

1 **Gonad characterization and reproductive seasonality in *Siphonaria lessonii* (Gastropoda:**
2 **Heterobranchia) from the Southwestern Atlantic Ocean**

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12 **Abstract.** Histological characterization of the hermaphrodite gonad and gametogenesis
13 seasonality were investigated in a population of *Siphonaria lessonii* from the coast of Buenos Aires
14 Province (37°16'S 56°58'W). Monthly analysis of the frequency of gametogenic stages, number
15 and mean oocyte area size were used to determine reproductive events over a 2-year period (June
16 2012 - May 2014). Female and male gametes were observed simultaneously within acini of adult
17 individuals and continuously throughout the period studied. Oogenesis commenced in the
18 beginning of austral autumn with gonad characterized mainly by proliferation of female cells.
19 From this moment oocyte gradually increased in number and area until spring when a large number
20 of individuals were found in evacuation. The same trend was also observed from early gonad
21 maturation to advanced stages indicating that gonad development presented a close relation with
22 oocytes frequency and area. Spermatogenesis was also observed as a continuous process
throughout the year although spent acini were more frequent from November until February.

23 Reproductive seasonality and gametogenesis were associated with changes in temperature and
24 duration of the daylight period.

25

Keywords: gametogenesis, siphonariid, hermaphroditism, histology, environmental variables

26

27 *Siphonaria lessonii* Blainville, 1827 is an herbivore gastropod commonly found inhabiting hard
28 substrata of the upper and midlittoral zones and the only pulmonate species that occurs along the
29 coast of Buenos Aires Province in Argentina (Olivier & Penchaszadeh 1968). Siphonariids are
30 iteroparous and very frequent in warm to tropical regions especially in the southern hemisphere
31 (Hodgson 1999). They are hermaphrodites, like all pulmonate gastropods, with internal
32 fertilization and most species lay eggs in gelatinous masses usually cemented to the substratum
33 (Chambers & McQuaid 1994; Pal & Hodgson 2005). *S. lessonii* has a wide geographic distribution
34 in southern South America, from the Uruguayan department of Rocha to Cape Horn, including
35 Malvinas (Falkland) Islands, in the southwestern Atlantic Ocean, through the Beagle Channel and
36 Magellan Strait extending north to Chiloé Island (Chile) in the Pacific Ocean (Güller, Ituarte &
37 Zelaya 2015).

38 In temperate climate zones, the reproductive behavior of marine mollusks is directly related to
39 environmental factors and seasonal climate variation (Giese & Pearse 1974). The reproductive
40 potential of a species, concerning sexuality and presence of hermaphroditism, is genetically
41 determined with strong influence of environmental conditions such as temperature, intensity and
42 period of daylight, that ultimately influence the intensity and period (s) of reproduction (Lucas
43 1965). Seasonality in reproduction of marine invertebrates is usually related to storage of mature
44 gametes and the subsequent spawning, which consequently results in a meaningful variation of
45 oocyte size frequency in all adult individuals of a population over a brief period (Olive, 1992).

46 Information regarding growth, mortality, recruitment and reproduction are valuable data to analyze
47 structure and dynamics of biological communities (Underwood 1979).

48 *Siphonaria lessonii* is one of the most abundant species and the dominant herbivore in the intertidal
49 zone of Buenos Aires province (Tablado, López Gappa & Magaldi 1994), feeding mostly on
50 diatom, chlorophyta and rodophyta algae (Bastida, Capezzani & Torti 1971). It co-occurs with the
51 dominant space competitor mytilid *Brachidontes rodriguezii* at mid- and low-intertidal levels
52 (Tablado & López Gappa 2001; Torroglosa & Gimenez 2018). Given that some bivalve species
53 depend upon the presence of algae to settle and recruit (Dayton 1971; Sousa 1984; Menge,
54 Lubchenco & Ashkenas 1986; Petraitis 1990), *S. lessonii* grazing activity may have an indirect
55 negative effect over populations of those species (Adami 2008).

56 Along Buenos Aires province coastline, extensive sandy beaches prevail over rocky bottom
57 beaches and cliffs. Artificial structures such as landing piers, used for recreational fishing,
58 introduced in sandy beaches like Villa Gesell provide a new substratum for *S. lessonii* settlement
59 and development, influencing not only the geographical distribution of this species but also the
60 ecological structure of the benthic community inhabiting these substrates.

