

Asexual reproduction in a population of *Holothuria difficilis* (Echinodermata; Holothuroidea) on Reunion Island

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Abstract

Asexual reproduction in *Holothuria difficilis* was monitored from May 2016 to May 2017 on Reunion Island's Saint Gilles Reef. The study population had a very low density level (0.12 ind. m⁻² for the study as a whole, with a maximum of 0.19 ind. m⁻²), in comparison with levels recorded elsewhere. Sparse distribution was another unique feature of that population, as the species is characterised by high-density aggregations. Asexual reproduction occurred within the population all year round, but a peak was observed during the transition between Southern Hemisphere summer and winter. The mean percentage of regenerating specimens in the population was 19.32%, with maximum levels barely exceeding 40%. These values are low in comparison to those recorded elsewhere in the same species.

Introduction

Many echinoderm species, including the sea cucumber *Holothuria (Platyperona) difficilis* (Semper, 1868), can reproduce both sexually and asexually via fission or fragmentation (Mladenov 1996). After an initial observation of transverse fission in preserved specimens by Benham (1912), a detailed description of the regeneration process in this species was proposed by Deichmann (1922) using the same type of material.

Holothuria difficilis is a small sea cucumber. The maximum length in a contracted specimen is 4 to 5 cm (Lawrence 1980; Lee et al. 2009) and 12 cm in an uncontracted individual (Rowe 1969; Samyn et al. 2006). The species is wide-spread in the Indo-Pacific Basin with an irregular distribution made up of large local concentrations. It is generally found in shallow water on coral-reef flats (Rowe and Doty 1977; Kerr 1994; Conand and Mangion 2003) or near the shore in intertidal zones (Lee et al. 2009).

Not a lot is known about the biology of this species' reproduction. The only study on asexual reproduction in *H. difficilis*, carried out on One Tree Island (OTI) in Australia's Great Barrier Reef, showed that this reproduction mode occurred year-round, with a peak at the beginning of the Southern Hemisphere winter (Lee et al. 2009). The purpose of this article is to document the seasonal nature of asexual reproduction in an *H. difficilis* population on a Reunion Island reef.

Materials and methods

These observations were made on the west coast of Reunion Island (21°07'S, 55°32'E), located in the south-western Indian Ocean about 680 km east of Madagascar, on the Saint Gilles-La Saline fringing reef (Figure 1A). This reef, which is 7.6 km long with a maximum width of about 500 m and an average depth of less than 1.5 m, is divided into two parts by L'Ermitage Pass. The site selected for this study, located in the southern part of the reef, is situated about 80 m from the reef front and 425 m from the shore (21°5'45.47''S and 55°13'48.72''E; 21°5'44.68''S and 55°13'47.64''E) (Figure 1B). Its depth varies from 0.3 to 0.8 m depending on the tide. It is characterised by a sandy-rubble substrate covered by a few damaged coral mounds and a great deal of coral rubble in the form of a slab (Figure 1C). It is subject to trade-wind swells throughout the year, which are stronger during the Southern Hemisphere winter (from May to October), and sometimes violent Southern Hemisphere swells (Tourand et al. 2013). This reef is included at the edges of the Reunion Island Natural Marine Reserve's marine protected area (GIP-RNMR) and the study site is in a Level 2a enhanced protection zone.

Length measurements were used rather than weight in spite of sea cucumbers' contractibility since hydrodynamics at the site made weighing impracticable and it was not possible to collect observed specimens to weigh on the shore and then return to the site.

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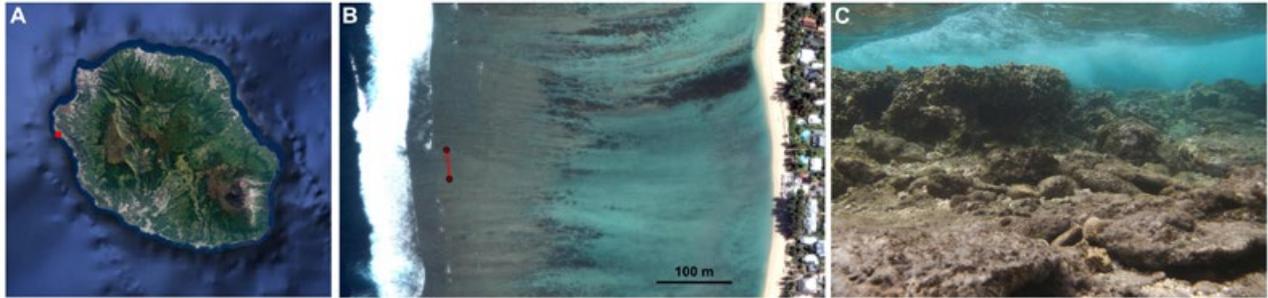


