

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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4 Iván Hernández-Ávila<sup>1,3\*</sup>, Marie-Anne Cambon-Bonavita<sup>2</sup>, Jozée Sarrazin<sup>1</sup>, Florence Pradillon<sup>1\*</sup>

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6 <sup>1</sup>Ifremer, REM/EEP, Laboratoire Environnement Profond, F 29280 Plouzané, France.

7 <sup>2</sup>Univ Brest, Ifremer, CNRS, Laboratoire de Microbiologie des Environnements Extrêmes, F  
8 29280 Plouzané, France.

9 <sup>3</sup>Permanent address: Facultad de Ciencias Naturales, Universidad Autónoma del Carmen,  
10 Ciudad del Carmen, Mexico

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12 \*Corresponding author: [ihernandez@pampano.unacar.mx](mailto:ihernandez@pampano.unacar.mx), [florence.pradillon@ifremer.fr](mailto:florence.pradillon@ifremer.fr)

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14 ORCID :

15 IHA : 0000-0002-6722-5226

16 MACB : 0000-0002-4076-0472

17 JS : 0000-0002-5435-8011

18 FP : 0000-0002-6473-6290

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21 **Abstract**

22 *Rimicaris exoculata* is a dominant species of deep Mid-Atlantic Ridge (MAR) vent fields and  
23 inhabits areas close to vent emissions at depths below 2000 m. Its high abundance and strong  
24 genetic connectivity along the MAR point at a remarkable ability to produce dispersing larval  
25 stages. However, the reproduction of this species long remained enigmatic because brooding  
26 females were rarely observed. Here, we describe the population structure and reproduction of *R.*  
27 *exoculata* at the Snake Pit and TAG vent fields (3600m depth) for the months of January-  
28 February. We observed major differences in population structure between habitats within a single  
29 vent field: females widely dominate the large swarms near active venting while inactive  
30 peripheries are inhabited by large males. Low temperature diffusion zones are mainly colonized  
31 by small juveniles of *R. chacei* instead of *R. exoculata*. Size structure of populations from dense  
32 active areas is polymodal at both fields, suggesting discontinuous recruitment. Male and female  
33 sizes did not vary across habitats and vent fields, with sexually mature female being slightly larger  
34 than males. In contrast to previous studies, hundreds of ovigerous females were observed at both  
35 vent fields, suggesting seasonal reproduction. Proportion of ovigerous females among sexually  
36 mature females were similar between vent fields (36.7 %). However, reproductive output was  
37 lower at TAG, where ovigerous females had smaller size-specific fecundity and egg size, and  
38 more aborted broods. Broods were colonized by the dirivultid copepod *Stygiopontius pectinatus*  
39 at both vent fields, apparently without deleterious effect on egg development. In the light of the  
40 observed variability in *R. exoculata* population structure, we propose a hypothetical scenario  
41 depicting its mating system and brooding behavior, and discuss more generally intraspecific  
42 interactions during its benthic life stages.

43 **Key words:** Life cycle, population structure, reproduction, *Rimicaris exoculata*, habitat  
44 variability, hydrothermal vents

## 45        **1. Introduction**

46            The fragmentary and ephemeral nature of deep-sea hydrothermal vent ecosystems is  
47 challenging for animal populations connectivity and resilience. Visually dominant species are  
48 usually endemic to these ecosystems, and large scale distribution and connectivity between  
49 populations were observed for some of them (Thaler et al. 2011, Teixeira et al. 2012, Beedessee  
50 et al. 2013). Advances in understanding mechanisms and processes related to reproduction,  
51 dispersal, recruitment and structure of vent populations are still hampered by the difficulties to  
52 upscale our observations temporally and/or spatially due to the cost and technical challenges of  
53 deep-sea studies. Remaining gaps in our understanding of vent species life histories may hide  
54 complex population structures that have not yet been acknowledged, even in some well-studied  
55 iconic species such as vent shrimps. Indeed, a recent study in another vent crustacean, the  
56 anomuran crab *Kiwa tyleri*, revealed complex life stages distribution affecting local population  
57 structures and reflecting life history of the species (Marsh et al. 2015).

58            The alvinocarid shrimp *Rimicaris exoculata* is a visually dominant species at hydrothermal  
59 vents of the Mid-Atlantic Ridge (MAR), especially in fields located at depths below 2000 m. This  
60 species lives close to the vent emission in dense aggregations of thousands of individuals per  
61 square meter (Desbruyères et al. 2000, Copley et al. 2007, Gebruk et al. 2010) and many aspects  
62 of its biology have been studied since the discovery of MAR hydrothermal vents in 1985 (Zbinden  
63 & Cambon-Bonavita 2020). The relationship of this shrimp with the dense and diverse symbiotic  
64 microbial communities hosted in its cephalothoracic cavity and gut have been extensively studied,  
65 demonstrating a clear trophic dependency of the shrimp on its symbionts, and pointing at tight  
66 regulation along its life cycle (Corbari et al. 2008, Ponsard et al. 2013, Jan et al. 2014, Le Bloa et  
67 al. 2020). However, the information available about reproduction and population biology is still  
68 scattered and sometimes contradictory. In terms of population structure, major differences in  
69 shrimp densities have been found at the Broken Spur vent field depending on the levels of

70 hydrothermal activity, including the decrease of adult densities in vent areas not directly exposed  
71 to vent emission (Copley et al. 1997). Habitats at the base of vent edifices were suggested to  
72 serve as nurseries for juvenile recruitment due to their high densities in these areas (Komai and  
73 Segonzac 2008). However, it remained unclear whether variations in adult density between  
74 habitats also involved variations in population structures. Samples collected at various MAR vent  
75 fields by Shank et al. (1998) showed a female-biased sex ratio, but the association of sex ratio  
76 with a particular habitat or temporal variation was not tested by additional sampling. In addition,  
77 patches of juveniles have been reported in adult aggregations (Shank et al. 1998, Copley et al.  
78 2007) but their distribution and proportions in the populations remain undetermined. Other  
79 aspects of population structure, such as size structure, are based on single sample, pooled  
80 samples or preliminary analyses (Gebruk et al. 1997, Vereshchaka 1997).

81         The reproduction of *R. exoculata* also has many intriguing gaps. Although oocyte size  
82 frequency suggests a lack of seasonality in the reproduction (Ramirez-Llodra et al. 2000, Copley  
83 et al. 2007), very few brooding specimens have been collected since the species description  
84 (Williams & Rona 1986, Ramirez-Llodra et al. 2000, Gebruk 2010, Guri et al. 2012). The lack of  
85 brooding females also contrasts with the large densities and strong genetic connectivity observed  
86 in MAR vent fields (Teixeira et al. 2012), which must be supported by a large larval pool. Egg size  
87 has been estimated in only two studies (Williams & Rona 1986, Ramirez-Llodra et al. 2000) and  
88 this size estimation was used to infer that these shrimp should have planktotrophic larvae with  
89 long planktonic duration. However, recent morphological evidence on hatching larval stages  
90 lacking functional mouth structures led to the revision of the former hypothesis and proposition  
91 that these larvae are lecithotrophic early in their dispersive planktonic phase (Hernandez-Avila et  
92 al. 2015). In addition, the realized fecundity (988 eggs per brood) was estimated on only one  
93 specimen due the lack of samples (Ramirez-Llodra et al. 2000).

94           Very few data concerning the distribution of *R. exoculata* populations at different spatial  
95 scales or their interactions in vent ecosystems are available in the literature. At vents, several  
96 studies have shown that variations of physical and chemical conditions of vent emission shape  
97 the structure of communities dominated by bathymodiolin mussels or siboglinid tube worms at  
98 various spatial and temporal scales (Sarrazin et al. 1997, 2015, Cuvelier et al. 2009, 2014).  
99 Variations in community structure are observed between edifices at the field-scale (Desbruyères  
100 et al. 2000, 2001, Sarrazin et al. 2020) as well as between habitats within the same edifice  
101 (Cuvelier et al. 2009, Sarrazin et al. 2015) highlighting the high level of complexity of these  
102 ecosystems.

103           In addition to variations at community level, vent fauna also show variations in their  
104 population structures. These variations are linked to differences in habitats occupied by the  
105 different life stages for motile organisms (Shank et al. 1998, Marsh et al. 2015), changes in  
106 population structure between vents (Nye et al. 2013) or habitats (Copley & Young 2006, Marsh et  
107 al. 2015) and various scales of temporal variations (Copley et al. 1997, 1999, 2007, Gebruk et al.  
108 2010, Cuvelier et al. 2011). Although less studied, there is evidence that populations of dominant  
109 species exhibit spatial and temporal variations at small scales. For instance, Copley et al. (1999)  
110 proposed that short-term changes in *R. exoculata* population density close to vent emission could  
111 be associated with tidal variation, as observed in other mobile vent species (Lelièvre et al. 2017).  
112 Variations in population structure most likely reflect the complex life cycles of vent species with  
113 different reproductive strategies, variations in physiological tolerance and resource use by the  
114 different developmental stages, and their interactions with environmental variations.

115           As one of the dominant species at MAR vent fields below 2000 m depth, *R. exoculata*  
116 plays a major role at ecosystem level, and its population biology could have important implications  
117 for the resilience, structure and biomass of these vent communities (Desbruyères et al. 2000,  
118 2001). In January-February 2014, large numbers of ovigerous females were observed at two vent

119 fields of the MAR, TAG and Snake Pit, during the BICOSE cruise. In addition, striking variations  
120 in the densities of adult and juvenile shrimps locally led us to hypothesize local variations in  
121 population structure. In this study, we tested such hypothesis and analyzed both population  
122 structure and reproductive features of ovigerous *R. exoculata* females in a series of samples  
123 collected in visually distinct shrimp assemblages. We untangled different levels of spatial variation  
124 in reproductive parameters and population structure and used this information to propose a  
125 scenario depicting interactions of shrimps with their conspecifics and their environment that would  
126 explain the observed distribution patterns, and provide clues on some aspects of their  
127 reproductive behavior and life cycle.

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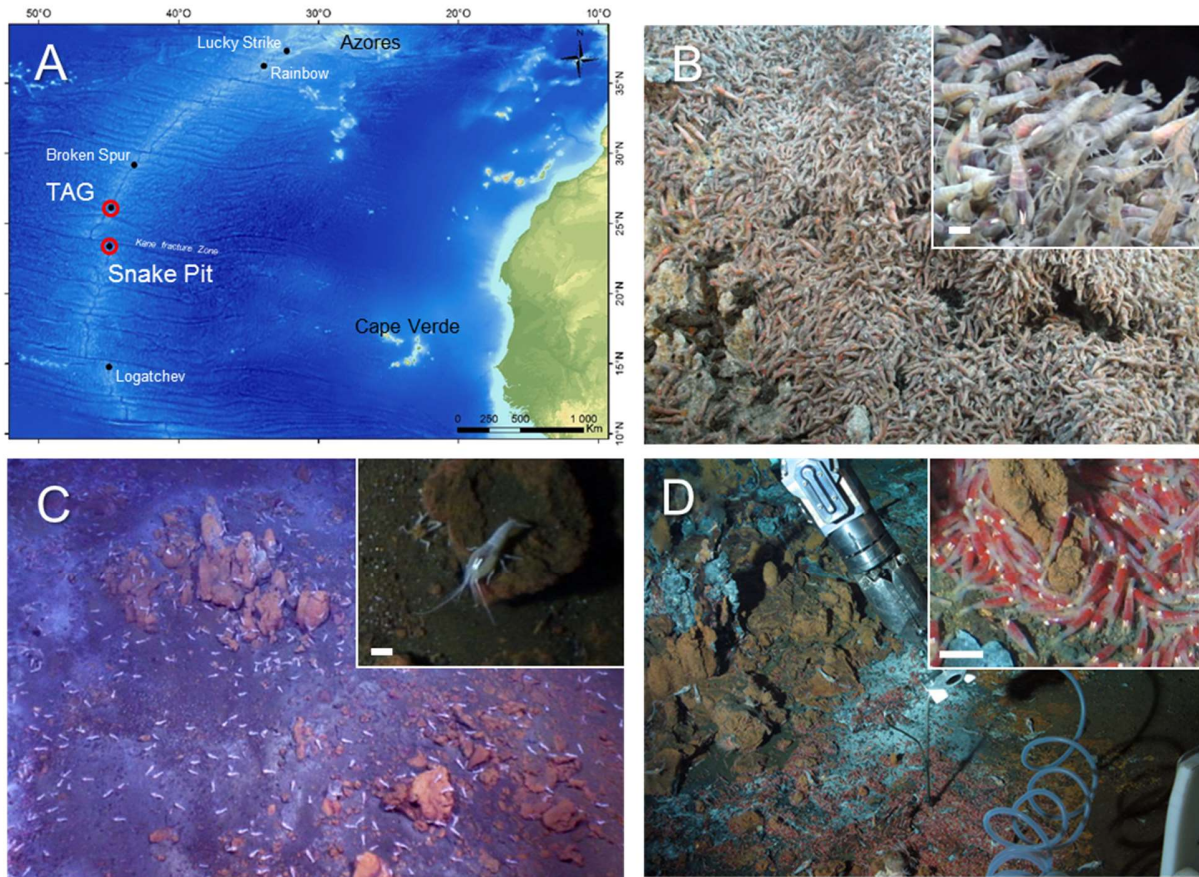
## 129 **2. Material and Methods**

