

RELATIONSHIP BETWEEN RESPIRATORY PUMPING AND OXYGEN CONSUMPTION IN *APLYSIA DEPILANS* AND *APLYSIA FASCIATA*

BY MIRIAM LEVY, YAIR ACHITUV AND ABRAHAM J. SUSSWEIN
Department of Life Sciences, Bar-Ilan University, Ramat Gan, 52 100, Israel

Accepted 28 June 1988

Summary

Respiratory pumping in *Aplysia* is a well-characterized behaviour controlled by identified neurones, but its function is unknown. To gain insight into the function of this behaviour, respiratory pumping and oxygen consumption were examined under identical conditions, in *Aplysia fasciata* Poiret and in *A. depilans* Gmelin. *A. fasciata* is found in less turbulent environments than is *A. depilans*, suggesting that control of respiratory pumping may differ in the two species.

Rates of respiratory pumping and oxygen consumption were poorly correlated. The basal rate of respiratory pumping was similar in both species and was not significantly dependent on animal mass, but the resting rate of oxygen consumption was higher in *A. depilans* than in *A. fasciata* and was an inverse function of animal mass in both species. Brief, moderate hypercapnia led to an increase in oxygen consumption in both *Aplysia* species. In *A. fasciata*, the increase was much greater. Increase in oxygen consumed was not accompanied by changes in the rate of respiratory pumping. Longer, more severe periods of hypercapnia led to *decreases* in oxygen consumption in both *Aplysia* species, and an *increase* in the rate of respiratory pumping. Decreased oxygen consumption was more gradual in *A. fasciata*. Severe hypoxia produced a decrease in the rate of oxygen consumed, and an increase in the rate of respiratory pumping.

Introduction

The marine gastropod *Aplysia* has been extensively utilized as a preparation for examining the neural basis of behaviour and behavioural plasticity (Kandel, 1976, 1979), but the relationship between behaviour and adaptation to environment in this genus has been little studied (but see Kupfermann & Carew, 1974). Such investigations could provide insight into the functions subserved by neural circuits underlying specific behaviours, and could predict variables modulating them.

The circuit controlling the gill is the most thoroughly analysed in the *Aplysia* nervous system. Gill contractions occur during two behaviours: (1) the gill withdrawal reflex, a defensive response elicited by mechanical stimulation of the siphon or mantle shelf, and (2) spontaneous respiratory pumping (Kupfermann &

Key words: *Aplysia*, respiratory pumping, oxygen consumption.

Kandel, 1969; Peretz, 1969; Kupfermann *et al.* 1974). Respiratory pumping is a fixed action pattern affecting a number of different organs (Koester *et al.* 1974; Kupfermann *et al.* 1974; Perlman, 1979; Jahan-Parwar & Fredman, 1978; Sawada *et al.* 1981). During respiratory pumping, the siphon and gill contract, the heart is inhibited and the parapodia close over the mantle shelf. Respiratory pumping is associated with widespread neural activity in the abdominal and pleural ganglia caused by firing of a neurone commonly termed interneurone II (Kandel *et al.* 1967; Koester *et al.* 1974). In the isolated nervous system, this activity is observed spontaneously at intervals of 30 s to 10 min (Byrne, 1983). Recently, a number of cells have been identified that contribute to interneurone II activity (Byrne & Koester, 1978). Of particular importance are the L25 cells, a group of spontaneously active, electrically coupled neurones in the abdominal ganglion, which seem to function as command cells and pattern generators for respiratory pumping (Byrne, 1983).

However, the function of respiratory pumping remains unclear. Koester *et al.* (1974) suggested that the movement serves a respiratory function, by irrigating the gill with a flow of fresh water through the mantle cavity and over the gill while contraction of the gill pumps haemolymph through the relaxed heart. Kupfermann & Carew (1974) found that *Aplysia* in tide pools of stagnant water are more likely to use respiratory pumping movements than are animals in more open waters. Koester *et al.* (1979) found that the rate of respiratory pumping is higher in *Aplysia* maintained in unaerated aquaria than in animals in a well-aerated environment. Finally, Croll (1985) showed that a rise in CO₂ tension signalled by lowering the environmental pH induces an increase in respiratory pumping rate. Spraying low-pH sea water over the chemosensory ospharidium induces respiratory pumping (Croll, 1985).

If the function of respiratory pumping is to ventilate the gill, it is possible that this behaviour is regulated by variables that also regulate the rate of oxygen consumption. In many animals, the rate of ventilatory movements is generally controlled by factors similar to those affecting the rate of oxygen consumption (Newell, 1979; Schmidt-Nielsen, 1983). To explore whether the same variables modify rates of oxygen consumed and respiratory pumping, both were measured under identical conditions.

The experiments measured oxygen consumed and respiratory pumping in two *Aplysia* species, *A. fasciata* and *A. depilans*. Achituv & Susswein (1985) have shown that *A. depilans* is more common in rougher waters, whereas *A. fasciata* is dominant in quieter waters. Based on differences in the distribution, it was suggested that the species should differ in their sensitivity to conditions of respiratory deprivation (Achituv & Susswein, 1985). *A. fasciata* should be better able to withstand respiratory stress. If the function of respiratory pumping is to ventilate the gill, it is possible that a compensatory increase in pumping rate in response to respiratory stress would be more pronounced in *A. fasciata* than in *A. depilans*.

Our data show that *A. fasciata* is better than *A. depilans* at withstanding

respiratory stress. Also, the role of respiratory pumping and oxygen consumption were poorly, or even negatively correlated.

Materials and methods

Animals

Aplysia fasciata and *A. depilans* were collected along the Mediterranean coast of Israel (Susswein *et al.* 1987). Animals were maintained in 940-l holding tanks filled with natural Mediterranean sea water at 16–17°C. One week prior to use in an experiment, animals were transferred to 20-l aquaria kept at $20 \pm 0.5^\circ\text{C}$. Salinity was regulated at 4%. Lighting was maintained at a schedule of 12 h:12 h L:D. Experiments were performed during daylight hours. Animals were fed *Ulva lactuca* until the day of the experiment.

