Influence of heterotrophic feeding on the sexual reproduction of *Pocillopora verrucosa* (Scleractinia, Pocilloporidae) in aquaria

By:

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As the candidate's supervisors we have/have not approved this thesis/dissertation for submission.

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ABSTRACT

Corals are able to source autotrophically-produced carbon since they have symbiotic unicellular dinoflagellates embedded in their tissue. However, they are also known to be heterotrophic feeders and able to ingest a variety of food sources, such as bacteria, particulate organic matter and zooplankton. Recent research has shown that heterotrophic feeding has a marked effect on both maintenance and growth in corals by providing mainly a nutritional source of nitrogen and phosphorus. Nevertheless, no study has yet been undertaken on the interactions between feeding and sexual reproduction in corals. This study examines the effects of heterotrophic feeding on the sexual reproduction of *Pocillopora verrucosa* in aquaria. Rotifers were used as live food source at two concentrations (LFC = low feed colonies) = 5×10^2 organisms/L; (HFC = high feed colonies) = 15×10^2 organisms /L) and an unfed control (UC = unfed colonies) was added for comparison. Three replicates of five colonies were used for each food concentration and control. Rotifers were distributed among the nine aquaria four times per week for three hours. Histological sections of coral polyps were prepared to monitor the development of gametogenic stages and the fecundity of the colonies. The number and size of oocyte, and spermary stages were determined in each polyp. Both fed and starved colonies proved to be simultaneous hermaphrodites and broadcast spawners. The gametogenesis period was short and occurred from October to December 2007. No spawning event was observed in the aquaria. However, the disappearance of mature oocytes in samples collected in January 2008 suggested that spawning took place between December 2007 and January 2008. Heterotrophic feeding had a strong effect on reproduction in *P.verrucosa*. The results showed that both the proportion of polyps with gametes and the reproductive effort were lower in the fed than in starved colonies. It is likely that an energetic trade-off occurred between reproduction and other metabolic functions. However, oocytes were bigger in fed corals compared with the unfed controls. Several hypotheses are proposed to explain these metabolic/energy distribution patterns.

Key words: Scleractinia • *Pocillopora verrucosa* • Reproduction • Heterotrophic feeding • Aquaria.

PREFACE

The experimental work described in this dissertation was carried out in the School of Biological & Conservation Sciences, University of KwaZulu-Natal, Durban, from January 2007 to May 2009, under the supervision of Professor Renzo Perissinotto, Professor Michael H Schleyer and Mrs. Alke Kruger.

The study represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, this is duly acknowledged in the text.

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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

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I. BACKGROUND

Coral reefs are among the most productive, diverse and complex ecosystems in the world (Odum and Odum 1955) supporting almost one third of the world's marine fish species (Newton *et al.* 2007). They provide many goods and services such as recreational activities and coastal protection (Moberg and Folke 1999). The recreational value of reefs is enormous (Pendleton 1995). In the Caribbean alone, the income from tourism was US$ 8 900 000 000 in 1993 (Dixon *et al.* 1993). Driml (1994) estimated that the financial value of tourism in the Great Barrier Reef was AUS$ 682 000 000 annually. They are also a valuable source of food for local communities (Bruckner 2000). More than 100 countries, representing approximately tens of millions of people, depend on coral reefs for part of their livelihood or for part of their protein intake (Salvat 1992). Indeed, reef-related fisheries constitute approximately 10% of the total fish consumed by humans (Smith 1978; Pauly et *al.* 2002). For example, coral reef fisheries in Philippines provide livelihood for more than a million fisherman who contribute almost US$ 1 billion annually to the country's economy (White *et al.* 2000). Coral reefs are also important in coastal protection from currents, waves and storms. In addition, they are essential in maintaining the biological diversity and constitute an important spawning, nursery and breeding areas (Moberg and Folke 1999).

Nevertheless, with the increase of anthropogenic pressures (*e.g.* mining for lime production, destructive fisheries, poaching, pollution, SCUBA diving etc.), coral reefs worldwide are in serious decline (Bellwood *et al.* 2004), 1% annually before 1997 and 2% annually between 1997-2003 (Bruno and Selig 2007). In addition, the harvest of corals for the international trade, apart from causing direct damage, has altered the species composition of some coral reef (Bellwood *et al.* 2004). Since the late 1980s, the number of home aquaria containing living corals has increased dramatically (Delbeek 2001). In 1985, the annual, global import value of marine fishes and invertebrates was estimated at US$24-40 million (Wood 1985). By 1992, this had this had reached US$360 million. The import value of corals in South Africa for 1998 was R601598 (64290kg), whereas the export value was R965354 (Stuttaford 1994). As a consequence, the number of colonies collected in the wild is increasing every year, despite the fact that hard corals are listed in the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES, Appendix II). To date, 60 % of coral reefs are in danger (Bruckner 2000), 30% are considered as severely damaged and over 25% of the reefs around the world have already died (Bellwood *et al.* 2004). Worldwide, human impacts have strongly affected the regenerative capacity of coral

reefs (Bellwood *et al.* 2004). Such loss of resilience makes coral reef ecosystems unbalanced and more vulnerable to natural disturbances. For example, conserving Southeast Asia reefs is estimated to cost \$23,100-\$270,000km^2 /year (Burke *et al.* 2002).

Coral farming would be an alternative to reduce the collective pressure on coral reef ecosystems. It would provide a sustainable supply for this intensive and growing trade and, therefore, could reduce the negative impact of collecting corals from their natural environment (Delbeek 2001). Fragmentation (asexual reproduction) is one of the easiest and most commonly used techniques to propagate corals (Borneman 2001). Most coral farms are capable of producing tens of thousands of fragments per year (Delbeek 2001). Recently, over 150 species of Scleractinia, 100 species of Octocorallia, and all available Zoantharia are being propagated regularly by asexual means (Borneman 2001). The majority of branching corals, *e.g*. *Acropora* spp. and *Pocillopora* spp. can be simply fragmented by cutting off a branch and then gluing it onto substrata. Corals with massive skeletons, *e.g*. *Goniopora* spp., *Platygyra* spp., *Porites* spp. can be fragmented by cutting the colonies into pieces using chisels or saws (Borneman 2001). Many soft corals, *e.g. Lobophytum* spp., *Sarcophyton* spp., *and Sinularia* spp. are also very easy to propagate. Most can be sliced with a razor or cut with sharp scissors to produce branch fragments, which are then fixed to substrate with glue, adhesive or fishing line. However, in some cases less than half of the transplanted pieces of coral survive and grow over long term (Delbeek 2001). This is mainly due to the still limited knowledge available on critical life history parameters of coral species and their required culture conditions (Delbeek 2001). The high mortality of cultured transplants is expensive and limits the productivity of the farms (Calfo 2001). Moreover, it requires the continuous replenishment of stock material and, therefore has a negative impact on coral ecosystems (Delbeek 2001). In addition, all offspring are genetically identical to the parent. Asexual populations are therefore clones, with little or no genetic diversity (Levinton 2001).

Sexual reproduction may play an increasing role in supplying the marine aquarium trade and reef restoration programs (Petersen *et al.* 2008). The propagation of corals by sexual reproduction would provide a sustainable supply for this growing trade. Therefore, the negative impact of collecting them from the wild could be strongly reduced. Understanding the sexual reproduction of corals, both in aquaria and in their natural environment, would also provide an alternative solution to reduce the harvest of wild specimens while playing a crucial role in conserving endangered species. The advantages of such an approach are: 1) the

potential discovery of new techniques for culturing hard corals (Heyward *et al.* 2002); 2) large-scale production of new colonies that would provide an adequate supply to aquarists (Ellis 1999); and 3) propagation and reseeding of reef habitats with young cultured colonies (Yates and Carlson 1992; Delbeek 2001; Petersen and Tollrian 2001; Amar and Rinkevich 2007). Moreover, sexual reproduction increases adaptive potential by providing continuously new combinations of genes and genetic variation due to chromosome crossover during meiosis (Giese and Pearse 1974). Transfer of genes increases intragenic variation but also produces new combinations of alleles on different chromosomes. Genetic variation may also increase resistance to diseases and resilience in bleaching events (Levinton 2001; Grottoli *et al.* 2006). These advantages can be assumed to be beneficial for the propagation of corals cultured in aquaria. However, in spite of spawning events having been reported in captivity, very little is known about how to initiate and control this sensitive metabolic process (Delbeek 2001).

The energy available for sexual reproduction depends on the percentage of energy allocated for growth and other physiological functions (Rinkevich 1989; Ward 1995; Rinkevich 1996). Scleractinian corals feed in two ways. They are able to source autotrophically-produced carbon (Muscatine *et al.* 1985) thanks to symbiotic unicellular dinoflagellates, known as zooxanthellae that live embedded in their tissue. Photosynthetic products are translocated from these symbiotic algae to the host tissue and are used to satisfy at least the basic metabolic requirements of corals (Falkowski *et al.* 1984). Muscatine *et al*. (1985) found that photosynthetically fixed carbon supplies the coral host with up to 140% of its daily energy demands. This nutrition is also used by the coral for the synthesis of new cells and extracellular products such as mucus. In the Great Barrier Reef, the genus *Acropora* exudes up to 4.8 litres of mucus per square metre of reef area per day (Wild *et al.* 2004).

However, it was found by Muscatine and Porter (1977) that photosynthetic products are deficient in nitrogen, phosphorus and amino acids, which are essential in gametes production. This suggests that other nutrient sources may interact in the reproductive process of corals. Heterotrophy has been recognized as an important factor providing major source of nitrogen and phosphorus to scleractinian corals (Harrison and Wallace 1990; Ferrier-Pagès et al. 2003; Houlbrèque *et al.* 2003). Numerous studies have demonstrated that corals are opportunistic feeders, able to ingest a wide variety of food items including dissolved organic (Grover *et al*. 2008) and inorganic compounds (Grover *et al.* 2008), free amino acids (Ferrier

1991; Anthony 1999; Hoegh-Guldberg and Williamson 1999), particulate organic matter (Anthony 1999; Anthony and Fabricius 2000), bacteria (Muscatine 1973; Sorokin 1973) zooplankton (Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003) and phytoplankton (Muscatine 1973).

A previous study performed on the annual heterotrophic feeding cycle of *Klyxum digitatum*, showed that feeding activity is correlated with the cycle of gametogenesis (Hartnoll 1975). In addition, more recent studies have focused on the effect of nutrient enrichment on coral sexual reproduction. In some cases, it has been shown that an abundance of nutrients is responsible for the increase in number (Amar and Rinkevich 2007) and size of oocytes (Ward and Harrison 2000; Bongiorni *et al.* 2003) as well as the viability of planulae (Amar and Rinkevich 2007) . For instance, Ward and Harrison (2000) demonstrated that corals exposed to elevated level of phosphorus produced more oocytes and spermaries than control colonies. They also observed no reduction in the mean oocyte number per polyp over the reproductive season. Reduction in oocyte number during gametogenesis is due to the oosorption of developing oocytes that may provide nutrients for the remaining oocytes (Harrison and Wallace 1990). Since colonies were given additional phosphorus in their experiments, they hypothesized that oosorption of young oocytes may not have been necessary. Thus, certain nutrients such as phosphorus may inhibit sexual reproduction of coral.