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### 131 **2.1. Sampling**

132 *Rimicaris exoculata* were collected at the Snake Pit (SP, 23°22.1'N 44°57.1'W, 3470 m  
133 depth) and TAG (26°08.2'N, 44°49.5'W, 3620 m depth) vent fields on the MAR (Fig. 1a) during  
134 the BICOSE cruise (DOI: 10.17600/14000100) from January 10<sup>th</sup> to February 11<sup>th</sup>, 2014. The two  
135 vent fields are approximately 310 km away, and separated by the Kane Fracture Zone. At Snake  
136 Pit, six samples were collected in dense shrimp aggregations on the walls of active chimneys of  
137 the Beehive site, close to fluid emissions (therein Active Emission Habitat/AEH) (Table 1, Suppl.  
138 Fig. 1). At TAG, three samples were collected in the AEH of Active Mound (Fig. 1b, Suppl. Fig. 1)  
139 and two samples were collected in dense aggregations of small alvinocaridid juveniles settled in  
140 flat areas of diffuse flow (Diffuse Emission Habitat/DEH) herein termed “nurseries” (Fig. 1d, Suppl.  
141 Fig. 2). Additionally, three other samples were collected at the base of the TAG mound, where no  
142 active emission was visible (Inactive Emission Habitat/IEH) and with adult shrimps scattered over

143 large areas (Fig. 1c, Suppl. Fig. 2). These different types of habitats occurred within meters or  
144 tens of meters from each other.



145  
146 Figure 1. Sampled vent fields and habitats. A) North Atlantic regional map with TAG and Snake  
147 Pit vent fields on the Mid Atlantic Ridge (Amante, C. and B.W. Eakins, 2009.  
148 doi:10.7289/V5C8276M). B) *R. exoculata* swarms in the active emission habitat (AEH). C)  
149 Inactive emission habitat (IEH) with scattered *R. exoculata* adults (white spots). D) Diffuse  
150 emission habitat (DEH), showing red aggregation of small juveniles. B-D : Pictures from the TAG  
151 vent field, Ifemer/ROV Victor 6000/BICOSE2014. Scale bars in close-up views in insets: 1 cm.

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153

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155 Table 1. Shrimp sample details, including temperature ranges measured within centimeters from  
 156 the faunal sampling point when available. ND: no data available.

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

157

158 Shrimps were collected with the suction sampler of the Remotely Operated Vehicle (ROV)  
 159 Victor6000. In AEH and DEH, the tip of the sampler was pointed as close as possible to the  
 160 individuals and maintained immobile during sampling to avoid as much as possible disturbing the  
 161 aggregation. The suction was activated for a few seconds in order to collect individuals from a  
 162 restricted area. Due to the low shrimp density in IEH, samples were obtained from larger areas  
 163 covering a few m<sup>2</sup>. In addition, 37 specimens caught with a shrimp-trap deployed in IEH were  
 164 included in the analyses. In total 3 445 specimens of *R. exoculata* were examined (Table 1).

165 Temperature measurements were conducted along with shrimp collections. Records were  
 166 obtained either from discrete measurements with the submersible temperature probe prior to  
 167 sampling, or from time-series measurements with autonomous temperature probes (WHOI-MISO  
 168 low temp-ONSET®) deployed within the shrimp aggregation for a few days prior to sampling.



169

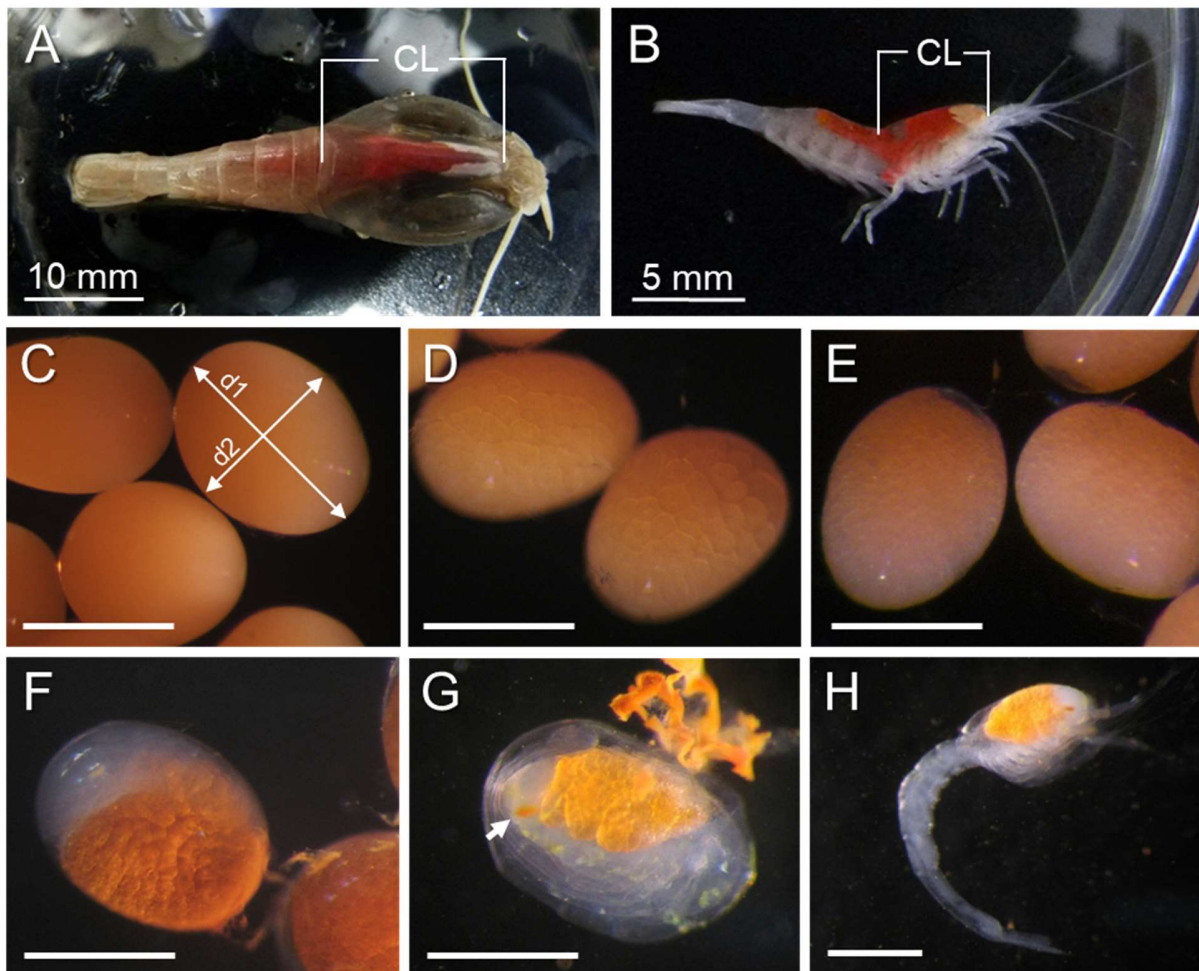
## 170 **2.2. Identification and measurements**

171 Specimens were initially identified and classified as juveniles, subadults or adults, in  
172 accordance with Komai & Segonzac (2008). The identification of the smaller juveniles from DEH  
173 was further assessed by DNA analyses because their morphology did not fit completely the  
174 description of early juvenile stages of *R. exoculata* available at the time of this study (Komai &  
175 Segonzac, 2008). Thirty-six juveniles from each DEH sample and 5 juveniles from AEH samples  
176 were used for molecular identifications (Table 1 & Suppl. Table 1).

177 Sex was identified in adults by the occurrence of the “appendix masculina” on the second  
178 pleopod in males, and the shape of the endopod of the first pleopod (Komai & Segonzac, 2008).  
179 Since these sexual characters appear during the transition from subadult to adult stage, sex of  
180 juvenile and subadult specimens could not be determined. Brooding females are characterized  
181 by the presence of embryos held between their pleopods under the abdomen, and by  
182 modifications of their pleopods (addition of setae to maintain the brood). Hatched females  
183 (females just after larval release but prior to molt) were identified by their modified pleopods.  
184 Brooding and hatched females are referred to as ovigerous females (following Nye et al. 2013).  
185 Young small adults and subadults resemble females (*i.e.* lack of appendix masculina) and also in  
186 some cases lack gonadal tissue that could be used for sex determination. In order to estimate the  
187 minimal size for confident determination of sex, a subsample of adult and subadult shrimps in  
188 small size classes (7 to 15 mm carapace lengths) were dissected to verify macroscopic evidence  
189 of gonadal tissue and estimate the onset size of sexual differentiation (OSD, see section 2.4,  
190 equivalent to the “minimum sexable size” of Anger & Moreira, 1998).

191 Carapace length (CL) was measured from the posterior margin of the ocular shield to the  
192 mid-posterior margin of the carapace in adults and subadults (Fig. 2a), with a precision of 0.1 mm.

193 In juvenile stages, CL was measured from the anterior tip of the rostrum to the posterior margin  
194 of the carapace (Fig. 2b). Morphological changes between juvenile and adult stages (rostrum  
195 reduction and development of the ocular shield) may introduce a bias in our measurements but  
196 this was small compared to the total length of the carapace, and had little impact on size frequency  
197 distributions and size comparisons.



198

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

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

219           During examination of female broods, we found dirivultid copepods between the embryos  
220   or attached at the base of the pleopods. They were identified to the species level through  
221   barcoding (Suppl. Table 1).

222

### 223           **2.3. Genetic identifications**

224           DNA was extracted from shrimp juveniles and copepods using the CTAB method (Doyle  
225   1990) on muscle tissue or on whole specimens for copepods. A section of the cytochrome oxidase  
226   I gene (COI) was amplified in a 50  $\mu$ L solution of 1X reaction buffer, 2 mM MgCl<sub>2</sub>, 0.25 mM dNTP,  
227   1.2 units of Taq polymerase and 0.6 mM of each primer (LCOI1490 and HCOI2198, Folmer et al.  
228   1994). Amplifications were performed as following: initial denaturation (5 min at 95°C), 40 cycles

229 including denaturation (1 min at 94°C), annealing (1 min at 52 °C) and extension (2 min at 72 °C),  
230 followed by a final extension of 7 min at 72°C. All PCR amplifications were conducted on a  
231 GeneAmp PCR system 9700 (Applied Biosystems). PCR products were purified and sequenced  
232 by Macrogen, Inc. (Netherlands) using the amplification primers. We aligned our sequences using  
233 MUSCLE (Edgar 2004), along with a set of alvinocaridid and other shrimps sequences. For our  
234 dirivultid copepod sequence, a subset of the sequences obtained by Gollner et al. (2011) was  
235 used for comparison. Neighbor-joining trees were constructed using Geneious R8 software  
236 (Kearse et al. 2012) using a HKY evolutionary model of nucleotide substitution. Robustness of  
237 the inferred trees was evaluated using bootstrap method with 1000 replicates.

