ACTA ADRIATICA
INSTITUT ZA OCEANOGRAFIJU I RIBARSTVO — SPLIT
SFR JUGOSLAVIJA

Vol. XVIII, No. 7

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SPLIT 1976

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INTRODUCTION

Animals and plants on rocky shores are distributed into strips or zones one above the other. Each zone is characterized by features of its own, forming a distinctive biological belt. A standard pattern of zonation is often best displayed on fairly smooth continuous rocky slopes whether steep or gradual, and it is best evident where wave action is neither absolutely maximal nor yet minimal. In addition to waves and tides, various other factors are known to exert a direct influence on the patterns of zonation. Among the factors are local features of topography, habitat selection, competition, and a number of physical factors which include temperature, desiccation, salinity and light.

The influence of temperature on intertidal animals is believed to determine their distribution on rocky shores. Huntsman and Sparks (1924) correlated the resistance to heat of marine animals with the environmental temperature, while Gowanloch and Hayes (1926) and Broekhuysen (1940) succeeded in correlating the lethal temperature of various intertidal animals with the distribution of animals on the shore.

Desiccation is regarded as one of the factors modifying the influence of temperature on the distribution of marine animals on rocky shores. The influence of desiccation on intertidal animals, therefore, depends primarily on the nature of the rocky shore and the position occupied by the different animals on the shore. Gowanloch and Hayes (1926) found that high-level species of *Littorina* had a higher resistance to desiccation than low-level species. The same conclusion was arrived at by Broekhuysen

(1940) working on intertidal gastropods from South African shores and by Micallef (1966) working on British trochids.

Salinity constitutes yet another important factor influencing intertidal zonation. The importance of salinity in intertidal animal distribution results from the fact that while varying salinities presumably never occur in the open sea, they may be encountered on rocky shores with possible lethal effects. Gowanloch and Hayes (1926), Broekhuysen (1940) and Micallef (1966) found that intertidal molluscs showed a graded resistance to prolonged submergence at different salinities and correlated the resistance exhibited by the animals with their vertical distribution.

In Malta, as elesewhere in the Mediterranean, *Patella caerulea* prefers horizontal or gently sloping surfaces which tend to be found at the level of the lower mediolittoral subzone. These places abound in small rock pools, and it is here that *P. caerulea* is generally observed. On the other hand, *Patella lusitanica* prefers vertical slopes which tend to be found at the level of the upper mediolittoral subzone. The upper mediolittoral zone coincides with the highest level of submergence by waves or by the rising of a calm sea.

METHODS AND MATERIALS

Limpets of the species *Patella caerulea* and *Patella lusitanica* were collected from the shore in Malta throughout the year. Only limpets relatively free of algal growth were collected. Prior to experimentation the limpets were »short-term acclimated«. They were placed at 10°C for 4 to 5 days prior to experimentation. The acclimation temperature of 10°C was adopted after Micallef (1966). The limpets were kept unfed during the acclimation period; only adults of the same size and displaying a response to pricking of the mantle skirt were used for experimentation.

Fifty percent mortality was taken to represent the average estimate to the resistance temperature, desiccation and different salinities of the two species. Limpets not displaying a response to pricking of the mantle skirt during a recovery period of 48 hours were considered to be dead. These procedures were adopted after Fraenkel (1960, 1961, 1966).

To determine the effect of high temperature in water, pyrex beakers of 500 ml capacity were filled with filtered seawater to a level of 0.5 cm from the brim and placed in a constant temperature bath (Townson and Mercer Type E. 270 Standard Bath) with temperature regulation to 0.01°C. Each beaker was covered by an inverted Petri dish to prevent any water vapor from escaping, thereby maintaining the salinity of the water in each beaker constant. One hour was allowed for full thermal equilibration of the water to take place. Acclimated limpets were placed in groups of five per beaker at the test temperature. At each temperature, different groups were subjected to varying exposure periods until 50 percent mortality started occurring.

