

Sublethal effects provoked by the presence of the predator *Nucella crassilabrum* (Lamarck) upon the mussel *Perumytilus purpuratus* (Lamarck) in Chile

Efectos subletales provocados en el mitflido *Perumytilus purpuratus* (Lamarck) por la presencia del depredador *Nucella crassilabrum* (Lamarck) en Chile

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ABSTRACT

Biotic interactions in marine invertebrates were analyzed under laboratory conditions. An oxygen debt was observed for the intertidal mussel *Perumytilus purpuratus* (Lamarck) in contact with the gastropod predator *Nucella crassilabrum* (Lamarck). This response was detected after a short contact with the snail. The oxygen debt was similar to that determined after anoxia (N₂ supply). Mussels maintained in the presence of whelks and control animals without predators, were sequentially subjected to high temperatures during 2 h of air exposure. The mortality rate was higher in experimental individuals (previously in contact with predators) than in controls. Experimental animals (previously exposed to predators) experienced greater water losses (weight drop) than controls, after high emersion temperature treatment. The results suggest that predators could be the cause of mortality not only when organisms are directly consumed, but also due to cumulative sublethal effects.

Key Words: desiccation, *Nucella*, oxygen debt, *Perumytilus*, predation, sublethal effects.

RESUMEN

En el presente trabajo se analizaron interacciones bióticas en dos invertebrados marinos, bajo condiciones de laboratorio. Se observó débito de oxígeno en el mitflido intermareal *Perumytilus purpuratus* (Lamarck) al ser puesto en contacto con el gastrópodo depredador *Nucella crassilabrum* (Lamarck). Esta respuesta fue detectada luego de un período de 30 minutos con el caracol. El débito de oxígeno fue similar al observado luego de anoxia (burbujeo de nitrógeno en inmersión). Mitflidos mantenidos en presencia del caracol y ejemplares controles (en ausencia del depredador), fueron sometidos posteriormente a altas temperaturas de emersión durante 2 h. La mortalidad fue mayor en el grupo experimental (previamente en contacto con *Nucella*) que en los ejemplares controles. Los animales experimentales del bivalvo (previamente expuestos al depredador) experimentaron mayor pérdida de agua (disminución del peso corporal) que los animales controles, luego de ser expuestos a alta temperatura en emersión. Los resultados sugieren que los depredadores pueden causar mortalidad en las presas, no sólo por consumo directo de ellas, sino también debido a efectos subletales.

Palabras clave: desecación, *Nucella*, débito de oxígeno, *Perumytilus*, efectos subletales de depredación.

INTRODUCTION

Explanations of the distribution and abundance patterns of marine organisms inhabiting coastal areas have emphasized the action of biotic factors such as predation (Paine 1974, Menge 1976), herbivory (Lubchenco 1978, 1983), and interspecific competition (Connell 1961, 1975, Dayton 1971, Menge & Sutherland 1987). Abiotic

factors such as temperature, desiccation and exposure to wave action, have also been considered (Doty 1946, Stephenson & Stephenson 1972, Newell 1979). These factors have been evaluated individually, measuring the effects that are easily identifiable and clearly reflected by variations in mortality rates. However, the consequences of sublethal effects operating sequentially have not been considered. If the factors that produce

sublethal effects (factors affecting the animal's fitness) operate one after another their individual effect can be increased. Thus, when these factors operate individually, they may not affect the survival of sessile organisms, but when they operate in sequence, they could be an important cause of mortality.

Various biochemical and physiological properties have been used to assess the sublethal effects of different environmental factors (Ivanovici & Wiebe 1981). The adenine nucleotid concentration has been successfully employed in diagnostic studies evaluating the response of organisms to changes in the environment (Rainer et al. 1979, Zarogian et al. 1982). In the intertidal zone, temperature, desiccation and low oxygen availability have proved to be the factors that cause greatest physiological stress in sessile invertebrates (Newell 1979). However, there is little information about the physiological or biochemical effects generated by biotic factors such as nonlethal contact with predators or competitors.

