

Abundance And Active Patch Selection Modulate Reproductive Connectivity And Fitness of Pea Crabs Living On Sand Dollars

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Abstract

Connectivity is paramount for population stability, but the mechanisms underlying the distribution of populated patches and how they affect reproductive connectivity and individual fitness remain elusive. Here, we mapped the distribution of sand dollars – as habitat patches for obligate-commensal pea crabs – at several sites. At occupied patches, we assessed whole-crab population structure and the fitness of ovigerous females. While sand-dollar supply did not limit the size of crab populations, overall crab abundance limited reproductive connectivity and the potential for offspring production. However, except for sites of extremely low and high connectivity, crab aggregations at sand-dollar clusters countervailed the overall random distribution of sand-dollar populations, greatly enhancing the reproductive potential of whole-crab populations. Crab interactions, likely controlled by larger females, added to reproductive connectivity by increasing the frequency of mating pairs in hosts. Differently from the population-level case, effects of crab abundance on individual fitness were dual and only detectable when abundance was lowest (positive) or highest (negative), so that fitness remained high at intermediate crab abundance, decreasing when it became either too low (e.g. Allee effects) or too high (e.g. energetic costs of intraspecific competition). This study indicates that connectivity may affect different levels of biological organization in specific ways.

Introduction

Environmental heterogeneity is commonplace in virtually all terrestrial and aquatic habitats, profoundly affecting the abundance and distribution of species [1]. Key ecological resources, mostly food and shelter [2, 3, 4], are usually found in discrete habitat patches where individuals aggregate and closely interact, thus forming local populations. At spatial scales large enough to include several of such patches, populations typically show a consistent structure, persisting over time through individual dispersals connecting populated habitat patches [5, 6]. Connectivity among localized populations within the operational metapopulation ultimately reduces intraspecific competition at crowded patches [7, 8], while preventing population density at marginal ones to decrease beyond critical thresholds [9, 10]. However, dispersal among already occupied patches and colonization of new areas [11, 12] also imply on higher mortality rates, as individuals need to travel over unsuitable habitat where, for instance, food supply is very limited [13, 14] or predation risk is very high [15, 16]. Therefore, populated habitat patches within all potential habitats in the metapopulation are expected to be closer to each other than would be anticipated based on habitat quality and carrying capacity alone.

Despite the relatively well documented role of connectivity on the overall abundance and stability of animal populations [17, 5], little is known on how individual dispersals may in the first place affect the spatial arrangement of occupied habitat patches and thus increase the rate of conspecific interactions. Particularly relevant is the case of mating interactions, as several populations may be restrained by negative density-dependent effects (e.g. Allee effects [18, 19]), in which overall offspring production is limited by the scarcity of encounters between potential mates [20, 21]. While shortage of mating encounters may eventually impact species that form relatively large and localized populations, with episodic dispersal events, it may become absolutely critical in species forming populations of only a few individuals, which strongly rely on frequent interchange. Therefore, any patterns of individual dispersals promoting smaller distances between populated habitat patches may greatly contribute to overall population connectivity and reproductive output. Moving beyond the population level, the effects of reproductive connectivity on individual fitness are at the best of our knowledge unknown. The most intuitive prediction is that the more frequent the encounter rate between potential mates, the higher the chances of individuals to maximize their offspring production. However, this may not be the case for animals that exploit very specific and small habitat patches. On one hand, those species often defend their territory for a chance to attract a suitable mate [22, 23, 24], but on the other they also frequently face strong intraspecific competition [25, 26], potentially reducing the capacity of individuals to allocate resources for reproduction.

Connectivity in populations of marine benthic invertebrates may be viewed as a combined result of processes operating at two different spatial scales. Most species undergo an indirect life cycle, with the release of free-swimming larvae that usually spend days to weeks in the water column and disperse over distances of a few to tens of km [27]. While most studies had emphasized large-scale connectivity through larval dispersal [28, 29], less attention has been paid to smaller-scale connectivity involving the movements of adults, which can be complex and underlie important reproductive processes (as in decapod crustaceans [30, 31, 32]). The case of the obligatory association between pea crabs and sand dollars is especially interesting for the study of metapopulation dynamics, because sand-dollar hosts provide a hard substrate and food resources for crabs [33], constituting island habitats amidst the otherwise unsuitable sandy seabed. The tight relationship with sand-dollar hosts shapes the mating systems of pea crabs [34]. Unlike most other brachyurans, females are larger than males [24, 35] and therefore more likely to defend their territory and expel any crabs from their host. Still, there is no evidence of durable pair bonds and monogamy for most pea crab species, with frequent dispersals from one host to another [36, 35], especially when the distance between both is small [37, 34]. Females may greatly benefit from multiple mating [38] as they can use sperm from up to six different males in a single brood [39] and produce brood sizes from 66 to 97% of whole-body weight (compared to brood constraints around 10% for other brachyuran crabs [40]). Therefore, restricted access to reproductive males may greatly restrain the potential for egg production owing to sperm limitation [41, 42].

In this study, we focus on populations of pea crabs *Dissodactylus crinitichelis* Moreira, 1901 [43], living on sand dollars *Encope emarginata* (Leske, 1778) [43]. The carrying capacity of sand dollars is apparently restricted to four adult crabs, with density-dependent processes regulating dispersal rates to an alternative host [35]. The mating system is not elaborate [35], with males probably engaging in simple mate searches [44], as observed in other pinnotherid species [45, 33]. Joint chemical cues released by healthy sand-dollars hosting conspecifics are likely used by crabs while shifting hosts and searching for mates [46]. We used distribution maps (Supplementary Fig. S1) at nine different sites along a 16-km long coastline (Supplementary Fig. S2) to first test whether crabs equally colonize potential hosts or alternatively occupy sand-dollar clusters, as expected by chemical mediation. The population structure of crabs at occupied sand dollars was assessed to elucidate possible mechanisms underlying the formation of non-random distribution patterns. We then used connectivity metrics based on Incidence Function Modeling (IFM, Moilanen and Nieminen, 2002) [47] to test whether crab colonization patterns actually enhance reproductive connectivity at occupied sand-dollars compared to vacant ones, and finally test whether the accessibility of females by searching males alter the fitness of the former, by assessing deviations from expected reproductive outputs. A final analysis using overall sand-dollar and crab population parameters as independent variables, and spatial distribution patterns and connectivity estimates as dependent ones, was used to put into context the potential role of crab gregarious behavior in the determination of reproductive connectivity and its effects on individual fitness.

