Table 2. The weights of the bursa and body at hatching of EA and TP chicks (P_1), offspring from EA chicks (F_1), and offspring from reciprocal crosses of EA and TP chicks (F_1). The numbers in parentheses refer to the number of birds.

P_1		F_1					
		Hatch 1		Hatch 2		Hatch 3	
Bursa (mg)	Body (g)	Bursa (mg)	Body (g)	Bursa (mg)	Body (g)	Bursa (mg)	Body (g)
			Testosterone propionate				
19.3±10 (20)	44.2±4.8						
			Ethyl alcohol				
59.9±14.0 (20)	45.6±3.8	43.0±8.8 (96)	38.4±3.9	42.3±11.7 (54)	41.7±3.7	44.3±10.0 (79)	41.1±3.0
			TP males × EA females				
				42.2±14.4 (21)	38.6±2.2	41.9±10.0 (68)	41.0±2.5
			*EA males × TP females**				
		39±10.6 (39)	38.3±2.9	36.8±8.5 (39)	39.2±3.8	34.2±9.7 (39)	39.1±3.6

* Within hatches the bursa means of this group are significantly different from all other bursa means.

with EA females. No significant differences in F_1 bursa weights were found in chicks whose dams hatched from EA dipped eggs (Table 2). However, chicks produced by dams that hatched from TP dipped eggs (EA × TP) showed a significant reduction in bursa weight in all three hatchings.

The reduced size of the bursa in the F_1 and F_2 generations of both trials may have resulted from a gene or chromosomal change, or a change transmitted through the cytoplasm occurring in the P_1 chicks. The results of the reciprocal matings (Table 2) suggest that a cytoplasmic change had occurred. This change could be inherited through the cytoplasm, in which case the reduction in the size of the bursa would be expected to remain as a stable effect, or as a dauermodification—a temporarily inherited alteration produced by environmental means. Many generations will need to be studied to determine the stability of the bursa change. The influence that TP exerts on the embryo's bursa is reflected in a reduced capacity of the hatched chicken to produce circulating antibody (*2; 6*).

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Thermoperiodism in Sea-run Cutthroat Trout (Salmo clarki clarki)

Abstract. *Thermal tolerances from square-wave cycled temperature acclimation were compared with those from constant acclimation temperatures. A response to the maximum temperature of the square-wave cycle occurred. The acclimation thermoperiod was varied from one-fourth to twice the 24-hour cycle, and a relative maximum of tolerance resulted for the 24-hour thermoperiod. This suggests physiological adaption to natural 24-hour thermoperiods.*

Thermal acclimation and tolerance studies have been conducted more commonly with constant acclimation temperatures (*1*). Fewer workers (*2–7*) have considered cycled temperature acclimation more like that occurring in natural environments.

Of these, Brett (*2*) compared field with laboratory temperature tolerances and acclimation in *Ameiurus* and found that tolerances in the field would have had corresponding laboratory acclimation temperatures more like the maxima than the average of the environmental temperatures. Fry *et al.* (*3*) gave evidence that fish were better able to withstand upper extremes when subjected to fluctuating rather than constant acclimation. Lowe and Heath (*4*) were able to obtain higher tolerances with fish subjected to cyclic variation of temperature in the field over a 3-month season than with any form of constant temperature acclimation. They also found that fish cycle themselves behaviorally so that near-lethal temperatures are preferred for limited periods of the day, a characteristic well known in reptiles (*8*). Hillman (*5*) showed that in tomatoes damage caused by constant light could be obviated by cycling the temperature. Hubbs (*6*) had better development for fish from habitats with greater thermal fluctuation when using controlled fluctuating temperatures in the laboratory.