II. INTRODUCTION

Sexual reproduction, including annual spawning, fertilization, embryogenesis and settlement is well documented for many corals species (Fadlallah 1983; Schlesinger and Loya 1985; Babcock *et al.* 1986; Sier and Olive 1994; Kruger and Schleyer 1998; Carlon 1999). The reproductive modes of corals are diverse and are generally classified in four patterns. Scleractinian corals can be hermaphroditic, having both testes and ovaries in the same polyp, or gonochoric with separate sexes (Harrison and Wallace 1990) Moreover, they can be broadcast-spawners, where polyps package oocytes and sperm together within gamete bundles that are then ejected through the polyp mouth into the water column. They can also be brooders, with fertilization occurring internally and larval development taking place within the parent for several weeks before release (Carlon 1999). Corals within the same species may have different reproductive modes in different zoogeographic locations (Szmant 1986). For example, *Pocillopora damicornis* broods both asexual planulae and broadcast-spawns gametes in Western Australia (Ward 1992) but is only a broadcast spawner in the Eastern Pacific (Glynn *et al.* 1991).

Sexual reproduction in scleractinian corals appears to be dictated by several environmental factors, such as seawater temperature, solar irradiance and lunar cycle (Giese and Pearse 1974; Harrison *et al.* 1984; Penland *et al.* 2004). Glynn *et al.* (1991) found that reproductive activity in Eastern Pacific corals was stimulated by warm sea temperatures. For example, in Costa Rica and Panama, up to 50% of all *Pocillopora elegans* and *P. damicornis* were fecund during warmer periods (Giese and Pearse 1974). Similar studies provide evidence that optimal temperatures control gametogenesis, spawning and planula release in many coral species (Jokiel and Guinther 1978; Szmant-Froelich and Pilson 1980). However, in other tropical and subtropical localities, a few species such as *P. damicornis* become gravid during the cool season (Schlesinger and Loya 1985; Szmant 1986). Moreover, Stimson (1978) observed that the timing of gametogensis and spawning was not consistent between populations of the same species on the east coast of Australia. These inconsistencies suggest that other factors may be involved in the process (Mendes and Woodley 2002). Jokiel and Guinther (1978) suggested that variations in both solar irradiance and prey availability may be key factors in the regulation of coral reproduction. These obviously constitute the main regulators of the two different nutritional strategies used by scleractinian corals: autotrophic and heterotrophic feeding.

Although they gain the majority of their carbon requirement through their zooxanthellae (Muscatine *et al.* 1985), and despite the small size of their polyps, most pocilloporid corals are voracious opportunistic feeders (Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003). For example, *P. damicornis* is capable of feeding on fine suspended particulate matter (Anthony 1999) as well as on zooplankton (Palardy *et al.* 2006). In tank studies, *Stylophora pistillata* has demonstrated a capacity to feed on a wide range of food sources including dissolved free amino acids (Grover *et al.* 2008) bacteria including cyanobacteria, flagellates (Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003) and zooplankton (Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003)

Recent studies on *S. pistillata* have shown that heterotrophic feeding is of great importance for both the maintenance and growth of corals (Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003). Ferrier-Pagès *et al*. (2003) and Houlbrèque *et al*. (2003) found that feeding on zooplankton increases the growth rate of the skeleton as well as the amount of tissue synthesized. Heterotrophic feeding has also been shown to increase photosynthesis by enhancing the number of zooxanthellae per host cell (Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003). However, the relative importance (and/or dependence) of feeding on sexual reproduction is still unclear, as no descriptive or quantitative data are available on the effects of food on the reproductive effort in corals.

Pocillopora verrucosa (Fig. 1) is a branching scleractinian also named “cauliflower or knob-horned" coral because of the structure of the branches and the appearance of the colonies when their polyps are expanded (Veron 1986; Borneman 2001). The polyps (Fig. 2) are small but very prominent and fuzzy (Borneman 2001). The colonies are generally brown and grow to a maximum height of 25cm and less than 50 cm in diameter (Riegl 1993) . The branches are 1 to 2 cm thick and are characterised by large verrucae that are regular in size (Veron 1986). This species is one of the most common species of the family on local reefs. It extends in distribution from the Red Sea, eastwards to Hawaii and around Australia, except in the north (Veron 1986). It is also common along the east coast of Africa north of Transkei until 31°S; 33°E (Riegl 1993).

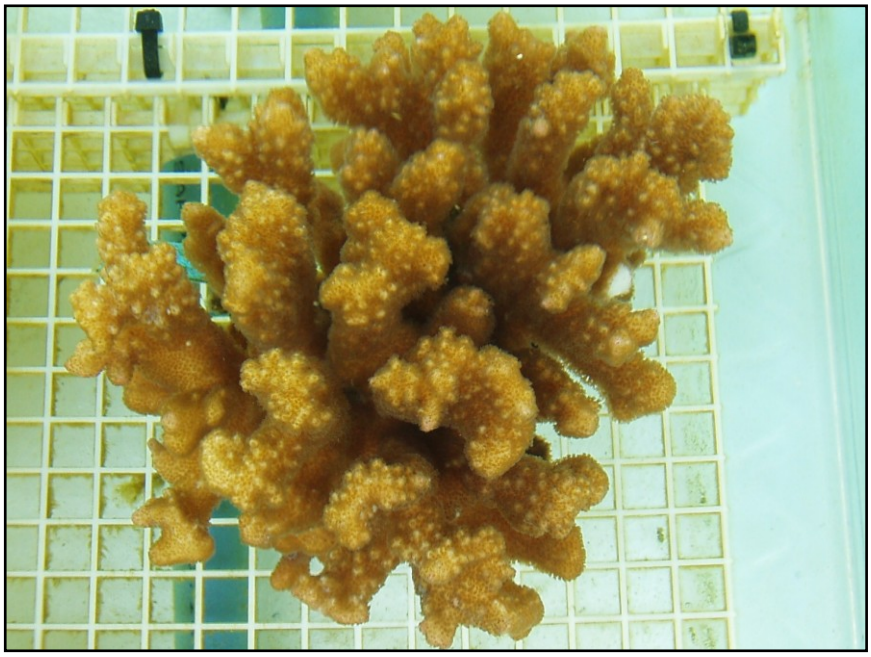

Figure 1. A live *Pocillopora verrucosa* colony on a plastic tray.

Figure 2. Polyp of *Pocillopora verrucosa* (×90).

Pocillopora verrucosa is broadcast spawner in KwaZulu-Natal, South Africa (Kruger and Schleyer 1998), in the Red Sea (Schlesinger and Loya 1985) and in the Maldives (Sier and Olive 1994) but is a brooder in Eniwetok, eastern Australia (Stimson 1978). The gametogenic cycle is short and occurs generally over a four-month period with male and female gametes maturing simultaneously in the polyps (Schlesinger and Loya 1985; Sier and Olive 1994; Kruger and Schleyer 1998). In each polyp, 12 gonads are embedded in 12 mesenteries. The maximum of six ovaries and six spermaries are arranged alternatively around the periphery of the polyp (Sier and Olive 1994).

Pocillopora verrucosa was chosen for a study on the effects of feeding on its reproduction for several reasons: it is the most common species of the family on local reefs (Kruger and Schleyer 1998) and its life-history seems to favour widespread dispersal of its larvae (Ridgway *et al.* 2001). It has a short period of reproductive activity of four months (October to January) during spring and summer in KwaZulu-Natal (Kruger and Schleyer 1998). Moreover the species seems to be resistant and well-adapted to marginal conditions, *e.g.* low seawater temperature at high latitude, strong wave action, considerable surge and sediment movement on the reefs (Schleyer 2000). In addition, the species is found on upper reef slopes, as well as in deep water and lagoons (Veron 1986), and therefore, may have a high tolerance to variations in light intensity.

Objectives of the study

The aim of this work was to investigate the effect of rotifers (*Brachionus* sp.) as a food source on the reproduction of a typical scleractinian coral, *Pocillopora verrucosa*. The experimental approach was designed to establish whether food affects the reproductive effort of this species. This was determined by monitoring the gametogenic cycle and the mean diameter and number of oocytes and spermaries that developed under different feeding regimes, using histological techniques. The main question of this work was as follows: Does heterotrophic feeding have a positive effect on the reproductive effort of *P. verrucosa*, increasing 1) the number of gametes as well as 2) their size as a result of an increase in prey concentration?

III. MATERIAL AND METHODS

III. 1. Coral collection

Colonies of the hard coral, *P. verrucosa*, were collected in June 2007 from Vetches Pier (29°52'2.08''S; 31°3'15.52''E; Fig.3), a small artificial reef situated in front of the Oceanographic Research Institute (ORI) at uShaka Marine Park in Durban, KwaZulu-Natal (South Africa). This reef, measuring approximately 80 m × 20 m, consists of sandstone boulders accreted by oyster and mussel shells (Schleyer 1981). The depth varies between 1 and 1.8 m, the annual mean temperature is 21.8°C and the mean salinity is 35‰ (Schleyer 1984). A total of 18 colonies between 358 and 400 mm in diameter were randomly sampled by SCUBA diving at an average depth of 1 to 1.5 m. They were carefully detached from the natural substratum and placed in individual plastic bags to prevent any damage during transportation. The corals were then placed in aquaria on plastic trays in natural seawater for approximately two months. During this period the colonies were monitored for necrosis, mortality, disease and damage. No signs of stress response such as a moderate polyp swelling, extensive color changes or extensive mucus secretions were observed during the two months after collection.

Figure 3. Vetches Pier, Durban Harbour, KwaZulu-Natal, with coral collection site highlighted by white circle (Google Earth, 2009).

III. 2. Experimental set-up

The experimental system was established in a glass house under natural daylight. Water temperature in the aquaria was maintained at 25°C by heaters in the sump, while ambient air temperature was controlled by air conditioning units set at 25°C. A recirculating system consisting of nine aquaria was used. Aquaria were grouped into three separate sub-systems (Fig.4) of three aquaria, each containing 150 l of seawater supplied by a submersible pump (AQUAH$_2$O™ APH-8000 powerhead), at a flow rate of 8000 l per hour. Each 150 l tank was equipped with its own pump (AQUAH$_2$O™ APH-2500 powerhead) to ensure adequate water circulation.