This study suggests that nonlethal contact with predators can produce detrimental sublethal effects in intertidal sessile organisms. In addition, the study tests the hypothesis that when biotic and physical factors (temperature) operate sequentially in time, they can become potential sources of mortality.

MATERIALS AND METHODS

Animals

The mussel *Perumytilus purpuratus* (Lamarck) dominates the mid-upper intertidal zone in Chile (Paine et al. 1985). Mussels 3.0-3.5 cm long were collected in Metri Bay (41 °36'S; 72 °42'W) and then maintained in the laboratory in circulating and well-aerated seawater during 1-3 days, at 15 °C, without feeding. The gastropod *Nucella crassilabrum* (Lamarck) is an abundant species in southern Chile, cohabiting with the mussel at the same intertidal height (Dye 1991), with high densities at the middle intertidal level of Metri Bay (5-30 individuals/m²; between 1-4

m of tidal height). The snail was maintained in the laboratory as *P. purpuratus*.

Oxygen consumption of Perumytilus purpuratus

The oxygen consumption in immersion was measured with a WTW-530 oxygenmeter (0.01 mg/l of sensitivity), as described previously (Vial et al. 1992). Mussels were exposed during either 30 min or 4 h to the following experimental conditions: 5 mussels in the presence of 5 snail specimens in a 1 l glass aquarium, with aerated and 0.45 µm filtered seawater (15 °C); 5 mussels in a 1 l aquarium, supplied with a constant bubble of N₂, for 4 h (anaerobic condition in immersion); 5 mussels in emersion during 4 h (75-90 % RH, 15 °C); 5 mussels in a 1 l aquarium (15 °C) with aerated and filtered seawater (control). After these periods, mussels were removed from the aquarium, and placed in a second 1 l aquarium, with a new supply of filtered and well-aerated seawater (at the same temperature): "rest" condition. After increasing periods of reimmersion at this "rest" condition, mussels were placed individually in the oxygenmeter chamber (155 ml), and the oxygen consumption was monitored during 20-40 min periods (in 0.45 µm filtered and well-aerated seawater). *N. crassilabrum* did not consume any mussel specimen during the experimental treatments applied. The oxygen consumption was standardized considering the dry weight of the total soft tissue of the mussel, determined in each case by a regression equation (Vial et al. 1992).

Sequential exposure to predators and high emersion temperatures

Mussels were assigned to two groups: "experimental group", kept in 5 l aquarium for 2 h (mussels in aerated circulating seawater) and exposed to the predator *Nucella crassilabrum* (equivalent number of specimens); the "control group" was kept under identical conditions, but in the absence of whelks.

Survival to high temperatures during a 2-h period of aerial exposure was determined for mussels previously in contact with the predator *Nucella crassilabrum* (2 h) and for

the control animals. The experiments were conducted in a thermal chamber at temperatures ranging from 36 °C to 56 °C, with 60% RH. After exposition to high emersion temperatures, the animals were reimmersed in aerated, circulating seawater, at the acclimation temperature, for a period of 24 h. Survival of experimental and control groups under these conditions was evaluated after this 24 h reimmersion period.

The water loss of specimens exposed to a high emersion temperature (44 ± 1 °C) (experimental or control group) was calculated by weighing individuals before and after this treatment.

Statistical comparisons were performed by the Student t-test and the chi-square test.

RESULTS

Oxygen debt of Perumytilus purpuratus after exposure to Nucella crassilabrum

In the laboratory valve closure of the mussel *P. purpuratus* occurred a few minutes after exposure to the predator *N. crassilabrum*. As a result of valve closure, respiration of mussels previously exposed to the snail was low at the beginning of the "rest" period (Fig. 1; Table 1). Nevertheless, when the mussels opened their valves again, respiration increased, exceeding that of control individuals (without previous exposition to the gastropod). This oxygen debt was even observed after 4 h exposure to the presence of *N. crassilabrum* (Fig. 2). The oxygen debt was similar in magnitude to that observed after 4 h of anoxia (N₂ support in immersion). After 4 h of emersion, the mussel showed no oxygen debt during the entire reimmersion period (Fig. 2).

