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Nocturnalism in Sea Hares: Has UV Light Played a Role in its Evolution?

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Abstract. The possibility that UV light has played a role in the evolution of nocturnalism in sea hares was tested with *Aplysia dactylomela*. This species inhabits shallow-water regions of coral reefs worldwide. Such shallow habitats are subjected to strong solar effects, as evidenced by noticable bleaching in certain algal species. This study investigates possible deleterious effects of UV light on food consumption, growth, spawn production, hatching rates and percentages of eggs, and blood-glucose levels in a Jamaican population of *A. dactylomela*. Animals were caged under plastic sheets which allowed, respectively, penetration of all light wavelengths including UV, penetration of all wavelengths except UV, and penetration of no wavelengths. Animals exposed to UV wavelengths produced less spawn and had higher blood-glucose levels, suggestive of greater stress, than ones exposed to light with UV blocked out. Animals kept under total light block had highest growth (but not significantly so) accompanied by lowest spawn production, and exhibited lowest blood-glucose levels. Rates of food consumption did not differ significantly between the three treatments, nor did hatching times of the larvae. The deleterious effects of UV light leading to reduced fecundity and increased energy expenditure through stress could be plausible forces favouring selection of nocturnalism in this sea hare species.

Introduction

Aplysia dactylomela is a comparatively large, active, seaweed-eating marine gastropod which, in areas of coral reefs, inhabits shallow inshore (1–2 m depth) regions of sand/coral rubble and outcroppings of dead coral. It is nocturnal in such regions, spending the nighttime hours mate-seeking, copulating, and feeding. At or near dawn it searches for protective crevices or hides under rocks, where it spends the daylight hours digesting food and laying eggs (Carefoot and Taylor 1988).

There are several commonly accepted theories regarding the function of nocturnalism which include avoidance of daytime competitors, avoidance of predators, and minimization of desiccation. These theories, as yet untested in *Aplysia*, seem inapplicable because competitors like herbivorous fish and sea urchins are usually either spatially or temporally displaced from sea hares in tropical regions, adult sea hares possess an array of toxic body parts and secretions (see Carefoot 1987) which deter predators, and desiccation is not a factor. While a recent comparison of daytime versus nighttime energy budgets in *A. dactylomela* (Carefoot and Taylor 1988) showed that a 12-h daytime quiescent period was energetically advantageous, it did not explain why the species was active at night and not during the day. In fact, such periodism of activity has been observed in all *Aplysia* species studied thus far (Susswein et al. 1983; Susswein 1984; Carefoot 1989, 1991), and seems to be related to a need for a quiescent period to digest the large amounts of poor-quality food customarily eaten. A final idea, avoidance of light or, more specifically, avoidance of potentially harmful ultraviolet wavelengths of light through nocturnalism, is both applicable and testable.

From Jerlov's studies in the 1950's, we know that ultraviolet light in the UV-B (280–320 nm) and UV-A (320–400 nm) bands can penetrate quite deeply into seawater. Fleischmann (1989), for example, recorded 10% of surface UV radiation in the 300–400 nm band at 25 m depth in Discovery Bay,

Jamaica. In the 1–2 m depth-range inhabited by *Aplysia dactylomela* in Discovery Bay, over 60% of this band of UV light was present. Strong penetration of UV-B radiation into clear oceanic water has been shown by Jerlov (1950) in the Sargasso Sea (attenuation of only 14%·m^{-1}) and by Smith and Baker (1979) in the Gulf of Mexico(15–20%·m^{-1}), and even in coastal waters can be substantial (Lenoble 1956; Wood 1987, 1989).

Effects of solar UV radiation on the marine ecosystem are becoming increasingly well known, and include inhibition of phytoplankton productivity and motility (Lorenzen 1979; Bühlmann et al. 1987; Döhler and Stolter 1986; Häder 1986; Häder and Häder 1988, 1991; Hobson and Hartley 1983), reduced growth and survival of kelp sporophytes (Wood 1987), inhibition of egg release by kelp gametophytes (Lüning 1981), death of salmon (McArdle and Bullock 1987), reduced growth of corals (Jokiel and York 1982; see also Häder and Worrest 1991), and reduced survival of coral-reef epifauna (Jokiel 1980). In addition, exposure to natural levels of UV light leads to increased amounts of protective UV-absorbing compounds in corals ("S-320" substance: Shibata 1969; Jokiel and York 1982; Dunlap and Chalker 1986; Dunlap et al. 1986) and seaweeds (Wood 1987, 1989), and of certain "photoadaptive" enzymes (e.g., superoxide dismutase, which can inactivate O_2^- radicals produced from UV-mediated reactions) in zooxanthellae of sea anemones (Lesser and Shick 1989).