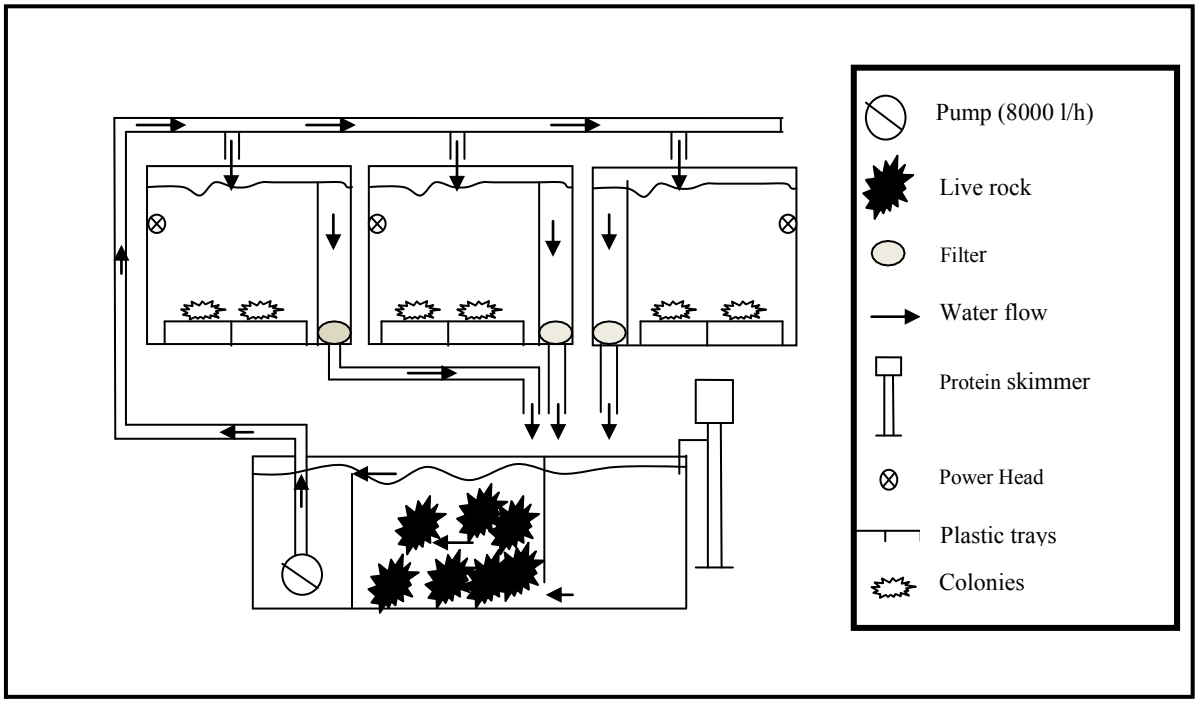

Figure 4. Diagrammatic representation of holding system for *Pocillopora verrucosa* consisting of three glass tanks connected to a biological filtration system.

Seawater quality was monitored in the system three times per week and levels of ammonium, nitrate, nitrite, phosphate, calcium and salinity were kept constant in each aquarium and between aquaria, in order to avoid bias (Table 1). Aquarium kits, such as "Calcium Marine Aquarium Test Lab (Red Sea)" and "Marine Lab (Red Sea)" were used for this purpose (Table 1). Release of nutrients may cause a rapid deterioration in the quality of water, if the filtration system is not efficient and specifically adapted. The water was thus

continuously filtered and passed through a bio-filter (live rock) and a protein skimmer (WEIRPRO™ SA-2013). The biological filtration was effected by pieces of dead coral colonized by algae and bacteria, which contributed to water purification. Noxious products such as ammonia, nitrates and phosphate were assimilated by the algae and anaerobic bacteria in the live rock (Borneman 2001). Ammonia is usually oxydated to nitrite and nitrate by the bacteria. Protein skimming was effected by stripping the water with air bubbles in a purification column (Delbeek 1994). Organic molecular compounds, such as proteins and particulate organic matters surrounding the air bubbles are attracted to the air-water interface. The surfactant compounds accumulated by the air bubbles then form a brown skin at the water surface which can be easily removed from the water. The glass tanks were isolated from each other with glass fibre wool and fine cotton filters inserted at the base of each outlet pipe where the water was returned to the sump (Borneman 2001). In addition, each individual aquarium was cleaned several times per week in order to avoid algal growth.

Table 1. Water quality parameters recorded in the experimental system.

Water parameters	**Value (± SD)**
Light intensity (µmol photons m^{-2} s^{-1})	776.69 (± 240.65)
Salinity (ppm)	1.025 (± 0.80)
pH	7.93 (± 0.20)
Temperature (°C)	25.69 (± 0.20)
Ca^{2+} ($mg.l^{-1}$)	425 (± 26.35)
No_3^- ($\mu M.l^{-1}$)	0
NH_3/NH_4 ($\mu M.l^{-1}$)	0.13 (± 0.20)
Po_4^- ($\mu M.l^{-1}$)	0.13 (± 0.13)

III. 3. Food source

An unidentified local rotifer species, *Brachionus* sp. was used as food source. This was obtained from a commercial hatchery and cultured in aquaria under natural daylight (Hagiwara 1989). Rotifers are used extensively in aquaculture because of their high fecundity rate and ease of culturing (Hagiwara 1989). Rotifers were selected in this study for several

reasons: 1) they are relatively small, ranging in size from 0.06-1.00 mm; 2) they are slow swimmers (in contrast with *Artemia*), maintaining their position in the water column (easier to be caught by the polyps); 3) they can be cultured at high densities; 4) They reproduce rapidly, making them available in large quantities within a short period (2 days); and 5) these nutrients include highly unsaturated fatty acids (Lubzens *et al.* 1989).

In this study, they were cultured in a 150 l container filled with seawater. The water was maintained at 26°C with a pH of 8 and under a specific gravity between 1.007 and 1.014. In addition, one liter of green water and 10 mg of baker's yeast were added daily to obtain mass production during the entire experiment (Fu *et al.* 1997). When rotifers reached their peak growth in the tank, the entire culture volume was filtered through a net and the rotifers collected on the mesh.

III. 4. Feeding experiments

Before the start of the feeding experiments, the heterotrophic feeding behavior of *P. verrucosa* was investigated. Individual colonies were placed in water with an excess of freshly collected rotifers in a one liter glass jar at 26°C. Feeding was observed under a stereomicroscope at ×40 magnification for 20 minutes and photographic recordings were taken.

During the feeding studies colonies were divided into three groups and fed as follows: 1) high feed corals (HFC) receiving 15×10^2 organisms l^{-1}; 2) low feed corals (LFC) that received 5×10^2 organisms l^{-1}; 3) and unfed corals (UC) that were not offered any food item during the experiments. The latter were used as controls. There were three replicates for each concentration. The nine aquaria were randomly allocated for each treatment, with each replicate containing six colonies. The different concentrations of prey were added to the aquaria three times per week for three hours (Ferrier-Pagès *et al.* 2003). During feeding, the submersible pumps were switched off in order to optimize prey capture and avoid nutrient recycling. The feeding experiment started two months before the reproductive season (October 2007) and was conducted until January 2008.

III. 5. Experimental procedure

Branch fragments of *P. verrucosa*, 3 to 5 cm in length, were collected from the experimental colonies during the reproductive season from October 2007 to February 2008

(Kruger and Schleyer 1998). One branch per colony was removed and fixed in 4% formalin in sea water. The branch fragments were then decalcified with 1-3% hydrochloric acid and immediately transferred to a 70% ethanol solution (Wallace 1985). The procedure of decalcification is summarized in Table 2.

Table 2. Decalcification procedure of coral tissue according to Wallace (1985).

Chemicals	**Time**
4% Formalin	1 week
1-3% Hydrochloric acid	1 week
70 % Ethanol	1 week

Histological sections were prepared to ascertain the various gametogenic stages. Tissues containing a minimum of five polyps per decalcified fragment were dissected using ultra-fine pins (Elephant Ento pins, size 00) under a dissecting microscope. These were dehydrated in a series of ethanol baths, cleared with xylene and then embedded in histological paraffin wax. The procedure for the dehydration and the wax embedding is presented in Table 3.

Table 3. Embedding procedure used for coral tissues in this study (Kruger *pers. comm*.)

Chemicals	**Time (h)**
50% Ethanol	2
70% Ethanol	2
90% Ethanol	2
100 % Ethanol	2
100 % Ethanol	1
Xylene	3
Histology paraffin wax	3
Histology paraffin wax	3
Histology paraffin wax	1

Table 4. Staining procedure for coral tissues used in this study (Kruger *pers. comm*.)

Chemicals	**Time**
Xylene	10 min
100% Ethanol	9 sec
100% Ethanol	9 sec
90% Ethanol	9 sec
70% Ethanol	9 sec
50 % Ethanol	9 sec
Tap water	9 sec
Ehrlich's haematoxylin	20 min
Tap water	9 sec
Warm tap water (30°C)	Until tissue turns purple blue
Tap water	9 sec
Eosin solution	1.20 min
Tap water	9 sec
50 % Ethanol	9 sec
70% Ethanol	9 sec
90% Ethanol	9 sec
100% Ethanol	9 sec
100% Ethanol	9 sec
Xylene	10 min
Xylene	10 min

Cross sections 7 µm thick were cut using a microtome. One in every four sections (28 µm), was mounted on frosted glass slides and then stained with Ehrlich's haematoxylin and eosin solution (Table 4). The respective composition of these stains is presented in Tables 5 and 6.

Table 5. Chemical composition of Ehrlich's haematoxylin stain (Drury and Wellington 1967).

Chemicals	Quantity
Haematoxylin powder	2 g
Ethanol absolute	100 ml
Glycerol	100 ml
Distilled water	100 ml
Acetic acid	10 ml
Potassium aluminium sulphate	15 g
Sodium iodate	0.3 g

Table 6. Chemical composition of eosin solution (Mahoney 1966).

Chemicals	Quantity
Eosin yellow powder	1g
Distilled water	100 ml

Gametes in serial sections were examined in selected polyps under a compound microscope at different magnifications (×200, ×400, ×1000) and photographed using NIS Element software (NIKON©). The number of gametes and their size were recorded for each gametogenic stage using image analysis (Image Pro Plus 6.0 Media Cybernetics Inc.). Only the oocytes and spermaries that were sectioned at their maximum diameter were measured. The size (in units of μm) of oocytes and spermaries was estimated by calculating the mean value of the maximum and minimum diameters at a right angle. The total oocyte volume was calculated by multiplying the oocyte volume ($(4/3)\ \pi r^3$; r equal radius) with the oocyte number. Total oocyte volume is used as an approximation to determine the total volume taken by oocytes in the polyps. The description of each gametogenic stage was based on the classification published by Glynn *et al.* (1991) and Kruger and Schleyer (1998). A sub-sample of five polyps per colony and per month analyzed for each feeding treatment. Average values of the number, size and volume of gametes are cited per polyp and per feeding treatment. In total, 450 polyps were examined in histological sections taken from the 18 colonies. The experiment was conducted during a single reproductive season, from October 2007 to February 2008.

III. 6. Statistical analysis

The effects of heterotrophic feeding on the size, number and volume of both oocytes and spermaries were analyzed using multivariate ANOVA. The normality and homogeneity of variance were assessed using the Kolmorogov–Smirnov statistical test. When a significant effect was detected, a Fisher LSD post-hoc test was used to locate significant differences between feeding treatments. All tests were carried out using STATISTICA 6.1 (Stat Soft Inc.). Data were expressed as means ± standard deviation (SD) or mean ± standard error (SE). Moreover, a nested analysis was performed on the number and volume of oocytes in order to ascertain the potential effect of tanks on the reproductive dynamics of *P. verrucosa*.

IV. RESULTS

P. verrucosa proved to be an efficient predator, despite having small polyps (Fig.5). Individual colonies (5 polyps) were able to catch and ingest two to six rotifers within a 20 minute period. The corals exhibited two main capture behaviors. The first type was reliant on sensory perception: polyps that came in contact with the food trapped their prey with their nematocysts and drew them back to their mouths where they were ingested (Fig. 5b). The second involved passive capture: polyps formed mucus webs that were extruded from the oral cavity to entrap the prey. When the mucus had captured enough food, it was pulled with the food back into the mouth. With this feeding mode, one single polyp was able to catch more than 10 prey at a time (Fig. 5).

