Remarkable Consistency of Larval Release in the Spermcast-Mating Demosponge *Amphimedon queenslandica* (Hooper and van Soest)

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Abstract: Many marine invertebrates, including many sponge species, reproduce by spermcast spawning, in which sperm released externally disperse in the water column to fertilize eggs retained internally by the maternal adult. The population consequences of a sexual reproduction mode that depends upon uptake of free spermatozoa from dilute suspension in the water column are not yet well understood. In the spermcast-spawning tropical demosponge *Amphimedon queenslandica*, we observed continuous fertilization and development in healthy maternal brood chambers. This results in a constant release of larvae into the water column. On average in our study population on Heron Island reef, a hermaphroditic adult will have 45 potential sperm donors available within a 4 m radius to fertilize the eggs retained within its brood chambers. A single adult may brood more than 300 embryos at one time, and all stages of development are always represented. These data can be explained by adult sponges releasing a steady trickle supply of sperm into the water column, perhaps in combination with the existence of a mechanism for sperm storage or post-fertilization developmental stasis in this species.

Keywords: Sponge, Amphimedon queenslandica, reproduction, Heron Island, hermaphrodite, fertilization.

INTRODUCTION

In marine habitats, indirect external fertilization is an extremely common reproductive strategy in many invertebrate phyla. Most often, external fertilization is achieved via broadcast spawning, in which both male and female gametes are released into the water column [1]. Many sessile marine invertebrates mate using a variation on this strategy - termed either egg-brooding or spermcast mating - in which only sperm (but not eggs) are released into the water column [2-5]. Spawned sperm subsequently disperse to fertilize eggs that are retained internally by the maternal adult, usually in brood chambers, in a manner analogous to internal pollination events in plants [6]. Spermcast mating is particularly common among poriferans, cnidarians (including some corals), bryozoans, ascidians and many algae [2, 6], making it a widespread and important reproductive strategy in the ocean.

The consequences of spermcast mating for population persistence, diversity and structure are not well understood. It is unclear whether populations can be maintained solely by sexual reproduction that depends on the release, dispersal and opportunistic uptake of free spermatozoa likely to be present only in dilute suspension, or whether supplementary asexual reproduction may be required [e.g., 3]. Provision of larval colonization required for population maintenance over time first requires that successful fertilization events occur. This will depend upon factors such as distance from paternal contributors, population density, and water flow regime [7-11], all of which are widely considered to directly effect sperm availability [but see 12]. Studies to date have reported variously that sperm may be limiting or extremely abundant [for examples of each, see 2, 3, 8, 12-16], and have demonstrated that sperm availability and transport patterns are likely to vary significantly in space and time, making it extremely difficult to accurately estimate field fertilization levels [17].

In various marine invertebrates, sperm longevity and dispersal potential, measured either by distance travelled from the source or time spent in the water column, is highly variable. Nonetheless, most studies are in agreement that most fertilizations occur nearer, rather than further from, the sperm source. In the sea urchin, *Strongylocentrotus droebachiensis*, 95% of individuals were successfully fertilized across a distance of 0.2 m in a current flow of 0.2 m/s, but only 40% were fertilized at 2m [10]. In the marine hydroid, *Hydractinia echinata*, successful fertilizations were recorded at distances of up to 10 m in current flow rates approaching 0 m/s, although the majority of fertilization occurred within 3 m [11]. Sperm longevity in the colonial ascidian *Botryllus schlosseri* has been recorded as high as 72 h [18], although most fertilizations in the field still occur by sperm from nearby sources [19]. In the brooding demosponge *Crambe crambe*, genotyping was used to infer a mean dispersal distance of just 35 cm, with isolation by distance acting over small scales due to the short dispersal of sperm and larvae [20].

In poriferans, mature sperm are not well studied [but see 21, 22], and there are few morphological details from which to infer likely extent of sperm swimming ability and hence dispersal distance. The flow-through water current generated by sponge choanocytes is used both to filter out food particles and to concentrate dilute sperm from the water column [2, 21]. Indeed, sperm released from conspecific individuals are transported into a recipient sponge in exactly the same manner as a food particle. Once inside, the donor sperm is phagocytosed by a choanocyte, which loses its flagellum and collar to become an amoeboid cell.

