Artificial shelters and marine infectious disease: no detectable effect of the use of casitas to enhance juvenile *Panulirus argus* in shelter-poor habitats on a viral disease dynamics

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Casitas, low-lying artificial shelters that mimic large crevices, are used in some fisheries for Caribbean spiny lobsters (*Panulirus argus*). These lobsters are highly gregarious and express communal defense of the shelter. Scaled-down casitas have been shown to increase survival, persistence, and foraging ranges of juveniles. Therefore, the use of casitas has been suggested to help enhance local populations of juvenile *P. argus* in Caribbean seagrass habitats, poor in natural crevice shelters, in marine protected areas. Following the emergence of Panulirus argus virus 1 (PaV1), which is lethal to juveniles of *P. argus*, concern was raised about the potential increase in PaV1 transmission with the use of casitas. It was then discovered that lobsters tend to avoid shelters harboring diseased conspecifics, a behavior which, alone or in conjunction with predatory culling of diseased lobsters, has been proposed as a mechanism reducing the spread of PaV1. However, this behavior may depend on the ecological context (i.e., availability of alternative shelter and immediacy of predation risk). We conducted an experiment in a lobster nursery area to examine the effect of the use of casitas on the dynamics of the PaV1 disease. We deployed 10 scaled-down casitas per site on five 1-ha sites over a reef lagoon (casita sites) and left five additional sites with no casitas (control sites). All sites were sampled 10 times every 3–4 months. Within each site, all lobsters found were counted, measured, and examined for clinical signs of the PaV1 disease. Mean density and size of lobsters significantly increased on casita sites relative to control sites, but overall prevalence levels remained similar. There was no relationship between lobster density and disease prevalence. Dispersion parameters (m and k of the negative binomial distribution) revealed that lobsters tended to avoid sharing natural crevices, but not casitas, with diseased conspecifics. These results confirm that casitas provide much needed shelter in seagrass

habitats and that their large refuge area may allow distancing between healthy and diseased lobsters. On eight additional sampling times over two years, we culled all diseased lobsters observed on casita sites. During this period, disease prevalence did not decrease but rather increased and varied with site, suggesting that other factors (e.g., environmental) may be influencing the disease dynamics. Using scaled-down casitas in shelter-poor habitats may help efforts to enhance juvenile lobsters for conservation purposes, but monitoring PaV1 prevalence at least once a year during the first few years would be advisable.

1 **Artificial shelters and marine infectious disease: no detectable effect**

2 **of the use of casitas to enhance juvenile *Panulirus argus* in shelter-**

3 **poor habitats on a viral disease dynamics**

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Abstract

Casitas, low-lying artificial shelters that mimic large crevices, are used in some fisheries for Caribbean spiny lobsters (*Panulirus argus*). These lobsters are highly gregarious and express communal defense of the shelter. Scaled-down casitas have been shown to increase survival, persistence, and foraging ranges of juveniles. Therefore, the use of casitas has been suggested to help enhance local populations of juvenile *P. argus* in Caribbean seagrass habitats, poor in natural crevice shelters, in marine protected areas. Following the emergence of Panulirus argus virus 1 (PaV1), which is lethal to juveniles of *P. argus*, concern was raised about the potential increase in PaV1 transmission with the use of casitas. It was then discovered that lobsters tend to avoid shelters harboring diseased conspecifics, a behavior which, alone or in conjunction with predatory culling of diseased lobsters, has been proposed as a mechanism reducing the spread of PaV1. However, this behavior may depend on the ecological context (i.e., availability of alternative shelter and immediacy of predation risk). We conducted an experiment in a lobster nursery area to examine the effect of the use of casitas on the dynamics of the PaV1 disease. We deployed 10 scaled-down casitas per site on five 1-ha sites over a reef lagoon (casita sites) and left five additional sites with no casitas (control sites). All sites were sampled 10 times every 3–4 months. Within each site, all lobsters found were counted, measured, and examined for clinical signs of the PaV1 disease. Mean density and size of lobsters significantly increased on casita sites relative to control sites, but overall prevalence levels remained similar. There was no relationship between lobster density and disease prevalence. Dispersion parameters (m and k of the negative binomial distribution) revealed that lobsters tended to avoid sharing natural crevices, but not casitas, with diseased conspecifics. These results confirm that casitas provide much needed shelter in seagrass habitats and that their large refuge area may allow distancing between healthy and diseased lobsters. On eight additional sampling times over two years, we culled all diseased lobsters observed on casita sites. During this period, disease prevalence did not decrease but rather increased and varied with site, suggesting that other factors (e.g.,

48   environmental) may be influencing the disease dynamics. Using scaled-down casitas in shelter-

49   poor habitats may help efforts to enhance juvenile lobsters for conservation purposes, but

50   monitoring PaV1 prevalence at least once a year during the first few years would be advisable.

51   **Introduction**

52   The Caribbean spiny lobster (*Panulirus argus*) is a valuable resource that sustains numerous

53   industrial and artisanal fisheries in the wider Caribbean region (Wahle, Linnane & Harrington,

54   2020; Doerr, 2021). Juveniles of *P. argus* dwell in shallow reef lagoons and embayments where

55   marine vegetation (seagrasses and macroalgal beds) abound. Marine vegetation provides

56   settlement habitat for postlarvae of *P. argus* and protection for the smaller juveniles; however,

57   juvenile lobsters eventually outgrow the protection afforded by the vegetation and seek nearby

58   crevice-type shelters before migrating as subadults to the coral reef habitats where the adults

59   live (Butler, Steneck & Herrnkind, 2006; Briones-Fourzán & Lozano-Álvarez, 2013).

60   Caribbean spiny lobsters are gregarious, with multiple individuals sharing crevice-type

61   dens. Aggregated lobsters express group defense, increasing the per capita survival (Eggleston

62   & Lipcius, 1992). Because the risk of predation is high for juveniles of *P. argus*, they greatly

63   depend on available crevice shelters for survival (Smith & Herrnkind, 1992; Behringer et al.,

64   2009). Shallow hard-bottom habitats may abound in potential shelters for juvenile spiny lobsters

65   in the form of large sponges, coral heads, solution holes, rocky outcrops, ledges, and crevices.

66   In contrast, soft-bottom habitats such as seagrass meadows are typically poor in crevice

67   shelters (Sosa-Cordero et al., 1998; Briones-Fourzán & Lozano-Álvarez, 2001; Behringer et al.,

68   2009), potentially causing local demographic bottlenecks for *P. argus* (Arce et al., 1997; Butler &

69   Herrnkind, 1997; Caddy, 2008).

70   Because lobsters will also take refuge in many types of man-made structures, several

71   highly productive Caribbean fisheries for *P. argus* have long used low-lying, flat-topped artificial

72   shelters called "pesqueros" in Cuba, "condos" in the Bahamas, and "casitas" in Mexico and

73   elsewhere (Briones-Fourzán, Lozano-Álvarez & Eggleston, 2000; Cruz & Phillips, 2000; Doerr,

74   2021). Concern has been raised about casitas potentially causing overexploitation, resulting in

75   their ban in some fisheries (e.g., Florida, USA) (Ross, Butler & Matthews, 2022). However,

76   overexploitation is more likely to occur where open-access fisheries conditions exist (Caddy,

77   2008; Doerr, 2021). In the casita-based Cuban and Mexican fisheries, which have regulations

78   such as the use of limited and enforceable spatial access rights, casitas have increased the

79   carrying capacities of the managed areas (Sosa-Cordero, Liceaga-Correa & Seijo, 2008;

80   Headley et al., 2017).

81   From the conservation viewpoint, some authors have expressed concern that the use of

82   casitas may alter the local benthic habitats or biological communities, or affect the lobsters

83   themselves. However, lobsters have a broad diet that includes many types of invertebrates

84   including clams with chemosynthetic bacteria that live in seagrass habitats (Higgs, Newton &

85   Attrill, 2016), and there has been little measurable impact of casitas on the abundance of the

86   invertebrate fauna on which lobsters feed (Vidal & Basurto, 2003; Nizinski, 2007), or on the

87   benthic habitats where casitas are deployed (Ross, Butler & Matthews, 2022). There is also no

88   evidence that casitas negatively affect subadult and adult *P. argus* (Gittens & Butler, 2018).

