TABLE 1

Growth of Control and Oil-Exposed Larval and Postlarval Lobsters

Stage	*Initial wt (mg)*[a]	*Final wt (mg)*[a]	Q_G *(cal)*[c]	$\dot{Q}_G$ *(cal/h)*[c]
I–II–B[b] control	0·90 ± 0·05	1·70 ± 0·05	3·24 ± 0·05	0·039
oil-exposed	0·90 ± 0·05[f]	1·55 ± 0·05[d]	2·63 ± 0·04[d]	0·027
II–III–B[b] control	1·70 ± 0·05	3·70 ± 0·05	7·00 ± 0·10	0·083
oil-exposed	1·70 ± 0·05[f]	3·50 ± 0·05[e]	6·30 ± 0·10[d]	0·065
IV–V–B[b] control	7·5 ± 0·3	15·1 ± 0·1	23·8 ± 0·2	0·28
oil-exposed	7·5 ± 0·3[f]	14·3 ± 0·1[d]	21·3 ± 0·2[d]	0·22

[a] Mean of 6 replicates ±1 SE.

[b] Exposed as stages I, II and IV, respectively, and molted to subsequent stage during exposure.

[c] Calories channeled to growth before molt to subsequent stage; based on caloric content at beginning and end of exposure and molting time of 84 h for control larvae and 96 h for oil-exposed larvae.

[d] $P < 0{\cdot}01$. [e] $P < 0{\cdot}05$. [f] Not significant.

increases in the lipid content of oil-exposed lobsters, there were no significant differences in biochemical composition between control and oil-exposed lobsters (Table 2).

Analysis of total lipids of control and oil-exposed larval and postlarval lobsters is presented in Table 3. For stage II-B lobsters the greater percentage of lipids is found in the polar lipid pool. In stages III-B and V-B there are consistent decreases in the polar lipid pool and increases in the neutral lipid pool. The ratio of neutral lipids to polar lipids is 3–5 times higher in postmetamorphic animals, possibly reflecting the importance of long-term energy stores in postlarval animals. Analyses of neutral lipid and polar lipid content of control and oil-exposed lobsters indicate significant ($P < 0{\cdot}01$) reductions in neutral lipid pools of stage III-B and stage V-B lobsters and significant ($P < 0{\cdot}05$) increases in the polar lipid pools of stage V-B lobsters; for stage II-B lobsters, no significant differences in either lipid pool were observed.

Analysis of lipid class composition of control and oil-exposed larval and postlarval lobsters is presented in Table 4. Oil-exposed lobsters had consistently lower levels of triacylglycerols (the major lipid energy store) and higher levels of sterols than control lobsters. There were no consistent differences in polar lipid classes between control and oil-exposed lobsters, although there were slight increases in phosphatidylcholine among stage larval stages, they remained significantly lower ($P < 0{\cdot}01$) than control values (Fig. 2). Although respiration rates were significantly reduced among oil-exposed stage IV larvae, the pattern of respiratory changes was similar to the pattern observed for control larvae with the exception of a delay in the decline in respiration rate associated with the pre-molt condition (Fig. 3); at the 80 h sampling time, oil-exposed larvae had not yet proceeded to molt stage D_3. Following transfer of oil-exposed lobsters to clean seawater, respiration rates were maintained at reduced levels up to 7 days following transfer (Fig. 3).

Ammonia excretion rates were generally not significantly different between control and oil-exposed lobsters. Exceptions were observed for stage II larvae at 72 h and with the molt to stage III larvae at 96 h ($P < 0{\cdot}01$), for stage III larvae at both the 24 h and 72 h sampling times ($P < 0{\cdot}01$) (Fig. 2), and for stage IV larvae at 80 h with the delay to molt stage D_3 (Fig. 3). Following transfer to clean seawater, ammonia excretion rates were significantly reduced ($P < 0{\cdot}01$) among stage V lobsters (Fig. 3).

Calculated O:N ratios were reduced among all larval stages exposed to oil (Fig. 2), indicating an increased dependence on protein catabolism and a possible impairment of lipid metabolism in comparison with control larvae. Following transfer to clean seawater, O:N ratios were restored to control values although the magnitude of both respiration rate and ammonia excretion rates was reduced (Fig. 3).