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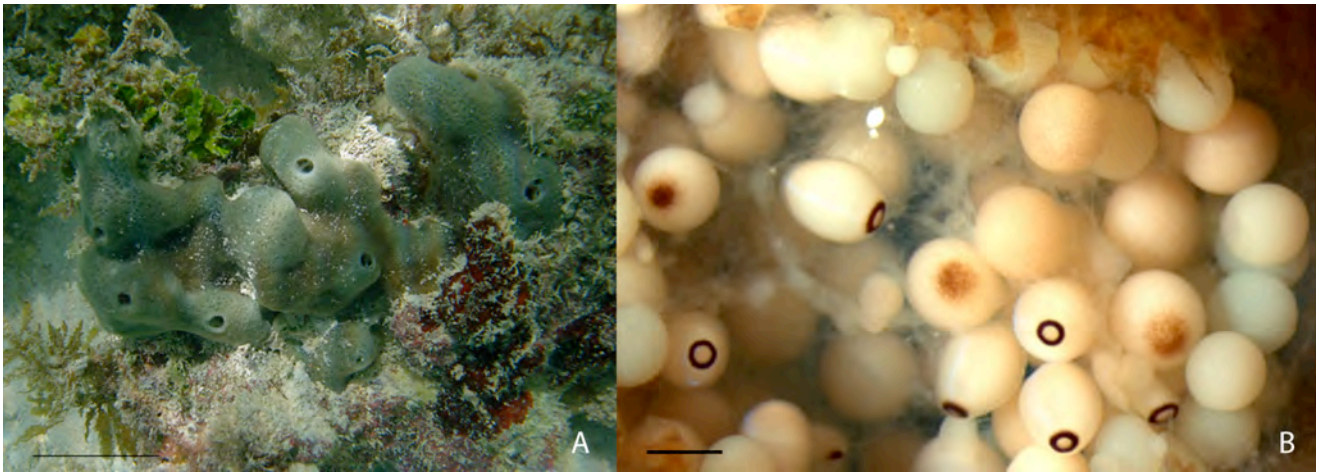

Fig. (1). A. Large adult *Amphimedon queenslandica* growing on coral rubble on the reef flat, Heron Island Reef (scale bar: 5 cm). **B**. Embryos and larva packed together inside a maternal brood chamber. Several different developmental stages can clearly be identified, including white, brown, spot and ring (scale bar: 500 μM).

Phagocytosed sperm are transferred to unfertilized eggs, probably in the vicinity of the brood chambers, where fertilization is thought to occur [3, 21-25].

The marine demosponge *Amphimedon queenslandica* (Fig. **1A**) is a hermaphroditic sessile animal that mates via spermcast mating [26]. At any time of the year, *A. queenslandica* adults are observed to contain up to twenty discrete brood chambers that are packed with embryos and larvae of varying stages of development (Fig. **1B**) [26-28]. Sperm release in this species has never been observed and effectively nothing is known about the location and timing of spermatogenesis, sperm biology, release or longevity. The exact time required for development from fertilization through to mature free-swimming larva is unknown, but is estimated from observations of embryos removed from their mother to be in the order of three weeks (M. Adamska and B. Degnan, unpubl. data). Several deve-lopmental stages are clearly and consistently recognizable, providing an excellent framework for studies of development and larval release. The full genome sequence of *A. queenslandica* is being made available courtesy of the Joint Genome Institute, US Dept of Energy, so that this species is now being recognized as an emerging model organism [26]. Full genome sequence will also facilitate the rapid identi-fication of microsatellite loci that can be developed as tools for genotyping analyses, and thus give insight into consequences for *A. queenslandica* of spermcast mating as a reproductive strategy.

Here we contribute to limited existing data on spermcast spawning in natural populations by documenting the extent and patterns of larval supply in *A. queenslandica*. We use exhaustive characterization of brood chamber contents to document the numbers and distribution of the different developmental stages in multiple maternal adults, and discuss the results in relation to availability of sperm as indicated by availability of potential fathers.

