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Ivan Hernandez, Marie-Anne Cambon-Bonavita, Jozee Sarrazin, Florence Pradillon. Population structure and reproduction of the alvinocaridid shrimp *Rimicaris exoculata* on the Mid-Atlantic Ridge: Variations between habitats and vent fields. *Deep Sea Research Part I: Oceanographic Research Papers*, 2022, 186, 103827 (14p.). 10.1016/j.dsr.2022.103827 . hal-04203815

HAL Id: hal-04203815

<https://hal.science/hal-04203815>

Submitted on 22 Jul 2024

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1 **Population structure and reproduction of the alvinocaridid shrimp *Rimicaris exoculata* on**
2 **the Mid-Atlantic Ridge: variations between habitats and vent fields**

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20

21 **Abstract**

22 The shrimp *Rimicaris exoculata* is the most conspicuous component of vent communities
23 developing around hydrothermal fluid emissions below 2000 m on the northern Mid-Atlantic Ridge
24 (nMAR). Its high genetic connectivity suggests a remarkable ability to produce dispersing larval
25 stages. However, so far brooding females have been rarely observed and reproduction remained
26 enigmatic. Spatially complex population structures related to the heterogeneity of local habitat
27 conditions are described for many vent species, this information being fundamental to gain a
28 better understanding of their life history. Here our aim was to assess such complexity along with
29 reproductive development in *R. exoculata* populations within two vent fields, TAG and Snake Pit
30 (3620m and 3470m depth respectively). We compared samples collected in January-February
31 2014 among visually distinct assemblages with different degrees of exposure to vent fluids. Dense
32 aggregations located near active venting included mostly females and immature individuals, while
33 inactive peripheries harbored low density assemblages of large males. Small juveniles gathering
34 around low temperature diffusions belonged to another species, *Rimicaris chacei*. One third of
35 the sexually mature females were ovigerous at the two vent fields during our sampling period,
36 with lower fecundities and egg sizes in the TAG population. Overall, the observed shrimp
37 distribution patterns were consistent across both vent fields, although a high degree of
38 heterogeneity in population structure was observed locally within dense aggregations, probably
39 reflecting micro-scale variations in environmental conditions. Our results thus highlight spatially
40 complex population structures where *R. exoculata* females brood eggs within dense aggregations
41 exposed to vent fluids, while peripheral inactive areas may be important mating grounds for
42 adults. In addition, we suggest temporal variability in reproductive activity, increasing in the winter
43 season, which questions potential seasonality in a deep-sea species.

44 **Key words:** Life history, population structure, reproduction, *Rimicaris exoculata*, habitat
45 variability, hydrothermal vents.

46 **1. Introduction**

47 At deep-sea hydrothermal vents, the persistence of endemic species at regional scale
48 depends on their ability to disperse and colonize their extremely patchy and locally dynamic
49 habitat. High levels of genetic connectivity between spatially distant vent populations have been
50 observed for many of the visually dominant species (e.g. Thaler et al. 2011, Teixeira et al. 2012,
51 Beedessee et al. 2013). More generally, vent communities are expected to exhibit attributes of
52 resilience facing environmental instability, rooted in the fact that they thrive in naturally highly
53 dynamic habitats (Van Dover, 2014). However, how they would cope with additional challenges
54 caused by human activities in the deep-sea remains difficult to assess because we still lack
55 knowledge on fundamental aspects of their life cycle, demographic connectivity and underlying
56 colonization mechanisms (Van Dover et al. 2018).

57 The complex and dynamic nature of vent systems generates local patchiness in physico-
58 chemical conditions which influences composition of communities at different temporal and spatial
59 scales (Sarrazin et al. 1997, 2015, Desbruyères et al. 2000, 2001, Cuvelier et al. 2009, 2014). At
60 the species level, population densities and structures are also influenced by local conditions,
61 resulting in spatio-temporal variations. Life stages of a species may occupy different areas
62 characterized by contrasting environmental features at the scale of a single edifice (Cuvelier et
63 al. 2011, Nye et al. 2013, Marsh et al. 2015, Husson et al. 2017). Segregation of juveniles in
64 specific areas were observed in *Bathymodiolus azoricus* mussels (Cuvelier et al. 2009, 2011) and
65 *Rimicaris exoculata* shrimps (Shank et al. 1998), two species dominating vent communities along
66 the northern Mid Atlantic Ridge (nMAR). In the case of *B. azoricus*, spatial segregation was related
67 to the degree of exposure to vent fluids, juveniles being in areas with lower hydrothermal influence
68 (Cuvelier et al. 2011, Husson et al. 2017). Segregation in different habitats according to sex or
69 reproductive status (e.g. brooding females) was also reported in vent decapods (Nye et al. 2013,
70 Marsh et al. 2015). In addition, the chaotic mixing of vent effluents with seawater as well as tidal

71 forcing generate further variability in populations on short temporal scales (Copley et al. 1999).
72 Thus, the interplay between habitat variability and the life cycle of vent species, in which
73 physiological and resource requirements differ with developmental stages, may result in complex
74 population structures. Such complexity must be addressed to better understand vent species life
75 history.

76 On the nMAR, the alvinocarid shrimp *Rimicaris exoculata* (Williams and Rona, 1986) lives
77 close to vent fluid emissions in dense aggregations of thousands of individuals per square meter
78 (Segonzac et al. 1993, Copley et al. 1997, 2007, Gebruk et al. 2000). Its population biology could
79 thus have important implications for the structure, biomass and resilience of the local communities
80 (Gebruk et al. 1997, Desbruyères et al. 2000, 2001). Many aspects of its biology have been
81 investigated (Zbinden and Cambon-Bonavita, 2020), including trophic dependency on bacterial
82 symbionts at the adult stage (Ponsard et al. 2013), regulation of the symbiotic association along
83 the host's life cycle (Corbari et al. 2008, Le Bloa et al. 2020), thermal tolerance (Ravaux et al.
84 2019) as well as sensory abilities (Zbinden et al. 2017, Ravaux et al. 2021). However, population
85 structure and reproduction of *R. exoculata* are less well understood, and available information is
86 often based on single or pooled samples, or on preliminary analyses (e.g. Gebruk et al. 1997,
87 2000, Vereshchaka, 1997). Striking variations in shrimp densities at the TAG and Broken Spur
88 vent fields were related to the availability of substratum exposed to hydrothermal fluids (Copley
89 et al. 1997, 1999), but accompanying variations in population structures were never
90 characterized. Population samples collected at various MAR vent fields showed female-biased
91 sex ratios (Shank et al, 1998), but the association of such bias with a particular habitat or to
92 temporal variations was not evaluated. Habitats located at the base of vent edifices were
93 suggested to serve as nurseries (Gebruk et al. 2000, Komai and Segonzac 2008), and patches
94 of juveniles have also been reported in adult aggregations (Shank et al. 1998, Gebruk et al. 2000,
95 Copley et al. 2007), but their size structure was not defined. Finally, brooding females were

96 virtually absent from studied populations in different vent fields (Williams and Rona, 1986, Gebruk
97 et al. 1997, Ramirez-Llodra et al. 2000, Copley et al. 2007), except at Logatchev in March 2007
98 (Gebruk et al. 2010, Guri et al. 2012), which questions their spawning strategy.

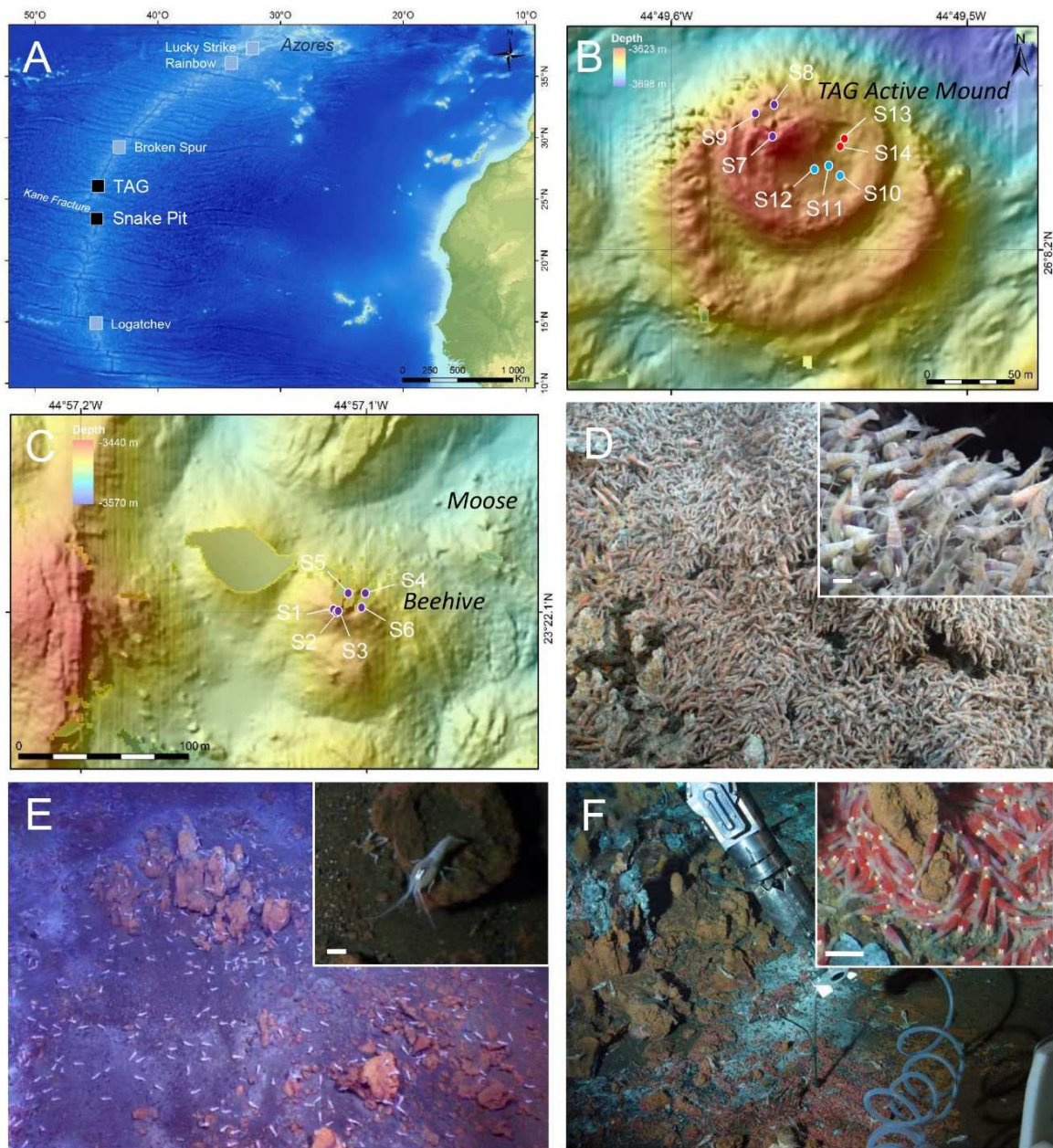
99 In January-February 2014, we explored *R. exoculata* populations from two nMAR vent
100 fields, TAG and Snake Pit. Striking spatial variations in densities of adults and juveniles, similar
101 to those reported in previous studies, and many brooding females were observed. We
102 hypothesized that the visually contrasting shrimp assemblages reflected differences in population
103 structures and habitat conditions. The objectives of the present study are: 1) to compare
104 population structures and local environmental conditions between visually distinct shrimp
105 assemblages, 2) to evaluate regional scale variation by comparing visually similar shrimp
106 assemblages across vent fields, as well as local heterogeneity by comparing randomly collected
107 samples of the same type of assemblage within a single field, and 3) to assess the frequency and
108 distribution of brooding females in the different assemblage types, as well as their fecundities and
109 synchrony to better understand the spawning strategy of the species. This allows us to propose
110 a scenario depicting interactions of shrimps with their conspecifics and their environment at
111 different life stages, and provide clues on some aspects of their reproductive behavior.

