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The Effect of Temperature and Thermal Distribution on Glycolysis in Two Rockfish Species *(Sebastes)*

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Abstract

Studies on the effects of temperature on the activities of Embden-Meyerhof (EM) glycolysis and the hexose monophosphate shunt (HMP) in fishes have dealt mainly with exotic and/or acclimated fishes. This study reports the effects of short-term reductions in temperature on EM and HMP activity in two closely related species of temperate fishes (*Sebastes* spp.) and its possible relation to the thermal distribution of the species. Thermal distribution data were collected by SCUBA for *S. mystinus* and *S. serranoides* in King Harbor, Redondo Beach, California, USA. Activities of the pathways were determined in liver-tissue studies, using glucose-^{14}C and liquid scintillation techniques following the method of Hochachka (1968) with modifications. The data were analyzed by distribution-free methods. Tissue studies indicated HMP activity in both species at lowered temperature (5°C), but only in *S. serranoides* at 15°C. Results indicate that *S. mystinus* is capable of instantaneous temperature compensation, possibly related to its tendency to occupy cold waters.

Introduction

The pathways of glucose metabolism and their activities are well known in most higher vertebrates. In fishes, while the pathways themselves are well defined, their activity and control, especially by environmental parameters, are less clear. Much of the work in this area has concentrated on the activities of the Embden-Meyerhof (EM) pathway and the hexose monophosphate shunt (HMP).

Brown (1960) discovered that the HMP is slightly active in the carp *Salvelinus fontinalis* but does not make a major contribution to glucose oxidation. Hochachka and Hayes (1962) demonstrated the activation of the HMP under conditions of "cold-acclimation" in the trout. Furthermore, in studies using three species of air-breathing, South American fishes, Hochachka (1968) also found that at elevated, non-acclimated temperatures the activity of the HMP increases.

Most of the work concerning the effect of temperature on the pathways of glucose oxidation has been performed with "acclimated" fishes, while relatively little has dealt with the short-term response of fishes to non-acclimatization temperatures[1]. The response of poikilothermic organisms to these two thermal regimes is believed to be different in both the regulatory strategy and nature of the response. A review of the field is given by Kinne (1975).

This study was undertaken to determine whether or not liver tissues of two closely related, temperate species of fish with different thermal distributions display specific, temperature-related differences in the oxidation of α-D-glucose.

Materials and Methods

Two species of rockfish (*Sebastes* spp.) were used in this study. The blue rockfish *S. mystinus* is found commonly from

[1] Although for the purposes of this study the terms acclimation and acclimatization are synonomous, the former is technically inappropriate to describe the state of the fishes used in the experiments reported here (Fry, 1967). Consequently, acclimatization will be used unless reference to published work requires acclimation; in which case the term will be enclosed in quotation marks.

Point Santo Tomas, Baja California, to the Bering Sea in depths ranging to 92 m (Miller and Lea, 1972). This species is primarily a plankton feeder which supplements its diet with plants and small fish (Miller and Geibel, 1973). The specimens of *S. mystinus* used in this study ranged in age from AG 0-AG 4, based on otoliths taken from sacrificed specimens. The AG designation identifies the age group to which the specimens belong and corresponds approximately to the age of an animal in years.

The olive rockfish *Sebastes serranoides* is common between San Benito Islands, Baja California and Redding Rock, Del Norte County, California. This species is found to depths of 155 m (Miller and Lea, 1972). *S. serranoides* feeds mainly on planktonic organisms and small fish (M. Love, personal communication). Specimens of *S. serranoides* used in this study ranged in age from AG 2-AG 5.

Data used for compiling temperature distributions of the two species were gathered over the 9-month period June, 1974 - February, 1975 by SCUBA. Sampling was done in King Harbor Marina, Redondo Beach, California at a station established by Dr. J.S. Stephens, Jr. of Occidental College. A single sample was defined as one transect, run in a straight line, at constant depth, for a timed 5-min interval. Samples were taken at depth intervals of 3 m. Sampling was begun at a depth of about 1.5 m and continued to the deepest available substrate. During transect runs, divers recorded the number of fish of each species as well as the water temperature at the beginning and end of each transect.