Responses of mussels to high emersion temperatures after being exposed to the predator

When *Perumytilus purpuratus* was exposed to high temperatures during emersion, differences were observed in the mortality between individuals previously exposed to predators (experimental) and control specimens. The mortality rate was greater in ex-

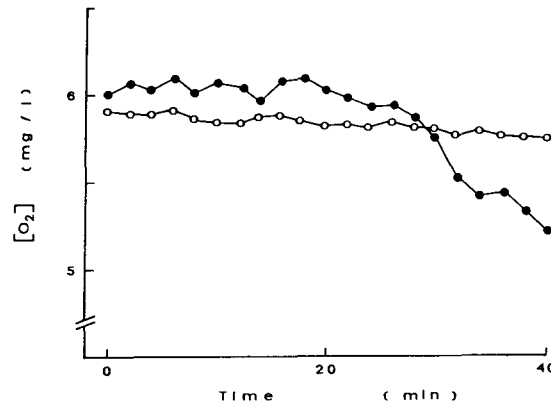


Fig. 1: Oxygen consumption of *Perumytilus purpuratus* previously exposed to *Nucella crassilabrum* for 30 min. After exposure to the snail, each mussel was immediately placed in the oxygenmeter chamber, and the oxygen concentration was measured during 40 min. (●) Mussel previously in contact with *N. crassilabrum*; (o) Control mussel.

Consumo de oxígeno de *Perumytilus purpuratus* expuesto previamente a *Nucella crassilabrum* por 30 min. Luego de la exposición al caracol, cada mitilido fue colocado inmediatamente en la cámara del oxigenómetro y la concentración de oxígeno fue medida durante 40 min. (●) Mitilido previamente en contacto con *N. crassilabrum*; (o) Control.

TABLE 1

Oxygen consumption of experimental specimens of *Perumytilus purpuratus* during the first 20 min and the second 20 min in the oxygenmeter chamber, immediately after exposure to the experimental conditions (contact with predator)

Consumo de oxígeno de especímenes experimentales de *Perumytilus purpuratus* durante los primeros 20 minutos y los segundos 20 minutos en un oxigenómetro, inmediatamente después de la exposición a condiciones experimentales (contacto con depredador)

Oxygen consumption of <i>P. purpuratus</i> in immersion (ml O ₂ / h / g dry wt)	
0-20 min	20-40 min
0.175 ± 0.070	0.976 ± 0.173*

(Mean ± SE, n = 4)

* t = 3.43; P < 0.05, with respect to control respiration value, Fig. 2 (0.383 ± 0.086, n = 10).

perimental organisms, reaching 100% at 45 °C (Fig. 3). The total mortality of *P. purpuratus* exposed to this experimental treatment (35-56 °C during 2 h of emersion) was 48.8 % for the control group, and 67.1 %, for

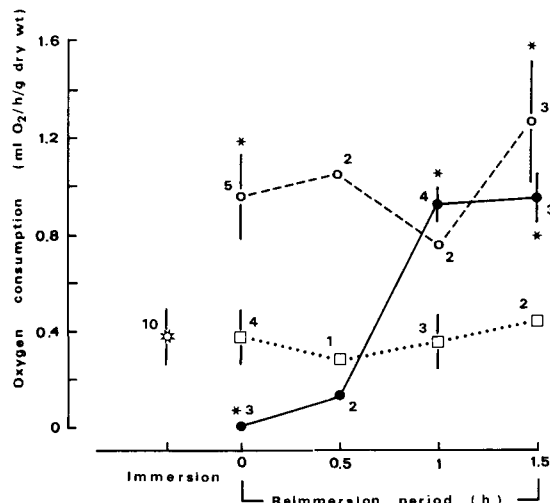


Fig. 2: Oxygen consumption of *Perumytilus purpuratus* previously exposed to the predator *Nucella crassilabrum* during 4 h, to anoxia and to emersion. After this period, mussels were reimmersed ("rest" condition). (mean \pm SE; n indicated at each point).