112 **2. Material and Methods**

113 **2.1. Sampling**

114 *Rimicaris exoculata* were collected at the TAG (26°08.2'N, 44°49.5'W, 3620 m depth) and
115 Snake Pit (SP, 23°22.1'N 44°57.1'W, 3470 m depth) vent fields on the nMAR (Fig. 1A) during the
116 BICOSE cruise (DOI: 10.17600/14000100) from January 10th to February 11th, 2014. The two vent
117 fields are separated by 300 km of oceanic ridge and a major transform fault (Kane Fracture)
118 shifting its axis by 150 km. Fourteen spatially discrete samples were collected from shrimp
119 assemblages at both vent fields. At TAG, 3 samples were collected in dense shrimp aggregations

120 swarming on Active Mound (Table 1, Fig. 1B&D, Fig. S1F-H Appendix B) and 2 samples were
121 collected in aggregations of small red alvinocaridid juveniles at the base of the mound, which are
122 herein called “nurseries” (Table 1, Fig. 1F, Fig. S2D-E Appendix B). Additionally, 3 other samples
123 were collected at the base of the TAG active mound, where adult shrimps were scattered over
124 large areas (Table 1, Fig. 1E, Fig. S2A-C Appendix B). At Snake Pit, 6 samples were collected in
125 dense shrimp aggregations on the walls of active chimneys of the Beehive site (Table 1, Fig. 1C,
126 Fig. S1A-E Appendix B). Distance between each sample within each vent field varied from one
127 meter to tens of meters (Fig. 1B-C).



128

129 **Figure 1.** Sampled vent fields and shrimp assemblages. A) North Atlantic map (Amante, C. and
 130 B.W. Eakins, 2009. doi:10.7289/V5C8276M) with the main active vent fields known on the Mid
 131 Atlantic Ridge and our study sites denoted with black squares ; B) Location of the samples
 132 collected on TAG active mound ; C) Location of the samples collected on the Beehive edifice of
 133 Snake Pit field ; B-C : colours denote assemblage types : dense aggregations in purple, scattered
 134 assemblages in blue, nurseries in red ; D) Dense *R. exoculata* aggregation ; E) Scattered *R.*
 135 *exoculata* assemblage ; F) Nursery of small juveniles ; D-F : Pictures from TAG vent field, scale
 136 bars in close-up views : 1 cm.

137 Shrimps were collected with the suction sampler of the Remotely Operated Vehicle (ROV)
 138 Victor6000. In dense aggregations and nurseries, the tip of the sampler was placed in contact
 139 with individuals and maintained immobile during suction to avoid disturbance as much as
 140 possible. The suction was activated for a few seconds in order to collect individuals from a small
 141 area (less than 0.05 m²). Scattered shrimps were sampled by sweeping the tip of the suction
 142 sampler over seafloor areas of a few m². This larger sampling area was necessary to gather a
 143 sufficient number of individuals. In addition, 37 individuals caught with a baited (with fresh fish
 144 meat) shrimp-trap deployed for 5 days on inactive substratum at TAG (S12) were included in the
 145 analyses. A total of 3 445 specimens were examined (Table 1, Hernández-Ávila et al. 2021).

146 **Table 1.** Shrimp sample details, including temperature ranges measured within centimeters from
 147 the assemblage sampling point when available. ND: no data available.

Vent Field	Dive	Sample	Sample size (ind.)	Juveniles for genetics	Assemblage type	Depth (m)	T (°C)
SP	PI01-564-Aspi3	S1	143		dense	3463	3.5-22
SP	PI01-564-Aspi4	S2	390		dense	3463	3.5-22
SP	PI01-564-Aspi5	S3	808		dense	3463	3.5-22
SP	PI05-568-Aspi1	S4	391		dense	3465	10-21
SP	PI05-568-Aspi4	S5	110		dense	3468	4-18
SP	PI05-568-Aspi6	S6	364	1	dense	3472	ND
TAG	PI08-571-Aspi2	S7	600	4	dense	3626	8-14
TAG	PI10-573-Aspi1	S8	207		dense	3624	6-33
TAG	PI10-573-Aspi2	S9	161		dense	3627	3-30
TAG	PI10-573-Aspi5	S10	18		scattered	3635	2.4-2.8
TAG	PI10-573-Aspi6	S11	38		scattered	3635	2.4-2.8
TAG	PI12-575-Nasse 2	S12	37		scattered	3634	2.4-2.8
TAG	PI08-571-Aspi1	S13	77	36	nursery	3637	2.8-5.3
TAG	PI12-575-Aspi2	S14	101	36	nursery	3637	2.8-5.3

148

149 Temperature measurements were conducted along with shrimp collections. Records were
 150 obtained either from discrete measurements with the submersible temperature probe prior to

151 sampling, or from time-series measurements with autonomous temperature probes (WHOI-MISO
152 low temp-ONSET®) deployed within the shrimp aggregation for a few days prior to sampling.

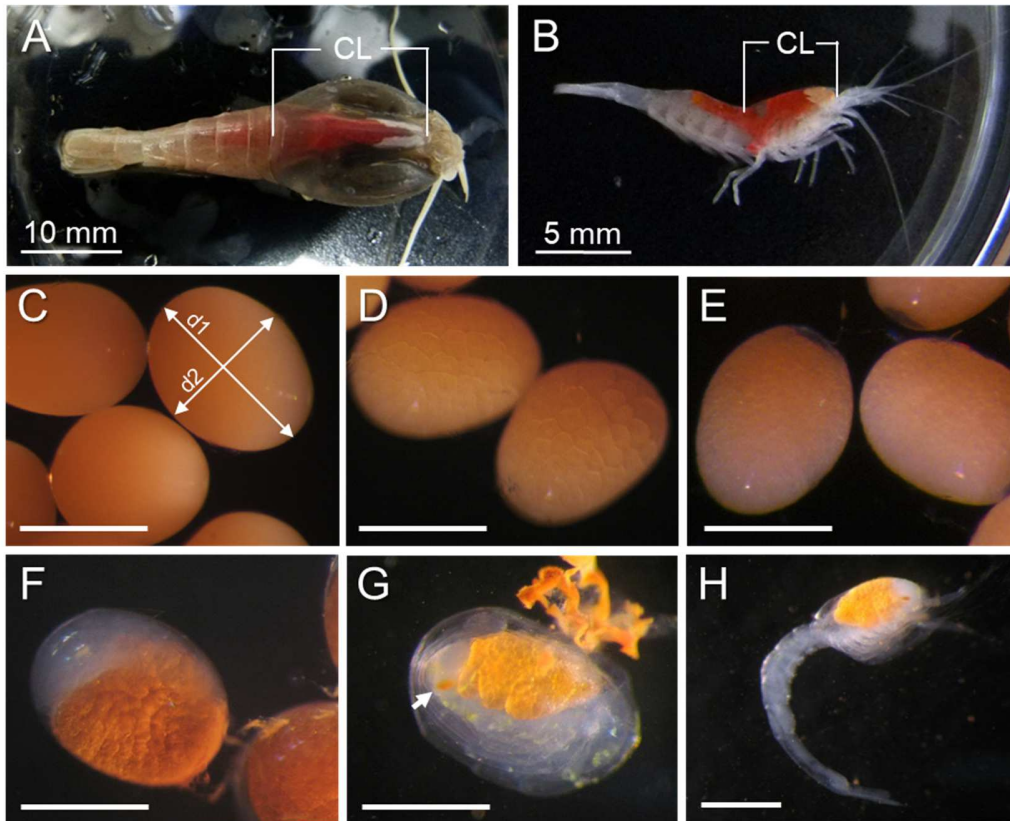
153 **2.2. Identification and measurements**

154 Specimens were identified and classified as juveniles, subadults or adults, in accordance
155 with Komai and Segonzac (2008), and using the size of the onset of sexual determination (OSD,
156 Suppl. data Appendix A) to sort subadults from small adult females which are morphologically
157 nearly identical. Individuals resembling adult females with sizes < OSD were considered as
158 subadults. The identification of the smaller juveniles from nurseries was further assessed by DNA
159 analyses because their morphology did not fit completely the description of early juvenile stages
160 (stage A) of *R. exoculata* available at the time of this study (Komai and Segonzac, 2008). Thirty-
161 six juveniles from each nursery and 5 juveniles from dense aggregations were used for molecular
162 identifications (Table 1, Table S1 Appendix B).

163 Sex was identified in adults by the occurrence of the "appendix masculina" on the second
164 pleopod in males, and the shape of the endopod of the first pleopod (Komai and Segonzac, 2008).
165 Since these sexual characters appear during the transition from subadult to adult stage, sex of
166 juvenile and subadult specimens could not be determined. Brooding females were characterized
167 by the presence of embryos held between their pleopods under the abdomen, and by
168 modifications of their pleopods (addition of setae to maintain the brood). Hatched females
169 (females just after larval release but prior to molt) were identified by their modified pleopods.
170 Brooding and hatched females were referred to collectively as ovigerous females (following Nye
171 et al. 2013). We also defined a size of effective sexual maturity (ESM) representing the minimal
172 size at which females spawn (Suppl. data Appendix A). Females larger than ESM were called
173 sexually mature females.

174 Carapace length (CL) was measured from the posterior margin of the ocular shield to the
175 mid-posterior margin of the carapace in adults and subadults (Fig. 2a), with a precision of 0.1 mm.
176 In juveniles, CL was measured from the anterior tip of the rostrum to the posterior margin of the
177 carapace (Fig. 2b). Morphological changes between juvenile and adult stages (rostrum reduction
178 and development of the ocular shield) may introduce a bias in our measurements but this was
179 small compared to the total length of the carapace, and had little impact on size frequency
180 distributions and size comparisons.