The question was raised as to whether UV light might be affecting *Aplysia dactylomela* when we observed that the seaweed *Acanthophora spicifera* had been sun-bleached in the same shallow-water areas inhabited by this animal. With the view, then, that nocturnalism in sea hares may have evolved in response to UV light, an experiment was undertaken in Discovery Bay, Jamaica, to test for possibly deleterious effects of natural levels of UV light in *A. dactylomela*. We predicted that exposure to UV light would be stressful to the sea hares, resulting in higher blood-glucose levels, lower food consumption, and lessened body growth, spawn production, and spawn vitality.

Materials and Methods

Sea hares of 150–400 g live wt were collected from shallow coral-rock areas near the Discovery Bay Marine Laboratory, Jamaica, in May 1991. They were sorted according to size and divided into three groups of 12 individuals, each group of 230 g mean live wt. These groups were subjected to different treatments in the field: 1) exposure to all light wavelengths including UV (designated UVTRANSP), 2) exposure to all wavelengths except UV (UVBLOCK), or 3) exposure to no direct light at all (TOTALBLOCK).

The caging arrangements were as follows. The 12 individuals in each group were randomly subdivided into six pairs, and each pair assigned to a mesh basket (25 × 15 × 12 cm height, 0.6 cm plastic mesh). The six baskets per treatment were suspended from the undersurface of PLEXIGLAS® acrylic plastic sheets (0.6 cm thick, 100 × 40 cm) of compositions which allowed different light wavelengths to be transmitted. Hinged panels of the same material (15 cm height) prevented unscreened light from entering from the sides and ends of each sheet. The baskets could be removed for weighing and feeding. Each unit of six baskets plus supporting sheet and hinged panels was buoyed at the water surface, such that the supporting sheet was at the air/water interface, and the whole assembly was moored in 1 m water depth. Even in brisk wave chop the animals were never more than 10–15 cm below the sea surface. Basket positions were changed daily to randomize possible edge effects. It should be noted that there was no true replication in this design, as each treatment group of baskets was clustered under a single plastic sheet. However, we believe that the problems inherent in this type of pseudoreplication would have been minimized by randomizing the basket positions under each sheet daily.

The light transmission characteristics for each screening material were, for UVTRANSP: all UV-visible radiation above 275 nm transmitted (included all UV-A and UV-B radiation), for UVBLOCK: no UV or short-wavelength violet light below 450 nm transmitted and, for TOTALBLOCK: no UV or visible light transmitted (data from Cadillac Universal Plastics, Inc., Day International Ltd.). Light (400–700 nm) penetration to 15 cm depth was measured at 1200 h on 5 June 1991 in full sun as 88% under UVTRANSP, 87% under UVBLOCK, and 27% (representing bottom reflection and scatter) under TOTALBLOCK.

Body weight was recorded for each animal at Day 0, and thereafter at 5-d intervals, over the 25-d duration of the experiment. Spawn weights were measured daily and selected pieces cultured individually to determine hatching times and hatching percentages (expressed as percentage of capsules that opened and released larvae). To correct for different body weights in each treatment, and because spawn from a given animal in a basket could not be identified, spawn outputs were expressed per kg live body wt per basket (N = 6 for each treatment).

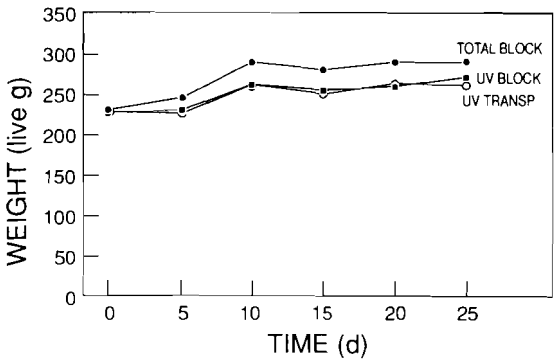

Fig. 1. Change in weight of *Aplysia dactylomela* subjected to differences in type and amount of light. Each point represents the mean of 12 animals. UVTRANSP: exposure to all light wavelengths including UV; UVBLOCK: exposure to all wavelengths except UV; TOTALBLOCK: no exposure to direct light.