238

#### 239 **2.4. Data analysis**

240 Onset of sexual differentiation (OSD) was estimated using a similar procedure as what  
241 was used for determination of the size of sexual maturity in Crustacea (Wenner et al. 1974).  
242 Proportions of specimens with gonad tissue were estimated for size classes between 7-15 mm  
243 CL (larger specimens with clear sex differentiation were not included). The proportion of  
244 specimens with gonad differentiation were plotted against size classes and fitted to the logistic  
245 equation:

$$246 \quad P_{sd} = \frac{1}{1+e^{(a-b*CL)}}$$

247 where  $P_{sd}$  is the proportion of shrimps with gonad differentiation, CL is the carapace  
248 length, and  $a$  and  $b$  are constants. The size at which 50 % of the individuals exhibit sexual  
249 differentiation denoted by gonad development was considered as an estimation of OSD (Suppl.  
250 Fig. 3). Subadults were subsequently identified as individuals resembling adults with sizes < OSD.

251 Similarly, the size of effective sexual maturity (ESM) for females was estimated from the  
252 proportion of ovigerous females per size class, corrected by the maximum proportion of ovigerous  
253 females (King 2007). We applied the previous logistic equation, substituting  $Psd$  by the corrected  
254 proportion of ovigerous females ( $Povf$ ) on the equation. The ESM was estimated by the size at  
255 which  $Povf$  is 50% (Suppl. Fig. 4).

256 For each sample, juvenile ratio, subadult ratio and sex ratio (F:M) were estimated.  
257 Deviation from a sex ratio of 1:1 was tested with a  $\chi^2$  test, using Yates correction in samples with  
258 few specimens ( $n < 30$ ). In order to determine the variations in sex ratio within each habitat at  
259 each vent field, we performed a heterogeneity  $\chi^2$  test (Zar 2010). Variations in the proportions of  
260 females with size  $>$  ESM and the proportions of ovigerous females between vents were tested  
261 using  $\chi^2$  test.

262 Size class structures were analyzed for each sample, estimating kurtosis and skewness  
263 for size class aggregation and deviation from the mean, respectively (Zar 2010). Although  
264 histograms were elaborated denoting juveniles, subadults, males and females, size structure  
265 comparisons were performed including all specimens. Normality tests were performed for each  
266 sample using the Shapiro-Wilk test (Zar 2010). Discrete size cohorts in the samples were  
267 identified using the statistical package `mixdist` (MacDonald 2003) running also in R<sup>TM</sup>. The  
268 goodness of fit of the identified size cohorts was verified using  $\chi^2$  test. The analyses were  
269 performed for each sample collected in the AEH and compared to verify the consistency of  
270 identified cohorts. Identification of cohorts in other habitats were not performed due to insufficient  
271 sample size.

272 For AEH samples, differences in body sizes associated with sex and vent fields were  
273 tested using multifactorial analysis of variance (ANOVA), after  $\log_{10}$  transformation. For this  
274 analysis, samples were nested at the factor vent field, representing the variation in body size

275 between samples collected within the same vent field. Similarly, an ANOVA test was performed  
276 in order to examine differences in male body sizes between habitats (AEH vs DEH) at the TAG  
277 vent field. In this case, samples were nested at the factor habitat which represents the small-scale  
278 variation. Data normality and homoscedasticity were tested using  $\chi^2$  for frequency distribution and  
279 Levene test respectively (Underwood 1997, McGuinness 2002).

280 In crustaceans, brood size increases proportionally with female size (Oh & Hartnoll 2004).  
281 In order to compare reproductive outputs at the two vent fields, we estimated the relation between  
282 realized fecundity (number of embryos per brood) and female size (CL) using regression  
283 analyses, after  $\log_e$  transformation. The difference in size-specific fecundity between vent fields  
284 was tested using a t-test analysis. Variations in embryo size associated with parental female,  
285 embryo stage and vent fields were analyzed with a multifactorial ANOVA test. For this analysis  
286 the factor parental female was considered as nested to the combination of vent field and embryo  
287 stage.

288

### 289 **3. Results**

290

#### 291 **3.1. *Rimicaris* shrimp populations at TAG and Snake Pit in January 2014**

292 Overall, 3445 individuals from 14 samples collected at both vent fields were examined.  
293 Among these, we determined developmental stage and sex for 3388 individuals and measured  
294 3379 individuals (missing data are due to body damages preventing accurate measurements or  
295 sex/stage determination). The global dataset includes 1925 females (56.8%), 292 males (8.6%),  
296 882 subadults (26.1%) and 289 juveniles (8.5%). Global sex-ratio clearly deviates from 1:1 ( $\chi^2$ ,  
297  $df=1$ ,  $p < 0.01$ ). Of the 1925 females, 136 (7.1%) are either brooding eggs (125), or have recently  
298 hatched larvae (11).

299           Smaller specimens are early juveniles (CL = 4.4 mm), whereas the largest one is a female  
300 with 24.4 mm CL (but being an outlier specimen as other largest females reach ~20.6 mm CL).  
301 Size-range of the juveniles varies between 4.4 and 10.3 mm, and overlaps subadult size range  
302 (7-9.9 mm CL) (Suppl. Table 2). Subadult maximal size was determined by our estimation of the  
303 onset size of sexual differentiation (OSD = 10 mm, Suppl. Fig. 3). The OSD value is also  
304 consistent with the size of the smaller adult male found in our samples (CL = 9.9 mm). Although  
305 the size of some juveniles exceeds the OSD size, they are morphologically distinct from  
306 subadults: their rostrum is not completely reduced, and their carapace not fully inflated.

307           Overall, size ranges of males and females are similar with respective CL ranges = 9.9-  
308 19.1 mm and 10 -24.4 mm (Suppl. Table 2). However, the average size of males was higher than  
309 the average size of females, with 15.1 mm CL and 12.5 mm CL respectively (t-test= 20.71,  $p <$   
310 0.001). Most ovigerous females exhibit large sizes, with CL ranging from 12 mm to 20.6 mm  
311 (average size: 16.5 mm CL). We estimate the size at effective sexual maturity (ESM) at 15.1 mm  
312 CL for females (Suppl. Fig. 4). Hereafter, females with size  $\geq$  15.1 mm CL are called sexually  
313 mature females.

314

## 315           **3.2. Variation in population structure across habitats and vent fields**

316

### 317           **3.2.1. Different types of habitats**

318           Visually, striking differences characterized shrimp assemblages when we collected our 14  
319 samples. Most samples (9) were collected among dense aggregations of actively swimming  
320 shrimps gathering around vigorous fluid emissions (Fig. 1-B, Suppl. Fig. 1). In these aggregations,  
321 we recorded steep temporal variations in temperature with maximum varying from 14 to 33°C  
322 (Table 1). Resting shrimps scattered on inactive sulfide substratum at the periphery of dense

323 aggregations were collected 3 times at TAG (Fig. 1-C, Suppl. Figs. 2 A-C). In this habitat, no fluid  
324 exit was visible and temperature was low and stable, with a maximum of 2.8°C (Table 1), whereas  
325 ambient seawater temperature was 2.6°C. At TAG, aggregations of very small individuals  
326 characterized by their bright red color were sampled around diffusions of translucent fluids exiting  
327 from very small chimneys or cracks (Fig. 1-D, Suppl. Figs. 2 E, F). Temperatures among those  
328 young individuals varied from 2.8°C to 5.3°C (Table 1). We thus defined 3 types of aggregations  
329 characterized by different temperature regimes, with visually distinct shrimp assemblages in terms  
330 of density, size and behavior. These are called Active Emission Habitat (AEH), Inactive Emission  
331 Habitat (IEH) and Diffuse Emission Habitat (DEH).

### 332 3.2.2. *Variations in population structure between habitats (TAG)*

333 At TAG, we observe striking differences in terms of population structure, size-frequency  
334 distribution, and reproductive features among the three habitats. In AEH, with 71% of females  
335 and 8,5% of males, sex-ratio is clearly biased for females (8.4:1). Although sex ratios are  
336 significantly different among AEH samples from TAG ( $\chi^2_{het}= 50.05$ ,  $df= 2$ ,  $p< 0.001$ , Table 2), all  
337 of them are significantly female biased ( $\chi^2$ ,  $df= 1$ ,  $p< 0.02$  in all cases, Table 2).

338 In contrast, in IEH, 90.3% of the individuals are males, while females represent only 6.5%  
339 of the shrimps collected. Sex ratio is significantly biased for males (1:14) and in each TAG IEH  
340 samples ( $\chi^2$ ,  $df= 1$ ,  $p\leq 0.002$  in all cases, Table 2).

341 Subadults represent 17.5% of the individuals on average in AEH (Table 2), with  
342 significant variation between samples (from 3% to 23.2% of the individuals in each sample,  $\chi^2_{het}=$   
343 16.4,  $df= 2$ ,  $p< 0.001$ ), but they are rare in IEH with only two individuals collected overall.

344



345 Table 2. *R. exoculata* specimens and sex ratios at different vent fields and habitats. J: juveniles,  
 346 <OSD (onset of sexual differentiation): subadults, F: females (non-brooding); OF: ovigerous  
 347 females; M: males.

Active Emission Habitat (AEH)										
Vent field	Sample	J	<OSD	F	OF	M	n	F : M	$\chi^2$	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
Inactive Emission Habitat (IEH)										
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
Diffuse Emission Habitat (DEH)										
TAG	S13	77	0	0	0	0	77			
TAG	S14	101	0	0	0	0	101			

348

349 Ovigerous females are almost exclusively observed in AEH at TAG (only one hatched  
 350 female observed in IEH and none in DEH), representing 11.7 % of the females on average, with  
 351 strong variations between samples (from 8.4% to 25.6%). These variations reflect both variations  
 352 in the proportion of sexually mature females among all females (22.4% on average, with variations  
 353 between samples: 13.3% to 64.9%), and, to a lesser extent, variations in the proportion of

354 ovigerous females among sexually mature females (36.8 % on average, with variations between  
355 samples: 29.8% to 43.6%).

356 Juveniles are not abundant in AEH (less than 3% of the total population, Table 2, no  
357 significant heterogeneity between samples:  $\chi^2_{het}= 3.64$ ,  $df= 2$ ,  $p= 0.602$ ), and are rare in IEH with  
358 only one early stage juvenile collected. In contrast, DEH samples are exclusively composed of  
359 early stage juveniles. Although they were not collected, few large adult individuals (both *R.*  
360 *exoculata* and *R. chacei*) were observed crawling around these nurseries (*in situ* observations  
361 and Fig. 1-D).

### 362 3.2.3. Variation in population structure between vent fields (AEH)

363 AEH Snake Pit samples exhibit similar population structure to those from TAG, with a  
364 strong dominance of females. Females, males, subadults and juveniles represent respectively  
365 57.2%, 5.9%, 33 % and 3.9% of the overall population. Sex-ratio is female-biased (9.8:1 overall)  
366 and similar to the ratio observed in AEH at TAG. Like in TAG AEH samples, sex-ratio vary  
367 significantly among AEH samples at Snake Pit ( $\chi^2_{het}= 20.06$ ,  $df= 5$ ,  $p= 0.001$ , Table 2), but all  
368 are significantly female-biased ( $\chi^2$ ,  $df= 1$ ,  $p\leq 0.01$  in all cases).