For the effect of high temperature in air, empty beakers of 500 ml capacity were immersed to the brim in a constant temperature bath by means of lead weights. Each beaker was covered by an inverted Petri dish and sufficient time was allowed for thermal aquilibration of the air inside the beaker with the water in the constant temperature bath to take place. Wet acclimated limpets were placed on perspex discs and then placed in beakers. Five specimens were placed in each beaker. A few drops of water were added to each beaker to ensure a saturated atmosphere thus minimizing the desiccation factor. Evaporation, however, took place to some extent from the shell surface. Shells were always dry after each test. Limpets were introduced in each beaker with maximum speed to avoid any temperature changes. The temperature inside each beaker was checked periodically with a thermistor probe and thermometer (Yellow Springs Tele-Thermometer Model 43TZ). As in the previous method, different groups of limpets were subjected to different exposure periods at each test temperature until 50 percent mortality started occurring.

At the end of the test period the following procedure was adopted for both experiments. Limpets were withdrawn from the beakers by means of a long stainless steel forceps, transferred to a tank containing wellaerated seawater at room temperature and left there for half an hour, after which a preliminary inspection of the animal was conducted. The tank was then placed in a constant temperature room and left there for 48 hours at 10°C. In order to avoid contamination of the water by any dead limpets periodic inspection of the animals was carried out during the recovery period. The number of surviving and dead limpets at the end of the recovery period was recorded.

The measurements of oxygen consumption were made on groups of five limpets. The animals were first thermally equilibrated, generally overnight, at the test temperature. Oxygen consumption in moist air, or in Millipore filtered seawater, was measured by means of a specially designed volumetric respirometer (Bannister, Bannister and Micallef, 1969). The rates of oxygen consumption were expressed as microliters oxygen per gram wet flesh weight per hour ($\mu Lo_2/g/hr$).

To determine resistance to desiccation, the animals were shaken and dried on a filter paper to remove excess water prior to experimentation. Ten limpets were each placed facing upwards, or downwards, in a weighing bottle, weighed and placed in a cabinet-desiccator. The humidity inside the desiccator was maintained at 65 percent by means of 100 ml of 9.7N, sulphuric acid placed in a beaker in the desiccator. The desiccator was kept in a constant temperature room at 18°C during the period of experimentation. The temperature and humidity inside the desiccator were periodically checked on a thermohygrometer (Griffin and George Ltd., Catalog No. L31—925) placed inside the desiccator. The limpets were subjected to the experimental conditions for varying periods of time until 50 percent mortality started to occur. At the end of each test period the limpets were taken from the desiccator and each limpet was quickly weighed to avoid any uptake of moisture from the atmosphere. All weight determinations were carried out on a Mettler H5 analytical balance. After the animals were weighed at the end of experiment, they were placed in seawater at 10°C and the number of limpets recovering within a period of 48 hours was noted.

To determine the resistance to high and low salinities, groups of ten acclimated limpets were placed in circular polythene basins and the temperature maintained at 18°C. Millipore-filtered seawater of salinity 37.2‰ was used to prepare dilute and concentrated seawater. Dilutions of the seawater were prepared by mixing it with glass-distilled water to make solutions ranging from 20 to 80 percent by volume. Concentrated solutions of the seawater of up to 140 percent by volume were prepared by evaporation in a hot air oven at a temperature of 65°C (Micallef, 1966). One liter of the required seawater was used for each group of limpets. The water was changed on alternate days, and aeration was applied for about 15 minutes twice daily. Before the aeration the animals were examined individually for their condition. Each limpet was lifted to the surface and limpets not displaying a response to pricking of the mantle skirt were removed and transferred to normal seawater. After 24 hours in normal seawater, these limpets were again tested. The number of dead limpets was recorded, the experiment was continued until 50 percent mortality started occurring.