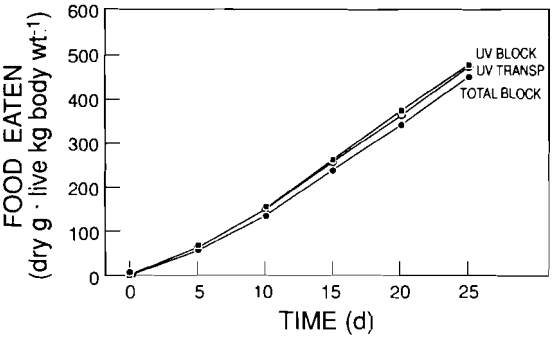

Fig. 2. Cumulative food consumption by *Aplysia dactylomela* in the UV-experiment. Data points represent mean of N=6. Treatment designation same as in Fig. 1.

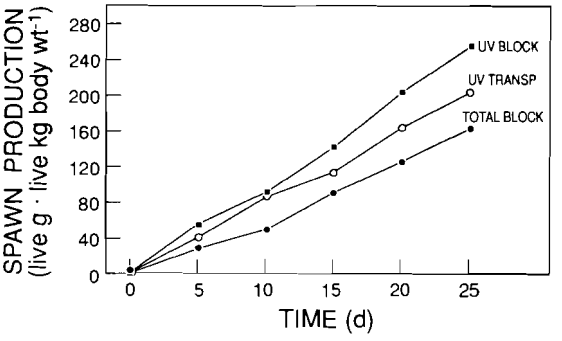

Fig. 3. Cumulative spawn production by *Aplysia dactylomela* in the UV-experiment. Data points represent mean of N=6. Treatments same as in Fig. 1.

Food, consisting mainly of the red alga *Acanthophora spicifera*, but including some *Centroceras clavulatum*, was provided daily. Uneaten remnants were collected daily and their weights subtracted from amounts provided to give fresh amounts eaten. To correct for different water contents of the two foods used and for different sizes of animals in the different treatments, food consumption was expressed as equivalent dry weight eaten per live kg animal (after drying of selected pieces at 70° C to constant weight). Blood samples were drawn from selected animals (N = 6) in each treatment at Day 0, and at 8-d intervals thereafter, and analysed for glucose concentration (following the method in Carefoot 1991). Sampling was done at 1000 h on each designated day, a time when animals were metabolically quiescent (Carefoot 1991). Where time-effects were involved, data were analysed by Repeated Measures ANOVA's followed by Tukey's Multiple Comparisons Tests (TMCT). Other data, such as between-treatments comparisons of hatching times and percentages (after arcsine transformation), were analysed by ordinary ANOVA and TMCT.

Results

Fig. 1 shows weight changes in field animals over 25 d. TOTALBLOCK animals grew more than those in the other treatments, but differences were not significant ($F_{2,198} = 0.50$, $p = 0.61$, ANOVA). Time-effects were highly significant ($F_{5,198} = 31.85$, $p < 0.001$), with the data segregating into two statistically homogenous subsets: Days 0–5 and the remainder ($p < 0.05$, TMCT). The flat portions of the growth curves are likely explained by all extra food energy being converted into spawn, but we have no explanation for the Days 5–10 rise, as there was no obvious change in amounts of spawn being produced during this time (see later).

No significant difference was found in amount of food consumed among treatments (Fig. 2; $F_{2,75} = 1.148$, $p = 0.34$). A significant time effect was evident ($F_{4,75} = 168.7$, $p < 0.001$), with the data segregating into three homogenous subsets, the first representing Days 0–5 (62 dry g alga eaten·live kg body $wt^{-1} \cdot 5d^{-1}$); the second, Days 5–10 (83); and the third, Days 10–25 (107–109; $p < 0.05$, TMCT). These differences likely resulted from an initial several day delay before we were able to give the animals enough food to match their appetites. Once *ad libitum* feeding conditions were reached, food consumption rates became more even. Higher rates of food consumption should have been reflected in higher growth and/or spawn production but this was not evident (see Figs. 1 and 3). During the last five days of the experiment, each pair of sea hares was eating an amount of fresh food equivalent to 22% of their live body wt per day.