61 Although numerous reports described biological and ecological aspects of *S. lessonii* in the Buenos
62 Aires coast (Olivier *et al.* 1966; Bastida *et al.* 1971; Lopez Gappa, Tablado & Magaldi 1993;
63 Lopez Gappa, Tablado & Magaldi 1996; Tablado & López Gappa 2001; Nuñez, Ocampo &
64 Cledón 2014), the characterization of the hermaphrodite gonad and the seasonal reproduction
65 remains unknown for this species. Therefore, the present study aims to histologically characterize
66 the morphology of *S. lessonii* hermaphrodite gonad and describe both oogenesis and
67 spermatogenesis. We also investigate the seasonality of its reproduction in a population inhabiting
68 Villa Gesell's fishing pier through chronological evaluation of gametogenesis and its relation to

69 variation in oocyte frequency. Finally, we analyze these results in comparison to environmental
70 factors.

71

72

MATERIALS AND METHODS

73

74 **Sample collection and histology**

75 Thirty adult ranging from 9.0 to 21.0 mm total shell length, which corresponds to sexually
76 developed specimens of *S. lessonii* were haphazardly collected monthly by hand during diurnal
77 low tides from June 2012 to May 2014 (except March 2014) at the landing pier of Villa Gesell
78 beach (37°16'S 56°58'W), Buenos Aires Province. Individuals collected were transported in
79 containers at temperature range between 9°C to 11°C, in order to slow down metabolism and
80 reduce stress. In the laboratory, individuals were measured (shell length) with a Vernier caliper
81 (± 0.01 mm) and the hermaphrodite gonad (HG) was removed and fixed in aqueous Bouin's
82 solution for 12 hours. After fixation, tissues were dehydrated in ascending concentrations of
83 ethanol to 96%. Tissues were embedded in paraffin wax and sections of 5 μ m thickness were cut
84 using a Leica microtome and later stained with hematoxylin and eosin. Sections were examined
85 under light microscope (Zeiss Axiostar) and photographed with a digital camera (Canon G10).

86

87 **Hermaphrodite gonad development and gametogenic cycle**

88 To describe the gametogenic cycle in both sexes, five different sections of each individual gonad
89 were randomly selected and classified independently according to gonad development stages
90 determined through histological observations. This same procedure was used to estimate the
91 frequency and size of female gametes. Oocytes with visible nucleus and nucleoli were classified
92 according to gametogenesis development, quantified and measured (area, μ m²). Images of each

93 section at magnification 400 were taken using the software AxioVision 4.8.2. and used to analyze
94 the relation between oocyte frequency and area, gonad development stages and monthly variation.
95 Two methods were employed to study gonad maturation in relation to oocyte frequency and area:
96 the first referred to an assay on distribution of oocyte size classes; and the second consisted in
97 examining the variation in oocyte area.

98

99 **Oocyte stages**

100 Oogenesis was classified into three development stages in order to analyze gametogenesis
101 quantitatively. Previtellogenic oocytes (PVO) were characterized by small basophilic cells and
102 lack of yolk granules with relative small cytoplasm area compared to nuclear area, sometimes
103 containing two nucleolus. Early vitellogenic oocytes (EVO) presented irregular shape with an
104 increase in cytoplasm area with few yolk granules. Late vitellogenic oocytes (LVO), also
105 considered ripe oocytes, presented large cytoplasm area packed with yolk granules.

106

107 **Environmental variables**

108 Environmental variables (atmospheric temperature, sea surface temperature and daylight period)
109 were accessed for correlation with gametogenic processes. Photoperiod (daylight) was calculated
110 as mean monthly day length, using data obtained on daily sunrise and sunset register for Mar del
111 Plata (nearest site), provided by Servicio de Hidrografia Naval de Argentina, available online
112 (<http://www.hidro.gob.ar>). Sea surface temperature (SST) was obtained freely from the website
113 register on Villa Gesell's coast (<http://seatemperature.info>), and atmospheric temperature (ATM)
114 from INTA (Argentina Nacional Institute of Technology) available online
115 (<http://siga2.inta.gov.ar>). Monthly mean temperature for SST and ATM were calculated by
116 determining the average value between minimum and maximum daily records.