181 Embryos were removed from the abdomen of brooding females, counted and staged. We
182 classified embryonic developmental stages into 3 categories, similar to those defined by Nye et
183 al. (2013) for *R. hybisae*. Early stage embryos encompass freshly laid fertilized eggs without
184 cellular division, and dividing eggs until the blastula stage (Fig. 2C-D). Mid stage starts with
185 gastrulation when a clear region differentiates at one pole of the embryo and extends to the end
186 of the naupliar development (Fig. 2E-F). Late stage includes post-naupliar development, when
187 eye pigmentation becomes visible, abdomen appears clearly separated from the rest of the body
188 with yolk in the cephalothorax, and appendages are fully lengthened and appear curled around
189 the cephalothorax (Fig. 2G). For each brood, ten embryos were randomly selected and both their
190 maximum and minimum diameters were measured at a precision of 0.03 mm under a
191 stereomicroscope with a graduated ocular. The volume of embryos was estimated according to
192 Oh and Hartnoll (2004), considering a spheroid volume as $v = (4/3) \pi r_1 r_2^2$, where r_1 and r_2 are half
193 of the maximum and minimum axis, respectively. This estimation has a precision of 1.6×10^{-5}
194 mm^3 .



195

196 **Figure 2.** Size measurements of different life stages of *Rimicaris exoculata*. A) Adults and
 197 subadults. B) Juveniles. C-H) Embryos and hatched larvae. Early developmental stage: C)
 198 Fertilized egg (with position of measurements for maximum (d1) and minimum (d2) diameters),
 199 D) Blastula stage; Mid developmental stage: E) Early gastrulation, F) Nauplius stage; Late
 200 developmental stage: G) Pre-hatch embryo (arrow indicates eye spot). H) Hatching Zoea larva.
 201 CL: carapace length. Scale bars C-H: 500 μ m.

202 **2.3. Genetic identifications**

203 DNA was extracted from muscle tissues of juveniles using the CTAB method (Doyle 1990).
 204 A section of the cytochrome oxidase I gene (COI) was amplified in a 50 μ L solution of 1X reaction
 205 buffer, 2 mM $MgCl_2$, 0.25 mM dNTP, 1.2 units of Taq polymerase and 0.6 mM of each primer
 206 (LCOI1490 and HCOI2198, Folmer et al. 1994). Amplifications were performed as follows: initial
 207 denaturation (5 min at 95°C), 40 cycles including denaturation (1 min at 94°C), annealing (1 min
 208 at 52 °C) and extension (2 min at 72 °C), followed by a final extension of 7 min at 72°C. All PCR

209 amplifications were conducted on a GeneAmp PCR system 9700 (Applied Biosystems). PCR
210 products were purified and sequenced by Macrogen, Inc. (Netherlands) using the amplification
211 primers. Accession number of each sequence is provided in Table S1 (Appendix B). We aligned
212 our sequences using MUSCLE (Edgar, 2004), along with a set of alvinocaridid and other shrimp
213 sequences. A neighbor-joining tree was constructed using Geneious R8 software (Kearse et al.
214 2012) using a HKY evolutionary model of nucleotide substitution. Robustness of the inferred tree
215 was evaluated using bootstrap method with 1000 replicates.

216 **2.4. Data analysis**

217 For each sample, juvenile ratio, subadult ratio and sex ratio (F:M) were estimated. Deviation
218 from a sex ratio of 1:1 was tested with a χ^2 test, using Yates correction in samples with few
219 specimens ($n < 30$). In order to assess the variability in ratios between samples of a same type
220 of assemblage at each vent field, we performed a heterogeneity χ^2 test (Zar, 2010). Variations in
221 the proportions of sexually mature females or ovigerous females between vent fields were tested
222 using χ^2 test.

223 Size class structures were analyzed for each sample, estimating kurtosis and skewness for
224 size class aggregation and deviation from the mean, respectively (Zar, 2010). Although
225 histograms were elaborated denoting juveniles, subadults, males and females, size structure
226 comparisons were performed including all specimens. Normality tests were performed for each
227 sample using the Shapiro-Wilk test (Zar, 2010). Discrete size cohorts in samples from dense
228 aggregations were identified using the statistical package mixdist (MacDonald, 2003) running in
229 R™. The goodness of fit of the identified size cohorts was verified using χ^2 test. Identification of
230 cohorts in other types of assemblages were not performed due to insufficient sample size.

231 For samples from dense aggregations, differences in body sizes associated with sex and
232 vent fields were tested using multifactorial analysis of variance (ANOVA), after \log_{10}

233 transformation. For this analysis, samples were nested at the factor vent field, representing the
234 variation in body size between samples collected randomly within dense aggregations within the
235 same vent field. Similarly, an ANOVA test was performed in order to examine differences in male
236 body sizes between assemblage types (dense vs scattered) at the TAG vent field. In this case,
237 samples were nested at the factor assemblage type. Data normality and homoscedasticity were
238 tested using χ^2 for frequency distribution and Levene test respectively (Underwood, 1997,
239 McGuinness, 2002).

240 The difference in size-specific fecundity between vent fields was tested using a t-test
241 analysis. Variations in embryo size associated with parental female, embryo stage and vent fields
242 were analyzed with a multifactorial ANOVA test. For this analysis the factor parental female was
243 considered as nested to the combination of vent field and embryo stage.

244 **3. Results**

245 **3.1. *Rimicaris* shrimp populations at TAG and Snake Pit in January 2014**

246 Among the 3445 individuals collected in our samples, we determined developmental stage
247 and sex for 3388 individuals and measured 3379 individuals (missing data are due to body
248 damages preventing accurate measurements or sex/stage determination). The global dataset
249 included 1925 females (56.8%), 292 males (8.6%), 882 subadults (26.1%) and 289 juveniles
250 (8.5%). Global sex-ratio clearly deviated from 1:1 (χ^2 , df= 1, $p < 0.01$). Of the 1925 females, 136
251 (7.1%) were either brooding eggs (125), or had recently hatched larvae (11).

252 Smaller specimens were early juveniles (CL = 4.4 mm), whereas the largest one was a
253 female with 24.4 mm CL (but this was an outlier since the next largest females were ~20.6 mm
254 CL). Size-range of the juveniles was 4.4 to 10.3 mm, and overlapped the subadult size range (7-
255 9.9 mm CL) (Table S2 Appendix B). The onset of sexual differentiation (OSD) was estimated at

256 10 mm CL (Suppl. data Appendix A), which is consistent with the size of the smallest adult male
257 found in our samples (CL = 9.9 mm). Although the size of some juveniles exceeded the OSD size,
258 they were morphologically distinct from subadults: their rostrum was not completely reduced, and
259 their carapace not fully inflated.

260 Overall, size ranges of males and females were similar with respective CL ranges = 9.9-
261 19.1 mm and 10-24.4 mm (Table S2 Appendix B). However, the average size of males was
262 greater than the average size of females, with 15.1 mm CL and 12.5 mm CL respectively (t-test=
263 20.71, $p < 0.001$). Most ovigerous females exhibited large sizes, with CL ranging from 12 mm to
264 20.6 mm (average size: 16.5 mm CL). We estimated the size at effective sexual maturity (ESM)
265 at 15.1 mm CL for females (Suppl. data Appendix A).

266 **3.2. Variation in population structure across assemblage types and vent fields**

267 *3.2.1. Habitat characteristics of shrimp assemblages*

268 In dense aggregations, the substratum was completely covered by shrimps, sometimes with
269 multiple layers of individuals. Vent fluids were visibly bathing these assemblages and we recorded
270 steep temporal variations in temperature with maxima varying from 14 to 33°C (Table 1). On
271 inactive sulfide substratum at the periphery of dense aggregations, we visually estimated shrimp
272 densities around 10 individuals.m⁻². These scattered individuals were collected only at TAG,
273 although similar assemblages were also observed at Snake Pit. In this habitat, no fluid exit was
274 visible and temperature was low and stable, with a maximum of 2.8°C (Table 1), whereas ambient
275 seawater temperature was 2.6°C. At TAG, aggregations of very small individuals characterized
276 by their bright red color were sampled around diffusions of translucent fluids exiting from very
277 small chimneys or cracks. Temperatures in these nurseries varied from 2.8°C to 5.3°C (Table 1).
278 The 3 types of assemblages with visually distinct shrimp densities, sizes and behavior were thus
279 also characterized by different temperature regimes reflecting the local exposure to vent fluids.

280 3.2.2. *Variations in population structure between assemblage types (TAG)*

281 At TAG, we observed striking differences in terms of population structure, size-frequency
282 distribution, and reproductive features between the three types of assemblages. In dense
283 aggregations, with 71% of females and 8.5% of males, sex-ratio was clearly biased towards
284 females (F:M=8.4:1). Although sex ratios were significantly different between dense aggregation
285 samples from TAG ($\chi^2_{het}= 50.05$, $df= 2$, $p< 0.001$, Table 2), all of them were significantly female
286 biased (χ^2 , $df= 1$, $p< 0.02$ in all cases, Table 2). In contrast, in scattered assemblages, 90.3% of
287 the individuals were males, while females represented only 6.5% of the shrimps collected. Sex
288 ratio was significantly male-biased overall (1:14) and in each sample (χ^2 , $df= 1$, $p\leq 0.002$ in all
289 cases, Table 2). Subadults represented 17.5% of the individuals on average in dense
290 aggregations (Table 2), with significant variation between samples (from 3% to 23.2% of the
291 individuals in each sample, $\chi^2_{het}= 16.4$, $df= 2$, $p< 0.001$). Subadults were rare in scattered
292 assemblages with only two individuals collected overall.

293 Ovigerous females were almost exclusively observed in dense aggregations at TAG (only
294 one hatched female observed among scattered assemblages and none in nurseries),
295 representing 11.7 % of the females on average, with strong variations between samples (from
296 8.4% to 25.6%). These variations reflect both variations in the proportion of sexually mature
297 females among all females (22.4% on average, with variations between samples: 13.3% to
298 64.9%), and, to a lesser extent, variations in the proportion of ovigerous females among sexually
299 mature females (36.8 % on average, with variations between samples: 29.8% to 43.6%).

300 Juveniles represented less than 3% of all shrimps in dense aggregations (Table 2, no
301 significant heterogeneity between samples: $\chi^2_{het}= 3.64$, $df= 2$, $p= 0.602$), and were rare in
302 samples from scattered assemblages with only one early stage juvenile collected. In contrast,
303 nursery samples were exclusively composed of early stage juveniles. Although they were not

304 collected, a few large adult individuals (both *R. exoculata* and *R. chacei*) were observed crawling
 305 around these nurseries (Fig. 1F, Fig. S2D-E Appendix B).

306 **Table 2.** *R. exoculata* specimens and sex ratios in the different samples. J: juveniles, <OSD
 307 (onset of sexual differentiation): subadults, F: females (non-ovigerous); OF: ovigerous females;
 308 M: males.