Cumulative spawn production over the 25-d experimental period is shown in Fig. 3. Treatment effects were highly significant ($F_{2,75} = 7.60$, $p = 0.005$), with most spawn being laid by UVBLOCK and least by TOTALBLOCK animals (means of 51 and 33 live g spawn · live kg animal^{-1} · 5-d^{-1}, respectively. TMCT disclosed two statistically homogenous subsets representing UVBLOCK and UVTRANSP/TOTALBLOCK). No time effects were evident.

Hatching times of spawn (8.5–9.6 d at 25–26° C), shown in Table 1, were not significantly different among the three treatments ($F_{2,28} = 2.13$, $p = 0.14$). Interestingly, TOTALBLOCK animals exhibited smaller percentage hatching (66%) than either UVBLOCK or UVTRANSP ones (81 and 95%, respectively), but the differences were not significant ($F_{2,26} = 2.14$, $p = 0.13$).

Changes in blood-glucose levels are shown in Fig. 4. There were highly significant treatment effects ($F_{2,60} = 12.20$, $p = 0.001$), with the data segregating into three non-overlapping subgroups ($p < 0.05$, TMCT). Overall means (± S.D. in μg glucose · ml hemolymph^{-1}) were: UVTRANSP = 30.1 ± 7.8, UVBLOCK = 25.8 ± 6.8, and TOTALBLOCK = 21.5 ± 9.1. The UVTRANSP value compares favourably with a value of 30.8 μg glucose · ml^{-1} recorded previously in a field population of *Aplysia dactylomela* in Discovery Bay at 0900h (Carefoot 1991). There was a significant time-effect ($F_{3,60} = 6.53$, $p = 0.001$), with levels being lower on Days 16–24 than on Days 0–8 (21.4–22.8, and 29.5–29.6 μg · ml^{-1}, respectively; $p < 0.05$, TMCT).

Discussion

Our predictions regarding the effect of solar UV radiation on *Aplysia* were supported to the extent that blood-glucose levels were higher, suggestive of greater stress, and spawn production lower, in animals exposed to direct sunlight with UV. However, rates of food consumption did not differ between the three treatment groups, nor did rates of body growth differ significantly between animals exposed to solar radiation with and without UV. There are two possibilities to consider with respect to the role of UV light on *Aplysia* behavior: the first, as already noted, is that UV light plays a proximate role in directly stressing the animals and, the second, is that the animals simply use UV light as a cue to tell them that it is time to find a daytime shelter in which to rest and digest their food. In this case, the elevated blood glucose would simply be a consequence of greater activity as the animals seek shelter.

Adult *Aplysia* with access to copulatory partners allocate most of their growth energy to production of spawn. Conversely, in the absence of egg production or spawning stimuli, energy is predominately allocated to somatic growth. Thus, it was not unexpected that the treatment group showing most somatic growth (TOTALBLOCK; see Fig. 1, but note that the difference was not significant) would be the one laying least spawn, but the causes for this were unclear. Does absence of direct light inhibit conversion of food energy into spawn in sea hares? *Aplysia dactylomela* and *A. juliana*, both nocturnal, are known to lay most eggs during daytime (Sarver 1978; Carefoot and Taylor 1988). This might suggest that either light, even if shaded, stimulates laying, or it might simply be that while resting during daytime the sea hares engage in egg-laying as well as some copulatory behaviour which does not require them to leave their protective hideaways.

The treatment groups exposed to direct light (UVBLOCK and UVTRANSP) showed an almost identical pattern of growth (Fig. 1). Spawn production by UVTRANSP animals was significantly less than that by UVBLOCK animals (Fig. 3), suggesting an inhibitory effect of UV light. Since food consumption was almost identical in these two treatments, the extra energy accruing to the UVTRANSP group (represented by the difference in spawn production) was presumably allocated to locomotory activity or other stress-related responses related to exposure to UV light. The basis for this belief is the significantly higher level of blood-glucose in the UVTRANSP group as compared with the others (Fig. 4).

