

## Reproduction Effort in the Nudibranch *Phestilla sibogae*: Calorimetric Analysis of Food and Eggs<sup>1</sup>

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**ABSTRACT:** *Phestilla sibogae*, a nudibranch living on corals of the genus *Porites*, is rarely found on the reef at Kaneohe Bay, Hawaii, although *Porites compressa* is a dominant coral there. This is probably due to massive predation on juveniles and adults. Such predation pressure would force this species to put high effort into reproduction. In this work I found that *P. sibogae* laid eggs amounting to up to 17% of their body weight each day. Furthermore, based on a 100% conversion efficiency for ingested coral tissue, 51–78% of the calories each individual ate daily were channeled into egg production. Photosynthetic activity of zooxanthellae in the nudibranch's tissue suggests that the algae may provide some of the energy required by the animal's metabolism.

THE NUDIBRANCH *Phestilla sibogae* Bergh, 1905 feeds exclusively on corals of the genus *Porites* (Harris 1975, Rudman 1981) and is thought to occur throughout the entire Indo-Pacific region (Rudman 1981). At Kaneohe Bay, Oahu, Hawaii, the nudibranchs are rarely found, although *Porites compressa* is a dominant coral on the reef (R. A. Kinzie, E. Cox, and C. Hunter, pers. comm.). However, *P. sibogae* become pests when colonies of *Porites compressa* are kept in running seawater tanks in the absence of predators. This suggests that the population of *P. sibogae* on the reef is controlled by massive predation. The most obvious protective strategy employed by *P. sibogae* is camouflage and hiding. There are no active defense mechanisms, such as nematocysts, in this species (Rudman 1981). These observations support the suggestion of Harris (1975) that: "Predation on nudibranchs may be another factor influencing longevity. To grow fast and reproduce as rapidly as possible before being eaten may be far more important than the availability of the food source." It is known that the juveniles of *P. sibogae* exhibit fast growth (faster than that of a similar species, *Phestilla melanobranchia*

[Harris 1975]), and that upon reaching maturity, growth slows and reproduction (egg-laying) becomes continuous (Harris 1975, Rudman 1981).

The animals settle, live, and lay eggs on their food source—colonies of *Porites*. They ingest and digest both animal tissue and the zooxanthellae present in the coral (Rudman 1981). Observations showing zooxanthellae in the cerata of *P. sibogae* were reported by Kawaguti (1943), Rudman (1982), as well as in the present work. Zooxanthellae might have more than one function in the host tissue. They provide protective coloration, could be digested (Rudman 1981), and if photosynthetically active can translocate photosynthesis products to the host, as reported for other nudibranch species (Hoegh-Guldberg and Hinde 1986, Hoegh-Guldberg et al. 1986, Kempf 1984).

In this investigation I first estimated how much of the energy derived from eating coral tissue is channeled into reproduction (i.e., egg production) on a daily basis (reproductive effort) and then examined whether or not the zooxanthellae present in the cerata of the *P. sibogae* were photosynthetically active.

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### MATERIALS AND METHODS

At the Hawaii Institute of Marine Biology (on Coconut Island, in Kaneohe Bay, Oahu),

*P. sibogae* establish populations on colonies of *Porites compressa* kept in running seawater tanks. Observations and experiments were done on animals collected from these populations.

Experiments were run in running seawater chambers, 1.5 liters in volume. Two chambers were used, each containing two individuals of *P. sibogae* and a piece of living *Porites compressa*. The animals used were of different sizes, ranging on average from 1847 mg to 3546 mg in total mass for pairs in each experiment. Once every 24 hr the animals were taken from the chamber, placed on a filter for 30 sec to let the water drain, weighed, and put back in the chamber. The eggs laid during the preceding 24-hr period were collected, and the surface area of the coral eaten was measured by marking the area on a transparent plastic film, cutting it out, and weighing it. The area was then calculated from a calibration of plastic film pieces of known area and weight.