Figure 1. A) Reunion Island (red dot = location of the Saint Gilles-La Saline reef). B) Study site and transect position (red line) close to the reef crest. C) Typical habitat of *Holothuria difficilis* on the reef of Saint-Gilles-La Saline (Reunion Island). This habitat is characterised by the presence of slab-shaped rubbles scattered on a sandy-detrital substrate, and strong hydrodynamics.

A 40 m x 5 m (200 sq. m) transect parallel to the reef front was marked out on a site where *H. difficilis* aggregations had previously been observed. It was monitored once a month in the late morning from 15 May 2016 to 15 May 2017. Observations were done via snorkelling. All the specimens observed in the transect were measured and photographed *in situ*. The following morphological features were then recorded: 1) specimen status: whole specimen (W), or regenerating individual (R), and 2) state of contraction determined by the body's appearance: uncontracted (nc) or contracted (c). In regenerating specimens, the following features were also recorded: 1) nature of the regenerating part when that could be determined: anterior end regenerating the posterior end [anterior part] (AP), posterior end regenerating the anterior end [posterior part] (PP), or specimens for which the anterior-posterior axis could not be determined (P ?) and, 2) the length of the regenerating part.

All the individuals resulting from fission observed had begun to regenerate their missing parts. All the observed specimens were hidden in coral rubble, which was carefully removed and then put back into place after observation. None of the animals were removed from their locations.

It is easy to differentiate between Ws (Figure 2A) and Rs (Figure 2B) due to the yellowish-orange

colour of the regenerating part, which stands out against the even, dark-brown colour of Ws. They are also easy to distinguish from juveniles, which have an even, fairly light orange to greenish-yellow colour (Figure 2C).

In addition, random two-hour explorations from the shore to the breakers were carried out once a month by two observers at six different sites (three north of L'Ermitage Pass and three south of it), to allow species density and distribution estimates, along with possible sightings of regenerating specimens.

Results

H. difficilis' very low density on Saint-Gilles-La Saline Reef, which is atypical for the species, limited quantitative analysis of the measurements taken on the transect since out of 13 samplings, only 5 provided observations of 30 or more specimens (May 2016 and February–May 2017). In addition, it was impossible to estimate the number of specimens counted repeatedly during successive samplings, but the wide variations in W densities, and, to a lesser degree, R densities, suggest that such bias was minor. It was also impossible to estimate recruitment, emigration and immigration rates in the transect or mortality rates since population numbers did not remain constant.



Figure 2. A) Whole individual, ≈ 3.5 cm. B) Fission product regenerating its anterior part (PP), ≈ 2 cm. C) Juvenile, ≈ 1.75 cm. Scale bars = 1 cm.

Population structure

Sizes

Some 326 specimens were measured during this study, including 263 Ws and 63 Rs. Some 86.8% were contracted ($n = 283$), and 13.2% were uncontracted ($n = 43$). The prevalence of contracted specimens in the sample was probably due to stress linked to removing the rocks the animals were hiding under, thereby exposing them to both light and the currents. There were relatively few small or large specimens: 4.91 % were under 2.0 cm in length ($n = 16$) and 9.20 % were over 5.0 cm ($n = 30$) (Figure 3A).

The Ws' median size was 3.3 cm, while the Rs' was 3.0 cm (Figures 3A–B). Whatever the regenerating part (i.e. AP, PP and P?), Rs were significantly smaller than Ws but were of similar sizes within that category (Figure 3B). For the Ws, and for the

Rs, contracted specimens were significantly smaller than uncontracted ones (Figure 3C), so contraction does affect specimen size. For that reason, including contracted-specimen measurements does lead to a bias in estimates of the population's average specimen size. The average specimen size of uncontracted animals in the total population was 4.61 ± 1.42 cm ($n = 43$), for W-nc 5.13 ± 1.43 cm ($n = 29$) and for R-nc 3.59 ± 0.75 cm ($n=14$) (Figure 3C).