Dense aggregations										
Vent field	Sample	J	<OSD	F	OF	M	n	F : M	χ^2	p
Snake Pit	S01	1	8	109	1	20	139	5.5:1	62.308	<0.001
Snake Pit	S02	14	175	181	2	9	381	20.3:1	157.688	<0.001
Snake Pit	S03	6	177	522	29	53	787	10.6:1	410.603	<0.001
Snake Pit	S04	16	188	174	0	6	384	29:1	156.8	<0.001
Snake Pit	S05	26	22	39	1	20	108	2:1	6.667	0.01
Snake Pit	S06	21	143	158	22	19	363	9.5:1	130.256	<0.001
Total								9.8:1	904.264	<0.001
								Heterogeneity	20.056	0.001
TAG	S07	19	138	380	35	23	595	18:1	350.831	<0.001
TAG	S08	1	6	163	25	7	202	26.7:1	168.005	<0.001
TAG	S09	6	23	58	20	51	158	1.5:1	5.651	0.017
Total								8.5:1	472.441	<0.001
								Heterogeneity	50.046	<0.001
Scattered assemblages										
TAG	S10	1	0	2	0	15	18	1:7.5	9.941	0.002
TAG	S11	0	2	2	1	33	38	1:16	25	<0.001
TAG	S12	0	0	1	0	36	37	1:35	33.108	<0.001
Total								1:16	67.6	<0.001
								Heterogeneity	0.449	0.993
Nurseries										
TAG	S13	77	0	0	0	0	77			
TAG	S14	101	0	0	0	0	101			

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312 **3.2.3. Variation in population structure between vent fields (dense aggregations only)**

313 Samples in dense aggregations from Snake Pit exhibited similar population structure to
314 those from TAG, with a strong dominance of females. Females, males, subadults and juveniles
315 represented respectively 57.2%, 5.9%, 33 % and 3.9% of the overall population. Sex-ratio was
316 female-biased (9.8:1 overall) and similar to the ratio observed in TAG dense aggregations. Like
317 in TAG, sex-ratio varied significantly among dense aggregation samples at Snake Pit ($\chi^2_{het}=$
318 20.06, df= 5, p= 0.001, Table 2), but all were significantly female-biased (χ^2 , df= 1, p \leq 0.01 in all
319 cases).

320 Overall, subadults were more abundant in Snake Pit samples than in TAG samples,
321 representing almost a third of the population. However, their proportion varied strongly between
322 samples (from 5.8% to 45.9% of the individuals, $\chi^2_{het}= 164.9$, df= 5, p< 0.001). Like in TAG dense
323 aggregations, the proportion of juveniles in Snake Pit samples was generally low, except in one
324 sample where they reached 24.1% of the individuals, resulting in significant heterogeneity
325 between Snake Pit samples ($\chi^2_{het}= 89.32$, df= 5, p< 0.001).

326 Although the proportion of ovigerous females among sexually mature females was similar
327 between vent fields (36.6 % on average at Snake Pit, $\chi^2= 0.003$, p= 0.956), the proportion of
328 sexually mature females among all females was significantly lower in Snake Pit (11.7 %) than in
329 TAG (22.4 %) dense aggregation samples ($\chi^2= 34.24$, p< 0.001), resulting in lower proportion of
330 ovigerous females overall (4.4% of all females).

331 *3.2.4. Variations in size frequency distributions among assemblage types and vent fields*

332 Overall, reflecting the differences in sex and stage distributions between types of
333 assemblages, size distributions also differed. While nurseries hosted very small shrimps, almost
334 none were found in other assemblage types. Mostly large individuals inhabited scattered
335 assemblages. In dense aggregations, shrimp sizes varied over a wide range, overlapping slightly
336 both size ranges of shrimps in nurseries and scattered assemblages (Fig. 3, Fig. S3 Appendix B).

337 Size frequency distributions varied between samples and assemblage types, both in terms
338 of kurtosis and skewness (Fig. 3). General trends in dense aggregation size frequency
339 distributions include bias towards small sizes (skewness= 0.945) and slightly leptokurtic
340 distribution (kurtosis= 0.423). In some samples, the distribution was clearly non-unimodal. In
341 others, deviation in skewness and kurtosis suggested a mixture of cohorts. Based on a size cohort
342 analysis of each sample, we visually identified 5 different cohorts overall in dense aggregations
343 (Table 3). The first comprised juveniles and smaller subadults, the second subadults and small
344 adults, and the last 3 cohorts consisted only of adults, with sexually mature females in the last
345 two. In both vent fields, the cohorts corresponding to juveniles, subadults and small adults
346 represented an important proportion of the population of dense aggregations. In individual
347 samples, cohort number varied between 2 to 4 (Table 3, Fig. S4 Appendix B).

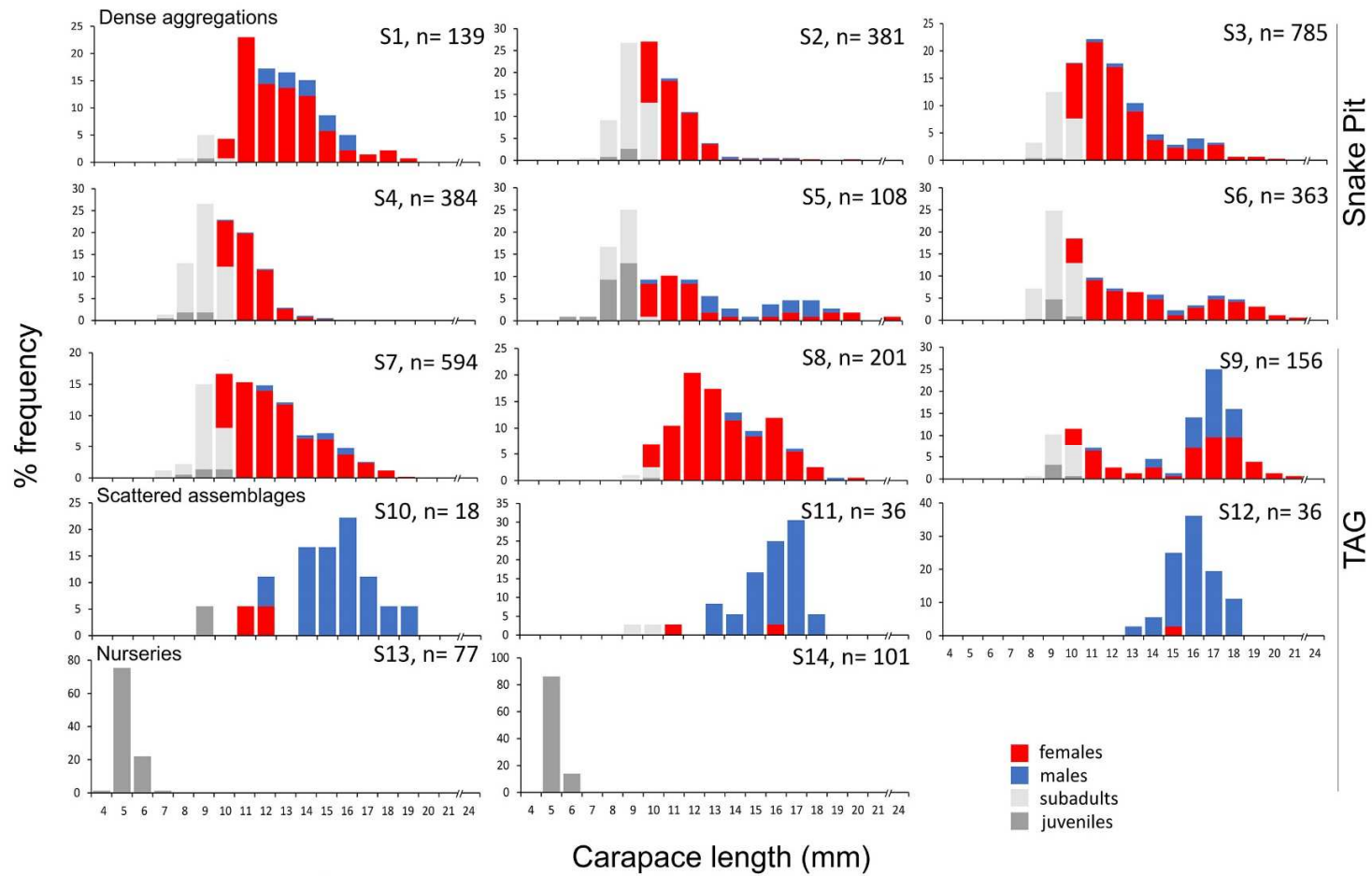
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355 **Figure 3.** Size class structure of *R. exoculata* from each sample collected in different assemblages at Snake Pit and TAG vent fields.

356 **Table 3.** Identified cohorts in dense shrimp aggregations at the Snake Pit and TAG vent fields.
 357 Mean and standard deviations are shown for each sample, proportion of each cohort are in
 358 brackets. Correspondence of cohorts between samples was visually defined but not statistically
 359 tested. χ^2 denote the deviation of the sample from the cohort estimation. ns: non-significant.

Cohort:	1	2	3	4	5	χ^2
Snake Pit						
S1		11.45±1.75 (0.59)	13.24±2.03 (0.41)			12.93ns
S2	8.86±0.87 (0.61)	10.78±1.06 (0.37)		16.14±1.58 (0.02)		3.058ns
S3	9.14±0.89 (0.33)	11.25±1.09 (0.54)		15.49±1.50 (0.13)		11.16ns
S4	8.45±0.77 (0.50)	10.47±0.96 (0.48)	12.92±1.18 (0.02)			1.995ns
S5	8.30±0.87 (0.52)	11.40±1.19 (0.28)			17.28±1.80 (0.20)	15.32ns
S6	8.70±0.70 (0.47)	10.44±0.84 (0.17)	12.81±1.03 (0.17)		17.11±1.38 (0.19)	3.247ns
TAG						
S7	9.83±1.40 (0.59)		13.38±1.90 (0.41)			25.11*
S8		10.12±0.83 (0.15)	12.17±1.00 (0.51)	15.39±1.27 (0.34)		3.556ns
S9	8.98±0.53 (0.21)	10.50±0.62 (0.11)	13.37±0.79 (0.06)		16.73±0.99 (0.62)	8.230ns

360 *Despite the deviation of the size structure from the cohort model ($p= 0.0485$), it was the closest
 361 model to the observed data

362 Male and female body sizes in dense aggregations were significantly different between
 363 samples at each vent field, indicating heterogeneity in sizes within this type of assemblage
 364 (ANOVA, $p < 0.001$, Table S3 Appendix B). Body sizes also varied significantly with sex (ANOVA,
 365 $p < 0.001$, Table S3 Appendix B), males being larger than females at each vent field (Table S2
 366 Appendix B). Size distribution of ovigerous females clearly departed from the rest of the females,
 367 with sizes similar to the male average size in TAG and even larger than the male average size in
 368 Snake Pit. Although males and females tended to be smaller in Snake Pit than in TAG dense
 369 aggregations, size differences were not significant between the two vent fields for each sex
 370 (ANOVA, $p= 0.083$, Table S3 Appendix B). In contrast, ovigerous females were slightly larger in

371 Snake Pit than in TAG dense aggregations, but this variation between the two vent fields was not
372 statistically significant (ANOVA $F = 1.649$, $p = 0.246$, $df_2 = 6$).