Experimental chamber and procedures

All experiments were performed at $20 \pm 0.5^\circ\text{C}$. At the start of experiments animals were lightly blotted and then weighed. This mass was used for calculating rates of oxygen consumption (see below). After being weighed, animals were transferred to the test chamber. Observations were begun immediately upon placing the animals into the chamber, without a period for equilibration of the animals with the apparatus, since under rest conditions (21% O_2 , pH 7.8) no difference was seen in oxygen consumed or in respiratory pumping rate between the first 10 min and subsequent times (see below). The test chamber was a 1500-ml clear Plexiglas cylinder (Fig. 1) with fitted openings through the chamber lid allowing entry of electrodes for measurement and regulation of pH and oxygen tension. Tubes through the lid permitted bubbling of different mixtures of gases into the water and addition of NaOH. At the start of experiments measuring the rate of oxygen consumed, the chamber was sealed and no further oxygen was bubbled into the water. The change in O_2 tension in the water was used to monitor oxygen utilized by the animals. In other experiments, water was bubbled with air, or an air and N_2 mixture, for a few hours prior to the experiment and throughout the experiment itself. A magnetic stirrer was situated underneath the animals, separated from the *Aplysia* by a plastic barrier with holes. Although care was taken to prevent disturbance of the animal by the stirrer or by bubbles, in some cases such disturbances occurred, and elicited respiratory pumping movements. These experiments were discarded.

Regulation of oxygen tension

In experiments in which the oxygen tension in the water was varied, a mixture of 2.8% O_2 and 97.2% N_2 instead of air was bubbled into the water (Fig. 1).

Regulation of CO_2 tension

Carbon dioxide tension of the ambient sea water was maintained by bubbling

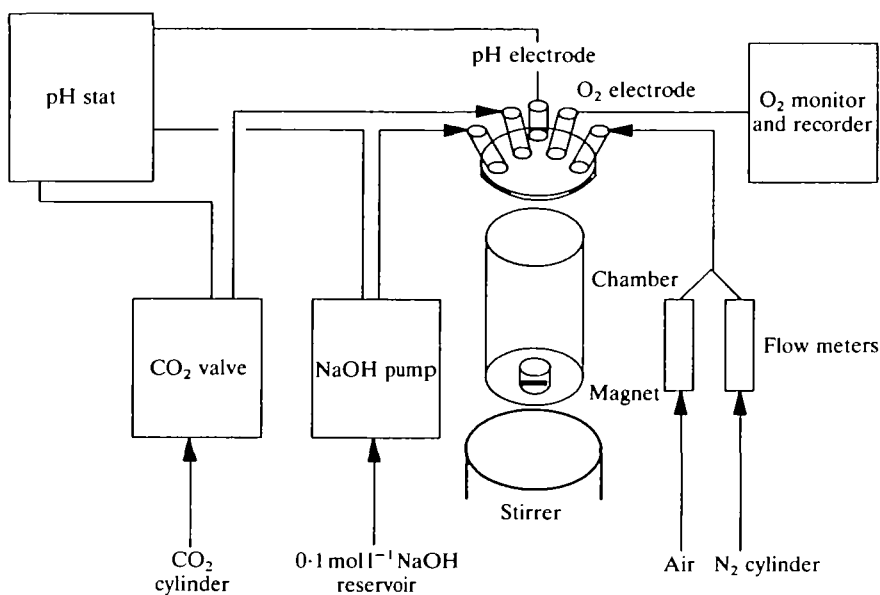


Fig. 1. The experimental arrangement. Animals were kept for 40 min in an experimental chamber that could be sealed. pH and oxygen tensions were measured by pH and oxygen electrodes that were inserted through openings in the chamber lid. pH was regulated by controlling the flow of CO₂ and NaOH into the chamber, as a result of feedback from the pH electrode. O₂ tension was modified by bubbling different mixtures of air and N₂ into the chamber. A magnetic stirrer at the bottom of the chamber ensured constant mixing of the water.

CO₂ into the water. CO₂ was measured by using pH as a monitor. A pH electrode in the water allowed measurement of the pH to an accuracy of ± 0.1 unit. pH was adjusted using a pH regulator (New Brunswick Instruments) that provided feedback control of the pH in the experimental chamber. The regulator added CO₂ to the water when pH was too high, and released drops of 0.1 mol l⁻¹ NaOH when pH was too low.

Measurement of oxygen tension

In experiments measuring rates of oxygen consumed, the change in oxygen tension in the water as a result of respiration was measured using a Clark-type oxygen electrode (YSI 5331). Output of the electrode was amplified, and the signal was recorded by a pen recorder.

To bring the sea water for experiments to equilibrium with ambient air (21 % O₂), the water was stirred for 24 h before the experiment. To calibrate the oxygen electrode, the output of the electrode in fully saturated sea water was measured, and this was assigned a value of 100%. The oxygen concentration that is equivalent to this value was determined by referring to published tables (Grasshoff, 1976). The output of the electrode equivalent to zero oxygen was determined by placing the electrode in a saturated solution of Na₂SO₃ (Daven-

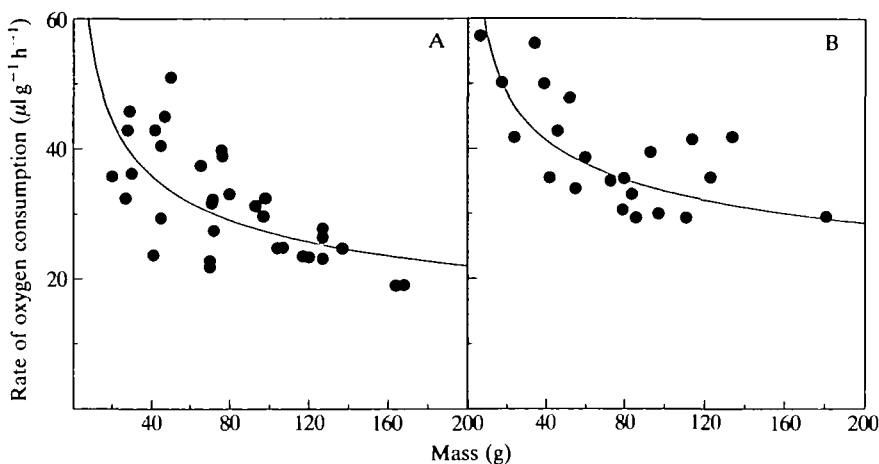


Fig. 2. Relationship between animal mass and oxygen consumed, in *Aplysia fasciata* (A) and *A. depilans* (B). A significant negative correlation was observed between mass and rate of oxygen consumption in both *Aplysia* species. For all masses, rate of oxygen consumption was higher in *A. depilans* than in *A. fasciata*. Best-fit regression lines fitting the formula $\text{rate} = ax^b$ are drawn.

port, 1973). Periodically, oxygen contents of the water were also checked by Winkler titration (Fox & Wingfield, 1938).