89   Because lobsters over a broad size range commonly occupy large commercial casitas (~2 m² in

90   area, 15 cm in height) (Lozano-Álvarez, Briones-Fourzán & Phillips, 1991; Sosa-Cordero et al.,

91   1998; Candia-Zulbarán et al., 2012), other authors have cautioned against the use of casitas in

92   nursery habitats because they may function as ecological traps for juveniles (Gutzler, Butler &

93   Behringer, 2015). However, it has been shown that shelter scaling is important for survival of

94   lobsters and for the enhancement of juveniles of *P. argus* in shelter-poor seagrass habitats

95   (Eggleston et al., 1990; Arce et al., 1997; Briones-Fourzán et al., 2007).

96   Based on the general positive or neutral effects of casitas on lobsters and their habitats,

97   the use of scaled-down casitas has further been suggested to enhance juvenile lobsters in

98   marine protected areas (Sosa-Cordero et al., 1998; Briones-Fourzán, Lozano-Álvarez &

99   Eggleston, 2000; Briones-Fourzán et al., 2007; Briones-Fourzán & Lozano-Álvarez, 2013). But

100   in 1999-2001, a previously unknown disease emerged in populations of juvenile Caribbean

101   spiny lobsters. The disease is caused by Panulirus argus virus 1 (PaV1) (Shields & Behringer,

102   2004), a member of the newly established family Mininucleoviridae (Subramanian et al., 2020).

103   PaV1 can be lethal for juveniles of *P. argus* ($\leq$ 50 mm carapace length, CL), for which this virus

104   shows predilection (Shields & Behringer, 2004; Li et al., 2008).

105   PaV1 can be transmitted by contact and through water, at least over distances of 1–2 m,

106   raising concerns about the potential increase in transmission with the use of casitas (Butler,

107   Behringer & Shields, 2008; Behringer & Butler, 2010; Behringer et al., 2012). However, *P. argus*

108   express behavioral immunity, i.e., a tendency to avoid odors emanating from infected

109   conspecifics (Behringer, Butler & Shields, 2006; Candia-Zulbarán et al., 2015), which may help

110   reduce the transmission of the disease (Anderson & Behringer 2013; Butler et al., 2015). Yet,

111   Lozano-Álvarez et al. (2008) observed high levels of cohabitation between healthy and diseased

112   lobsters in experimental scaled-down casitas deployed over shelter-limited seagrass habitats.

113   These authors hypothesized that, on these habitats, lobsters make a trade-off between avoiding

114   disease and avoiding predation risk, and that the large shelter area provided by casitas may

115   reduce physical contact among healthy and diseased lobsters. Upon testing these hypotheses,

116   Lozano-Álvarez et al. (2018) found that both the availability of alternate shelter and immediacy

117   of predation risk modulate the expression of behavioral immunity in *P. argus*. They also found

118   that healthy lobsters tended to be segregated from co-occurring diseased lobsters in casitas,

119   although this distancing decreased with increasing number of lobsters in a casita. In a different

120   study, distribution parameters of lobsters in large commercial casitas were generally not

121   affected by the presence of diseased conspecifics; rather, investment in disease avoidance by

122   lobsters appeared to be partially modulated by local habitat features (Briones-Fourzán et al.,

123   2012).

124   In addition to behavioral immunity reducing transmission of PaV1, Butler et al. (2015)

125   also contemplated the possibility of predatory culling of diseased lobsters, which has been

126   shown to reduce the spread of pathogens in some systems, but not in others (review in Lopez &

127   Duffy, 2021). Culling diseased individuals during surveillance or fishing operations has also

128   been suggested as a means to reduce disease transmission if the culled individuals are

129   disposed of at land (Behringer et al., 2012). However, whether culling is an efficient way to

130   manage marine diseases is still a matter of debate (Groner et al., 2016; Behringer et al., 2020;

131   Glidden et al., 2022).

132   The studies supporting the suggestion to use scaled-down casitas to enhance juvenile

133   lobsters in shelter poor habitats were conducted before the full establishment of PaV1, but as

134   juvenile lobsters are more susceptible to PaV1 than adults, further investigation is required on

135   the potential effects of casitas on the dynamics of the PaV1 disease. We addressed this issue

136   via a field experiment consisting of two stages. In the first stage, we examined the relationship

137   between the expected increase in density of juvenile lobsters with scaled-down casitas and the

138   prevalence of PaV1 disease, and compared the patterns of shelter occupancy by lobsters as

139   related to disease between casitas and natural crevices. We did not expect disease prevalence

140   to increase with the use of casitas despite increasing lobster density given the complex but

141   flexible behavioral responses of *P. argus* under different ecological contexts. In the second

142   stage, we tested whether the systematic culling of diseased lobsters from casita sites altered

143   the probability of disease and whether such changes were consistent among sites.

144   **Materials & Methods**

145   **Study area**

146   The study was conducted in the reef lagoon of the Puerto Morelos Reef National Park, a marine

147   protected area located on the northern part of the Mexican Caribbean coast. The reef lagoon

148   (centered at 20°52'07'' N, 86°51'40'' W) extends from the shore to the coral reef tract, which lies

149   at ~500 m to 2000 m from the shore. Maximum depth within the reef lagoon is 5 m (Fig. 1). No

150   lobster fishing is allowed within the reef lagoon.

151   The Puerto Morelos reef lagoon has been extensively studied since the early 1990s

152   (e.g., Rodríguez-Martínez et al., 2010; Caballero-Aragón et al., 2022). Based on its vegetation,

153   the lagoon is divided into a narrow coastal fringe (50–100 m in width), a broad mid-lagoon zone,

154   and a back-reef lagoon zone. The present study took place in the mid-lagoon, which

155   encompasses the greatest part of the lagoon, and the back-reef lagoon. In the mid-lagoon, the

156   sandy sediments tend to be deeper and the seagrass biomass and height are generally greater,

157   but with substantial temporal and spatial variation. In the back-reef lagoon, seagrass meadows

158   have generally less biomass, shorter leaves, and a less dense canopy because the sediment

159   layer is thinner and hard substrate is more abundant (van Tussenbroek, 2011; Zarco-Perelló &

160   Enríquez, 2019). Biomass of drift algae tends to be greater on the mid-lagoon than on the back-

161   reef lagoon zone (Van Tussenbroek, 2011; Lozano-Álvarez, Meiners & Briones-Fourzán, 2009).

162   Although the reef lagoon constitutes a nursery area for juveniles of *P. argus* (≤50 mm CL),

163   shelter is a limiting factor for the larger juveniles (Briones-Fourzán et al., 2007) because crevice-

164   type shelter is scarce and over-dispersed in the reef lagoon (Briones-Fourzán & Lozano-

165   Álvarez, 2001).

166   **Experimental design**

167   Permits to conduct this study were issued by Comisión Nacional de Acuacultura y Pesca

168   (DGOPA.12019.031108.3134, DGOPA-06695.190612.1737, and PPF/DGOPA-259/14). The

169   experimental design followed that of Briones-Fourzán & Lozano-Álvarez (2001) and Briones-

170   Fourzán et al. (2007). Briefly, 10 experimental sites were delimited in the reef lagoon (Fig. 1).

171   Each site measured 100 m × 100 m (= 1 ha), an area that exceeds the daily home range of

172   juveniles of *P. argus* (<1 to ~20 m) (Butler, Steneck & Herrnkind, 2006; Lozano-Álvarez,

173   Meiners & Briones-Fourzán, 2009). To ensure the independence of data, all sites were

174   separated from each other and from the reef tract by distances of 200 m to 600 m, which

175   exceed the movement range of juveniles ≤70 mm CL (Briones-Fourzán et al., 2007). In August

176   2009, we deployed 10 scaled-down casitas per site on five randomly chosen sites (sites 2, 4, 5,

177   7, and 8 in Fig. 1, hereafter "casita sites"), whereas the other five sites remained without casitas

178   (sites 1, 3, 6, 9, and 10 in Fig. 1, hereafter "control sites"). Casita dimensions were 1.1 m² in

179   area × 4 cm in entrance height and 8 cm in inner height (Fig. 2A-C). On each site, the 10

180   casitas were randomly deployed, but leaving a distance of at least 20 m between adjacent

181   casitas. To facilitate working within each site, wood stakes were installed every 10 m throughout

182   the site area.

183   **Experimental stage A**

184   Stage A was designed to examine the potential effect of casita deployment on prevalence of the

185   PaV1 disease as well as the occupancy patterns of shelters (both casitas and natural crevices)

186   by lobsters as related to disease. Briones-Fourzán et al. (2007) found that density and mean

187   size of lobsters significantly increased on casita sites relative to control sites, but their study

188   ended in 2002, when PaV1 was just emerging. Because the present study was conducted after

189   several years of increasing prevalence of PaV1 (Lozano-Álvarez et al., 2008), in addition to

190   clinical prevalence of PaV1 we also compared mean density and size of lobsters between casita

191   sites and control sites.