373 Size frequency distributions in scattered assemblages were leptokurtic (kurtosis 0.12 to
374 1.99) and biased towards larger sizes (skewness -0.45 to -1.52). Males exhibited no difference in
375 body sizes between dense aggregations and scattered assemblages, although significant
376 difference was detected between samples of a given type of assemblage (ANOVA, $p < 0.001$,
377 Table S4 Appendix B). Females also exhibited similar size ranges between assemblage types,
378 although the low number of females collected in scattered assemblages prevented statistical
379 comparisons.

380 3.2.5. Juvenile distribution in dense aggregations and nurseries

381 Juvenile sizes in dense aggregations were similar between vent fields, only showing
382 differences between samples (ANOVA $F_{vents} = 5.2001$, $p = 0.057$, $df_2 = 7$, $F_{samples} = 2.643$, $p = 0.015$,
383 $df_1 = 7$, $df_2 = 101$). However, juveniles from nurseries were much smaller (ANOVA $F_{vents} = 804.91$,
384 $p < 0.001$, $df_2 = 3$, $F_{samples} = 1.580$, $p = 0.195$, $df_1 = 3$, $df_2 = 199$) and formed a distinct cohort from that
385 of dense aggregations. Based on morphological features described in Komai and Segonzac
386 (2008), we suspected that juveniles from nurseries were possibly a mixture of *R. exoculata* and
387 *R. chacei*. COI sequences from juveniles with various morphological features in nurseries were
388 all consistent with *R. chacei* affiliation, whereas sequences from juveniles in dense aggregations
389 were consistent with *R. exoculata* affiliation (Fig. S5 Appendix B).

390 3.3. Reproductive features

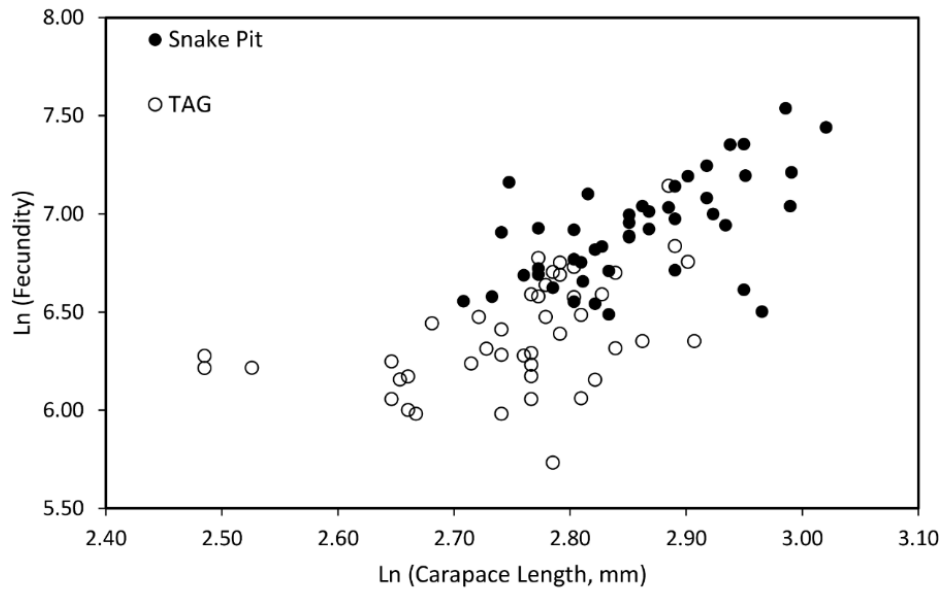
391 3.3.1. Fecundity

392 Of the 125 brooding females collected, 36 had obviously lost part of their broods, not
393 because of hatching (embryos were clearly not yet fully developed), but rather due to either

394 abortion or more probably loss during sampling. These were not included in our fecundity
395 analyses.

396 Fecundities varied from 304 eggs in a female from TAG with 16.2 mm CL, to 1879 eggs
397 in a female from Snake Pit with 19.8 mm CL. The largest brooding females were observed at
398 Snake Pit (with 1704 eggs and 20.5 mm CL for the largest), while the smallest brooding females
399 were from TAG (with 500 and 532 eggs for the two smallest -12 mm CL- individuals). The overall
400 average fecundity was 833 eggs, and was higher among Snake Pit brooding females (1045 eggs,
401 with an average CL of 17.4 mm) than among TAG ones (616 eggs, with an average CL of 15.9
402 mm). As expected, a positive correlation was observed between carapace length of the females
403 and fecundity (Pearson correlation, $R= 0.682$, $t\text{-test}= 8.71$, $p< 0.001$) (Fig. 4). We consider that
404 more data are necessary to estimate accurate linear regression models of fecundity and compare
405 differences between populations of the two vent fields or with other alvinocaridid shrimps.

406 Overall size-specific fecundity ranged from 19 to 95 embryos.mm⁻¹ CL. The size-specific
407 fecundity of females did not change with the developmental stage of the brood (Early vs Mid
408 stage, $t\text{-test}= 0.98$, $p= 0.164$, $df=57$; Mid vs Late stage, $t\text{-test}= 0.17$, $p= 0.432$, $df=57$), but was
409 significantly lower in females from TAG (39 ± 10 embryos.mm⁻¹ CL) than in females from Snake
410 Pit (59 ± 13 embryos.mm⁻¹ CL) ($t\text{-test}=8.16$, $p<0.001$, $df=87$). In addition, more females with
411 damaged broods were observed at TAG.

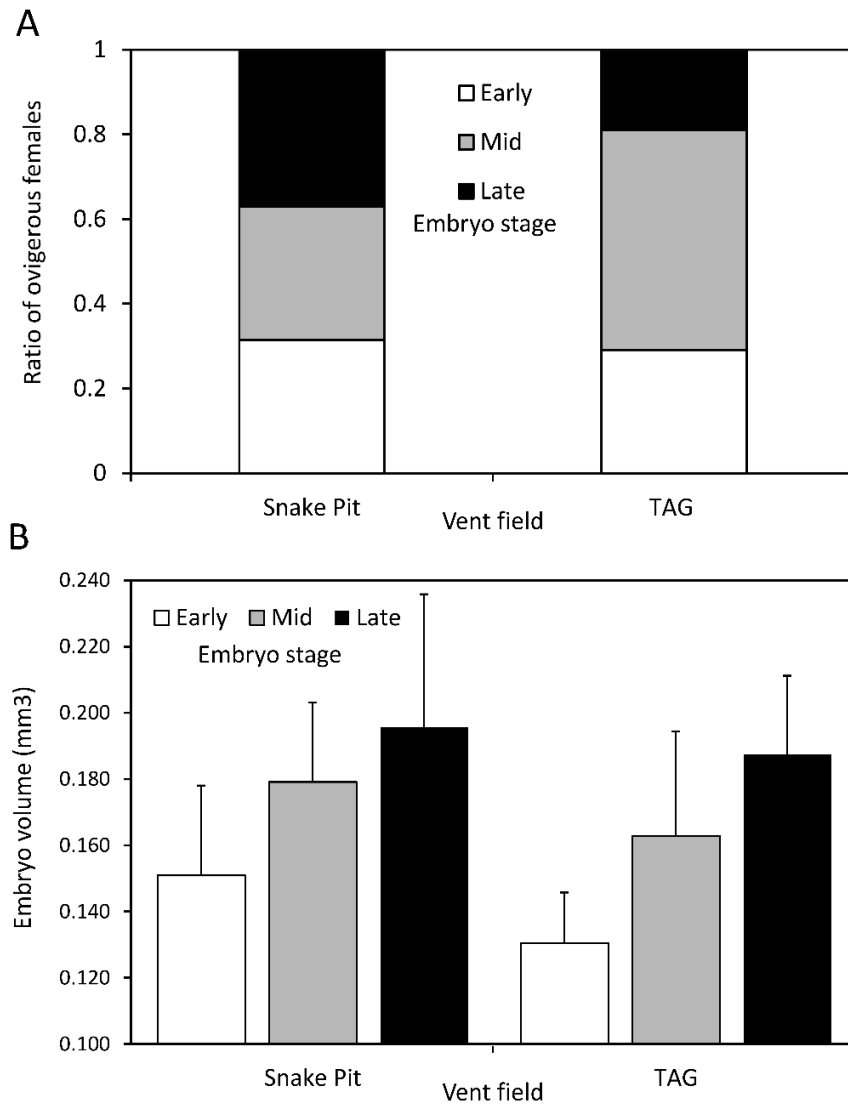


412
 413 **Figure 4.** Relationship between fecundity and size in *R. exoculata* from TAG and Snake Pit vent
 414 fields.

415 **3.3.2. Reproductive synchrony**

416 Within each individual brood examined, all eggs were at the same developmental stage
 417 (early, mid or late), except for occasional dead embryos or non-fertilized eggs. However, embryos
 418 at all developmental stages were observed in females from each vent fields, showing a lack of
 419 synchrony between females. Overall, the distribution of brood stages was different between vent
 420 fields ($\chi^2= 7.097$ $p=0.014$), with variability between samples of a given vent field (Fig. S6 Appendix
 421 B). At each vent field, a third of the females carried early stage broods, however at Snake Pit late-
 422 stage brooding and hatched females were slightly more frequent (37%) than at TAG (19%). At
 423 TAG, most of the brooding females were at the mid stage (51.9%) (Fig. 5A).

424



425

426 **Figure 5.** Characteristics of *R. exoculata* broods at TAG and Snake Pit vent fields. A. Proportion
 427 of broods with eggs at each developmental stage (including hatched females as having late
 428 broods). B. Sizes of individual embryos at different developmental stages.

429 *3.3.3. Egg sizes and development*

430 The volume of the eggs within the brood of each female showed significant heterogeneity
 431 due to inter-individual variations, developmental stage of the broods, and vent fields (Table S5
 432 Appendix B). Despite individual variations, a clear trend of egg volume increase with
 433 developmental stages was observed at each vent field (Fig. 5B). The ratio between the minimum

434 and maximum diameters of embryos decreased along their development, with minimum diameter
435 being on average 0.87 of the maximum diameter in early stage, and 0.76 of the maximum
436 diameter in the late stage. Eggs thus become more elongated at the end of embryonic
437 development, which may reflect an increase of the embryo polar axis, the distribution of the
438 structures inside the envelope and water uptake.

