Lactate dehydrogenase during the larval development of *Cancer irroratus*: Effect of constant and cyclic thermal regimes[1]

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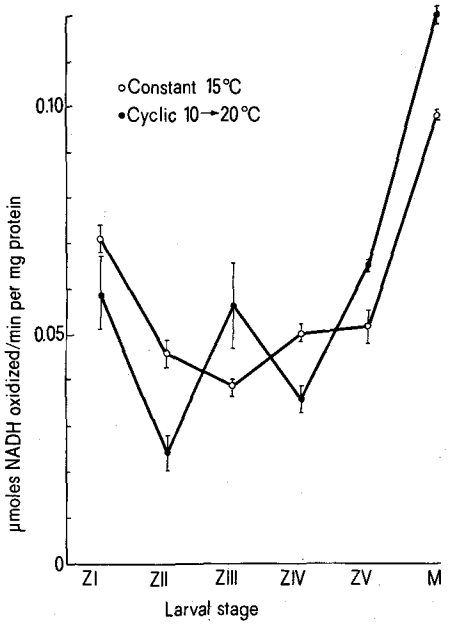
Summary. The activity of the key glycolytic enzyme, lactate dehydrogenase in the larval stages of *Cancer irroratus* was differentially affected by the daily cyclic and constant temperatures. Enzyme activity was significantly enhanced in the fifth zoeal and megalops stages cultured under the cyclic regime.

Pelagic larvae of marine invertebrates experience diurnal, spatial and temporal changes in temperature during their pelagic existence. Development of reliable laboratory techniques for culture of invertebrate larvae has led to recent studies on the effects of varying temperatures on larval development, survival and metabolism. Larvae of the brachyuran crab, *Cancer irroratus*, have been cultured under both constant and cyclic temperatures. Larvae cultured under cyclic thermal regimes show higher survival throughout larval development, altered developmental rates, and enhanced tolerance to exposure to thermal extremes[3]. In addition, metabolic-temperature studies indicate that respiratory metabolism in *C. irroratus* larvae is enhanced when cultured under cyclic thermal conditions[4]. Rate/temperature curves of oxygen consumption of *C. irroratus* larvae show zones of thermal insensitivity which are extended in the upper range in larvae cultured under cyclic temperature regimes[4]. It is clear that a variety of physiological processes are affected by these alterations in thermal conditions. In this communication, we report changes in the activity of the key glycolytic enzyme, lactate dehydrogenase (EC 1.1.1.27), in *C. irroratus* cultured under cyclic and constant temperatures.

Activities of lactate dehydrogenase determined from crude cell-free homogenates of *Cancer irroratus* larvae cultured under cyclic and constant temperatures. Activity is expressed in μmoles NADH oxidized/min mg Lowry protein. Assay temperature was 15 °C

State	Constant 15 °C	Cyclic 10–20 °C	Cyclic/constant
Zoea-I	71.2	58.6	0.82
Zoea-II	46.1	24.5	0.53
Zoea-III	38.8	56.4	1.45
Zoea-IV	50.2	35.8	0.71
Zoea-V	51.6	103.7	2.01
Megalopa	95.1	119.7	1.26



Lactate dehydrogenase activity in *C. irroratus* larvae cultured under cyclic and constant thermal regimes I–V, zoeal stages; M, megalopa.

Egg masses were removed from ovigerous female *C. irroratus* collected in Narragansett Bay, Rhode Island. Newly hatched zoeae were cultured at constant 15 °C and at a 10–20 °C diurnal cycle as previously described[3]. Lactate dehydrogenase activity was determined in crude cell-free homogenates prepared from pooled larva samples from the zoeal and the megalops stages. The activities of lactate dehydrogenase during the larval development of *C. irroratus* are depicted in the figure. The activity patterns for both experimental groups are essentially U-shaped with highest enzyme activities in the beginning and the end of larval development. A U-shaped pattern in the activities of a number of glycolytic enzymes was observed during the larval development of the fruit fly, *Drosophila pseudoobscura*[5]. The activity of glutamic oxaloacetic transaminase in the crab, *Rithropanopeus harisii*, shows a U-shaped pattern during larval development. The changes in lactate dehydrogenase activity in the present study probably reflect changes in overall glycolytic activity during *C. irroratus* development.

Thermal conditions have an influence on the activity of lactate dehydrogenase in *C. irroratus* larvae (table). Enzyme activities in larvae cultured under the cyclic thermal regime are substantially higher at the later 2 larval stages. This enhanced enzyme activity is paralleled by elevated oxygen consumption in the last 3 stages of larval development in animals cultured under the cyclic regime[4]. It is likely that the elevation of lactate dehydrogenase activity in this species is mediated by increased quantities of a pre-existing isoenzyme, as zoeal lactate dehydrogenase is electrophoretically monomorphic on starch gel[7] indicating the absence of a complex isoenzymic system. Enzymes of

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7 Horizontal starch gel electrophoresis of tissue extracts prepared from individual *C. irroratus* zoea.

the hexose monophosphate shunt show quantitative rather than qualitative changes during thermal acclimation in the blue crab, *Callinectes spaidus*[8]. It is of interest that the muscle and hepatopancreas of the crayfish, *Cambarus bartoni*, respond differentially to thermal acclimation. Lactate dehydrogenase activity increases in muscle but decreases in the hepatopancreas following cold acclimation[9].