192   Briones-Fourzán et al. (2007) estimated a persistence of 80.9 ± 17.8 d (mean ± SD) for

193   juvenile lobsters on casita sites and of 40.7 ± 10.3 d on control sites. Therefore, between

194   November 2009 and April 2012, we conducted 10 samplings every 3–4 months to minimize the

195   possibility of serial correlation of data. Using scuba, samplings consisted of surveying the entire

196   area of each site for lobsters, including beneath casitas and in all pre-existing shelters, such as

197   hard coral heads, sponges, soft corals, outcrops, solution holes and all kinds of crevices. The

198   largest external diameter of a natural shelter is considered a good approximation to the shelter

199   area it provides (Childress & Herrnkind, 1997; Briones-Fourzán & Lozano-Álvarez, 2001);

200   therefore, pre-existing shelters were categorized into small (<25 cm across the largest external

201   diameter), medium (25–50 cm), or large (>50 cm). Lobsters were extracted from their crevice or

202   casita with hand nets and visually examined for clinical (macroscopic) signs of PaV1 infection

203   (milky hemolymph, visible through the translucid membrane between the cephalothorax and

204 abdomen, and a reddish discoloration of the clear marks over the exoskeleton, Fig. 2D) (Shields

205 & Behringer, 2004; Huchin-Mian et al., 2008). Lobsters with these signs are hereafter referred to

206 as "diseased" (Montgomery-Fullerton et al., 2007; Li et al., 2008; Candia-Zulbarán et al., 2012;

207 Huchin-Mian et al., 2013). Specificity and sensitivity of the macroscopic determination of PaV1

208 estimated against endpoint PCR were 1.0 and 0.5, respectively, both in the Puerto Morelos reef

209 lagoon, where mostly juvenile lobsters are found (Candia-Zulbarán, Briones-Fourzán & Lozano-

210 Álvarez, 2019), and in Bahía de la Ascensión, where lobsters span a greater size range

211 (Huchin-Mian et al., 2013). Therefore, in these areas, for every visibly diseased lobster there is

212 another subclinically infected lobster (i.e., lobsters carrying the virus but without having

213 developed the disease). However, for the sake of simplicity all lobsters with no clinical signs of

214 PaV1 are hereafter referred to as "healthy". The carapace length (CL, mm) of lobsters was

215 measured from the inter-orbital notch to the rear end of the carapace with Vernier calipers.

216 Examination and measurement of lobsters was conducted in situ (underwater) to avoid

217 exposure to air and to reduce handling stress. After data collection, all lobsters were carefully

218 returned to their previous shelter or casita. For each site and sampling time, disease prevalence

219 was estimated as the number of diseased lobsters over the total number of lobsters × 100.

220 **Experimental stage B**

221 Experimental stage B was intended to examine whether culling all diseased lobsters found on

222 each sampling date altered overall prevalence levels relative to stage A, and whether such

223 changes were consistent among sites. For these purposes, from September 2012 to January

224 2015 we conducted eight additional samplings every 3–4 months. On each of these samplings,

225 all lobsters with clinical signs of PaV1 were culled (i.e., removed and taken to land). Stage B

226 was conducted exclusively on casita sites. Control sites were not considered in this stage

227 because of their extreme paucity of lobsters (see Results).

228 **Data analyses**

229 **Experimental stage A**

230   *Lobster density and mean size*— The data on lobster density (previously transformed to Log (N

231   + 1) to increase homogeneity of variances) and lobster size were separately subjected to

232   repeated measures analysis of variance using a General Lineal Model (GLM) approach. The

233   main (fixed) factor was site type (with two levels, casita sites and control sites), whereas time

234   (10 samplings between November 2009 and April 2012) was the repeated measure.

235   *Relationship between lobster density and disease prevalence.* Initially, we had planned to use a

236   logistic regression analysis in which the binary response variable would be the

237   absence/presence of clinical signs of PaV1, to examine the effect of site type and sampling time

238   (categorical factors) on the probability of finding diseased lobsters (Quinn & Keogh, 2002).

239   Unfortunately, the complete lack of lobsters or the absence of diseased lobsters on one or more

240   control sites on several sampling times (Table S1) precluded the use of this analysis, as the

241   model required data on lobsters with and without clinical signs of PaV1 in all levels of both

242   factors. Instead, we used correlation analyses to examine whether disease prevalence tended

243   to increase with lobster density (lobsters ha^{-1}). For this analysis, we considered the data from all

244   casita sites and control sites on which the number of lobsters on a given sampling time was ≥5

245   (Putt et al., 1988). Separate analyses were performed for casita sites, for control sites, and for

246   all sites together. We further compared disease prevalence between site types with a Mann-

247   Whitney test.

248   *Occupancy of shelters as related to diseased individuals* — Preliminary analyses revealed that

249   very few small and medium pre-existing shelters were occupied by lobsters, both on casita sites

250   (0.6% and 7.0%, respectively) and on control sites (3.9% and 16%, respectively), and that in

251   most cases these shelters, when occupied, harbored a solitary lobster. In contrast, 33.0% of all

252   large pre-existing shelters on casita sites, and 37.2% on control sites, were occupied by one or

253   multiple lobsters. Therefore, only large pre-existing shelters (hereafter "crevices") were

254   considered for comparison with lobster distribution in casitas.

255   To determine whether the pattern of distribution of lobsters among crevices and casitas

256 varied as a function of the presence/absence of diseased lobsters in the shelter, we followed the

257 model selection approach used by Briones-Fourzán et al. (2012) for commercial casitas, based

258 on the negative binomial distribution (NBD) of the number of lobsters per shelter. The following

259 procedure was separately applied to casitas and crevices on casita sites, and to crevices on

260 control sites, but is only explained for casitas.

261 The NBD is defined by two parameters: m, which is the average number of lobsters per

262 casita (or crevice) and k, which is a dispersion parameter. As k tends to infinity, the distribution

263 approaches a random distribution and the data can be modeled as a Poisson process, whereas

264 as k tends to zero, the distribution becomes more clumped (White & Bennets, 1996). Model

265 selection uses a likelihood ratio testing framework to identify the best model out of a set of

266 competing models to explain selected parameters for a given set of samples (Burnham &

267 Anderson, 2002). We separated the total number of casitas examined over experimental stage

268 A into two samples: one consisting of casitas containing exclusively healthy lobsters, and one

269 consisting of casitas containing healthy + diseased lobsters. We then used a set of four

270 candidate models to compare parameters of the NBD of lobsters over our sampling times

271 (White & Eberhardt, 1980). The general (most parameterized) model, $\{k_v, m_v\}$, predicts that all

272 samples v differ in m and k. The other three models, which have fewer parameters, are $\{k, m_v\}$:

273 samples have a common k but different m; $\{k_v, m\}$: samples have a different k but a common m,

274 and $\{k, m\}$: all samples have a common k and a common m (the reduced model) (White &

275 Eberhardt, 1980; White & Bennets, 1996). The "best" model would be that with the lowest

276 Akaike Information Criterion corrected for sample size (AICc, see Burnham & Anderson, 2002).

277 However, delta AICc (Δ_i) and the Akaike weight (w_i) provide better measures of the strength of

278 evidence for each model. Δ_i is the AICc of a given model minus the AICc of the best model

279 (whose Δi is set to zero), whereas w_i represents the ratio of the Δ_i of a given model relative to

280 the whole set of models (the w_i from all models sum to 1) and thus represents the "probability"

281 of each model given the data. Akaike weights also provide a basis for model averaging, a

282   procedure that allows the entire set of models to be used to compute a weighted average for

283   each parameter and its corresponding unconditional variance (Hobbs & Hillborn, 2006).

284   The analysis was done with the software EcoMeth 6.1 (Kenney & Krebs, 2002), which

285   includes a modification of the computer program originally developed by White & Eberhardt

286   (1981) and White & Bennets (1996). The output from this software provides, for each model, the

287   goodness of fit to the NBD, maximum likelihood and AIC values, and the corresponding

288   estimates of m and k with their respective variances. These estimates allow the computation of

289   AICc, Δ_i, and w_i, as well as the model averaging procedure (see Burnham & Anderson, 2002;

290   Hobbs & Hillborn, 2006).

291   **Experimental stage B**

292   Data from experimental stage B were used to analyze the effects of culling and site on the

293   probability of finding disease. When the response variable is binary (e.g., presence/absence of

294   clinical signs of PaV1), the appropriate test is a logistic regression analysis, which is a type of

295   generalized linear model (Quinn & Keough, 2002). The predictors (categorical factors) in the

296   model were experimental stage and casita site. Site was included in the model to account for

297   the potential effects of local habitat characteristics. Experimental stage had two levels (stage A,

298   with no culling, and stage B, with culling), with stage A as the baseline (reference) level. Site

299   had 5 levels (corresponding to the 5 casita sites), with site 8 as the reference level. This

300   analysis revealed whether culling all diseased lobsters throughout experimental stage B altered

301   disease probability relative to stage A and whether these changes were consistent among

302   casita sites.