117

118 **Statistical analysis**

119 All statistical analyses were performed using the software Statistical Statsoft (version 7). Data
120 normality and variance homogeneity were calculated using Shapiro-Wilks and Levene tests,
121 respectively. One-way ANOVA was used to assess monthly differences in the frequency of oocyte
122 maturation stages. To assess the monthly variation in oocyte mean frequency and area, Kruskal
123 Wallis nonparametric analysis was performed. Pearson Product Moment Correlation (PPMC) was
124 used to test relationships between late vitellogenic oocyte frequency and area (μm^2), and
125 environmental parameters, daylight (hours), SST ($^{\circ}\text{C}$) and ATM ($^{\circ}\text{C}$). Significance level was
126 predetermined for $p < 0.05$.

127

128

129 **RESULTS**

130

131 **Hermaphrodite gonad structure and development**

132 A total of 297 adult individuals were histologically analyzed with size range of 9.0 – 21.6 mm
133 shell length (mean \pm SD = 14.3 \pm 2.89). The hermaphrodite gonad (HG) of *S. lessonii* is a
134 multifollicular organ situated in the posterior-dorsal part of the body under the shell apex, from
135 medial to the right side of the organism and next to the digestive gland. It has a globular to irregular
136 lobed shape with a yellow-orange color that may vary from beige to a brownish orange depending
137 on its development. Mature individuals had both oocytes and sperm simultaneously within acini
138 of the hermaphrodite gonad, with male germ cells developing more centrally and closer to the
139 hermaphrodite duct while female cells develop peripherally in the HG. Maturation of gametes

140 within acini occurred from edge to center with oocytes growing and gradually detaching
141 themselves from the acinar wall and filling the acinus lumen (Figure 1).

142 After careful histological examination of *S. lessonii* hermaphrodite gonad, female and male were
143 analysed separately. A five development stage criteria (Figure 2) was established for female
144 gametes: proliferation, growth, pre-evacuation, evacuation and post-evacuation. In proliferation
145 (Fig. 2A), acini were characterized by the generation of oogonia and mainly the presence of small
146 PVO attached to the acinar wall. During this stage, EVO may be present, but not LVO. In growth
147 (Fig. 2B), EVO and few LVO begin to fill the acini integrally along with PVO. Pre-evacuation
148 (Fig. 2C) was characterized by the full occupation of the acini by LVO, which expand the acini
149 area reducing the connective tissue between them. In the evacuation stage (Fig. 2D), oblong
150 oocytes were observed with cytoplasm packed with yolk and some becoming detached from the
151 acinar wall and filling the lumen, ready to be evacuated to the hermaphrodite duct. In post-
152 evacuation (Fig. 2E, F), acini were characterized by large empty spaces, presence of atresic oocytes
153 and gametogenic remains, and in some cases a few developing PVO and non-evacuated oocytes.
154 Male gametogenesis was classified into four different development stages (Figure 3). Early
155 development stage (Figure 3A) was characterized by acini containing mainly spermatocytes. In
156 the differentiation stage (Fig. 3B), acini contains mostly spermatocytes and spermatids. In the
157 mature stage (Fig. 3C), acini was displayed mainly spermatids and spermatozoa. Finally, after
158 evacuation of mature spermatozoa, the acini had a few spermatogenic remains and empty spaces
159 in the lumen, corresponding to the spent stage (Fig. 3D).

160

161 **Seasonal reproduction**

162 Gametogenesis was observed as a continuous process during the studied period with a year round
163 production of both female and male gametes by adult individuals. Examination of the HG

164 development showed variation in the relative frequency of female gametogenic stages (Fig.4A).
165 In the first year, high frequency of post-evacuation acini were observed from December 2012 to
166 April 2013, whilst in the second year, post-evacuation stage was higher from December 2013 to
167 February 2014, concluding two months earlier than in the previous year. Notwithstanding, few
168 post-evacuation sections were also observed in preceding months. Proliferation of female cells was
169 highest in April and May 2013 and February and April 2014, followed by an increase in frequency
170 of growth and pre-evacuation stages, successively, from June to November in 2012 and 2013.
171 The monthly distribution of spermatogenesis is summarized in Figure 4B. Gametogenesis in the
172 male portion was also continuous with individuals recorded in post-evacuation stage throughout
173 the 2 years study period, showing higher frequencies by the end of spring and beginning of
174 summer. Initiation of gametogenesis, with individuals in early development stage, took place in
175 summer with peaks in March 2013 and February 2014. Differentiation of male germ cells occurred
176 more frequently from autumn to spring. Evacuation, represented the sections analyzed, were
177 observed all year round but in higher frequencies during winter and spring.

