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Strategies, patterns and environmental cues for reproduction in two temperate haliclonid sponges

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ABSTRACT: The reproductive biology of 2 haliclonid sponges was examined over a 2 yr period. Histological samples of *Haliclona* sp. 1 (green *Haliclona*) and *Haliclona* sp. 2 (brown *Haliclona*) from tagged and haphazardly sampled individuals of both species were examined using light microscopy. Interest in these 2 sympatric species is high, due to the potent and unique bioactive compound (salicylihalamide A) they produce, hence the need to understand the reproductive biology of both species to ensure their proper conservation and management. Green *Haliclona* is viviparous, with both gonochoric and hermaphroditic individuals in the sponges sampled. Brown *Haliclona* is also viviparous and is clearly a gonochoric species. Only decreasing wave height showed a significant correlation to gametogenesis, but the onset and progression of reproduction in both species coincided with increases in water temperature and photoperiod. Oogenesis for both species extended from November to April. Spermatogenesis extended over 3 mo for green *Haliclona* (January to March) and 4 mo for brown *Haliclona* (January to April). Embryogenesis within brood chambers started in January and extended over 4 mo in green *Haliclona* and 5 mo in brown *Haliclona*. The parenchymellae larvae of green *Haliclona* were observed in tissue samples for 2 mo compared to 4 mo for brown *Haliclona*. The reproductive output of each species was similar; however, female reproductive output at 2.9% (green *Haliclona*) and 2.4% (brown *Haliclona*) of the mesohyl was much lower than that of other viviparous species. Male reproductive output (2.3 and 2.4% for green and brown *Haliclona*, respectively) compares favourably to that of oviparous species. The high reproductive output of males and the timing of reproduction in both species of sponge appears to help in reducing the risk of unsuccessful fertilisation and lower the probability of larvae being released into unfavourable conditions.

KEY WORDS: *Haliclona* · Sexual · Fecundity · Gametogenesis

INTRODUCTION

Sponges often dominate reef fauna, particularly in temperate regions (Fromont 1999). They inhabit all areas of the oceans from tropical (De Weerdt 2000) to polar (Barnes 1995) waters, from intertidal zones (Barnes 1999) to the deep sea (Witte 1996). Their presence in these habitats may, in part, be related to their successful application of a variety of reproductive strategies (Simpson 1980), from asexual reproduction such as fragmentation (i.e. mechanical separation of parts) (Wulff 1991), budding (Corriero et al. 1998), or formation of gemmules (Fell 1995) to sexual reproduction including viviparity (Ilan 1995), oviparity (Mariani et al. 2000), gonochorism (Witte et al. 1994) and various forms of hermaphroditism (Simpson 1984, Riesgo et al. 2007). Initiation and regulation of sponge reproduction has been linked to environmental factors, including water temperature (Simpson 1980, Fromont 1994b, Witte et al. 1994, Fell 1995, Corriero et al. 1998, Ereskovsky 2000, Mercurio et al. 2007), food availability (Simpson 1980, Witte 1996), photoperiod and lunar

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phase (Simpson 1980, Fromont 1994a, Usher et al. 2004). Despite broad biological knowledge, understanding of the role of reproduction in the structuring and maintenance of sponge populations is limited (but see Whalan et al. 2005, Zilberberg et al. 2006, Riesgo et al. 2007, Whalan et al. 2007).

While the reproductive biology of many sponge taxa has been well documented in the northern hemisphere (e.g. Elvin 1976, Barthel 1986, Wapstra & van Soest 1987, Ilan & Loya 1990, Ilan 1995, Tsurumi & Reiswig 1997, Corriero et al. 1998, Ereskovsky 2000, Lepore et al. 2000, Mercurio et al. 2007, Riesgo et al. 2007), fewer studies have been undertaken in Australia (Fromont 1994a,b, Fromont 1999, Usher et al. 2004, Whalan et al. 2005, 2007), particularly for temperate species (Fromont 1999, Usher et al. 2004, Whalan et al. 2005). Further, many studies do not examine how reproductive biology influences sponge population dynamics. The paucity of knowledge may be due to the difficulties of studying reproduction where the target organism does not have designated gonads (Bergquist 1978). In addition, understanding the reproductive biology of some species is complicated by the co-occurrence of both sexual and asexual reproduction within the same populations (Battershill & Bergquist 1990, Maldonado & Uriz 1999).

As sponges are now being recognised as one of the richest sources of marine natural products (Munro et al. 1999, McClintock & Baker 2001, Paul et al. 2006), commercial demand for secondary metabolites may place increasing pressure on harvesting wild populations. Maintenance of a population is intimately linked to the reproductive strategy employed (Doherty 1994). Therefore, knowledge of the reproductive biology of the targeted organism is essential for the effective management and conservation of the organism's population.

The overall aim of the present study was to provide a detailed description of the reproductive biology of 2 sympatric sponge species (*Haliclona* sp. 1 and *Haliclona* sp. 2) that have received considerable attention from natural product chemists due to the production of a potent cytotoxic natural product, salicylihalamide A (Erickson et al. 1997). Sponges of *Haliclona* sp. 1 (hereafter green *Haliclona*; Western Australian Museum Voucher Z37485) have a massive morphology, with large chimney-like oscules (Abdo 2007). *Haliclona* sp. 2 (hereafter brown *Haliclona*; Western Australian Museum Voucher Z37486) individuals have a main body supported by a short basal stalk, with a submassive mound shape and numerous small oscules (Abdo 2007). This study was aimed to determine: (1) mode of reproduction, (2) sequence and synchrony of reproductive development, (3) relationships between size and levels of reproductive output, and (4) environmental cues of reproduction.

MATERIALS AND METHODS

Sampling procedure. The reproductive biology of *Haliclona* sp. 1 (green *Haliclona*) and *Haliclona* sp. 2 (brown *Haliclona*) was investigated at Hamelin Bay (34.20ºS, 115.04ºE) on the southwest coast of Australia from November 2004 to May 2006. No sampling was undertaken between June 2005 and October 2005 due to the closure of the boat access point during winter. Samples of tagged individuals (N = 15 for each species) and haphazardly selected individuals (N = 53 for green *Haliclona*, N = 47 for brown *Haliclona*) were taken monthly in 2004/2005, but only the tagged individuals were sampled in 2005/2006. Comparisons between reproductive periods using periodic regression (detailed below) indicate that the pattern of reproduction reported here was not influenced by the reduction in sampled individuals or by repeated sampling of tagged individuals.

The size of tagged sponges was determined using an underwater stereo camera to capture a series of stereo images of each sponge (Abdo et al. 2006). Each set of images was then processed in the laboratory to obtain a volume estimate for each sponge (Abdo et al. 2006).