Growth data of control and oil-exposed larval and postlarval lobsters are presented in Table 1. All oil-exposed lobster larvae showed significant delays ($P < 0{\cdot}01$) in molting to subsequent larval stages. At the end of the 96 h exposure period, control lobsters had developed to intermolt stage C (Aiken, 1973) of the subsequent larval or postlarval stage, whereas oil-exposed larvae had only developed to stage A or B, the early post-molt stages. The mean time to molt was 84 h ($\pm 1{\cdot}0$ h) for control larvae and 96 h ($\pm 2{\cdot}0$ h) for oil-exposed larvae. In addition to delays in molt, reduced growth rates were also observed among oil-exposed lobsters, as they were significantly smaller after the molt than control animals (Table 1). Differences in caloric content of lobsters sampled at the beginning and end of the exposure period were used to calculate the energy channeled to growth during a specific molt (Q_G) and on an hourly basis during the molt ($\dot{Q}_G$). In all cases the energy available for growth was reduced among oil-exposed lobsters (Table 1), presumably as a result of the failure to catabolize energy-rich lipid substrates. With the exception of slight

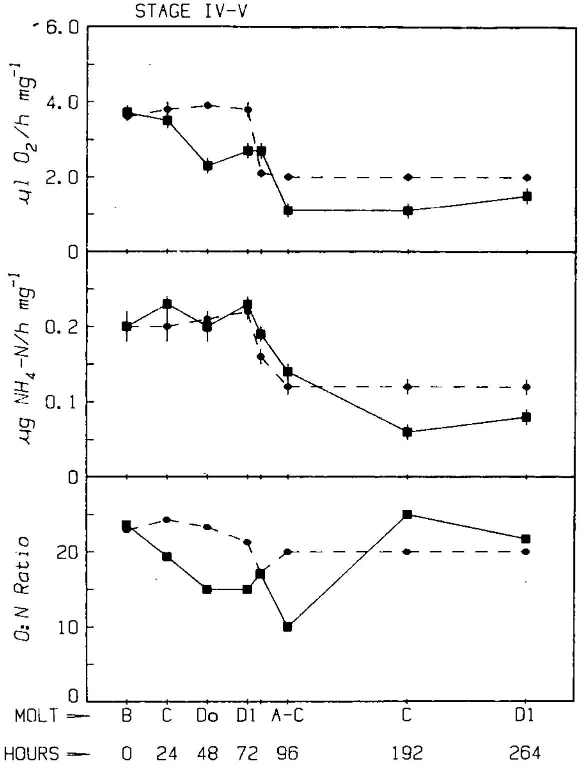


Fig. 3. Respiration rates, ammonia excretion rates and O:N ratios of stage IV and V lobsters exposed to 0·25 ppm South Louisiana crude oil for 96 h at 20 °C and maintained for 1 week following exposure to clean seawater. ●, control values; ■, oil-exposed values. Mean of 8 replicates, bars represent 1 standard error. Mark between 72 and 96 h represents 80 h sampling time.

rates among all larval stages and the first postlarval stage, with slight increases occurring immediately after molting in each larval stage (Fig. 2) and decreases just prior to molting to stage V (Figs 2 and 3). The calculated O:N ratios decreased slightly with each larval stage and were at the lowest value just prior to molting to stage V (Figs 2 and 3). These results indicate that the larval stages are dependent on the catabolism of mixed substrates for energy needs with an increased dependence on protein catabolism during the later stages of development.

With oil exposure respiration rates were significantly reduced after only 24 h of exposure for stage I ($P < 0{\cdot}01$) and stage II ($P < 0{\cdot}05$) larvae. After 72 h, respiration rates of all larval stages were significantly reduced ($P < 0{\cdot}01$), and although they increased at 96 h with the molt to subsequent