METHODS

All field sampling, observation and collection of *Amphimedon queenslandica* material was conducted at Shark Bay on Heron Island Reef, southern Great Barrier Reef (23° 26' S, 151° 55' E). Adult *A. queenslandica* at this site range in size from approximately 1cm^2 to 112 cm^2 surface area (Fig. **1A**), and are relatively easy to remove non-destructively from the field and to maintain in aquaria. Mature, free-swimming larvae can be reliably induced to emerge from the adult by placing adults in still water warmed by the sun, as described in Degnan *et al.* [26].

At the Shark Bay field site, we used a haphazard method to assign four *A. queenslandica* adults as maternal individuals A - D. We conducted exhaustive searches around each of the assigned maternal adults (A-D) within a predetermined 4 m radius, locating all adult sponges that could serve as a potential source of sperm. *A. queenslandica* attach to hard substrate, so our search involved examining every rock or coral rubble within the search area. After all surrounding adults were located in the field, the four designated maternal adults were removed from the benthos using a hammer and chisel, and transported in seawater to the laboratory at the Heron Island Research Station (HIRS). In the laboratory, we dissected each maternal adult by serial slicing to locate and set aside all brood chambers present within the sponge. Each brood chamber slice was then exhaustively dissected to extract every individual embryo and larva separately as described in Degnan *et al.* [27]. The developmental stage – designated as white, brown, cloudy, spot, ring, or larva, that in sequence cover the full developmental progression from egg to mature swimming larva (see Fig. **1B**; for details, see [27]) - was recorded for each.

To estimate variation in larval release among individuals in the population and over time, we also removed 10 adult sponges from the field site by using a hammer and chisel to gently dislodge the coral rock on which the sponge was fixed without touching or stressing the sponge in any way. These sponges were transported back to the HIRS, where they were maintained in outdoor flow-through aquaria. Small taps fitted to the water outlets feeding each aquarium enabled us to control flow rates to between 0.8 and 1.0 L/min at all times. Water temperature was measured hourly between 10 am and 3 pm for one week, and was found to closely reflect field temperatures (data not shown); in general, while ambient seawater was flowing through the aquaria,

temperature remained quite constant, reacting only very slowly to air temperatures changes during the day.

On alternate days for a period of 22 days, water flow was switched off between 12 noon and 3 pm to allow still water, warmed by the sun, to induce larval spawning in all aquaria [as described in 26]. Larvae spawned from adults in each aquarium were collected and counted between 2 pm and 3 pm on each of these days. Upon release, *A. queenslandica* larvae tend to swim towards the surface, although they become progressively negatively photo- and geo-tactic over the first few hours post-release [26, 29]. As a result, recently-released larvae tended to aggregate in the upper corners of aquaria and can be collected quite easily using a sucker cup as described in Degnan *et al.* [26]. Thorough scans of each aquarium ensured that the majority of released larvae were collected and counted during each spawning event, so that counts could be directly compared across individual sponges and across days of the experiment. The intake pipe for the flow-through seawater system is located on the inner reef flat on southern side of Heron Island Reef, immediately in front of the HIRS. The nearest *A. queenslandica* individuals are more than 500 m away from this intake, and we consider it extremely unlikely that *A. queenslandica* sperm enter the aquarium system. We are thus confident that the aquarium-maintained adults would have had contact with little or no new sperm contribution once they were removed from the field site and placed in aquaria. In any case, very small numbers of sperm introduced by the intake would be insufficient to bias results, given the large numbers of embryos and larvae recorded in this experiment (see Results for details).

Additional adults, collected from the field and maintained in aquaria as described above, were sacrificed and dissected at regular intervals. On days 7, 14, 21, 28, 34 and 41 post-removal from the field, two of these sponges were randomly selected for dissection so that all embryos could be extracted and staged. This regular dissection allowed us to track the number of embryos and larvae at each stage of development for a period of almost 6 weeks, and thus to infer length of time required for development from fertilized egg through to mature larva.