Reproductive elements in some species of sponge can either occur throughout the mesohyl (Bergquist 1978) or be isolated in certain areas (Fromont 1999), and a pilot study was undertaken to determine the distribution of reproductive elements within the mesohyl for both species of *Haliclona* studied (Whalan et al. 2007). Tissue was taken from the sponge surface (top 5 cm), from the middle of the mesohyl and from basal regions of reproductive individuals (N = 10 from each species). A 1-way ANOVA (Zar 1999) confirmed that reproductive elements were not confined to specific regions, with no significant differences in the numbers of elements between regions of the sponge for both green *Haliclona* ($F = 0.55$, df = 2, $p = 0.581$) and brown *Haliclona* ($F = 0.09$, df = 2, $p = 0.917$). Data were checked for normality and homogeneity of variance.

Histology. In the main study, tissue samples were collected by divers on SCUBA by cutting a small piece of tissue (approximately 4 cm^3) haphazardly from each individual. The excised tissue was placed into histology cassettes and fixed in gonad fixative (FAACC—100 ml = 10 ml of 10% formaldehyde solution, 5 ml of 5% glacial acetic acid, 1.3 g of calcium chloride dihydrate and 85 ml of tap water) after collection (Fromont 1999, Whalan et al. 2007). After 48 h, samples were transferred into 70% ethanol until histological preparation.

Using a histological tissue processor, samples were run through an alcohol and xylene sequence, and impregnated under vacuum with paraffin wax. Samples were blocked in paraffin wax and sectioned at

8 µm with a microtome. Tissue sections were stained with haematoxylin & eosin, mounted and examined by light microscopy to identify reproductive elements (Fromont 1999, Whalan et al. 2007).

Determination of reproductive output (RO). Using a digital camera (Olympus C5050) mounted onto a light microscope (Olympus CH2), digital images were captured of the reproductive elements within each tissue section. Digital imaging software (ReproMeasure by SeaGIS: www.seagis.com.au) was used to calculate the diameters and area of reproductive elements. The area of the tissue section was also determined to allow the calculation of the reproductive output (RO) for each sponge sampled, and the number of reproductive elements per mm^2.

RO was calculated by determining the total area of reproductive elements within the total area of sectioned tissue (i.e. percentage of reproductive elements per mm^2). Total reproductive output for each sponge (RO_t) was calculated by converting RO to a volume (multiplying the RO by the thickness of the section, i.e. 8 µm), then multiplying this by the volume of the sponge (Whalan et al. 2007).

Reproductive elements were classified as oocytes, embryos, larvae, or spermatocysts. The relatively low numbers of oocytes, embryos and larvae allowed the counting and measurement (i.e. area and diameter) of all the reproductive elements within each tissue section (Whalan et al. 2007). As spermatocysts had a much greater abundance, the total number of spermatocysts was determined by counting all spermatocysts within 5 separate haphazardly selected fields of view (FOV). The counts from each FOV (equivalent to 1 mm^2) were then averaged for the tissue section (Whalan et al. 2007). Measurements of spermatocyst diameter and area were made of 10 spermatocysts from each of the 5 FOV and averaged for each tissue section. Oocytes were only measured when sectioned through the nucleus, whereas embryos, larvae and spermatocysts were measured if sectioned medially. Following Whalan et al. (2007), the accuracy of RO as a measure of reproductive output was examined by analysis of the correlation (using Pearson's correlation coefficient r; Zar 1999) between RO and the total number of reproductive elements. A significant correlation was observed between the RO and total number of reproductive elements for both green *Haliclona* (males [including those with oocytes]: $r = 0.918$, $p < 0.001$; females: $r = 0.917$, $p < 0.001$) and brown *Haliclona* (males: $r = 0.931$, $p < 0.001$; females: $r = 0.894$, $p = < 0.001$). This indicates that RO is a valid measure of reproductive output for both species (Whalan et al. 2007).

Physical data. Water temperatures were recorded at Hamelin Bay using 2 Onset StowAway Tidbit temperature loggers, which were sampled at hourly intervals. These data were averaged to give monthly temperature for the sampling period. Photoperiod data were obtained by calculating the total hours of sunlight from sunrise and sunset times (for Hamelin Bay) obtained from Geosciences Australia (www.ga.gov.au/geodesy/astro/sunrise.jsp), and were averaged over each month. Wave height data were obtained from the Western Australian Department of Planning and Infrastructure's Coastal Data Centre (www.dpi.wa.gov.au/imarine/13461.asp). Hourly measurements of total wave height were averaged to give mean total wave height for each month over the study period.

Statistical analysis. Periodic regression was used to determine the cyclical patterns of RO for each reproductive period (Year 1: 2004/2005; Year 2: 2005/2006) separately for each sponge species (Batschelet 1981, deBruyn & Meeuwig 2001). In periodic regression, the independent variable is an angular representation of time, where the yearly data is divided into 360° to give each month an angular equivalent, θ (deBruyn & Meeuwig 2001). The transformed data are then analyzed by simple linear regression (deBruyn & Meeuwig 2001). This technique was used because it is more powerful, robust and less sensitive to missing data, which is common in the cyclical data recorded in reproduction studies (Batschelet 1981, deBruyn & Meeuwig 2001). The slopes of the periodic regression analysis were compared using a *t*-test (Zar 1999) to determine if there was a difference in the RO between years for each sponge species. Data were checked for normality prior to analysis.

To determine any correlations between reproductive output (both for RO and RO_t) and sponge size (Whalan et al. 2007), Pearson's correlation coefficient was calculated for both male and female sponges (Zar 1999). *t*-tests were used to test for differences in the sizes of sponges with oocytes and embryos, compared to those which did not produce female reproductive elements for each species separately (Zar 1999). Data were checked for normality prior to analysis. Pearson's r was also used to test for correlations between the percentage of reproductive sponges and RO (pooled for males and females) for each sponge species, with water temperature, photoperiod and total wave height. Bonferroni corrections were applied for the correlation analyses.

RESULTS

Reproductive cycle

Green *Haliclona* was viviparous, brooding both eggs and larvae, with examples of gonochorism and hermaphroditism. Female sponges were reproductive from November through April. Male sponges were reproductive for 3 mo (January to March) (Fig. 1a). During