Results

Correlations among sand-dollar and crab population parameters

Most overall variables of crab and sand-dollar populations were not significantly correlated (Table 1), including a lack of correlation between host and crab abundance (which was actually a negative, not positive, numerical trend). The only correlations that were significant were those between host abundance and occupancy ratio (negatively), and between host demand (n.crabs/sand-dollar) and crab abundance (Table 1).

Table 1

Collinearity matrix for overall population parameters of crabs (*Dissodactylus crinitelis*) and their sand-dollar hosts (*Encope emarginata*). Correlation coefficients in bold indicate statistical significance ($p < 0.05$).

			(1)	(2)	(3)	(4)	(5)
	Mean	sd					
Host abundance (1)	229.22	160.50	-				
Occupancy ratio (2)	0.34	0.19	-0.74	-			
Crab abundance (3)	72.00	49.19	-0.36	0.56	-		
Prop. Females (4)	0.47	0.12	0.27	-0.20	-0.36	-	
N.crabs/sand-dollar (5)	0.44	0.27	-0.61	0.67	0.32	-0.41	-

Spatial distributions of sand dollars

Several abiotic factors, not addressed in this study, have likely influenced sand-dollar abundance and distribution patterns at the sampled sites. However, in all sampled sites, except for Barreiros and Araçá, the spatial distribution of the subset of occupied sand dollars clearly differed from the distribution of the whole sand-dollar population (Supplementary Fig. S1, Fig. 1). Except for Araçá, and for a very small difference, whole populations were remarkably distributed at random, while the subsets of occupied sand dollars were always clearly clumped (Fig. 1). Sand dollars hosting crabs were typically 0.3 to 0.5 m closer to their nearest neighbor compared to expected distances for random positions, except for Barra Velha South and North, where occupied sand dollars were even more aggregated, 0.9 to 1.3 m closer than expected, respectively (Fig. 1). Barra Velha North was also the site where the overall abundance of sand dollars and crabs, and the proportion of females were the lowest (Supplementary Table S1). Likewise, Barra Velha South ranked very low in all those three parameters (6th, 6th and 7th, respectively).

Population structure of crabs

The number of adult crabs in sand dollars ranged between one and three, with a much higher frequency of sand dollars hosting a single crab (75.4%), compared to those hosting two (22.8%) or three (1.8%, Fig. 2). The overall sex ratio departed from the 1:1 ratio ($\chi^2 = 7.13$, $p = 0.008$), with a higher proportion of males (0.55) than females (0.45, Fig. 2). From all possible combinations of males and females for any given sand dollar, only two were not observed in the field [1 male plus 2 females (1M2F) and 3 females (3F)]. The most obvious deviations from theoretical frequencies, assuming random distribution of crabs among occupied sand dollars and independent occurrence of males and females, were a lower frequency of solitary males and females, a much higher frequency of sand dollars hosting a sexual pair (1M1F), and a much lower frequency of sand dollars occupied by two females (2F; Fig. 2). The indent in Fig. 2 groups the different combinations that allowed testing our predictions. Results show that (i) males were more likely than females to coexist with other individuals of the same sex ($\chi^2 = 15.69$, $p < 0.01$), and (ii) from all sand dollars populated by two or three crabs, those hosting at least a male and a female (*M*F) were more frequent than expected by chance ($\chi^2 = 38.21$, $p < 0.01$).

The same predictions were tested in all sites separately, but test power was restrained by low sample size (number of crabs). Even so, departures followed the same directions when detected (Supplementary Fig. S3). Prediction (i) was held at Araçá ($\chi^2 = 7.97$, $p < 0.01$; Fig. S3g), and prediction (ii) was supported at Armação North ($\chi^2 = 20.65$, $p < 0.01$; Fig. S3b), Barreiros ($\chi^2 = 6.93$, $p < 0.01$; Fig. S3d), Praia Preta ($\chi^2 = 8.01$, $p < 0.01$; Fig. S3h), and Praia Grande ($\chi^2 = 10.26$, $p < 0.05$; Fig. S3j). Likewise, in all sites where the sex-ratio differed significantly from the 1:1 proportion, males outnumbered females. This was the case at Armação South ($f_0 = 0.70$, $\chi^2 = 13.76$, $p < 0.01$; Fig. S3c), Araçá ($f_0 = 0.66$, $\chi^2 = 10.57$, $p < 0.001$; Fig. S3g) and Praia Grande ($f_0 = 0.61$, $\chi^2 = 3.85$, $p < 0.05$; Fig. S3j).

Connectivity estimates for hosting reproductive populations

At most sites, reproductive connectivity was higher for occupied sand dollars compared to vacant ones (Fig. 3). This difference was statistically significant at five sites, Armação South ($z_U = 3.33$, $p = 0.0009$, Fig. 3b), Barra Velha North ($z_U = 2.66$, $p = 0.008$, Fig. 3d), Barra Velha South ($z_U = 1.99$, $p = 0.047$, Fig. 3e), Praia Preta ($z_U = 2.74$, $p = 0.006$, Fig. 3g) and Praia Grande ($z_U = 3.56$, $p = 0.0004$, Fig. 3i), almost corresponding to the five sites where spatial distributions of all sand dollars and occupied ones differed the most (Fig. 1). The exception is Saco Grande, which ranked 4th in spatial distribution differences but showed an inverted numerical difference of reproductive connectivity, higher at vacant sand dollars (Fig. 3h). At this site, the average z-scores, computed for the whole pool of occupied sand dollars across sites ($Z_{C_{occ}}$), was the lowest (-0.92, Supplementary Table S2). Interestingly, the other case of inversion was observed at Armação North (Fig. 3a), where $Z_{C_{occ}}$ was the highest (+2.07, Supplementary Table S2).

Connectivity estimates on individual fitness

The allometric model fitted well size (CW) to fecundity relationships (Supplementary Fig. S4, Supplementary Table S3), except for Armação South (Fig. S4b, Table S3), where the size range of ovigerous females (5.0 to 6.1 mm) was probably too narrow for a proper analysis. Mean body size [4.8 mm at Armação North (Fig. S4a) to 6.9 mm at Barra Velha North (Fig. S4d)] and average fecundity [233 eggs at Armação North (Fig. S4a) to 587 at Barra Velha South (Fig. S4e)] showed substantial variation across sites. This was also the case of intercepts (0.84–34.50, mean 10.7) and allometric coefficients (1.2–3.3, mean 2.3, Table S3), leading to substantial differences in size-adjusted fecundity, for instance higher at Saco Grande (Fig. S4h) compared to Barreiros (Fig. S4c). Those cross-site differences should however be viewed with caution because sites were surveyed only once and at different times, therefore not controlling for any possible temporal effects within the breeding season such as those caused by temperature changes, or the dynamics of breeding cohorts.

