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MICROCALORIMETRIC MONITORING DURING ADAPTATION TO HYPO- AND HYPEROSMOTIC MEDIA OF FIDDLER CRABS *UCA PUGILATOR*

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INTRODUCTION

The semiterristic fiddler crabs, *UCA PUGILATOR* (BOSC), live along the American Atlantic coast under the influence of the tide (TEAL 1958). Under this cyclic living conditions the crabs are exposed to a variety of salinity changes in the surrounding medium (tide, evaporation, dilution by rain and river waters, etc.). Due to this fact these animals have developed effective mechanisms to regulate their hemolymph concentration and consequently are known to be excellent osmoregulators (JONES 1941, POTTS 1954, GRENN, HARSH, BARR and PROSSER 1960, EVANS, COOPER and BOGAN 1976, BALDWIN and KIRSCHNER 1976, GRASZYNSKI, UNVERZAGT and BIGALKE 1979). In all these papers methods are used which allow insights into special aspects of the different adaptational processes, for example measurements of hemolymph ion concentrations or the determination of the activity of transport ATPase. But these methods are not suitable ones to describe the adaptational processes of the whole animal relatively undisturbed by the experimental treatment. Measurements of oxygen consumption neither are completely satisfactory because respirometry is in principle an integrative method. As the physiological adaptation to salinity changes is achieved by energy consuming processes like the active uptake or extrusion of ions these adaptational processes will be followed by changes in the metabolic rate which inevitably is accompanied by a change in heat production. So, measuring the heat production rate by calorimetry should be a most convenient method to observe all those alterations linked to osmoregulative regulation.

FORREST 1969, LAMPRECHT and SCHAARSCHMIDT 1974, LAMPRECHT 1980 and LAMPRECHT 1983 pointed out, that tere are some essential advantages compared to the convential, i.e. the up to now most used methods. Calorimetry is a non integrative, quantitative and qualitative method to monitor abolut values of a system under low stress conditions time continuous. Also it doesn't involve any direct

operation in the system itself. The calorimetric method monitors actual rates and not an integrated increasing or decreasing fuction as manometry does. Calorimetrically recorded power-time-curves show significantly more details as curves of manometric or polarographic experiments do. The structure in the calorimetric curves can be used to determine phases of high locomotive activity without any direct observation of the animal.

On the other hand all processes proceeding in the system are connected with a heat production and will also be monitored by the calorimeter. Only in comparison with other methods typical structures in power-time-curves can be associated to specific reactions, with one exception: The heat production by locomotion. High locomotive activity produces sharp peaks which rise clearly over the level of heat production during phases of "normal" metabolic activity.

MATERIAL and METHOD

Animals: Fiddler crabs of specie *UCA PUGILATOR* (BOSC) were obtained from GULF SPECIMEN Co., Panacea, Florida USA. 25 crabs were randomly taken from two shipments of about 1000 animals each. The female crabs' weight ranged between 0.81g and 1.51g (male crabs: 1.23g to 1.90g). The obvious anatomic difference between male and female crabs, the claw, which is about 40% of male crabs' body weight, is expected to be inert in the context of extracellular osmoregulation. The crabs lived under 21±1°C laboratory conditions, illuminated 12h a day.

Water composition: In all experiments synthetic "normal" sea water (SW) was used. "Normal" SW (100% SW) consists of 36g salt (TROPIC MARINE) per 1000ml of tap water, the other SW sollutions contained 12g (33% SW) and 60g (166% SW) salt per 1000ml respectively.

Keeping conditions: During the experiments the crabs were held in plastic boxes (l×w×h: 12×25.5×11cm). Each box contained 120ml SW and was covered with a glas plate to protect against extensive evaporation. The boxes were placed in an angel of about 5°. The maximum water level was 11cm, with one third of the bottom being left dry. The water temperature was 20±1°C in darkness and 26±1°C during illumination. The crabs were held in two groups of about 25 animals in 100% SW.