369 Overall, subadults are more abundant in Snake Pit samples than in TAG samples,  
370 representing almost a third of the population. However, their proportion varies strongly between  
371 samples (from 5.8% to 45.9% of the individuals,  $\chi^2_{het}= 164.9$ ,  $df= 5$ ,  $p< 0.001$ ). Like in TAG AEH,  
372 the proportion of juveniles in Snake Pit samples is generally low, except in one sample where  
373 they reach 24.1% of the individuals, resulting in significant heterogeneity between Snake Pit  
374 samples ( $\chi^2_{het}= 89.32$ ,  $df= 5$ ,  $p< 0.001$ ).

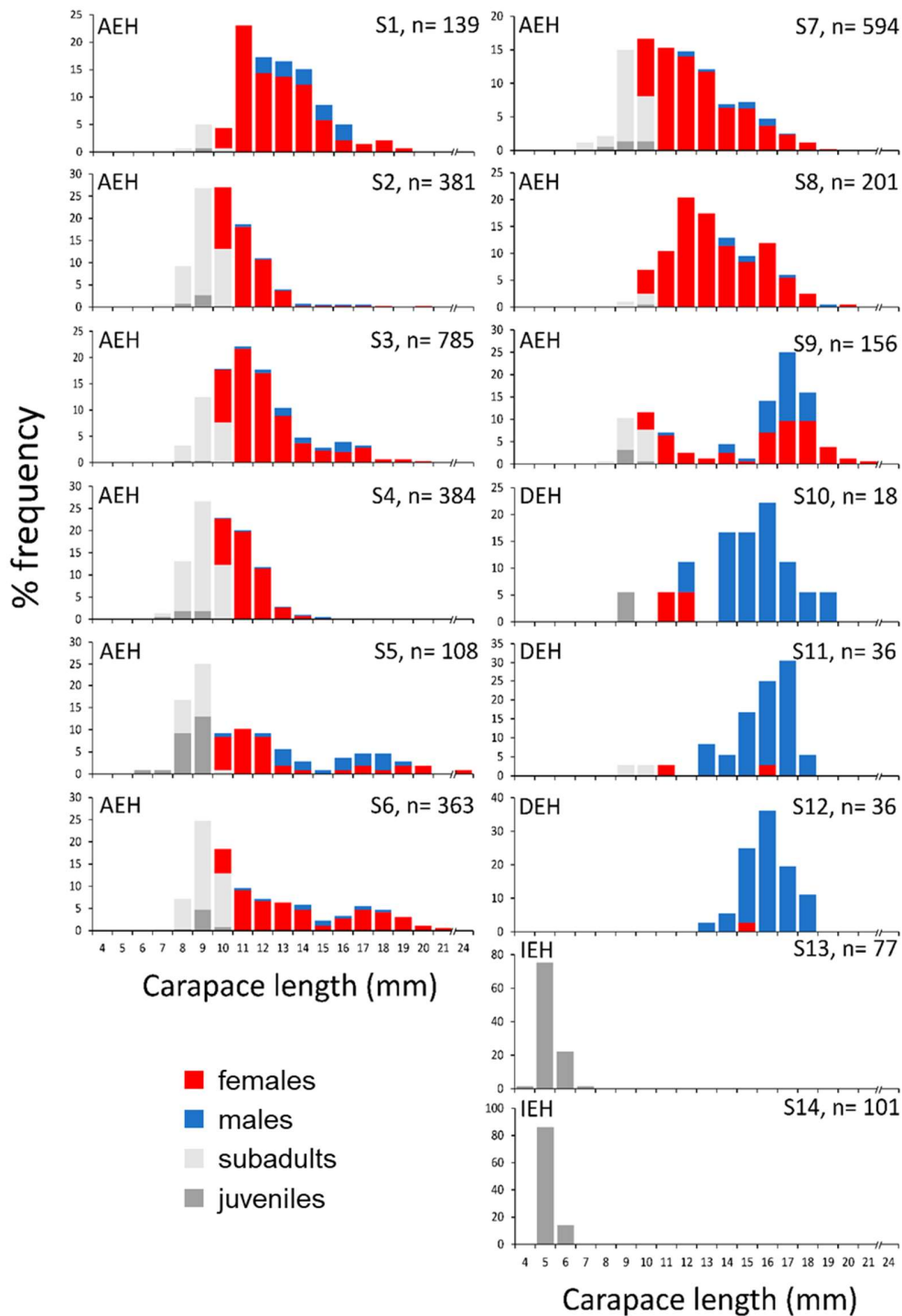
375 Although the proportion of ovigerous females among sexually mature females is similar  
376 between vent fields (36.6 % on average at Snake Pit,  $\chi^2= 0.003$ ,  $p= 0.956$ ), the proportion of

377 sexually mature females among all females is significantly lower in Snake Pit (11.7 %) than in  
378 TAG (22.4 %) AEH samples ( $\chi^2= 34.24$ ,  $p < 0.001$ ), resulting in lower proportion of ovigerous  
379 females overall (4.4% of all females).

#### 380 *3.2.4. Variations in size frequency distributions among habitats and vent fields*

381 Overall, reflecting the differences in sex and stage distributions between habitats, size  
382 distributions also differed between habitats. While DEH host very small shrimps almost not  
383 represented in other habitats, mostly large individuals inhabit IEH. In AEH, shrimp sizes vary over  
384 a wide range, overlapping slightly both size ranges of shrimps in DEH and IEH (Fig. 3, Suppl. Fig.  
385 5).

386 Size frequency distributions vary among samples and habitats in both vent fields, both  
387 in terms of kurtosis and skewness (Fig. 3). General trends in AEH size frequency distributions  
388 include bias towards small sizes (skewness= 0.945) and slightly leptokurtic distribution (kurtosis=  
389 0.423). In some samples, the distribution is clearly non-unimodal. In others, deviation in skewness  
390 and kurtosis suggests a mixture of cohorts. Based on a size cohort analysis, 5 different cohorts  
391 are identified overall in AEH (Table 3). In individual samples, cohort number vary between 2 to 4  
392 (Table 3, Suppl. Fig. 6), with some correspondence observed between samples across the two  
393 vent fields. Overall in AEH, we identify one cohort of juveniles and subadults, one cohort of  
394 subadults and small adults (<12 mm CL) and three cohorts of adults, two of which include females  
395  $\geq$  ESM. In both vent fields, the cohorts corresponding to juveniles, subadults and small adults  
396 represent an important proportion of the population of AEH.



397

398 Figure 3. Size class structure of *R. exoculata* in different habitats of the Snake Pit (left side) and  
 399 TAG (right side) vent fields. AEH active emission habitat, DEH diffuse emission habitat, IEH  
 400 inactive emission habitat.

401 Table 3. Identified cohorts in AEH at the Snake Pit and TAG vent fields. Mean and standard  
 402 deviations are shown for each sample, proportion of each cohort are in brackets.  $\chi^2$  denote the  
 403 deviation of the sample from the cohort estimation. ns: non-significant.

Cohort:	1	2	3	4	5	$\chi^2$
<b>Snake Pit</b>						
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
<b>TAG</b>						
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

404 \*Despite the deviation of the size structure from the cohort model ( $p=0.0485$ ), it was the closest  
 405 model to the input data

406

407 Male and female body sizes in AEH are significantly different among samples, indicating  
 408 small scale variations in sizes, within habitats at each vent field (ANOVA,  $p < 0.001$ , Suppl. Table  
 409 3). Body sizes also vary significantly with sex (ANOVA,  $p < 0.001$ , Suppl. Table 3), males being  
 410 larger than females at each vent field (Suppl. Table 2). Size distribution of ovigerous females  
 411 clearly departed from the rest of the females, with sizes similar to the male average size in TAG  
 412 and even larger than the male average size in Snake Pit. Although males and females tend to be  
 413 smaller in Snake Pit than in TAG AEH, size differences are not significant between the two vent  
 414 fields for each sex (ANOVA,  $p=0.083$ , Suppl. Table 3). In contrast, ovigerous females are slightly

415 larger in Snake Pit than in TAG AEH, but this variation between the two vent fields was not  
416 statistically significant (ANOVA  $F = 1.649$ ,  $p = 0.246$ ,  $df_2 = 6$ ).

417 Size frequency distributions of IEH samples are leptokurtic (kurtosis 0.12 to 1.99) and  
418 biased towards larger sizes (skewness -0.45 to -1.52). Males do not exhibit different body sizes  
419 between AEH and IEH, although significant differences are detected between samples of a given  
420 habitat (ANOVA,  $p < 0.001$ , Suppl. Table 4). Females also exhibit similar size ranges between  
421 both habitats, although the low number of females collected in IEH prevents statistical  
422 comparisons.

#### 423 3.2.5. Juvenile distribution in AEH and DEH: detection of *Rimicaris chacei* nurseries

424 Juvenile sizes at the AEH are similar between vent fields, only showing differences  
425 between samples (ANOVA  $F_{vents} = 5.2001$ ,  $p = 0.057$ ,  $df_2 = 7$ ,  $F_{samples} = 2.643$ ,  $p = 0.015$ ,  $df_1 = 7$ ,  $df_2 =$   
426 101). However, juveniles from DEH are much smaller (ANOVA  $F_{vents} = 804.91$ ,  $p < 0.001$ ,  $df_2 = 3$ ,  
427  $F_{samples} = 1.580$ ,  $p = 0.195$ ,  $df_1 = 3$ ,  $df_2 = 199$ ) and form a distinct cohort from that of AEH. Based on  
428 morphological features described in Komai & Segonzac (2008), we suspected that juveniles from  
429 DEH were possibly a mixture of *R. exoculata* and *R. chacei*. Molecular identification using COI  
430 barcode reveals that all sequences of these juveniles are consistent with *R. chacei* affiliation,  
431 whereas juveniles from AEH (with clear *R. exoculata* juvenile morphology according to Komai &  
432 Segonzac 2008) are consistent with *R. exoculata* affiliation (Suppl. Fig. 7).

433

### 434 3.3. Reproductive features

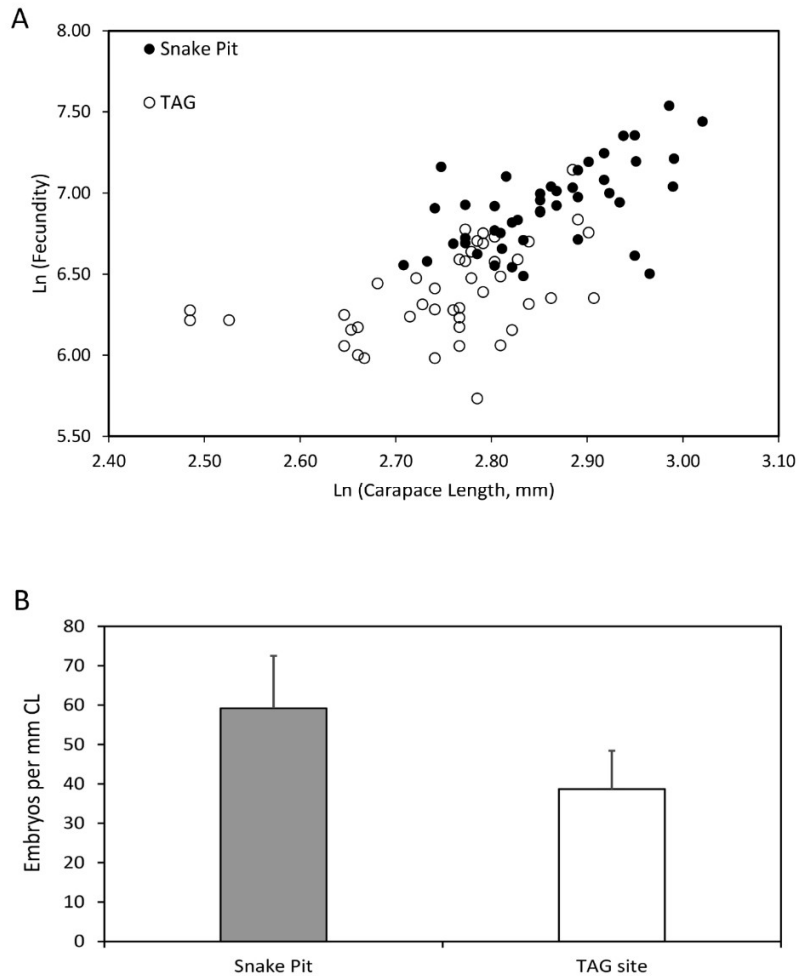
435

#### 436 3.3.1. Fecundity

437           Of the 125 brooding females found in our samples, 36 had obviously lost part of their  
438 broods, not because of hatching -since embryos were clearly not yet fully developed- but rather  
439 due to either abortion or more probably lost during sampling. These are not included in our  
440 fecundity analyses.