Still, we assumed that any connectivity effects on individual fitness would be largely consistent over the time we undertook fieldwork. The relationship of connectivity (i.e. a proxy for the frequency of mating interactions through male searching) and female individual fitness (i.e. the departure from expected fecundity) was highly variable across sites (Fig. 4). In most cases effects were slight, with slope values (b_f) ranging from -5.80 to 8.28 (Supplementary Table S4), but in two cases effects were large and significant ($p < 0.05$), negative at Armação North ($b_f = -20.4$, $R = 0.48$; Fig. 4a) and positive at Barra Velha North ($b_f = 12.1$, $R = 0.74$; Fig. 4b)

Overall relationships of host and crab population parameters on connectivity and its effects on individual fitness

The best models explaining the variation of all three dependent variables examined (i.e. aggregation index, reproductive connectivity and connectivity effects on individual fitness) included only a single factor (Table 2). In all cases, both the full model and parameter coefficients were significant. Host demand, as the number of crabs per sand dollar, explained 78% of the variation of the aggregation index of occupied sand dollars, and crab abundance explained 93% and 46% of the variation of the reproductive connectivity of occupied sand dollars and the variation of connectivity effects on fitness, respectively. Occupancy ratio was left out of the list of independent variables owing to its correlation with other two predictors (Table 1).

Table 2

Summary results of stepwise backward multiple regression analyses testing the overall effects of host abundance (the abundance of sand dollars), crab abundance, proportion of female crabs, and host demand (crab abundance / host abundance) on the aggregation index and reproductive connectivity of occupied hosts, and on the effects of connectivity on individual fitness (estimated as the slope of connectivity vs. fitness relationships, see Fig. 5). Significance of overall selected models and respective predictors are specified in bold ($p < 0.05$).

	Aggregation index (R_e/R_o)			Reproductive connectivity (ZC_{occ})			Connectivity effect on fitness (b_j)		
	Coefficient	t	p	Coefficient	t	p	Coefficient	t	p
All effects									
Intercept	2.7316	2.19	0.094	-1.0990	-1.26	0.276	15.8528	0.80	0.469
Host abundance	0.0000	-0.03	0.981	0.0001	0.08	0.939	0.0200	0.86	0.439
Crab abundance	-0.0003	-0.07	0.944	0.0196	6.81	0.002	-0.1546	-2.35	0.078
Prop. females	0.7520	0.44	0.685	-0.5144	-0.43	0.690	-26.6822	-0.98	0.384
N.crabs/sand-dollars	-2.4835	-2.75	0.051	-0.2070	-0.33	0.759	11.2556	0.79	0.476
	multiple r^2	$F_{4,4}$	p	multiple r^2	$F_{4,4}$	p	multiple r^2	$F_{4,4}$	p
	0.794	3.84	0.110	0.936	14.69	0.012	0.657	1.92	0.272
Backward stepwise solution									
Intercept	3.1183	11.77	< 0.001	-1.4129	-8.20	< 0.001	11.3662	2.39	0.048
Host abundance	-	-	-	-	-	-	-	-	-
Crab abundance	-	-	-	0.0196	9.76	< 0.001	-0.1353	-2.43	0.045
Prop. females	-	-	-	-	-	-	-	-	-
N.crabs/sand-dollars	-2.6257	-5.01	0.002	-	-	-	-	-	-
	multiple r^2	$F_{1,7}$	p	multiple r^2	$F_{1,7}$	p	multiple r^2	$F_{1,7}$	p
	0.782	25.06	0.002	0.932	95.25	< 0.001	0.459	5.93	0.045

Those three main relationships are plotted in Fig. 5. The negative relationship between host demand and aggregation of occupied sand dollars (Fig. 5a), and the positive relationship between crab abundance and reproductive connectivity (Fig. 5b) are strong and compelling. The negative linear relationship between crab abundance and the effects of connectivity on individual female fitness is weaker, though significant. It is interesting to note, however, that the positive effects at Barra Velha North and the negative effects at Armação North, which were the only significant trends in Fig. 4, showed up in extreme positions in Fig. 5c.

Discussion

Connectivity among discrete population patches - a key process in metapopulation dynamics - strongly depends on the spatial distribution of both occupied and vacant habitat patches, as well as dispersal efficiency [48]. Here we show that a

combination of early colonization and later adult movements promotes a clustered distribution of occupied habitat patches, which in almost all cases contrasts with a remarkable random distribution of all patches that can be potentially colonized. Any mechanisms involving active habitat selection would thus enhance average connectivity and possibly the persistence of populated patches [5, 49, 50]. This translated to a higher reproductive connectivity of occupied patches compared to vacant ones, with likely higher encounter rates between males and females at five out of the nine study sites. While gregarious behavior apparently favors the reproductive potential at the population level, effects at the individual level appear to be dual. Effects on individuals are generally weak, but at the sites where overall crab abundance is very high and very low, connectivity effects are respectively negative and positive, indicating that infrequent access to potential mates may indeed restrain brood size, but also that crowding likely elicits strong intraspecific competition, ultimately impacting egg production. Individual fitness is therefore apparently maximized at average crab density.

The aggregation index of occupied hosts was negatively correlated to the demand of sand dollars; that is, when crabs are too scarce for the number of available hosts, occupied sand dollars become way much closer than expected for a random distribution. A more clustered distribution in sparser crab populations may enhance per capita reproductive rate and also reduce predation risk [51, 52]. Other biological mechanisms granted to symbionts and commensals as these pea crabs, such as the ability of localizing and recognizing conspecifics and hosts by chemical cues dissolved on water [53, 54], can also mediate communication among populations in such a small system. As reported in other studies, chemical attraction in marine systems is effective even when the concentration of water-borne cues is very low [55, 56], so that the few crabs present in a thin population may effectively cluster around the limited spots where host colonization already took place (as observed in Barra Velha North and South). On the contrary, when crabs are more abundant and scattered, the concentration of chemical cues are likely above any response threshold over the entire site [55], and individuals colonize available sand dollars at more variable distances from cue sources, especially when they are in short relative supply (as observed in Araçá and Praia Grande). Those chemically driven dynamics may set up an advantageous trade-off for pea crab populations, decreasing crowding within sand dollar hosts when overall crab abundance is exceedingly high, and favoring persistence at sites of low crab abundance by ensuring connectivity at the few spots where the occupancy ratio remains high.