No mortality occurred amongst the *P. verrucosa* colonies used in the experiments. After two months in the aquaria, new tissue had grown over collection scars and areas of dead skeleton. Moreover, no sign of stress response such as a moderate polyp swelling, extensive color changes or extensive mucus secretions was observed during the three weeks after collections. In addition, an analysis performed on the mean number of oocytes per polyp and oocyte total volume per polyp showed that there was no significant difference between the various experimental aquarium systems ($p > 0.05$).

IV. 1. Effect of feeding on the gametogenic cycle

P. verrucosa was found to be a simultaneous hermaphrodite (Fig. 6) with a short annual reproductive cycle in aquaria under different feeding regimes. Female and male gametes were located in the same polyp on separate septa (Figs. 6a, 7a). In gravid polyps, a maximum of six ovaries and six spermaries were distributed alternately, linked to their respective mesenterial filaments.

IV. 1. 1. Oogenesis

In both fed and starved colonies, oocyte development involved four distinct stages, as described by Kruger and Schleyer (1998). Stage I oocytes or oogonia (Figs. 6a, 6d), were located in clusters forming an ovary within the mesenterial filament. Both nuclei and nucleoli were clearly visible but no cytoplasm had developed yet. Stage II oocytes (Figs. 6b-d) were also packed in the ovary, but this time, were characterized by an accumulation of cytoplasm.

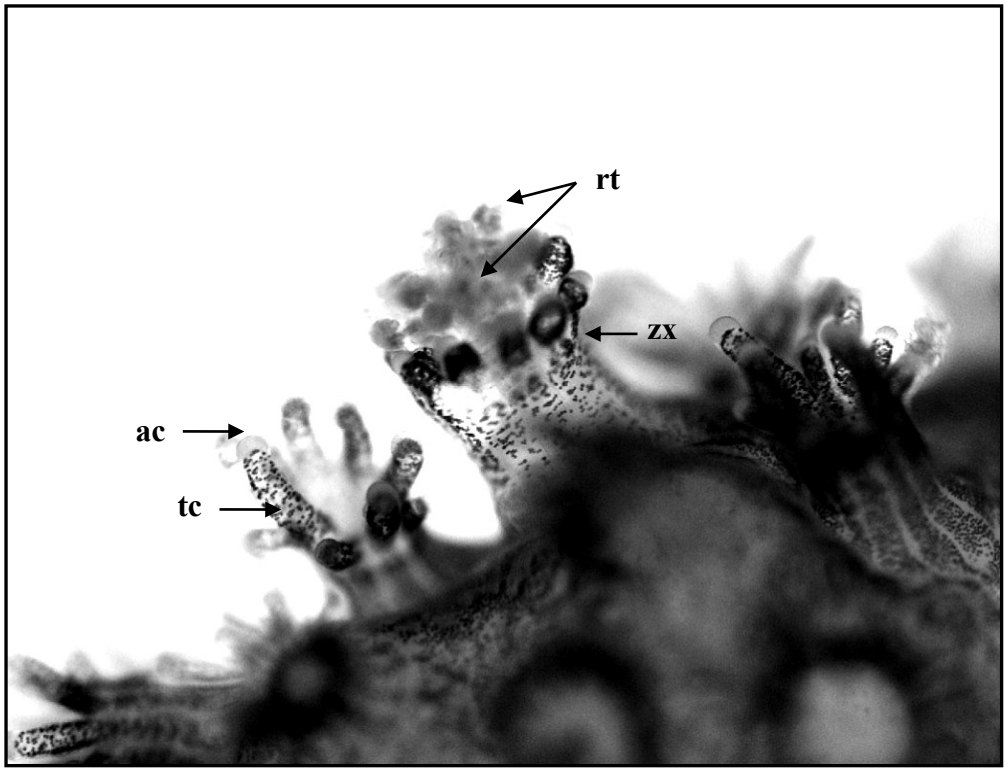

Figure 5. *Pocillopora verrucosa* (× 90). Feeding polyps with expanded tentacles. The individual in the middle is ingesting several rotifers (*Brachionus* sp.). ac, acrosphere; rt, rotifer; tc, tentacle; z, zooxanthellae.

The nuclei and nucleoli were stained with more contrast and their outlines were more uniform. Stage III oocytes (Figs. 6d-e) were spherical and surrounded by a vitellogenic matrix. The cytoplasm was filled with white lipid droplets and the nuclei were located in the middle of the oocyte. Stage IV oocytes (Fig. 6f) were surrounded by a purple membrane, probably an artifact of histology. Their cytoplasm contained an enlarged vacuole and had many zooxanthellae. Nuclei were detached from the vitellogenic filament and had migrated to the periphery of the oocyte.

IV. 1. 2. Spermatogenesis

The four stages of spermary development that could be differentiated in the three feeding treatments can be described as follows. The earliest stage (stage I, Fig. 7b) consisted of small clusters of stem cells surrounded by a thick layer of spermatogonia in the mesoglea. Stage II (Fig. 7c) spermaries were spherical, exhibiting a greater affinity for haematoxylin and containing clusters of spermatocystes with large nuclei. Stage III (Figs. 7d-e) spermaries contained more numerous spermatids that were smaller in size and had condensed nuclei. Lumina were visible in some spermaries and flagella (or tails) were discernible in some spermatids. Stage IV (Fig. 7f) spermaries were easily recognizable in that the spermatozoa were much denser, more numerous but smaller than in the previous stages and exhibited a distinct flagellum. All spermaries were bigger and filled a large proportion of the polyps in this stage.

IV. 1. 3. Oocyte development

Oocytes were found from October to December 2007 in colonies under the three feeding treatments (Fig. 8). They were also observed in January but in negligible numbers. The reproductive cycle of the HFC (Fig. 8c) was shorter as no oocytes remained in the polyps by January 2008. In all feeding treatments, Stage I to II oocytes were dominant during the first two months of the gametogenic cycle and peaked in November 2007. Stage III oocytes were observed between November 2007 and January 2008 in the UC treatment but only from November to December 2007 in both LFC (Fig. 8b) and HFC (Fig. 8c). Mature oocytes (stage IV) were found between November 2007 and January 2008 inside polyps of the UC and LFC and peaked in December 2007 in these treatments. However, they were found only between November 2007 and December 2008 in the HFC treatment

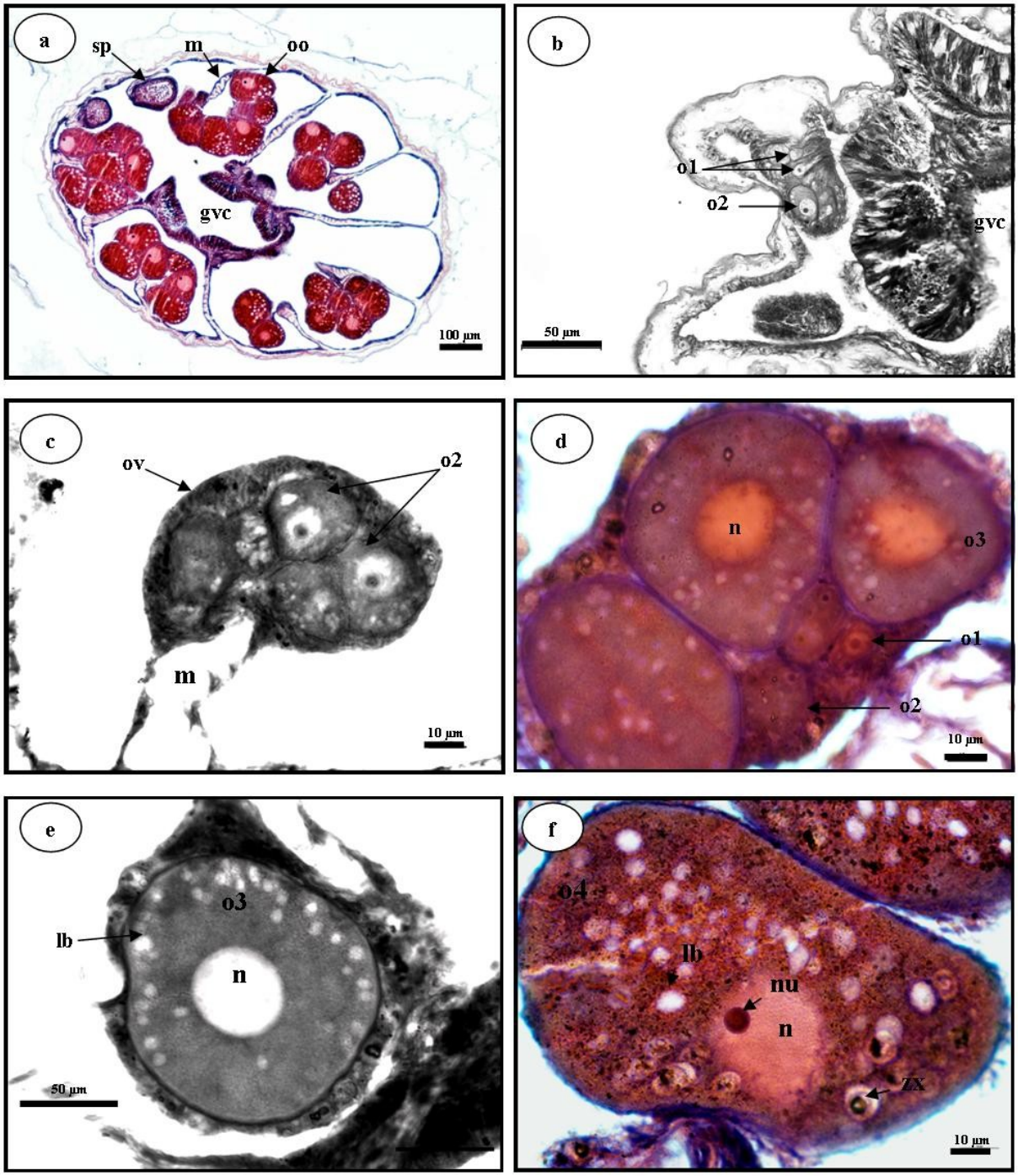

Figure 6. Histological sections of *Pocillopora verrucosa* polyps showing oocyte development. **(a)** Structure of oocytes and spermaries. **(b)** Ovary containing stage I and II oocytes. **(c)** Ovary containing stage II oocytes, nucleus and nucleoli are visible. **(d)** Oocytes stage I, II and III embedded in the same mesentery. **(e)** Oocyte stage III. **(f)** Mature oocyte (stage IV) containing zooxanthellae and exhibiting cytoplasm filled with lipid droplets (Note purple-blue oocyte membrane). gvc, gastrovascular cavity; lb, lipid droplet; m, mesentery; n, nucleus; nu, nucleolus; oo, oocyte; o1,2,3, and 4, oocyte stage I, II, II and IV respectively; ov, ovary; sp, spermary.