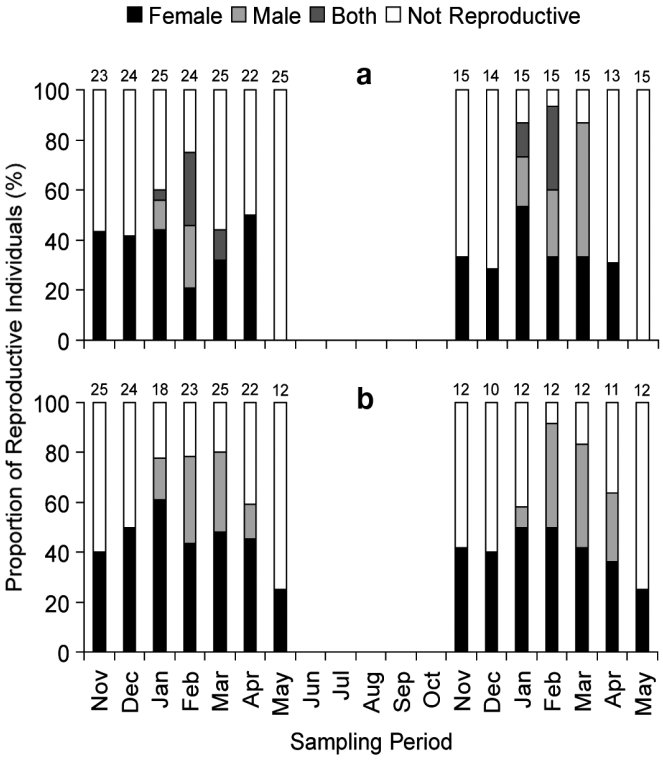

Fig. 1. *Haliclona* spp. Proportion of female, male and non-reproductive sponges for: (a) green and (b) brown *Haliclona*. No sampling took place between June and October. Numbers above each bar represent the total numbers of sponges sampled for each month

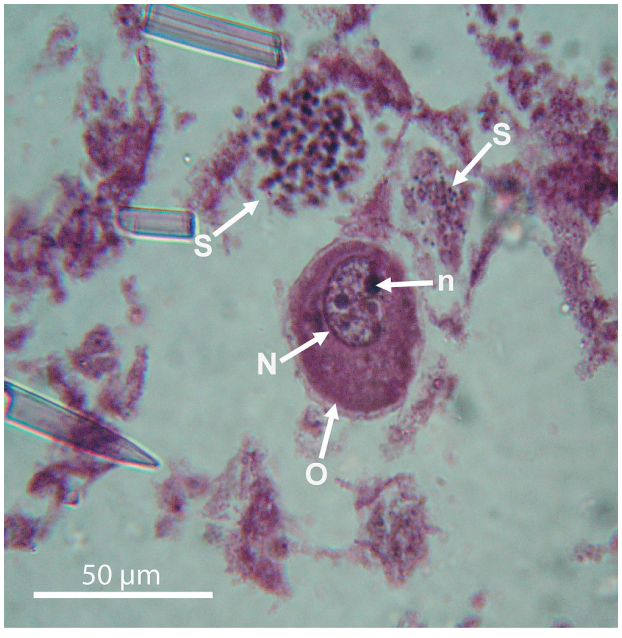

Fig. 2. *Haliclona* sp. 1. Oocytes present in male sponges of green *Haliclona* between January and March. S: spermatocyst; O: oocyte; N: nucleus; n: nucleolus of oocyte

this period several male sponges were found to have oocytes and spermatocysts present within their tissue (Fig. 2); however these sponges were never observed to produce embryos or larvae. No reproductive individuals were present after April (Fig. 1a).

Brown *Haliclona* was also viviparous, developing male and female reproductive elements in separate individuals and clearly showing this species to be gonochoric. Female and male reproductive elements were never observed in the same individual during the study period. Female sponges were reproductive over the entire sampling period (November to May) and male sponges were reproductively active from January to April (Fig. 1b). In brown *Haliclona*, reproduction was still evident when sampling ceased in May. No samples were taken from June to October, and interpretations are based on the 7 mo (from both years) during which samples were taken.

Reproductive development

Spermatogenesis

Spermatogenesis in both species occurs asynchronously (within an individual) throughout the mesohyl, but is synchronous within a spermatocyst. Also, spermatogenesis was asynchronous among individuals during the 3 mo spermatocysts were present in the mesohyl. Spermatic cysts were observed in green *Haliclona* from January until March and were spherical in shape, with mean cyst diameter measuring 26.3 ± 1.7 µm (±SE) (Fig. 3a). In brown *Haliclona*, spermatic cysts were observed from January through to April and were also spherical in shape, with a mean diameter of 26.3 ± 2.8 µm (±SE) (Fig. 3b).

Oogenesis, embryogenesis and larval development

Green *Haliclona* had consistent timing and periods of oogenesis, embryogenesis and larval development over both reproductive periods (Fig. 4a). Oogenesis occurred from at least November, extending asynchronously both within and among individuals until April. Oocyte size remained similar over this period, with a mean oocyte diameter of 51.1 ± 2.7 µm (mean ± SE) (ranging from 45.1 to 59.1 µm). Oocytes were spherical to elliptical in shape, with a large nucleus and nucleolus (Fig. 5a). Oocytes present in sponges producing spermatocysts were significantly (*t*-test: $t = -5.05$, df = 72, $p < 0.01$) smaller in mean (±SE) size (36.9 ± 1.4 µm) than those observed in female sponges (54.9 ± 2.4 µm).

Embryogenesis occurred within brood chambers throughout the sponge mesohyl, indicating migration of

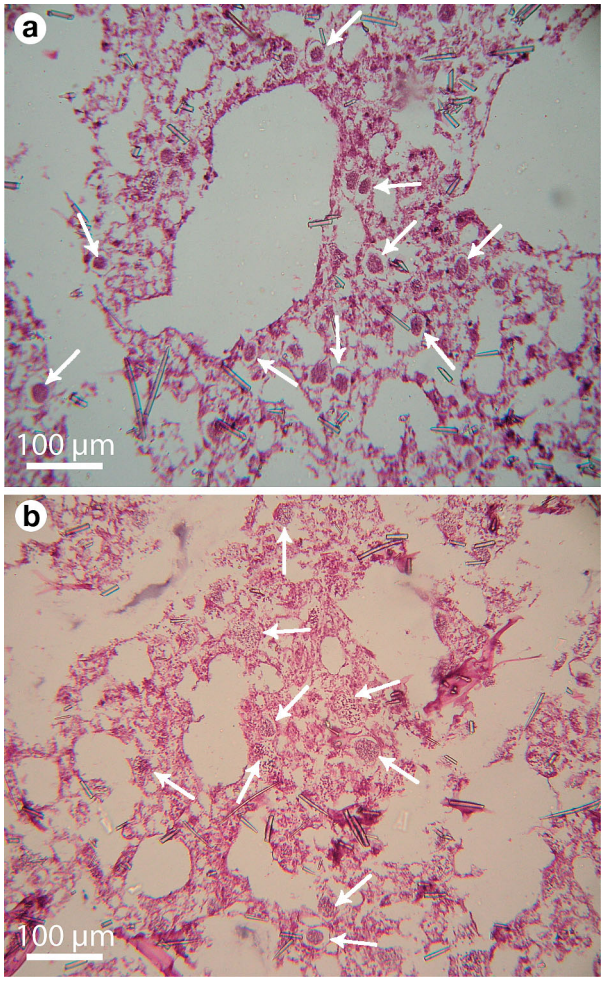

Fig. 3. *Haliclona* spp. Spermatocysts present in male sponges of: (a) green and (b) brown *Haliclona*. Arrows indicate spermatocysts