RESULTS

The four designated maternal adults (A to D) selected for analysis were separated in Shark Bay by as little as 25 m, and as much as 106 m, from each other. Exhaustive searches of all potential fathers around these 4 adults revealed a high degree of patchiness, and an overall population density that was higher than expected based on previous anecdotal observations (Table **1**). Density estimates across the four sampled areas ranged from 18 to 95 adults per 50 m^2, with a mean of 46; lowest density was observed in the area with most sand (around Adult C), consistent with the fact that *A.*

Table 1. Characteristics of the 4 m Radius Area (50 m^2) Around each of the Four Designated Maternal Adult Sponges in Shark Bay, Heron Reef

Maternal Adult	Nos. Potential Fathers in 4 m Radius	Habitat Description
A	33	Mostly rock and rubble; little sand
B	94	Equal proportion rock, rubble, sand
C	17	Mostly sand, scattered rock, rubble
D	34	Equal proportion rock, rubble, sand

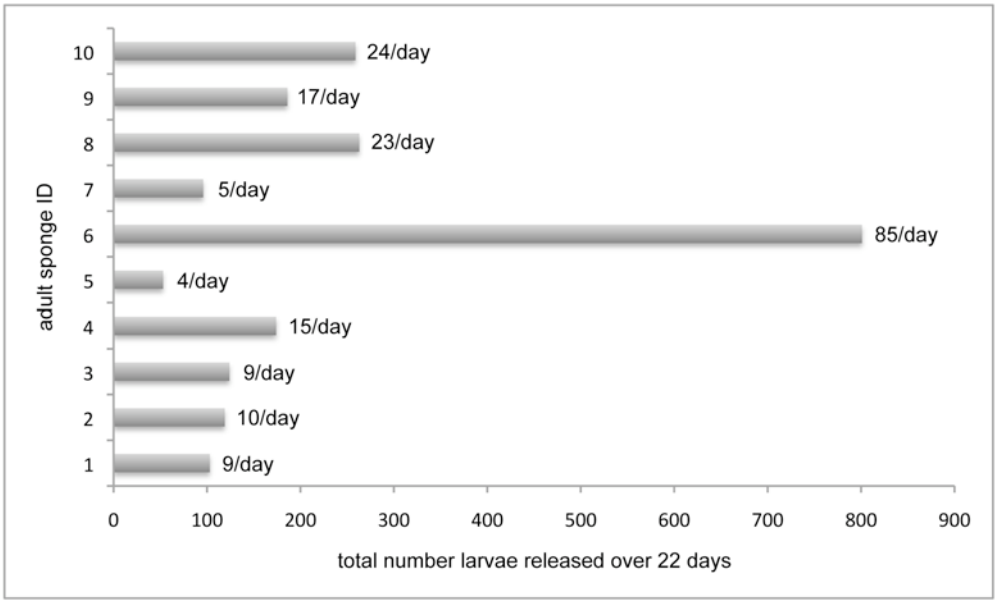

Fig. (2). Total number of larvae released from each of 10 adult sponges maintained in aquaria for 22 days; spawning and counting was undertaken every second day throughout the experimental period. Number alongside each bar denotes median number of larvae released on each of the 11 counting days.

Table 2. Exhaustive Counts of all Embryos and Larvae Contained within all Brood Chambers of Four Adult Sponges Removed from the Field and Dissected Immediately. Relative Proportions of Developmental Stages Differ among Brood Chambers within a Sponge ($P < 0.01$) but Not among Different Sponges ($P > 0.05$)