441           Fecundity varies from 304 eggs in a female from TAG with 16.2 mm CL, to 1879 eggs in  
442 a female from Snake Pit with 19.8 mm CL. The largest brooding females are observed at Snake  
443 Pit (with 1704 eggs and 20.5 mm CL for the largest), while the smallest brooding females are from  
444 TAG (with 500 and 532 eggs for the two smallest -12 mm CL- individuals). The average fecundity  
445 is overall of 833 eggs, and is higher among Snake Pit brooding females (1045 eggs, with an  
446 average CL of 17.4 mm) than among TAG ones (616 eggs, with an average CL of 15.9 mm). As  
447 expected, a positive correlation is observed between carapace length of the females and fecundity  
448 (Pearson correlation,  $R= 0.682$ ,  $t\text{-test}= 8.71$ ,  $p< 0.001$ ) (Fig. 4A). We consider that more data are  
449 necessary to estimate accurate linear regression models of fecundity and compare differences  
450 between populations or with other alvinocaridid shrimps.

451           Overall size-specific fecundity ranges from 19 to 95 embryos.mm<sup>-1</sup> CL. The size-specific  
452 fecundity of females does not change with the developmental stage of the brood (Early vs Mid  
453 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 is  
454 significantly lower in females from TAG ( $39 \pm 10$  embryos.mm<sup>-1</sup> CL) than in females from Snake  
455 Pit ( $59 \pm 13$  embryos.mm<sup>-1</sup> CL) ( $t\text{-test}=8.16$ ,  $p<0.001$ ,  $df=87$ ) (Fig. 4B). Finally, more females with  
456 damaged broods are observed at TAG, although we could not identify the cause of loss (abortion  
457 or sampling).



458

459 Figure 4. Fecundity in *R. exoculata* from TAG and Snake Pit vent fields. A. Number of embryos  
460 related with female size (carapace length). B. Size-specific fecundity, number of embryos per unit  
461 of size (mm of carapace length).

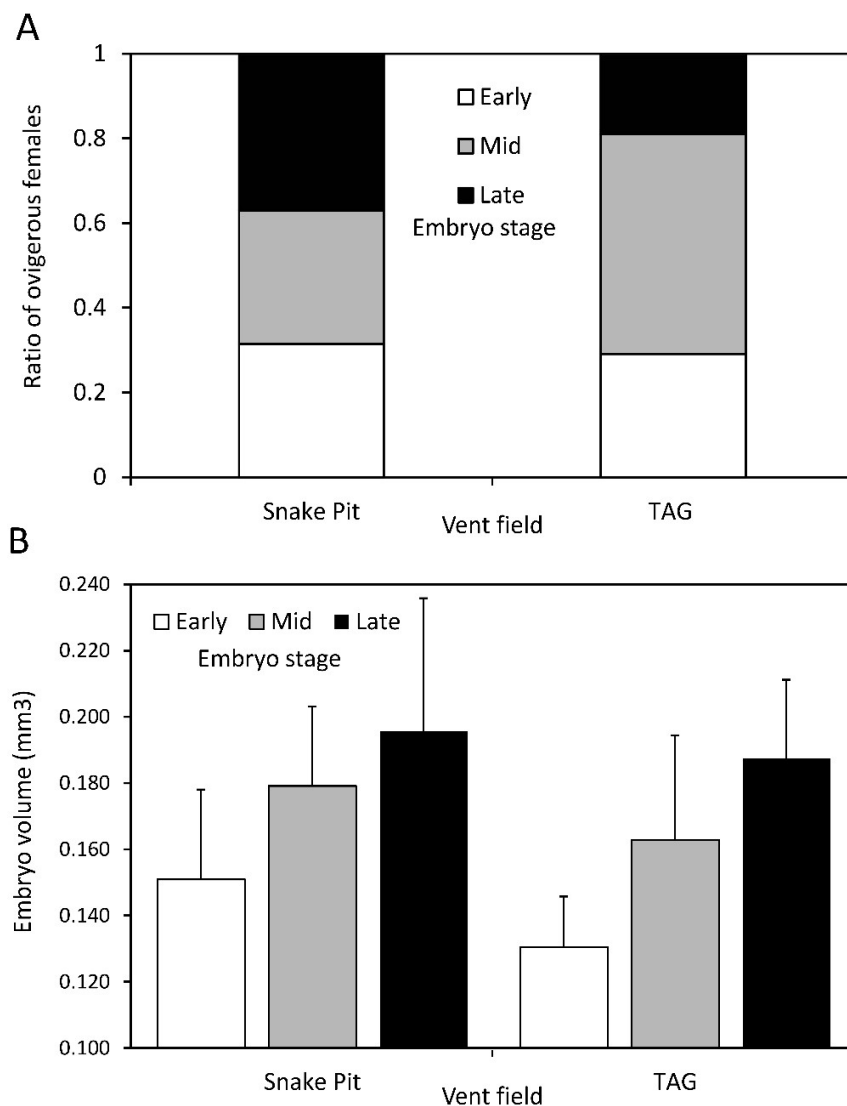
462

### 463 3.3.2. Reproductive synchrony

464 Within each individual brood examined, all eggs are at the same developmental stage  
465 (early, mid or late), except for occasional dead embryos or non-fertilized eggs. However, embryos  
466 at all developmental stages are observed in females from both vent fields, showing a lack of  
467 synchrony between the broods of different females. Overall, the distribution of brood stages are  
468 different between vent fields ( $\chi^2= 7.097$   $p=0.014$ ), with some variability between samples of a



469 given vent field (Suppl. Fig. 8). At both vent fields, a third of the females carry early stage broods,  
470 however at Snake Pit late-stage brooding and hatched females were slightly more frequent (37%)  
471 than at TAG (19%). At TAG, most of the brooding females are at the mid stage (51.9%) (Fig. 5A).  
472



473  
474 Figure 5. Characteristics of *R. exoculata* broods at TAG and Snake Pit vent fields. A. Proportion  
475 of broods with eggs at each developmental stage (including hatched females as having late  
476 broods). B. Sizes of individual embryos at different developmental stages.

477

### 478 3.3.3. *Egg sizes and development*

479 The volume of the eggs within the brood of each female shows significant heterogeneity  
480 due to inter-individual variations, developmental stage of the broods, and vent fields (Supp. Table  
481 5). Despite individual variations, a clear trend of egg volume increase with developmental stages  
482 is observed at both vent fields (Fig. 5B). The ratio between the minimum and maximum diameters  
483 of embryos decreases along their development, with minimum diameter being on average 0.87  
484 of the maximum diameter in early stage, and 0.76 of the maximum diameter in the late stage.  
485 Eggs thus become more elongated at the end of embryonic development, which may reflect an  
486 increase of the embryo polar axis, the distribution of the structures inside the envelope and water  
487 uptake.

488 At each stage, embryos in TAG broods are smaller than in Snake Pit broods (Figure 5B).  
489 The volume of early stage embryos is  $0.151 \pm 0.027 \text{ mm}^3$  at Snake Pit, and  $0.131 \pm 0.015 \text{ mm}^3$   
490 at TAG ( $t\text{-test}= 8.386$ ,  $p < 0.001$ ,  $df=242$ ). At mid-stage, the volume of embryos increases to  $0.179$   
491  $\pm 0.024 \text{ mm}^3$  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  
492 the late stage, the volume of embryos reaches  $0.196 \pm 0.040 \text{ mm}^3$  at Snake Pit and  $0.187 \pm 0.024$   
493  $\text{mm}^3$  at TAG ( $t\text{-test}= 2.458$ ,  $p= 0.007$ ,  $df= 238$ ).

### 494 3.3.4. *Copepod occurrence within broods*

495 The copepods collected from the brooding females were identified as *Stygiopontius*  
496 *pectinatus* using molecular barcodes (Suppl. Fig. 9). These copepods appear to be truly  
497 associated with eggs because they are found deep inside the broods usually attached to the setae  
498 at the basis of the pleopods. In addition, no copepods were found on the abdomen of non-  
499 brooding females. At Snake Pit, 33 % of the brooding females are colonized with copepods,

500 whereas only 20 % of them are infected at TAG. The proportion of infested females tends to  
501 increase between early (10%) and late (40%) stage broods ( $\chi^2= 6.540$ ,  $p= 0.038$ , Suppl. Figure  
502 10). This trend is observed at each vent field and for each sample. In infested broods, the number  
503 of copepods per brood vary between 1 and 5, and appears to be higher at Snake Pit (2 copepods  
504 per brood in average, range: 1-5 copepods) than at TAG (1.1 copepods per brood in average,  
505 range: 1-2 copepods). Similarly, the average number of copepod per brood tends to increase with  
506 brood age from 1 copepod/brood in average in early broods to 2 copepod/brood in average in late  
507 broods.

508

#### 509 **4. Discussion**

510 In January-February 2014, samples of *Rimicaris exoculata* populations at TAG and Snake  
511 Pit vent fields revealed strong female-biased sex ratios (87% females among adults overall). Sex  
512 ratio biases were also striking locally, reflecting abrupt changes in population structure among  
513 habitats. Dense shrimp assemblages crawling next to high temperature fluid exits (AEH)  
514 consisted mainly of females and immature individuals, whereas shrimps observed scattered in  
515 the cold and stable periphery of active vents (IEH) were almost only adult males. At TAG, near  
516 low temperature diffusion areas (DEH), gathering of very small juveniles absent from AEH, and  
517 previously hypothesized to be early juveniles of *R. exoculata*, were indeed *R. chacei* nurseries.  
518 Ovigerous females were observed in larger proportion than ever reported so far for the species,  
519 representing about a third of the sexually mature females in AEH. Overall, these patterns were  
520 consistent across both vent fields, although a high degree of heterogeneity in population structure  
521 was observed at small spatial scales, between samples from a given habitat. Ovigerous females  
522 were more abundant at TAG, reflecting a higher proportion of sexually mature females. However,

523 lower fecundities, smaller eggs as well as a higher proportion of aborted broods, were observed  
524 in ovigerous females from TAG, suggesting a lower individual reproductive effort.

525

#### 526 **4.1. Spatial variation in *R. exoculata* population structure and reproduction**

527 Our study describes strongly biased sex ratios in *R. exoculata*, where sexes appear to  
528 segregate between different habitat types. Similar evidence has been previously reported by  
529 Shank et al. (1998) in samples collected from active chimney walls of several MAR vent fields,  
530 where females were found in much larger numbers than males. Although variations in sex ratios  
531 between the different vent fields were observed, variations within vent fields could not be  
532 assessed because unique samples were collected in each field (Shank et al. 1998). In 2007,  
533 shrimps collected in AEH at Logatchev during the Serpentine cruise also showed a female-  
534 dominated population (I. Hernández-Ávila, unpublished data). Population structure of shrimps in  
535 IEH has not been reported previously, probably because individuals scattered at the periphery of  
536 dense aggregates have been considered as remains of the main populations observed in AEH,  
537 rather than individuals preferentially occupying a specific and separated habitat. Therefore,  
538 similarly to our observations, variations in sex ratios observed in previous studies could indeed  
539 reflect small-scale local variations, rather than variations between vent fields.