Manometry: The manometric experiments were performed with a differential respirometer (GILSON, IGP-14). In contrast to most traditional manometric apparatus (e.g. WARBURG-equipment) this one works with constant pressure in the reaction chambers. The chambers volume (140ml) is regulated by an piston, compensating the consumed gas volume to keep the pressure constant. In the chamber is a small tub to take up 20% KOH, absorbing the produced carbon dioxid.

Calorimetry: The heat production rates were measured with two microcalorimeters, modell E.CALVET (SETARAM, Lyon, France) with in three "reaction chambers"

each having a volume of 100ml. The sensitivity of the calorimeters was about 60µV/mW. The signals were recorded on two four-channel recorders (Typ BD5, KIPP& ZONEN, Delft). The recorded curves were integrated with a polarplanimeter to determine total heat production and from this the heat production rate. A typical adjustment was 500mV full range and 60mm/h paper speed. A detailed description is given by CALVET and PRAT 1956, HEMMINGER and HÖHNE 1979 and for the application to small animals by Lamprecht 1980 and 1983. The calorimeter chambers offered the possibility to measure the oxygen consumption (Portable O_2 and Temp. Meter, BECKMAN Instruments, Irvine USA) and heat production at the same time. Such an arrangement of simultaneously recording has the advantage of detecting anaerob metabolic phases.

EXPERIMENTS

The investigation consisted of three parts (A, B and C):

A: Heat production after adaptation to different salinities. This experiment took 10 days and includes three phases: 1.preadaptation, 2.adaptation and 3. measurement of heat production. The two pays preadaptation phase (1.) was only carried out for 166% SW. The adaptation (2.) took six days. This period of time was chosen as GRASZYNSKI, UNVERZAGT and BIGALKE (1979 a and b) reported that the specific activity of Na^+-K^+-ATPase had reached a new plateau within this time span.

On the first Day the crabs were fed. The water in the holding boxes was changed four times during the experiment. Phase 3. (measurement of heat production) started on the 9th day. The heat production was recorded twice 24h at 20°C. There were 15ml SW and one fiddler crab in the sample chamber. The reference chamber contained only 15ml SW. Recording started after a temperature adjustment of 1.5h. 21 female and six male *UCA* were examined.

B: Heat production during adaptation to different salinities. Experiment B took 13 days and also consisted of three phases. 1.prephase, 2.measurement of heat production for 24h under 100% SW conditions and 3.measurement of heat production during adaptation. In the prephase (1.) the fiddler crabs were hold in 100% SW. The crabs were fed only the first day. During the experiment the water in the holding boxes was changed four times. During the adaptation and recording phase (3.) the heat production was recorded continuously for six days at different SW concentrations. Nine female crabs were examined.

C: Oxygen consumption measurement. In experiment C the oxygen consumption of eight female *UCA* at 25°C was determined in darkness. A GILSON differential respirometer was used. The system was prewarmed for 0.5h. The oxygen consumption was examined for 1.5h.

RESULTS and DISKUSSION

Except two animals all *UCAs* in the experiment survived in all used sea water (SW) concentrations, i.e. the chosen change of concentrations corresponds to the range of the genetically fixed reaction limits. The results of BALDWIN and KIRSCHNER (1976) also didn't show an increasing mortality at 10% and 175% SW respectively.

The heat production (Tab.1) after adaptation to 33% and 166% SW significantly differed from the control group in normal 100% SW. The mean heat production was about two times higher than under normal conditions. With increasing heat production the range of variation also increased (with multiplication factor 1.5 to 2).

The comparison of the 95%-α-confidence intervals (Tab.1) and the graphical illustration (Fig.1) show a significat difference between the 33 and 166% SW conditions and normal 100% SW. The confidence intervals do not overlap.

Tab.1: Calorimetric determined heat production at 20°C and 33, 100 and 166% sea water (SW) and heat production calculated from oxygen consumption at 25°C and 100% SW (fourth column).