178

179 **Oocyte size and distribution among gonadal stages**

180 From a 215 *S. lesonii* histologically analyzed for this purpose, 12775 oocytes were counted,
181 measured and classified according to their development. Means, standard deviation, minimum and
182 maximum oocyte area (μm^2) were calculated for each development stage Table 1. The mean
183 number of oocytes, calculated by number of oocytes per section, and mean oocyte area size
184 presented variation among gonadal development stages, with an increase in both frequency and
185 size from Proliferation stage (3.7 and $226.8 \pm 165.1 \mu\text{m}^2$) to Evacuation stage (12.0 and $1007.6 \pm$
186 $988.73 \mu\text{m}^2$), and sequentially decreased in Post-evacuation stage (4.0 and $726.7 \pm 823.1 \mu\text{m}^2$).
187 Furthermore, the distribution of oocyte size classes showed that the number of small oocytes (0-

188 400 μm^2) also decreased from Proliferation to Evacuation while the frequency of larger oocytes
189 ($>1000 \mu\text{m}^2$) increased along with gametogenesis development (Fig. 5).

190 Non-parametrical Kruskal-Wallis analysis revealed significant variation in oocyte size among
191 gonad development stages regarding the stages PVO ($H= 123.01, df= 4, p<0.001$), EVO ($H=$
192 $42.89, df= 4, p<0.001$) and LVO ($H= 22.48, df= 3, p<0.001$). Low values were found in
193 Proliferation for PVO ($168.4 \pm 78.7 \mu\text{m}^2$) and EVO ($520.5 \pm 172.5\mu\text{m}^2$) sizes. Significantly higher
194 mean value was observed for PVO in Evacuation stage ($209.1 \pm 86.0 \mu\text{m}^2$), while EVO and LVO
195 were found higher in both Pre-evacuation ($628.9 \pm 209.7\mu\text{m}^2$ and $2111.2\pm 854.2\mu\text{m}^2$) and
196 Evacuation ($620.9 \pm 207.4 \mu\text{m}^2$ and $2135.0 \pm 857.5 \mu\text{m}^2$), respectively.

197

198 **Oocyte stages size frequency and area**

199 Data analyzed for oocyte number and area were determinate by the mean oocyte in each
200 microscopic section. Oocytes in the three stages were observed continuously throughout the two
201 years study period, although presenting different monthly frequencies. During winter and spring
202 gonads presented higher mean number of PVO and EVO, while LVO number started to increase
203 during winter reaching highest values in the beginning of spring to subsequently decrease until the
204 end of summer. Significant variation in monthly mean number of oocytes (Fig. 6A) was
205 determined through the Kruskal-Wallis analysis (PVO, $H= 265.4, df= 23, p<0.001$; EVO $H=$
206 $351.8, df= 23, p<0.001$; LVO= $441, 2, df= 23, p<0.001$). Significant higher mean value were
207 recorded in September 2013 ($9.6 \pm 4.7 \mu\text{m}^2$) and August ($9.7 \pm 5.0 \mu\text{m}^2$) for PVO, while EVO
208 was higher in September 2013 ($6.6 \pm 3.3 \mu\text{m}^2$). LVO was higher in October 2012 ($7.2 \pm 6.6 \mu\text{m}^2$)
209 and in September 2013 ($6.6 \pm 3.1 \mu\text{m}^2$), whereas April 2013 ($0.1 \pm 0.34 \mu\text{m}^2$) and April 2014 (0.2
210 $\pm 0.5 \mu\text{m}^2$), presented the lowest values during the study period.

211 The area of previtellogenic oocytes ranged from 35.4 to 486.42.5 μm^2 ($202.0 \pm 85.4 \mu\text{m}^2$), early
212 vitellogenic oocytes from 264.69 to 1252.40 μm^2 ($617.1 \pm 205.1 \mu\text{m}^2$) and late vitellogenic oocytes
213 from 888.7 to 6843.4 μm^2 ($2062.3 \pm 850.1 \mu\text{m}^2$).
214 The variation in the mean oocyte area (μm^2) for the three oogenesis stages is presented in Fig. 6B.
215 the analysis of the monthly variation in mean oocyte using the Kruskal-Wallis analysis showed
216 significant differences for all stages described (PVO, $H=144.3$, $df= 22$, $p<0.001$; EVO, $H= 66.1$,
217 $df= 22$, $p<0.001$; LVO $H= 100.2$, $df= 22$, $p<0.001$). LVO presented significant high mean values
218 were observed for December 2013 ($2218.7 \pm 643.2 \mu\text{m}^2$), August 2013 ($2259.1 \pm 582.3 \mu\text{m}^2$),
219 September 2013 ($2171.2 \pm 325.6 \mu\text{m}^2$), January 2014 ($2291.4 \pm 657.0 \mu\text{m}^2$), November 2012
220 ($2202.9 \pm 445.3 \mu\text{m}^2$), June 2012 ($2202.5 \pm 409.5 \mu\text{m}^2$) and May 2014 ($2482.5 \pm 1122.3 \mu\text{m}^2$).