Measurement of respiratory pumping movements

Respiratory pumping movements were observed visually without any specialized equipment, and were readily recognized in both *Aplysia* species. The time of occurrence of a pumping movement was noted to the nearest minute.

Results

Effects of animal mass

In many animals resting metabolic rate is an exponential function of body mass (Schmidt-Nielsen, 1983; Newell, 1979). To determine whether similar relationships occur in *Aplysia*, the resting rates of oxygen consumption and respiratory pumping were determined in *A. depilans* and *A. fasciata* of different sizes over a period of 40 min.

Effect on oxygen consumption

Oxygen consumption was negatively correlated with animal mass in both *Aplysia* species. For *A. fasciata*, the best-fit exponential curve was: oxygen consumed = $10.95 \times \text{mass}^{-0.30}$ ($r^2 = 0.49$). For *A. depilans*, the best-fit exponential curve was: oxygen consumed = $95.83 \times \text{mass}^{-0.23}$ ($r^2 = 0.53$) (Fig. 2).

To determine whether differences between species and mass were significant, animals were divided into three mass ranges (5–50 g, 51–100 g and greater than

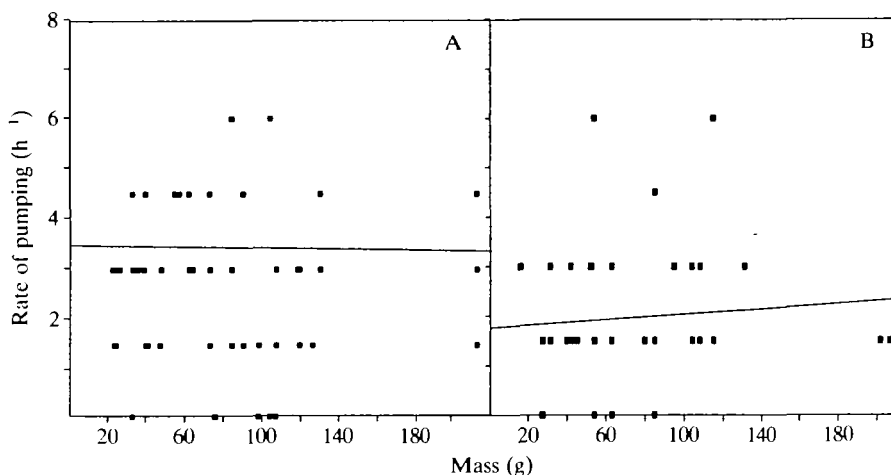


Fig. 3. Relationship between animal mass and rate of respiratory pumping, in *Aplysia fasciata* (A) and *A. depilans* (B). No correlation was observed between mass and respiratory pumping in either species. Furthermore, the rate of pumping was very low in both species. For *A. fasciata*, $r = -0.07$. For *A. depilans*, $r = 0.04$. The least mean square linear regression line is drawn.

100 g) and a two-way analysis of variance was performed. Significant effects due to animal mass and to species difference were seen ($F = 18.8$, $P < 0.0001$, 5 and 53 df for the effect due to mass; $F = 21.5$, $P < 0.0001$, 1 and 53 df for the effect due to species).

Effect on respiratory pumping movements

If respiratory pumping subserves a steady-state ventilatory function, species difference and animal mass might be expected to affect this behaviour in a manner similar to effects on rate of oxygen consumed. Under conditions identical to those at which oxygen consumption was measured, but on different individuals, the number of respiratory pumping movements was counted. In contrast to the rate of oxygen consumption, the resting rate of respiratory pumping was found to be similar in both *Aplysia* species, and was not significantly correlated with animal mass (Fig. 3).

Effects of respiratory stress

In many animals, respiratory stresses such as hypercapnia and hypoxia produce compensatory increases in ventilation rate and in oxygen consumed (Schmidt-Nielsen, 1983; Newell, 1979). One would expect that compensation for these conditions should also be evident in the two *Aplysia* species examined. Furthermore, compensation might be more marked in *A. fasciata* than in *A. depilans*.

In the present experiments, the effects of hypercapnia and hypoxia on oxygen consumption and respiratory pumping were examined. Hypercapnia was induced by bubbling CO_2 into the water, while oxygen tension was maintained at resting

Table 1. Effect of CO₂ tension on oxygen consumption

Source	ANOVA			
	df	ss	F-value	P
Species	1	0.020	0.02	0.888
pH	5	584.533	112.91	0.0001
Time	3	112.540	36.213	0.0001
Species × pH	5	137.380	26.54	0.0001
Species × time	3	4.939	1.59	0.190
pH × time	15	30.748	1.98	0.0154
Species × pH × time	15	21.481	0.80	0.6747

levels by constantly bubbling air into the experimental chamber. Hypoxia was generated by bubbling a mixture of O₂ and N₂ into the water, while CO₂ tension was unchanged. To minimize effects of animal mass, experiments were restricted to *Aplysia* weighing 100–200 g.

Effects of hypercapnia

The effect of differences in CO₂ tension was monitored throughout a 40-min period in the test chamber. CO₂ was monitored by fixing the pH of the water (see Materials and methods). Experiments were performed at six levels of pH: 7.8 (control), 7.5, 7.0, 6.5, 6.0 and 5.5. For analysis of the data, the 40-min observation period was divided into four equal periods, and either oxygen consumed or number of respiratory pumping movements for each animal for each 10-min period was calculated. From 9 to 20 individuals were tested at each level of pH.

Effect on oxygen consumption. To determine whether significant differences in oxygen consumed could be attributed to species differences, to different levels of CO₂, to different lengths of time exposed to conditions of hypercapnia or to interactions among these variables, a three-way analysis of variance was performed (Table 1). There were significant differences in oxygen consumed as a function of CO₂ tension and as a function of time under conditions of hypercapnia. There was also a difference in oxygen consumed between the two *Aplysia* species at different degrees of hypercapnia and at different times at different levels of CO₂.