Maternal Adult	Brood Chamber	Developmental Stage						Total
		White	Brown	Cloud	Spot	Ring	Larva	
A	1	2	3	0	0	1	1	7
	2	17	7	2	5	9	2	42
	3	10	3	0	5	2	3	23
	total	*29*	*13*	*2*	*10*	*12*	*6*	*72*
B	1	42	17	6	24	14	24	127
	2	15	12	6	12	12	6	63
	3	1	1	0	1	3	3	9
	4	8	2	4	11	3	4	32
	total	*66*	*32*	*16*	*48*	*32*	*37*	*231*
C	1	34	12	14	11	18	20	109
	2	17	12	6	11	22	24	92
	3	20	23	3	20	33	9	108
	total	*71*	*47*	*23*	*42*	*73*	*53*	*309*
D	1	10	2	0	2	3	1	18
	2	2	0	1	2	2	0	7
	3	6	2	0	2	0	0	10
	total	*18*	*4*	*1*	*6*	*5*	*1*	*35*
TOTAL		184	96	42	106	122	97	654
% mean		28.2%	14.7%	6.4%	16.2%	18.7%	14.8%	100%

queenslandica attaches only to hard substrates.

The four adults removed from the field were of almost identical size, at approximately 15 cm^2 surface area. Perhaps surprisingly, then, the total number of embryos and larvae found in each of them was highly variable, ranging from 35 to 309 (Table **2**). Among brood chambers located within a single sponge, progeny numbers were not usually evenly distributed at the time of sampling (e.g., adult A cf. Adult C) (Table **2**), and the relative proportions of each developmental stage differed significantly among chambers ($P < 0.01$, chi-square test of homogeneity, df = 12). In contrast to this variability, the relative proportions of each developmental stage were strikingly consistent across all four adult sponges ($P > 0.05$, chi-square test of homogeneity, df = 18), regardless of the total number of progeny present. More embryos generally were at the white stage than at any other stage; the remaining stages are fairly equally represented, except for the cloud stage which consistently is found in lowest numbers both within individual brood chambers and within an entire adult sponge.

Among 10 individual adult sponges removed from the field and maintained in aquaria, we observed a very striking variation in number of larvae spawned over a 22 day counting period. Totals ranged from a low of 53 (adult ID 5; median 4 per day) to a high of 801 (adult ID 6; median 85 per day) larvae spawned from a single sponge (Fig. **2**). Such an extreme variation was completely unexpected given that sponge 5 and sponge 6 were very similar in overall size; both were approximately 18 cm^2 in surface area. The enormous larval output of individual sponge 6 was consistent with the discovery, upon dissection at the end of the 22 days, of the presence of 20 discrete brood chambers, even then still containing a total of 719 embryos at different developmental stages. By comparison, when dissected at the end of the experiment, sponge 5 contained only 2 brood chambers and a total of 28 embryos.

The mean number per adult of larvae that were released every two days from sponges in experimental aquaria fluctuated throughout the 22 day counting period, ranging from a low of 9 to a high of 36 (Fig. **3**). There was a slight trend towards decreasing numbers as the experiment progressed, but this trend was not significant. Thus we did not observe a depletion of larvae by the end of the 22 day period, indicating that time for completion of development from fertilization through to mature larva is longer than 3 weeks.

To infer more exactly the time required for development through to mature larva, additional adults maintained in aquaria, and spawned on the same alternate days, were then dissected on days 7, 14, 21, 28, 34 and 41. This allowed us to track the number of embryos and larvae at each stage of development for a period of almost 6 weeks. Two indivi-