Crab distribution patterns clearly affected reproductive connectivity. Gregarious behaviors are usually associated with adjustments in mating systems [44], which mediate competitive interactions while maximizing mating success. The frequency of sand dollars with only one adult crab, either a male or a female, is lower than expected, while sand dollars hosting a potential mating pair are more frequent than predicted by the null model assuming random distributions and independent occurrence of males and females. This suggests that lonely crabs are prone to leave their hosts to find a potential mate. In addition, the frequency of females (but not males) sharing their hosts with crabs of the same sex is lower than expected, indicating some sort of agonistic behavior that would ensure the winning female a higher mating success (as observed in pipefish [57]). This would set a selective pressure for large female size, which is likely on course as pinnotherid females, unlike most brachyurans, are larger than males. In the case of *Dissodactylus crinitichelis* females attain a size 12% larger than males [35], which suggests they play a major role in controlling the population structure within sand dollars.

The combined effects of gregarious behaviors, setting the spatial distribution of occupied sand dollars, and interactions among individuals, determining the population structure at each host, ultimately modulate the reproductive connectivity of each sand dollar. At most sites, crab dispersals and interactions contribute to an increase of potential encounters between males and females, as reproductive connectivity was generally higher for occupied sand dollars compared to vacant ones. When connectivity z-scores were computed for the whole sample instead ($Z_{c_{occ}}$), not separately for each site, we found that the two exceptional sites where empty sand dollars were the most connected ones (although without statistical support) were sites where occupied hosts were on average either the less or the most connected. This suggests that the capacity for crabs to actively reach and colonize more proximate sand dollar hosts, as to increase the chances of male –

female interactions, is lower when baseline reproductive connectivity is either very low, owing to an unfavorable combination of (high) distance among potential hosts, (low) crab abundance and (more) unbalanced sex-ratio (Saco Grande, Table S2), or very high, owing to an exceptional density of potential hosts (Armação South, Table S2), leaving no margin to active improvement of reproductive connectivity through crab movements. Therefore, except for those two extreme conditions, active selection of habitat patches may not only contribute to an increased dispersal rate (as host shifts), but also to an increased reproductive output of whole populations through an intensification of encounters between males and females (i.e. effective connectivity [58, 59, 60]). Independently of within-site differences between vacant and occupied sand dollars, the overall reproductive connectivity of crab populations is tightly related to overall crab abundance. Since there was no evidence of habitat limitation (i.e. no correlation between overall sand dollar and crab abundance), limited recruitment and / or high benthic mortality are the most likely processes controlling the size of *Dissodactylus crinitichelis* populations in the study area.

By modulating reproductive connectivity, crab distributions also affected individual fitness. Results showed that increased potential for encounter rates do not necessarily translate into increased fitness. In seven out of the nine sampled sites, reproductive connectivity effects on individual fitness were actually weak, indicating that large effects are only expected to take place under unusual circumstances. Again, crab abundance is apparently the most relevant variable, since connectivity effects on fitness were strong only at the sites where crab abundance was the highest (Armação North) and the lowest (Barra Velha North). Although seasonality may have played an important role on fecundity variation among sites, we still found a relationship between population connectivity and individual fitness of females. Also, both Armação North and Barra Velha North were sampled during the summer of 2020, suggesting that the significant (and opposite) trends at those sites were not an outcome of seasonality. In Armação North the effect was negative and very strong ($b_f = -20.4$), with less connected females producing broods 20% above the expected size (with one individual reaching > 60%), and more connected females producing broods nearly 40% smaller than expected. Given the wide variation in fitness and the higher number of observations below 0% (Fig. 4a), the trend suggests that most females may be facing strong intraspecific competition and resource limitation for egg production. This result is consistent with previous studies suggesting that interference competition for resource use may cause decline in reproductive success [61, 62, 26]. In contrast, the effect of reproductive connectivity was positive in Barra Velha North. Although significant, the effect in this case was not so strong ($b_f = +12.1$), with fitness varying within a slightly narrower range (-25% to +20%) and observations more symmetrically distributed around 0% (Fig. 4d). This result is consistent with sperm limitation caused by restricted access to suitable mates [41, 42].

Altogether, these results indicate that habitat supply is not limiting the size of pea crab populations in the study area, but crab abundance, in turn, is clearly restraining reproductive connectivity and thus the potential for offspring production. However, except for extreme cases of low and high connectivity, patterns of crab aggregations and eventual within-host interactions greatly enhance the reproductive potential, given the natural spatial distribution of sand dollars. This may be key for the long-term stability of thin crab populations scattered in sparse sand dollar hosts. Contrary to the reproductive potential of whole crab populations, crab abundance plays a dual indirect influence on individual fitness through contrasting connectivity effects. Individual fitness, measured as the percent deviation from expected egg production, is probably maximum at intermediate crab densities, not too low as to limit mate encounters, and thus sperm transfer, but also not too high, as to impose severe interspecific competition in the confined host habitat. This study highlights the need for a broader understanding of connectivity effects, beyond its role on basic demographic parameters and considering different levels of biological organization.

Methods

Study sites, fieldwork and laboratory procedures

We conducted this study along the São Sebastião Channel, in the State of São Paulo, Brazil (Supplementary Fig. S2). From November 2018 to August 2019, several preliminary diving surveys were undertaken along the margins of the channel, with depth varying from approximately 2 to 10 m, to find populations of the sand dollar *Encope emarginata*. After this searching period, nine sites were established for mapping (Fig. S2c): Armação North (3 m), Armação South (3 m), Barreiros (2 m), Barra Velha North (2 m), Barra Velha South (2 m), Araçá (3 m), Praia Preta (3 m), Saco Grande (4 m) and Praia Grande (8 m depth). We expected that despite abiotic differences among sites and seasonality crab population distribution would differ from sand dollar distribution.