439 At each stage, embryos in TAG broods were smaller than in Snake Pit broods (Fig. 5B).
440 The volume of early stage embryos was $0.151 \pm 0.027 \text{ mm}^3$ at Snake Pit, and $0.131 \pm 0.015 \text{ mm}^3$
441 at TAG ($t\text{-test}= 8.386$, $p < 0.001$, $df=242$). At mid-stage, the volume of embryos increased to 0.179
442 $\pm 0.024 \text{ mm}^3$ at Snake Pit and $0.163 \pm 0.032 \text{ mm}^3$ at TAG ($t\text{-test}= 3.719$, $p < 0.001$, $df= 116$). In
443 the late stage, the volume of embryos reached $0.196 \pm 0.040 \text{ mm}^3$ at Snake Pit and 0.187 ± 0.024
444 mm^3 at TAG ($t\text{-test}= 2.458$, $p= 0.007$, $df= 238$).

445 **4. Discussion**

446 Populations of *Rimicaris exoculata* sampled in January-February 2014 at TAG and Snake
447 Pit vent fields revealed strikingly biased sex ratios, reflecting abrupt changes in population
448 structure among different shrimp assemblages. Dense aggregations next to high temperature
449 fluid exits consisted mainly of females and immature individuals, whereas shrimps observed
450 scattered in the cold and stable periphery of active vents were almost only adult males. At TAG,
451 assemblages of very small juveniles around low temperature diffusions were *Rimicaris chacei*
452 nurseries, whereas *R. exoculata* juveniles were found in the denser aggregations. Ovigerous
453 females were observed in larger proportion than ever reported so far for this species, representing
454 about a third of the sexually mature females in dense aggregations. Overall, these patterns were
455 consistent across both vent fields, although a high degree of heterogeneity in population structure
456 was observed at small spatial scale, across dense aggregations. Ovigerous females were more
457 abundant at TAG, reflecting a higher proportion of sexually mature females. However, lower

458 fecundities and smaller eggs were observed in ovigerous females from TAG, suggesting a lower
459 individual reproductive effort.

460 **4.1. Spatial variability in *Rimicaris* population structure**

461 *4.1.1. Sex segregation in different types of assemblages*

462 At TAG, we showed that dense aggregations of *R. exoculata* shrimps on active chimneys
463 and scattered individuals occurring in peripheral areas with no visible fluid exits have very different
464 population structures. Although they were reported in previous studies (Copley et al. 1997, 1999,
465 Segonzac et al. 1993), scattered shrimps were not included in demographic studies so far,
466 probably because they have been considered as remains of the main populations. Our
467 observations show that these scattered shrimps represent a truly specific assemblage consisting
468 mainly of large males, whereas females and immature individuals which constitute the bulk of the
469 population in dense aggregations are rarely observed at the periphery. At Snake Pit, dense
470 aggregations were also dominated by immature individuals and females, and scattered shrimps
471 were observed in inactive peripheries nearby the main active edifices. Although these shrimps
472 were not collected at Snake Pit, they were visually mirroring scattered assemblages from TAG
473 and comprised mostly large adults. We thus expect these shrimps, as well as scattered individuals
474 reported from other vent fields (Segonzac et al. 1993, Copley et al. 1997, 1999) to be also mainly
475 males. Complex population distributions with local variations in densities and structure are likely
476 general features of *R. exoculata* populations along the MAR.

477 Differentially distributed males and females with locally unbalanced sex-ratios have been
478 reported in several crustaceans from deep-sea vents and seeps. For instance, populations of
479 *Rimicaris hybisae* at the Cayman Trough vents have a sex ratio in favor of females close to the
480 fluid emissions, while more dispersed populations are dominated by males at their peripheries,
481 with some degree of local variability (Nye et al. 2013). At hydrothermal vents of the East Scotia

482 Ridge, the chirostyliid crab *Kiwa tyleri* exhibits similar -but inverse- patterns: areas close to vent
483 fluids emission are occupied by dense male-dominated aggregations, whereas females are more
484 numerous at the periphery (Marsh et al. 2015). In brine pools of the Gulf of Mexico, *Alvinocaris*
485 *stactophila* shows overall female-biased sex ratio with most males locating in areas exposed to
486 sulphidic and hypoxic conditions, whereas large reproductive females occupy less exposed areas
487 (Copley and Young 2006). Biased sex ratios, with small scale variability associated to
488 environmental conditions thus appear to be a common feature of populations of vent and seep
489 decapods, although the distribution of each sex within the fluid exposure gradient depends on
490 species.

491 4.1.2. Patchiness in dense aggregations

492 At a smaller scale, within dense *R. exoculata* aggregations of each vent field, we also
493 observed heterogeneity in population structures, with significant variations in the proportions of
494 the different life stages between our samples. Brooding females were found in significant numbers
495 in 5 out of 9 samples whereas they were almost absent from the 4 remaining, suggesting they
496 have a patchy distribution across those dense aggregations. Similarly, juveniles occurred in
497 significant proportion in only one sample from Snake Pit, again reflecting a patchy distribution of
498 this life stage. Patchiness within dense shrimp aggregations was already suggested in
499 populations of Logatchev and other vent fields because gatherings of juveniles among adults are
500 visually conspicuous (Gebruk et al, 2010, Shank et al, 1998). Our results confirm the spatial
501 heterogeneity in the distributions of the different life stages across dense aggregations, with local
502 segregation patterns. Similar spatial segregation between benthic life stages at vents were
503 reported in *Bathymodiulus* mussels (Husson et al. 2017) and in chirostyliid crabs (Marsh et al.
504 2015). These patterns have been related to local habitat conditions, life stage physiological
505 requirements or tolerances, as well as resource use (Husson et al. 2017). In *R. exoculata* dense

506 aggregations, additional observations are necessary to refine small-scale population structures
507 and their links with local environmental gradients and resource availability.

508 4.1.3. *Distinct nurseries for R. exoculata and R. chacei*

509 *Rimicaris* juveniles were reported from dense aggregations on vent chimney walls and
510 isolated patches at the base of edifices, in Logatchev (Gebruk et al. 2000), and other vent fields
511 along the nMAR (Shank et al. 1998). They were identified as *R. exoculata* (Shank et al. 1998) or
512 mixtures of both *R. exoculata* and *R. chacei* co-occurring in the same patches (Komai and
513 Segonzac 2008). Our molecular data demonstrated that small juveniles in isolated low
514 temperature nurseries were all *R. chacei*. In contrast, juveniles in dense aggregations belonged
515 to *R. exoculata*, suggesting that juveniles of both species segregate in distinct habitats, with
516 different temperature conditions. Aggregations of small juveniles of *R. chacei* were also observed
517 around fluid diffusions at the periphery of TAG and Snake Pit vent fields during following cruises
518 in 2017 and 2018 (Methou et al. 2020). A more systematic examination of morphological
519 characters along with molecular characterization led to a redefinition of the juvenile stages of each
520 *Rimicaris* species (Methou et al. 2020). In our 2014 samples, we identified all stages defined by
521 Methou et al. (2020), *R. chacei* juveniles all being in isolated low temperature nurseries, while *R.*
522 *exoculata* juveniles at all stages occurred in dense aggregations.

523 The size difference between *R. chacei* and *R. exoculata* juveniles in our samples suggests
524 that we may miss earlier benthic stages for the latter, assuming a similar settlement size between
525 the two species. However, a single *R. exoculata* pelagic post-larva was caught in nets towed
526 within the axial valley of the MAR 200-1000 m above the Broken Spur vent field in August and
527 September 1995, probably as it was approaching its recruitment place. It had a size similar to
528 those of our benthic *R. exoculata* juveniles (28 mm total length) in dense aggregations, whereas
529 post-larvae of *R. chacei* caught at the same time were smaller (13-23 mm) (Herring and Dixon

530 1998) and similar to our *R. chacei* juveniles from low temperature nurseries. This implies a larger
531 settlement size in *R. exoculata*, and undermines the possibility that we missed an earlier benthic
532 stage that would settle elsewhere or at another period. Isotopic analyses conducted on *R. chacei*
533 juveniles from isolated nurseries and on *R. exoculata* juveniles within dense aggregations at our
534 study sites also support a higher settlement size for the latter (Methou et al. 2020). Smallest
535 juveniles of each species harbor an isotopic signature reflecting nutrition on material of
536 photosynthetic rather than chemosynthetic origin, which suggests that both recently left the
537 pelagic realm to start their benthic life, settling at different sizes. Juveniles of each species thus
538 not only differ in their settlement habitats but also in their settlement size, which probably affects
539 their post-settlement life history.

540 **4.2. Reproductive development in *R. exoculata***

541 *4.2.1. Temporal variability in spawning*

542 Despite three decades of sampling at MAR hydrothermal vents, reports of *R. exoculata*
543 brooding females were extremely rare (Gebruk et al. 1997, Ramirez-Llodra et al. 2000) except in
544 March 2007 on the Logatchev vent field (Gebruck et al. 2010), but very few individuals were
545 collected at that time (Komai and Segonzac 2008, Guri et al. 2012). In January-February 2014,
546 7.1% of the females collected in our samples were brooding eggs or had just released their larvae.
547 This apparently low proportion essentially reflects the high proportion of small females below the
548 size of sexual maturity. Indeed, a third of the sexually mature females were ovigerous at each
549 vent field. In addition, many of the two remaining thirds exhibited bright pink well developed
550 gonads dorsally, suggesting that these females were ready to spawn (FP personal observation
551 on our samples as well as on *in situ* video, not quantified). The occurrence of large numbers of
552 ovigerous females, observed at the same period at two vent fields 300 km apart, and contrasting
553 with all previous collections along the nMAR (see 1985-2014 collection compilation, Table 4)

554 indicates that spawning activity varies temporally in *R. exoculata*, with a certain degree of
 555 synchrony at regional scale. Such pattern may possibly suggest spawning periodicity in *R.*
 556 *exoculata*. At this stage, additional observations are needed to assess its temporality, geographic
 557 extent, as well as possible drivers.

558 **Table 4.** Occurrence of brooding females in *Rimicaris exoculata* samples from different cruises
 559 on the nMAR since 1985.

Site	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rainbow 36°14'N						1998 ^{5, 7}	1997 ³ 2002 ¹⁰	2005 ⁷	2005 ¹⁰	1998 ¹⁰		
Broken Spur 29°10'N							1997 ³ 2002 ¹⁰		1994 ^{2, 10} 1996 ^{2, 10}			
TAG site 26°08'N	2014*	2014* 2018 ¹¹	2018 ¹¹			2002 ¹⁰	1997 ³	1985 ¹ 2005 ⁷	1994 ⁵		2004 ⁶	
Snake Pit 23°22'N	2014*	2014* 2018 ¹¹	2018 ¹¹			2002 ¹⁰	1997 ³ 2001 ⁷	2003 ¹⁰			1995 ⁷	
Logatchev 14°45'N			2007 ⁷⁻⁹				1997 ^{3, 4} 2001 ⁷				1998 ¹⁰	

560 ¹Williams and Rona (1986), ²Gebruk et al. (1997), ³Shank et al. (1998), ⁴Gebruk et al (2000), ⁵Ramirez-
 561 Llodra et al. (2000), ⁶Copley et al (2007), ⁷Komai & Segonzac (2008), ⁸Gebruk et al. (2010), ⁹Guri et
 562 al. (2012), ¹⁰Lunina and Vereshchaka (2014), ¹¹Methou et al. (2019), ***this study**. Reports included
 563 here examined several tens to several hundred specimens. Color code: blue: no brooding female
 564 reported; green: statement of “rare” brooding females or 1-2 brooding females reported; pink:
 565 statement of “many” brooding females or at least 10 brooding females in more quantitative reports.