RESULTS

The effect of high air and water temperatures on *Patella caerulea* and *Patella lusitanica* is shown in Figure 1. *P. lusitanica* was found to be more tolerant than *P. caerulea* to high air and water temperatures. Two features of interest can be observed in Figure 1a) both limpets showed a greater

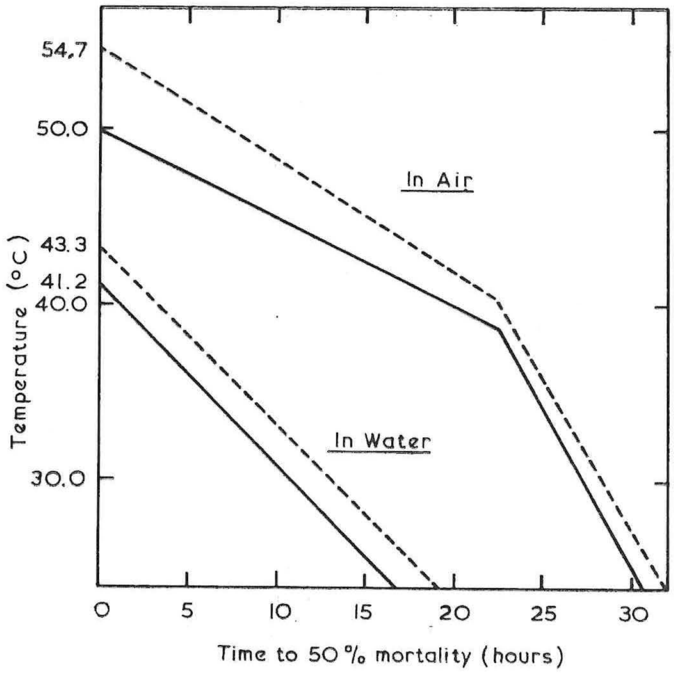

Fig. 1 — Median mortality temperature as a function of time of exposure for Patella caerulea (solid lines) and Patella lusitanica (interrupted lines) in air and in water.

resistance to high temperatures in air than in water, and b) a discontinuity in the time-temperature relationship for air temperature was obtained for both limpets. The higher resistance to air temperatures suggests that these can maintain a lower body temperature than the environmental temperature. The probable mechanism is evaporative cooling. With regard to the discontinuity, it is suggested that these represent the stage or point of heat coma. Above this temperature, therefore, the survival of the animals is as much dependent on resistance to heat as on resistance to desiccation.

The internal state of *P. caerulea* and *P. lusitanica* under temperature stress is indicated from the oxygen consumption of the two limpets in air and in water. Figures 2 and 3 show that both limpets increased their rate of oxygen consumption with temperature in air and in water between 10° and 30° except that in *P. caerulea* the rate of oxygen consumption decreased above 25°C in water. From the two figures it can also be seen that whereas *P. caerulea* has a higher rate of oxygen consumption in water than in air, *P. lusitanica* has a higher rate of oxygen consumption in air than in water. The respiration results obtained indicate that the two limpets show a respiratory adaptation corresponding to their respective level on the shore.

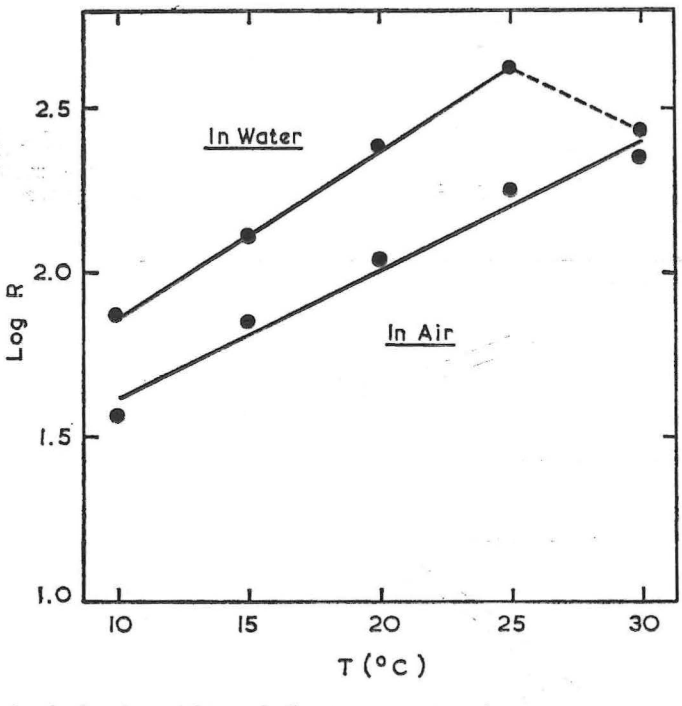

Fig. 2 — Plot of the logarithm of the mean rate of oxygen consumption (R = μL/g/hr of Patella caerulea against temperature (T). The straight lines have been fitted by the method of least squares.