303   ## Results

304   **Experimental stage A**

305   *Lobster density and size* — Lobster density was significantly affected by site type and sampling

306   time, but not by their interaction (Table 1). On average, lobster density was about eight times as

307   high on casita sites (overall mean ± SD: 72.4 ± 46.3 lobsters ha^{-1}) as on control sites (8.7 ± 1.7

308 lobsters ha⁻¹), but with substantial temporal variation on both site types (Fig. 3A). Similarly,

309 lobster size was significantly affected by site type and sampling time, but not by their interaction

310 (Table 1). The mean size of lobsters was generally higher on casita sites (overall mean ± SD:

311 30.8 ± 3.3 mm CL) than on control sites (23.6 ± 2.8 mm CL), but varied over time on both site

312 types (Fig. 3B).

313 *Relationship between lobster density and disease prevalence* — Throughout experimental stage

314 A, there were 50 data on lobster density and disease prevalence for casita sites (5 sites × 10

315 sampling times), but only 34 data for control sites, because 16 data corresponded to sites with

316 0–4 lobsters on a given sampling time and hence could not be included (Table S1). Throughout

317 experimental stage A, overall disease prevalence was 15.9% ± 7.4% on casita sites (overall

318 mean ± SD, N = 50) and 14.4% ± 4.0% on control sites (N = 34). These values were not

319 significantly different (Mann-Whitney test, U = 694.5, p = 0.157). The correlation between log-

320 transformed lobster density and disease prevalence was not significant on casita sites ($r = -$

321 0.217, N = 50, *p* = 0.130) (Fig. 4A), on control sites (*r* = 0.009, N = 34, *p* = 0.962) (Fig. 4B), or

322 on both types of sites together (*r* = 0.005, N = 89, *p* = 0.962) (Fig. 4C).

323 *Occupancy of shelters as related to disease* — Throughout experimental stage A, we examined,

324 500 casitas (50 casitas × 10 sampling times) and 215 crevices on casita sites, and 495 crevices

325 on control sites. Few casitas and crevices contained only diseased lobsters. However, most

326 casitas (45.4%) harbored heathy + diseased lobsters, followed by casitas with only healthy

327 lobsters (34.8%), whereas 11.2% casitas had no lobsters (Table 2). In contrast, most crevices in

328 both casita sites and control sites harbored zero lobsters (67.5% and 62.8%, respectively),

329 followed by crevices occupied exclusively by healthy lobsters (22.3% and 28.7%, respectively),

330 whereas crevices harboring both healthy + diseased lobsters were scarce (5.1% and 3.6%,

331 respectively) (Table 2). On casita sites, the maximum number of occupants was 23 for casitas

332 with healthy lobsters, 107 for casitas with healthy + diseased lobsters, 10 for crevices with

333 healthy lobsters, and 5 for crevices with healthy and diseased lobsters. On control sites,

334   crevices with healthy lobsters and crevices with healthy + diseased lobsters had a maximum of

335   8 and 5 lobsters, respectively.

336   In all cases, the NBD fitted well the distribution of lobsters. On casita sites, $\{k, m_v\}$ and

337    $\{k_v, m_v\}$ were the best models for casitas as well as for crevices (Table 3). Both models

338   estimated a different m for each sample but the former estimated a common k for all samples,

339   whereas the latter estimated a different k for each one. Therefore, we proceeded with model

340   averaging. Casitas with healthy + diseased lobsters had a higher m (7.08 ± 0.49 lobsters per

341   casita, mean ± SE) than casitas with healthy lobsters (4.07 ± 0.34 lobsters per casita) (Fig. 5A),

342   whereas k did not differ significantly between both casita samples (0.75 ± 0.08 and 0.72 ± 0.06,

343   respectively) (Fig. 5B). Throughout casita sites, crevices with healthy lobsters had a higher m

344   (0.62 ± 0.13 lobsters per crevice) than crevices with healthy + diseased lobsters (0.31 ± 0.10

345   lobsters per crevice), whereas k did not differ significantly between both crevice samples (0.19 ±

346   0.04 and 0.15 ± 0.05, respectively). Therefore, on casita sites, both m and k were higher in

347   casitas than in crevices; that is, casitas in general harbored more lobsters, but the distribution of

348   lobsters was more clumped in crevices.

349   On control sites, by contrast, model $\{k_v, m_v\}$ was by far the best fit to the data on lobster

350   distribution, with a w_i of 0.999 (Table 4), with crevices with healthy lobsters having higher values

351   of m (0.61 ± 0.06 lobsters per crevice) and k (0.43 ± 0.07) than crevices with healthy + diseased

352   lobsters (m = 0.16 ± 0.05 lobsters per crevice; k = 0.11 ± 0.03) (Fig. 5A and 5B).

353   **Experimental stage B**

354   On the five casita sites, we examined 5714 lobsters in total, 3619 during experimental

355   stage A and 2095 during stage B. Of the total lobsters observed in stage B, all diseased lobsters

356   (442 in total) were culled (Table S1). The logistic regression analysis showed that the probability

357   of finding diseased lobsters was higher in stage B (odds ratio: 1.2) relative to stage A and varied

358   significantly with site (Table 4). Compared to site 8 (the reference site), the probability of finding

359   diseased lobsters was overall higher on site 4 (odds ratio: 1.31), and lower on site 7 (odds ratio:

360   0.71), but did not differ significantly on sites 2 and 5 (odds ratio: 1.1 and 0.9, respectively)

361   (Table 4). Disease prevalence on casita sites varied with sampling time in both stages but

362   tended to be higher throughout stage B (Fig. 6A). However, relative to stage A, average disease

363   prevalence during stage B was significantly higher only on casita sites 2 and 4 (Fig. 6B).

364   **Discussion**

365   We examined the potential effects of scaled-down casitas for juvenile lobsters on the dynamics

366   of the PaV1 disease. Due to the larger aggregations of lobsters with increasing lobster density,

367   disease prevalence could be expected to increase over time on casita sites, where there were

368   on average eight times as many lobsters as on control sites, but we found no apparent

369   correlation between lobster density and disease prevalence. These experimental results are

370   akin to results from different simulated scenarios of host spatial structure and avoidance of

371   diseased lobsters by healthy conspecifics, which showed no increase in transmission or

372   persistence of PaV1 with increasing density of lobsters (Dolan, Butler & Shields, 2014).

373   Under experimental conditions, healthy lobsters avoid shelters harboring diseased

374   lobsters (Behringer, Butler & Shields, 2006, Candia-Zulbarán et al., 2015), but whether and to

375   what extent this occurs in natural conditions likely depends on the ecological context (Butler et

376   al., 2015; Lozano-Álvarez et al., 2018). Predation risk for juveniles of *P. argus* is ever present

377   (Smith & Herrnkind, 1992; Butler, Steneck & Herrnkind, 2006), especially in seagrass meadows

378   where crevice shelters are scarce (Briones-Fourzán & Lozano-Álvarez, 2001). In the present

379   study, 11% casitas, but ~65% crevices, did not harbor any lobsters, reflecting the smaller

380   effective refuge area provided by crevices (even the larger ones) relative to casitas. In these

381   circumstances, lobsters may avoid shelters with limited space already occupied by diseased

382   conspecifics, although this would increase their predation risk (Anderson & Behringer, 2013).

383   But if casitas are deployed in those habitats, their large refuge area may allow segregation of

384   healthy and diseased lobsters (Lozano-Álvarez et al., 2018). Gutzler, Butler & Behringer (2015)

385 cautioned that a concentration of small lobsters in casitas may increase the abundance of large

386 piscine predators. However, lobsters and predators typically cohabit in casitas on account of

387 their large refuge area (Lozano-Álvarez & Spanier, 1997; Sosa-Cordero et al., 1998; Lozano-

388 Álvarez et al., 2010; Briones-Fourzán et al., 2012; Ross, Butler & Matthews, 2022). Moreover,

389 competition for scarce shelter between lobsters and other taxa, including predators, can be

390 reduced if shelter availability increases, e.g., with casitas (Lozano-Álvarez et al., 2010; Briones-

391 Fourzán et al., 2012).

392   The analyses of lobster distribution among casitas and crevices yielded interesting

393 results. The mean number of lobsters (m) was higher in casitas than in crevices, but the

394 distribution of lobsters was more clumped (k was closer to zero) in crevices than in casitas.

