A. chiajei* 4 ind. $core^{-1}$	n = 6 500 ind. m^{-2} *A. filiformis* 4 ind. $core^{-1}$ *A. chiajei* 2 ind. $core^{-1}$
	High density	n = 6 1000 ind. m^{-2} *A. filiformis* 12 ind. $core^{-1}$	n = 6 700 ind. m^{-2} *A. chiajei* 8 ind. $core^{-1}$	n = 6 1000 ind. m^{-2} *A. filiformis* 8 ind. $core^{-1}$ *A. chiajei* 4 ind. $core^{-1}$
Loch Etive	Low density	n = 6 250 ind. m^{-2} *A. filiformis* 3 ind. $core^{-1}$	n = 6 175 ind. m^{-2} *A. chiajei* 2 ind. $core^{-1}$	n = 6 250 ind. m^{-2} *A. filiformis* 2 ind. $core^{-1}$ *A. chiajei* 1 ind. $core^{-1}$
	Medium density	n = 5 500 ind. m^{-2} *A. filiformis* 6 ind. $core^{-1}$	n = 6 350 ind. m^{-2} *A. chiajei* 4 ind. $core^{-1}$	n = 5 500 ind. m^{-2} *A. filiformis* 4 ind. $core^{-1}$ *A. chiajei* 2 ind. $core^{-1}$
	High density	n = 4 1000 ind. m^{-2} *A. filiformis* 12 ind. $core^{-1}$	n = 6 700 ind. m^{-2} *A. chiajei* 8 ind. $core^{-1}$	n = 4 1000 ind. m^{-2} *A. filiformis* 8 ind. $core^{-1}$ *A. chiajei* 4 ind. $core^{-1}$

Fig. S4: Morphological measurements made on *A. filiformis* and *A. chiajei* individuals during image analysis (ImageJ, version 1.46r, Schneider et al., 2012, doi: 10.1038/nmeth.2089); i) Arm length, measured as a line directly through the centre of the limb, where the total length of all five limbs was averaged for analysis. ii) Disc diameter, measured as the largest of the five distances across the lines of symmetry, from the disc as its widest point to the base of the opposite arm.

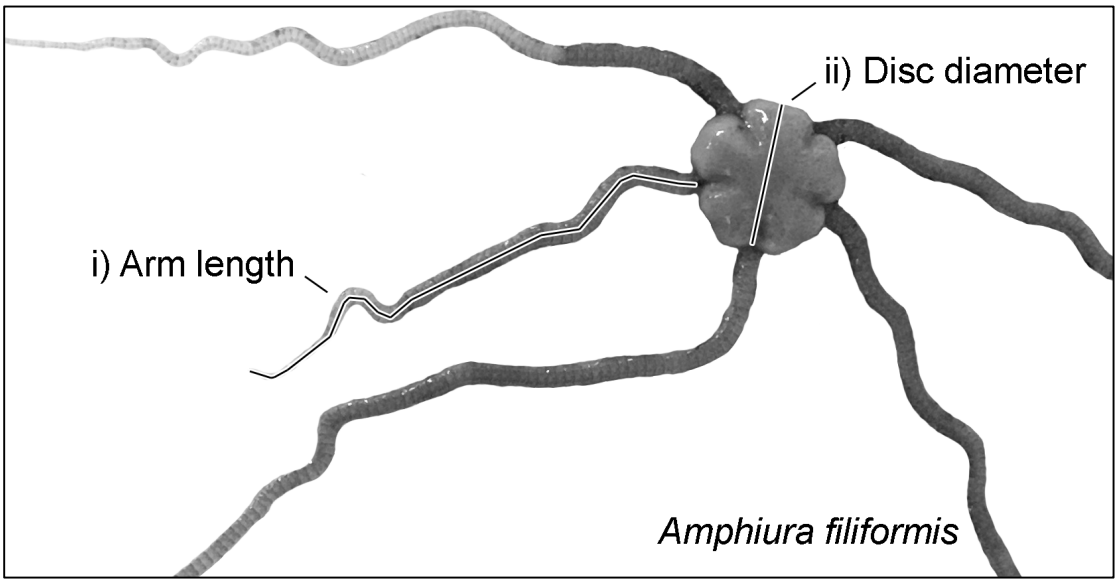


Fig. S5: Pairs plot of morphological variables of mean arm length (mm), disc diameter (mm) and wet weight biomass (g) of *A. chiajei* and *A. filiformis,* shown against the Pearson correlation coefficient on the inverse panel.