Elevated blood-glucose levels in *Aplysia* arise in at least three ways: during locomotory or other physical activity, from presence of food in the gut, or through stress associated with air exposure, elevated temperature, low salinity, perforation of the gut, and electrical shock (Ram and Young 1992; Carefoot 1993). Since all treatment groups ate more or less equally (Fig. 2), differences in amount of food in the gut can be dismissed as a probable cause of higher blood-glucose level in one group over another. This leaves locomotory activity or stress as most likely causes. These factors are not unrelated. *Aplysia*, like any other animal when stressed, will increase energy expenditure through activation of sensory systems and acceleration of enzyme turnover and blood circulation, to enter a state of metabolic preparedness for "flight or fight". Unfortunately, the physical arrangement of the cages did not permit easy observation of locomotory behaviour, either not allowing clear view because of wave

Table 1. Hatching times and hatching percentages for spawn of *Aplysia dactylomela* in the treatment groups indicated (designation same as in Fig. 1). Water temperature 25–26° C.

Treatment	N	Hatching Time (days, $\bar{x}$ ± S.D.)	Hatching Percentage (%, $\bar{x}$ ± S.D.)
UVBLOCK	10	9.0 ± 1.3	81 ± 19
UVTRANSP	10	9.6 ± 1.3	95 ± 16
TOTALBLOCK	10	8.5 ± 1.1	66 ± 32

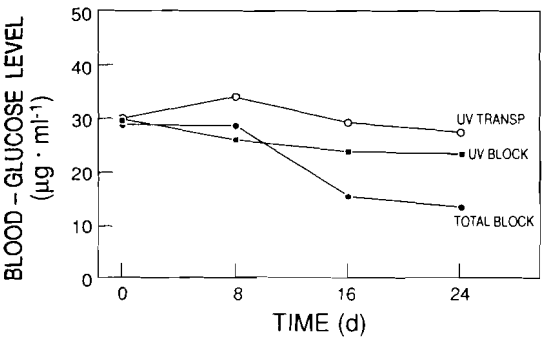

Fig. 4. Changes in blood-glucose levels of *Aplysia dactylomela* in response to different light treatments. Each point represents the mean of N=6. Treatments same as in Fig. 1.

wash or because of the huge volume of seaweed provided as food. Even if increased locomotion had occurred, however, it would likely have resulted from some sort of stress leading to attempted flight as shown in other studies (Willan 1979; DiMatteo 1981). This could explain the higher sustained blood-glucose levels in the two treatment groups exposed to direct light (UVTRANSP and UVBLOCK) as compared with the TOTALBLOCK treatment group; the animals may have been more active simply because they were searching for daytime cover. Diminished blood-glucose levels over time, apparent in all groups though greatest in the TOTALBLOCK treatment group, could indicate a lessening locomotory response as the animals became more accustomed to their surroundings. Even if it could be shown that locomotion was not involved in raising blood-glucose levels, light itself (not just UV) would have to be implicated as a stress because exposure to it in two of the treatments led to elevated blood-glucose levels. Finally, since the UVTRANSP treatment produced significantly higher blood-glucose titre in the sea hares than did the UVBLOCK treatment, the conclusion must be that exposure to UV light was, indeed, stressful.

This experiment has shown on two counts that UV light is deleterious to *Aplysia dactylomela*: the first in reduced fecundity; the second, in presumed extra energy expenditure through stress. Either could be plausible forces favouring selection of nocturnalism in the species. Whether other nocturnal species of sea hares show these same responses and how diurnal species tolerate or avoid such effects of UV light, are questions for future research.