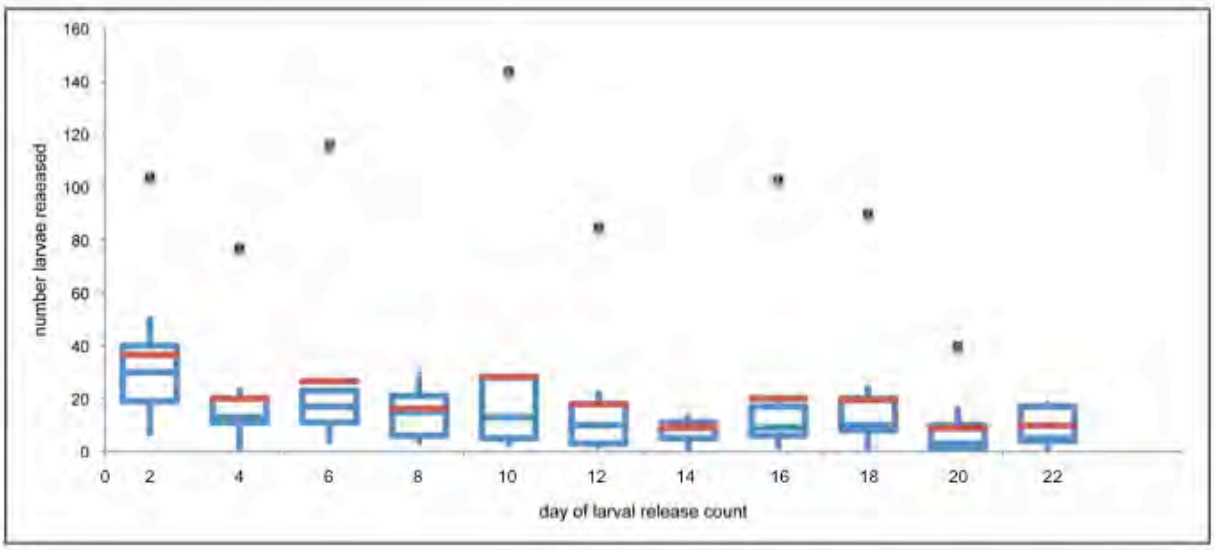

Fig. (3). Mean number (red line) of larvae released from 10 adult sponges maintained in aquaria, counted every second day from days 2 to 22. Extreme outlier data points for several days, representing adult #6, are shown.

duals were sacrificed on each of days 7 though 34. On day 41, all remaining adults left in aquaria were dissected; there were five adults in total. Interestingly, two of these adults still had intact brood chambers, but the chambers were completely empty; that is, no eggs, embryos or larvae were observed. These data were used to record changes in proportions of embryo and larval stages change over this period, under the assumption of no new sperm entering the system. Proportions of various developmental stages were compared against those in the four designated maternal adults scored immediately upon removal from the field (Table **2**); proportions in these individuals are considered as those 'expected' under a scenario of continuing new fertilizations. We used a chi-square goodness-of-fit test to compare observed distributions of developmental stages through each week (weeks 1 – 6) of the aquarium experiment with expected distributions based in field-collected adults (week 0) (Table **3**). There was no significant difference between distribution of developmental stages in week 0 and week 1 adults ($P > 0.05$; $df = 5$), but distributions in all subsequent weeks (2 through 6) were significantly different ($P < 0.05$) from those in week 0.

DISCUSSION

In the ocean, reproductive success is heavily influenced by population structure, and specifically by distribution of breeding adults, because this will have a direct influence on the chance of fertilization of gametes. Broadcast spawning species usually release clouds of sperm into the water column at specific times, to coincide with release of eggs, resulting in intermittent periods of high and low sperm densities. By comparison, most spermcast spawners are thought to release a constant trickle of sperm throughout

Table 3. Percentages (with Actual Counts Provided in Parentheses) of Different Developmental Stages in Adult Sponges Dissected Over a Period of Six Weeks of Maintenance in Aquaria, Under a Scenario of Sperm Limitation. On Day 7, Observed Percentages were not Significantly Different from those Expected Based on Field-Collected (Day 0) Adults (chi-square, df = 5, P > 0.05, ns). From Day 14 Onwards, Observed Percentages were Consistently Significantly Different (chi-square, df = 5, *P < 0.05). In chi-squared Goodness-of-Fit Tests, Day 0 Percentages were Used as 'Expected' Values. *n* = Number of Adults Dissected on each of the Days