566 Previous studies suggested continuous reproduction in *R. exoculata*, based on
 567 asynchronous ovarian development observed among females collected in summer at the
 568 Rainbow vent field and in autumn at TAG (Ramirez-Llodra et al. 2000, Copley et al. 2007). At that
 569 time, the lack of brooding females was explained by their migration out of dense aggregations
 570 towards areas with less exposure to harmful vent fluids to protect their eggs. Our observations
 571 suggest that brooding females actually remain in dense aggregations, but brooding activity may
 572 be restricted in time which could explain the lack of observations during other months. Additional

573 observations of ovarian development at different periods, taking into account the size of females,
574 and perhaps also their distribution in the mixing gradient of vent fluid and seawater, are needed
575 to better understand the gametogenic cycle of the species. In *Alvinocaris stactophila* from cold
576 seeps in the Gulf of Mexico, females exhibit increasing oocyte size in gonads throughout the year,
577 and ovigerous females are observed mainly in February-March (Copley and Young 2006). In *R.*
578 *hybisae* from vents in the Cayman Trough, winter spawning was also suggested, but the effect of
579 spatial population structure and need to expand temporal coverage was recognized (Nye et al.
580 2013). As for *R. hybisae*, we need to expand the temporal breath of observations on *R. exoculata*
581 reproduction to better constrain its spawning seasonality and assess its degree of variability.

582 While most vent species along the MAR were suggested to reproduce continuously,
583 seasonal spawning and recruitment was shown for *Bathymodiolus azoricus* mussels from the
584 Menez Gwen vent field near the Azores (Colaço et al. 2006). The relatively shallow depth of
585 Menez Gwen (850m), where the influence of seasonal phytoplankton variation is still significant
586 compared to deeper sites provide a driver for this seasonality, as also suggested for *A. stactophila*
587 at seeps of similar depth in the Gulf of Mexico (Copley and Young 2006). A sexual pause was
588 observed in *Bathymodiolus puteoserpensis* occurring at sites below 2000m (Tyler and Young
589 1999), suggesting that seasonal spawning is also possible at depth, and that both environmental
590 drivers and phylogenetic constraints may be important. In Alvinocarididae, both continuous and
591 seasonal reproduction have been reported (Copley and Young, 2006, Ramirez-Llodra et al.,
592 2000). In *R. exoculata*, polymodal size structures suggest discontinuous recruitment, which may
593 also reflect periodic spawning. However, the effect of larval dispersal dynamics, recruitment
594 temporality and post-settlement growth rates and mortality all remain to be assessed before any
595 supported conclusion can be drawn.

596 4.2.2. *Reproductive effort in R. exoculata at TAG and Snake Pit*

597 Size at first reproduction represents a key life-history parameter reflecting the life-time
598 investment in reproduction of a species (Anger and Moreira 1998). In Alvinocaridid species, the
599 size at first reproduction varies between 50% (*R. hybisae*, Nye et al. 2013) and 60% (*A. muricola*,
600 Ramirez-Llodra and Segonzac 2006, *A. stactophila*, Copley and Young 2006, *M. fortunata*,
601 Ramirez-Llodra et al. 2000) of the maximal size of the species. *R. exoculata* thus falls within the
602 range of the family, with the smallest brooding females measuring 12 mm CL, which represents
603 50% of its maximal size. However, few females between 12 mm CL and 15 mm CL (i.e. < ESM)
604 were brooding eggs in our samples (3.5%) and likely represent 'premature' specimens, while
605 many more become sexually mature (36.5%) when they reach 15.1 mm CL (ESM), which
606 represents 62% of the species maximal size. Although a later onset of reproduction might limit life
607 time investment in reproduction for an iteroparous species (Ramirez-Llodra et al. 2000), favoring
608 reproduction to the largest individuals which produce the largest broods might be advantageous
609 and help to maximize energy investment in reproduction.

610 Fecundity in *R. exoculata* was on average of 833 eggs/female, ranging from 304 to 1879
611 eggs/female. This is in agreement with fecundities found in the few brooding females available
612 before our study (Ramirez-Llodra et al. 2000). In addition, *R. exoculata* fecundity is similar to that
613 reported for *R. hybisae* (max 1707 eggs/female, Nye et al. 2013), but lower than that of *R. chacei*
614 (2510 eggs for a female from Lucky Strike, Ramirez-Llodra et al. 2000). Egg sizes of *R. exoculata*
615 were consistent with those reported previously: 0.145 mm³ for early stage eggs from TAG females
616 (Ramirez-Llodra et al. 2000), which is within the range reported here at similar stage (0.131-0.151
617 mm³ at TAG and Snake Pit respectively). Other *Rimicaris* species have smaller eggs: 0.08 mm³
618 for *R. hybisae* (Nye et al. 2013), and 0.09 mm³ for *R. chacei* (Ramirez-Llodra et al. 2000). With
619 fecundities in the lower range of those reported for its genus, and eggs sizes in the upper range,
620 *R. exoculata* may have a specific strategy of higher parental investment per egg.

621 *R. exoculata* reproductive outputs differed between TAG and Snake Pit populations. Indeed
622 both realized fecundities and egg sizes were lower at TAG. Although we cannot completely
623 exclude a sampling effect on the observed fecundities at TAG, or variations in fertilization success
624 between vent fields, the difference in realized fecundity most probably reflects the larger body
625 sizes of brooding females at Snake Pit, and the positive allometric variation of fecundity with
626 female size. Similarly, differences in fecundity between *R. hybisae* females of two vent fields in
627 the Cayman Trough were primarily attributed to the large size differences observed between
628 shrimps of the two fields (Nye et al. 2013). The lower egg size at TAG, however, suggests that
629 additional factors, such as food availability or environmental challenges (fluid toxicity, temperature
630 stress), may also contribute to differences in reproductive investment between populations of the
631 two fields.

632 Both fields are at similar depth, 300 km apart along the ridge, and regional factors are
633 unlikely to provide environmental heterogeneity that could be linked to the different reproductive
634 investment. We hypothesize that local environmental factors associated with vent emissions are
635 more likely to affect *R. exoculata* reproductive effort. Shrimp tolerance to metallic elements and
636 dissolved gases in vent fluids depends on detoxification processes through metallothioneins,
637 antioxidants (Gonzalez-Rey et al. 2007) and metabolic activities of their symbiotic bacteria (Jan
638 et al. 2014, Cambon-Bonavita et al. 2021). Higher concentrations in some metallic elements in
639 hydrothermal fluids could force the shrimps to allocate more metabolic energy to detoxification
640 processes, at the expense of reproductive functions. TAG fluids have higher iron, copper and
641 manganese concentrations than those from Snake Pit (Desbruyères et al. 2000, Schmidt et al.
642 2008, Charlou et al. 2010), which could explain the lower reproductive output of shrimps at this
643 vent field. However, both bioenergetics and vent processes are complex and physiological
644 tolerance of reproductive shrimps must be experimentally tested (e.g. August et al. 2016), along
645 with a more detailed exploration of local vent chemistry.

646 **4.3. Life history traits of *R. exoculata* males and females**

647 Adult males and females of *R. exoculata* exhibit different distributions, which suggests
648 distinct life history traits. Although spatial sex segregation is common in vent decapods, usually
649 opposite trends have been reported, where males occupy areas closer to high temperature fluids
650 or with steeper chemical gradients, whereas brooding females locate preferentially in areas with
651 milder conditions (Perovitch et al. 2003, Copley and Young, 2006, Marsh et al. 2015).

652 *4.3.1. Brooding in vent fluids*

653 Brooding *R. exoculata* females were observed within dense aggregations at Logatchev
654 (Gebruk et al. 2010, Guri et al. 2012). At TAG and Snake Pit, all brooding females we observed
655 or collected were crawling in dense aggregations bathed in vent fluids. Only *R. hybisae* at vents
656 in the Cayman Trough has also been observed brooding eggs in habitats exposed to
657 hydrothermal influence (Nye et al. 2013).

658 Ovigerous females with broods at all developmental stages including hatching zoea were
659 found in dense aggregations, suggesting that females of *R. exoculata* remain in areas exposed
660 to vent fluids during the whole brooding period until they release the larvae. Exposure of embryos
661 to high temperatures may accelerate their development and shorten the brooding period, while
662 heat stress may also challenge normal development. *In vitro* incubations of embryos of
663 *Shinkaicaris leurokolos*, an alvinocaridid species inhabiting vent areas exposed to hydrothermal
664 fluids in Okinawa Trough, showed increased developmental rates with increasing temperature
665 and optimal growth at temperatures within the range experienced by adults *in situ* (10-20°C,
666 Watanabe et al. 2016). Embryos of *S. leurokolos* hatched within 9-12 weeks at 10°C and 3-4
667 weeks at 20°C (Watanabe et al. 2016). Considering the habitat of brooding females in *R.*
668 *exoculata*, eggs are likely to hatch within a few weeks following spawning. Another observation in
669 favor of a short brooding period is that ovigerous females did not appear to suffer excessive

670 mineral load on their carapace (FP personal observation). In *R. exoculata*, very frequent molting
671 - every 10 days - may regularly eliminate mineral precipitations resulting from vent fluid exposure
672 and overgrowing cephalothoracic symbiotic bacterial communities (Corbari et al. 2008). Since
673 molting is interrupted during the brooding period in decapods (Correa and Thiel 2003), a long
674 incubation within vent fluids would certainly result in highly mineralized cephalothoracic surfaces,
675 which were not observed in our ovigerous females.

676 Brooding within vent fluids may also sustain the bacterial community observed on egg
677 envelopes (Methou et al. 2019), and thus participate in symbiont transmission to the young shrimp
678 through infestation of the larvae (Hernandez Avila et al. 2015, Methou et al. 2019). Another
679 speculative hypothesis would be that mothers could imprint their offspring with vent signature by
680 bathing their eggs within vent fluids during embryonic development, which would later help them
681 return to suitable vent habitats after dispersal. Such homing process might involve the strongly
682 developed higher brain centers of *R. exoculata* enabling the memory and navigation skills
683 necessary to locate suitable recruitment sites (Machon et al. 2019), as well as their sensory
684 abilities to detect vent environments (Ravaux et al. 2021).