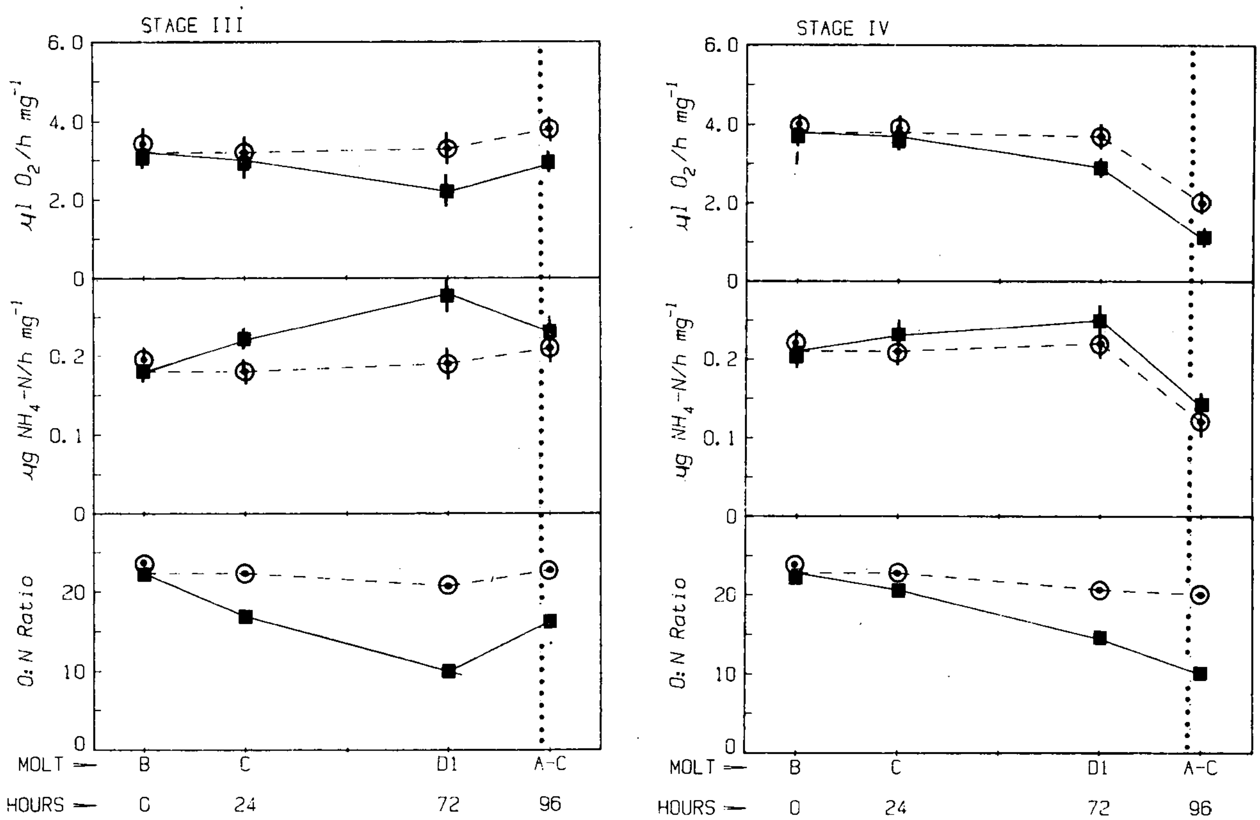
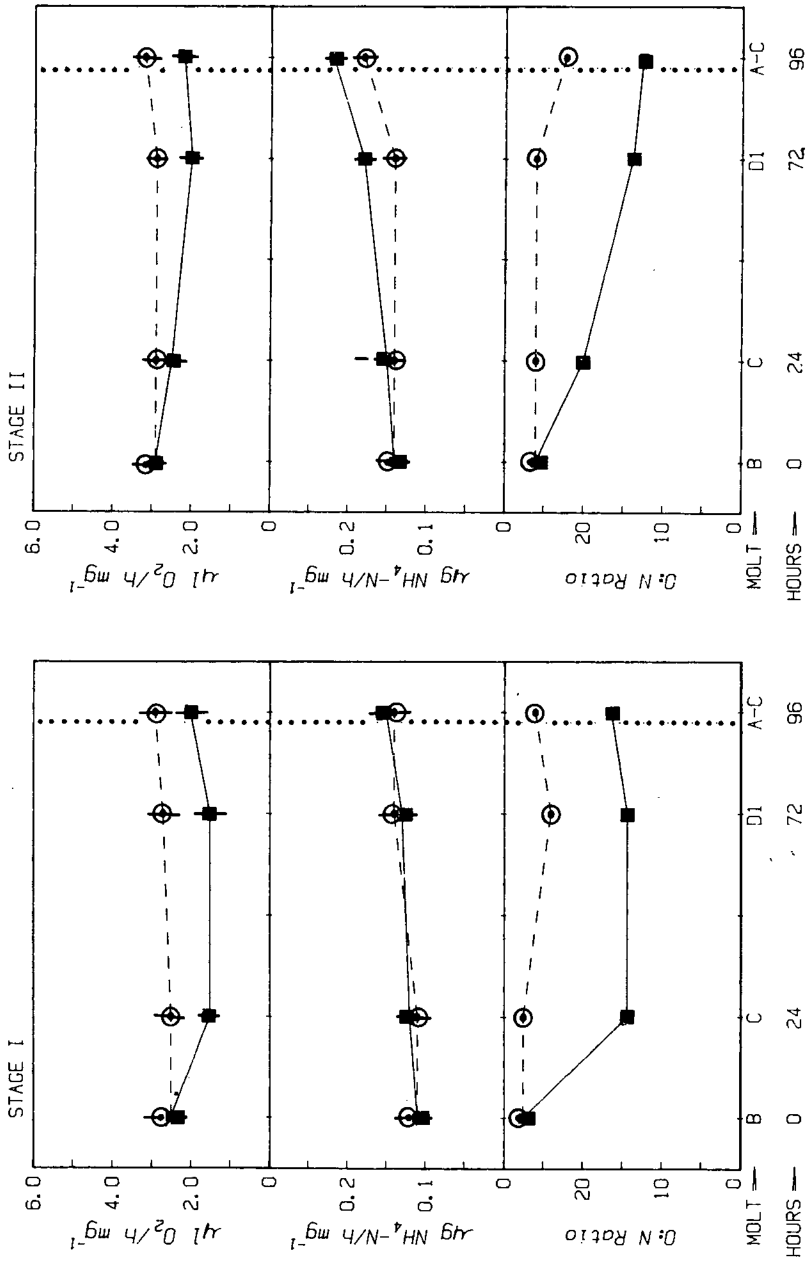