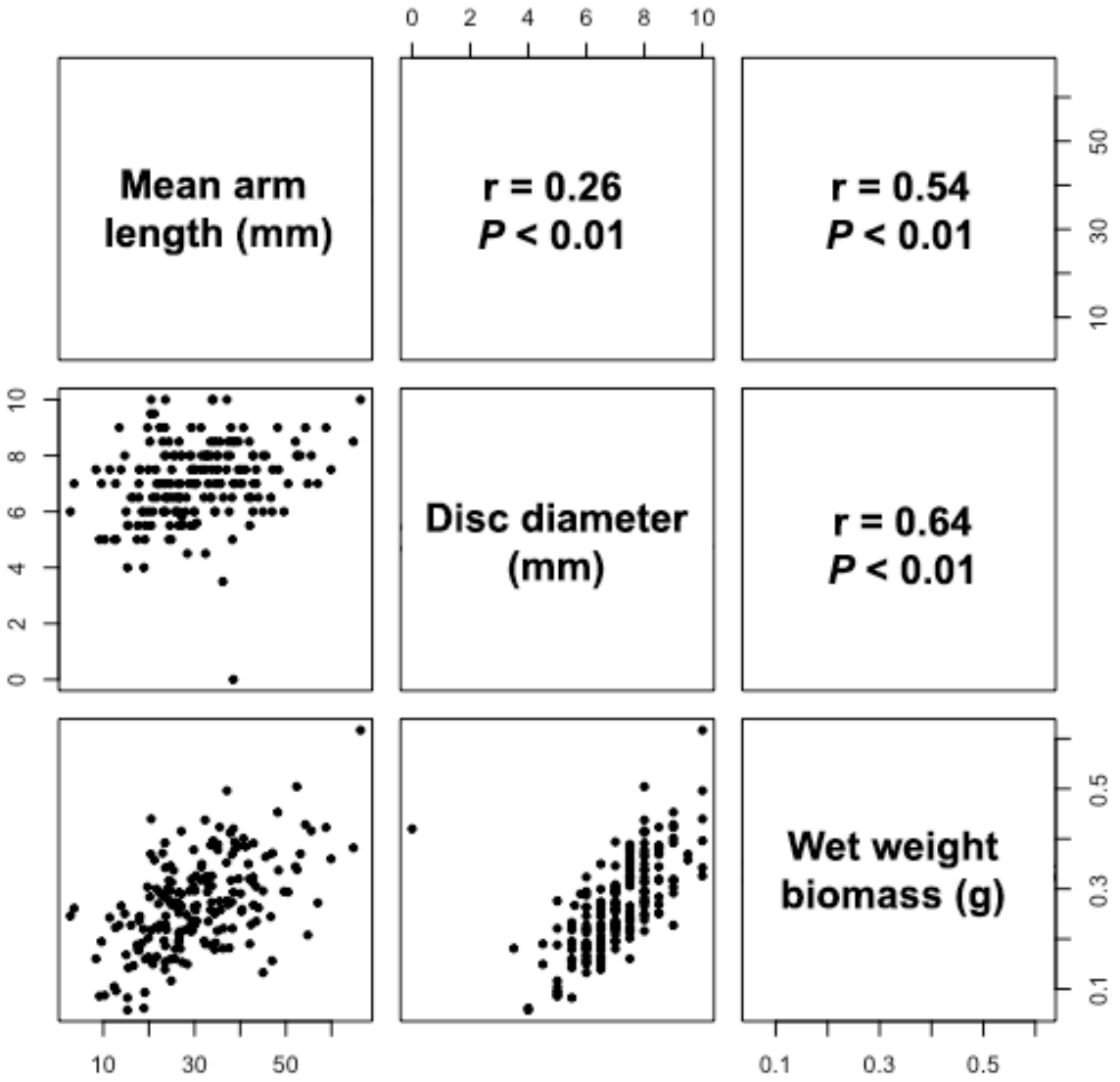
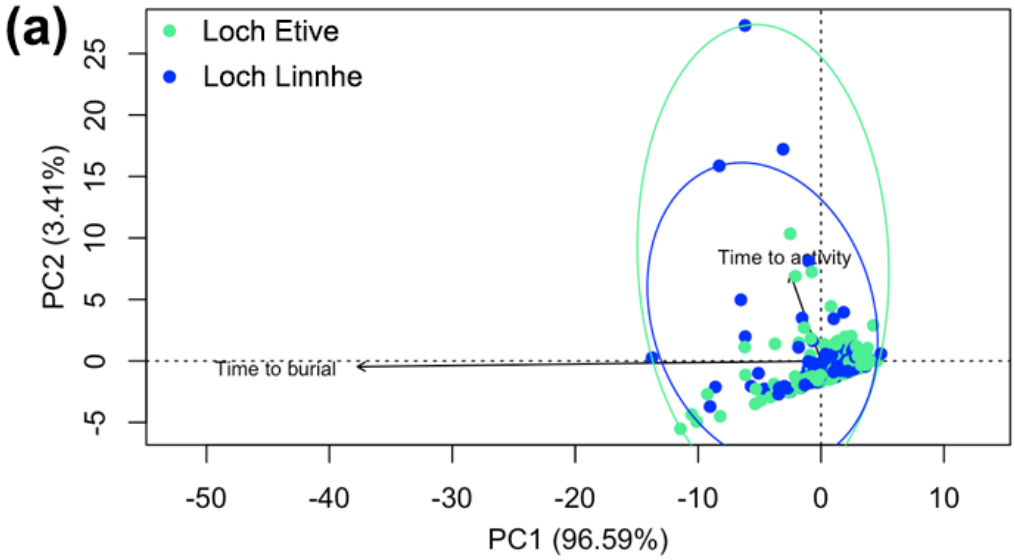