At pH 7.8 oxygen consumption was not changed through the 40-min test period, in either *Aplysia* species (Figs 4 and 5). As was seen previously (Fig. 2), the resting rate of oxygen consumption was higher in *A. depilans* than in *A. fasciata*. In *A. fasciata*, a decrease in pH from 7.8 to 7.5 and 7.0 led to an increase in oxygen consumption throughout the 40-min test period. From pH 7.0 to 5.5 oxygen consumption declined. In contrast, in *A. depilans* an increase in oxygen consumption over baseline values was seen only at pH 7.5 and only in the first 20 min; thereafter, the rate of oxygen consumption progressively declined. A particularly striking finding was that under conditions of low pH, especially during the third

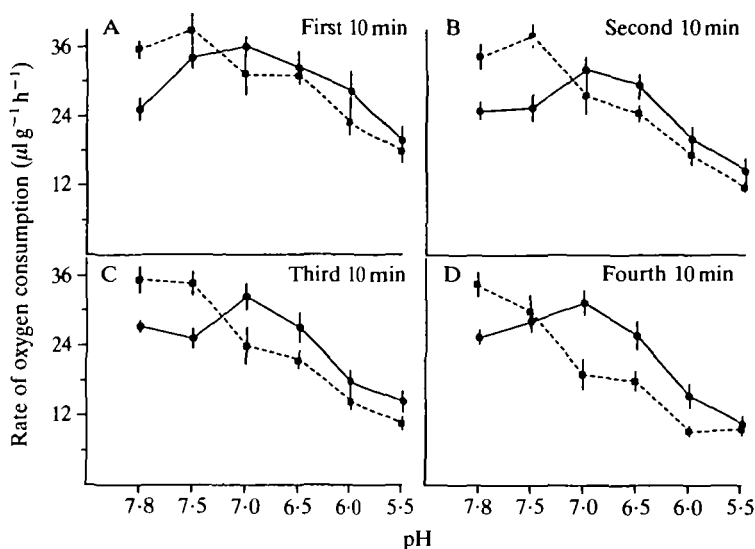


Fig. 4. The rate of oxygen consumption in *Aplysia depilans* (■) and *A. fasciata* (●), as a function of different levels of pH. Data were analysed by dividing the 40-min period in the test chamber into four 10-min periods. Data are shown separately for each of these periods (A–D). In *A. fasciata*, in all four 10-min periods, a decrease in pH up to pH 7.0 caused a compensatory increase in oxygen consumed. More drastic reduction of pH produced a decline in oxygen consumption. In contrast, in *A. depilans* an increase in oxygen consumption was seen only with a decrease to pH 7.5, and only in the first two 10-min periods. Standard errors are shown.

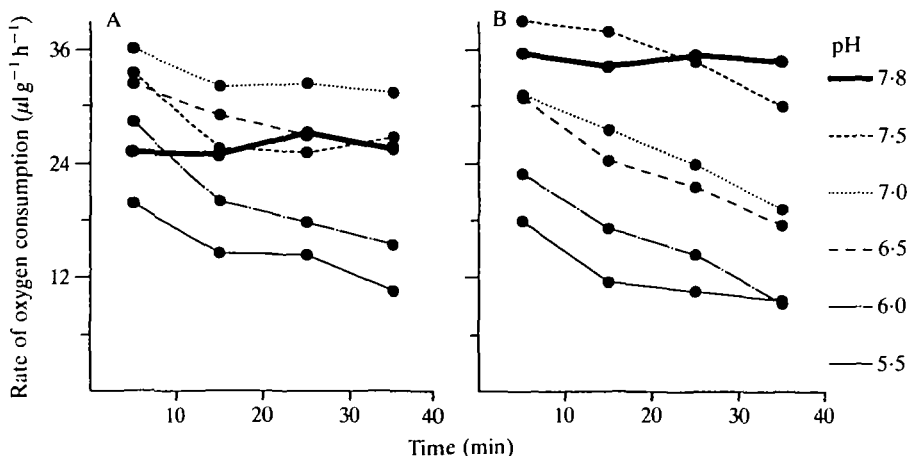


Fig. 5. Rate of oxygen consumption by *Aplysia fasciata* (A) and *A. depilans* (B) as a function of time in the test chamber, at six different levels of pH. Data are identical to those in the previous figure, but are replotted to show the effects of time more clearly. At all levels of pH below 7.8, a decline in rate of oxygen consumption is seen as a function of time.

Table 2. Effect of CO₂ tension on rate of respiratory pumping

Source	ANOVA			
	df	ss	F-value	P
Species	1	223.627	29.69	0.0001
pH	5	2842.433	72.25	0.0001
Time	3	469.751	19.90	0.0001
Species × pH	5	588.167	14.95	0.0001
Species × time	3	32.166	1.36	0.253
pH × time	15	892.957	7.57	0.0001
Species × pH × time	15	0.00	0.00	1.000

and fourth 10-min intervals, the rate of oxygen consumption was much higher in *A. fasciata* than in *A. depilans*, reversing the relationship between the two species under rest conditions.

Effect on respiratory pumping movements. To determine whether respiratory pumping is also affected by different levels of hypercapnia, in a separate set of experiments the rate of pumping movements was tested under conditions similar to those above (Table 2). There were significant differences in the rate of respiratory pumping as a function of difference in species, of different levels of CO₂ and at different times after hypercapnia had been initiated. There were also significant differences in the rate of pumping at different levels of pH in the two *Aplysia* species, and at different levels of pH at different times in the test chamber.

The data indicate that a decrease in pH leads to an increase in the rate of respiratory pumping movements. However, in contrast to the effect on oxygen consumption, a sharp threshold was found for this effect in both *Aplysia* species (Fig. 6). For pH 7.8, 7.5 and 7.0, the rate of respiratory pumping movements was unchanged throughout the 40-min test period. At pH 6.5 and below, progressively larger increases in the rate of respiratory pumping movements were seen. As the time under conditions of hypercapnia increased, the rate of pumping movements declined.

It is possible that the effects of pH are indirect and are caused by a net decrease in O₂ in the water. Thus, a decrease in pH leads to increased oxygen consumption and a net decrease in oxygen in the chamber (see above), and a decrease in oxygen concentration can itself affect respiratory pumping (see below). However, this explanation is unlikely, since the experiment was performed in a relatively large experimental chamber, and the decrease in O₂ was minimal. Also, the largest effects on respiratory pumping were seen at levels of pH at which relatively little oxygen is extracted from the ambient water, and no effects on respiratory pumping were seen at levels of pH producing large changes in the rate at which oxygen is consumed.