395 However, m was much higher in casitas co-occupied by healthy + diseased lobsters than in

396 casitas harboring only healthy lobsters, even though k was similar in both casita samples. In

397 conjunction, these findings confirm the paucity of pre-existing shelters throughout the Puerto

398 Morelos reef lagoon and that casitas do allow for the cohabitation of healthy and diseased

399 lobsters. Values of m and k were also generally not affected by the presence of diseased

400 lobsters in large commercial casitas throughout Bahía de la Ascensión (Briones-Fourzán et al.,

401 2012).

402   In contrast with casitas, cohabitation of healthy and diseased lobsters was less common

403 in crevices, and m was smaller in crevices harboring healthy + diseased lobsters than in those

404 harboring only healthy lobsters. These results confirm the tendency of healthy lobsters to avoid

405 sharing natural crevice-type shelters with diseased conspecifics (Butler et al., 2015), likely

406 because they provide a smaller space than casitas. Similarly, in the lower Florida Keys (USA),

407 significantly more lobsters were found in casitas than at either coral heads or low relief

408 hardbottom (Ross, Butler & Mattews, 2022).

409   Although healthy lobsters tend to avoid diseased lobsters, the latter maintain their

410 gregarious behavior (Behringer & Butler, 2010). Lozano-Álvarez et al. (2018) found that the

411   tendency of healthy lobsters to stay away from diseased lobsters beneath casitas decreased as

412   the number of lobsters per casita increased, suggesting that the large refuge area provided by

413   casitas allows the segregation between healthy and diseased lobsters only to a certain point.

414   This segregation will depend both on the number of lobsters occupying the casita and on the

415   gregarious behavior expressed by the diseased lobsters (Lozano-Álvarez et al., 2018), which is

416   why we tested whether culling diseased individuals may favor aggregation of lobsters and

417   reduce prevalence levels.

418   Contrary to expectation, the overall level of prevalence on casita sites increased during

419   experimental stage B, when culling was performed, relative to stage A. However, we only culled

420   all overtly diseased lobsters, which are the most infective (Li et al., 2008), every 3–4 months, a

421   period during which some subclinically infected lobsters likely developed the disease and other

422   healthy lobsters could have become infected. Although subclinically infected lobsters are as

423   abundant as clinically infected lobsters (Candia-Zulbarán, Briones-Fourzán & Lozano-Álvarez,

424   2019), they are not equally avoided by healthy conspecifics (Candia-Zulbarán et al., 2015).

425   Therefore, culling more often than we did could potentially yield different results (Groner et al.,

426   2016). On the other hand, selective predation on infected prey does not always reduce infection

427   prevalence (Lopez & Duffy, 2021), and some models have shown that when the most heavily

428   infected individuals in a population are culled, disease prevalence may increase due to

429   persistence of less virulent strains of the parasite which are able to establish in sparser

430   populations (Bolzoni & de Leo, 2013; Behringer et al., 2020). Indeed, many authors do not

431   consider culling an effective way to manage marine infectious diseases because of the dearth of

432   knowledge on the relative importance of other environmental drivers and mechanisms of

433   transmission and dispersion of pathogens in the ocean, and on the spatial scales at which

434   infective stages and host larvae may travel (reviewed in Groner et al., 2016; Shields, 2018;

435   Glidden et al., 2022). In the absence of this information, culling as a potential mechanism to

436   manage the PaV1 disease remains contentious.

437   During stage B, when culling was conducted, the probability of finding diseased lobsters

438   varied with site, but was significantly higher in only two of the five casita sites relative to stage A,

439   supporting the notion that small-scale habitat and community characteristics (e.g. habitat

440   complexity, types of substrate, species diversity) can play important roles in disease ecology

441   (Small & Pagenkopp, 2011; Lafferty, 2017; Davies, Briones-Fourzán & Lozano-Álvarez, 2019).

442   In the Florida Keys, high variability in disease prevalence in individual sampling sites was also

443   common (Behringer et al., 2011; Butler et al., 2015). In Bahía de la Ascensión, disease

444   prevalence was consistently higher on more vegetated sites, suggesting that vegetation could

445   act as an environmental reservoir of PaV1 (Briones-Fourzán et al., 2012; Davies, Briones-

446   Fourzán & Lozano-Álvarez, 2019), as it does for other pathogens (Small & Pagenkopp, 2011).

447   However, in the relatively narrow Puerto Morelos reef lagoon, vegetation is highly dynamic due

448   to hurricanes, herbivore pressure, and inputs of nutrients from anthropogenic sources

449   (Rodríguez-Martínez et al., 2010; van Tussenbroek, 2011; Caballero-Aragón et al., 2022). Also,

450   the possible existence of other animals acting as reservoirs for PaV1 cannot be excluded

451   (Davies, Briones-Fourzán & Lozano-Álvarez, 2019; Davies et al., 2020).

452   In the Puerto Morelos reef lagoon, average prevalence of the PaV1 disease increased

453   from 2.5% in 2001 to 10.5% in 2006 (Lozano-Álvarez et al., 2008), to 15–20% between 2009

454   and 2015 (the present study). It further remained around 16–20% between 2016 and 2022,

455   suggesting that it has leveled off (Davies et al., 2020; P. Briones-Fourzán, personal

456   observations). This also appears to be the case in Bahía de la Ascensión, where the average

457   prevalence remained around 5% between 2008–2010 (Candia-Zulbarán et al., 2012) and 2016–

458   2017 (Davies, Briones-Fourzán & Lozano-Álvarez, 2019). In both locations, real prevalence is

459   estimated to be at least twice as high (Bahía de la Ascensión: Huchin-Mian et al., 2013; Puerto

460   Morelos: Candia-Zulbarán, Briones-Fourzán & Lozano-Álvarez, 2019). The higher prevalence

461   levels in the Puerto Morelos reef lagoon reflect that the local population of lobsters consists

462 mostly of juveniles ≤ 50 mm CL, which are the most susceptible to PaV1, whereas in Bahía de

463 la Ascensión the local population spans from juveniles to adults.

Conclusions

465 The present study suggests that conservation efforts to enhance juvenile lobsters using scaled-

466 down casitas in shelter-poor habitats is a viable option. The use of casitas did not increase

467 PaV1 prevalence and culling clinically infected lobsters, at least with the periodicity that we

468 used, did not decrease disease prevalence. On the contrary, prevalence was higher throughout

469 the culling period. These results suggest that other factors, such as small-scale habitat and

470 community characteristics, may be influencing disease dynamics. Therefore, upon using casitas

471 to enhance juvenile *P. argus*, previous baseline surveys would be advisable (Shields, 2018) as

472 well as monitoring prevalence levels at least once a year during the first few years (e.g., Davies

473 et al. 2020).

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Figure 1

Study area.