Developmental Stage	Day on which Adults were Dissected						
	Day 0 (n = 4)	Day 7 (ns) (n = 2)	Day 14 * (n = 2)	Day 21* (n = 2)	Day 28* (n = 2)	Day 34* (n = 2)	Day 41* (n = 5)
White	27.3 (174)	31.7 (242)	41.2 (331)	32.8 (336)	40.2 (475)	46.1 (126)	47.6 (90)
Brown	15.1 (96)	16.5 (126)	36.3 (292)	42.1 (432)	25.1 (296)	40.3 (110)	6.9 (13)
Cloud	6.6 (42)	6.4 (48)	1.5 (12)	4.5 (46)	1.9 (22)	1.5 (4)	0.0 (0)
Spot	16.7 (106)	15.3 (117)	8.0 (64)	12.3 (126)	9.9 (117)	5.5 (15)	11.1 (21)
Ring	19.2 (122)	18.0 (137)	5.8 (47)	5.8 (60)	8.3 (98)	1.8 (5)	0.0 (0)
Mature larva	15.1 (96)	12.1 (93)	7.2 (58)	2.5 (26)	14.6 (172)	4.8 (13)	34.4 (65)

reproductive months of the year [2, 30], resulting in a constant supply of dilute sperm in the water column. The population consequences - for population persistence, diversity and structure - of a reliance on release, dispersal and opportunistic uptake of free spermatozoa present only in dilute suspension are not well understood. In the demosponge *A. queenslandica,* the presence of multiple brood chambers packed with embryos and larvae of all developmental stages clearly demonstrates that successful fertilizations occur frequently and regularly enough to enable a constant provision of larval colonization.

Regular and frequent fertilizations depend upon factors such as distance from paternal contributors, population density, and water flow regime [7-11, but see 12], all of which directly effect availability of sperm for maternal adults to receive. In our Shark Bay population, we report a mean density of 46 (range 18 to 95) individuals per 50 m^2; on average, then, a hermaphroditic adult sponge will have 45 potential sperm donors available within a 4 m radius to fertilize the eggs retained within its brood chambers (Table **1**). Variation in density is generally associated with the relative proportion of hard substrate (rocks, coral rubble) versus sand substrate in a particular locale, because *A. queenslandica* attaches only to hard substrates. In broadcast mating species such as the sea urchin *Strongylocentrotus droebachiensis* [10], and the marine hydroid, *Hydractinia echinata* [11], fertilization success decreases as distance separating two adults increases, such that near neighbours are considered to dominate fertilization events [16]. When local sperm sources are unavailable, however, *Botryllus schlosseri* is successfully fertilized from distant paternal contributors [31]; similarly, Lasker *et al*. [8] reported that 20% of octocoral progeny did not match paternal contributors in their immediate study area. Together, these observations indicate that the likelihood of successful fertilization events occurring between two intraspecific individuals generally is expected to decrease as distance between them increases, due to dilution of gametes in the water column. Further, paternal contributors that are larger, located closer to maternal adults and have greater sperm production are predicted to achieve greater fertilization success [16, 31, 32; but see 33].

Among maternal adults, we do not find any correlation between size of sponge and total number of progeny contained within brood chambers. The adults we removed from the field were all of approximately the same size (15 cm^2), and yet the number of progeny they contained varied dramatically, from 35 to 309 (Table **2**). This indication of large intraspecific variation in larval production was substantiated by the huge variation among aquarium-maintained adults in the number of mature larvae released. In this case, although 9 out of 10 adults each released between 53 and 263 larvae across a 22 day period, the remaining adult was a standout supplier, releasing a huge 801 larvae in the same period (Figs. **2**, **3**). Given that all 10 sponges were collected from the same geographic population and were maintained in aquaria under nearly identical temperature and water flow conditions, we conclude that the extent of variation in larval output observed among these adults is representative of that which exists naturally within the Shark Bay population. Whether this extensive variation in larval output translates into the same degree of variation in successful larval recruitment into the population was not tested under the current project, but would have important implications for genetic diversity and evolution of the population.

In the field, there also was no direct correlation between number of progeny and number of potential fathers in the 4 m radius. The adult sponge containing the highest number of progeny (309) was surrounded by the lowest number of potential fathers, with only 17 in its 4 m radius. In contrast, the adult with the lowest number of progeny – just 35 – had twice as many (34) potential fathers nearby. Together with our anecdotal observation that all developmental stages are always present in a healthy brood chamber, these data strongly suggest either that sperm supply is not limited in this population, or that a mechanism exists for sperm storage and/or developmental stasis. We suggest that factors other than sperm availability are responsible for the observed variation in reproductive output among individuals.