The average size of all the specimens observed during the study was 3.51 ± 1.07 cm, with a median of 3.2 cm, a maximum in July 2016 (4.27 ± 1.66 cm, $n = 11$) and a minimum in May 2017 (2.98 ± 1.07 cm, $n = 32$) (Figure 3D).

Only five juveniles, all under 2 cm in length, were observed during the random explorations while none were spotted in the transect.

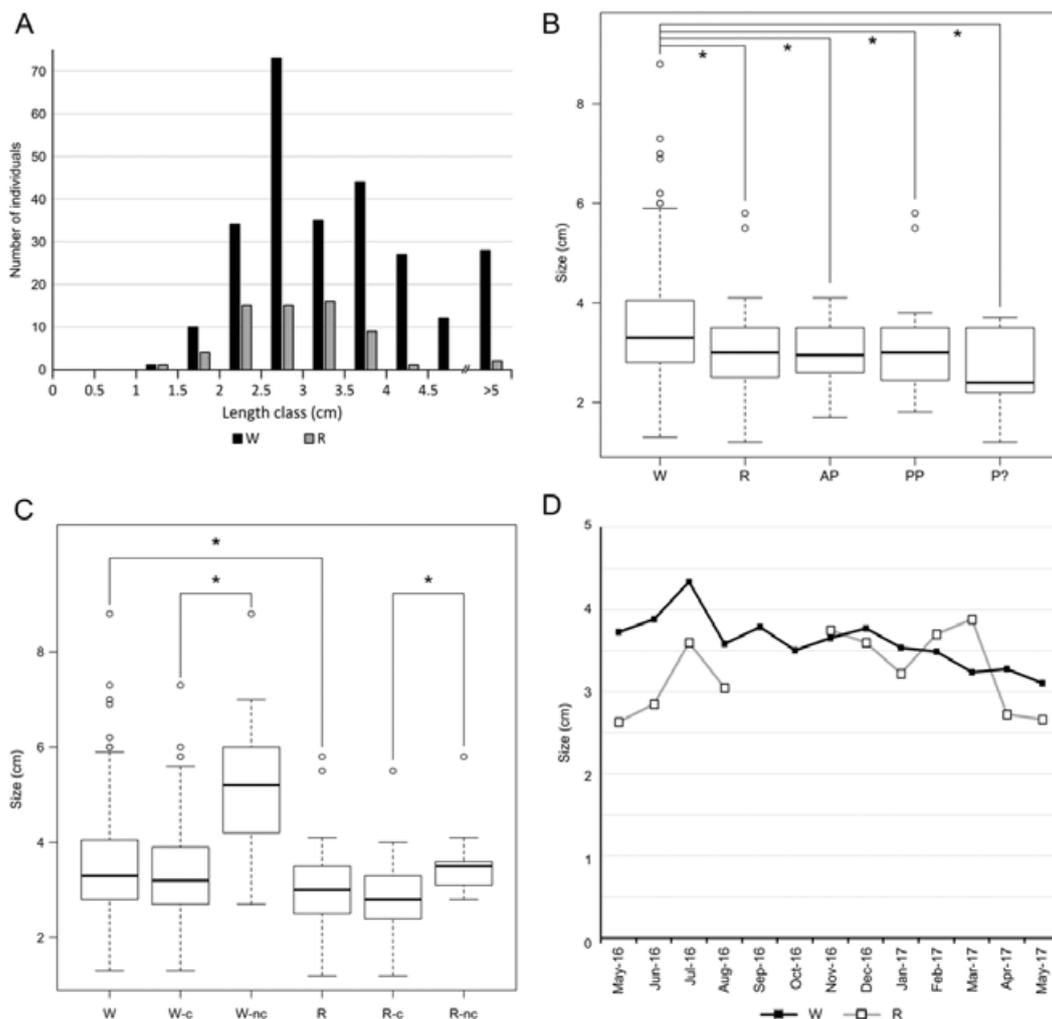


Figure 3. A) Size frequency distribution of whole (W, black bars) and fissioned individuals (R, grey bars) on the transect over the 13-month study. B) Fissioned individuals, (R, AP, PP, and P?) were significantly smaller than whole individuals (W). C) Contraction status (c = contracted, nc = uncontracted). D) Average size variations over the study period. No data are available for Rs in September and October 2016 (no specimens observed). * indicates a significant difference at $p < 0.05$.