540 Female-biased sex ratios have been reported in several vent crustaceans, often  
541 associated with different spatial patterns among sexes. For instance, Nye et al. (2013) reported  
542 that populations of *Rimicaris hybisae* from the Cayman Trough have a sex ratio in favor of females  
543 close to the vent emissions, while more dispersed populations are dominated by males at their  
544 peripheries, with some degree of local variability. In brine pools of the Gulf of Mexico, *Alvinocaris*  
545 *stactophila* shows overall female-biased sex ratio with males preferentially locating on the outer  
546 part of mussel beds (Copley & Young 2006). At hydrothermal vents of the East Scotia Ridge, the

547 chirostylid crab *Kiwa tyleri* exhibits similar -but inverse- patterns: areas close to vent fluids  
548 emission are occupied by dense male-dominated aggregations, whereas females are more  
549 numerous at the periphery (Marsh et al. 2015). *A. muricola* inhabiting cold seeps off Congo also  
550 revealed a globally female-biased sex ratio, with significant local variation observed between  
551 limited numbers of samples (Ramirez-Llodra & Segonzac 2006). Other vent shrimps such as the  
552 Hippolytidae *Lebbeus virentova* from the Cayman Trough also exhibit female-biased sex ratio,  
553 but spatial variation was not reported (Nye & Copley 2014).

554           Immature individuals including juveniles were observed in AEH and IEH. However, both  
555 habitats were occupied by apparently different life stages, with very small juveniles in IEH,  
556 whereas AEH harbored larger juveniles and subadults. Small juveniles of IEH had inconsistent  
557 morphologies with a mixture of characters described for both early juveniles of *R. exoculata* (stage  
558 *A sensus* Komai & Segonzac 2008) and juveniles of *R. chacei*. Barcoding of these IEH juveniles  
559 revealed that they were indeed all *R. chacei*, whereas larger juveniles in AEH belonged to *R.*  
560 *exoculata*. This observation has been repeated in 2017 and 2018, with large aggregations of small  
561 juveniles of *R. chacei* observed around vent diffusion areas at the periphery of TAG and Snake  
562 Pit vent fields (Methou et al. 2020). A more systematic examination of morphological characters  
563 along with molecular characterization led to a redefinition of the juvenile stages of both *Rimicaris*  
564 species (Methou et al. 2020). Our nurseries here are thus specific of *R. chacei*, whereas juveniles  
565 of *R. exoculata* appear to be found only in AEH, where they grow to the subadult and adult stages.  
566 Although this is not shown in details here, juveniles corresponding to the 2 juvenile stages of *R.*  
567 *exoculata* described in Methou et al. (2020) occurred in our AEH samples. Variations in the  
568 proportions of juveniles and subadults in AEH may hide further structuration at very small spatial  
569 scales, perhaps corresponding to environmental gradients associated with vent fluid and  
570 seawater mixing. Additional observations are necessary to elucidate such microscale population  
571 structure within AEH.

## 572 **4.2. Reproductive efforts of *Rimicaris* and potential environmental effects**

573 The size for sexual differentiation (OSD) is 10 mm, about half of the maximal size of the  
574 species (not considering our single outlier). While the OSD parameter has practical value for sex  
575 separation in studies of population structure, size at first reproduction represents a key life-history  
576 parameter reflecting the life-time investment in reproduction of a species (Anger & Moreira 1998).  
577 In Alvinocaridid species, the size at first reproduction varies between 50% (*R. hybisae*, Nye et al.  
578 2013) and 60% (*A. muricola*, Ramirez-Llodra & Segonzac 2006, *A. stactophila*, Copley & Young  
579 2006, *M. fortunata*, Ramirez-Llodra et al. 2000) of the maximal size of the species. *R. exoculata*  
580 thus falls within the range of its family, with the smallest brooding females measuring 12 mm CL,  
581 which represents 58% of its maximal size (20.6 mm CL, not considering our outlier). However,  
582 few females between 12 mm CL and 15 mm CL (i.e. < ESM) were brooding eggs in our samples  
583 (3.5%) and likely represent 'premature' specimens, while much more become sexually mature  
584 (36.5%) when they reach 15.1 mm CL (ESM), which represents 73% of the species maximal size  
585 (excluding outliers). Shrimps of the family have been reported as iteroparous (Copley & Young  
586 2006, Nye et al. 2013, Ramirez-Llodra et al. 2000), and a later onset of reproduction might limit  
587 life time investment in reproduction. On the other hand, since large females have larger broods,  
588 favoring reproduction to the largest individuals might be advantageous and help to maximize  
589 energy investment in reproduction.

590 Fecundity in *R. exoculata* was on average of 833 eggs/female, ranging from 304 to 1879,  
591 with brood sizes increasing with female sizes, which is common in carideans (Corey & Reid 1991).  
592 Previous counts on the rarely collected ovigerous females fall within this range. Ramirez-Llodra  
593 et al. (2000) reported a female from Snake Pit (Microsmoke cruise, November 1995) with 988  
594 eggs (16.4 mm CL) and a female from TAG (Bravex cruise, September 1994) with 836 eggs (17.7  
595 mm CL). *R. exoculata* appears to have fecundities similar to those reported for *R. hybisae*  
596 (maximal fecundity of 1707 eggs, Nye et al. 2013) but lower than fecundities of *R. chacei* (2510

597 eggs reported in a female collected in June 1994 at Lucky Strike, Ramirez-Llodra et al. 2000).  
598 Egg sizes of *R. exoculata* are also consistent with the previous report of Ramirez-Llodra et al.  
599 (2000): early stage (blastula) embryos had a volume of about 0.145 mm<sup>3</sup> (volume are calculated  
600 hereafter from diameters given in reference papers, with the spheroid formula used in this paper)  
601 at TAG, within the size range we observed in this study at similar developmental stage: 0.131  
602 mm<sup>3</sup> at TAG and 0.151 mm<sup>3</sup> at Snake Pit. Eggs of *R. exoculata* are larger than those of *R. hybisae*  
603 (0.08 mm<sup>3</sup>, Nye et al. 2013), or those of *R. chacei* (0.09 mm<sup>3</sup>, Ramirez-Llodra et al. 2000). They  
604 are also larger than the eggs of *A. muricola* (0.1 mm<sup>3</sup>, Ramirez-Llodra & Segonzac 2006) and  
605 similar in size to the eggs of *M. fortunata* (0.13 mm<sup>3</sup>, Ramirez-Llodra et al. 2000). *R. exoculata*  
606 thus stands among the Alvinocarididae shrimps with the largest eggs, perhaps reflecting a specific  
607 strategy of higher parental investment per egg.

608         We observed significant variations in reproductive outputs between *R. exoculata* from TAG  
609 and Snake Pit. With lower realized fecundities, perhaps partly due to higher post-spawning losses,  
610 and lower egg sizes, the reproductive effort of individual females at TAG indeed appears to be  
611 reduced compared to that of females from Snake Pit. The difference in fecundity could be  
612 explained by the larger body sizes of brooding females at Snake Pit, and the positive allometric  
613 variation of fecundity with female size. Similarly, differences in fecundity between *R. hybisae*  
614 females of two vent fields in the Cayman Trough were primarily attributed to the large size  
615 differences observed between shrimps of the two fields (Nye et al. 2013). However additional  
616 factors, such as food availability, environmental challenges (fluid toxicity, or temperature stress),  
617 or variations in fertilization success may also contribute to fecundity differences between both  
618 vent fields.

619         The smaller size of the embryos carried by females from TAG compared to those from  
620 Snake Pit suggests that the reproductive investment per egg is lower at TAG, further contributing  
621 to the lower reproductive output observed at this vent field. Both vent fields are located

622 approximately 310 km apart nearly at the same depth, and regional factors are unlikely to provide  
623 environmental heterogeneity that could explain the different investment in reproductive efforts.  
624 We thus hypothesize that reproduction differences between the two populations are more likely  
625 related with local environmental factors associated with vent emissions. Shrimp tolerance to  
626 metallic elements and dissolved gases in vent fluids depends on detoxification processes through  
627 metallothioneins, antioxidants (Gonzalez-Rey et al. 2007) and metabolic activities of their  
628 symbiotic bacteria (Jan et al. 2014). Higher concentrations in some metallic elements in  
629 hydrothermal fluids could force the shrimps to allocate more metabolic energy to detoxification  
630 processes, at the expense of reproductive functions. For instance, TAG fluids have higher iron,  
631 copper and manganese concentrations than those from Snake Pit (Desbruyères et al. 2000,  
632 Schmidt et al. 2008, Charlou et al. 2010), which could explain the lower reproductive output of  
633 shrimps at this vent field. However, both bioenergetics and vent processes are complex and these  
634 hypotheses must be explored with experimental approaches testing physiological tolerance of the  
635 shrimps (e.g. August et al. 2016) in relation with more detailed exploration of local vent chemistry.

636 *Stygiopontius pectinatus* copepods are known to inhabit the cephalothoracic cavity of *R.*  
637 *exoculata* (Gollner et al. 2010, Humes 1996, Ivanenko et al. 2006). They belong to the  
638 Siphonostomatoidea, an order of copepods comprising many cases of parasitic lifestyles.  
639 *Stygiopontius* copepods of the Juan de Fuca Ridge graze bacteria in alvinellid tubes (Limen et al.  
640 2008) and *S. pectinatus* are believed to feed on microbial communities developing in the  
641 cephalothoracic cavity of *R. exoculata* (Gollner et al. 2010, Humes 1996). Here, they appear to  
642 also inhabit broods, which could similarly provide shelter and food, since the egg envelopes are  
643 colonized by bacteria (Methou et al. 2019). We observed a trend of increasing copepod infestation  
644 with brood aging, which also correlates with the development of microbial communities on egg  
645 envelopes, but could also simply be the result of cumulative probability of encounter with time.  
646 The infestation of Alvinocaridid broods with small meiofaunal organisms has been briefly reported



647 for some brooding females of *A. muricola* in cold seeps of the Gulf of Guinea (Ramirez-Llodra &  
648 Segonzac 2006). Small nematodes *Chomodorita* sp. nov. were reported among the eggs of *A.*  
649 *muricola*, and were associated with the presence of decaying embryos, with one instance of  
650 predatory behavior (the nematode was partly inside the embryo) reported. In *R. exoculata* broods,  
651 although a more extended survey would be needed to statistically assess the impact of *S.*  
652 *pectinatus* copepods on egg development, our observation did not reflect a negative effect, as  
653 higher infestation rates were found at Snake Pit where broods had more numerous, larger and  
654 healthier eggs.

#### 655 **4.3. Spawning seasonality in *R. exoculata*?**

656 Despite three decades of sampling at MAR hydrothermal vents, brooding females were  
657 very rarely reported in *R. exoculata* (Vereshchaka 1997, Ramirez-Llodra et al. 2000). The only  
658 exception until 2014, was the observation of brooding females on structures of the Logatchev vent  
659 field in March 2007, and the collection of a few of them (Komai et Segonzac 2008, Gebruck et al.  
660 2010, Guri et al. 2012). In January-February 2014, large numbers of ovigerous females were  
661 observed crawling among dense shrimp aggregates on sulfide structure walls. In our samples,  
662 the overall abundance of ovigerous female was relatively low (7.1% of the sampled females) but  
663 this probably reflects the low proportion of females reaching the size of sexual maturity. In fact,  
664 while a third of the sexually mature females were ovigerous, many of the two remaining thirds  
665 exhibited bright pink well developed gonads suggesting they were nearly ready to spawn  
666 (observation not quantified, seen in our samples and on *in situ* video acquisition). This high  
667 proportion of ovigerous females among sexually mature ones, consistent across two vent fields  
668 310 km apart, and contrasting with all previous collections along the MAR (see 1985-2014  
669 collection compilation, Table 4) suggests temporal variations in reproductive activity of *R.*  
670 *exoculata* with a possible spawning season in winter.