Studies were made with sea-run *Salmo clarki clarki* to compare laboratory-cycled temperature with constant-temperature acclimation and to determine the effect of cycle length (thermoperiod) on temperature tolerance (*9*). Yearling hatchery fish of spawn taken from adults ascending the Nooksack River, Whatcom County, Washington, February 1962, were used (*10*). Control for the square-wave acclimation patterns (Fig. 1*A*) was within ± 0.05° C of the designated temperatures (*11*). The square-wave pattern was used since in its case the major difference between cycled and constant acclimation is the alternating nature of the pattern; the shape of the particular temperature-time curves of natural environments would be an added variable. Three aquaria at a time could be cycled independently with various cycle periods as shown (Fig. 1*A*) while two or more control aquaria were held at constant temperatures. There were 15 fish per 4 gallons of filtered hatchery water (samples were occasionally reduced by escape or accident). Hatchery food was fed three to four times daily, and the photoperiod was 24 (12:12) hours, coinciding with the 24-hour thermoperiod. Fish were held at 15°C for one week before experiments were begun (except for acclimation samples at 10°C). The *p*H varied from 7.1 to 8.2, and oxygen was kept near saturation throughout the experiments.

The measurement of temperature tolerance was the CTM (critical thermal maximum or maxima) as defined by Lowe and Vance (*12*). The methods for obtaining them were similar to those formerly used (*13*). The heating rate for the CTM was 0.4 ± 0.1°C per minute in a glass chamber the same

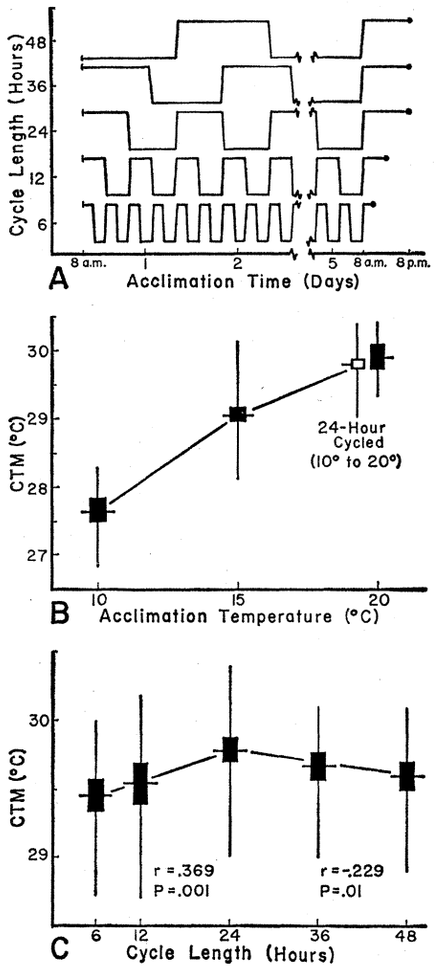

Fig. 1. Temperature acclimation and critical thermal maximum in sea-run cutthroat trout. *A*, square-wave patterns of cycled (10° to 20°C) acclimation. *B*, regression of CTM to *constant* temperature acclimation (black graphs), showing the relation of CTM for 24-hour cycled acclimation (open graph) to this regression. *C*, relation of CTM to acclimation cycle length (see *A* above). The CTM are Dice-Leraas graphs of mean ± two S.E. (rectangles), and range (vertical lines).

size as the acclimation aquaria. The CTM response point was loss of coordination, resulting in swimming on the back; it was measured to the nearest tenth degree Celsius with a Schultheis rapid-adjusting thermometer calibrated by a National Bureau of Standards certified thermometer. All fish revived after the CTM determinations.

The points of the square-wave cycles at which CTM determinations were made are indicated in Fig. 1*A* by the termination of each cycled pattern. Thus, fish from the 6- and 12-hour thermoperiods were tested at the end of their high temperature phase, while the other three groups were tested after 12 hours at the high temperatures. Tests showed that CTM did not vary with phase during 24-hour or shorter thermoperiods.

The results in Table 1 and Fig. 1 were obtained from experiments conducted between 18 December 1962 and 11 March 1963. Replications are denoted in Table 1. The Dice-Leraas graphs in Fig. 1 indicate the mean, ± two standard errors and the range. In Fig. 1*B* the CTM for the cycled samples are not significantly different ($P = .25$) from the CTM for the constant acclimation samples at 20°C, even though the average acclimation temperature for the cycled samples is 15°C. Thus, the fish respond to the upper extremes rather than the means of the thermoperiod, which corroborates by laboratory experiment what Brett (*2*) found earlier from comparing thermal tolerances of field-acclimated fish with the tolerance-acclimation regression for *Ameiurus* in the laboratory. While the difference in CTM between 15°C constant and cycled acclimation is only 0.7°C, this may correspond to a fifty percent or more increase in resistance time (*12, 13*), which could be significant in withstanding the peak of the daily temperature curve of the stream. Thus, conditioning by the upper thermal extreme could act as a "preadjustment" to a sudden rise in temperature.