Densities

The study population’s average density fluctuated greatly over the course of the study (Fig. 4A). It averaged about 0.12 ind. m⁻² (n = 326) over the 13 months, with a maximum observed in May 2016 (0.19 ind. m⁻², n = 39) and a minimum in July 2016 (0.06 ind. m⁻², n = 11). A second peak occurred in September 2016 (0.13 ind. m⁻², n = 26), following a sharp drop in numbers from May to July and preceding another decrease through to November. A constant increase in density was observed from December 2016 to April 2017 and in April the density was 0.18 ind. m⁻² (n=36). The average density for Ws over the course of the study was 0.1 ind. m⁻² (n = 263) and for Rs, it was 0.02 ind. m⁻² (n = 63) (Figure 4A).

The qualitative estimates made during the random explorations suggested that *H. difficilis* could be found in very low densities in most reef flat zones near the reef front. Aside from some rare one-to-two metre zones where densities were higher (about 5 ind. m⁻²), all the observed specimens were isolated, often located more than 20 m from each other.

Asexual reproduction

No specimens undergoing fission, wound healing or internal organ regeneration (i.e. without any visible regeneration) were observed either in the transect or during random explorations. The average percentage of Rs was 19.32% of the study population, with rates of more than 20% in May, June and November 2016 (41.02%, 42.85% and 26.66, respectively) (Figure 4B), and in April and May 2017 (22.22% and 28.12%, respectively). In September

and October 2017, no Rs were observed in transect (Fig. 4A–B), although three Rs were observed during the random explorations. For the Rs, there were 32 PP, 26 AP and five specimens whose anterior-posterior axis could not be determined. On average, the original part accounted for 77.65 ± 12.23% of the body length in Rs (77.54% ± 14.72% in R-nc, n = 14) (Figure 4C). On average, the original part of the PPs was 76.82% ± 14.53% of the body length (78.14 ± 20.08% in PP-nc, n = 6), for APs it was 79.60 ± 9.22% (78.63 ± 12.28% in AP-nc, n = 8) (Figure 4C). The average length of the original part was 2.57 ± 0.41 cm in AP-nc (n = 8) and 3.03 ± 1.28 cm in PP-nc (n = 6).

Discussion

Population structure

Size

The sample population’s average specimen size (3.51 ± 0.7cm) changed little over the study period. It was 4.61 cm ± 1.42 cm (n = 43) in uncontracted specimens, which is similar to Bakus’ (1968) estimated average size at Enewetak Atoll, i.e. 4 cm, and the maximum size recorded by Lawrence (1980) in other populations of the same atoll (4 cm contracted). In contrast, size distribution between Reunion Island and OTI differed greatly. On OTI, Lee et al. (2009) observed a population dominated by specimens under 2 cm in length and mainly composed of regenerating specimens, whereas on Reunion Island, more than 95% of the animals measured were over 2 cm in length in a population composed of one-fifth regenerating specimens. In addition, Lee et al. (2009) estimated that the