From January 2019 to March 2020, a distribution map with the position of individual sand dollars was obtained at each site. Divers first defined a 15 x15 m square area, which was delimited with marked ropes (at every 1 m) to assist a close scrutiny of the whole area. For that, two pairs of divers used gridded 1 X 1 m PVC quadrats to define sand dollar positions with a resolution of 0.25 m². Each sand dollar was also checked for the presence of adult *Dissodactylus crinitchelis* crabs (carapace width, CW, larger than 3.0 mm), which were removed from hosts and placed in separate Eppendorf labelled tubes. In the laboratory, all crabs were sexed and measured (CW to the nearest 0.1 mm) and the eggs of all ovigerous females were counted under a dissecting microscope.

Numerical and statistical analyses

Correlations among sand-dollar and crab population parameters – A collinearity matrix for the variables (i) host abundance (the abundance of sand dollars), (ii) occupancy ratio (the proportion of sand-dollars hosting crabs), (iii) crab abundance, (iv) proportion of female crabs, and (v) host demand (crab abundance / host abundance) was calculated for a better understanding of the relationships between crabs and their hosts, and also to select the candidate independent variables that might affect crab connectivity and its potential influence on individual fitness (see below).

Spatial distributions of sand dollars - For each site map, we obtained a matrix of distances between all sand dollars using the ImageJ 1.x software [63], and calculated ΔR , i.e., the difference between the observed (R_o) and the expected (R_e) average nearest neighbor distance (NND) of sand dollars for a random distribution, and the respective standard error of this difference (S_R [64]). R_e was corrected for the absence of boundary strips. Separate 95% confidence intervals (CIs) around ΔR estimates were calculated for the distribution of all sand dollars, and the subset of sand dollars hosting an adult crab population. Cases where CIs included zero indicated random spatial distributions, and cases where CIs spanned only positive and only negative values indicated uniform and clumped distributions, respectively. For sand dollars hosting crabs, uniform distributions would support crab repulsion through interference competition, while clumped distributions would favor connectivity and the formation of adult crab aggregations.

Population structure of crabs – The number of adult crabs populating sand dollars is often low [35], so that all combinations of males and females can be easily listed. Here, this number varied from 0 to 3, and therefore the population structure in any given occupied sand dollar necessarily fell in 1 out of only 9 possibilities [i.e., from 1 male (1M) to 3 females (3F)]. The expected frequencies of all 9 sexual combinations were calculated in two steps. First, we determined the expected frequency of sand-dollars occupied by 1, 2 and 3 crabs, regardless of their sex, using a zero-truncated Poisson distribution [65],

$$g(k; \lambda) = P(x = k | x > 0) = \frac{\lambda^k}{(e^\lambda - 1)k!}$$

where k is population size (number of crabs, from 1 to 3) and λ is the average estimate of the number of crabs per sand dollar, using the whole sample (including unoccupied sand dollars).

Second, we determined the share of each sexual combination, within each population size, based on the binomial distribution,

$$f(n, k, p) = \binom{k}{n} p^n (1 - p)^{k-n}$$

where n is the number of males, p is the estimated proportion of males and $(1-p)$ the estimated proportion of females. The resulting null model thus assumes random distribution of crabs among occupied sand dollars and independent occurrence of males and females.

Overall departures from expected values were examined using a chi-square goodness-of-fit test for the whole dataset and for each site separately. We then tested (i) whether females are less likely than males to share their host with crabs of the same sex, and (ii) whether the frequency of sand-dollars hosting a sexual pair is higher than could be advanced by chance. For the first we tested the dependence of 'sex' (male, female) and 'number of individuals' (1, > 1) in 2 X 2 contingency tables, and for the second we ran a goodness-of fit test for the subset of sand-dollars hosting 2 or 3 crabs, to assess whether the formation of heterosexual pairs is more frequent than expected. In both cases we used the chi-square statistic when expected frequencies were all ≥ 5 , and the Fisher exact test when this condition could not be met. Critical p -values were adjusted using the Bonferroni correction for multiple comparisons.

Connectivity estimates – First, we intended to test whether the potential for hosting a reproductive population differed between empty and occupied sand dollars. We used a connectivity measure based on the Incidence Function Model (IFM), following the recommendations of Moilanen and Nieminen (2002) [47]. We considered that individual crab movements over 3–4 weeks, comprising at least two reproductive cycles and eventually host shift and mate search, would usually not exceed 2.5 m away from the focal sand-dollar host. Therefore, only potential hosts within this buffer radius were considered to comprise the metapopulation around any given focal sand-dollar. When focal sand-dollars were close to margins and the buffer radius partially lied off the sampling square area, connectivity estimates were corrected proportionally. These estimates (C_i) aimed to measure the likelihood of sexual pairings, assuming that the reproductive value of each crab is inversely related to (i) the distance from the focal sand dollar and (ii) the proportion of its sex within the buffer radius, and were calculated as:

$$C_i = \sum_{j=1}^n \exp(-d_{ij}) \times 2(n_{mj}\hat{p}_f + n_{fj}\hat{p}_m)$$

where n is the number of occupied sand dollars within the metapopulation, d_{ij} is the distance of the reference sand dollar j to the focal sand-dollar i , n_{mj} and n_{fj} are the number of males and females in the reference sand dollar, and \hat{p}_m and \hat{p}_f are the proportion of males and females in the potentially interacting crab metapopulation, respectively. Connectivity values were converted to z -scores (z_c), separately for each site, and compared between empty and occupied sand dollars using the U Mann-Whitney procedure owing to several cases of strong right skewness. Because sample size was in most cases large ($n > 20$) p -values were computed for obtained U statistics using the normal distribution approximation (z_U). Aligned to the expectations explained above, in '*Spatial distributions*', occupied sand dollars would be less connected in the case of crab repulsion through agonistic interactions, but more connected if interactions ultimately lead to the proximity of potentially mating pairs.

For the case of *individual fitness*, we focused only on sand dollars hosting an ovigerous female. The goal here was to estimate how often focal breeding females would be accessed by searching males within the buffer radius, and then test the correlation between this connectivity measure and a proxy of fitness based on egg production. We anticipated that female reproductive output would be highest when searching males would not be too numerous as to compete with females for resources, but also not too low as to reduce mating frequency to the point of sperm limitation [41, 42]. Connectivity here was calculated using the same rationale above, but accounting only for the number of potential mates and their distance to focal ovigerous females:

$$C_i = \sum_{j=1}^n \exp(-d_{ij}) \times (n_{mj})$$

Estimates of fitness were obtained by fitting the allometric model to size (= CW) vs. fecundity data [66] for each site, separately, and then measuring the relative residual (in %) of each fecundity estimate from its expected value. A negative correlation between connectivity and fitness (i.e. a negative slope, $b_f < 0$) would suggest that searching males would negatively impact females, as through exploitative competition, while a positive one ($b_f > 0$) would indicate that mating opportunities are scarce, so that an increased frequency of male accesses ultimately leads to enhanced female fitness by increasing offspring size.