In addition, the thermoperiods were varied from 6 to 48 hours, according to Fig. 1*A* over a 5-day interval of acclimation. It is seen in Fig. 1*C* that the highest value of the CTM occurs for the 24-hour thermoperiod and that the CTM diminish in either direction by regressions where $P = .001$ and .01. If the 6-hour or 48-hour cycled CTM are placed on the CTM-acclimation temperature regression in Fig. 1*B*, they lie about half way between the 15°C constant and the 24-hour cycled CTM values. Since these differences in thermoperiod affect the CTM response by this amount (that is, 50 percent), the maximum response that occurs for the 24-hour cycle may represent a significant advantage.

The response to the extreme rather than the mean of the thermoperiod is attributed by Brett (*2*) to the gain of tolerance at high temperatures being much faster than the rate of loss of tolerance at low temperatures, and therefore a form of summation occurs over several thermoperiods. This explanation may also account for the maximum response seen for the 24-hour thermoperiod in Fig. 1*C*, since in 36- and 48-hour thermoperiods there is greater time at low temperatures for tolerance loss than in the 24-hour thermoperiod. While Brett (*2*) shows no drop in resistance time for the first 2 days at a low temperature, experiments conducted by the author show a significant drop in CTM during the first 24 hours at a low temperature, though less than what would be gained in going to a high temperature in the same time interval.

Table 1. The relation of critical thermal maxima to constant and cycled temperature acclimation. Experiments with groups of 12 to 16 fish replicated one to five times.

Acclimation thermoperiod	CTM (deg C) ($\bar{x} \pm s_{\bar{x}}$)	Experiments (No.)	Total fish (No.)
	Cycled (10° to 20°C)		
6-hr	29.44 ± 0.06	3	39
12-hr	29.53 ± 0.07	3	40
24-hr	29.77 ± 0.04	5	65
36-hr	29.66 ± 0.05	2	28
48-hr	29.59 ± 0.05	3	40
	10°C		
Constant	27.63 ± 0.08	2	29
	15°C		
Constant	29.06 ± 0.05	6	90
	20°C		
Constant	29.88 ± 0.09	5	64

The lower CTM values for the 6- and 12-hour samples can also be explained on the basis of acclimation rate. Data for resistance time (*2, 14*) and CTM acclimation rates (*13*) show that the preponderance of gain occurs in the first day or two at the new high temperature. The pattern of gain with time is sigmoid; a lag occurs in the early stages, such that samples at 3 and 6 hours are disproportionately lower in the lag phase than the 12-hour samples. Therefore, the 6- and 12-hour thermoperiods could represent the lagging in rate of gain of their respective 3- and 6-hour exposures to 20°C compared to the rate of gain for the 12-hour exposure of the 24-hour thermoperiod. It is also possible that other circadian physiological mechanisms of adjustment are involved.

What is perhaps remarkable in a broader view is that the mechanisms involved have resulted in a maximum effect for the natural 24-hour thermoperiod. The results of Hubbs showing a developmental response to fluctuating temperatures and the work of Hillman relating thermoperiod to photoperiod broadens the significance of thermoperiod beyond considerations of tolerance presented here. That these effects are probably physiological adaptations to habitats with 24-hour thermal variations may be easily considered.

That such cycle responses are probably genetic and not derived from cycled conditioning during ontogeny is established by the noncyclic nature of the temperature of the hatchery water, which comes from a depth of 27.5 m in Lake Whatcom. It is of further interest that adults of this variety live in the more stenothermal, less cyclic marine environments, while only the young are normally obligated to the cyclic stream temperatures (*15*).