Location of the 10 experimental sites on the Puerto Morelos reef lagoon. Each site measured 1 ha (100 m × 100 m). Control sites (orange squares: sites 1, 3, 6, 9 and 10) had no casitas. Casita sites (blue squares: sites 2, 4, 5, 7 and 8) had 10 casitas each. Black areas denote coral reefs. Inset denotes location of Puerto Morelos (yellow dot). Isobaths are in meters. Figure modified from Briones-Fourzán et al. (2020).
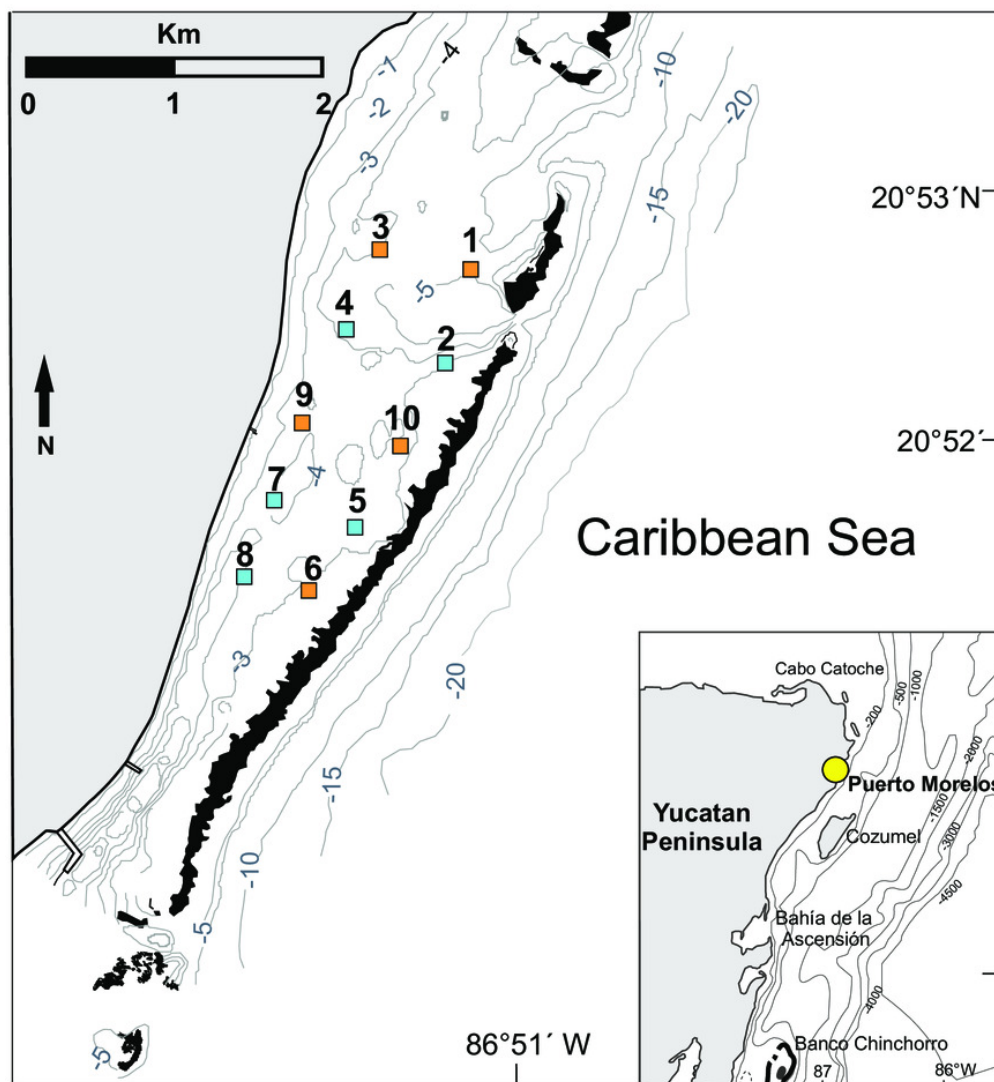
Figure 2

Experimental casitas and clinical signs of PaV1.

(A) An experimental casita (1.1 m² in area, 4 cm entrance height, 8 cm inner height) deployed on the bottom. (B) A casita lifted to show its frame. (C) Lobsters sheltering beneath a casita. (D) A healthy lobster (left) and a diseased lobster (right) exhibiting clinical signs of PaV1: milky hemolymph (red arrow) and a reddish discoloration of the exoskeleton. Photo credits: Fernando Negrete-Soto.
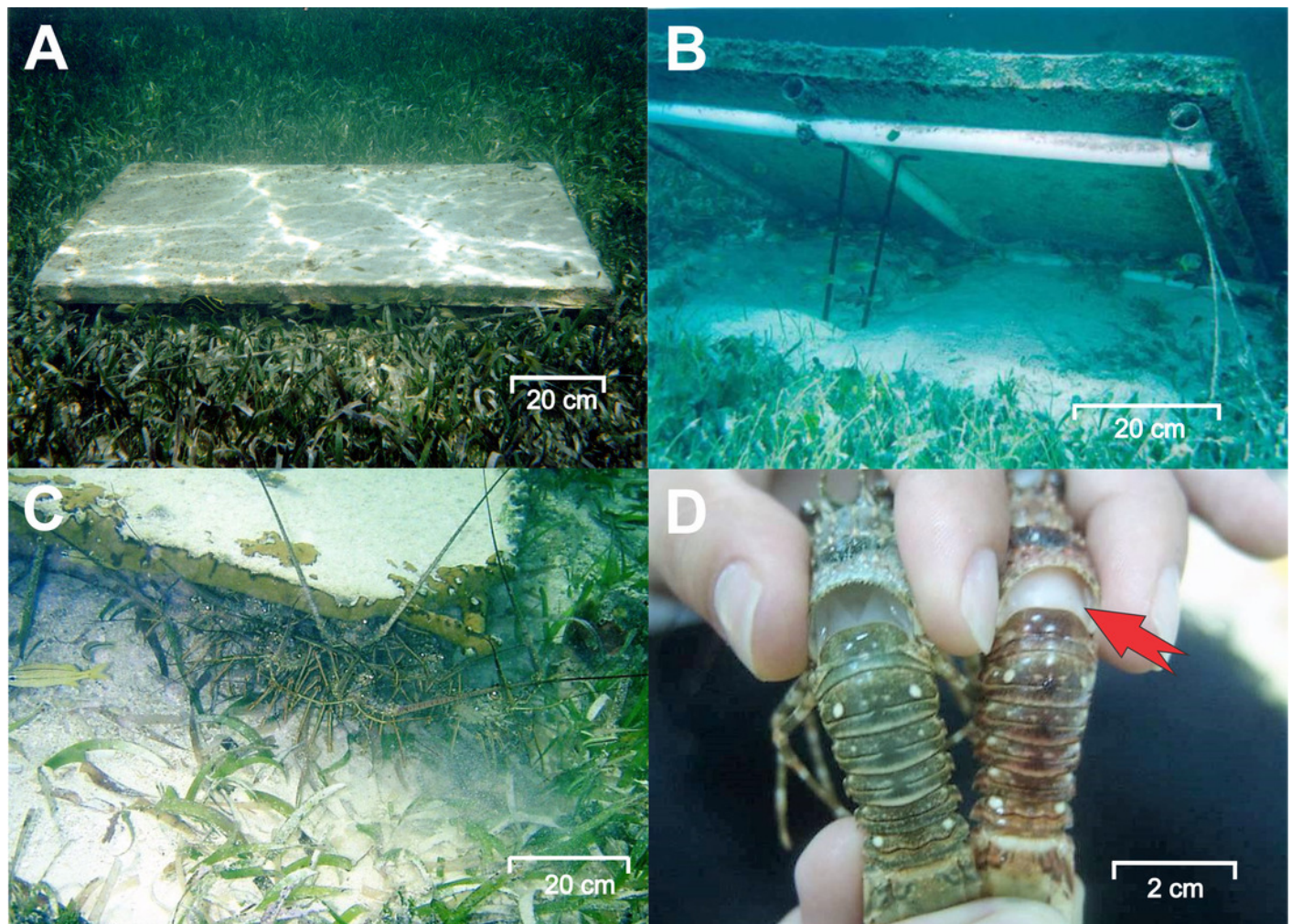
Figure 3

Mean lobster density and lobster size.