- (●) Contact with predator in immersion.
- (.) Emersion condition
- (o) Anoxic condition (N₂ supply in immersion)
- (*) Control respiration in immersion
- * Values statistically different in relation to the control; P < 0.05.

Consumo de oxígeno de *Perumytilus purpuratus*, previamente expuesto al depredador *Nucella crassilabrum* durante 4 h, a anoxia y a emersión. Luego de este período, los mitilidos fueron reimmersed (condición de "reposo"). (media \pm ES; n indicado en cada punto).

- (●) Condición de inmersión en contacto con depredador
- (.) Condición de emersión
- (o) Condición de anoxia (burbujeo de N₂)
- (*) Respiración control en inmersión
- * Valores estadísticamente significativos en relación al control; P < 0,05.

the experimental group (χ^2 : 10.43; P < 0.05; df: 49); the LT50 was 2 °C lower for the experimental group (Fig. 3).

The high mortality rates of *P. purpuratus* observed in the experiments could be attributed to water loss during exposure to high emersion temperatures. Dead organisms of the control group after 70 min of emersion at 44 °C showed a higher water loss than the surviving organisms (Table 2; Fig. 4), suggesting that desiccation could be the originating factor (as well as the consequence) of death. This result was similar between dead and surviving specimens of the experimental group (data not shown).

Individuals that had previously been exposed to predators exhibited a significantly higher water loss than the control animals (Fig. 4). Higher water loss was also detected in dead organisms of the experimental group than dead specimens of the control group (110, 120 and 170 min of emersion at 44 °C, Fig. 4). This suggests that animals previously exposed to predators remained with their valves open during emersion for longer periods and thus this was reflected in higher water losses. The same result could be explained by an earlier death of experimental specimens than that of the control group. The fact that experimental surviving animals showed higher water losses than the surviving animals of the control group (Fig. 4), provide an evidence supporting the first alternative.

DISCUSSION

Little evidence exists indicating that *Nucella crassilabrum* is an active predator of *Perumytilus purpuratus* in the intertidal zone of southern Chile (Dye 1991). However, field observations during low tide, gave evidence of consumption of *P. purpuratus* by *N. crassilabrum*. In a previous work it was established that the mussel *P. purpuratus* closes its valves when it perceives the presence of *Nucella*, as an escape mechanism from predation, inducing an hypoxic condition (Vial et al. 1992). Several mechanisms have been identified whereby organisms perceive the presence of predators, thus obliging them to close their valves or opercula (Bullock 1953, Philips 1975). Fig.1 shows that after a period as short as 30 min of exposure to the predator *Nucella*, an oxygen debt has been observed. The magnitude of this oxygen debt was similar to that obtained after anoxia (N₂ bubbled seawater, Fig. 2). The oxygen debt for *P. purpuratus* exposed to *Nucella* was similar in magnitude to the values reported previously for different marine invertebrates submitted to emersion or anoxic conditions (Widdows et al. 1979, Widdows & Shick 1985, Meinardus-Hager & Gäde 1987). After 4 h of emersion, no oxygen debt was observed for *P. purpuratus*. This result contrasts with the oxygen debt observed previously for