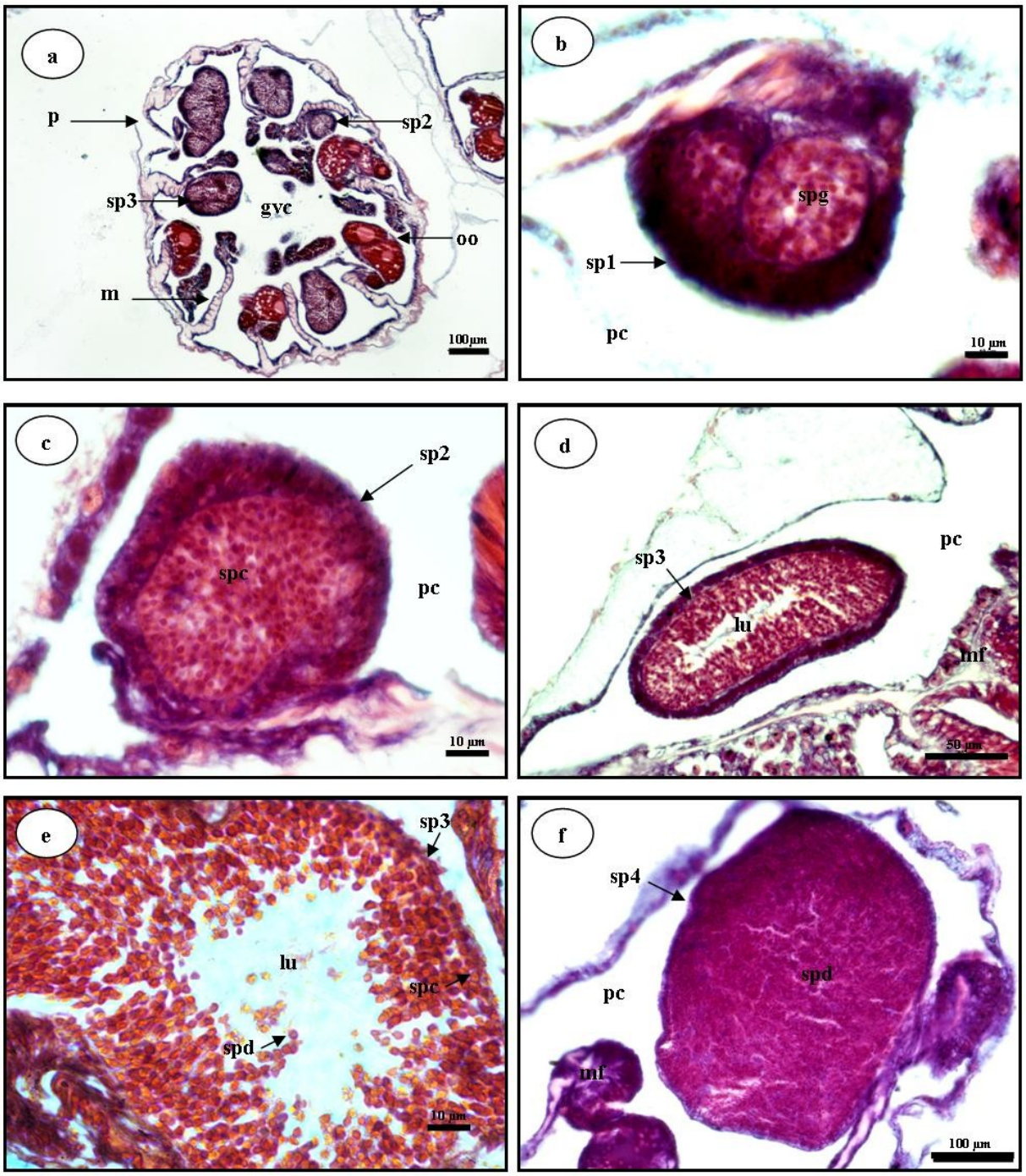

Figure 7. Histological section of *Pocillopora verrucosa* polyps showing spermary development. **(a)** spermaries arrangement into a polyp. **(b)** Immature spermary stage I. **(c)** Spermary stage II containing immature spermatocytes. **(d)** Spermary stage III. **(e)** Spermary stage III: immature spermatocytes densely packed at the periphery and mature spermatids concentrated near the centre. **(f)** Mature spermary stage IV, spermatids densely packed throughout spermary. gvc, gastrovascular cavity; lu, lumen; m, mesentery; oo, oocyte; p, polyp; pc, polyp cavity; spc, spermatocytes; sp1, 2, 3, and 4, spermary stage I, II III and IV respectively; spd, spermatid; spg, spermatogonia.

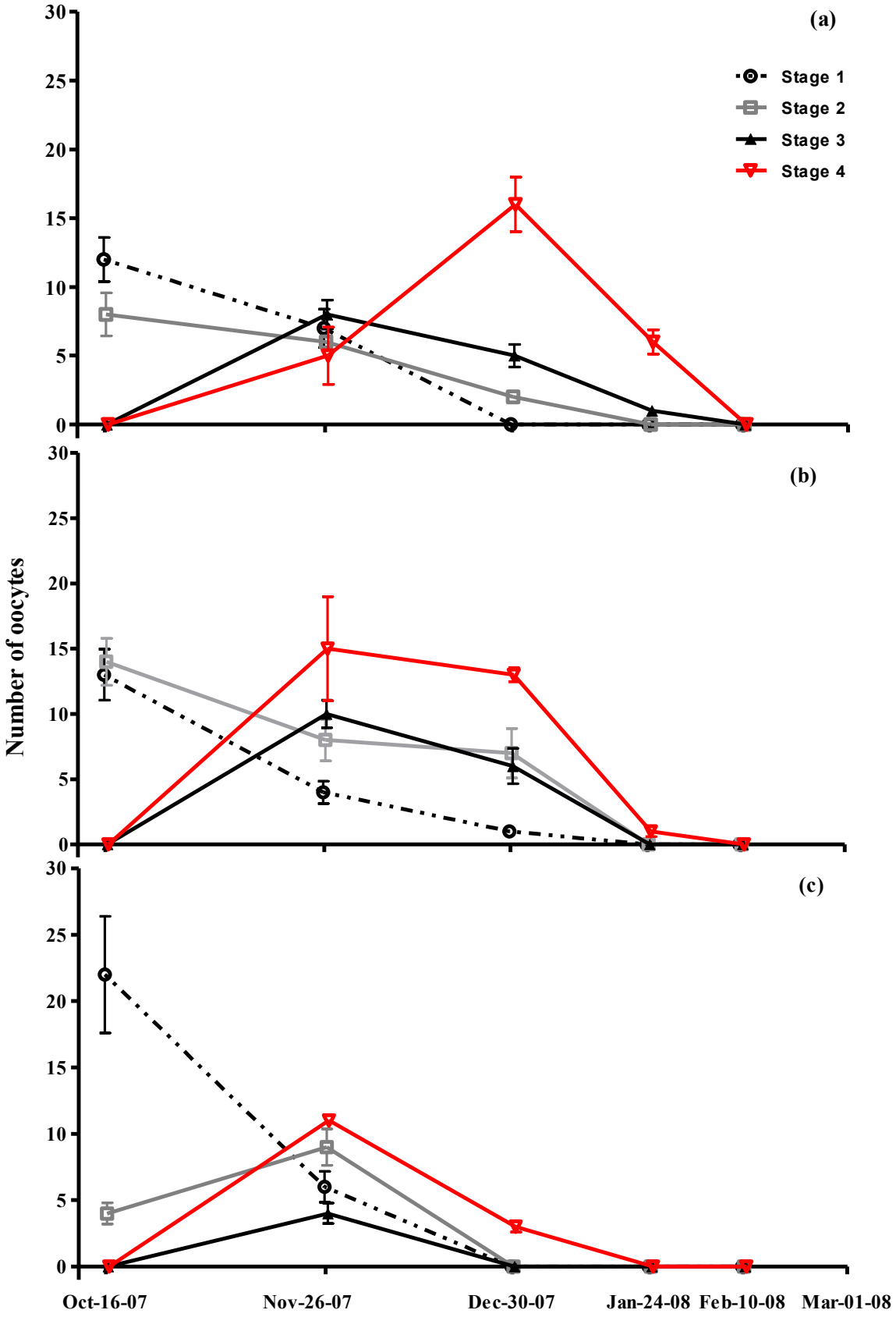

Figure 8. Monthly mean number (±SE) of oocytes per polyp in the different developmental stages of *P. verrucosa* polyps under different feeding regimes. **(a)** UC, unfed controls; **(b)** LFC, 5×10^2 *Brachionus* sp.l^{-1}; **(c)** HFC, 15×10^2 *Brachionus* sp.l^{-1}.

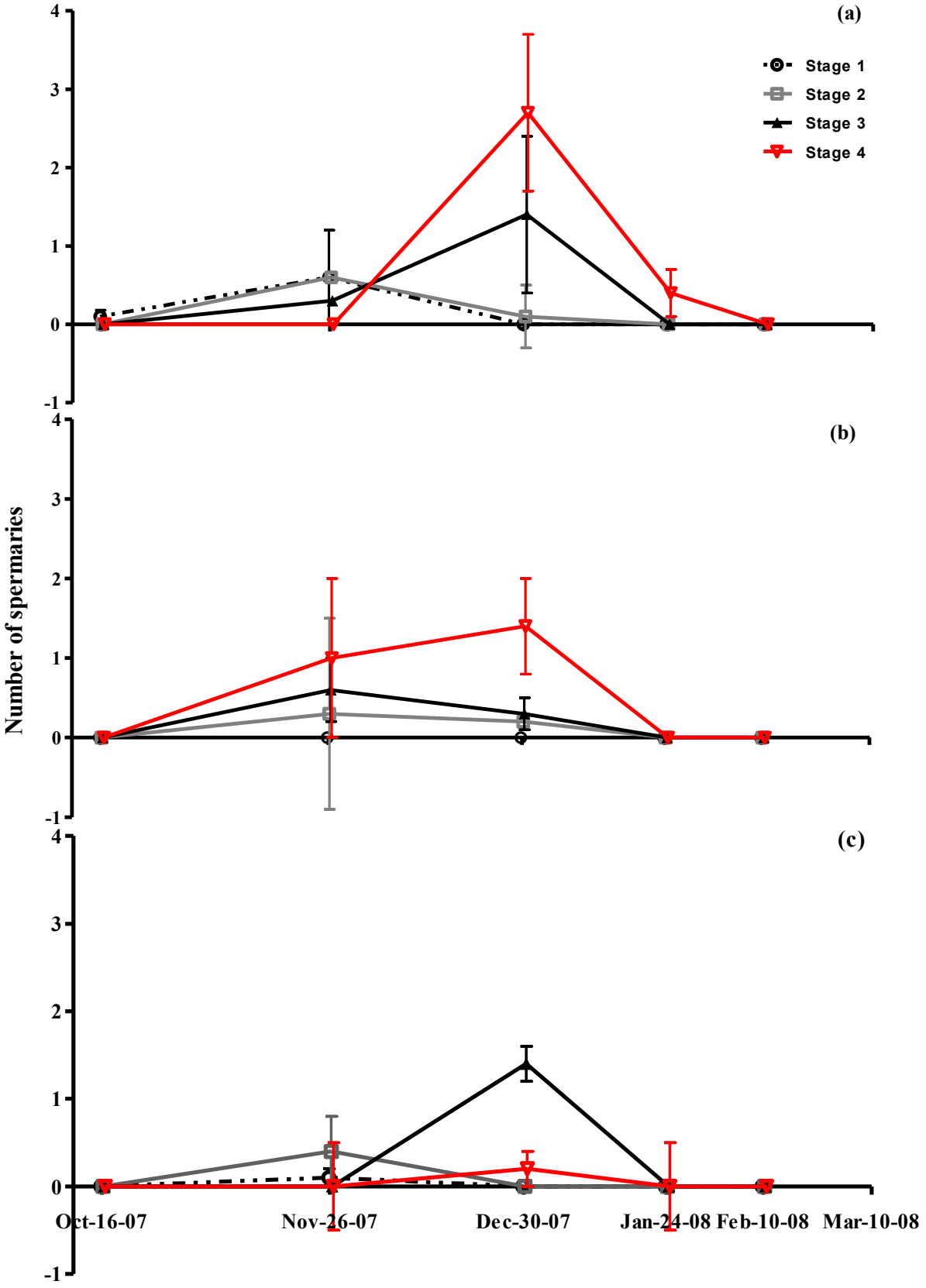

Figure 9. Monthly mean number (±SE) of spermaries per polyp in the different developmental stages of *P.verrucosa* polyps. **(a)** UC, unfed controls; **(b)** LFC, 5×10^2 *Brachionus* sp. l^{-1}; **(c)** HFC, 15×10^2 *Brachionus* sp. l^{-1}.