(A) Lobster density (Log (N + 1)) and (B) lobster mean size (carapace length, mm) on casita sites (blue columns) and control sites (orange columns) throughout experimental stage A. Error bars are 95% confidence intervals.
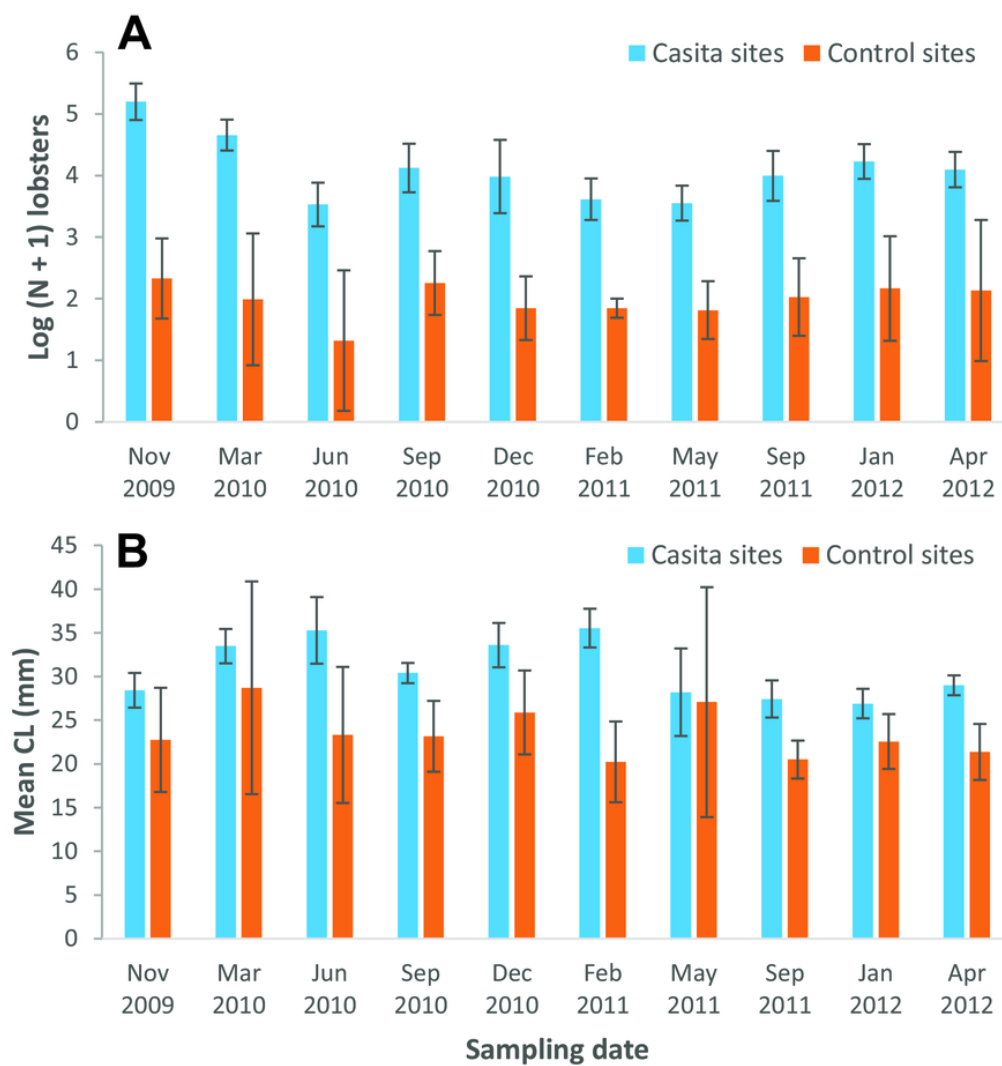
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Figure 4

Lobster density versus disease prevalence.

Relationship between lobster density (Log (N + 1) lobsters ha^{-1}) and prevalence of PaV1 disease (percentage of diseased lobsters) in (A) casita sites (blue dots), (B) control sites (orange dots), and (C) all sites throughout experimental stage A.
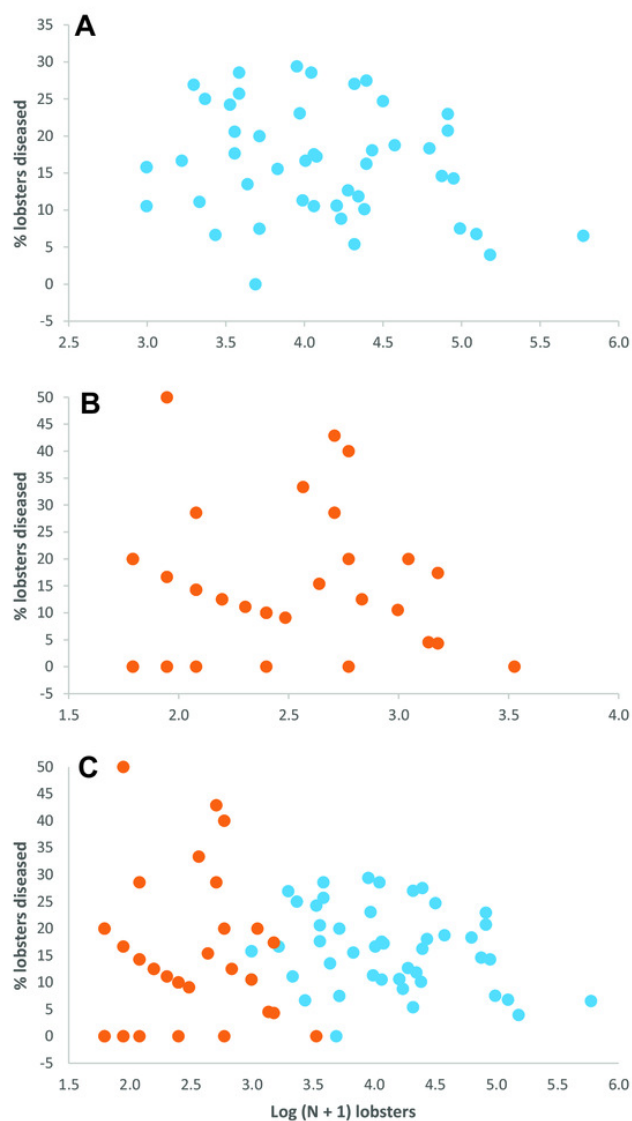
Figure 5

Parameters of the negative binomial distribution.

Comparison of (A) m (mean number of lobsters per casita or crevice) and (B) k (dispersion parameter) for casitas and crevices on casita sites, and for crevices on control sites, occupied exclusively by healthy lobsters (green columns) or co-occupied by healthy and diseased lobsters (brown columns). Error bars are 95% confidence intervals.
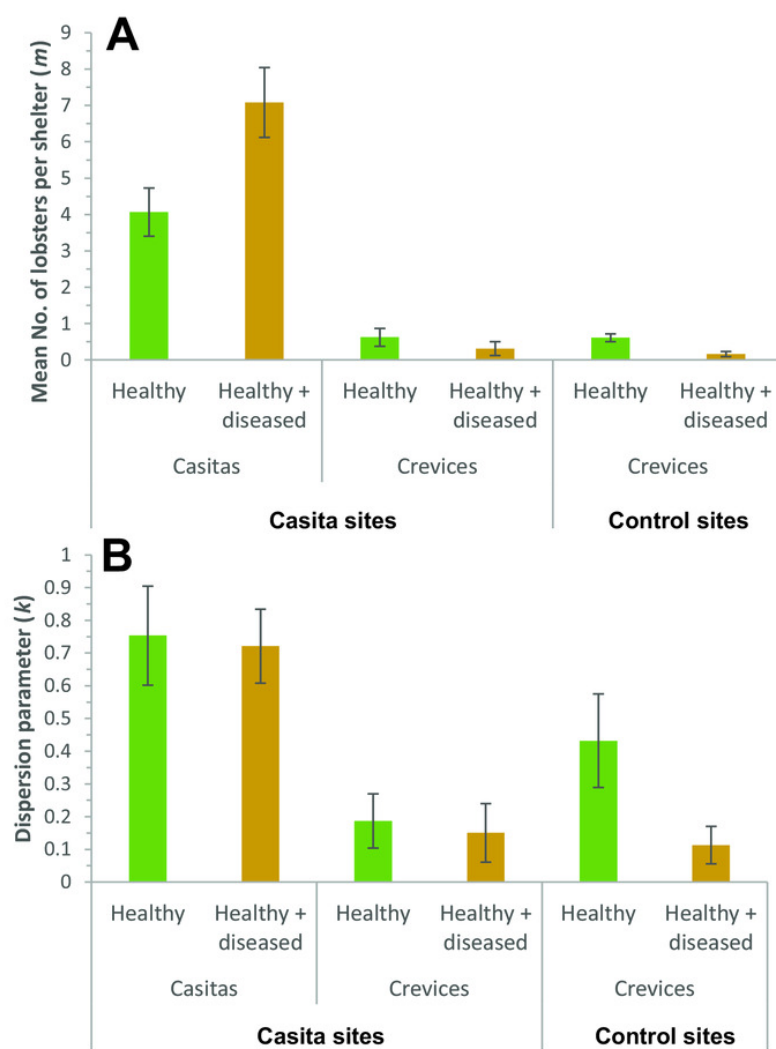
Figure 6

Disease prevalence on casita sites by experimental stage.