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References

Bühlmann B, Bossard P, Uehlinger U (1987) The influence of longwave ultraviolet radiation (u.v.-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton. J Plankton Res 9:935–943

Carefoot TH (1987) *Aplysia*: Its biology and ecology. Oceanogr Mar Biol Ann Rev 25:167–284

Carefoot TH (1989) A comparison of time/energy budgeting in two species of tropical sea hares *Aplysia*. J Exp Mar Biol Ecol 131:267–282

Carefoot TH (1991) Blood-glucose levels in the sea hare *Aplysia dactylomela*: interrelationships of activity, diet choice and food quality. J Exp Mar Biol Ecol 154: 231–244

Carefoot TH (1992) Stress effects on blood-glucose levels in sea hares. Mar Biol (in press)

Carefoot TH, Taylor BE (1988) Sea hares in coral rubble: the significance of nocturnal grazing. Proc 6th Int Coral Reef Symp 2:7–13

DiMatteo T (1981) Investigation into interspecific encounters of the sea hare *Aplysia dactylomela* Rang, 1828. The Veliger 24:72–75

Döhler G, Stolter H (1986) Impact of UV-B (290–320 nm) radiation on photosynthesis-mediated uptake of ^{15}N-ammonia and ^{15}N-nitrate of several marine diatoms. Biochem Physiol Pflanzen 181:533–539

Dunlap WC, Chalker BE (1986) Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. Coral Reefs 5:155–159

Dunlap WC, Chalker BE, Oliver JK (1986) Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J Exp Mar Biol Ecol 104:239–248

Fleischmann EM (1989) The measurement and penetration of ultraviolet radiation into tropical marine water. Limnol Oceanogr 34:1623–1629

Häder D-P (1986) Effects of solar and artificial UV irradiation on motility and phototaxis in the flagellate, *Euglena gracilis*. Photochem Photobiol 44:651–656

Häder D-P, Häder MA (1988) Ultraviolet-B inhibition of motility in green and dark bleached *Euglena gracilis*. Curr Microbiol 17:215–220

Häder D-P, Häder M (1991) Effects of solar and artificial U.V. radiation on motility and pigmentation in the marine *Cryptomonas maculata*. Envir Exp Bot 31:33–41

Häder D-P, Worrest RC (1991) Effects of enhanced solar ultraviolet radiation on aquatic ecosystems. Photochem Photobiol 53:717–725

Hobson LA, Hartley FA (1983) Ultraviolet irradiance and primary production in a Vancouver Island fjord, British Columbia, Canada. J Plankton Res 5:325–331

Jerlov NG (1950) Ultra-violet radiation in the sea. Nature 166:111–112

Jokiel PL (1980) Solar ultraviolet radiation and coral reef epifauna. Science 207:1069–1071

Jokiel PL, York Jr. RH (1982) Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. Bull Mar Sci 32:301–315

Lenoble J (1956) Sur la pénétration du rayonnement ultraviolet dans les eaux méditerranéennes. C R Acad Sci 243:1781–1783

Lesser MP, Shick JM (1989) Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. Mar Biol 102:243–255

Lorenzen CJ (1979) Ultraviolet radiation and phytoplankton photosynthesis. Limnol Oceanogr 24:1117–1120

Lüning K (1981) Egg release in gametophytes of *Laminaria saccharina*: induction by darkness and inhibition by blue light and U.V. Br Phycol J 16:379–393

McArdle J, Bullock AM (1987) Solar ultraviolet radiation as a causal factor of 'summer syndrome' in cage-reared Atlantic salmon, *Salmo salar L.*: a clinical and histopathological study. J Fish Dis 10:255–264

Ram JL, Young ES (1992) Shock induces a long-lasting elevation of blood glucose in *Aplysia*. Experientia 48: 14–18

Sarver DJ (1978) The ecology and energetics of *Aplysia juliana* (Quoy and Qaimard, 1832). Ph.D. thesis, University of Hawaii. pp 125

Shibata K (1969) Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. Plant Cell Physiol 10:325–335

Smith RC, Baker KS (1979) Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem Photobiol 29:311–323

Susswein AJ (1984) Effects of food deprivation upon behavioral patterns and time budgeting of *Aplysia fasciata*. Behav Neural Biol 42:127–133

Susswein AJ, Gev S, Feldman E, Markovich S (1983) Activity patterns and time budgeting of *Aplysia fasciata* under field and laboratory conditions. Behav Neural Biol 39:203–220

Willan RC (1979) The ecology of two New Zealand opisthobranch molluscs. Ph.D. thesis, University of Aukland. pp 198

Wood WF (1987) Effect of solar ultra-violet radiation on the kelp *Ecklonia radiata*. Mar Biol 96:143–150

Wood WF (1989) Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (gigartinales) to ultraviolet radiation. Aquat Bot 33:41–51