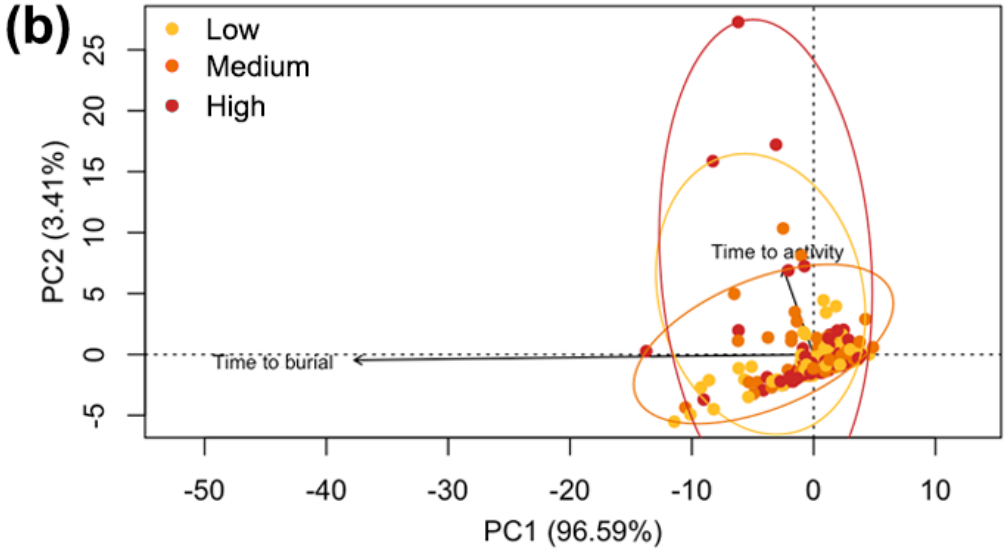