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Inhibition by 5-Iododeoxyuridine of the Oncogenic Effects of Adenovirus Type 12 in Hamsters

Abstract. *Subcutaneous tumors induced in newborn hamsters by type 12 adenovirus were suppressed when 0.5 mg of 5-iodo-2′-deoxyuridine (IUdR) was given at the same subcutaneous site as the virus. Although one injection of IUdR given immediately after the virus was effective, additional injections on subsequent days reduced further the number of hamsters developing tumors. These effects of IUdR are especially interesting since replication of infectious adenovirus 12 cannot be demonstrated in the hamsters at any time before or after tumor development.*

The suppressive effects of 5-halogenated pyrimidine deoxyribonucleosides on replication of DNA viruses (*1–4*) suggested that they might also influence induction of neoplasia by certain oncogenic DNA viruses, such as adenovirus types 12 and 18 (*5–8*). The activity of 5-iodo-2′-deoxyuridine on herpes simplex and vaccinia viruses both in vivo (*1, 2*) and in vitro (*2, 3*), and the effect on adenovirus multiplication in vitro (*4*) made this drug a logical choice for the studies reported herein in which a strain of type 12 adenovirus was used that leads to the induction of tumors in newborn hamsters with greater regularity than does type 18 (*6, 7, 9*). The tumors produced by adenoviruses in hamsters are of particular interest, since no infectious virus, but only incomplete replication of virus, can be demonstrated in those hamsters that develop neoplasms (*8*).

We used a pool of human adenovirus type 12 which contained approximately 10^7 $TCID_{50}$'s (tissue culture infectious doses 50 percent effective, per 0.1 ml). This was derived from the prototype strain of adenovirus 12 supplied by us to the Viral and Rickettsial Registry of the American Type Culture Collection. Virus (0.04 ml, undiluted) was inoculated intraperitoneally or subcutaneously, as described previously (*8*). Hamsters in the last stages of pregnancy were supplied from the colony of the National Institutes of Health, and were held in isolated quarters. Two or three litters, containing approximately eight young each, were used routinely for each test group; they were injected with virus within 24 hours after birth (*6*). All hamsters were inspected daily for tumors, illnesses, and deaths. Only those hamsters surviving at the time of first occurrence of tumors in the experiment (aproximately 30 days) were included for tabulation of results.

The 5-iodo-2′-deoxyuridine (IUdR) was dissolved in twice-distilled sterile water at a concentration of 5.0 mg per 1.0 ml; after passing it through a Millipore filter it was then maintained at room temperature until use. Each dose that was inoculated consisted of 0.1 ml of solution (0.5 mg of IUdR).

We conducted two separate experiments. In the initial experiment, five groups of hamsters (two litters per group) were subjected to different regimens of virus and IUdR inoculations. Two groups were given virus subcutaneously and three, intraperitoneally. In the second experiment, four test groups (two litters each) were given virus subcutaneously and one group, intraperitoneally. In each experiment we included control hamsters which received only the virus.

No definite evidence of toxicity attributable to IUdR was noted in the newborn hamsters, except possibly when multiple injections of the drug were given intraperitoneally. In one experiment, 13 out of 16 hamsters given a single injection of virus and repeated doses of IUdR by this route on alternate days died before the onset of grossly observable tumors; however, in another experiment done in the same way, the majority survived this period of therapy. The overall 30-day survival rates of the groups given IUdR subcutaneously compared favorably with untreated, but virus-injected, groups, and with our general experience with uninjected controls. However, in the one litter which was given virus subcutaneously and multiple subcutaneous injections of IUdR, in the second experiment, there was high mortality during the pre-tumor period.

Tumors produced by intraperitoneal injections were difficult to evaluate for a number of reasons. The presence of multiple small tumors, or even single tumors with a diameter as great as

Table 1. The effect of 5-iodo-2-deoxyuridine (IUdR) on the induction of subcutaneous tumors in hamsters.

Injections of IUdR	No. in test	No. with tumors *	No. dead with tumors *
Virus (SC)† plus IUdR (SC)			
Immediately, plus 9 or 10 additional doses	37	8	5
One dose immediately, or 2 hours later	34	14	10
24 hours later, plus 9 additional doses	11	6	5
Control			
None	28	26	15

* 90-day period of observation. †Subcutaneous.