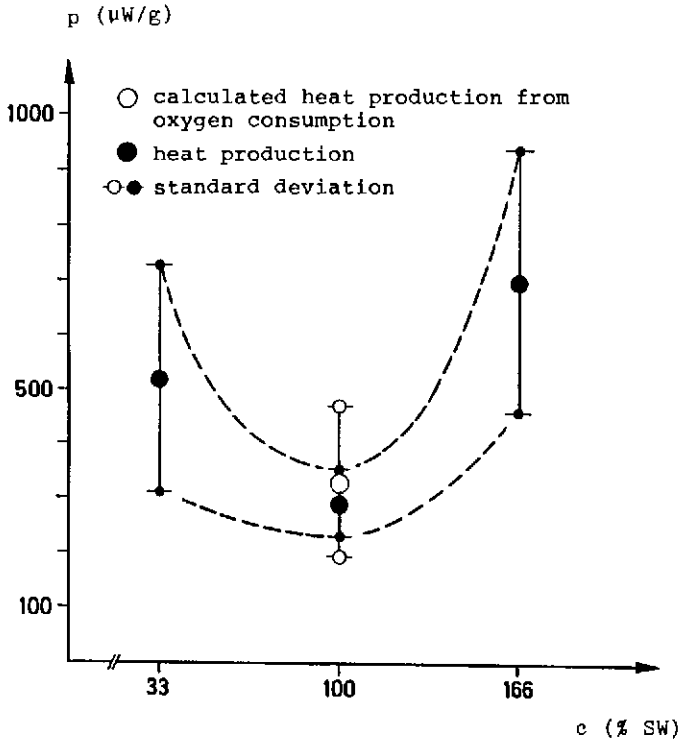
% SW (sea water)	33	100	166	100
number of animals	7	8	6	8
mean weight (g)	1.28±0.18	1.17±0.25	1.35±0.19	1.15±0.21
mean heat production (µW/g)	520±207	293±62	700±238	325±141
95%-α-confidence-interval	367-673	250-336	510-890	227-423

The increase of the metabolic rate after placing the *UCAs* into 33% and 166% SW (experiment B) was not proportional to time. After large increase on the first day in 33% SW the heat production decreased until the third day. On the 4th day the heat production increased again up to the level of the first day. After a small decrease at the first day an increase of nearly the same amount followed in 166% SW. A high increase was observed at the third day in this concentration.

One of the main problems in calorimetric measurements with intact animals is unambiguously to distinguish between the heat production caused by locomotion and the heat production af the basal metabolic reactions. The measured values for heat production during five days adaptation to 33 and 166% SW were splitted by using empiric quartiles (HODGES, KRECH and CRUTCHFIELD, 1975). This method was used to convert the high amount of data into tendenzy-diagrams over the time. We also hoped that the produced diagrams would provide some indication of a possible circadiane rhyth .

The values of the "low" and "high" classes are represented as means for all animals for each day in Fig. 2, 3 and 4. The mean of minima and maxima are shown

too. The means of "low" and minima can possibly be interpreted as the basal metabolic rate, the means of "high" and maxima as heat production caused by locomotion and basal metabolic rate. The high increase of heat production during adaptation to 166% SW is in correspondence to our observations of high locomotive activity in experiment A.



F ig.1: Heat production as a function of sea water (SW) salt concentration at 20°C after one week adaptation to concentrations as indicated.

The small difference between the graphs of minima to "low" and maxima to "high" supports our expectations that minima and "low" represent the metabolic baseline and maxima and "high" can be regarded as indicators for the locomotive avtivity. With regard to "basal metabolism" we calculated the following values: a) 33% SW: 137µW/g; b) 100% SW: 65µW/g and c) 166% SW: 254µW/g.