685 4.3.2. *Mating in the periphery*

686 At TAG, half of the males of our sampling set were collected in the inactive periphery,
687 suggesting that they spend a significant part of their time there, and raising questions on the
688 underlying driving factors. It is unlikely that males stay permanently at the periphery because they
689 need to supply their symbiotic bacteria with reduced compounds of the vent fluids to ensure their
690 nutrition (Ponsard et al. 2013). Alternative diet on food items (particles, microbial mats) picked on
691 the substratum in inactive areas is unlikely because the morphology of their cephalothorax and
692 chelipeds is not suited for such feeding strategy (Segonzac et al. 1993). Peripheral areas also
693 harbor more predators, such as *Maractis rimicarivora* anemones (Fautin and Barber 1999, Copley

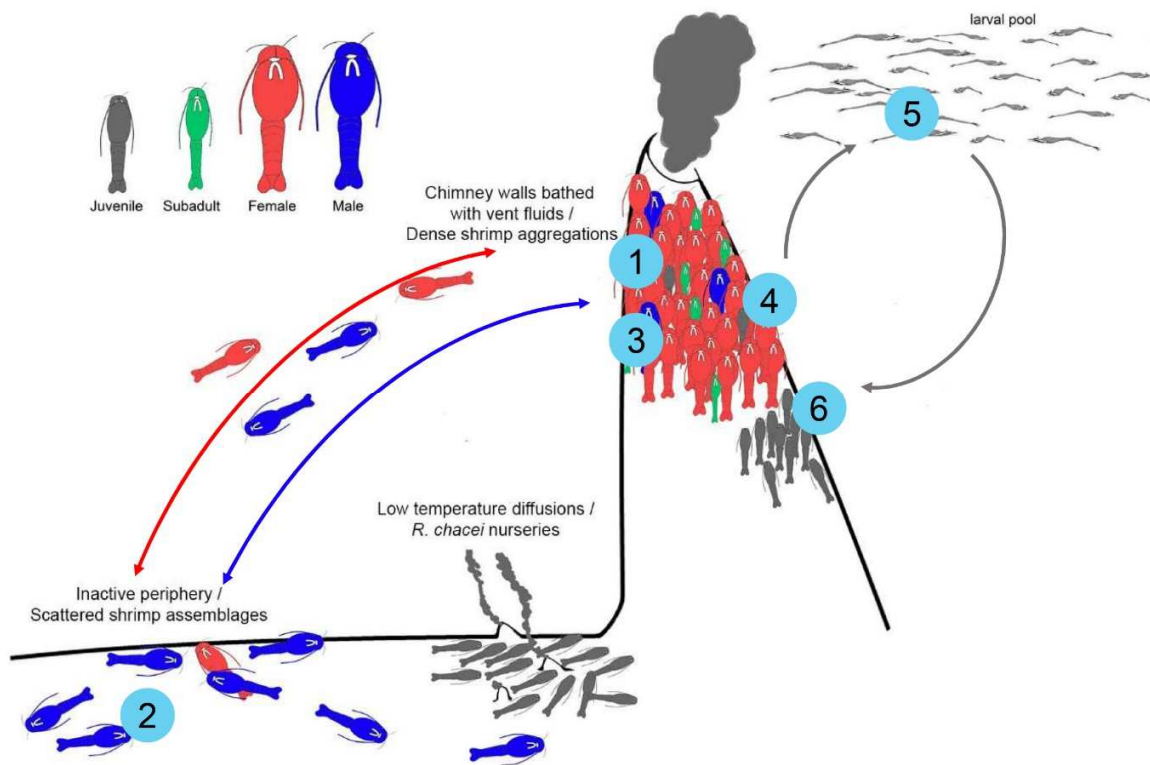
694 et al. 2007) that are excluded from dense aggregations where environmental conditions are
695 probably too harsh. The migration of *R. exoculata* males to inactive areas is therefore not likely
696 driven by trophic needs and expose individuals to increased predatory risk. The hypothesis of a
697 reproductive behavior remains a plausible explanation. In Caridean shrimps, females molt before
698 egg extrusion, and mating occurs following this pre-spawning molt (Bauer 2004). Vent shrimps
699 may migrate briefly towards a milder habitat for molting to avoid exposure to vent fluids while they
700 are most vulnerable. Location in peripheral areas might be advantageous for males to reach
701 females just after their pre-spawning molt and mate successfully.

702 In crustaceans, body sizes and male weaponry have been associated with the mating
703 system (Correa & Thiel 2003, Baeza & Thiel 2007, Asakura 2009). In free-living caridean shrimps,
704 larger males are usually observed in mating systems that involve sexual competition for females
705 or precopulatory mate guarding (Bauer 1996, Correa & Thiel 2003, Asakura 2009). In contrast,
706 the lack of sexual competition in “pure searching” mating systems or long-term mate (monogamy
707 or semi-monogamy) is generally associated with similar or smaller-sized males (Bauer 1996,
708 Correa & Thiel 2003). In *R. exoculata*, sexually mature females, i.e. those actually involved in the
709 courtship and mating processes, have similar or slightly larger sizes than males. In addition, *R.*
710 *exoculata* lacks sexual dimorphism in secondary characters associated with male competition
711 (e.g. increase in cheliped size and cephalotorax). Thus, a “pure searching” model where males
712 search for receptive females just after their reproductive molt using mostly tactile signals or
713 pheromones (Bauer 1976, 1996) could better describe the mating system in *R. exoculata*. In
714 several instances, we observed *R. exoculata* pairs at the periphery of dense shrimp aggregations,
715 with a male (supposedly) clutching a female (sometimes identified by its pink dorsal gonads) with
716 its walking legs (Video S1 AppendixB-C). Such behavior is typical of the mating process described
717 by Bauer (1976) for *Heptocarpus pictus*, a caridean shrimp exhibiting a “pure searching” mating
718 model.

719 **4.4. A life-history scenario for *Rimicaris* shrimps at vents**

720 Our observations on the distribution of the sexes and life-stages of *R. exoculata* at vents,
721 and our hypotheses on the drivers of these distributions can be synthesized in a possible life-
722 history scenario (Fig. 6). Juveniles appear to settle directly among their older conspecifics, in
723 areas bathed with hydrothermal fluids, forming patches within dense aggregations, and thus
724 contributing to the heterogeneity of these assemblages. Such gatherings of juveniles might be
725 the result of discrete settlement events and/or reflect different tolerance or physiological needs of
726 new settlers compared to those of older individuals. After settlement, *R. exoculata* juveniles
727 remain in dense aggregations where they grow to the subadult and adult stages. When they reach
728 sexual maturity, adults could migrate in less active parts of vent fields for mating and spawning.
729 Sexually mature males would reach a position where they could “more easily” encounter sexually
730 receptive females that move at the periphery for their pre-spawning molt. After mating and egg
731 extrusion, females would return to dense aggregations to brood their eggs, while the fate of males
732 remains uncertain. Males may however return to dense aggregations at some point to fulfill their
733 nutrition needs. After an incubation period of a few weeks on chimney walls, brooding females
734 would release zoea larvae (Hernández-Ávila et al. 2015). These larvae would disperse within
735 bathypelagic waters, developing and feeding for a while on pelagic food items until they reach a
736 large post-larval stage and return to a benthic and chemosynthetic life style at vents. The benthic
737 life of the conspecific *R. chacei* may follow a different scenario as settlement occurs in the herein
738 so-called nurseries that form a distinct habitat from that of *R. exoculata*. Further observations by
739 Methou et al. (2022) indeed highlight a different post-settlement trajectory resulting in much
740 smaller adult populations for this species.

741



Life stage	Habitat	Observation	Reference
1 Growth & maturation	Chimney walls	All stages from juveniles to adults are present, few males.	This study.
2 Mating & spawning	Periphery	Many adult males, rare females, mating behavior observed.	This study.
3 Brooding	Chimney walls	Brooding females with embryos at all stages.	Guri et al. 2012, this study.
4 Larvae release	Chimney walls	Late broods and hatched females are present, zoe larvae collected near adult populations.	Hernandez-Avila et al. 2015, this study.
5 Larval dispersal	Bathypelagic	<i>Rimicaris</i> post-larval stages collected 200-1000 m above vents.	Herring & Dixon 1998
6 Recruitment	Chimney walls	Most juveniles observed in dense aggregations.	Methou et al. 2020, this study.

742

743 **Figure 6.** Schematic scenario depicting life history and habitat use of *Rimicaris exoculata* through
 744 its life cycle at vents on the nMAR.

745

746 **5. Conclusion**

747 This study revealed complex population structures in *R. exoculata*, with spatial variations in
748 life-stage distributions between and within visually distinct shrimp assemblages of a single vent
749 field. This complexity is probably the result of habitat heterogeneity at small spatial scales and
750 different physiological and nutritional requirements along the shrimp life cycle. Males and females
751 contrasting spatial distributions resulted in strongly biased sex ratios locally, and could be related
752 to their reproductive strategy involving mating at the periphery of dense aggregations and
753 incubation of the eggs within vent fluids.

754 The physiological and nutritional flexibility allowing male holobionts to cope with conditions
755 that may be deleterious for their symbiotic communities remains to be assessed. Spatial
756 distributions of the early stages, reflecting changes in their physiological tolerance and/or
757 resource use, are also probably related to symbiont proliferation and their gradual transition
758 towards a chemosynthetically derived nutrition. A more detailed analysis of the interplay between
759 environmental conditions, shrimp life stages as well as the development of their symbiotic
760 community as they grow is needed to better understand the factors driving the observed *in situ*
761 distributions.

762 Finally, future studies should provide insights into temporal stability of the spatial patterns
763 we observed. In particular, temporal variability in reproductive activity needs a more thorough
764 assessment through repeated observations at our study sites and other vents along the mid-
765 Atlantic ridge. Such studies are required to accurately estimate seasonally in reproduction of
766 deep-sea species, and identify the underlying drivers.

767

768

769 **Credit author statement:**

770 Iván Hernández-Ávila : Conceptualization, methodology, formal analysis, investigation, writing –
771 original draft.

772 Marie-Anne Cambon-Bonavita : Resources, supervision, writing – review & editing.

773 Jozée Sarrazin : Resources, writing – review & editing.

774 Florence Pradillon : Conceptualization, methodology, resource, supervision, writing – review &
775 editing.

776

777 **Acknowledgment**

778 This research was supported by Ifremer REMIMA program, EU Seventh Program for
779 Research, Technological Development and Demonstration Activities, MIDAS grant 603418,
780 FGMA PhD grant E-223-85-2012-2 to IHA and Campus France grant 796045K to IHA. We also
781 thank the captain and crew of the oceanographic cruise BICOSE-2014 (DOI:
782 10.17600/14000100), as well as the pilots of the ROV Victor 6000. We also thank M. Segonzac
783 for his help on juvenile sorting during the BICOSE cruise.

784

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