For determination of the caloric content of the coral, tissue was removed from freshly collected colonies using a Water Pik washing device (Johannes & Wiebe 1970). Distilled water was used with the Water Pik to obtain a sample as free of salt as possible. The volume of homogenate collected from a known surface area of coral was recorded. The homogenate was frozen, freeze-dried, and weighed. Weight of dry tissue per square centimeter was calculated. Dried tissue was used to determine ash content (500°C for 3 hr [Paine 1971]). Pellets of dry homogenate weighing 6.57–8.11 mg were used for calorimetric analysis.

The eggs, collected each day, were rinsed with distilled water, placed on a piece of filter paper for 10 min to let the water drain, and weighed for wet mass. Additional eggs, collected from the reservoir of animals, were used to establish the caloric content of the eggs. The eggs were rinsed, dried, and weighed for wet mass as described. Freeze-dried eggs were used for dry mass and ash content (after combustion for 3 hr at 500°C) measurements. Pellets weighing 4.38–18.11 mg were used in calorimetry.

Caloric values for eggs and coral tissue were determined using a Phillipson microbomb

calorimeter (Phillipson 1964). Samples of coral tissue were high in ash content, as reported in previous investigations (Glynn and Krupp 1986, Richmond 1987). Eggs were unexpectedly high in ash content. Similar results have been reported by Todd (1979) for spawn samples of nudibranchs. Because of the high ash contents, an endothermy correction factor of 0.14 cal/mg ash was applied (Paine 1966; see also Todd 1979). Respiration of the animals first in the dark and then in the light ( $45 \mu\text{E m}^{-2} \text{s}^{-1}$ ) was measured with an oxygen probe (Strathkelvin Instruments) in a 225-ml chamber containing filtered (through 0.5- $\mu\text{m}$  filter) water and using one animal at a time.

## RESULTS

The results of four experiments are summarized in Table 1. The average mass of eggs laid daily ranged from 157.5 mg to 571.3 mg and was correlated to animal mass ( $r = 0.78$ ). The ratio between body mass and eggs mass ranged from 0.17 in experiment 3 to 0.08 in experiment 4. This ratio was the same in experiments 1, 2, and 3 ( $P = .05$ , SNK test), but it was significantly lower ( $P = .05$ ) in experiment 4. The area of coral eaten daily by the nudibranch pairs ranged from 2.7  $\text{cm}^2$  (experiment 1, day 4) to 12.7  $\text{cm}^2$  (experiment 3, day 1). There was no correlation between animal weight and area of coral eaten ( $r = 0.18$ ). Calculations of the caloric contents of eggs were based on the calorimetric analysis of eggs collected from the reservoir of animals and resulted in a factor of  $6.22 \pm 1.2$  cal/mg dry mass. The caloric area specific content of *Porites compressa* was determined to be  $69.5 \pm 7.53$  cal/ $\text{cm}^2$ . The ratio between calories eaten and calories in the eggs ranged from 0.78 in experiment 2 to 0.25 in experiment 4. Calculation of this ratio assumed that all coral tissue and zooxanthellae ingested were utilized (i.e., assimilation of 100%). The ratios were not significantly different for experiments 1 to 3, but were significantly lower in experiment 4 ( $P = .05$ , SNK test).