Overall relationships of host and crab population parameters on connectivity and its effects on individual fitness – The sand-dollar and crab variables examined for collinearities (see above), except for ‘occupancy ratio’ which was related to other two predictor variables (see results), were used here as independent variables in stepwise backward multiple regression analyses to indicate potential processes underlying site-to-site differences on the aggregation index (R_e/R_o) and reproductive connectivity of occupied sand-dollars ($Z_{C_{occ}}$ calculated from average C_i values at each site), and on the effects of female connectivity, as their access to searching males, on individual fitness (b_f).

Declarations

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AUTHOR CONTRIBUTIONS

JAS and AAVF designed the study, acquired and analyzed the data, interpreted results and wrote the paper. Both authors approved the submitted version and agreed to be personally accountable for their own contributions.

DATA AVAILABILITY

The datasets generated during the current study are available from the corresponding author on reasonable request.

ADDITIONAL INFORMATION

The authors declare no competing interests.

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Figures

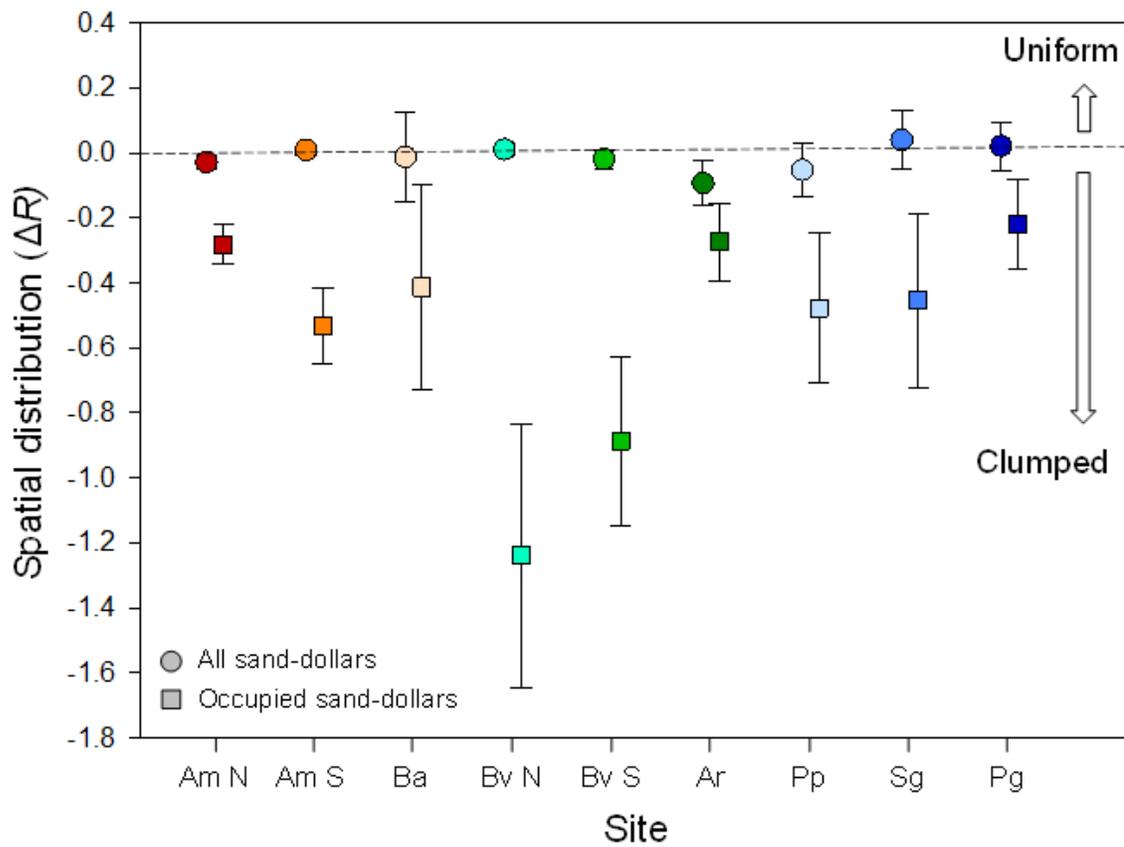


Figure 1

Spatial distributions of sand-dollars at sampling sites – Average differences, in meters, between observed (R_o) and expected (R_e , for a random pattern) nearest neighbor distances for all individuals, and for only those hosting crab populations in each sampling site. At $\Delta R = 0$ populations, marked with a dashed line, distributions are distributed at random. Error bars stand for 95% CIs. Site abbreviations as in figure 1.