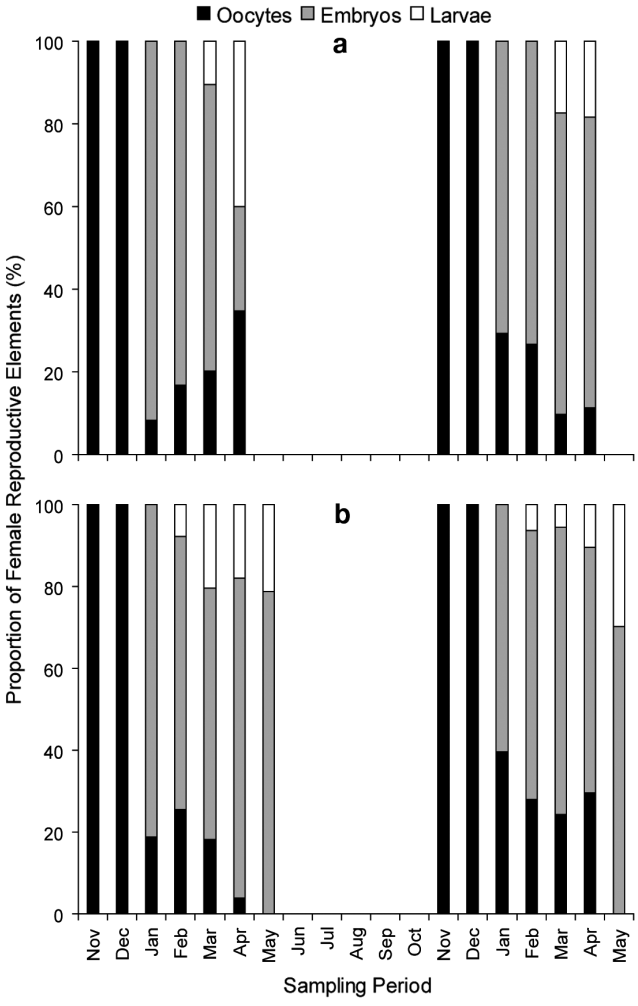

Fig. 4. *Haliclona* spp. Timing of oogenesis, embryogenesis and larval development in: (a) green and (b) brown *Haliclona*. No sampling took place between June and October

oocytes to these reproductively active regions. Embryos were first observed in January (Figs. 4a & 5b). Embryo development was asynchronous within and between brood chambers among individual sponges (Fig. 5c). The mean (±SE) diameter of embryos increased from January (130.9 ± 14.1 µm) to a maximum mean size (454.9 ± 36.0 µm) in March. Embryos decreased in mean (±SE) size (299.1 ± 60.4 µm) in April, and no embryos were present in May (Table 1, Fig. 4a).

The first parenchymellae larvae were observed in March and were present within brood chambers for 2 mo (Fig. 4a). Green *Haliclona* larvae had a mean (±SE) length of 1041 ± 22 µm and could be distinguished from embryos due to differentiation of the larval epithelium of columnar cells (Fig. 5d).

The timing of oogenesis, embryogenesis and larval development in brown *Haliclona* was also similar for both years over the sampling period (Fig. 4b). Oogenesis extended from November until April throughout the mesohyl. Oocyte size was also consistent over this period with a mean (±SE) oocyte diameter of 67.3 ± 2.2 µm (ranging from 61.1 to 75.5 µm), with a large nucleus and nucleolus (Fig. 6a). Embryogenesis in brown *Haliclona* extended from January through to May (Fig. 4b), with several stages of development present within a brood chamber. Brood chambers were found throughout the sponge mesohyl, and similarly oocytes appear to have migrated into brood chambers as observed for green *Haliclona* (Fig. 6b,c). Embryogenesis was asynchronous within and between brood chambers, as well as among individuals. Embryo mean (±SE) size increased from 262.8 ± 40.3 µm in January to a peak during March (474.5 ± 67.5 µm) (Fig. 6b). Embryos decreased in size to a mean (±SE) of 297.5 ± 51.2 µm in May. Brown *Haliclona* parenchymellae larvae were first observed in February, and were present within brood chambers until May, with a mean (±SE) length of 1097 ± 31 µm (Figs. 4b & 6d).

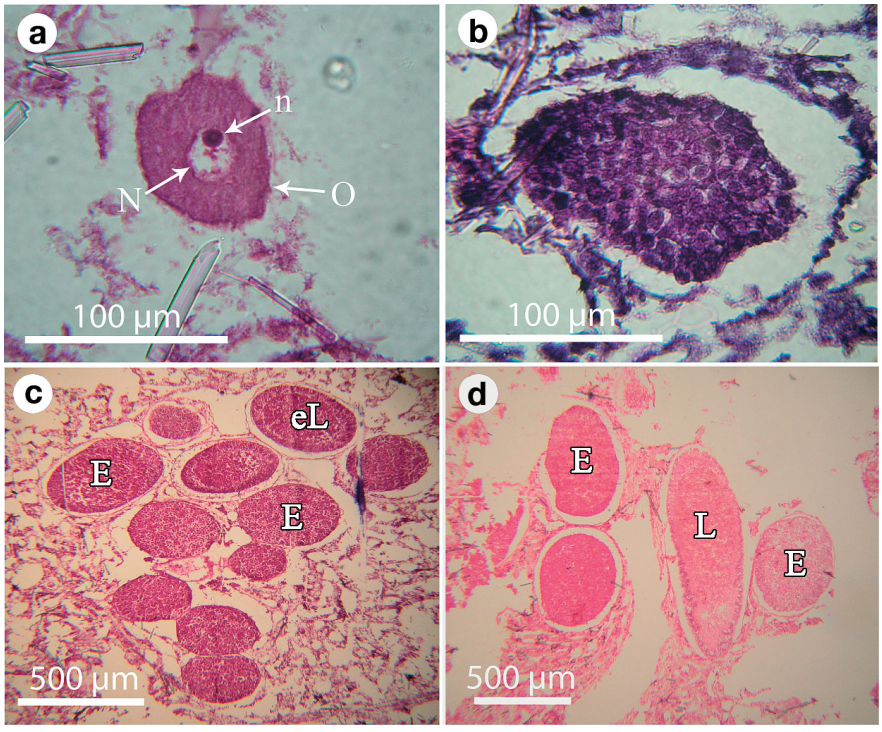

Fig. 5. *Haliclona* sp. 1. (a) Oogenesis, (b) early embryos, (c) brood chamber with different stages of embryogenesis and (d) larvae. N: nucleus; n: nucleolus; O: oocyte; L: larvae; E: embryo; eL: early larval stage

Table 1. *Haliclona* spp. Density of propagules and percentage of reproductive tissue (i.e. reproductive capacity) for green and brown *Haliclona*. NS: no sampling