Fig. S6: Biplots of ordination with Principle Component Analysis (PCA) in the *vegan* package in *R* (Oksanen et al., 2017; URL: https://CRAN.R-project.org/package=vegan). Plots show the multivariate behavioural traits 'Time to (begin) activity' and 'Time to (complete) burial' of individuals and the (**a**) Population (**b**) Density and (**c**) Species identity treatments under which these individuals were maintained. Arrows indicate the variation of the traits across all individuals, while coloured ellipses encircle the individuals belonging to each treatment group.


(a)
Loch Etive
Loch Linnhe
Time to activity
Time to burial
PC2 (3.41%)
PC1 (96.59%)
25
20
15
10
5
0
-5
-50
-40
-30
-20
-10
0
10


(b)
Low
Medium
High
Time to activity
Time to burial
PC2 (3.41%)
PC1 (96.59%)
25
20
15
10
5
0
-5
-50
-40
-30
-20
-10
0
10

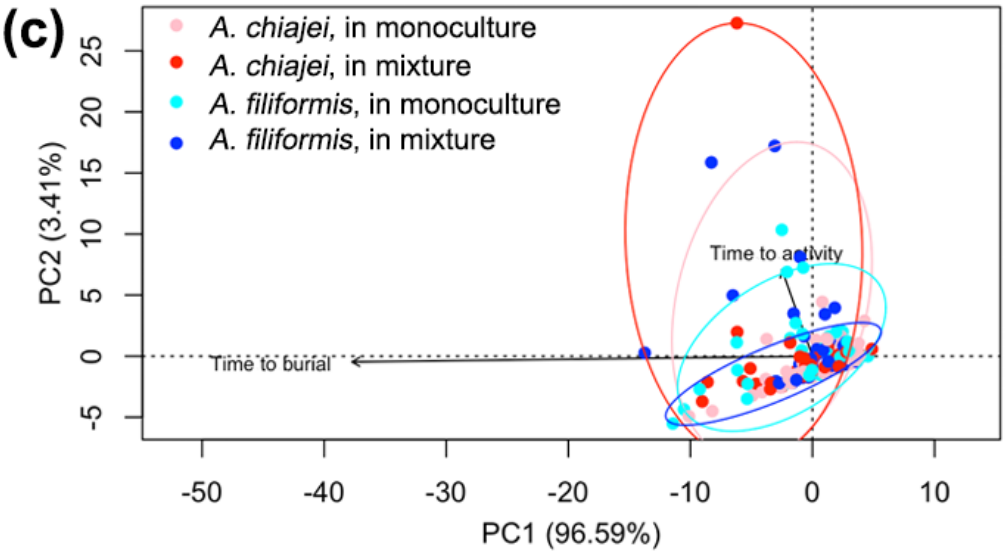

(c)
A. chiajei, in monoculture
A. chiajei, in mixture
A. filiformis, in monoculture
A. filiformis, in mixture
Time to activity
Time to burial
PC2 (3.41%)
PC1 (96.59%)
25
20
15
10
5
0
-5
-50
-40
-30
-20
-10
0
10

Fig. S7: Mean arm length (mean ± SE) (cm) of *Amphiura chiajei* and *Amphiura filiformis* originating from two populations in Loch Etive (56°27'34.20"N, 5°20'29.28"W) and Loch Linnhe (56°29'49.6"N, 5°29'56.2"W), Scotland, UK. Analysis showed a significant difference in average arm length between species (ANOVA: $F_{1,188} = 14.996$, $P < 0.001$) and populations (ANOVA: $F_{1,188} = 4.033$, $P = 0.046$).