As shown in Fig. 2 and 4 an increase of the metabolic rate can be observed when the animals are adapted to low and high salt concentrations even if one considers only the "low" values which may be accepted as representing the basal metabolic rate. One of the puzzeles of our investigation is whether this elevated basal metabolic rate represents the activation of transport processes. This does not seem to be the case. POTTS (1954) evaluated which part of the total

energy consumed by an animal is used for osmotic work. In the crayfish *POTAMOBIUS FLUVIATILUS*, the crab *ERIOCHEIR SINENSIS* and the lamellibranch *ANODONTA CYGNEA* the increase of total energy is up to 13% in fresh water. Using his nomograms we calculate for *UCA PUGILATOR* in 33% and 166% SW an augmentation of energy of 5 and 10%.

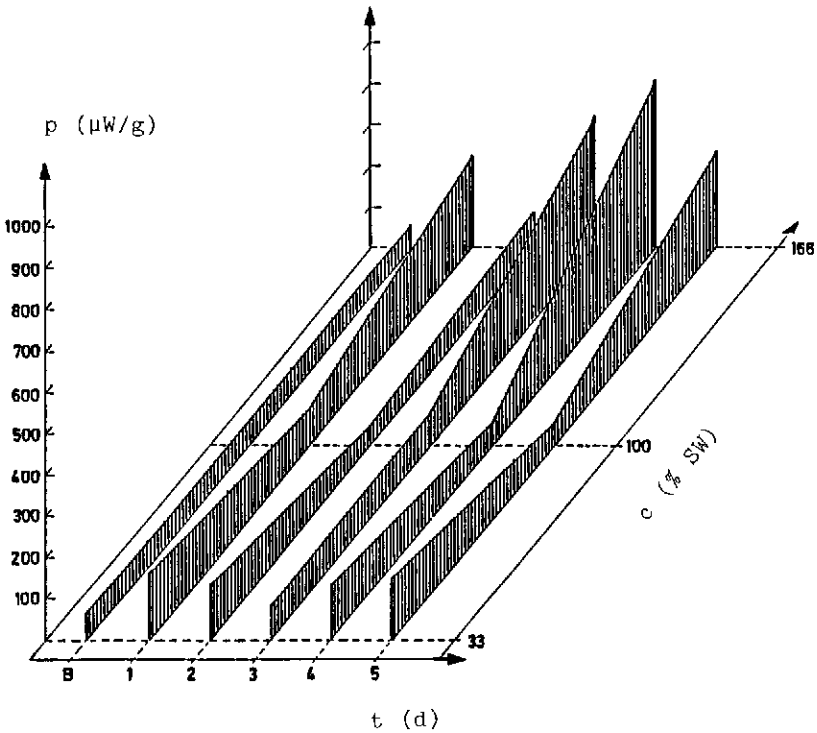


Fig.2: Relationship between different sea water (SW) salt concentrations and "low" heat production during and adaptation period of five days. B is basal value of heat production under 100% SW condition.

Even if our experimental conditions differ from those partly theoretical conditions of POTTS (1954) most of the values calculated by him seem to be to small to be observed in our investigation. Therefore, we conclude that the increase of the basal metabolic rate represents a quite complex adaptational answer of the animal composed of elevated transport rates, augmented synthetic activity and increased readiness in general.

The standard deviation was not calculated. We can assume a high variance of the estimate as consequence of the small sample size. In all experiments we couldn't detect any evidence for the existence of rhythmic metabolic activity or anaerob metabolic phases.