		Spermatocysts (no. mm^{-2})	Oocytes (no. mm^{-2})	Embryos (no. mm^{-2})	Larvae (ind. mm^{-2})	% ♂ Tissue	% ♀ Tissue
Green *Haliclona*							
2004	Nov		0.042 (±0.012)				0.006 (±0.002)
	Dec		0.062 (±0.029)				0.006 (±0.002)
2005	Jan	43.86 (±5.94)	0.018 (±0.003)	0.195 (±0.045)		2.34 (±0.37)	1.18 (±0.277)
	Feb	22.06 (±2.53)	0.026 (±0.005)	0.132 (±0.026)		1.19 (±0.14)	1.69 (±0.344)
	Mar	27.27 (±16.36)	0.031 (±0.006)	0.108 (±0.029)	0.016 (±0.005)	0.72 (±0.41)	1.47 (±0.385)
	Apr		0.028 (±0.004)	0.020 (±0.002)	0.032 (±0.007)		0.48 (±0.156)
	May						
	Jun–Oct	NS	NS	NS	NS	NS	NS
	Nov		0.051 (±0.008)				0.01 (±0.003)
	Dec		0.084 (±0.022)				0.02 (±0.010)
2006	Jan	19.04 (±5.60)	0.033 (±0.009)	0.080 (±0.031)		0.87 (±0.32)	0.24 (±0.115)
	Feb	45.14 (±2.40)	0.048 (±0.018)	0.133 (±0.031)		2.06 (±0.12)	1.23 (±0.439)
	Mar	18.18 (±3.07)	0.025 (±0.005)	0.185 (±0.021)	0.044 (±0.015)	0.48 (±0.12)	2.92 (±0.524)
	Apr		0.014 (±0.007)	0.087 (±0.032)	0.022 (±0.003)		1.43 (±0.440)
	May						
	Mean (overall)	29.26 (±4.99)	0.039 (±0.006)	0.112 (±0.02)	0.029 (±0.006)	1.28 (±0.05)	0.89 (±0.06)
Brown *Haliclona*							
2004	Nov		0.064 (±0.012)				0.009 (±0.002)
	Dec		0.043 (±0.007)				0.007 (±0.001)
2005	Jan	18.13 (±5.47)	0.028 (±0.005)	0.123 (±0.051)		1.04 (±0.49)	1.28 (±0.602)
	Feb	22.16 (±3.36)	0.055 (±0.009)	0.143 (±0.030)	0.017 (±0.003)	1.55 (±0.26)	2.36 (±0.804)
	Mar	12.82 (±3.03)	0.033 (±0.015)	0.112 (±0.024)	0.037 (±0.017)	0.73 (±0.22)	2.16 (±0.646)
	Apr	19.30 (±2.54)	0.005 (±0.002)	0.094 (±0.029)	0.022 (±0.005)	0.69 (±0.14)	1.65 (±0.475)
	May			0.082 (±0.047)	0.022 (±0.007)		1.38 (±0.897)
	Jun–Oct	NS	NS	NS	NS	NS	NS
	Nov		0.049 (±0.015)				0.02 (±0.005)
	Dec		0.084 (±0.034)				0.03 (±0.015)
2006	Jan	14.80 (±3.89)	0.064 (±0.021)	0.097 (±0.025)		0.18 (±0.12)	1.24 (±0.348)
	Feb	44.21 (±5.51)	0.049 (±0.014)	0.115 (±0.041)	0.011 (±0.005)	2.39 (±0.42)	2.20 (±0.748)
	Mar	14.83 (±1.90)	0.027 (±0.011)	0.077 (±0.026)	0.006 (±0.000)	0.90 (±0.18)	1.52 (±0.531)
	Apr	11.39 (±0.85)	0.015 (±0.000)	0.031 (±0.011)	0.005 (±0.000)	0.39 (±0.13)	0.63 (±0.342)
	May			0.014 (±0.008)	0.006 (±0.000)		0.11 (±0.082)
	Mean (overall)	19.704 (±3.72)	0.043 (±0.006)	0.089 (±0.013)	0.016 (±0.004)	0.99 (±0.05)	1.04 (±0.09)

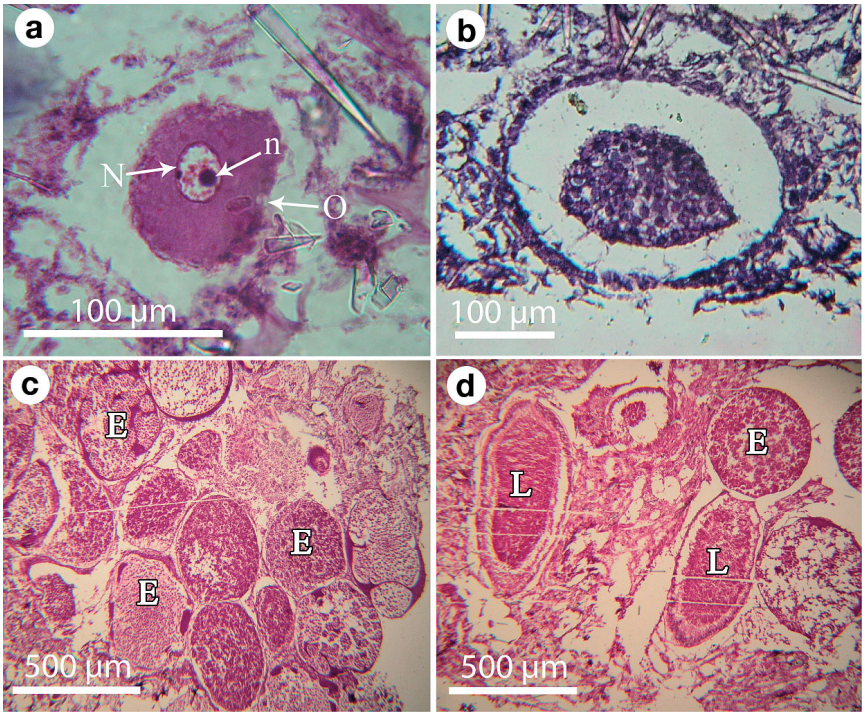

Fig. 6. *Haliclona* sp. 2. (a) Oogenesis, (b) early embryogenesis, (c) multiple stages of cleavage in brood chambers and (d) larvae. N: nucleus; n: nucleolus; O: oocyte; L: larvae; E: embryo

Reproductive output

RO was highest in males of green *Haliclona*, with a mean (±SE) of 1.28 ± 0.05% of the mesohyl devoted to gametes, whereas females had 0.89 ± 0.06% of their mesohyl devoted to reproductive elements (Table 1). Reproductive output was highest for green *Haliclona* from January to March, when both male and female RO was at its peak (except for the second year, when high female RO extended to April) (Table 1). The density of reproductive elements (number per square millimetre) follows a similar trend, with greater mean (±SE) numbers of male gametes (29.3 ± 5.0) compared to female elements (0.13 ± 0.02) per mm^2. Reproductive output did not differ significantly between years (*t*-test: $t = 1.463$, df = 10, $p < 0.05$) when the coefficients of the periodic regressions of RO for each reproductive period were compared. $RO_{Year\ 1} = -0.396 + 2.80(\pm 0.84)\text{cosine}(\theta) + 1.53(\pm 0.61)\text{sine}(\theta)$; $RO_{Year\ 2} = 0.248 + 2.27(\pm 0.80)\text{cosine}(\theta) + 1.98(\pm 0.59)\text{sine}(\theta)$.