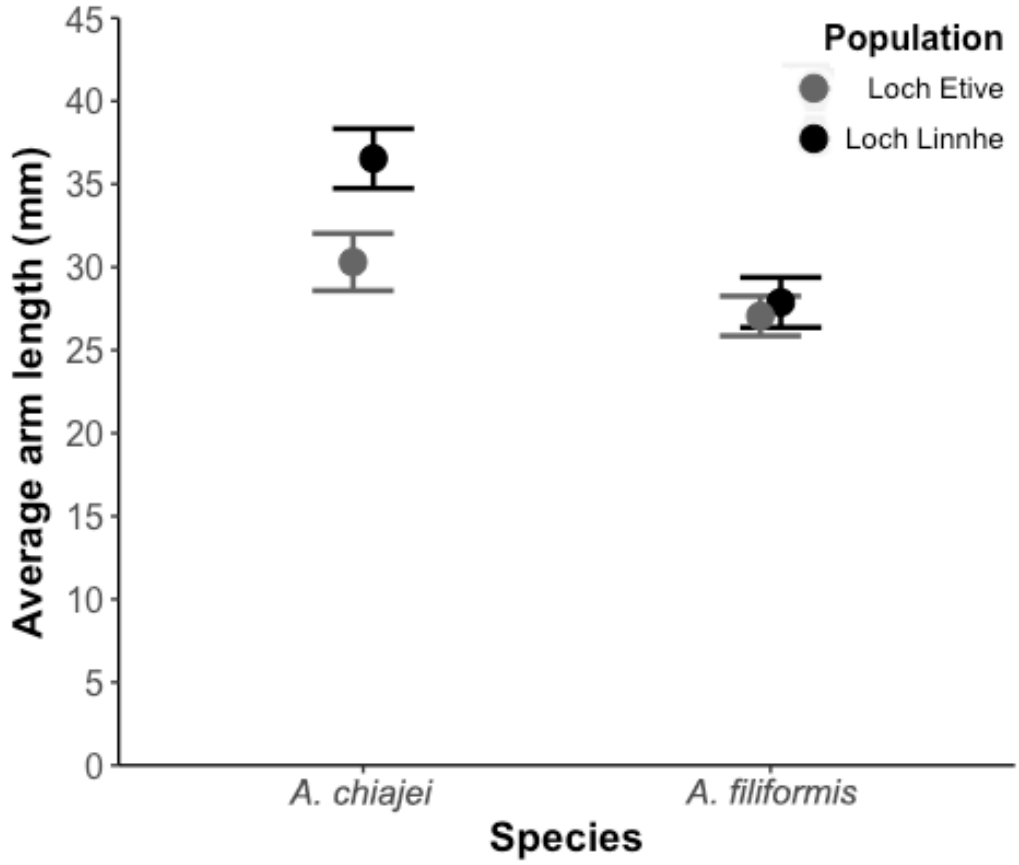


Table S4: Results table for ANOVA of coefficient of variance (CV) of behavioural traits i) time taken to begin behaviour and ii) time taken to complete burial, showing results for all terms in the minimum adequate model following model selection based on Akaike Information Criteria (AIC) from the context factors Density, Species Mixture Treatment, Population, and their interactions.

i) CV Time taken to begin behaviour

	Df	Sum of Squares	Mean Sum of Squares	F	*P*
Density	2	0.1672739	0.08363693	0.5775962	0.56340796
Population	1	0.3368041	0.33680407	2.3259671	0.13090038
Species Mixture Treatment	2	0.6637300	0.33186498	2.2918578	0.10722049
Density * Species Mixture Treatment	4	1.3859010	0.34647524	2.3927562	0.05677979
Residuals	86	12.4529489	0.14480173		

i) CV Time taken to complete burial

	Df	Sum of Squares	Mean Sum of Squares	F	*P*
Density	2	0.04847546	0.02423773	0.37345964	0.6895729
Population	1	0.00237216	0.00237216	0.03655071	0.8488795
Species Mixture Treatment	2	0.20612455	0.10306228	1.58800361	0.2108675
Density * Population	2	0.01378756	0.00689378	0.10622071	0.8993560

Density * Species Mixture Treatment	4	0.38815904	0.09703976	1.49520753	0.2117686
Population * Species Mixture Treatment	2	0.16478266	0.08239133	1.26950162	0.2867119
Density * Population * Species Mixture Treatment	4	0.44212026	0.11053006	1.70306876	0.1577676
Residuals	78	5.06224132	0.06490053		

Fig. S8: Coefficient of variation (CV; the ratio of standard deviation to the mean) (mean ± SE, n = 6) of the time (**a, c, e**) taken to begin activity and (**b, d, f**) complete burial for individuals of the species *Amphiura chiajei* and *Amphiura filiformis* maintained under differing (**a – b**) species mixture treatments and (**c – d**) densities, and (**e – f**) originating from different populations.