221

222 **Environmental variables and reproduction**

223 During the study period, daylight (DL) varied from 9.6 hours in June to 14.7 hours in December.
224 Extension in daylight period was closely followed by an increase in sea surface temperature (SST)
225 and atmospheric temperature (ATM), with a slight difference between sampling years. In the first
226 period (2012-2013) mean SST was 6.39 °C in July and 21.05 °C in January and mean ATM was
227 9.84 °C in July and 21.61 °C in February. In the second period (2013-2014) mean SST was 8.16
228 °C in July and 22.28 °C in December and mean ATM was 9.61 °C in August and 21.39 °C in
229 February (Fig. 6C). Statistical analysis showed a negative correlation between late vitellogenic
230 oocyte frequency and ATM ($n = 23$; $r = -0.55$; $P = 0.007$).

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232

233

233 **DISCUSSION**

234

235 Examination of the hermaphrodite gonad showed that *S. lessonii* is a simultaneous hermaphrodite
236 with female and male gametes developing at the same time within acini, which agrees with
237 previous studies in siphonariids species (Berry 1977; Simpson 1977; Hodgson, Bernard & Lindley
238 1991; Pal & Hodgson 2005). Protandric hermaphroditism, as suggested by Hubendick (1978),
239 based on the investigations of Marcus & Marcus (1960) on *S. hispida*, and Zischke (1974) on *S.*
240 *pectinata*, where a brief phase of protandris preceding simultaneous hermaphroditism was
241 described, was not possible to determine since this work focused only on larger individuals.

242 Analysis of monthly variation of the relative frequency of gametogenic stages, frequency of
243 oocytes stages and mean oocyte area (μm^2) indicates that the population from the Villa Gesell
244 landing pier presents an annual reproductive cycle. Gametogenesis of both female and male cells
245 was a continuous process throughout the two years study period. Female cells proliferation occurs
246 predominantly in April/May (beginning of autumn) when daylight is already decreasing, followed
247 by lower atmospheric and sea surface temperatures. From this moment, oocyte area gradually
248 increases until November/December (end of spring) coinciding with higher number of individuals
249 in evacuation and/or post-evacuation stages. In the period prior to the evacuation, the
250 hermaphrodite gonad of *S. lessonii* increases considerably in size, occasionally expanding to the
251 mantle cavity, comprehending the final maturation period of female gametes.

252 Furthermore, the current work also demonstrated that even though mature oocytes (LVO) could
253 be distinguished in adult individuals along the year, evacuation (spawning) occurred from winter
254 (June 2012 and August 2013) to the beginning of austral summer (February 2013 and January
255 2014), with peaks in spring months (October 2012 and December 2013), when relative frequencies
256 of individuals in evacuation were highest. This result coincides approximately with observations
257 by Olivier & Penchaszadeh (1968) on spawning of *S. lessonii* population in Mar del Plata rocky
258 shores, who reported egg masses from June to March. Yet, it is important to mention that during

259 autumn field observations, occasional egg masses could still be found cemented to the pier
260 substrate during low spring tides. Another mention can be noted, that the dominant space
261 competitor mytilid *Brachidontes rodriguezii* is in the beginning of the spawn events in February
262 to May (Torroglosa & Gimenez 2017)

263 Male gametogenic development was also continuous and synchronous with female process
264 throughout the period studied. Evacuation stage frequency increased during winter and spring,
265 followed by an increase in post-evacuation stage by the end of spring and throughout summer. Pal
266 & Hodgson (2005) reported that spermatogenesis in *S. serrata* occurs continuously with acini
267 presenting major activity during late winter and early spring although recording a lower frequency
268 of monthly spawning than in the present study. Despite de fact that these organisms can storage
269 foreign spermatophores in their spermatheca, it seems that *S. lessonii* also synchronizes evacuation
270 in order to maximize fertilization with the possibility of cross fertilization.