For use in experiments, individuals of both species were collected with gill nets. These nets were set and tended by divers who remained with the nets to remove specimens before they became irretrievably gilled. Upon return to the laboratory the fishes were held in salt-water (33 to 34‰) aquaria at 15°C±2C° for not more than 3 days before being used in experiments. During this time the fishes were not fed, and were maintained in an 8 h light:16 h dark photoperiod.

The pathways of glucose oxidation in liver samples from the two species were determined by the collection of respired $^{14}CO_2$ from oxidized glucose-1-^{14}C and glucose-6-^{14}C. Since in the HMP, CO_2 is produced exclusively from the C-1 position of 6-phosphogluconate, glucose-1-^{14}C which enters the HMP will generate $^{14}CO_2$ production while glucose-6-^{14}C will not. Alternatively, if labelled glucose, either glucose-1-^{14}C or glucose-6-^{14}C, enters the EM pathway it will result in the production of $^{14}CO_2$. This is due to the conversion of glucose to identical triose molecules in the glycolytic pathway. A full description of this method is given by Katz and Wood (1960). Their work showed that, due to the effects of recycling, this technique is quantitatively unreliable for determining the relative contributions of the HMP and EM pathways to overall glucose metabolism. The method is qualitatively acceptable, however, as an indicator of the activity or inactivity of the pathways in question. The method assumes that under normal metabolic conditions equal rates of $^{14}CO_2$ production should be obtained from the oxidation of both glucose-1-^{14}C (G1-^{14}C) and glucose-6-^{14}C (G6-^{14}C). Consequently, a ratio of the two rates should approximate unity. Deviations from unity can then be taken to indicate HMP activity.

The experimental procedure followed is essentially that of Hochachka (1968), but with modifications of the $^{14}CO_2$ collection system and tissue preparation. Tissue studies were carried out at two temperatures, 15° and 5°C. The first temperature represents the acclimatization temperature for the two rockfish species. The latter is a temperature which neither species encounters in nature. The $^{14}CO_2$ collection system was based on a system described by Dr. K. Bever of the University of Southern California (personal communication). A small air compressor was used to maintain constant pressure in a compressed-air storage cylinder. The compressed air was passed through a mixture of ascarite-drierite to remove CO_2 and water. The CO_2-free, dry air then passed through a Gilmont flowmeter and into a series of 6 needle valves of the type used for aquaria. Air passed from the valves through a length of surgical tubing (3 mm inner diameter) attached to a disposable hypodermic needle which penetrated a No. 2 rubber stopper and acted as the primary inlet to a 50-ml Erlenmeyer flask. The stopper was used to seal the flask. A second needle was used as the outlet through the stopper and was connected to an identical, secondary, arrangement in a 10-ml glass vial using a No. 000 rubber stopper. This vial contained 1 ml of hyamine hydroxide and a round, glass-fiber filter. This particular system was designed to accommodate 6 reaction flasks.

Flow rates in the reaction flasks, based on the rate from the flowmeter, were computed to be 65 ± 5 cm^3/min/flask. This rate represents a complete turnover of the atmosphere in the flasks approximately every 45 sec. The flasks were placed on a shaker inside a freezer modified to serve as a controlled tempera-

Table 1. Composition of marine teleost saline for tissue incubation

Salt	Concentration (mM)
NaCl	157.0
KCl	147.0
$MgCl \cdot 6\ H_2O$	1.5
$NaHCO_3$	5.0
$CaCl_2$	3.0
$NaHPO_4$	1.0
EDTA	1.0

ture chamber. Chamber temperature was thermostatically regulated by a YSI Thermistemp Model 71A temperature controller and periodically monitored using a YSI telethermometer model 44TD. Air lines and wiring passed through a small hole in the side of the chamber.