IV. 1. 4. Spermary development

The male reproductive cycle was shorter than that of the female with a lag of one month between spermary and oocyte development (Fig. 9). Spermaries of individual polyps from both fed and starved colonies were discernable from late November 2007 until January 2008. Stage I spermaries were rare in the polyp sections. They were only visible in November 2007 in both UC (Fig. 9a) and HFC treatments (Fig. 9c). No stage I spermaries were recorded in the LFC (Fig. 9b). Stage II spermaries were present from November to December 2007 only in the UC and LFC. Stage III spermaries were found from November to December 2007 in the UC and LFC treatments, while polyps from the HFC treatment exhibited stage III spermaries only in December 2007. Mature stage IV spermaries were observed from November 2007 to January 2008 in all three treatments, and peaked in December 2007.

IV.2. Effect of feeding on reproductive effort

IV. 2. 1. Fecundity

Histological analysis showed that the average percentage of polyps containing oocytes was significantly higher in the UC compared with the LFC and HFC treatments (ANOVA: df = 2, F = 37.70, $p< 0.05$; Fisher LSD post-hoc, $p< 0.05$; Fig. 10a). However, the percentage of polyps with spermaries did not differ significantly between feeding treatments (ANOVA: df = 2, F = 0.72, p> 0.05; Fisher LSD post-hoc, p> 0.05; Fig. 10b).

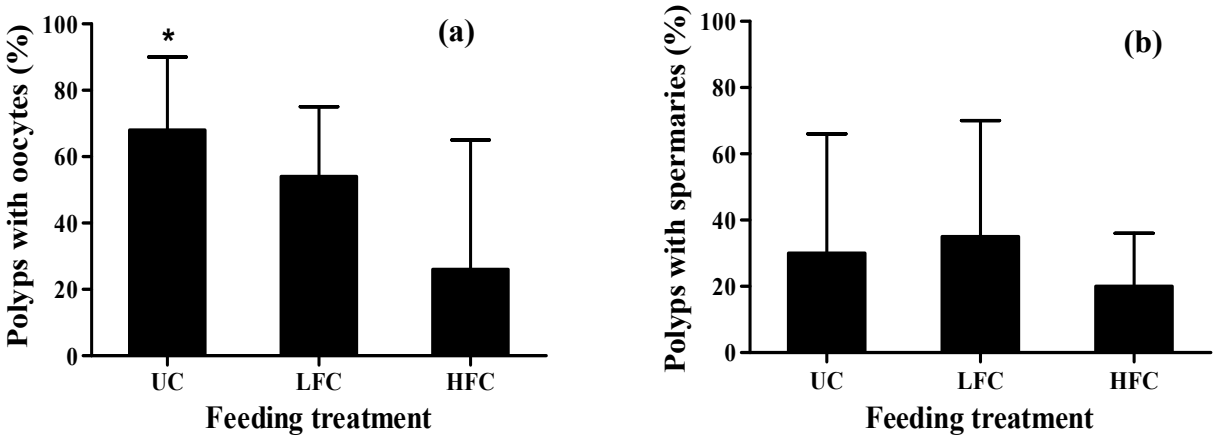

Figure 10. Average percentage (±SD) of polyps / colony containing **(a)** oocytes and **(b)** spermaries in colonies of *Pocillopora verrucosa* under the three feeding regimes. UC, unfed controls; LFC, 15×10^2 *Brachionus* sp. l^{-1}; HFC, 5×10^2 *Brachionus* sp. l^{-1}; *, significant difference (p< 0.05).

IV. 2. 2. Number of oocytes and spermaries

The mean number of oocyte per polyp and per colony (Fig. 11a; tables 12, 13) was significantly different between the three feeding regimes (ANOVA: df = 2, $F = 2.45$, $p < 0.01$). The UC treatment contained significantly more oocytes per polyp compared with the HFC treatment (Fisher LSD post-hoc, $p < 0.05$). There was however no significant difference between the UC and LFC treatments (Fisher LSD post-hoc, p = 0.96; Fig.11a).

The average number of mature oocyte (stage IV) per polyp and per colony recorded prior to spawning (December 2007) was significantly different between the three feeding treatments (ANOVA: df = 2, $F = 20.97$, $p < 0.01$). In particular, the number of oocytes was higher in the UC than in the HFC treatment (Fisher LSD post-hoc, $p < 0.05$). There was, however, no significant difference between UC and LFC (Fisher LSD post-hoc, $p < 0.05$; Fig.11a).

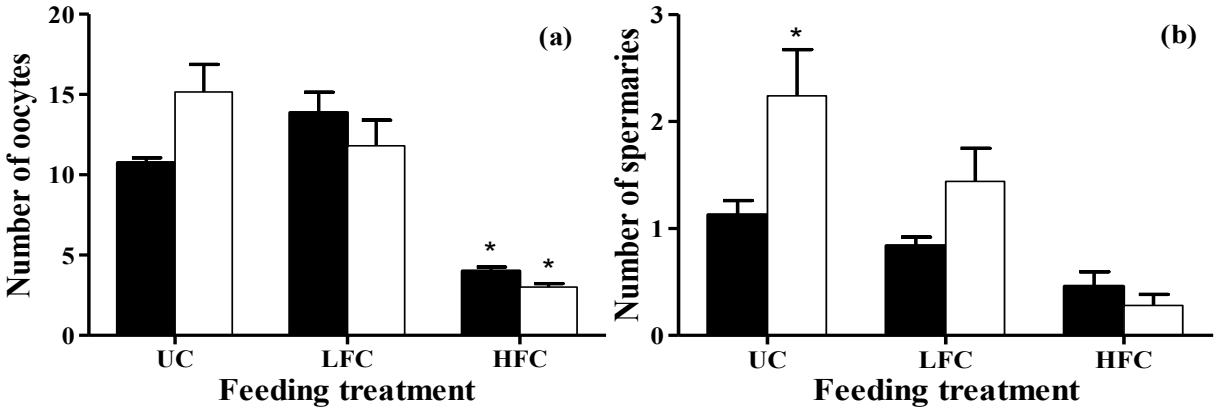

Figure 11. Reproductive effort of *P. verrucosa* in the feeding experiments (±SE). **(a)** Oocyte; **(b)** spermary. Black bars = total mean number of gamete (including stage I, II, III and IV per polyp measured for the entire gametogenic cycle from October to January); white bars = mean number of mature gamete (stage IV) per polyp and colony prior to the end of the gametogenic cycle (December 2007). In December 2007, the dominant stage was stage IV oocytes, ready to be released. UC, unfed controls; LFC, 5×10^2 *Brachionus* sp. 1^{-1}; HFC, 15×10^2 *Brachionus* sp. 1^{-1}.

No significant difference was found in the average number of spermary per polyp between the three feeding treatments (ANOVA: df = 2, $F = 1.75$, $p > 0.05$; Fig. 10b). Nevertheless, the mean number of mature stage IV spermaries recorded prior to spawning was

significantly different (ANOVA: df = 2, $F = 1.75$, $p < 0.01$) with more spermaries per polyp observed in the UC than in the other two treatments (Fisher LSD post-hoc, $p > 0.05$).

IV. 2. 3. Size of oocytes and spermaries

The mean size of stage I-IV oocytes (Table 7) par polyp was significantly different between the UC and both LFC and HFC (ANOVA $_{\text{stage I}}$: df = 2, $F = 2.45$, $p < 0.01$; ANOVA $_{\text{stage II}}$: df = 2, $F = 2.45$, $p < 0.01$; ANOVA $_{\text{stage III}}$: df = 2, $F = 2.45$, $p < 0.01$; ANOVA $_{\text{stage IV}}$: df = 2, $F = 2.45$, $p < 0.01$). Considering the entire gametogenic cycle, the mean oocyte size of each stage attained in the UC treatment was significantly smaller compared to that of both LFC and HFC treatments (Fisher LSD post-hoc, $p < 0.05$). However, the average oocyte size observed in the LFC and HFC treatments did not differ significantly (Fisher LSD post-hoc, $p > 0.05$). In contrast, the average size of stage I-IV spermaries (Table 8) measured in polyp sections was not significantly different between unfed and fed colonies ($p > 0.1$).

Table 7. Mean size ±SE **(n)** of oocyte stages per polyp present in the colonies of *P. verrucosa* in the feeding experiments. All sizes are in µm. UC, unfed controls; LFC, 5×10^2 *Brachionus* sp. l^{-1}; HFC, 15×10^2 *Brachionus* sp. l^{-1}. Numbers in bold refer to the number of oocytes measured.

Oocyte stage	Unfed corals	Low feed corals	High feed Corals
Stage I	10.82 ± 0.01 **(296)**	12.63 ± 0.02 **(206)**	12.99 ± 0.04 **(78)**
Stage II	20.96 ± 0.03 **(204)**	27.73 ± 0.02 **(396)**	29.84 ± 0.05 **(178)**
Stage III	39.60 ± 0.05 **(240)**	55.21 ± 0.04 **(311)**	53.66 ± 0.17 **(70)**
Stage IV	69.18 ± 0.03 **(484)**	89.03 ± 0.02 **(476)**	75.78 ± 0.07 **(93)**

Table 8. Mean size ±SE **(n)** of spermary stages per polyp present in the colonies of *P. verrucosa* in the feeding experiments. All sizes are in µm. UC, unfed controls; LFC, 5×10^2 *Brachionus* sp. l^{-1}; HFC, 15×10^2 *Brachionus* sp. l^{-1}. Numbers in bold refer to the number of spermaries measured.

Spermary stage	Unfed corals	Low feed corals	High feed Corals
Stage I	36.41 ± 0.60 **(19)**	44.86 ± 0.00 **(1)**	32.91 ± 1.60 **(3)**
Stage II	51.07 ± 0.38 **(21)**	57.65 ± 1.03 **(15)**	38.15 ± 0.90 **(12)**
Stage III	76.11 ± 0.33 **(52)**	100.12 ± 0.68 **(28)**	85.99 ± 1.93 **(6)**
Stage IV	132.22 ± 0.42 **(92)**	154.78 ± 0.52 **(73)**	126.97 ± 0.93 **(32)**

IV. 2. 4. Total oocyte volume

The mean values obtained for the total oocyte volume in the polyps (Fig.12a) were significantly different between the three feeding treatments (ANOVA: df = 2, $F = 9.238$, $p < 0.01$). Oocytes produced in the LFC were significantly more voluminous compared to both the UC and HFC (Fisher LSD post-hoc, $p < 0.01$). However no difference was obtained between UC and HFC (Fisher LSD post-hoc, $p > 0.05$).