Correlation between oxygen consumed and respiratory pumping. If the function of respiratory pumping movements is to ventilate the gill, one might predict that the rate of pumping and the rate of oxygen consumed would be regulated in

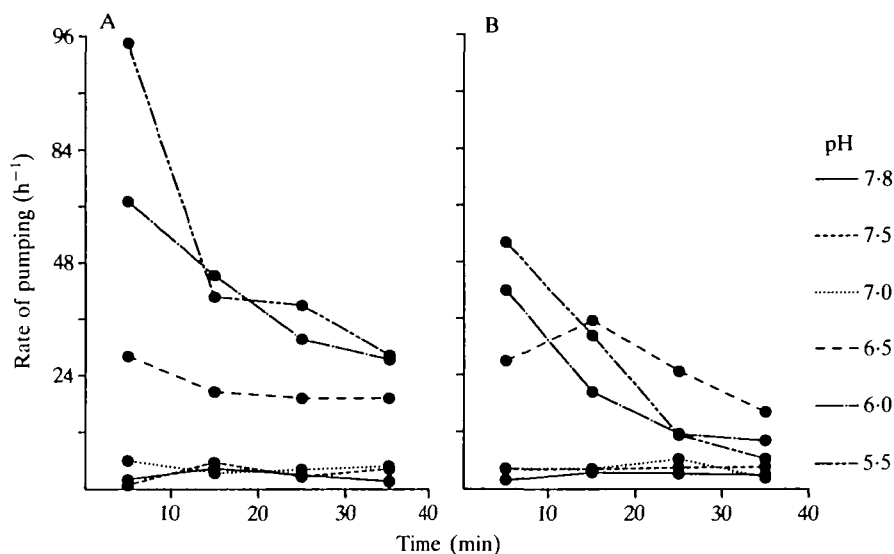


Fig. 6. The rate of respiratory pumping in *Aplysia fasciata* (A) and *A. depilans* (B) as a function of time in the test chamber, at six different levels of pH. No change in the rate of respiratory pumping was observed in either species from pH 7.8 to 7.0. Lower levels of pH produced large increases in the rate of respiratory pumping. As pH decreased, the rate of respiratory pumping increased. This effect was more pronounced in *A. fasciata* than in *A. depilans*. The rate of pumping decreased as a function of time in both *Aplysia* species.

tandem. The data indicate that both the rate of oxygen consumption and the rate of respiratory pumping are increased by a rise in CO₂ tension, but the increases do not occur at the same levels of pH or at the same times after hypercapnia had been initiated. To test directly whether the rates of oxygen consumption and respiratory pumping are regulated together, the mean rate of pumping for each 10-min interval of the 40-min test period, for each level of pH, were plotted against the mean rates of oxygen consumed measured under the same experimental conditions (Fig. 7). The two parameters were weakly, but significantly ($P < 0.05$), *negatively* correlated. The negative correlation presumably reflects the sharp increase in the rate of respiratory pumping when pH is 6.5 and below, when oxygen consumption is already in decline.

Effects of hypoxia

The effects of hypoxia were not explored as thoroughly as those of hypercapnia. In these experiments, animals of each species were placed in the experimental chamber for 40 min in sea water that had been equilibrated with 2.8% oxygen. As above, the observation period was divided into four equal periods, and oxygen consumed or number of respiratory pumping movements was calculated for each animal for each 10-min period. In these experiments, the number of pumping movements was not counted during the first 10 min of observation.

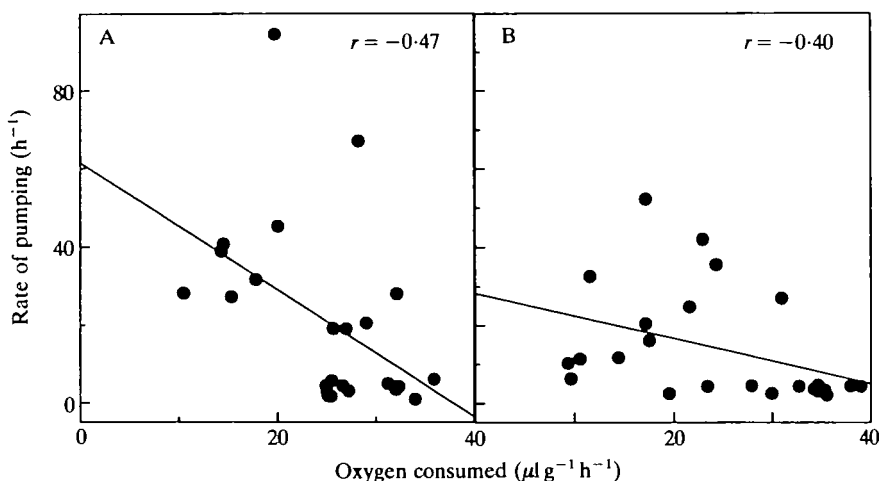


Fig. 7. Correlation between the rate of respiratory pumping and the rate of oxygen consumption in *Aplysia fasciata* (A) and *A. depilans* (B). Data are replotted from those in the three previous figures. The mean value of the respiratory pumping rate for each 10-min period in the test chamber, for each level of pH, was plotted against the corresponding value of oxygen consumption for the same period. A negative correlation was found between the two values. Least mean square regression lines are shown.

Table 3. *Effect of oxygen tension on rate of oxygen consumed*

Source	ANOVA			
	df	ss	F-value	P
Species	1	22.674	38.78	0.0001
Oxygen concentration (oxy)	1	529.166	905.06	0.0001
Time	3	12.449	7.10	0.0002
Species × oxy	1	34.455	58.93	0.0001
Species × time	3	0.692	0.39	0.7569
Oxy × time	3	15.111	8.62	0.0001
Species × oxy × time	3	1.842	1.05	0.3726

Effect on oxygen consumed. To test the relationship between the rate of oxygen consumption and differences in oxygen tension, species difference, time under conditions of hypoxia or interactions among these variables, a three-way analysis of variance was performed (Table 3). There were significant differences in oxygen consumed between the two species, between the two levels of oxygen tension and as a function of time in conditions of hypoxia. There were also significant differences between the two species at different levels of O_2 tension, and between different levels of oxygen at different times.