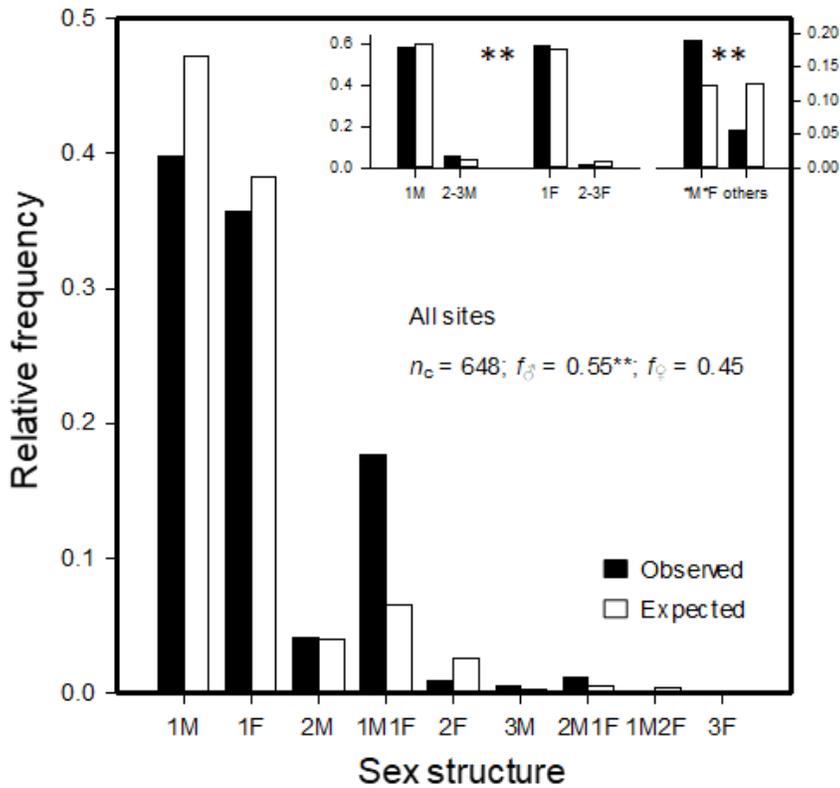


Figure 2

Population structure of pea crabs *Dissodactylus crinitichelis* - Overall (a) and site-specific (b-j) frequencies of all observed sexual combinations (M: male; F: female) in single sand-dollar hosts *Encope emarginata*. Expected frequencies, assuming random distribution of crabs among occupied sand dollars and independent occurrence of males and females, are given in solid color. Categories are grouped in the indent of plot (a) and in all single-site plots (b-j) as to test whether females have lower chance than males to coexist in the same host (left), and whether the occurrence of heterosexual pairs is higher than expected by chance for sand dollars hosting two crabs or more (right). n_c : total number of crabs, f_m : male relative frequency, f_f : female relative frequency, ns: non-significant, **: $p < 0.01$.

Potential to host reproductive populations

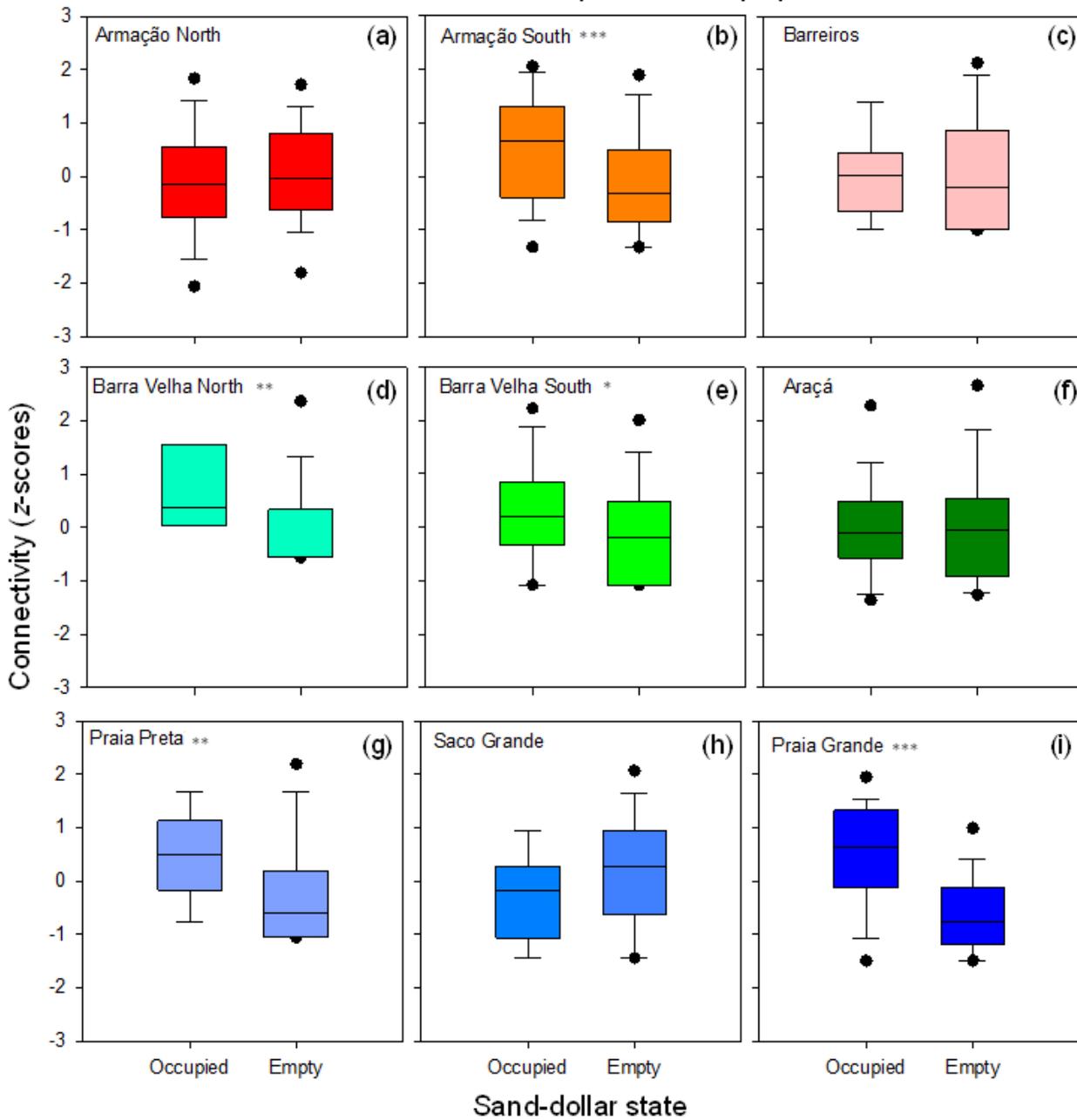


Figure 3

Connectivity estimates measuring the potential for hosting reproductive populations (male and female coexistence) in empty and occupied sand-dollars at the time of sampling in all study areas. Boxes show the median and the interquartile range, whiskers extend to 10th and 90th percentiles, and dots mark the 5th and 95th percentiles. Asterisk codes after site labels indicate statistical differences of connectivity between occupied and empty sand dollars: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0005$.