The mean values obtained for the total oocyte volume prior to spawning (December 2007; Fig.12b) was also significantly different between the three feeding regimes (ANOVA: df = 2, $F = 7.355$, $p < 0.01$). Oocytes measured in both UC and LFC, were significantly more voluminous compared to those packed in HFC. Moreover, the oocyte total recorded in LFC was significantly larger than that observed in UC (Fisher LSD post-hoc, $p < 0.05$).

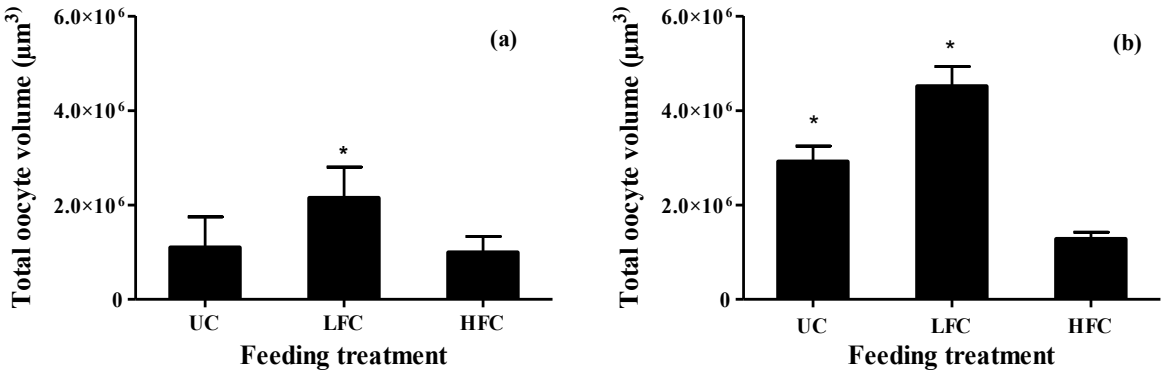

Figure 12. Total oocyte volume (±SE) per polyp recorded **(a)** during the entire gametogenic cycle **(b)** prior to spawning in December 2007. UC, unfed controls; LFC, 5×10^2 *Brachionus* sp. l^{-1}; HFC, 15×10^2 *Brachionus* sp. l^{-1}; *, significant difference ($p < 0.05$).

V. DISCUSSION

When exposed to different feeding regimes, *Pocillopora verrucosa* proved to be a simultaneous hermaphrodite and broadcast spawner in the aquaria. A similar reproductive pattern was observed previously in *P. verrucosa* in KwaZulu-Natal (Kruger and Schleyer 1998), the Maldive Islands (Sier and Olive 1994) and the Red Sea (Schlesinger and Loya 1985). In the current study, the reproductive period was short, occurring during spring and summer from October 2007 to January 2008. Oogenesis began in October 2007 and progressed until December 2007. Spermary development started one month later (November), but spermaries reached maturity simultaneously with the oocytes in December 2007. A similar, short reproductive season was also recorded for this species in all the studies mentioned above, *i.e.* KwaZulu-Natal (Kruger and Schleyer 1998), Red Sea (Fadlallah 1985) and Maldive Islands (Sier and Olive 1994). This reproductive strategy is known as annual hermaphroditic protogyny (Harrison and Wallace 1990) and is employed by many simultaneous hermaphroditic corals such as *Goniastrea australensis* (Kojis and Quinn 1982), *Acropora* spp. (Wallace 1985), *Montastrea annularis* (Szmant 1991), *Favites abdita* and *Leptoria phrygia* (Kojis and Quinn 1982). However, in the Red Sea population of *P. verrucosa*, oogenesis commences three months before spermatogenesis (Schlesinger and Loya 1985).

No spawning was observed in the experimental aquaria in this study. Nevertheless, the decrease in the number of mature oocytes and spermaries (stage IV) observed in late December 2007 suggests that spawning occurred between this period and January 2008. Moreover, no absorption of gamete was detectable in the histological sections of the polyps. This may be attributable either to the size of gametes, which may have been too small to be visible in the aquaria or to the time of spawning. If gametes were released during the night, they would have been removed by the protein skimmer or captured by the other filtration systems associated with the experimental setup.

Most of the mature stage IV *P. verrucosa* oocytes observed in the histological sections contained zooxanthellae in December 2007. This algal acquisition suggests that the oocytes were viable and attained maturity (Benayahu and Schleyer 1998). In other studies, mature oocytes of this species also contained zooxanthellae prior to spawning, *e.g.* in KwaZulu-Natal (Kruger and Schleyer 1998) and in the Maldives (Sier and Olive 1994). Hirose *et al*. (2000) found that in both *P. verrucosa* and *P. eydouxi*, oocytes took up zooxanthellae three to four

days before spawning. Species of the genera *Porites* and *Montipora* are also known to incorporate zooxanthellae into their oocytes a few weeks prior to spawning (Harrison and Wallace 1990), while the scleractinian coral *M. digitata* acquires endosymbiotic algae 24 h before spawning (Harrison and Wallace 1990).

A comparison of the fertility rate (percentage of polyps with gametes) in unfed (UC) versus naturally growing colonies (Kruger and Schleyer 1998) indicates that conditions in captivity may have positively affected the reproductive dynamics of the experimental *P. verrucosa*. Unfed colonies contained a higher percentage of polyps with gametes than colonies growing in the natural environment. This suggests that environmental conditions in the aquaria were adequate for coral growth, and the reproduction processes in the polyps were not stressed at any stage during the experiment. Moreover, *P.verrucosa* in aquaria produced the same number of gametes (Table 10) as those observed in the field (Kruger and Schleyer 1998). However, other studies have shown that colonies in the Maldive Islands (Sier and Olive 1994) and in the Red Sea (Fadlallah 1985) tend to invest more energy into reproduction than their KZN counterparts. The mean number of oocytes per polyp was higher in the Maldives (56.52 ±36.40) and the Red Sea (33.0 ± 10.7 to 93 ± 20.8) compared to those recorded in this study (10.75 ± 1.51) and that of Kruger and Schleyer (1998) (14.67 ± 8.12, Table 10). These comparisons are, however, only applicable to the mean number of oocytes produced during the entire reproductive season. Unfortunately, no data were found on the development of mature gametes into healthy larvae which would have provided an insight into reproductive viability (number of oocytes giving rise to planulae capable of settling and metamorphosing) of populations growing in their natural environment.

In this study, it was found that the proportion of polyps with gametes was significantly lower in fed compared with unfed colonies (Table 9). It could be argued that feeding reduced the fecundity of *P.verrucosa*. However, measurements of fertility alone are insufficient to draw conclusions concerning the effects of supplementary nutrition on the reproductive process itself (Loya *et al.* 2004). Measurements of other reproductive parameters, such as the mean number of gametes, total oocyte volume, percentage maturity and gamete size may provide more comprehensive and reliable information on the biological response of corals to nutritional supplementation (Ward and Harrison 2000; Loya *et al.* 2004). Indeed, a comparison of the mean number of oocytes per polyp and the total oocyte volume per polyp produced during the entire gametogenic cycle showed that the proportion of resources

invested in reproduction differed. Coral colonies used as unfed controls (UC) allocated more energy towards oocyte development than did those exposed to the highest food concentration (HFC). However, no significant effect of feeding was recorded in the average number of spermaries per polyp produced by the three groups exposed to different feeding regimes (Table 9).

Table 9. Summary of significant results presented in this study.

Parameters	**Significant differences**
Polyps with oocyte (%)	UC > LFC = HFC
Polyps with spermary (%)	UC = LFC = HFC
Total oocyte number	UC = LFC > HFC
Total spermary number	UC = LFC = HFC
Number of mature oocytes	UC = LFC > HFC
Number of mature spermaries	UC > LFC = HFC
Oocyte size (μm)	UC < LFC = HFC
Spermary size (μm)	UC = LFC = HFC
Total oocyte volume (N × Size)	LFC > UC & HFC; UC > HFC

In addition, to better understand the effect of feeding on reproduction, the stage IV oocytes and spermaries was compared just before the likely spawning event (December 2007). According to Loya *et al.* (2004) it is critical to determine if gametes reach a size that would enable fertilization. This gives accurate information concerning the "total energy invested" in reproduction. The results obtained in this study suggested that the increased nutrition may have reduced the reproductive outputs of *P. verrucosa* by acting as a stressor or inhibiting factor. The number of mature oocytes and spermaries recorded in polyps was significantly lower in fed colonies provided considerable supplementation compared to unfed controls (Table 9). The same reproductive response is observed when corals are exposed to high levels of nutrients, *e.g.* nitrite, nitrate, phosphate, orthophosphate and ammonium (Tomascik 1990; Ward and Harrison 2000; Harrison and Ward 2001). Loya *et al*. (2004) suggested that chronic eutrophication caused by fish cages deployed *in situ* in the Red Sea had a negative effect on the reproductive effort of another pocilloporid coral, *Stylophora pistillata*. The proportion of oocytes reaching maturity was lower in the colonies exposed to nutrient

enrichment, compared to those occurring under natural conditions. Similar reproductive patterns were recorded in Australia for two *Acropora* species, *A. longicyathus* and *A. aspera*, also exposed to high levels of nutrients (Ward and Harrison 2000). Moreover, *P. damicornis* in Australia and Hawaii failed to produce planulae when exposed to ammonium enrichment (Ward and Harrison 2000; Cox and Ward 2003). In contrast, Bongiorni *et al*. (2003) and Amar and Rinkevich (2007) found that nutrient enrichment had a positive effect on *S. pistillata* colonies in the Red Sea as it increased both the average number of oocytes per polyp and the number of polyps with developing testes. However, Loya *et al*. (2004) considered these results and conclusions unreliable, as the measurement of reproductive effort was not performed on the last reproductive stage (stage IV and planulae).

Results presented in this study suggest that a physiological trade-off may occur in the energy allocation in *P.verrucosa*. Heterotrophic feeding may enhance other physiological responses at the expense of reproduction when excess food is available. Growth rates and sexual reproduction are often described as competing biological processes (Stearns 1977), sharing the same energy resources (Harrison and Wallace 1990; Loya *et al.* 2004). Recent studies have revealed a positive effect of heterotrophy on coral growth (Kim and Lasker 1997; Witting 1999; Anthony and Fabricius 2000; Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003). Coral growth is a complex metabolic process that includes both tissue growth and skeletal calcification. Calcification involves the secretion of an organic matrix and the deposition of a $CaCO_3$ fraction (Houlbrèque *et al.* 2003). This implies that corals have to allocate a relatively high proportion of their energy budget to calcification (Jacques and Pilson 1980; Witting 1999; Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003). Houlbrèque *et al*. (2003) have demonstrated that calcification rates may be two to three times greater in polyps of the coral *S. pistillata* exposed to high levels of food, compared to polyps under less favorable conditions. In addition, similar experiments have shown that feeding has a strong effect on tissue growth in *S. pistillata*, increasing it by two to eight times above background rates (Ferrier-Pagès *et al.* 2003). In the same species, the maximum amount of carbon and nitrogen ingested from prey represents one third of the total needs for tissue growth (Ferrier-Pagès *et al.* 2003; Houlbrèque *et al.* 2003).