A particularly striking effect is that the rate of oxygen consumption of *A. depilans* is much lower than that of *A. fasciata* at low levels of oxygen tension, in

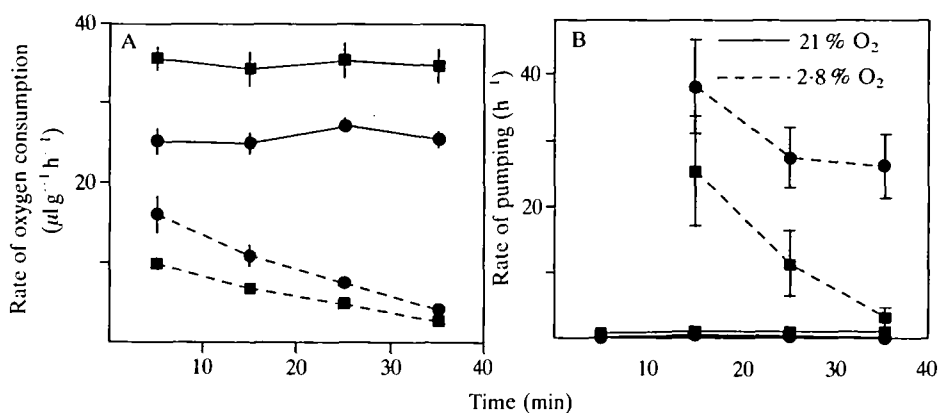


Fig. 8. (A) The rate of oxygen consumption in *Aplysia depilans* (■) and *A. fasciata* (●), as function of time in the test chamber, at two different levels of O_2 tension. Severe hypoxia led to a decline in the rate of O_2 consumption in both *Aplysia* species, but this effect was larger in *A. depilans*. In both species, the rate of oxygen consumption declined with time at low levels of oxygen tension. (B) The rate of respiratory pumping in both *Aplysia* species as a function of time in the test chamber, at the same two levels of O_2 tension. In this experiment, data were not gathered for the first 10 min under conditions of hypoxia. Severe hypoxia led to an increase in the rate of respiratory pumping in both *Aplysia* species, but this effect was larger in *A. fasciata*. In both species, the rate of pumping declined with time at low levels of oxygen tension. Standard errors are shown.

spite of a higher rate of oxygen consumption under conditions of normoxia (Fig. 8A).

Effect on respiratory pumping movements. To examine whether the decrease in oxygen consumption is accompanied by a decrease in respiratory pumping movements, this variable was measured in another set of experiments under identical conditions. We found that a decrease in oxygen tension produced an increase in the rate of respiratory pumping (Fig. 8B). There were significant differences in the rate of respiratory pumping between the two species, between the two levels of oxygen tension and among different times in conditions of hypoxia (Table 4). There were also significant differences between the two species at different levels of O_2 tension and between different levels of oxygen at different times.

Discussion

Control of oxygen consumption

Effect of mass

Oxygen consumption is higher in smaller *Aplysia* than in larger ones (Fig. 2). This presumably reflects an increased metabolic rate in smaller individuals. Similar findings are common in many other animals (Schmidt-Nielsen, 1983; Newell, 1979).

Table 4. Effect of oxygen tension on rate of respiratory pumping

Source	ANOVA			
	df	ss	F-value	P
Species	1	24.675	9.96	0.0019
Oxygen concentration (oxy)	1	357.391	144.20	0.0001
Time	3	48.911	9.87	0.0001
Species \times oxy	1	75.699	30.54	0.0001
Species \times time	3	0.000	0.00	1.0000
Oxy \times time	3	44.031	8.88	0.0002
Species \times oxy \times time	3	0.000	0.00	1.0000

Effects of hypoxia and hypercapnia

In both *A. depilans* and *A. fasciata* hypercapnia leading to a decrease in pH to 7.0–7.5 causes an increase, followed by a gradual decrease, in oxygen consumption. However, the increase is much more marked in *A. fasciata*. Severe, unphysiological conditions of hypercapnia (below pH 7.0), or unphysiological hypoxia (2.8% O₂), lead to an apparent inability to compensate, as shown by a decrease in oxygen consumption. The decrease is more gradual in *A. fasciata*. Since levels of pH below 7.0 do not generally occur in natural sea water (Newell, 1979), we have no evidence that a decreased ability to regulate ever occurs under physiological conditions, although it is possible that the response to 40 min of exposure to a pH of 6.5 or less elicits responses similar to longer periods of exposure to a more moderate decrease in pH.

The increase in O₂ consumption seen in both species of *Aplysia* as a function of a rise in CO₂ tension suggests that both species can be classified as respiratory regulators, rather than as conformers. In regulating animals, a decrease in O₂ tension leads to a compensatory maintenance in the rate of O₂ consumption, whereas in conformers the rate of oxygen consumption declines with declining O₂ tension (Prosser, 1973). The data indicate that *A. fasciata* is a more effective regulator than *A. depilans*. Although the maximal rate of oxygen consumption is similar in the two species, in *A. depilans* this rate is achieved at pH 7.5, whereas in *A. fasciata* it occurs at pH 7.0. At pH 7.8 (normal sea water), the rate of oxygen consumed in *A. depilans* is approximately 90% of the maximal rate, whereas in *A. fasciata* consumption is approximately 66% of maximum. As the time spent under conditions of low pH increases, the rate of oxygen consumed becomes dramatically higher in *A. fasciata* than in *A. depilans*. These data indicate that under steady-state conditions the rate of oxygen consumption in *A. depilans* is close to maximum, leaving relatively little 'headroom' to increase the rate of respiration in response to stress. In contrast, resting respiration in *A. fasciata* seems to be more efficient, allowing respiration to be increased under conditions of hypercapnia. Under conditions of severe hypoxia, the rate of decline in oxygen consumption is slower in *A. fasciata* than in *A. depilans*.

Possible correlates with different environments

The distribution of *A. fasciata* and *A. depilans* suggests that *A. fasciata* would be more resistant to conditions of respiratory stress (Achituv & Susswein, 1985). The present data support this prediction. *A. depilans* are more commonly found in open, well-oxygenated waters, whereas *A. fasciata* dominate calmer, more protected sites (Achituv & Susswein, 1985), although considerable overlap in distribution is found. Many morphological and behavioural features of the two *Aplysia* species are consistent with adaptation to differing environments. *A. depilans* has a lower profile and is a broader animal; the foot is wider and better adapted to holding onto a substrate in rough sea conditions. The internal shell protecting the delicate organs of the mantle cavity is more heavily calcified, and the stubby parapodia are joined to one another high up, providing additional protection to the gill and viscera (Eales, 1960). In contrast, *A. fasciata* has long parapodia that are joined to one another low down, permitting swimming by flapping the parapodia (Eales, 1960; Bebbington & Hughes, 1973). In previous studies, we have observed swimming in *A. fasciata* only in calm, protected environments or in the laboratory (Susswein *et al.* 1984); we have never observed swimming in *A. depilans*, although Bebbington & Hughes (1973) reported seeing it. *A. depilans* does not secrete ink in response to noxious stimuli (Eales, 1960), perhaps because the ink is rapidly washed away in rough water.