The tissue preparation was a mince. Livers removed from specimens were weighed and placed in ice-cold salt solution. It was necessary to use a composite of liver samples for any one flask since the liver size per fish was usually small. Consequently, each reaction flask contained liver tissue from more than one fish. Efforts were made to minimize differences between flasks as well as to keep sample sizes close to 0.5 g. Tissues were kept at ice-bath temperature whenever possible during preparation.

Since the animals used were marine fishes it was necessary to prepare an appropriate saline solution for use as the incubation medium and to keep excised livers cold. The solution was prepared according to the blood electrolyte composition of the kelp bass *Paralabrax clathratus* (Holmes and Donaldson, 1969). This species is sympatric with both of the rockfish species. The composition of the incubation medium is given in Table 1. EDTA (ethylenediaminetetraacetic acid) was added as a chelating agent and was necessary for the dissolution of the salts. The final solution was buffered at pH 7.2 using a 0.05 M sodium phosphate buffer.

Flasks were run in triplicate. Incubations at each temperature, 15° and 5°C, were carried out for 3 h after which time the preparation was killed by the addition of 5 ml of 10% trichloroacetic acid solution, and the system allowed to clear for 30 min. The glass-fiber filters were then removed from the vials and their radioactivity determined by a Beckman liquid scintillation counter, Model LS-100C.

Data analysis was accomplished on an IBM 370/125 computer using SPSS and ITF BASIC facilities. SPSS subprogram BREAKDOWN was used to analyze diver transect data. Output from the scintillation counter was fed into programs written in ITF BASIC to make appropriate background corrections and to standardize results from tissue studies to a per gram basis. A second BASIC program performed the "Fisher Test of Difference Scores" (Bradley, 1968). This is a distribution-free statistical test, equivalent to a test of the null hypothesis that two populations have either symmetric shapes and a common axis of symmetry or are identical.

Comparison of the temperature distributions was accomplished using the chi-square test for homogeneity (Blum and Rosenblatt, 1972). All two-tailed statistical tests were performed at the 0.05 α-level, i.e., for $\alpha \leq 0.05$ the null hypothesis was rejected. In the statistical analysis each flask represented a single sample.

Results

The mnemonics used in the presentation and discussion of experimental data are as follows ("Species" represents the source of the liver tissue while "Substrate" indicates the type of labelled glucose present in the incubation medium):

Mnemonic	*Sebastes* species	Substrate
MG1-^{14}C	*S. mystinus*	G1-^{14}C
MG6-^{14}C	*S. mystinus*	G6-^{14}C
SG1-^{14}C	*S. serranoides*	G1-^{14}C
SG6-^{14}C	*S. serranoides*	G6-^{14}C

Data from the SPSS compilation of diver records are represented by the relative frequency distributions of Figs. 1 and 2. Sample sizes are large and equal for both species (n=79). Interspecific comparison of the relative frequency distributions (juveniles and subadults) shows a significant difference between the two species ($\alpha \leq 0.01$). Subadults differ in their distribution from juveniles of the same species in both species ($\alpha \leq 0.005$). Interspecific comparison of the subadult distributions also indicates a significant difference ($\alpha \leq 0.005$).

Both species occur throughout the range of temperatures sampled, but *Sebastes serranoides* juveniles are more uniformly distributed than are juvenile *S. mysintus,* although not significantly so. The latter species tends to occupy colder waters. Interestingly, just the opposite pattern occurs in subadults of both