The reverse situation occurred in brown *Haliclona*, where females had the highest RO at 1.04 ± 0.09% (±SE) compared to males with 0.98 ± 0.05% of the mesohyl occupied by gametes. Reproductive output peaked in February for brown *Haliclona*, which coincided with peaks in both male (1.55% [Year 1] and 2.39% [Year 2]) and female RO (2.36% [Year 1] and 2.20% [Year 2]) (Table 1). The mean (±SE) density of reproductive elements was higher in males (19.7 ± 3.7) compared to females (0.138 ± 0.023). There was no significant difference (*t*-test: $t = 0.76$, df = 10, $p < 0.05$) in RO of brown *Haliclona* between years [$RO_{Year\ 1} = 0.410 + 1.90(\pm 0.59)\text{cosine}(\theta) + 2.13(\pm 0.43)\text{sine}(\theta)$; $RO_{Year\ 2} = -0.373 + 2.66(\pm 1.03)\text{cosine}(\theta) + 1.95(\pm 0.76)\text{sine}(\theta)$].

Size and reproductive output

The size of individuals of green *Haliclona* ranged from 301 to 6976 cm^3. The mean (±SE) size of male sponges was 3247 ± 954 cm^3 (range 301 to 6976 cm^3), compared to the mean (±SE) size of female sponges at 2473 ± 707 cm^3 (range 587 to 4724 cm^3). No significant correlation was found between male sponge size (including individuals producing oocytes) and RO ($r = 0.085$, $p = 0.828$). There was also no significant correlation between the size and RO of female green *Haliclona* ($r = 0.659$, $p = 0.155$). However, for both the male and female sponges there was a significant positive correlation between sponge size and RO_t (male sponges: $r = 0.928$, $p < 0.01$; female sponges: $r = 0.968$, $p < 0.01$). There was no significant difference in size between sponges that produced embryos and those that did not (*t*-test: $t = -0.76$, df = 13, $p = 0.460$).

Brown *Haliclona* individuals ranged in size from 37 to 868 cm^3. Male sponges had a mean (±SE) size of 324 ± 73 cm^3 (range 124 to 750 cm^3), whereas females had a mean (±SE) size of 316 ± 141 cm^3 (range 37 to 868 cm^3). No significant correlation was observed between the size of male brown *Haliclona* and their RO ($r = 0.157$, $p = 0.686$), or between the size of females and their respective RO ($r = -0.309$, $p = 551$). A signifi-

cant positive correlation was found between sponge size and RO_t for both the male and female brown *Haliclona* (males: r = 0.872, p < 0.01; females: r = 0.975, p < 0.01). There was also no significant difference between the size of female (i.e. produce oocytes) and male (i.e. no oocytes) sponges (*t*-test: *t* = –0.06, df = 13, p = 0.955).

Environmental cues

Although RO in both *Haliclona* species coincided with increasing water temperature (Fig. 7a), there was no statistically significant correlation between water temperature and the percentage of sponges that were reproductively active (green *Haliclona*: r = –0.15, $p_{\alpha=0.017}$ = 0.611; brown *Haliclona*: r = 0.02, $p_{\alpha=0.017}$ = 0.939) or the RO (green *Haliclona*: r = 0.244, $p_{\alpha=0.017}$ = 0.400; brown *Haliclona*: r = 0.159, $p_{\alpha=0.017}$ = 0.586). A significant drop in temperature was recorded in February (compared to January and March) (1-way ANOVA: *F* = 2539.19, df = 2, p < 0.01). It should be noted that the decrease in water temperature started at the end January and temperatures only began increasing after the beginning of March, which is not reflected in the monthly averages. The production of oocytes in males of green *Haliclona* also occurred during this time of decreasing water temperature (Fig. 8).

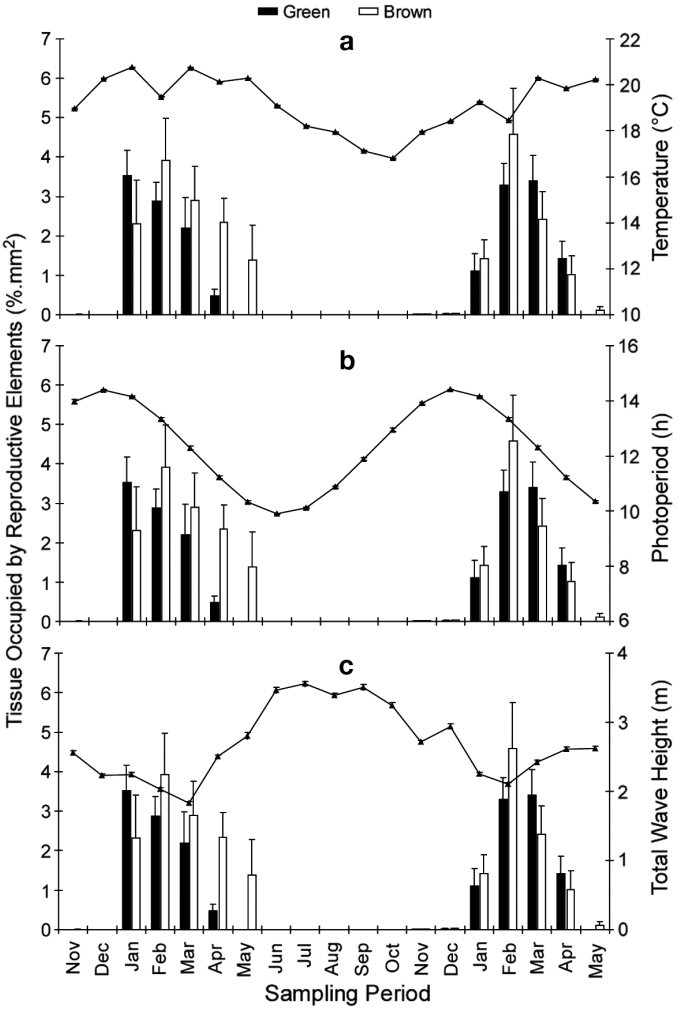

Fig. 7. *Haliclona* spp. Total reproductive capacity (i.e. % tissue occupied by reproductive elements) for green and brown *Haliclona*, with (a) water temperature, (b) photoperiod and (c) total wave height. Note temperature and photoperiod axes do not begin at zero; no sampling between June and October