The life cycles of the two species also support the hypothesis that *A. fasciata* are relatively resistant to conditions of respiratory stress. From February to September, seawater temperature along the Mediterranean coast of Israel progressively rises from 17 to 29°C. *A. fasciata* are commonly found throughout this period, whereas *A. depilans* can be found only when temperatures are cooler (Gev *et al.* 1983). It is possible that the effects of increased temperature are similar to the effects reported here of decreased pH and decreased oxygen tension.

*Control of respiratory pumping**Relationship to oxygen consumption*

Data on regulation of oxygen consumption may provide a means of examining the function of the neural circuitry controlling the *Aplysia* gill. If the function of respiratory pumping is to ventilate the gill, one might expect that the incidence of such movements should be well correlated with changes in the rate of oxygen consumption. As oxygen consumption rises and falls, the rate of gill contractions should also rise and fall. Coregulation of ventilatory movements and oxygen consumption is common in many invertebrates and vertebrates (Prosser, 1973; Newell, 1979; Schmidt-Nielsen, 1983).

We found that under different experimental conditions the two parameters are either uncorrelated or are *negatively* correlated. The data suggest that changes in the rate of respiratory pumping contribute relatively little to regulation of ventilation under normal conditions.

Basal rate of respiratory pumping

Although *A. depilans* have a significantly higher resting rate of oxygen consumption than *A. fasciata* (Fig. 2), the basal rate of respiratory pumping is similar in both species (Fig. 3). This rate ($2\text{--}3\text{ pumps h}^{-1}$) is much lower than would be expected if the movement served a steady-state ventilatory function. A previous report (Eberly & Pinsker, 1984) also noted that the basal rate of respiratory pumping movements is very low in *A. brasiliana*. Moreover, although the rate of oxygen consumed is an inverse function of animal mass (Fig. 2), the rate of pumping is unrelated to it (Fig. 3).

Effects of hypercapnia and hypoxia

Conditions of moderate hypercapnia, which lead to an increase in oxygen consumption (Figs 4, 5), do not affect spontaneous respiratory pumping rate (Fig. 6). The rate of these movements was seen to increase *only* with extreme conditions of hypercapnia and hypoxia (Fig. 8), which lead to a *decrease* in the rate of oxygen consumed. The external stimulus leading to increased pumping under conditions of severe hypercapnia is likely to be the decreased pH, rather than change in P_{CO_2} . Croll (1985) has shown that spraying acid onto the ospharidium leads to an increase in the rate of pumping.

Possible functions of respiratory pumping movements

It is possible that the function of respiratory pumping movements is to increase ventilation of the gill *only* under conditions of extreme respiratory stress, when more moderate compensatory changes not dependent on pumping are no longer effective, and the rate of oxygen consumed has begun to decline. Support for this possibility comes from the observation that when respiratory pumping is induced in response to extreme hypercapnia or hypoxia, its rate is higher in *A. fasciata* than in *A. depilans*.

It is also possible that the function of respiratory pumping movements is entirely unrelated to ventilation. The movement may function primarily to clear the gill and mantle of debris, as occurs in other invertebrates (Prosser, 1973; Yonge & Thompson, 1976), and may be analogous to a cough or a hiccup in vertebrates. Support for this hypothesis comes from the observation that respiratory pumping usually accompanies defaecation in *Aplysia* (unpublished observation). It is also possible that the movement is primarily defensive, and is performed in response to external threats or to a deteriorating environment. Support for this possibility comes from previous data by Pinsker and collaborators (Kanz *et al.* 1979; Eberly *et al.* 1981; Eberly & Pinsker, 1984), who have shown that, in freely behaving *Aplysia*, stimuli that evoke the defensive gill withdrawal reflex also activate the circuitry inducing respiratory pumping. A significant proportion of the evoked withdrawal reflex is due to the activation of this circuit (Kanz *et al.* 1979). Also, Clearly & Byrne (1986) have identified a higher-order interneurone that seems to function as an initiator or modulator of defensive reactions, such as inking, tail

withdrawal, gill withdrawal and inhibition of feeding. The cell also excites L25, the command cell for respiratory pumping movements (Cleary & Byrne, 1986). Finally, in *Notarchus* (a member of the same family as *Aplysia*) escape swimming movements occur in response to noxious stimuli (Martin, 1969). The movements are very similar to respiratory pumping, and clearly serve a defensive function. In a single preliminary experiment (unpublished observation), we placed a specimen of *Notarchus indicus* in low-pH sea water, and observed that escape swimming was elicited.

Previous studies on regulation of respiratory pumping

Croll (1985) concluded that respiratory pumping movements in *A. californica* are likely to function to ventilate the gill, since hypercapnia or decreased pH initiate an increase in the rate of these movements. Although our conclusions differ somewhat from his, our data largely support and extend his observations. Thus, Croll (1985) found that lowering the pH in the environment increased the rate of respiratory pumping. However, he examined larger step changes in pH than were examined here, for no more than 5 min, and therefore did not observe the sharp threshold increase in respiratory pumping rate that we observed. Also, Croll (1985) did not examine the effects of the same treatment on oxygen consumption and respiratory pumping, and therefore was not able to observe the differences in threshold and time course of respiratory pumping and oxygen consumption. He also found that hypoxia had no effect on the rate of gill contraction, whereas we found a clear effect. However, the hypoxia conditions of Croll (1985) were much less extreme than in the present study.

We dedicate this paper to the memory of Professor Harold M. Pinsker, who died in 1986. Harry was a good friend of one of us (AJS), and he is missed. Harry contributed much information about the function of gill movements in *Aplysia*, and we hope that he would have enjoyed this work. We also thank Dr M. Schwarz for comments on an earlier draft of the manuscript, Yisrael Ziv for useful advice throughout these experiments, Leah Mizrahi for technical assistance, and the Health Sciences Research Center of Bar-Ilan University and Israel Institute for Psychobiology – Charles E. Smith Family Foundation grant no. 17/88 for partial support.