271 Characterization of the gonad development stages through analysis of oocyte area distribution
272 demonstrated that frequency of larger oocytes increased alongside gonad development, with
273 greater presence of small cells (<400 μm^2) during the early stage of gonad maturation
274 (Proliferation) and the larger oocytes (>1000 μm^2) during advanced stages (Pre-evacuation and
275 Evacuation). The mean oocyte area also increased from proliferation to evacuation when oocytes
276 grew from 226.8 (\pm 165.1) μm^2 to 1007.6 (\pm 988.7) μm^2 . The same trend was already found by
277 measuring oocyte diameter in several gastropod species, such as *Buccinum isaotakii* (Ilano,
278 Fujinaga & Nakao 2003), *Megalobulimus abbreviatus* (Horn, Achaval & Zancan 2005),
279 *Anodontites trapesialis* (Calil & Mansur 2007) and *Melampus coffeus* (Maia, Rocha-Barreira &
280 Coutinho 2012). After evacuation, oocyte size decreased in Post-evacuation stage, although non-
281 evacuated mature oocytes could still be found within acini. The gametogenic cycle presented a
282 variation in number of oocytes among stages. In the beginning, proliferation stage was

283 characterized by a minor number of small (previtellogenic) oocytes. Then, the number and the area
284 of oocytes increases towards evacuation stage, when it reached its highest values. And after
285 evacuation, gonads showed a decrease in number and area of oocytes.

286 It is also important to mention that post-evacuation showed an increasing presence of PVO
287 compared to the evacuation stage, indicating the early regenerative capacity of the gonad after the
288 evacuation of female gametes.

289 Hodgson (1999) revealed that siphonariid species of temperate zones present seasonal spawnings
290 while those of tropical and sub-Antarctic zones are reproductively active all year round even
291 though some could exhibit peaks of spawning. This author also reported that in species with annual
292 spawning cycles, gametogenesis could be seasonal. The present population of *S. lesonii* showed
293 continuing gametogenic activity with a distinguish seasonality in its reproduction, even though
294 occasional spawns occurred out of this period. Creese (1980) observed that *S. denticulate* and *S.*
295 *virgulata* spawn from summer to autumn in the southern hemisphere despite the presence of
296 mature oocytes in hermaphrodite gonad during most of the year. Ocaña & Emson (1999) observed
297 seasonality in the spawning of *S. pectinata* in Gibraltar (northern hemisphere), comprehending
298 periods from the end of winter to end of summer (from March until September), with peaks in May
299 and June (Table 2).

300 Results suggest correspondence between mean oocyte number and area in relation to the gonadal
301 maturation throughout the period studied. Oocyte number and area showed low values for both
302 analyses in all three stages during autumn months when most gonads were in proliferation and
303 growth stages. In this sense, only PVO presented higher frequency during such period. In spring
304 gonad increased in size presenting a great number of larger oocytes, with this increment coinciding
305 with months when most individuals were in evacuation and post-evacuation.

306 Several studies reported a correlation between gametogenic development in marine invertebrates
307 and environmental factors, particularly temperature and food availability (Hirano & Inaba 1980).
308 Results indicated that seasonality in *S. lessonii* reproduction in Villa Gesell coincided with an
309 increase in atmospheric and sea surface temperatures along with extension in daylight period.
310 Gonad were in evacuation stages with fully developed oocytes and post-evacuation from spring to
311 austral summer, thus reproduction occurring during the hottest months. In a physiologically
312 stressful environment such as the intertidal rocky shore, high temperatures may expose egg masses
313 and developing embryos to severe desiccation during diurnal low tides to severe desiccation
314 (Przeslawski, Davis & Benkendorff 2005). Heat may also affect adult individuals, as suggested by
315 *S. japonica* mortality in Hong Kong (Liu, 1994) and starvation of *S. diamenensis* due to low food
316 availability (Quinn, 1988a), as algae productivity decreases during summer in Australian coast.
317 Furthermore, sea surface temperature may be linked with the success of the planktotrophic larval
318 development and survival, as larval period will be relatively short if nutrition and temperature are
319 optimal, and longer if conditions are sub-optimal (Pechenik 1984; Hoegh-Guldberg & Pearse
320 1995; Pechenik, Marsdenb & Pechenik 2003). In that sense, chance of planktotrophic larvae being
321 predated increases as a function of the smaller its size and the longer it remains in the water column
322 (Thorson, 1950). Additionally, Hirano & Inaba (1980) reported differences between spawning
323 periods in two populations of *S. japonica* when compared with Abe (1940) and suggested a
324 correlation with sea water temperature.