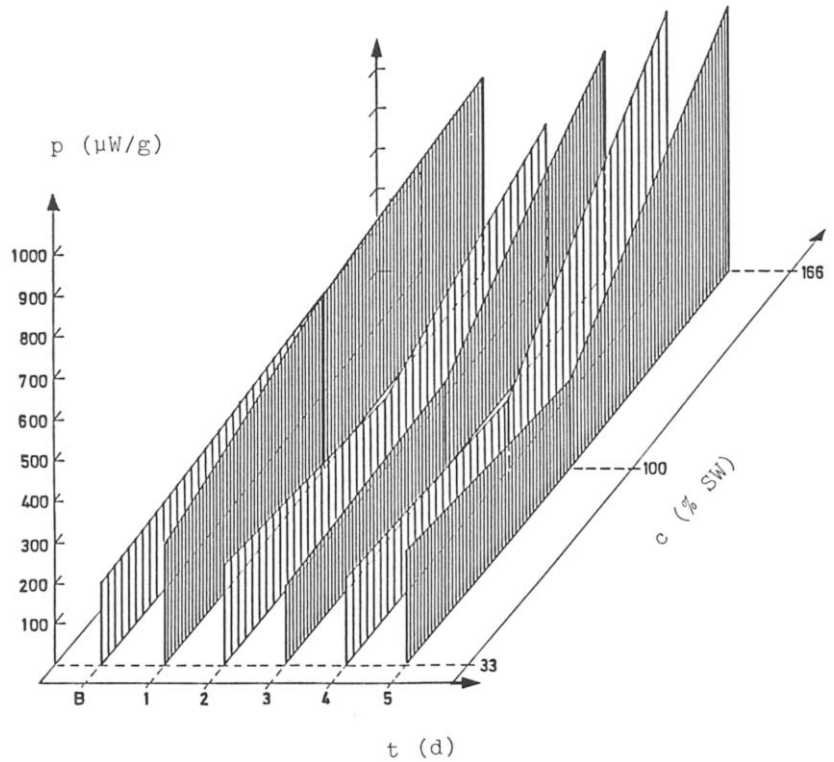
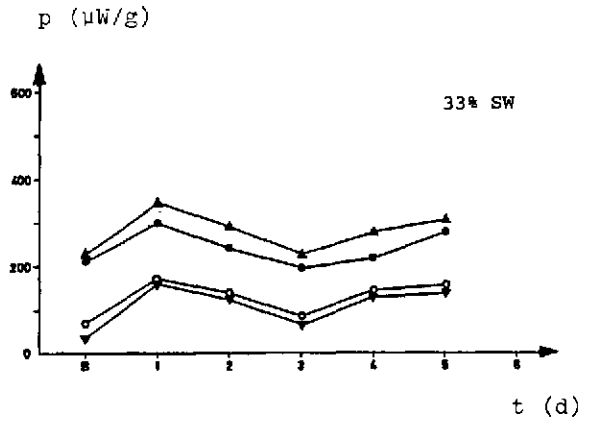


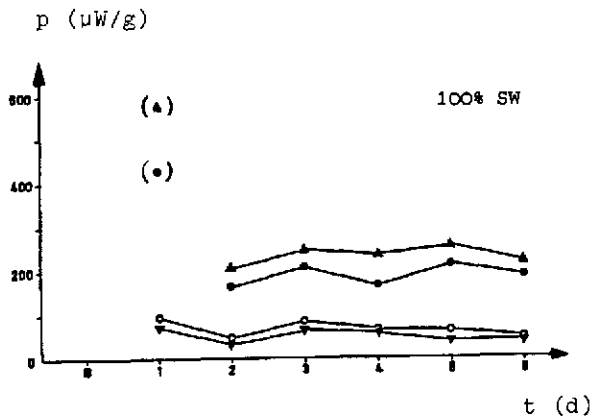
Fig.3: Relationship between different sea water (SW) salt concentrations and "high" heat production during an adaptation period of five days. B is the basal value of the heat production at 100% SW.

Experiment C, the measurement of the oxygen consumption of eight female fiddler crabs gave a result of 73±20µlO_2/g at 25°C. MACINTOSH (1978) found for some 1g heavy *UCA* species at 25°C the following values for oxygen consumption: 140µlO_2/gh (*DUSSUMIERI*), 122µlO_2/gh (*ROSEA*), 100µlO_2/gh (*FORCIPATA*), 68µlO_2/gh (*TRIANGUALRIS*). VERNBERG, GURAM and SAVORY (1978) observed an oxygen consumption of 95±14µlO_2/gh at 20°C after 14 days adaptation to constant temperature of *UCA PUGILATOR*.