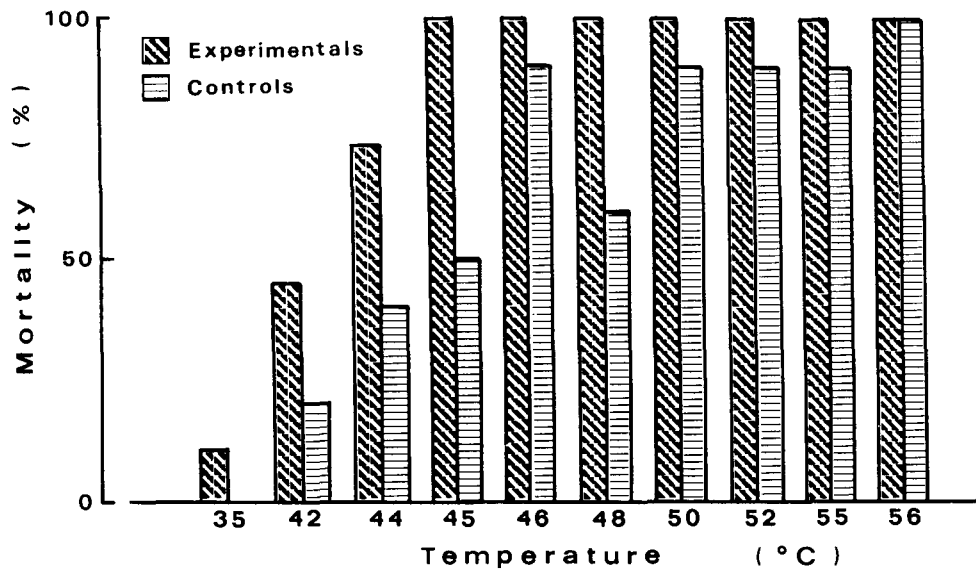


Fig. 3: Mortality rates (%) of *Perumytilus purpuratus* exposed to presence of the predator *Nucella crassilabrum* and of control animals (not exposed to predators), subjected to temperatures above 35 °C for a 2-h emersion period (60 % RH, in all cases). (n = 21-26 individuals at each temperature).

Tasas de mortalidad de *Perumytilus purpuratus*, expuesto a la presencia del depredador *Nucella crassilabrum* y de animales controles (no expuestos al depredador), sometidos a temperaturas sobre 35 °C por un período de 2 h en emersión. (n = 21-26 individuos a cada temperatura).

TABLE 2

Water loss of dead and surviving specimens of *Perumytilus purpuratus* of the control group, exposed to 44 °C in emersion (60% RH), for 70 min
 Pérdida de agua en especímenes muertos y sobrevivientes de *Perumytilus purpuratus* del grupo control, expuestos a 44°C en emersión (60 % HR), por 70 minutos

	n	Water loss (g / specimen)
Dead	27	0.640 ± 0.340
Survivors	16	0.040 ± 0.002

Mean ± SD; t = 9.17; P < 0.001

Mytilus edulis exposed to emersion for 5 h (Widdows & Shick 1985). Nevertheless, *P. purpuratus* aerial rates of respiration are higher than those of *M. edulis* (Widdows et al. 1979), or *M. chilensis* (Vial et al. 1992, Simpfendörfer et al. 1995). This difference in aerial respiration rates could explain the absence of oxygen debt after emersion for *P. purpuratus*.

In addition to valve closure, the subjection, perforation and manipulation mechanisms of predators (Carriker & Williams

1978, Carriker 1981), must also affect the energy levels of organisms that are not actually consumed. These organisms will experience a decrease in the amount of energy at their disposal to respond to other sources of perturbation or stress, which was apparent when the animals were exposed to high temperatures. There was a significant difference in the response to high temperatures between individuals that were previously in contact with predators and control animals kept without predators. Thus, individual sublethal effects acting upon the mussel without affecting survival can constitute a significant cause of mortality when operating sequentially.

Responses of organisms to high temperature regimes usually involve heat loss through evaporation, when relative humidity is high. Nevertheless, at low levels of relative humidity, desiccation is greater and the organisms must maintain their valves closed; this escape mechanism to desiccation generally requires energy. In *Perumytilus purpuratus*, a relationship between mortality and water loss could be established. The "contact with predators" and "high temperature" factors may cause sublethal effects.