The results for oxygen consumption are shown in Table 2. Oxygen consumption

TABLE 1  
SUMMARY OF DAILY MEASUREMENTS AND AVERAGES FOR FOUR EXPERIMENTS

EXP. NO.	DAY	ANIMAL MASS (mg)	EGGS MASS (mg)	CORAL EATEN (cm <sup>2</sup> )	EGGS WEIGHT		EGGS CALORIES	
					ANIMAL WEIGHT	CORAL CALORIES		
1	1	2,508	380	7.3	0.15	0.43		
	2	2,471	359	3.6	0.15	0.82		
	3	2,209	327	6.1	0.15	0.44		
	4	2,323	290	2.7	0.13	0.89		
Average		2,377.7	339	4.9	0.14	0.65		
SD		138.0	39.3	2.1	0.01	0.24		
2	1	3,634	577	4.7	0.16	1.02		
	2	3,286	514	8.1	0.16	0.53		
	3	3,720	673	6.7	0.17	0.78		
	Average		3,546.7	571	6.5	0.16	0.78	
SD		229.8	54.7	1.7	0.01	0.24		
3	1	2,196	367	12.7	0.17	0.24		
	2	1,990	297	4.1	0.15	0.61		
	3	2,130	419	6.1	0.20	0.57		
	4	2,174	477	6.0	0.22	0.66		
	5	3,090	414	7.2	0.13	0.48		
Average		2,316	395	7.2	0.17	0.51		
SD		440	67.2	3.3	0.04	0.16		
4	1	1,828	117	4.1	0.06	0.23		
	2	1,867	198	6.3	0.11	0.26		
Average		1,847	157	5.2	0.08	0.25		
SD		28	57.3	1.5	0.03	0.02		

NOTE: Animal mass determined at the beginning of a 24-hr period; eggs mass determined at the end of a 24-hr period. Results are for pairs of animals in each experiment.

TABLE 2  
RESULTS OF OXYGEN CONSUMPTION MEASUREMENTS  
Light = 45  $\mu\text{E m}^{-2} \text{s}^{-1}$

EXP. NO.	ppm O <sub>2</sub> /min	
	DARK	LIGHT
1	-0.073	-0.019
	$r^2 = 0.79$	$r^2 = 0.58$
2	-0.073	-0.037
	$r^2 = 0.80$	$r^2 = 0.96$
3	-0.021	-0.015
	$r^2 = 0.84$	$r^2 = 0.99$
4	-0.055	-0.017
	$r^2 = 0.94$	$r^2 = 0.78$
5	-0.025	-0.013
	$r^2 = 0.68$	$r^2 = 0.64$

was significantly lower when measured at 45  $\mu\text{E m}^{-2} \text{s}^{-1}$  than in the dark ( $P = .02$ , Mann-Whitney  $U$  test).

#### DISCUSSION

The proportion of resources (energy) that is diverted to reproduction should be in balance with other energy-demanding life activities (metabolism, respiration, growth). The proportion channeled into reproduction depends on the life history of the species and on environmental factors (Williams 1966). According to Miller (1962), nudibranchs can be separated into two groups. In the first are species that eat seasonal food and grow and reproduce quickly while their food source lasts. In the second group are species that eat food that is abundant throughout the year and that grow slowly and reproduce seasonally. *Phestilla sibogae* has characteristics of

both groups. Its food is available in all seasons; however, it grows quickly and reproduces constantly (Harris 1975, Rudman 1981). This could result from both evolutionary background and environmental factors.

An important environmental factor influencing life history is predation (Crowl and Covich 1990). *Phestilla sibogae* is rarely seen on *Porites* in the field, yet is abundant on this coral when kept in predator-free laboratory seawater tanks. This observation suggests massive predation on juveniles and adults in the field. The reproductive traits mentioned above for *P. sibogae* may well be determined by predation pressure on the adults and the lack of an active defense.

Such predation pressure would also influence the quantity of available energy that is channeled into reproduction. An increase in resources devoted to reproduction would result in decreased growth and survivorship and would reduce the future potential to reproduce (Williams 1966). When *P. sibogae* reaches maturity (as evidenced by the onset of egg-laying), growth rate slows (Harris 1975, Rudman 1981), and an animal lays an average of 1.5 egg masses a day for about 100 days (death occurs soon after the egg-laying ceases) (Harris 1975). As shown in the results reported here (experiments 1–3), each individual lays 14–17% of its body weight in eggs daily. Based on 100% conversion efficiency for ingested food, derived from eating coral, 51–78% of the energy is channeled into reproduction on a daily basis. Because this value is based on 100% conversion efficiency, the actual percentage of ingested energy invested in reproduction must be lower. In experiment 4 the numbers are significantly lower (0.08 of body weight and 0.25 of the energy), probably because these animals were smaller and still growing faster than larger animals, hence putting more energy into somatic growth.

