DIE-OFF RATE OF *STAPHYLOCOCCUS AUREUS* IN SEA-WATER

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All strains of coagulase positive *Staphylococcus aureus* are pathogens, causing a wide range of infections. They have been found to be shed by bathers under all conditions of swimming. Being salt tolerant they may survive in the marine environment, presenting a potential health hazard to bathers on crowded beaches.

The effect of solar radiation, temperature, salinity and predation on the survival of *S. aureus* was studied in laboratory experiments using 2^k factorial designs. Survival of studied bacterial group was expressed as T_{90}, the time required for a 90% reduction in bacterial number.

The number of *S. aureus* was determined by membrane filtration using a Baird-Parker Base Agar (Biolife). The culture of *S. aureus* for survival experiments was supplied by the Institute of Public Health, Split, Republic of Croatia.

T_{90} values under light conditions ranged from 8.0 to 11.9 hours, and under dark conditions from 115.1 to 422.5 hours. Survival of *S. aureus* was statistically significantly longer than survival of all faecal indicator groups (TUDOR *et al.*, 1990). An inverse relationship existed between survival of *S. aureus* and all studied factors. The largest negative effects were the main effects of solar radiation, temperature and predation, while the largest positive effects were the interactions between solar radiation and temperature, and between solar radiation and predation (Tab.1).

The results showed that solar radiation was the dominant factor in controlling the survival of *S. aureus*. The effect of temperature was also very important but partially obscured by the effect of solar radiation. The importance of predation in elimination of *S. aureus* from marine environments was established as statistically significant under experimental conditions. Effects of predation were more expressed under dark than under light conditions, indicating an interference effect between solar radiation and predation. Thus, solar radiation was detrimental not only to survival of *S. aureus* but also to survival and/or activity of predators inhibiting the effect of predation. Under natural conditions salinity was a less important factor controlling the persistence of *S. aureus*, suggesting their high salt tolerance.

Tab.1. Results of multifactor ANOVA comparing the main and 2-factor interaction effects of solar radiation (R), temperature (T), salinity (S) and predation (P) on the survival of *S. aureus*.

SOURCE OF VARIATION	SS	df	MS	F	P
MAIN EFFECTS	1.89 E5	4	4.72 E4	26.31	<0.005
R	1.17 E5	1	1.17 E5	64.96	<0.001
T	5.30 E4	1	5.30 E4	29.53	<0.005
S	5.18 E3	1	5.18 E3	2.89	n.s.
P	1.41 E4	1	1.41 E4	7.86	<0.05
2-FACTOR INTERACTIONS	7.32 E4	6	1.22 E4	6.80	<0.05
R x T	4.84 E4	1	4.84 E4	26.94	<0.005
R x S	4.62 E3	1	4.62 E3	2.57	n.s.
T x S	2.20 E3	1	2.20 E3	1.23	n.s.
R x P	1.29 E4	1	1.29 E4	7.19	<0.05
T x P	5.03 E3	1	5.03 E3	2.80	n.s.
S x P	1.26 E2	1	1.26 E2	0.07	n.s.
RESIDUAL	8.98 E3	5	1.80 E3		

n.s. - not significant (P>0.1)

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EFFECTS OF THE SEA WATER OSMOCONCENTRATION CHANGES ON OXIDATIVE PROCESSES IN ISOLATED GILL OF SHORE CRAB *CARCINUS MEDITERRANEUS* CSRN

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The gills of marine organisms play an important physiological role in respiration, osmoregulation and volume and acid-base regulation. Although the significance of the gills in ion transport functions in the Crustacean is evidently recognized (LUCU, 1990), the relationship between their respiratory and ion-regulatory functions has been pointed out only by few studies, not very recent (ENGEL, 1975).

The rate of oxygen consumption was measured in the posterior gills isolated from the shore crabs *Carcinus mediterraneus Csrn* collected at the coastline of the Istrian peninsula (Northern Adriatic). The gills from the intermoulting adult crabs were excised and perfused (LUCU and SIEBERS, 1986) before insertion into the oxygen chamber with an identical diluted solution in which OCR (oxygen consumption rate) was measured. Measurements were performed in a closed experimental system in the perpex chamber. A radiometer PO_2 electrode (E 5046) was protruded through the cover into the chamber and the OCR was measured by digital PHM 72 radiometer analyzer (Copenhagen) with technical details described by LUCU and PAVICIC (in prep.)

The main anearobic end product in Crustacea lactate (HILL *et al.*, 1991) was affected by the sea water osmoconcentration changes. The mean concentration of lactate in the blood was not changed significantly in the crabs acclimated to the sea water in the salinity range from 11.0 to 38 x 10^{-3}.

When gills were isolated from the crabs acclimated to various diluted sea water concentrations, the OCR was salinity-dependent and considerably increased as sea water concentration decreased. Prior to respiration measurement isolated gills were perfused by 2.5 x 10^{-3} mol l^{-1} ouabain dissolved in 50 per cent DSW (diluted sea water) and incubated in the respiration chamber. OCR was depressed by ouabain by approximately 30 per cent compared to the control solution (table 1). In the gills immersed in the N-methyl glucamine solution containing 250 mmol Cl^- l^{-1}, the OCR of the isolated preparation was close to zero, but only by the sodium substitution increased steadily. The OCR in which chloride plays a minor role, is a function of the sodium concentration changes. V_{max} was reached at 465 µl O_2 h^{-1} per gram gill wet weight and K_m at the sodium concentration of 16.8 mmol l^{-1}. Moreover, in the K-free and Ca-free saline, oxygen consumption of the excised gills was also reduced, supporting the indispensable role of the potassium and calcium ions in the respiration processes (table 1).

Dilute sea water		Physiological saline			
Control	Ouabain	Control	K-free	Ca-free	Mg-free
		(µl O_2 x h^{-1} per gram gill w.w.)			
526.19±26	365.78±36	593.41±43	362.47±33	478.15±44	573±48
	P < 0.01*		P < 0.01	P < 0.01	P > 0.05

Table 1. Effect of ouabain (2 x 10^{-3} mol l^{-1}) on gill respiration measured in the isolated posterior gills from *Carcinus mediterraneus* incubated in the DSW (260 mmol Cl/l). The gill was isolated from the crabs acclimated for 2 weeks in DSW. The mean respiration rate wase measured after it reached a steady-rate level in the isolated gills immersed in the control artificial saline (in mmol/l; NaCl, 260; KCl, 5; $MgCl_2$; $CaCL_2$, 4; HEPES, 5; pH, 8) and compared with the gill respiration rates measured in K-free, Ca-free and Mg-free saline. In the K,Ca and Mg-free solution appropriate ions were substituted by isoosmotic NaCl (N = 6 for each mean ± S.E.; * signigicantly different from corresponding control group). w.w. = wet weigh.

The results suggest that a portion of the energy liberated by the gill respiration is utilized by the gill Na,K ATPase enzyme complex maintaining Na and K concentration gradients between the extracellular and intracellular compartments.

In the Ca-free saline containing 0.1 mmol/l EGTA the OCR was reduced by about 19 per cent relative to the control Ca containing saline. ATP synthesis could be controlled by the supply of energy to the electron transport chain which is in turn controlled by cytosolic free calcium levels. It is known that in michocondria Ca is coupled with H pumping providing an electrochemical gradient or proton promotion force which is used to generate synthesis of ATP from ADP and P_i.50 per cent inhibited OCR by KCN was attained at 1 µmol l^{-1}. We suggest that electron transfert chain was blocked, and consequently oxidative capacity mediated via cytochrome oxidase activity was diminished.

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