325 *S. lessonii* is a simultaneous hermaphrodite species that presents a continuous gametogenic activity
326 in the Villa Gesell landing pier, producing both sperm and eggs throughout the year. Such
327 reproduction strategy, combined with a free-swimming planktotrophic veliger, allows this species
328 a successful establishment along de coastline of southwestern Atlantic Ocean. Several authors
329 (Marshall & McQuaid 1991; Marshall & McQuaid 1992; Gray & Hodgson 1997; Hodgson, 1999;

330 Marshall, Peter & Chown, 2004) attributed the colonization success of siphonariids to their
331 physiological (anaerobiosis, metabolic rate depression, thermal tolerance), ecological and
332 behavioral adaptations. The combination of these attributes makes *S. lessonii* a highly competent
333 species that is able to colonize recently introduced artificial structures with hard substrata in the
334 intertidal zone, enabling the expansion of its geographical distribution.

335

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506 Figure 1: Light micrograph transection of hermaphrodite gonad. **A** General view showing
507 peripheral distribution of oocytes and spermatozoa at the internal region; **B** Detail of oocytes
508 development in the distal region of acini. Abbreviations: OOC, oocyte; SCE, simple connective
509 epithelium; SPZ, spermatozoa. Scale bars **A**= 500 µm; **B** 100 µm.

510

511 Figure 2: Female gametogenic stages of *S. lessonii*. **A** Proliferation, mainly presence of oogonia
512 and pre-vitellogenic oocytes; **B** Growth, early vitellogenic and vitellogenic oocytes; **C** Pre-
513 evacuation, large vitellogenic oocytes compressed within acini; **D** Evacuation, vitellogenic oocytes
514 detached from acinar wall and free in the lumen; **E-F** Post-evacuation, large empty acini with
515 gametogenic remains and non-evacuated oocytes. Abbreviation: CT, connective tissue; OOG,
516 oogonia; PVO, pre-vitellogenic oocyte; EVO, early vitellogenic oocyte; LVO, late vitellogenic
517 oocyte; AO, atresic oocytes; RE, gametogenic remains. Scale bars **A, B** = 100 µm; **C-F**= 50 µm.

518

519 Figure 3: Male gametogenic stages. **A.** Early development, mainly spermatocytes. **B.**
520 Differentiation stage, numerous spermatogonia and spermatocytes in the periphery. **C.** Mature,
521 presence of spermatozoa. **D.** Spent, empty acini. Abbreviations: SPC, spermatocytes; SPD,
522 spermatids; SPZ, spermatozoa. Scale bars 100 µm.

523

524 Figure 4: Monthly frequency of gametogenesis stages in *S. lessonii* during the study period. **A.**
525 Female gonad. **B.** Male gonad.

526

527 Figure 5: Size-frequency distribution of the oocyte area (μm^2) for the different gonad development
528 stages in *S. lessonii*. n_{sec} , number of sections analyzed; n_{oos} , number of oocytes. Proliferation,
529 growth, pre-evacuation, evacuation and post-evacuation.

530

531 Figure 6: Monthly variation in mean oocyte frequency and area (per microscopic section) for
532 different oocyte development stages in *S. lessonii*. **A.** Mean number (\pm SD) of oocytes stages. **B.**
533 Mean (\pm SD) oocyte area (μm^2). **C.** Mean values (\pm SD) of atmospheric (ATM) and sea surface
534 (SST) temperatures; graph bars in the secondary axis bar indicate daylight period (h).
535 Previtellogenic oocytes (PVO); early vitellogenic oocytes (EVO); late vitellogenic oocytes (LVO).

536

537 **Table 1:** Summary of data on oocyte frequency and area for the hermaphrodite gonad
538 development stages. Proliferation, growth, pre-evacuation, evacuation and post-evacuation; n ,
539 number of oocytes; mean; SD, standard deviation; minimum and maximum.

540

541 **Table 2:** Summary of the spawning periods reported for *Siphonaria* sp. over latitudinal distribution
542 in the north and south hemispheres.