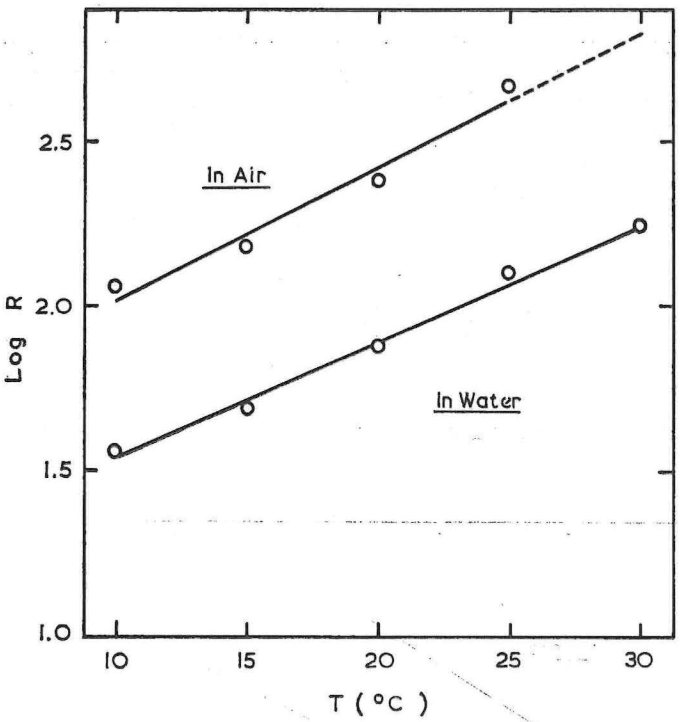

Fig. 3 — Plot of the mean rate of oxygen consumption (R= L/g/hr) of **P***atella lusitanica against temperature (T). The straight lines have been fitted by the method of least squares.*

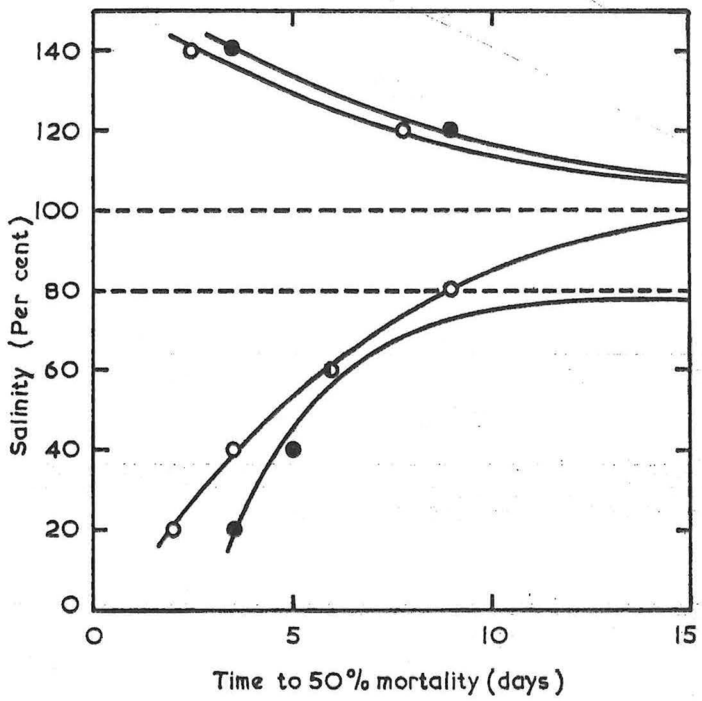

Fig. 4 — Percent mortality with time in groups of 10 Patella caerulea (O—O) and 10 Patella lusitanica (O—O) kep at a relative humidity of 56 percent and a temperature of 18°C. The curves have been fitted by inspection.