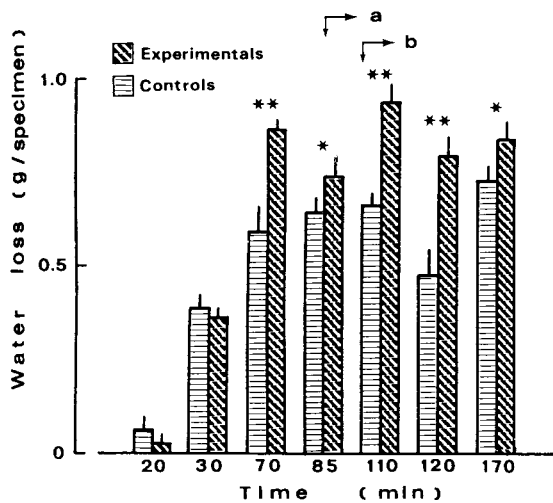


Fig. 4: Water loss at increasing periods of exposure to 44°C in experimental (2 h with *Nucella crassilabrum*) and control mussels.

(a) 100 % mortality for experimental group.

(b) 100 % mortality for control group.

(Mean + SD; n = 48).

* P < 0.05; ** P < 0.001.

Pérdida de agua en períodos crecientes de exposición a 44 °C en individuos experimentales (2 h con *Nucella crassilabrum*) y en controles.

(a) 100 % mortalidad en grupo experimental

(b) 100 % mortalidad en controles (media ± DS; n = 48)

* P < 0,05; ** P < 0,0011

However, mortality rates will increase significantly when these factors occur in sequence. This can be attributed to the fact that, previous to emersion, the experimental animals remained closed during the period of exposition to the predator (Vial et al. 1992). This involves an additional expenditure of energy, which would limit the organisms capacity to respond to the second sublethal factor of high temperature during emersion. A high tolerance was observed for the oyster *Crassostrea rhizophorae*, after exposition to high emersion temperatures: 100 % survival after 2-h exposition to 45° C in emersion, and after 5 h, at 42° C (Littlewood 1989). This high temperature emersion should cause mortality, when it occurs sequentially with a second sublethal factor. This type of interaction of biotic and abiotic factors can contribute to the comprehension of some variations observed in the distribution and abundance patterns of intertidal sessile organisms in their natural environment. In Metri Bay during the summertime,

temperatures of the rock during emersion are as high as 40 °C - 50 °C, and frequently exceed 45 °C. For this reason it can be assumed that predation should be the cause of mortality not only when organisms are directly consumed by predators, but also due to cumulative sublethal effects.

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LITERATURE CITED

- BULLOCK TH (1953) Predator recognition and escape responses of some intertidal gastropods in presence of starfish. *Behaviour* 5: 130-140.
- CARRIKER MR (1981) Shell penetration and feeding by naticacean and muricacean predatory gastropods: a synthesis. *Malacologia* 20: 403-422.
- CARRIKER MR & LG WILLIAMS (1978) The chemical mechanism of shell dissolution by predatory boring gastropods: a review and an hypothesis. *Malacologia* 17: 143-156.
- CONNELL JH (1961) The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* 42: 710-723.
- CONNELL JH (1975) Some mechanisms producing structure in natural communities. In: Cody M & J Diamond (eds) *Ecology and evolution of communities*: 460-490. Belnap Press, Cambridge, Massachusetts.
- DAYTON PK (1971) Competition, disturbance and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs* 41: 351-389.
- DOTY MS (1946) Critical tide factors that are correlated with the vertical distribution of marine algae and other organisms along the Pacific coast. *Ecology* 27: 315-328.
- DYE AH (1991) Feed preferences of *Nucella crassilabrum* and juvenile *Concholepas concholepas* (Gastropoda, Muricidae) from a rocky shore in southern Chile. *Journal of Molluscan Studies* 57: 301-307.
- IVANOVICI AM & WJ WIEBE (1981) Towards a working definition of "stress": a review and critique. In: Barret GW & R Rosemberg (eds) *Stress effects on natural ecosystems*: 13-27. John Wiley and Sons Ltd.
- LITTLEWOOD DTJ (1989) Thermal tolerance and the effects of temperature on air-gaping in the mangrove oyster, *Crassostrea rhizophorae*. *Comparative Biochemistry and Physiology* 93A: 395-397.
- LUBCHENCO J (1978) Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. *American Naturalist* 112: 23-39.
- LUBCHENCO J (1983) *Littorina* and *Fucus*: Effects of herbivores, substratum heterogeneity and plant escapes during succession. *Ecology* 64: 1116-1123.