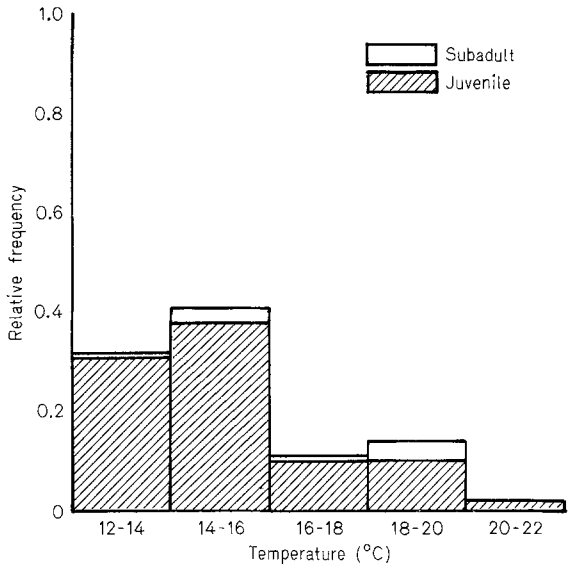

Fig. 1. *Sebastes mystinus*. Thermal distribution (N=79)

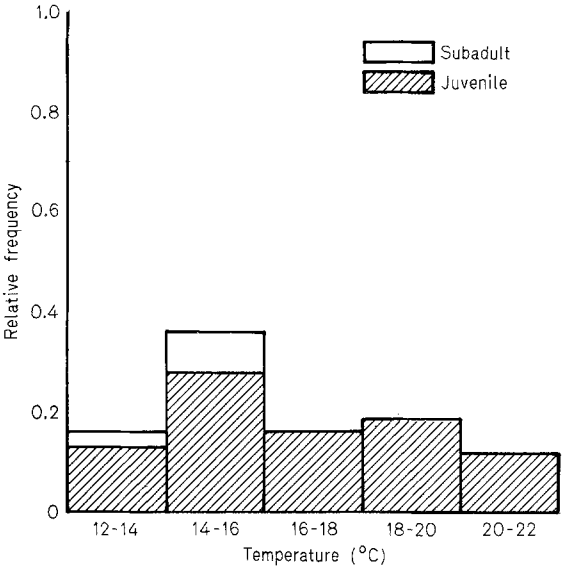

Fig. 2. *Sebastes serranoides*. Thermal distribution (N=79)

Table 2. *Sebastes mystinus*. Corrected scintillation output from oxidation of G6-^{14}C and G1-^{14}C at 15° and 5°C by minced liver

Date (1975)	G6-^{14}C (cpm/g)	G1-^{14}C (cpm/g)
15°C		
25 Jan.	981.77	1601.49
	1592.71	717.47
24 Feb.	678.82	2021.88
	516.94	1111.24
25 April	905.14	1013.44
	957.64	1413.58
$\bar{x}$	858.59	1411.20
s	388.16	434.37
5°C		
3 April	916.33	3105.27
	463.00	1516.96
	402.89	3522.00
9 April	456.88	841.83
	374.21	1060.94
	519.16	1198.65
4 June	117.01	214.61
	67.95	248.13
	112.48	270.88
$\bar{x}$	378.19	1320.19
s	267.72	1228.51

Table 3. *Sebastes serranoides*. Corrected scintillation output from oxidation of G6-^{14}C and G1-^{14}C at 15° and 5°C by minced liver

Date (1975)	G6-^{14}C (cpm/g)	G1-^{14}C (cpm/g)
15°C		
31 Jan	274.41	523.94
	368.20	813.34
	855.15	776.48
2 Mar	132.54	2745.48
	195.50	2962.07
	190.77	1832.77
22 Mar	265.51	2268.00
	334.90	1740.87
$\bar{x}$	327.12	1707.87
s	227.07	929.63
5°C		
23 Mar	180.77	812.83
	180.47	606.66
	254.98	605.90
10 April	97.18	463.08
	175.03	364.50
	132.11	1067.69
29 May	122.50	716.45
	319.58	400.66
	107.61	210.93
$\bar{x}$	174.48	583.19
s	72.94	259.91

Table 4. *Sebastes mystinus* (MG) and *S. serranoides* (SG). Matrix of α-values and ratios resulting from comparison of $^{14}CO_2$ production at 15°C (2-tailed values)[a]

	MG6-^{14}C	MG1-^{14}C	SG6-^{14}C	SG1-^{14}C
MG6-^{14}C	1.0	0.078	0.024	
	1.0	0.61	2.63	
MG1-^{14}C	0.078	1.0		0.483
	1.64	1.0		0.83
SG6-^{14}C	0.024		1.0	0.016
	0.38		1.0	0.19
SG1-^{14}C		0.438	0.016	1.0
		1.21	5.26	1.0

[a]Lower numeral is ratio value; upper is α-value. In computing ratios, numerator corresponds to the ordinate value of the matrix while the denominator is on the abscissa.