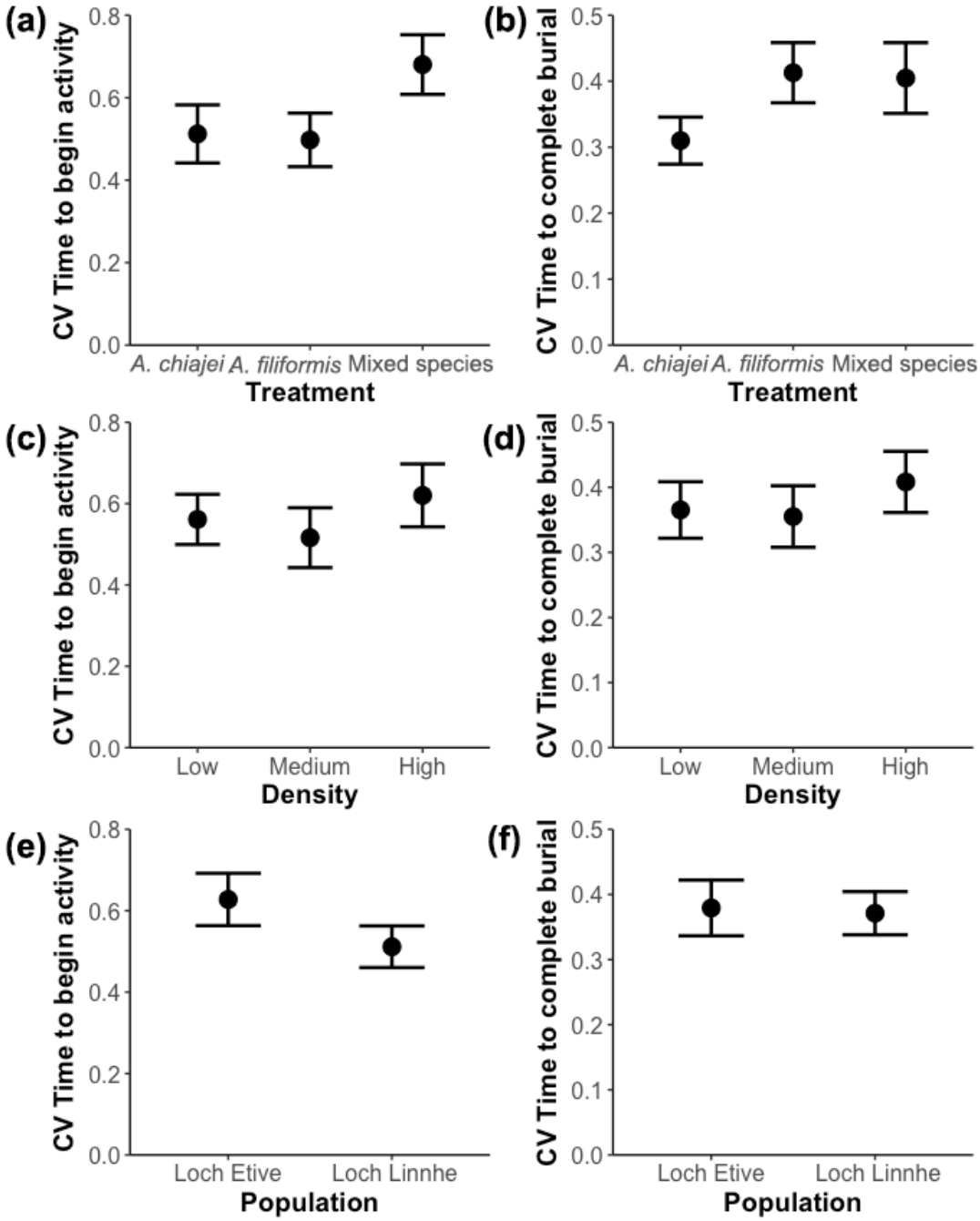


Fig. S9: Coefficient of variation (CV; the ratio of standard deviation to the mean) for the mean arm length of individuals of species *Amphiura chiajei and Amphiura filiformis,* showing a non-significant difference between densities and populations (ANOVA: $F_{1,94} = 0.02$, $P = 0.8836$).