(A) Prevalence of the PaV1 disease (percentage of diseased lobsters) in casita sites by sampling time throughout experimental stages A (blue columns) and B (gray columns). (B) Average percentage of diseased lobsters on individual casita sites during experimental stages A (blue columns) and B (gray columns) Error bars are 95% confidence intervals.
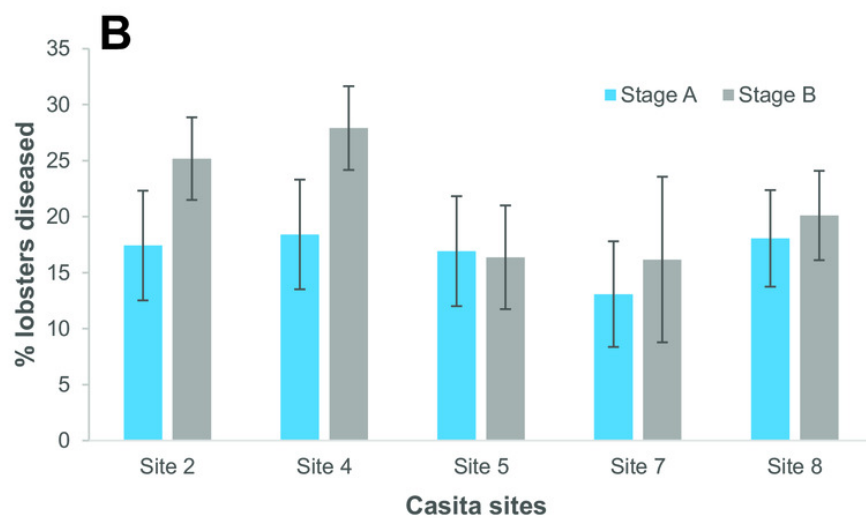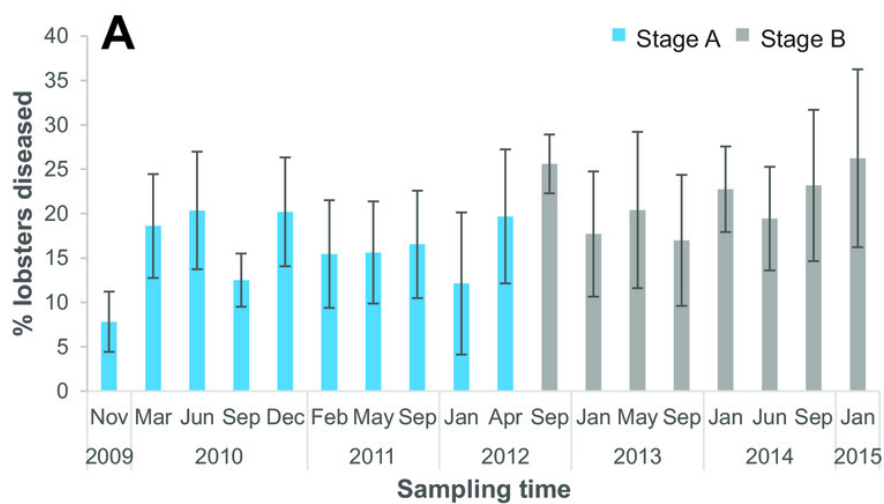
Table 1 (on next page)

Effect of casitas and time on lobster density and size.

Results of General Lineal Models testing for effects of site type (2 levels: casita sites and control sites) and Time (10 sampling dates, repeated measure) on density of lobsters (Log (N+1) lobsters per ha) and lobster size (carapace length, mm).

1 **Table 1: Effect of casitas and time on lobster density and size.**

2 Results of General Lineal Models testing for effects of site type (2 levels: casita sites and control

3 sites) and Time (10 sampling dates, repeated measure) on density of lobsters (Log (N +1)

4 lobsters per ha) and lobster size (carapace length, mm).

5

Effect	df	Lobster density			Lobster size		
		MS	F	p	MS	F	p
Intercept	1	921.177	320.952	<0.001	1061.678	5590.229	<0.001
Site type	1	112.931	39.347	<0.001	1.774	9.341	0.016
Error	8	2.870			0.190		
Time	9	1.420	6.616	<0.001	0.081	3.418	0.002
Time × Site type	9	0.346	1.614	0.127	0.041	1.726	0.099
Error	72	0.215			0.024		

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Table 2(on next page)

Occupancy of casitas and crevices.

Summary of data from casitas and crevices distributed on casita sites, and from crevices distributed on control sites, occupied by healthy lobsters, diseased lobsters, healthy + diseased lobsters, and no lobsters.

1 **Table 2: Occupancy of casitas and crevices.**

2 Summary of data from casitas and crevices distributed on casita sites, and from crevices

3 distributed on control sites, occupied by healthy lobsters, diseased lobsters, healthy + diseased

4 lobsters, and no lobsters.

5

Lobster condition	Casita sites				Control sites	
	Casitas	%	Crevices	%	Crevices	%
Healthy	172	34.4	48	22.3	142	28.7
Diseased	45	9.0	12	5.6	24	4.8
Healthy + diseased	227	45.4	11	5.1	18	3.6
No lobsters (empty)	56	11.2	144	67.0	311	62.8
Total	500	100	215	100	495	100

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Table 3(on next page)

Model selection for occupancy of casitas and crevices.

Results of model selection contrasting four models (based on parameters of the negative binomial distribution) (A) for casita sites, separately considering a set of two casita samples (casitas occupied exclusively by healthy lobsters and casitas co-occupied by healthy and diseased lobsters) and a set of two crevice samples (crevices occupied exclusively by healthy lobsters and crevices co-occupied by healthy and diseased lobsters), and (B) for control sites, considering a set of two crevice samples (crevices occupied exclusively by healthy lobsters and crevices co-occupied by healthy and diseased lobsters). AIC_c: Akaike information criterion adjusted for small sample size; ΔAIC_c: difference between each AIC_c and the smallest AIC_c; w_i: Akaike weight.

1   **Table 3: Model selection for occupancy of casitas and crevices.**

2   Results of model selection contrasting four models (based on parameters of the negative

3   binomial distribution) (A) for casita sites, separately considering a set of two casita samples

4   (casitas occupied exclusively by healthy lobsters and casitas co-occupied by healthy and diseased

5   lobsters) and a set of two crevice samples (crevices occupied exclusively by healthy lobsters and

6   crevices co-occupied by healthy and diseased lobsters), and (B) for control sites, considering a

7   set of two crevice samples (crevices occupied exclusively by healthy lobsters and crevices co-

8   occupied by healthy and diseased lobsters). AICc: Akaike information criterion adjusted for

9   small sample size; ΔAIC_c: difference between each AIC_c and the smallest AIC_c; wi: Akaike

10   weight.

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	Model	No. of parameters	Maximum likelihood	AIC_c	ΔAIC_c	w_i
(A) Casita sites						
Casitas	$\{k, m_v\}$	3	-1528.960	3063.964	0	0.6588
	$\{k_v, m_v\}$	4	-1528.603	3065.280	1.316	0.3412
	$\{k, m\}$	2	-1541.292	3086.606	22.642	0
	$\{k_v, m\}$	3	-1541.233	3088.510	24.546	0
Crevices	$\{k, m_v\}$	3	-291.230	588.528	0	0.5093
	$\{k_v, m_v\}$	4	-290.505	589.123	0.595	0.3782
	$\{k_v, m\}$	3	-293.333	592.734	4.206	0.0622
	$\{k, m\}$	2	-294.561	593.156	4.628	0.0503
(B) Control sites						
Crevices	$\{k_v, m_v\}$	4	-660.027	1328.104	0	0.9988
	$\{k_v, m\}$	3	-668.051	1342.132	14.028	0.0009
	$\{k, m_v\}$	3	-669.150	1344.330	16.226	0.0003
	$\{k, m\}$	2	-682.953	1369.921	41.817	0

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Table 4(on next page)

Logistic regression analysis.

Estimates for logistic regression analysis testing the effects of experimental stage (two levels: Stage A (without culling), Stage B (with culling of diseased lobsters); Stage A is the reference level) and casita site (five levels: Sites 2, 4, 5, 7 and 8; site 8 is the reference level) on the probability of finding diseased lobsters.

1

2 **Table 4: Logistic regression analysis.**

3 Estimates for logistic regression analysis testing the effects of experimental stage (two levels:

4 Stage A (without culling), Stage B (with culling of diseased lobsters); Stage A is the reference

5 level) and casita site (five levels: Sites 2, 4, 5, 7 and 8; site 8 is the reference level) on the

6 probability of finding diseased lobsters.

7

	Effect level	Estimate	Standard error	Wald statistic	p	Odds ratio (IC 95%)
Intercept		-1.570	0.039	1639.106	<0.001	
Stage	Stage B	0.179	0.039	21.337	<0.001	1.20 (1.11–1.29)
Site	2	0.097	0.071	1.905	0.168	1.10 (0.96–1.27)
Site	4	0.268	0.067	16.078	<0.001	1.31 (1.15–1.49)
Site	5	-0.130	0.077	2.852	0.091	0.88 (0.75–1.02)
Site	7	-0.341	0.096	12.578	<0.001	0.71 (0.59–0.86)

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