Table 5. *Sebastes mystinus* (MG) and *S. serranoides* (SG). Matrix of α-values and ratios resulting from comparison of $^{14}CO_2$ production at 5°C (2-tailed values)

	MG6-^{14}C	MG1-^{14}C	SG6-^{14}C	SG1-^{14}C
MG6-^{14}C	1.0	0.004	0.063	
	1.0	0.29	2.17	
MG1-^{14}C	0.004	1.0		0.084
	3.45	1.0		2.27
SG6-^{14}C	0.063		1.0	0.004
	0.46		1.0	0.30
SG1-^{14}C		0.084	0.004	1.0
		0.44	3.33	1.0

Table 6. Matrix of α-values and ratios resulting from comparison of $^{14}CO_2$ production at 15° and 5°C (2-tailed values)

	MG6-^{14}C	MG1-^{14}C	SG6-^{14}C	SG1-^{14}C
MG6-^{14}C	0.031			
	0.48			
MG1-^{14}C		0.914		
		1.03		
SG6-^{14}C			0.008	
			0.56	
SG1-^{14}C				0.039
				0.37

species. Subadult *S. serranoides* occur only in colder waters while subadult *S. mystinus* occur throughout the range.

While these species are obviously not very different from each other in normal thermal ranges and, therefore, acclimatization, the differences in distribution may yet represent specific differences in thermal optima as described by Hochachka and Somero (1973). That is, the two species may metabolize "normally" at different temperatures within their partially overlapping ranges. This has been shown to be true in two other members of the rockfish family by Wilson *et al.* (1974). The distributional data from the present study are not conclusive in this regard. Other factors, such as inter- and intra-specific competition may also be of importance but are not considered in this paper.

The data from the tissue studies indicate that temperature does, however, affect the species differently. Tables 2 and 3 list, in chronological order, the computer output of corrected scintillation data along with means and standard deviations for each series. Tables 4 and 5 present the results of the Fisher test of the data as well as ratios which indicate the direction of differences where they occur. These results indicate that at 15°C *Sebastes mystinus* is not metabolizing glucose via the HMP, although $^{14}CO_2$ is produced at a greater rate from G1-^{14}C than from G6-^{14}C. *S. serranoides*, however, is metabolizing glucose to a significant extent via the HMP. Interspecifically, substrate-rate comparisons indicate that species differences in HMP activity are due to the lower rate of G6-^{14}C metabolism by *S. serranoides*. At 5°C, rates of G6-^{14}C and G1-^{14}C metabolism are not different between species, but in both cases *S. serranoides* is metabolizing glucose at a lower rate. Of considerable interest is the fact that at 5°C HMP activity is significant in *S. mystinus* as well as in *S. serranoides*. From Table 6, which compares output rates at 15° and 5°C, it can be seen that the apparent change in *Sebastes mystinus* is due to the decreased output of $^{14}CO_2$ from G6-^{14}C at 5°C. In *S. serranoides* there is no difference between substrate rates, although both are lower at 5°C indicating a general reduction in metabolic rate with temperature. This may be the cause of the reduced rate obtained from MG6-^{14}C at 5°C. In contrast is the uniform rate obtained from MG1-^{14}C regardless of temperature. This last datum indicates that *S. mystinus* liver tissue is capable of instantaneous thermal compensation.