The conversion of respiratory values to energy values was performed with the assumption that during the oxidation of one mol glucose 2872kJ (LEHNINGER 1975) are dissipated.

DAME and VERNBERG (1978) reported for *UCA PUGILATOR* in the temperature range 15 to 25°C a Q_{10} ranging from 1.2 to 1.9. If we correct our results of experiment C (oxygen consumption at 25°C, 433±188µW/g) with an estimated mean Q_{10} of 1.55, we'll expect 325±144µW/g (Tab.1) at 20°C. In Fig.1 we can see, that this result is in good agreement with our calorimetric results.





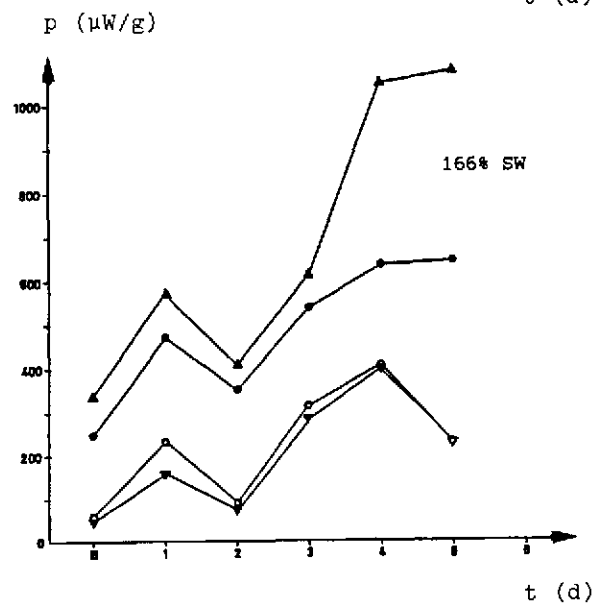


Fig. 4 : Relationship between three different Sea Water (SW) salt concentration and heat production during an adaptation period of five days. B is the base value of the heat production under 100% SW condition.

▲ Mean of MAXIMA
● Mean of "high"
o Mean of "low"
▼ Mean of MINIMA

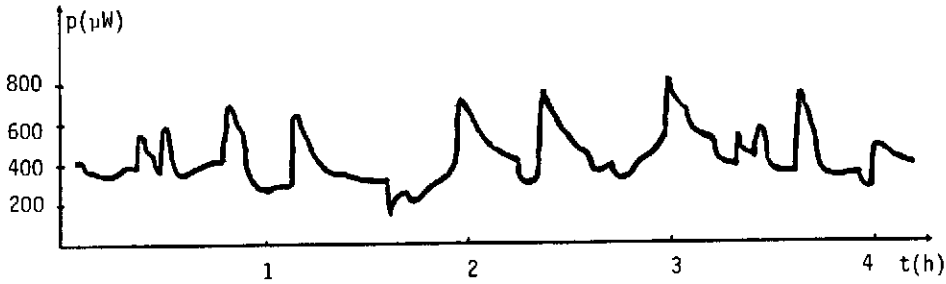


Fig.5: Typical power-time-curve of *Uca pugilator* of about 4h in 166% SW. The peaks indicate phases of raised locomotive activity.

SUMMARY

The heat production of *Uca pugilator* in Sea Water (SW) was measured with a batch calorimeter before, during and after adaptation to 33,100 and 166% SW. The heat production rate after adaptation was up to 100% higher in SW concentrations differing from "normal". The increase in heat production was not proportional to the time. Shortly after exposition to hyper- or hypotone media the crabs' basal metabolic rate ("low", Fig.3) was raised. The locomotive activity was specially raised in 166% SW. The increase in the observed heat production interpreted as basal metabolic rate was about 400%. The results from the comparison experiment, measurement of oxygen consumption, concurred adequate.

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