671

672

673 Table 4. Occurrence of brooding females in *Rimicaris exoculata* samples from different cruises  
 674 done on the MAR since 1985.

Site	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	No date
Rainbow						1998 <sup>8</sup> 2000 <sup>6</sup>	1997 <sup>4</sup> 2002 <sup>10</sup>	2005 <sup>8</sup>	2005 <sup>10</sup>	1998 <sup>10</sup>			
Broken Spur							1997 <sup>4</sup> 2002 <sup>10</sup>		1994 <sup>10</sup> 1996 <sup>10</sup>	1994 <sup>3,10</sup>			1994, 1996 <sup>3</sup>
TAG site	2014* 2014*, <sup>11</sup>					2002 <sup>10</sup>	1997 <sup>4</sup>	1985 <sup>1</sup>	1994 <sup>3</sup>				
Snake Pit	2014* 2014*, <sup>11</sup>					2002 <sup>10</sup>	1997 <sup>4</sup>	2003 <sup>10</sup>	1994 <sup>3</sup>			1995 <sup>8</sup>	
Logatchev			2007 <sup>8,9,11</sup>				2001 <sup>8</sup> 1997 <sup>4,5</sup>				2004 <sup>7</sup> 1998 <sup>10</sup>		
Mephisto						2006 <sup>8</sup>							

675 <sup>1</sup>Williams & Rona (1986), <sup>2</sup>Copley (Copley 1998), <sup>3</sup>Vereshchaka (1997), <sup>4</sup>Shank et al. (1998), 5.  
 676 Gebruk et al (2000), <sup>6</sup>Ramirez-Llodra et al. (2000), <sup>7</sup>Copley et al (2007), <sup>8</sup>Komai & Segonzac  
 677 (2008), <sup>9</sup>Gebruk et al. (2010), Guri et al. (2012), <sup>10</sup>Lunina & Vereshchaka (2014), <sup>11</sup>Methou et al.  
 678 (2019), \*this study. Non-direct estimations and too small samples were omitted. Color code:  
 679 blue: no brooding female reported; green: 1-2 specimens reported; pink: reported as “many  
 680 observed”<sup>8</sup> and few specimens collected<sup>8,9</sup>; red: > hundred specimens collected.

681

682 However, previous studies on *R. exoculata* reproduction suggested continuous  
 683 reproduction based on asynchronous ovarian development observed among females collected in  
 684 summer at the Rainbow vent field (Ramirez et al. 2000) and in autumn at TAG (Ramirez-Llodra  
 685 et al. 2000, Copley et al. 2007). Nonetheless, regardless of vent origin, oocyte maximum size was  
 686 significantly lower in summer than in autumn (Ramirez-Llodra et al. 2000). In addition, brooding

687 females were extremely rare in both studies (3 specimens totally reported in Ramirez-Llodra et  
688 al. 2000, collected in August, September and November). This thus might suggest an extended  
689 period of oocyte growth and maturation in summer and autumn, with rare occurrences of early  
690 spawning, followed with a winter period during which large proportion of sexually mature females  
691 spawn. Such scenario is similar to what is observed in *Alvinocaris stactophila* from cold seeps in  
692 the Gulf of Mexico (Copley & Young 2006). *A. stactophila* females exhibit increasing oocyte size  
693 throughout the year, with small previtellogenic oocytes in both ovigerous and non-ovigerous  
694 females in February-March, larger vitellogenic oocytes in summer with no ovigerous female  
695 observed and females with large vitellogenic oocytes and small previtellogenic oocytes in  
696 November. In the latter, females with large vitellogenic oocytes are large non-ovigerous females  
697 (but ready to spawn), while females with small previtellogenic oocytes were either ovigerous  
698 females or small bodied non-ovigerous females (Copley & Young 2006). Such pattern fits a  
699 seasonal spawning between November and March in *A. stactophila*, and appears very similar to  
700 what is observed in *R. exoculata*. In *R. hybisae* from vents in the Cayman Trough, winter spawning  
701 was also suggested (Nye 2013, Nye et al. 2013). In winter, most females had one cohort of small  
702 previtellogenic oocytes, while a smaller number of them had a cohort of large vitellogenic oocytes,  
703 and only a few had a cohort of intermediate size oocytes. Large and medium size vitellogenic  
704 oocytes were observed only in non-ovigerous females, while small size previtellogenic oocytes  
705 were observed both in ovigerous and non-ovigerous females, suggesting that while some females  
706 had already spawned, others had mature oocytes and would probably spawn shortly (Nye 2013,  
707 Nye et al. 2013). Inter-annual comparison of the proportion of brooding females between January  
708 and February further support winter spawning in *R. hybisae*, but the effect of spatial population  
709 structure and need to expand temporal coverage was recognized (Nye 2013). Although we did  
710 not dissect ovaries in our study, we noticed that many non-ovigerous females in the large size  
711 classes had conspicuous pink-orange gonads filled with large oocytes (visible through the  
712 transparent carapace) indicative of imminent spawning, whereas ovigerous females had shrank

713 whitish gonads, which reflects observations on ovaries of *R. hybisae* during the winter spawning  
714 period. As for *R. hybisae*, we need to expend the temporal breath of observations on *R. exoculata*  
715 reproduction to better constrain its spawning seasonality and assess its degree of variability.

716           Although reproductive traits of vent species were suggested to be phylogenetically  
717 constrained (Tyler & Young 1999), some Alvinocaridid shrimp species also exhibit continuous  
718 reproduction. *Alvinocaris muricola* (Ramirez\_Llodra & Segonzac 2006) from cold seeps off Congo  
719 is one such example, although temporally limited observations of asynchronous ovarian  
720 development could also hide seasonal patterns. In *Rimicaris chacei* from the MAR, additional  
721 observations could also challenge the current view of continuous reproduction based on time  
722 limited observations (Ramirez-Llodra et al. 2000). In contrast, *Mirocaris fortunata* exhibit many  
723 ovigerous females throughout the year, which is truly supporting continuous reproduction:  
724 Ramirez-Llodra et al. (2000) and Van Dover et al. (1996) reported many brooding females from  
725 May, June, July and September at Lucky Strike. We also repeatedly observed *M. fortunata*  
726 brooding females during multiple cruises at different periods of the year and different MAR vent  
727 fields: August 2013 and April 2015 at Lucky Strike (Momarsat cruise series), and January 2014  
728 at Snake Pit (BICOSE cruise). To conclude, Alvinocaridid reproductive strategies are diverse  
729 making it difficult to draw general inferences for the family.

730           Vent species are usually believed to reproduce continuously because of the absence of  
731 photoperiodic signals at depth coupled with the high and continuous productivity at vents that  
732 would obscure variations in surface-derived food supply. Seasonal reproduction in Alvinocaridid  
733 shrimps is however not an exception. On the MAR, *Bathymodiolus azoricus* mussels spawn in  
734 January (Colaço et al. 2006, Dixon et al. 2006), and this pattern has been related to the variability  
735 in the flux of particulate food material that might trigger seasonal reproductive activity (directly in  
736 planktotrophic larvae and/or indirectly in adults). On the East Pacific Rise, vent crabs *Bythograea*  
737 *thermydron* brood their offsprings in spring (Perovich et al. 2003), again possibly responding to

738 surface production cues. On the other hand, bythograeid crabs from Pacific-Antarctic Ridge vent  
739 sites were suggested to have continuous reproduction, suggesting biogeographic effects may  
740 induce variations in reproductive rhythms (Hilario et al. 2009). Similarly, the study of reproductive  
741 rhythms in other *Rimicaris* shrimps from other locations may provide insights into the mechanisms  
742 underlying spawning seasonality in *R. exoculata*.

743 In addition to seasonal reproduction, population size structure suggest discontinuous  
744 recruitment in *R. exoculata*, with similar patterns at both vent fields. Polymodal population  
745 structure was also noted by Gebruk et al. (1997) and Copley (1998). In addition, Shank et al.  
746 (1998) suggested discrete recruitment of juvenile cohorts based on the observation of red  
747 juveniles patches within adult swarms. Discontinuous recruitment may further suggest seasonal  
748 reproduction, although growth rates and recruitment temporality remain to be assessed before  
749 any supported conclusion can be drawn. *R. chacei* in nurseries appear to be recent recruits as  
750 they are similar to those captured in nets above vents (Herring & Dixon 1998). It is less clear for  
751 *R. exoculata* juveniles observed in active emission habitats, although the abundance of lipids and  
752 isotopic signatures suggest they also have recently experienced pelagic life and were feeding  
753 (Methou et al. 2020).

754

#### 755 **4.4. Brooding eggs in vent fluids**

756 The remarkable lack of *R. exoculata* brooding females in samples collected at various  
757 periods and locations, until this study, had been explained by the hypothesis of a migration of  
758 brooding females towards -yet to be found- areas with moderate environmental conditions in order  
759 to avoid exposure of their eggs to harmful vent fluids (Ramirez-Llodra et al. 2000). Such protective  
760 behavior of brooding mothers is indeed reported in many vent crustaceans. Brooding females of  
761 *B. thermydron* crabs on the East Pacific Rise were observed at the periphery of vents whereas

762 males and non-brooding females occupied areas with high fluid venting and temperatures  
763 (Perovitch et al. 2003). In Alvinocaridid shrimps, brooding females of *A. stactophila* locate  
764 preferentially in areas with more oxygen, and less toxic sulfur compounds than those occupied  
765 by males and non-brooding females (Copley & Young 2006). In the amphipod *Ventiella sulfuris*,  
766 adults migrate at the periphery of vents to mate and brood (Sheader & Van Dover 2007). In the  
767 anomouran crab *Kiwa tyleri*, brooding females also locate at the periphery in less-constraining  
768 conditions, whereas males remain close to vent fluid exits (March et al. 2015, Thatje et al. 2015).  
769 In *R. exoculata*, however, such scenario had already been challenged by the collection of a few  
770 brooding females in dense shrimp aggregates at Logatchev (Guri et al, 2012). Our study further  
771 strengthen these observations, brooding females from TAG and Snake Pit being exclusively found  
772 in dense shrimp aggregates where temperature records were higher, and never observed  
773 (including on video footage) in cold peripheral areas where males scattered.

774 Females of *R. hybisae* also appear to brood their eggs directly within aggregates crawling  
775 on chimney walls with no particular protection from vent fluids. Both *R. exoculata* and *R. hybisae*  
776 are rare examples of vent species that experience vent fluid exposure during embryonic  
777 development. Indeed, many species broadcast their eggs, in which case early development occur  
778 in the water column at some distance from the most extreme part of the vent fluid mixing gradient,  
779 and brooding species usually provide protective structures for the early development of their  
780 offsprings (e.g. Reynolds et al. 2010), or relocate into milder areas.

781 As all developmental stages were observed in broods of *R. exoculata*, including hatching  
782 zoea (Hernández-Ávila et al. 2015), females probably remain near vent fluid exits during the whole  
783 brooding period until they release their larvae. Exposure of embryos to high temperature may  
784 accelerate their development, and shorten the brooding period, while challenging normal  
785 development due to heat stress. *In vitro* incubations of embryos removed from brooding females  
786 of *Alvinocaris longirostris* and *Shinkaicaris leurokolos*, two Alvinocaridid species inhabiting

787 Okinawa Trough vent sites, showed increased developmental rates with increasing temperature  
788 for both species, with optimal growth at temperatures within the range experienced by their adult  
789 counterparts *in situ* (Watanabe et al. 2016). These authors showed that embryos of *S. leurokolos*,  
790 which lives near fluid exits, developed better between 10 to 20°C, and hatched within 9-12 weeks  
791 at 10°C and 3-4 weeks at 20°C. Considering the habitat of brooding females in *R. exoculata*, eggs  
792 are likely to hatch rather quickly, perhaps within a month following spawning. A relatively short  
793 brooding period in *R. exoculata* is also supported by the lack of macroscopic evidence of  
794 exceedingly high mineral deposits in the cephalothoracic cavity of ovigerous females. The short  
795 molt cycle of *R. exoculata* (10 days, Corbari et al. 2008) is believed to reset symbiotic communities  
796 of the cephalothoracic cavity sufficiently often to avoid impaired symbiosis due to mineral  
797 precipitation progressively overgrowing bacteria. In bearing-egg crustaceans, the molting cycle is  
798 interrupted during the brooding period until hatching (Correa & Thiel 2003). As females appear to  
799 spend the entire brooding period near vent fluids, a large extension of the molting cycle expose  
800 them to the risk of seeing their symbiotic bacteria drowned in mineral precipitations. However, as  
801 ovigerous females did not appear to suffer excessive mineral load of the cephalothoracic cavity,  
802 this observation also advocates for a relatively short brooding period.