Discussion and Conclusions

The reduction of EM glycolysis in *Sebastes mystinus* at 5°C may reflect a reduced need for ATP or other metabolites produced by this pathway consistent with a reduction in metabolism at low temperature. Hochachka and Somero (1973) postulated seasonal metabolic reorganization under conditions of changing "acclimation" temperature. By this hypothesis reorganization occurs in response to changing metabolic requirements. Under conditions of lowered temperature, in poikilotherms, a reduction in metabolic activity can occur as a result of imperfect cold-compensation thereby allowing the normal flow of carbon to be diverted to pathways used for growth and energy storage. For example, the HMP is generally believed to provide reducing power, as nicotinamide adenine dinucleotide phosphate (NADPH), for fatty acid synthesis and other biological reductions. This pathway is also used to generate nucleotides which form nucleic acids and play a major role in protein synthesis. The activity of this pathway at lowered "acclimation" temperatures (Hochachka and Hayes, 1962; Hochachka and Somero, 1973) is consistent with the concept of reduced metabolic energy requirements at lower temperatures. Under these conditions carbon flow can be diverted from direct, ATP-generating, EM glycolysis into pathways such as HMP which generate stored energy as triacylglycerols (fats).

During the experiments it was observed that both species had distinct deposits of white fat along the intestine and caecum. On inspection it appeared that the quantities of fat were much greater in *Sebastes serranoides* than in *S. mystinus*. The deposits in *S. serranoides* often filled the body cavity and were usually present along the loop of the intestine. In *S. mystinus* deposits occurred as small, spherical globules on the caecum and along the intestine. Since specimens collected later in the year showed considerably more fat, it may be that the globules represented initial deposition of storage fat.

In vertebrates, fatty acids synthesized in the liver may be converted to triacylglycerols at the depots (Bell *et al.*, 1965). These deposits were more extensive in *Sebastes serranoides* than in *S. mystinus*. In addition, it was found that the HMP was active in *S. serranoides* liver at both 15° and 5°C. These results indicate, relative to *S. mystinus*, an earlier, perhaps seasonal, shift or reorganization of glucose metabolism in *S. serranoides* directing carbon flow into fat synthesis. The earlier shift may be a specific difference since it was not observed in *S. mystinus* at 15°C. Such a specific difference might be attributable to the tendency of *S. mystinus* to occupy colder waters than *S. serranoides*. The difference may reflect a difference in acclimatization temperature and/or thermal sensitivity.

As described previously, acclimatization to a particular thermal regime may cause extensive metabolic reorganization to optimize metabolic function. A differential sensitivity to short-term reductions in temperature might be allied with such changes in acclimatization. In this case it appears that the lower acclimatization temperature of *Sebastes mystinus* enables this species to exploit short-term reductions in temperature by rerouting glucose through the HMP.

Work by Wilson *et al.* (1974) with other rockfish species, *Sebastes miniatus* and *S. auriculatus*, are consistent with such a theory. The data are not, as noted in their paper, statistically reliable, however, and cannot be taken as supportive evidence.

The HMP is believed to proceed normally at a low level in fishes (Hochachka and Clayton-Hochachka, 1973). The activity of the HMP in *Sebastes serranoides* at 15°C, the acclimatization temperature of the species, suggests that this condition is not "normal". Since the HMP plays a role in fat synthesis, any seasonal pattern of fat deposition should be associated with a seasonal change in metabolic pathways. Such changes could be keyed to changes in water temperature associated with seasonal changes at temperate latitudes. Sensitivity to short-term changes, as displayed by *S. mystinus*, could act as a trigger for more extensive metabolic changes in preparation for stressful changes in the internal or external environment of the organism.

There is no information on the existence or pattern of seasonality of fat deposition in rockfish. These experiments were designed only to determine HMP activity or inactivity and the effect of short-term reductions in temperature on the pathway of glucose oxidation. The data obtained are inadequate to define the nature of seasonality, but do strongly suggest the existence of seasonal metabolic reorganization in the two species studied.