References

- ACHITUV, Y. & SUSSWEIN, A. J. (1985). Habitat selection by two species of Mediterranean *Aplysia*: *A. fasciata* Poiret and *A. depilans* Gmelin (Mollusca: Opisthobranchia). *J. exp. mar. Biol. Ecol.* **85**, 113–122.
- BEBBINGTON, A. & HUGHES, G. M. (1973). Locomotion in *Aplysia* (Gastropoda, Opisthobranchia). *Proc. malac. Soc., Lond.* **40**, 399–405.
- BYRNE, J. H. (1983). Identification and initial characterization of a cluster of command and pattern-generating neurons underlying respiratory pumping in *Aplysia californica*. *J. Neurophysiol.* **49**, 491–508.
- BYRNE, J. H. & KOESTER, J. (1978). Respiratory pumping: neuronal control of a centrally commanded behavior in *Aplysia*. *Brain. Res.* **143**, 87–105.

- CLEARY, L. J. & BYRNE, J. H. (1986). Associative learning of the gill and siphon withdrawal reflex in *Aplysia*: Interneurons mediating the unconditioned response. *Soc. Neurosci. Abstr.* **12**, 397.
- ROLL, R. P. (1985). Sensory control of respiratory pumping in *Aplysia californica*. *J. exp. Biol.* **117**, 15–27.
- DAVENPORT, J. (1976). A technique for the measurement of oxygen consumption in small aquatic organisms. *Lab. Prac.* **25**, 693–695.
- EALES, N. B. (1960). Revision of the world species of *Aplysia* (Gastropoda, Opisthobranchia). *Bull. Br. Mus. nat. Hist. Zool.* **5**, 276–404.
- EBERLY, L. B., KANZ, J. E., TAYLOR, C. & PINSKER, H. M. (1981). Environmental modulation of a central pattern generator in freely behaving *Aplysia*. *Behav. neur. Biol.* **32**, 21–34.
- EBERLY, L. B. & PINSKER, H. M. (1984). Neuroethological studies of reflex plasticity in intact *Aplysia*. *Behav. Neurosci.* **98**, 609–630.
- FOX, H. M. & WINGFIELD, C. A. (1938). A portable apparatus for the determination of oxygen dissolved in a small volume of water. *J. exp. Biol.* **15**, 437–445.
- GEV, S., ACHITUV, Y. & SUSSWEIN, A. J. (1983). Seasonal determinants of the life cycle in two species of *Aplysia* found in shallow waters along the Mediterranean coast of Israel. *J. exp. mar. Biol. Ecol.* **74**, 67–83.
- GRASSHOFF, K. (1976). *Method of Sea Water Analysis*. Weinheim: Verlag Chemie.
- JAHAN-PARWAR, B. & FREDMAN, S. M. (1978). Control of pedal and parapodial movements in *Aplysia*. I. Proprioceptive and tactile reflexes. *J. Neurophysiol.* **41**, 600–608.
- KANDEL, E. R. (1976). *Cellular Basis of Behavior: An Introduction to Behavioral Neurobiology*. San Francisco: W. H. Freeman.
- KANDEL, E. R. (1979). *Behavioral Biology of Aplysia: A Contribution to the Comparative Study of Opisthobranch Molluscs*. San Francisco: W. H. Freeman.
- KANDEL, E. R., FRAZIER, W. T., WAZIRI, R. & COGGESHALL, R. E. (1967). Direct and common connections among identified neurons in *Aplysia*. *J. Neurophysiol.* **30**, 1352–1376.
- KANZ, J. E., EBERLY, L. B., COBBS, J. S. & PINSKER, H. M. (1979). Neuronal correlates of siphon withdrawal in freely behaving *Aplysia*. *J. Neurophysiol.* **42**, 1538–1556.
- KOESTER, J., DIERINGER, N. & MANDELBAUM, D. E. (1979). Cellular neuronal control of molluscan heart. *Am. Zool.* **19**, 103–116.
- KOESTER, J., MAYERI, E., LIEBESWAR, G. & KANDEL, E. R. (1974). Neural control of circulation in *Aplysia*. II. Interneurons. *J. Neurophysiol.* **37**, 476–496.
- KUPFERMANN, I. & CAREW, T. J. (1974). Behavioral patterns of *Aplysia californica* in its natural environment. *Behav. Biol.* **12**, 317–337.
- KUPFERMANN, I., CAREW, T. J. & KANDEL, E. R. (1974). Local, reflex and central commands controlling gill and siphon movements in *Aplysia*. *J. Neurophysiol.* **37**, 996–1019.
- KUPFERMANN, I. & KANDEL, E. R. (1969). Neuronal controls of a behavioral response mediated by the abdominal ganglion of *Aplysia*. *Science* **164**, 847–850.
- MARTIN, R. (1969). On the swimming behavior and biology of *Notarchus punctatus* Phillippi (Gastropoda, Opisthobranchia). *Publ. Staz. zool. Napoli* **35**, 61–75.
- NEWELL, R. C. (1979). *Biology of Intertidal Animals*, 2nd edn. Kent: Marine Ecological Survey.
- PERETZ, B. (1969). Central neuron initiation of periodic gill movements. *Science* **166**, 1067–1072.
- PERLMAN, A. (1979). Central and peripheral control of siphon-withdrawal reflex in *Aplysia*. *J. Neurophysiol.* **42**, 510–529.
- PROSSER, C. L. (1973). Oxygen: respiration and metabolism. In *Comparative Animal Physiology*, 3rd edn, vol. 1 (ed. C. L. Prosser), pp. 165–211. Philadelphia, London, Toronto: Saunders.
- SAWADA, M., BLANKENSHIP, J. E. & MCADOO, D. J. (1981). Neural control of a molluscan blood vessel, the anterior aorta of *Aplysia*. *J. Neurophysiol.* **46**, 967–986.
- SCHMIDT-NIELSEN, K. (1983). *Animal Physiology: Adaptation and Environment*, 3rd edn. Cambridge, London, New York, New Rochelle, Sydney: Cambridge University Press.
- SUSSWEIN, A. J., ACHITUV, Y. & MARKOVICH, S. (1987). *Aplysia* from shallow waters along the coasts of Israel. *Cah. Biol. mar.* **28**, 97–110.
- SUSSWEIN, A. J., GEV, S., ACHITUV, Y. & MARKOVICH, S. (1984). Behavioral patterns of *Aplysia fasciata* along the Mediterranean coast of Israel. *Behav. Neur. Biol.* **41**, 7–22.
- YONGE, C. M. & THOMPSON, T. E. (1976). *Living Marine Molluscs*. London, Glasgow: Collins.