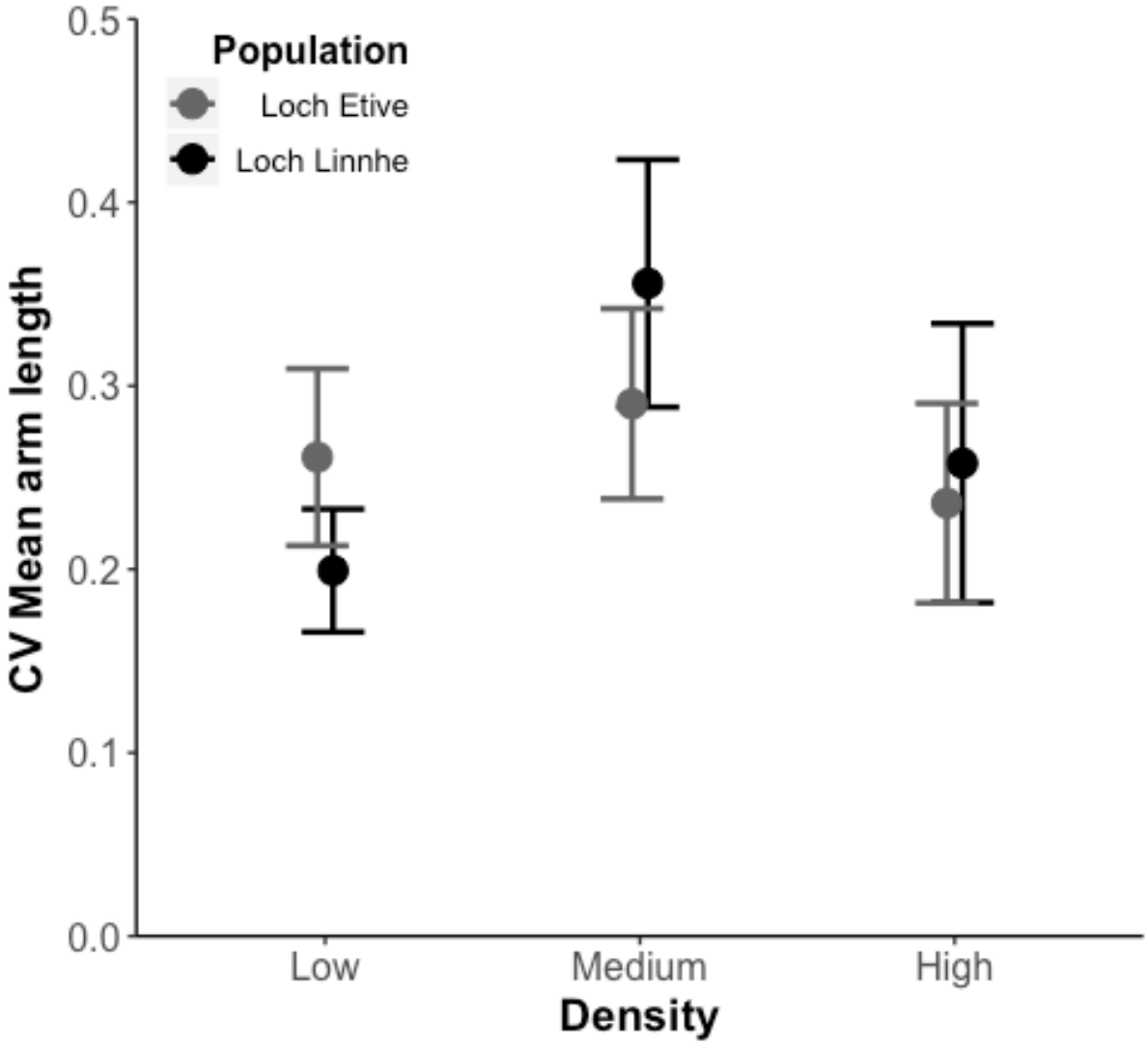


Fig. S10: Bioirrigation activity (mean ± SE) (Δ[Br−], mg L^{-1}) for *Amphiura chiajei* and *Amphiura filiformis* maintained under differing densities and originating from populations in either Loch Etive or Loch Linnhe, showing a non-siginificant interaction of density x population (ANOVA: $F_{2,89} = 2.24$, $P = 0.1120$).