Since the observed lethal temperature in air is higher in *P. lusitanica* than in *P. caerulea* and since at this temperature the effect of desiccation is also operative, it was expected that *P. lusitanica* would also have a higher resistance to desiccation than *P. caerulea*. Figure 4 shows that the time of 50 percent mortality was 36 hours in *P. caerulea* and 64 hours in *P. lusitanica* at a relative humidity of 65 percent and at a temperature of 18°C.

The resistance to high and low salinities obtained for the two limpets is shown in Figure 5. In Figure 5, the high salinity curves have been drawn to approach 100 percent (i. e., normal) salinity asymptotically. The low salinity curve for *Patella caerulea* has been drawn to approach 80 percent salinity asymptotically; that for *Patella lusitanica* has been drawn to approach 100 percent salinity asymptotically. It is seen that the two limpets had essentially the same resistance to high salinities, suviving indefinitely as the salinity decreased from high values to 100 percent salinity.

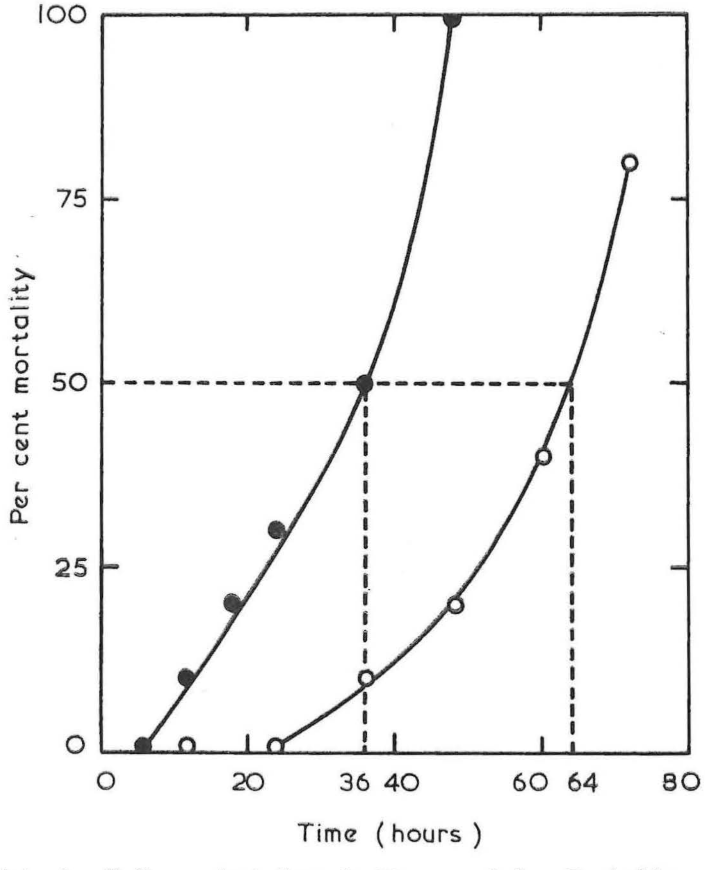

Fig. 5 — Plot of salinity against time to 50 percent for Patella caerulea (●—●) and Patella lusitanica (0—0). The curves through the plotted points have been drawn by inspection.

At low salinities, *P. caerulea* appeared to be more tolerant to low salinities than *P. lusitanica*. The time of 50 percent mortality was found to be indefinite in *P. caerulea* exposed to 80 percent salinity but was only 9 days in *P. lusitanica*. The greater ability of *P. caerulea* to survive at salinities somewhat below normal means that it is more tolerant than *P. lusitanica* to such dilution of the ambient seawater as the limpets may normally encounter on the shore.