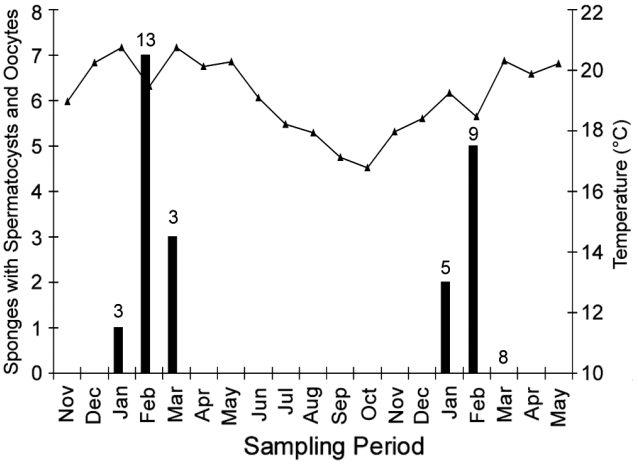

Fig. 8. *Haliclona* sp. 1. Number of male green sponges with oocytes and corresponding water temperature. Numbers above bars indicate total number of sponges with spermatocysts. Note temperature axis does not begin at zero, and sampling did not occur between June and October

Reproductive onset for each species coincided with the maximum photoperiod (Fig. 7b). No significant correlation was found for either species between photoperiod and the percentage of reproductive individuals (green *Haliclona*: r = 0.49, $p_{\alpha=0.017}$ = 0.074; brown *Haliclona*: r = 0.33, $p_{\alpha=0.017}$ = 0.243) or the RO (green *Haliclona*: r = 0.125, $p_{\alpha=0.017}$ = 0.677; brown *Haliclona*: r = –0.077, $p_{\alpha=0.017}$ = 0.794).

Increasing reproductive output coincided with a reduction in total wave height, and both variables were significantly correlated for green (r = –0.653, $p_{\alpha=0.017}$ = 0.011) and brown *Haliclona* (r = –0.690, $p_{\alpha=0.017}$ < 0.01). A significant correlation between total wave height and the percentage of reproductive individuals was also observed for green (r = –0.621, $p_{\alpha=0.017}$ = 0.018) and brown *Haliclona* (r = –0.720, $p_{\alpha=0.017}$ = 0.004) (Fig. 7c).

DISCUSSION

Reproductive development and sexuality

Both haliclonid species examined in the present study were viviparous and brooded eggs and paren-

chymellae larvae. These results are similar to studies of other species of Haplosclerida (Fromont 1994a,b, 1999) (Table 2). Viviparity is probably highly advantageous due to the protection of developing larvae, enhancing the chances of offspring survival and recruitment into suitable habitats (Ayling 1980, McEdward & Miner 2003). However, greater investment of energy reserves into fewer larvae generally decreases the output of larvae, and thus the numbers of potential recruits (McEdward 1997). Understanding this is particularly important for the correct management and conservation of potentially exploitable species, such as the haliclonid sponges examined here.

Brown *Haliclona* was clearly gonochoric, while green *Haliclona* appears to be hermaphroditic, as oocytes were observed within the tissue of some male sponges. Although gonochorism cannot be excluded in green *Haliclona*, as some individuals only showed the presence of either male or female reproductive elements during the period of study. This is consistent with other haplosclerids in which both gonochoristic and hermaphroditic species have been recorded (Simpson 1984, Fromont 1994a,b). The production of oocytes by males has been reported previously in several haplosclerid species (e.g. *Haliclona loosanoffi* and *H. permollis*) and in other orders of sponge (e.g. *Hymeniacidon sanguinea* and *Stelleta grubii*) (Elvin 1976, Fell 1976, Simpson 1984, Wapstra & van Soest 1987). The production of oocytes by males may be due to a proportion of the green *Haliclona* population being hermaphroditic, while other individuals within the population have a gonochoric habit. This has been reported previously (Lewandrowski & Fell 1981, Simpson 1984, Wapstra & van Soest 1987), as has the alternation between a mostly gonochoric and a mostly hermaphroditic population (Fell & Jacob 1979, Simpson 1984). However, the oocytes found in green *Haliclona* sponges that also produced spermatic cysts were never observed to develop into embryos, unlike hermaphroditic individuals of *Halichondria* sp. that did produce embryos (Fell & Jacob 1979). The 'abortive' oocytes observed in this study were significantly smaller than those produced by females, and it seems more probable that environmental factors may be influencing the physiology of those individuals. Sex determination in sponges is thought to be physiological rather than genetic (Simpson 1984). In the present study, oocytes in male green *Haliclona* were observed during a period of significant cooling of water temperature during February (which starts halfway through January and ends in the first half of March). This cooling is related to the Capes Current, which produces cold water upwelling at Hamelin Bay during the summer months (Gersbach et al. 1999, Pearce & Pattiaratchi 1999, Hanson et al. 2005). Oocyte development in males was also found in Elvin's (1976) study on the effect of temperature on *Haliclona permollis*, where oocytes in males and the presence of hermaphroditic individuals occurred after a period of cooling. It is unclear why only a proportion of males is influenced by this temperature change; possibly, individuals in the population are alternating between gonochorism and hermaphroditism (Fell & Jacob 1979). Manipulative experiments and continued monitoring of the population will confirm or disprove the relationship, but the data suggest that the production of oocytes in green *Haliclona* males is related to cooling water temperatures.

Table 2. Reproductive strategies and fecundity of various sponge species. Sponges examined in the present study are in bold. V: viviparous; O: oviparous; G: gonochoric; H: hermaphroditic; G/H: indeterminate sexuality

Species	Development	Sexuality	n	Fecundity				Source
				♂ (no. mm^{-2})	♂ (%)	♀ (no. mm^{-2})	♀ (%)	
Crambe crambe	V		50			7.62[a]		Uriz et al. (1998)
Geodia cydonium	O	G	10		0.07–0.21		0.004–0.2	Mercurio et al. (2007)
Haliclona amboinensis	V	G/H	3	0.7–1.5[a]		1.3–2.4[a]		Fromont (1994a)
Haliclona cymiformis	V	G/H	3	0.8–1.6[a]		0.5–0.8[a]		Fromont (1994a)
Green *Haliclona*	**V**	**G/H**	**68**	**18.2–45.1**	**0.48–2.34**	**0.04–0.25**	**0.01–2.92**	present study
Brown *Haliclona*	**V**	**G**	**62**	**11.4–44.2**	**0.18–2.39**	**0.04–0.21**	**0.01–2.39**	present study
Mycale contarenii	V		360			0.07–0.2	1.3–1.5	Corriero et al. (1998)
Niphates nitida	V	G/H	3	0.6–2.4[a]		1.6–3.2[a]		Fromont (1994a)
Rhopaloeides odorabile	V	G	181	4.6–14	0.72–2.97	0.01–0.13	0.02–1.03	Whalan et al. (2007)
Scopalina lophyropoda	V					1.25[a]		Uriz et al. (1998)
Tetilla sp.—deep		G	10			155		Meroz-Fine et al. (2005)
Tetilla sp.—shallow		G	10			19.3–88.2		Meroz-Fine et al. (2005)
Xestospongia bergquistia	O	G	3	5.9[a]		1.8–3.1[a]		Fromont (1994a)
Xestospongia exigua	O	G/H	3	7.2[a]		10[a]		Fromont (1994a)
Xestospongia testudinaria	O	G	3	9.7[a]		1.5–1.9[a]		Fromont (1994a)