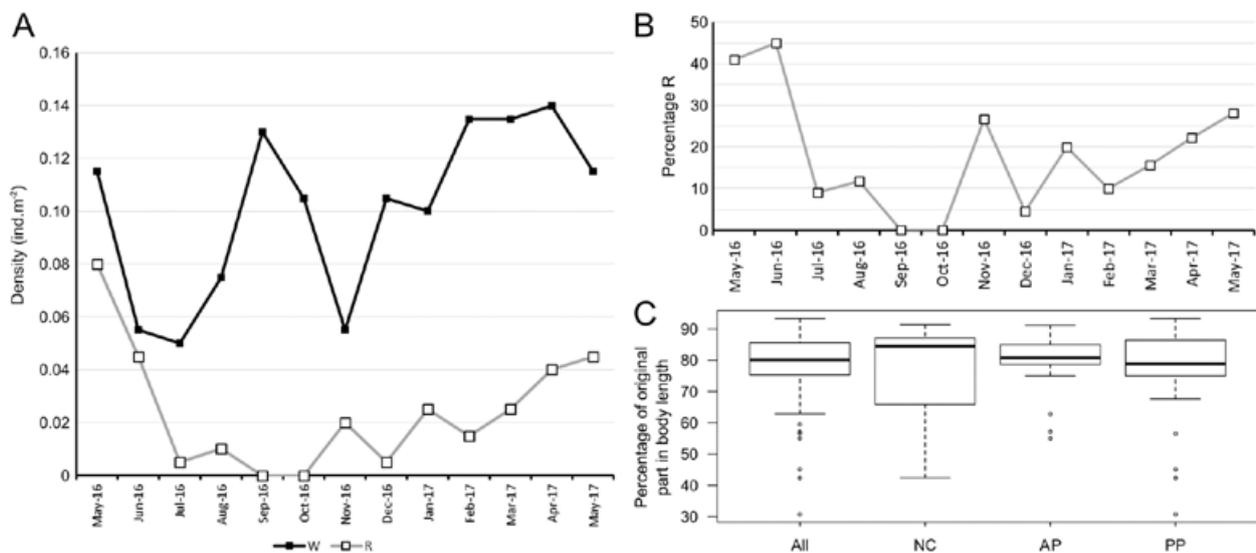


Figure 4. A) Distribution of the average density (individual per sq. m) of whole (W, black line) and fissioned individuals (R, grey line) on the transect. B) Distribution of the percentage of Rs in the population. C) Percentage of original part in body length.

maximum specimen length on OTI was 5 cm. In the study population on Reunion Island, specimens measuring more than 5 cm in length accounted for 9.20% of the population ($n = 30$) and the maximum sizes measured were 8.8 cm (nc) in the transect and 10.9 cm (nc) during the random explorations. These differences in size distribution may be linked to the low percentage of regenerating specimens observed on Reunion Island in comparison to those recorded on OTI or to a probably limited sexual recruitment. We did, in fact, only observe 11 out of 263 Ws (4.18%) of a size equal to or less than 2 cm, which suggests that recruitment from sexual reproduction is low or that the mortality rate for larvae and/or juveniles is high. Both assumptions are supported by the fact that only five juveniles were observed, during the random explorations, over the 13 months of the study.

In contracted Rs, the regenerating part was not contracted or only slightly so, and that specific characteristic was probably due to the short diameter of the regenerating longitudinal muscles as compared to those in the original section (Conand et al. 1997).

Habitat and density

H. difficilis' preferred habitat on Reunion Island was the same as the main one recorded elsewhere, i.e. on semi-exposed or exposed reef flats (Conand and Mangion 2003; Lee et al. 2009; Kerr 2014; Lawrence 1980) and near the reef front (Rowe and Doty 1977; Kerr 1994), as such habitats are quite shallow but experience strong hydrodynamics. The species' sparse spatial distribution on Saint Gilles-La Saline Reef did differ from the high-density-zone distribution recorded elsewhere.

The study population's density on Saint Gilles-La Saline Reef (0.12 ind./m²) was the lowest recorded one (Table 1). It is believed that the high densities recorded elsewhere in certain tropical sea cucumbers are linked to asexual reproduction, which may be able to maintain – and sometimes increase – population density (Emson and Mladenov 1987; Chao et al. 1993, 1994; Conand 1996; Uthicke 1997, 2001; Conand et al. 1998, 2002; Uthicke and Conand 2005; Lee et al. 2009; Pirog et al. 2017). Our observations suggest that on Reunion Island the population can maintain itself, even though at a very low density, through asexual reproduction since 1) its peak in May, 2016 was linked to the high density in Rs throughout the study (0.08 ind. m⁻², $n = 16$), 2) its peak in September 2016 could be linked to the growth in Rs observed in May 2016 (it happened at the same time as a peak in Ws and an absence of Rs), and 3) its peak in April 2017 was linked to the increased density of both species from January onwards. Moreover the similarity of the densities in May 2016 (0.19 ind. m⁻², $n = 39$) and April 2017 (0.18 ind. m⁻², $n = 36$), i.e. during periods of high density for Rs, suggest that the overall density is maintained over a one-year cycle through asexual reproduction, whose contribution may therefore compensate for mortality and emigration in this population.

Asexual reproduction

Regenerating specimens accounted for 19.32% of the study population over the 13 months, with two peaks of more than 40%. Deichmann (1922) calculated 68.29% for 123 sample specimens under the synonym *Actinopyga parvula*, and Lee et al. (2009, Figure 3A) calculated more than 30% in 9 of 10 samples spread

Table 1. Documented densities in *Holothuria difficilis*, with localities, sampling duration and references.