803 Brooding within vent fluids may also sustain the bacterial community including symbiotic  
804 lineages observed on egg envelopes (Methou et al. 2019), and may thus participate in symbiont  
805 transmission to the young shrimp (Hernandez Avila et al. 2015). Another speculative hypothesis  
806 would be that mothers could imprint their offspring with vent signature by bathing their eggs within  
807 vent fluids during embryonic development, which would later help them returning to vent habitats  
808 after dispersal. Such homing process might involve the strongly developed higher brain centers  
809 of *R. exoculata* enabling the memory and navigation skills necessary to locate suitable recruitment  
810 sites (Machon et al. 2019).

811

#### 812 **4.5. Mating system in *R. exoculata***

813 Spatial segregation of sexes in vent species has been related to reproductive processes  
814 needing specific habitats, but it is not known whether such spatial structuration remains  
815 throughout the year or is restricted to mating and brooding periods. Segonzac et al. (1993)  
816 observed dispersed *R. exoculata* - of unknown sex - at the periphery of Snake Pit in June 1988  
817 but more observations are needed to evaluate the temporal correlation between spatial  
818 distribution of sexes and reproductive processes. It is however unlikely that individuals resting in  
819 peripheral areas stay permanently in such habitats because of their need to supply their symbiotic  
820 bacteria with reduced compounds found in vent fluids (Ponsard et al. 2013). Moreover, the highly  
821 modified cephalothorax of *R. exoculata* adults hinder movement of the appendages, making any  
822 foraging or grazing behavior unlikely (Segonzac et al. 1993, Zbinden & Cambon 2020). Shrimps  
823 thus most likely fast when they are in IEH. Peripheral areas also harbor more predators, such as  
824 *Maractis rimicarivora* anemones (Fautin & Barber 1999, Copley et al. 2007), or fishes that are  
825 excluded from AEH, maybe because of the harsh vent conditions. The migration of *R. exoculata*  
826 males to inactive areas is therefore not likely driven by trophic needs or interspecific interactions  
827 (increased predatory risk), and given the current knowledge, the hypothesis of a reproductive  
828 behavior remains the best explanation. In addition, spermatophores at the gonopores of males  
829 were often observed in IEH, but never in males from other habitats (Pradillon, personal  
830 observation), suggesting a greater mating readiness in IEH. Mature caridean females molt just  
831 before egg extrusion (Bauer 2004), and vent shrimps may migrate briefly towards a milder habitat  
832 at this stage of increased vulnerability. Males in IEH would thus have better chances to encounter  
833 females just after their prespawning molt and mate successfully. After mating and egg extrusion,  
834 females would return to AEH and brood their eggs, while the fate of males remains uncertain.  
835 Males will however probably have to return to AEH at some point to fulfill their nutrition needs.



836           Body sizes and male weaponry in crustaceans have been associated with their mating  
837 system (Correa & Thiel 2003, Baeza & Thiel 2007, Asakura 2009). In free-living caridean shrimps,  
838 larger males are usually observed in mating systems that involve sexual competition for females  
839 or precopulatory mate guarding (Bauer 1996, Correa & Thiel 2003, Asakura 2009). In contrast,  
840 the lack of sexual competition in “pure searching” mating systems or long-term mate (monogamy  
841 or semi-monogamy) is generally associated with similar or smaller-sized males (Correa & Thiel  
842 2003, Bauer 1996). Although *R. exoculata* males were on average larger than females, this was  
843 due to the large proportions of small females. Considering only sexually mature females, i.e. those  
844 females actually involved in the courtship and mating processes, then sex size differences do no  
845 longer exist, and females are even slightly larger than males. In other vent carideans such as  
846 *Lebbeus virentova*, females tend to be larger than males (Nye & Copley 2014), and a similar trend  
847 has been proposed for the cold-seep alvinocaridid *A. muricola*, based on maximum sizes of males  
848 and females (Ramirez-Llodra & Segonzac 2006). In *R. hybisae*, males and females exhibit similar  
849 sizes (Nye et al. 2013). In addition, in *R. exoculata* there is a lack of sexual dimorphism in  
850 secondary characters associated with male competition (e.g. increase in cheliped size and  
851 cephalotorax). We thus hypothesize that a pure searching model could better describe the mating  
852 system in *R. exoculata*. In this model, males search for receptive females just after their  
853 reproductive molt using mostly tactile signals or pheromones (Bauer 1996, 1976). However,  
854 looking for tactile and chemical cues within a dense aggregation of congeners close to vent  
855 emissions does not seem optimal. A migration to the vent periphery to courtship and mate could  
856 increase the chance of male-female recognition and ensure mating success. In his description of  
857 the mating behavior of the Hippolytidae *Heptocarpus pictus*, a caridean species exhibiting a pure  
858 searching mating model, Bauer (1976) described a suite of events including one called “straddle”  
859 when the male clutches the female with his walking legs. We observed *R. exoculata* pairs with  
860 similar behavior several times *in situ* (in all habitats, but mainly at the periphery of AEH or in DEH)  
861 (Supplementary video 1), which further supports the pure searching mating model in this species.

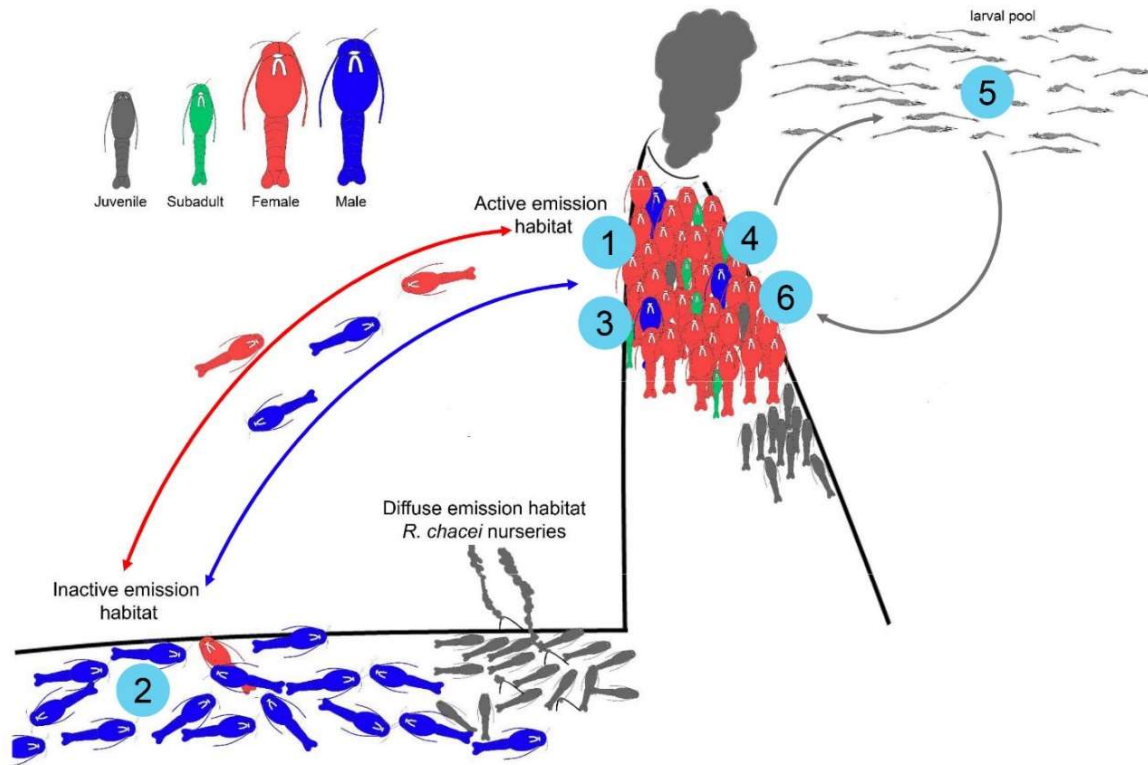
862

863 **4.6. A proposed life cycle scenario for *Rimicaris exoculata***

864 Our examination of population structure and reproductive features across different  
865 habitats allows us to draw a hypothetical scenario for the benthic phase of the life cycle of *R.*  
866 *exoculata* (Fig. 6). In our observations, juveniles of the species appear to recruit directly in dense  
867 aggregations crawling on vent active structures. Visually, they can be distinguished from adults  
868 and subadults by their bright red color. Although in this study “nurseries” near diffusion areas  
869 belonged to *R. chacei*, juveniles of *R. exoculata* were observed forming gatherings within dense  
870 assemblages (Pradillon, personal observation and Shank et al. 1998), perhaps related to small-  
871 scale environmental conditions within AEH. Considering all observations gathered so far in this  
872 study and others (Shank et al. 1998, Gebruck et al. 2000, Methou et al. 2020), juveniles found in  
873 AEH are the smallest observed and identified with confidence for *R. exoculata*. We cannot  
874 exclude that we are still lacking an earlier benthic stage, as smaller alvinocaridid juveniles -  
875 without species assignment – were caught in bottom trawls (Herring & Dixon 1998). However,  
876 isotopic signatures of the smaller benthic stages of *R. exoculata* reported by Methou et al. (2020)  
877 are reflecting a pelagic lifestyle and advocate for relatively recent recruitment of those stages.

878 After recruitment, juveniles shift towards a chemosynthetic nutrition, and grow to the  
879 subadult and adult stages. While small adults appear to stay within AEH as they grow, mature  
880 and reach reproduction size, sexually mature reproductive adults could migrate in less active parts  
881 of vent fields for mating. Sexually mature males would move there, reaching a position where they  
882 may more “easily” find sexually receptive females that move out of dense aggregations to molt,  
883 mate and extrude their eggs. No competitive or guarding behavior was observed in males, and  
884 their size broadly similar to the size of females, as well as their lack of weaponry, do not argue  
885 for competition. Instead, males appear rather inactive, and exhibit behavior resembling the “pure

886 searching” model where males contact females to find and mate with receptive females. Brooding  
887 would occur entirely within vent mixing gradients and last for a few weeks before release of zoea  
888 larvae. These larvae would then disperse and mix within bathypelagic waters, developing and  
889 feeding for a while on pelagic food items until they reach a large post-larval stage before returning  
890 to a benthic and chemosynthetic life style at vents.



Life stage	Habitat	Observation	Reference
1 Growth & maturation	AEH	All stages from juveniles to adults are present, but few males.	This study.
2 Mating & spawning	IEH	Many adult males, rare females, mating behavior observed.	This study.
3 Brooding	AEH	Brooding females with embryos at all stages.	Guri et al. 2012, this study.
4 Larvae release	AEH or closeby	Late broods and hatched females in AEH, zoe larvae collected within a few meters from adult populations.	Hernandez-Avila et al. 2015, this study.
5 Larval dispersal	bathypelagic	Alvinocaridid post-larval stages collected but not identified to the species level.	Herring & Dixon 1998
6 Recruitment	AEH	Most juveniles observed in AEH.	Methou et al. 2020, this study.

891

892 Figure 6. Proposed model of habitat use through the life cycle of the vent alvinocaridid shrimp  
 893 *Rimicaris exoculata*

894

895

896 **Credit author statement:**

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

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

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