[a]Data adjusted to consistent area unit (mm^{-2})

Environmental cues

Reproduction in both *Haliclona* species was significantly correlated with decreasing total wave height, although reproduction did coincide with increasing water temperature. The start of reproduction in both species corresponded to a peak in photoperiod. Peak reproductive activity and its association with environmental variables may have important consequences for the reproductive success of both species. Reproduction took place during periods of warm seawater temperatures (generally thought to be needed for spermatogenesis to occur; Simpson 1984) and lower wave action. The primary source of water motion arises from wave action, as tidal influences in the Hamelin Bay region are relatively small (i.e. maximum tidal range of approximately 80 cm). Therefore, reproduction during periods of lower wave action may reduce the risks of dilution of gametes and possible unsuccessful fertilisation as a consequence (Oliver & Babcock 1992). However, this is in contrast to the period of reproductive activity reported for the only other temperate haliclonid species studied in Australia, where development of embryos and larvae occurred during the austral winter (Fromont 1999).

Reproductive output

Reproduction in females of both green and brown *Haliclona* occurred over an extended period of 6 and 7 mo, respectively. Extended periods of oogenesis have been found for other sponge species (see Riesgo et al. 2007), and may relate to the source of investment (e.g. yolk self-synthesized by oocyte or provided by nurse cells) during the production of the oocytes (Riesgo et al. 2007). The period of oogenesis for both species may be longer than recorded here, as no sampling took place prior to November. However, in green *Haliclona* no evidence or reproductive activity was found after April, and oocytes were also not observed within brown *Haliclona* after April. This suggests that reproduction ceases over winter. Moreover, only a few studies have reported oogenesis to extend over most of the year (Riesgo et al. 2007).

Spermatogenesis extended over a period of 3 and 4 mo in green and brown *Haliclona*, respectively. This is a relatively long period compared to that in some species (in which it may only last a few weeks), but is similar to that reported for *Corticium candelabrum* and *Rhopaloeides odorabile* (Riesgo et al. 2007, Whalan et al. 2007). The extended periods of reproductive output observed for both sponge species studied here are likely to be beneficial in the maintenance of both populations by increasing the chances of successful fertilisation (i.e. in males) and release of offspring (i.e. by females) into favourable conditions.

The degree of parental care and its associated energy cost directly influences the reproductive success of an organism (Giese & Pearse 1974, McEdward 1997). The female reproductive capacities of both species studied here were similar; however, both had reproductive capacities lower than those reported for other haplosclerids and other viviparous sponges (Table 2). Further, the output of females in both haliclonid species was much lower than that in oviparous species (Table 2). Interestingly, a decrease in embryo size was observed for both species over the period of monitoring. This has been observed in other sponge species (e.g. *Corticium candelabrum*: Riesgo et al. 2007), and was suggested to result from a depletion of maternal energy reserves. Low maternal energy reserves may also account for the low female reproductive output observed here (Ramirez Llodra 2002).

Male reproductive output was similar for both species and higher than the fecundity of other viviparous species, except *Rhopaloides odorabile* (Table 2). The reproductive output of the species examined here is similar to that reported for some oviparous species (e.g. *Xestospongia berquista*, *X. exigua*, and *X. testudinaria*: Fromont 1994a). This high output and the asynchronous development both within and between individuals for each species (suggesting multiple spawning events) may reduce the risk of gamete dilution in the high-energy environment that the species inhabit and increase the chances of successful fertilisation. This is further evidenced by the asynchronous development of female reproductive elements in both species.

The consistency of reproductive output over both reproductive periods (as shown by periodic regression analysis) also suggests that the repeated sampling of tagged individuals had little impact on their reproductive output. Moreover, both species display positive growth (~3 and 5% mo^{-1} for green and brown *Haliclona*, respectively) during their summer reproductive period (Abdo unpubl. data).

Size and reproductive output

No relationship between size and reproductive output was found for either *Haliclona* species. This suggests that small individuals have the same level of reproductive output (per mm^2) as larger individuals. Early reproductive maturity would benefit the maintenance of each species population by increasing the supply of offspring and, in turn, the chances of successful recruitment. Early sexual maturity has been documented for several species (Fell et al. 1979, Meroz & Ilan 1995), but determining the age of sexual maturity

in sponges is difficult to evaluate due to their highly plastic and indeterminate growth patterns (Garrabou & Zabala 2001). In *Mycale fistulifera*, it was suggested that early sexual maturity helps maintain and increase the population in the face of limited space and aggressive competition (Meroz & Ilan 1995). However, a significant correlation was observed for males and females of both species when reproductive output was calculated based on the total volume of the individual, indicating that larger sponges have a greater total reproductive output. Whilst both species reach sexual maturity at an early stage, fecundity clearly increases with the size of sponges. Size-related reproductive output differs among sponge species, reported for some species (Uriz et al. 1995, Whalan et al. 2007), while being absent in others (Ayling 1980, Fromont 1994b). The patterns of reproductive output with body size, found here for both green and brown *Haliclona*, compare with those found for *Rhopaloeides odorabile* and *Crambe crambe* (Uriz et al. 1995, Whalan et al. 2007).

The data on reproductive mode, patterns, fecundity and environmental cues of reproduction in the present study provide valuable information on green and brown haliclonid sponge populations. Both species were found to be viviparous and showed clear gonochorism in brown *Haliclona*, but a mixed population of hermaphroditic and gonochorism individuals in green *Haliclona*. Both species' reproduction is closely timed with seasonal changes in environmental conditions, and their extended periods of gametogenesis are likely to increase the chances of fertilisation, offspring survival and recruitment. Further questions still require investigation to gain a complete understanding of the dynamics of each species' population. For example, the factors and processes influencing larval settlement and post-settlement survival are of particularly importance to completing our understanding of the dynamics of a sponge's population. The fundamental reproductive information provided by this study is particularly timely, as green *Haliclona* is potentially exploitable due to the bioactive compound it produces. Understanding how the sponges reproduce and what may influence their reproduction is critical in determining how each species' population is maintained, which is vital for their effective long-term management and conservation.

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