Effects on individual fitness

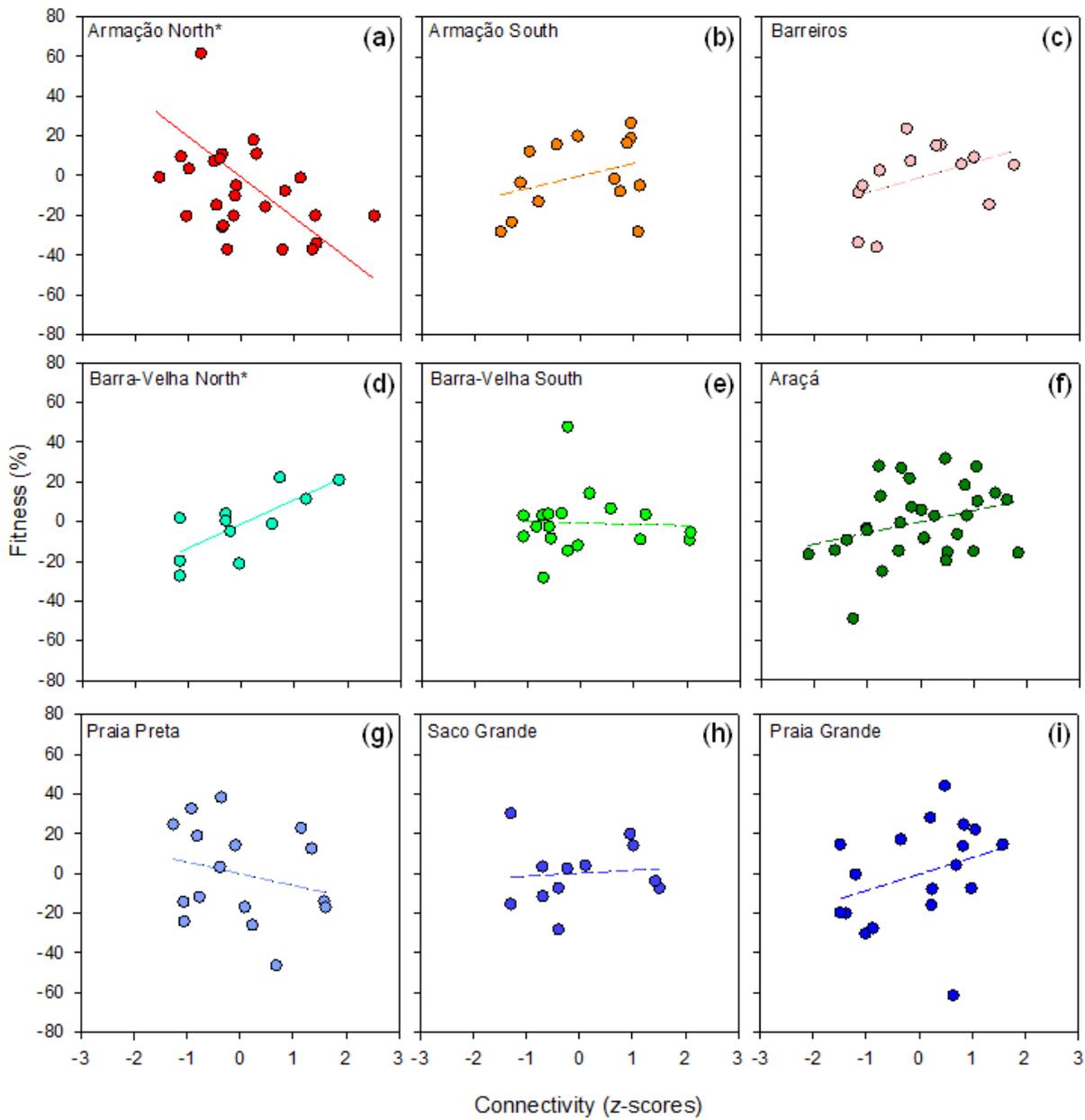


Figure 4

Relationships between connectivity metrics estimating the frequency of mating interactions and individual female fitness, as percent departures from expected fecundity. Significant relationships ($p < 0.05$), observed at sites Armação North and Barra Velha North, are shown as solid trend lines. More information on specific regression estimates are given in Table S3.

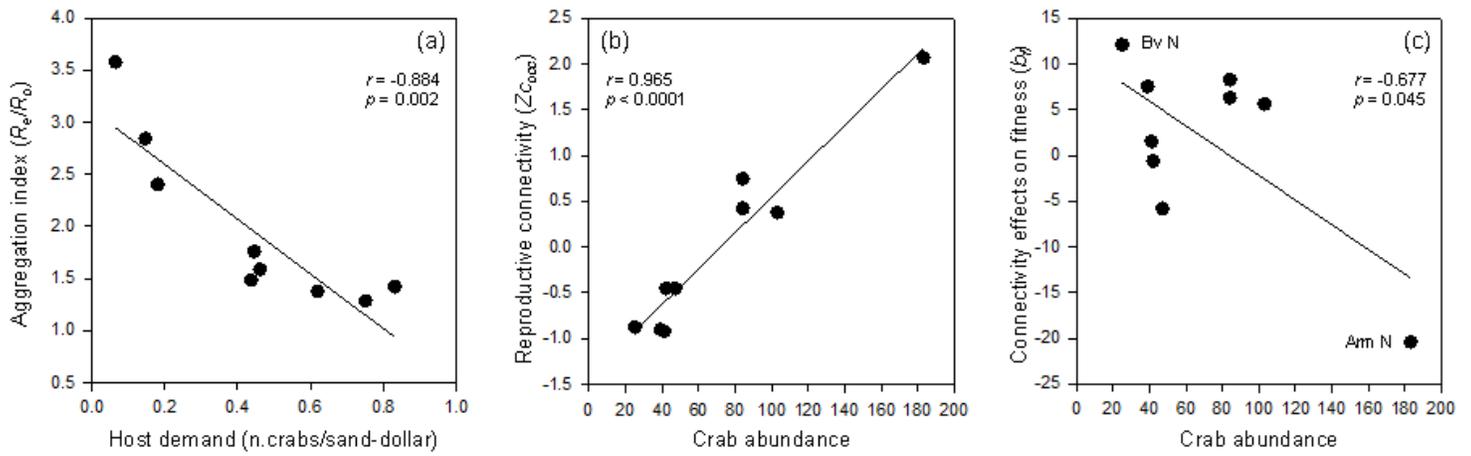


Figure 5

Linear trends between metrics relating overall population attributes and crab connectivity. (a) Negative relationship between host demand and the aggregation index (R_e/R_o) of occupied sand-dollars, (b) positive relationship between crab abundance and reproductive connectivity ($Z_{C_{occ}}$), and (c) negative relationship between crab abundance and the effects of connectivity on individual females fitness (β_f). The position of sampling sites Barra Velha North and Armação North, where connectivity to fitness relationships were extreme and significant, are indicated in panel (c).

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