The statistical analysis produced some unexpected results which have bearing on the method of analysis of $^{14}CO_2$ ratio data. The use of the G6-^{14}C/G1-^{14}C has often been used as an indicator of HMP activity as described in the "Materials and Methods" section. In comparing the results of the Fisher test, which has an asymptotic relative efficiency

(A.R.E.) of 1 compared to the Student *t*-test under normal conditions (Bradley, 1968), it was found that the ratio described is inadequate to distinguish a significant difference when the ratio is near 0.5. This is apparent from Table 4, in the comparison of MG6-^{14}C and MG1-^{14}C. In this case, from a subjective evaluation, the ratio, 0.61, might be taken to indicate a difference in the activity of the EM and HMP pathways. The Fisher test clearly indicates that at the 0.05 α-level the means are not significantly different. A similar result is observed for the 5°C comparison of SG6-^{14}C and MG1-^{14}C. This latter example is not an intraspecific comparison, and therefore serves only to aid in illustrating the imprecision of the ratio method as a means by which to differentiate mean $^{14}CO_2$ output when multiple samples are used. Although the Fisher test is limited when sample sizes are small ($n < 6$) it appears to be considerably more reliable for large ($n \geq 6$) samples. The G6-^{14}C/G1-^{14}C ratio seems at least roughly adequate, however, when the values used to compute it are "sufficiently different", i.e., when the ratio is not close to 0.5.

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Literature Cited

Bradley, J.V.: Distribution-free statistical tests, 388 pp. Englewood Cliffs, N.J.: Prentice-Hall 1968

Bell, G.H., J.N. Davidson and H. Scarborough: Text-book of physiology and biochemistry, 1140 pp. Baltimore: Williams and Wilkins 1965

Blum, J.R. and J.I. Rosenblatt: Probability and statistics, 549 pp. Philadelphia: W.B. Saunders 1972

Brown, W.D.: Glucose metabolism in carp. J. cell. comp. Physiol. *55*, 81-85 (1960)

Fry, F.E.J.: Responses of vertebrate poikilotherms to temperature. *In*: Thermiobiology, pp 375-406. Ed. by A.H. Rose. New York: Academic Press 1967

Hochachka, P.W.: Action of temperature on the branch points of glucose metabolism. Comp. Biochem. Physiol. *25*, 107-118 (1968)

- and B. Clayton-Hochachka: Glucose-6-phosphate dehydrogenase and thermal acclimation in the mullet fish. Mar. Biol. *18*, 251-259 (1973)
- and F.R. Hayes: Effect of temperature acclimation on pathways of glucose metabolism in the trout. Can. J. Zool. *40*, 261-270 (1962)
- and G. Somero: Strategies of biochemical adaptation, 358 pp. Philadelphia: W.B. Saunders 1973

Holmes, W.N. and E.M. Donaldson: The body compartments and distribution of electrolytes. *In*: Fish physiology, Vol. 1. pp 1-89. Ed. by W.S. Hoar and D.J. Randall. New York: Academic Press 1969

Katz, J. and H.G. Wood: The use of glucose-^{14}C for the evaluation of pathways of glucose metabolism. J. biol. Chem. *235*, 2165-2177 (1960)

Kinne, O. (Ed.): Marine ecology. Vol. II. Physiological mechanisms, Pt 1. 449 pp. London: Wiley Interscience 1975

Miller, D.J. and J.J. Geibel: Summary of blue rockfish and lingcod life histories; a reef ecology study; and giant kelp, *Macrocystis pyrifera*, experiments in Monterey Bay, California. Fish Bull. Calif. *158*, 1-137 (1973)

- and R.N. Lea: Guide to the coastal marine fishes of California. Fish Bull. Calif. *157*, 1-235 (1972)

Wilson, F.R., G.N. Somero and C.L. Prosser: Temperature-metabolism relations of two species of *Sebastes* from different thermal environments. Comp. Biochem. Physiol. *47(B)*, 485-491 (1974)

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