DISCUSSION

The effects of high temperatures on intertidal animals clearly depend on whether the temperatures are experienced in air or in water. The effect of high air temperatures is, however, complicated by the occurrence of evaporation, other events not being excluded. Limpets such as *Patella lusitanica* and *P. caerulea* do not hold the shell firmly pressed against the substrate. Evaporative cooling, therefore, can take place in these limpets from around the mantle edge. Experimental evidence in support of evaporative cooling in three representative intertidal animals has been obtained by Lewis (1963). The resistance to temperature of the limpets species investigated appears to agree with the distribution of *Patella* species described by Ebling (1968). The higher resistance in air found for *P. lusitanica* correlates with the fact that this limpet is found in exposed situations more often than *P. caerulea*.

The two limpets show a respiratory adaptation corresponding to that expected from their regime of exposure and submergence at their respective level on the shore. The results indicate that *P. lusitanica* is able to respire better in air than in water while the reverse happens in the case of *P. caerulea*. The present findings and those of Sandison (1966, 1967), Micallef (1966, 1967) and Micallef and Bannister (1967) appear to show that while the respiratory rates of intertidal animals may vary with temperature, their rates of respiration are different according to whether the animals are exposed or submerged.

The results obtained here confirm the earlier general observations made on marine molluscs by Gowanloch and Hayes (1926), Broekhuysen (1940), Micallef (1966) and Davis (1969) that higher level species have a greater resistance to desiccation than lower level species. The present findings indicate a correlation between resistance to desiccation and the position of the limpets on the shore for *P. caerulea* and *P. lusitanica*. The same correlation also applies to the salinity tolerances of the two limpets and the results obtained find a close parallel with the observations of Broekhuysen (1940) on South African prosobranchs. The tolerance of *P. lusitanica* to high salinities and its intolerance to low salinities suggests that it is not expected to inhabit rock pools. This conclusion was verified during visits to several rocky shores in Malta. The absence of *P. lusitanica* from rock pools is probably also due to the fact that it prefers an exposed environment with periods of submergence.

In conculsion it can be stated that a pattern of resistance to high temperatures in air and in water, desiccation, high and low salinities and respiration in air and in water has been obtained which account for the zonation of *P. caerulea* and *P. lusitanica.*

SUMMARY

The limpets *Patella lusitanica* and *Patella caerulea* have been observed to show adaptational differences which correlate with their position in the upper and lower parts, respectively, of the eulittoral zone in the Mediterranean.

The instantaneous lethal temperature in air and in water is higher in *P. lusitanica* than in *P. caerulea.* Both limpets have higher instantaneous lethal temperatures in air than in water.

P. lusitanica has a higher resistance to desiccation and a slightly higher body water than *P. caerulea.*

P. lusitanica and *P. caerulea* have essentially the same resistance to high salinities, but *P. caerulea* is more tolerant to low salinities than *P. lusitanica.*

The rate of oxygen consumption of *P. lusitanica* is about three times higher in air than in water, whereas the rate of oxygen consumption of *P. caerulea* in water is about twice that in air.

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KRATAK SADRŽAJ

Kod priljepaka *Patella lusitanica* i *Patella caerulea* zapažene su prilagodbene razlike koje su u korelaciji s njihovim položajem u gornjim, odnosno donjim djelovima eulitoralne zone u Mediteranu.

Trenutačna letalna temperatura na zraku i u vodi viša je za vrstu *P. lusitanica* nego za vrstu *P. caerulea*. Obe su vrste otpornije na visoke temperature zraka nego vode.

Vrsta *P. lusitanica* sadrži nešto više tjelesne vode i otpornija je na isušenje od vrste *P. caerulea*.

P. lusitanica i *P. caerulea* u istoj su mjeri otporne na visoku slanost ali je *P. caerulea* tolerantnija prema niskoj slanosti nego *P. lusitanica*.

Potrošnja kisika kod vrste *P. lusitanica* za oko tri puta je viša na zraku nego u vodi, dok je kod vrste *P. caerulea* u vodi za oko dva puta više nego na zraku.