Locality	Duration	Estimated density	Reference
Enewetak Atoll, Marshall Islands	1 month	1.4 to 32 ind. 900 cm ² . Maximum 130 ind. m ² (day) and estimated 200 ind. m ² at night	Bakus 1968
Enewetak Atoll, Marshall Islands	nd	324 ind. m ²	Lawrence 1980
Kosrae, Caroline Islands	3 months	572 ind. 100 m ² (abundance)	Kerr 1994
One Tree Island, GBR, Australia	October 2006 (duration nd)	102 ind. m ²	Lee et al. 2009
Fantome Island, GBR, Australia	nd	247 ind. m ²	Uthicke pers. obs., cited in Lee et al. 2009
Reunion Island	13 months (once a month)	0.12 ind. m ²	This study

over a period of 20 months, with peaks of more than 60%. So, the percentages of regenerating specimens recorded in the study population on Reunion Island appear to be low for this species.

Seasonal nature

The on-going presence of regenerating individuals observed in the transect and during the random explorations throughout the study period suggests that asexual reproduction of *H. difficilis* occurs year-round on Saint Gilles-La Saline Reef. However, monthly percentages of individuals produced by fission exceeding 20% of the population revealed two periods of higher intensity for that reproduction mode. The first, in May and June, formed a peak; the second was in November but the small sample size that month (11 Ws and 4 Rs) limits interpretation of those data. Asexual reproduction in *H. difficilis* on OTI also takes place year-round with a peak in the number of regenerating specimens in May, but unlike the study population on Reunion Island, the percentage of regenerating individuals was more than 50% from May to September, i.e. throughout Southern Hemisphere winter. However, peaks in the numbers of specimens that have begun regenerating their missing sections only indirectly indicates peak fission periods (Dolmatov 2014), as regeneration of internal organs takes place before the regenerating segment appears. The time needed for *H. difficilis* to regenerate its internal organs is not known but it is generally one to two months after induced fission in many species (Kille 1942; Uthicke 1997; Purwati et al. 2009; Dolmatov et al. 2012), though it can be more in others (Reichenbach and Holloway 1995). So fission peaks were probably before the month of May on both Reunion Island and OTI (where the observed specimens were in various stages of regeneration), i.e.; at the end of the Southern Hemisphere summer. As these two islands are located in similar latitudes in the Southern Hemisphere, it may be that abiotic factors that can promote asexual reproduction, such as seasonal variations of the photoperiod (Mladenov 1996; Conand et al. 2002) or ocean surface temperatures, which reach their maximums during that period (Donguy and Meyers 1996; Conand et al. 2007), are decisive for both populations.

AP/PP survival rates

The small difference in the numbers of AP and PP specimens observed (44.8% and 55.2%, respectively) suggests a relatively balanced survival rate for both parts, which agrees with Deichmann's sample composition (1922) that had 48.8% of APs and 51.2% of PPs. Similar survival rates are generally found in species whose fission site is near the middle of the body, e.g. *Stichopus chloronotus* (Conand et al.,

1998, 2002), *Holothuria atra* (Conand 1996; Thorne and Byrne 2013) in spite of regional differences (Uthicke 2001), or *H. parvula* (Emson and Mladenov 1987), whereas in other species the posterior section shows a much higher survival rate (see for review Thorne and Byrne 2013).

Fission site

The place on the body where fission occurs seems to be specific to each species (Purwati 2004; Dolmatov 2014) but this had not been documented for *H. difficilis*. Although no specimens in the fission or wound healing stages were observed during the study, our results allow us to suppose that fission may occur in this species near the middle of the body. Based on the average size of the original part in AP-nc and PP-nc specimens, whose lengths were not modified by contraction (2.57 ± 0.41 cm and 3.03 ± 1.28 cm, respectively), the average size of uncontracted animals before fission would be about 5.60 ± 1.69 cm [which would be in agreement with Lee et al.'s assumption (2009), suggesting that fission may only take place in this species once they have reached 4 to 5 cm in length]. This size would place the fission site at 46% of the body from the mouth. Such a fission site near the middle of the body is found in most species for which this issue has been documented (see for review Dolmatov 2014, Table 1).

Conclusion

This study is the second to document asexual reproduction in *H. difficilis*, a species whose biology is largely unknown. It showed some similarities with the first study, which took place in the Pacific (Lee et al. 2009), particularly in regard to the species' preferred habitat and the peak asexual reproduction period. Our study also uncovered certain differences, notably so in terms of the specimen size frequency distribution. The Reunion Island study population displayed two striking differences from all previous studies, i.e. a scattered distribution without any aggregations and very low densities, not at all in line with those that had been recorded for this species.

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