Three independent sets of data reported here are of particular interest; these are (i) consistent presence of all developmental stages in all adults surveyed, (ii) consistent proportional representation of stages among all adults surveyed, and (iii) quite constant release of larvae per adult per day. Importantly, these data together point to a steady stream of larval recruitment into this (or other recipient) population, suggesting that sexual reproduction via spermcast spawning alone is sufficient for population replenishment and maintenance in this species. We propose two non-exclusive explanations for this constant supply of larvae. First, adult sponges may release a steady trickle supply of sperm into the water column, such that sperm are continuously being taken up by recipient adults and fertilizations are occurring constantly. Sperm that are released as a constant trickle become part of a dilute sperm pool in the water column [8], and spermcast mating marine invertebrates are able to enhance fertilization success by combining filter feeding with sperm uptake [2, 21, 22]. High fertilization rates can occur when water is pumped directly over eggs [2, 4] or when sperm are accumulated around eggs retained in or on the maternal body [e.g., 34].

Second, there may exist in *A. queenslandica* a mechanism for sperm storage or for post-fertilization developmental stasis. Other marine invertebrates, such as the colonial ascidian *Diplosoma listerianum*, are known to have sperm storage sites, allowing for continued fertilizations even during periods of limited sperm availability [23, 35]. Sperm storage mechanisms facilitate extended contact between sperm and maternal tissue, thereby providing an opportunity for female cryptic choice. Sperm storage in conjunction with developmental stasis of unfertilized eggs - which can prolong egg viability [34] – could maintain constant larval release because normal developmental recommences only once stored sperm are released to allow new fertilizations to occur. As an alternative to sperm storage and developmental stasis of unfertilized eggs, eggs fertilized whenever sperm are extracted from the water column could be stored in developmental stasis, with normal development recommencing according to the rate at which mature larvae are released from the brood chamber. Either mechanism could account for the continued embryonic development and larval release observed in our aquarium experiment.

Neither total number of progeny per sponge, nor specific developmental stages, were evenly distributed among brood chambers within an individual, suggesting that there is no internal control to homogeneously fertilize eggs evenly among brood chambers. Rather, this is consistent with sperm intake on feeding currents, and haphazard localization to particular brood chambers. The presence of more white-stage embryos (28.2%; Table **2**) than any other stage is expected; in an *A. queenslandica* brood chamber, even a very healthy one, it is very difficult to distinguish between unfertilized white eggs and fertilized white stage early embryos, so that counts of "whites" are almost certain to incorporate both elements. Further, our anecdotal observations suggest that embryos spend longer in the white phase of development than they do in any other phase. We predict that the shortest phase of development is that represented by the 'cloud' phase, because we consistently find the lowest proportion (mean 6.4%) of embryos at this stage compared to any other; all remaining stages are generally similarly represented (mean 14 – 19%), suggesting that development proceeds through the brown, spot and ring phases at similar rates (Table **2**).

The larval release aquarium experiment might suggest that time for completion of development from fertilization through to mature larva is greater than our anticipated 21 days (see Introduction), because we do not see a significant decline in larval release numbers by this time, even in the absence of introduction of new sperm (Fig. **3**). In contrast, our dissected aquarium-maintained adults do show a decline in mature larvae numbers by 21 days, but these numbers then recover again in subsequent weeks, to a high at the end of week 7. There is almost a bimodal distribution in these data; as the brown stage declines from day 7 to day 21, the proportion of embryos in brown phase increases. This would be consistent with "new" embryos entering development as latter stages move through to mature larvae and are depleted, so as to maintain a non-interrupted provision of larvae. The increase in larvae again in the latter weeks could be the result of a spurt of new embryogenesis, brought about either by stored sperm being released for new fertilizations, or by the relaxing of developmental arrest of stored fertilized eggs. Based on the data at hand, however, we cannot reject the simple possibility that development to mature larva may take six weeks or more. We cannot, at this time, indicate whether sperm storage or developmental stasis mechanisms exist in *A. queenslandica*.

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