The changes in lactate dehydrogenase activity in *C. irroratus* larvae indicate that metabolic rate compensation is not directed at the mean temperature of the cycle (15 °C). If this were the case, enzyme activities and respiratory rates measured at 15 °C should be very similar in constant and cyclic temperature animals. Since animals cultured under a cyclic thermal regime are subjected to both a diurnal high and low temperature, adjustment may be cued to exposure to 1 particular thermal extreme. A uni-directional extension of the zone of thermal insensitivity of respiration was observed in *C. irroratus* cultured under cyclic thermal conditions[4]. The ontogeny of metabolic rate adjustment may be demonstrated by the observation that the divergence of lactate dehydrogenase activity did not occur until later in development. Lactic acid production has been demonstrated to be an important feature of metabolism in crab tissues[10]. The elevated lactate dehydrogenase activity observed in *C. irroratus* larvae cultured under cyclic temperatures indicates increased capacity for producing and dealing with lactic acid.

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Fractionation of mouse DNA by precipitation with F1 histone into fragments differing in their base composition[1]

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Summary. Several fractions of mouse DNA were obtained by gradual precipitation with histone F1. The analysis of their base composition revealed that histone interacted selectively with sequences of DNA rich in adenine plus thymine, regardless of the type of DNA molecules present in the DNA solution to be fractionated.

According to Šponar and Šormová[2], gradual dialysis of DNA and histone F1 solution from a high ionic strength to 0.15 M NaCl at neutral pH is accompanied by a selective interaction of histone with sequences of native DNA rich in adenine plus thymine. The effect was observed in artificial mixtures of bacterial DNAs differing in their base composition as well as in calf thymus DNA. In the latter case, a gradual accumulation of the GC-rich satellite DNA was noted in the supernatants obtained after centrifugation of the histone-DNA complexes. However, Płucienniczak et al.[3] found subsequently that DNA sequences precipitated by F1 within the range of 5–50% of the weight of the initial DNA, and presumably corresponding to the main calf thymus DNA, do not undergo fractionation despite their heterogeneity in base composition. This observation led to the assumption that main band DNA molecules of calf thymus DNA obtained by density gradient centrifugation regardless of their base composition, differ from some GC-rich fractions, possibly those of satellite DNA, in respect of the amount of specific areas to which the F1 histone attaches in the experimental conditions applied.

It was the aim of our work to investigate the course of fractionation of some other type of mammalian DNA by gradual precipitation with successive doses of F1. For this purpose the mouse DNA was chosen because the base

1 Acknowledgments. This research was supported by the Polish Government Grant No. PRBR-1317/13.
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Precipitation of histone F1-DNA complexes in 0.15 M NaCl and base composition of obtained DNA fractions

DNA fraction	1	2	3	4	5	6
Complex I	100	0.1	0.11 ± 0.02	11.4 ± 1.2	1.76 ± 0.09	1.79 ± 0.05
Complex II	80.0 ± 2.0	0.1	0.17 ± 0.02	15.0 ± 1.5	1.60 ± 0.09	1.62 ± 0.05
Complex III	58.0 ± 2.6	0.1	0.19 ± 0.02	13.9 ± 1.0	1.46 ± 0.09	1.51 ± 0.06
Complex IV	43.3 ± 4.2	0.2	0.41 ± 0.02	24.5 ± 0.7	1.35 ± 0.07	1.39 ± 0.05
Final supernatant	21.5 ± 2.0	–	0.59 ± 0.02	35.2 ± 2.9	1.24 ± 0.08	1.24 ± 0.06
Whole DNA	–	–	–	–	1.40	1.44

The results reported in columns 1, 3, 4, 5 und 6 are mean values from 3 experiments ± SD. Column 1: concentration of DNA solution before dialysis (μg/ml); column 2: weight ratio of F1-DNA before dialysis; column 3: the fraction of DNA in the precipitated complex, calculated at each step of the fractionation course from the content of DNA in the solution before dialysis and in the corresponding supernatant; column 4: the recovery of DNA in the precipitated complex expressed as the percentage of total DNA present in the initial solution to be fractionated; column 5: composition of 4 main bases in M% expressed as A + T/G + C coefficient; column 6: relative value of adenine and guanine content, expressed as A/G coefficient, measured by the distribution of radioactivity in purine bases of DNA prelabeled with sodium-C^{14}-formate by growing the L5178Y cells in vitro in the presence of this precursor.