In the present work, reproductive effort is defined as the percentage of body weight and of food intake put into production of eggs daily. In other studies the definition is different and therefore cannot be directly compared. Reproductive effort in nudibranchs was calculated by Todd (1979) as the ratio

between dry weight and caloric content in eggs (produced cumulatively during a long-term experiment) and the dry body weight and caloric content of the active female. Reproductive effort in terms of dry weight was 57.28–99.96% for *Onchidoris muricata* and 27.2–60.71% for *Adalaria proxima*, and in calories, 81.85–142.82% and 24.57–54.84% for the respective species. DeFreese and Clark (1983) also calculated the ratio between dry weight and caloric content of females' whole body and eggs for 11 species of nudibranchs. Their ratio was calculated for one event of egg-laying. The reproductive effort expressed in dry weight was 3–36.1%, while in caloric terms it was 9.1–36.6%. The reproductive effort as defined in this work was found to be 14–17% in body mass and 51–78% in calories channeled into reproduction daily.

Based on these high values, I conclude that the environmental conditions on the reef (being under massive predation pressure) force *P. sibogae* to "reproduce as rapidly as possible before being eaten" (Harris 1975).

While eating coral tissue, the animals ingest zooxanthellae. The algae can be digested (Rudman 1981). Personal observations during the present work showed that there are apparently living cells of zooxanthellae in the cerata. This has been reported by Kawaguti (1943), Rudman (1982), and Harris (1975). Oxygen consumption measurements show that consumption rate is lower in the light than in the dark, which suggests that the zooxanthellae in the animals are photosynthetically active and could contribute photosynthesis products to the nudibranch. Symbiosis between invertebrates and zooxanthellae where zooxanthellae contribute to energy intake of the animals by translocation of photosynthesis products is known in different groups. Symbiosis between zooxanthellae and nudibranchs has been described by Kempf (1984), Hoegh-Guldberg and Hinde (1986), Hoegh-Guldberg et al. (1986), Rudman (1982), and others. If *P. sibogae* get energy by translocation from zooxanthellae, it means that the ratio between energy intake and energy that goes to reproduction is not as high as indicated by the results of the present work, based only on energy intake from feeding on corals. This

“hidden” way of getting energy could also explain the high variability in the percentage of energy that goes to the eggs (51–78%) (from coral eating alone) and the low correlation between body size and area of coral eaten.

The amount of energy, if any, the animals get from translocation of photosynthetic products is not correlated to the size of the animal but rather to the characteristics of the algae population (number, chlorophyll per cell) and the amount of light to which they are exposed. Different concentrations of zooxanthellae in different individuals of a nudibranch species (*Pteraeolidia ianthina*) have been reported by Hoegh-Guldberg and Hinde (1986). In that work it was reported that 25–60% of photosynthetically fixed carbon is translocated to the animal. It has been shown that the algae have the same characteristics as zooxanthellae in shade-adapted corals (Falkowski and Dubinsky 1981, Porter et al. 1984) that are photosynthetically active in low light irradiance ( $I_c$  in 30–50  $\mu\text{E m}^{-2} \text{s}^{-1}$ ). If zooxanthellae in *P. sibogae* are shade adapted, they can photosynthesize even when the animal is hidden under a coral, as do zooxanthellae that live in coral tissue on the bottom side of some corals (unpublished data).

The results of this work give some clues about the life history of *P. sibogae* and raise questions about potential predators, the presence of the nudibranch on the reef, and the importance of zooxanthellae in the energy budget of *P. sibogae*.

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