- MEINARDUS-HAGER G & G GÄDE (1987) Recovery from environmental anaerobiosis and muscular work in the cockle, *Cardium tuberculatum*: oxygen debt and metabolic responses. *Journal of Experimental Zoology* 242: 291-301.
- MENGE BA (1976) Organization of the New England rocky intertidal community: role of predation competition and environmental heterogeneity. *Ecological Monographs* 46: 335-393.
- MENGE BA & JR SUTHERLAND (1987) Community regulations: variations in disturbance, competition and predation in relation to environmental stress and recruitment. *American Naturalist* 130: 730-757.
- NEWELL RC (1979) Biology of intertidal animals. Marine Ecological Surveys Ltd., Faversham, Kent. 757 pp.
- PAINÉ RT (1974) Intertidal community structure experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia* 15: 93-120.
- PAINÉ RT, JC CASTILLA & J CANCINO (1985) Perturbation and recovery patterns of starfish-dominated intertidal assemblages in Chile, New Zealand and Washington State. *American Naturalist* 125: 679-691.
- PHILIPS PM (1975) Distance chemoreception-triggered avoidance behaviour of the limpets *Acmaea (Collisella) limatula* and *Acmaea (Noteacmaea) scutum* to the predatory starfish *Pisaster ochraceus*. *Journal of Experimental Zoology* 191: 210-217.
- RAINER SF, AM IVANOVICI & VA WADLEY (1979) The effect of reduced salinity on adenylate energy charge in three molluscs (estuarine). *Marine Biology* 54: 91-99.
- SIMPFENDÖRFER RW, MV VIAL, DA LOPEZ, M VERDALA & ML GONZALEZ (1995) Relationship between the aerobic and anaerobic metabolic capacities and the vertical distribution of three intertidal sessile invertebrates: *Jehlius cirratus* (Darwin) (Cirripedia), *Perumytilus purpuratus* (Lamarck) (Bivalvia) and *Mytilus chilensis* (Hupé) (Bivalvia). *Comparative Biochemistry and Physiology* 111B: 615-623.
- STEPHENSON TA & A STEPHENSON (1972) Life between tide-marks on rocky shores. W.H. Freeman and Company, San Francisco. 425 pp.
- VIAL MV, RW SIMPFENDÖRFER, DA LOPEZ, ML GONZALEZ & K OELCKERS (1992) Metabolic responses of the intertidal mussel *Perumytilus purpuratus* (Lamarck) in emersion and immersion. *Journal of Experimental Marine Biology and Ecology* 159: 191-201.
- WIDDOWS J, BL BAYNE, DR LIVINGSTONE, RIE NEWELL & P DONKIN (1979) Physiological and biochemical responses of bivalve molluscs to exposure to air. *Comparative Biochemistry and Physiology* 62A: 301-308.
- WIDDOWS J & JM SHICK (1985) Physiological responses of *Mytilus edulis* and *Cardium edule* to aerial exposure. *Marine Biology* 85: 217-232.
- ZAROOGIAN E, J GENTILE, JF HELTSHE, M JOHNSON & AM IVANOVICI (1982) Application of adenine nucleotide measurement for the evaluation of stress in *Mytilus edulis* and *Crassostrea virginica*. *Comparative Biochemistry and Physiology* 71B: 643-649.