In the present study, no differences were found in the reproductive effort (*i.e.* average number of mature gametes produced) between unfed colonies (UC) and those fed with a low concentration of prey (LFC). This may suggest that energy allocation between reproduction

and other metabolic functions may be controlled by the level of food available in the environment. The amount of food ingested may act as a stimulus, inhibiting the reproduction process of corals when available in excess, and possibly stimulating it at lower levels. Ferrier-Pagès *et al*. (2000) have suggested that the direction of the biological response to food intake actually depends on the concentration of such foods. Moreover, allocation strategies may depend on the seasonal availability of food (Heino and Kaitala 1999). For example, pavonid corals undergo maximum growth during periods of low water temperature and high biological productivity, but reproduce during warm periods (Wellington 1982; Wellington and Glynn 1983). In addition, studies performed on the sexual reproduction of other broadcast-spawning corals have shown that gametogenesis occurs generally in summer (Fadlallah 1985; Babcock *et al.* 1986; Kruger and Schleyer 1998) during peaks of primary production (Table 11). In the current experiment, there is evidence of an interaction between reproduction and the availability of nutritional resources.

This study also revealed that heterotrophic feeding affected the size of oocytes in *P. verrucosa* (Table 7). Oocyte sizes were significantly larger in fed colonies than in the controls (Table 9). This suggests that *P. verrucosa* colonies exposed to higher nutrition may modify their life-history traits, by partitioning the resources allocated to the future offspring through reproduction. Therefore, the well-fed colonies produced fewer but more viable oocytes with greater dispersive potential than unfed corals (Stearns 1977). Nevertheless, some studies suggested that oocyte size alone does not reliably reflect the energy invested into reproduction (Thompson 1982; McEdward and Carson 1987; George *et al.* 1991). George *et al*. (1991) showed that starfish, fed with low food ration produced larger oocytes than those fed with high food ration but they have lower survival. In addition, Thompson (1982) considered that differences in organic composition of urchin oocytes of similar diameter may vary, when the urchins are fed different diets.

The potential effects of feeding on this pattern of reproduction in *P. verrucosa* reproduction were tested by comparing the total oocyte volume (Fig. 11) for each of the feeding treatments. One would expect an inverse relationship between oocyte number and oocyte size with similar reproductive outputs in terms of total oocyte volume between the fed and unfed colonies. However, no evidence of such a relationship was found in this study as both unfed and slightly fed colonies were more productive in terms of oocyte volume than those fed with a high concentration of rotifer prey (Table 9). Therefore, the positive effect of

food on oocyte size (Table 9) alone is insufficient to draw conclusions concerning variations in the reproductive traits of *P. verrucosa*.

Thus, these results have revealed that the effects of the ingestion of rotifers (*Brachionus* sp.) on the reproduction of *P. verrucosa* do not follow the hypotheses originally formulated for this study. Surprisingly, heterotrophic feeding reduced the fecundity in *P. verrucosa*, with the number of gametes decreasing with increasing prey concentration. Therefore, the provision of food for coral reproduction would seem inadvisable, especially in the production of young corals for reef restoration or the aquarium trade. However Petersen *et al.*(2008) found that the provision of food enhances the growth and survival of early sexually-derived coral recruits. This may contribute to the success of post-settlement stages at the time when they are most vulnerable.

VI. CONCLUSIONS

This work investigated the influence of heterotrophic feeding on sexual reproduction in the hard coral *Pocillopora verrucosa* in a recirculating aquarium system. A number of reproductive parameters were assessed to establish this. Initial investigations revealed that the reproductive mode, strategy and timing were the same as those employed by populations of *P. verrucosa* in different localities. Corals maintained under controlled conditions were more fecund compared than those growing in the wild. The results unequivocally showed that reproductive effort of *P. verrucosa* was reduced by feeding since the percentage of polyps with gametes and the number of oocytes per polyp was lower in fed colonies than in unfed controls. It was assumed that a trade-off occurred between growth and reproduction, with the energy from the heterotrophic feeding allocated to growth, rather than reproduction.

Nevertheless, this preliminary investigation on the effect of feeding on sexual reproduction in *P. verrucosa* was based on one reproductive season only. Further comparative studies are needed to test the validity of the results found in this experiment employing higher sample numbers.

In addition, it would be relevant to assess the growth rate in such experiments to estimate the trade-off between growth and reproduction in fed colonies. Extended studies are needed to determine how corals partition their resources when fed over a longer time scale. The use of methods such as natural stable isotope signal may provide an interesting means of identifying sources of carbon and nitrogen entering gamete production.

Table 10. Oocyte and spermary production by *Pocillopora verrucosa* in the Maldives, South Africa, the Red Sea and in aquaria (this study). ND, no data available.

Study site	Polyps with oocytes (%)	Polyps with spermary (%)	Mean no. of oocytes. (polyp^{-1} ±SD)	Mean no. of spermaries. (polyp^{-1} ±SD)	Mean no. of mature oocytes prior to spawning (polyp^{-1} ± SD)	Mean no. of mature spermaries prior to spawning (polyp $^{-1}$± SD)	Authors (year)
Maldive Islands	ND	ND	56.52 ± 36.40	ND	ND	ND	Sier and Olive (1992)
South Africa	59	27	14.67 ± 8.12	4.17 ± 1.34	18.9 ± ND	3.0 ± ND	Kruger and Schleyer (1998)
Red Sea	ND	ND	33.0 ± 10.7 to 93 ± 20.8	ND	ND	ND	Fadlallah (1985)
In aquaria (control =UC)	68	30	10.75 ± 1.51	1.13 ± 0.7	16 ± 8.54	2.24 ± 2.16	Séré (this study)

Table 11. Seasonality of coral reproduction and plankton productivity in some localities where corals are found. The corals listed are broadcast-spawners with one annual gametogenic cycle

Species	Location	Timing gametogenic cycle	Author (year)	Seasonality	Productivity (zoo/phytoplankton)	Author (year)
Pocillopora verrucosa	South Africa, Nine-mile Reef, KwaZulu-Natal (27°50S; 33°E)	Oct-Jan (1991-1993)	Kruger and Schleyer (1998)	Late spring- early summer	Peak in Oct-Mar (rain season)	Perissinotto. R, (2009) *pers. comm*.
P.verrucosa	Maldive Islands Galhu Falhu (4°N, 73°E)	Nov-Mar (1991-1992)	Sier and Olive (1994)	-	-	-
P.verrucosa	Saudi Arabia, Red Sea, Yanbu (4°N, 73°E)	Feb-May (1984-1985)	Fadlallah (1983)	Late winter- spring	Peak in March	Echelman and Fishelson (1990)
P. damicornis	South Africa, KwaZulu-Natal (29°S, 20°E)	Sep-Apr (2007-2008)	Massé (2009)	Late spring - summer	Peak in Oct-Mar (rain season)	Perissinotto. R, (2009) *pers. comm*
P. damicornis	Rottnest Island, Western Australia (32°S,115°E)	Sep-Apr (1988-1989)	Ward (1992)	Spring-summer	Peak in Oct-Feb	Furnas (2007)
P. damicornis	Rottnest Island, Western Australia (32°S,115°E)	Nov-Mar (1980-1982)	Stoddart and Black (1985)	Spring-summer	Peak in Oct-Feb	Furnas (2007)
Acropora spp. (11ssp.)	Magnetic island, GBR, Australia (19°S,146°E)	Jan-Feb to Oct (1983)	Babcock *et al*. (1986)	Summer-Spring	Peak in Oct-Apr	Furnas *et al*. (2005)
Galaxea fascicularis	Orpheus Island GBR, Australia (18°S,146°E)	Jun-Jul to Nov (1983)	Babcock *et al*. (1986)	Mid winter -Spring	Peak in Oct-Apr	Furnas *et al*. (2005)

Table 12. *Pocillopora verrucosa*. Mean number (± SD) of oocytes in *P. verrucosa* polyp's colony^{-1} treatment $^{-1}$

Feeding Treatment	Coral no.	Size (mm)	Average numbers of oocytes polyp^{-1}			
			Oct. 07	Nov. 07	Dec. 07	Jan. 08
UC	**1 UC**	356	12.8 (± 5.7)	16.8 (± 2.6)	19.6 (± 8.3)	-
	2 UC	389	17.0 (± 8.8)	12.8 (± 6.8)	-	-
	3 UC	400	-	17.0 (± 9.4)	28.4 (± 14.5)	-
	4 UC	357	-	11.2 (± 3.7)	16.6 (± 11.7)	5.2 (± 1.9)
	5 UC	350	16.6 (± 5.7)	16.4 (± 12.5)	4.2 (± 1.9)	6.6 (± 2.3)
	6 UC	397	-	8.6 (± 9.0)	28.4 (±11.8)	6.6 (± 3.4)
SFC	**1 SFC**	355	29.6 (± 6.8)	25 (± 11.3)	20.4 (± 11.8)	-
	2 SFC	359	2.6 (± 5.8)	17.8 (± 13.8)	30.2 (± 6.6)	-
	3 SFC	398	28.6 (± 11.3)	29.0 (± 17.7)	26.0 (± 11.3)	1.4 (± 0.9)
	4 SFC	351	-	43.2 (± 9.8)	2.8 (± 1.1)	-
	5 SFC	400	0.6 (± 1.3)	1.6 (± 8.6)	9.8 (± 4.5)	-
	6 SFC	356	-	-	0.2 (± 0.4)	-
LFC	**1 LFC**	382	5.2 (±11.6)	17.6 (± 6.7)	-	-
	2 LFC	368	-	20.0 (± 6.8)	-	-
	3 LFC	359	-	10.6 (± 3.6)	-	-
	4 LFC	400	-	-	-	-
	5 LFC	384	-	14.0 (±3.5)	-	-
	6 LFC	375	-	10.0 (±10.1)	2.6 (±0.9)	-

Table 13. *Pocillopora verrucosa*. Mean number (± SD) of spermaries in *P. verrucosa* polyp's colony^{-1} treatment^{-1}.

Feeding Treatment	Coral no.	Size (mm)	Average numbers of spermaries per polyp			
			Oct. 07	Nov. 07	Dec. 07	Jan. 08
UC	1 UC	356	0.4 (± 0.9)	2.7 (± 2.4)	4.0 (± 1.5)	-
	2 UC	389	-	3.9 (± 2.5)	5.0 (± 1.4)	-
	3 UC	400	-	-	4.7 (± 2.2)	-
	4 UC	357	-	0.2 (± 0.4)	4.0 (± 1.4)	0.6 (± 0.9)
	5 UC	350	-	-	-	-
	6 UC	397	-	0.2 (± 0.4)	4.2 (± 2.6)	1.6 (± 1.8)
SFC	1 SFC	355	-	1.8 (± 1.5)	2.9 (± 1.9)	-
	2 SFC	359	-	-	3.3 (± 0.8)	-
	3 SFC	398	-	0.8 (± 1.1)	0.2 (± 0.4)	-
	4 SFC	351	-	3.6 (± 2.4)	1.4 (± 1.7)	-
	5 SFC	400	-	1.4 (± 0.5)	1.4 (± 0.5)	-
	6 SFC	356	-	-	-	-
LFC	1 LFC	382	-	2.5 (± 2.1)	-	-
	2 LFC	368	-	2.2 (± 2.2)	1.7 (± 1.1)	2.4 (± 2.3)
	3 LFC	359	-	-	-	-
	4 LFC	400	-	-	-	-
	5 LFC	384	-	-	-	-
	6 LFC	375	-	-	0.4 (± 0.5)	-

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