Fig. 2. Respiration rates, ammonia excretion rates and O:N ratios of larval lobsters exposed to 0·25 ppm South Louisiana crude oil for 96 h at 20 °C. ○, control values, ■, oil-exposed values. Each point is the mean of 24 replicates, bars represent 1 standard error; dotted line indicates molt to next stage.


STAGE II
STAGE I
μl O₂/h mg⁻¹
6.0
4.0
2.0
0
μg NH₄-N/h mg⁻¹
0.2
0.1
0
O:N Ratio
20
10
0
MOLT
B
C
D1
A-C
HOURS
0
24
72
96

using the chloroform–methanol–water (80:35:3) system of Innis & Clandinin (1981); the neutral lipids were separated by an adapted version of the solvent system of Christie & Hunter (1979) which consisted of dichloroethane–chloroform–glacial acetic acid–isopropanol (92:8:0·1:0·03) (Sasaki, in preparation). Together these solvent systems differentiate the following lipid classes: sterols, steryl esters, mono-, 1,2-di- and triacylglycerols, free fatty acids, phosphatidyl (P.) choline, P. ethanolamine, P. serine, P. inositol and sphingomyelin.

Fatty acids were analyzed as fatty acid methyl esters (FAME) by packed column gas chromatography with a flame ionization detector. A 6 ft, 2·1 mm i.d. stainless steel column packed with 10% SP-2330 on Chromosorb 100/120 W AW (Supelco, Inc.) was used at 200°C with a nitrogen flow of 20 ml/min. The major lipid classes were separated for fatty acid analysis using thin-layer chromatography. FAME were formed from these classes by minor modifications of the boron trifluoride–methanol method of Metcalfe *et al.* (1966) after saponification with 0·5 N NaOH in methanol.

Statistical analyses

Differences among physiological and biochemical parameters measured from the oil-exposed and control lobsters were assessed by analysis of variance (Sokal & Rohlf, 1969).

RESULTS

Survival of larval lobsters exposed to 0·25 ppm South Louisiana crude oil was not significantly different from the survival of control animals and ranged from 41·3–51·1% (±1·5% SE) for stages I–III and 92·5–96·0% (±1·5% SE) for stage IV lobsters; the higher mortality observed among the early larval stages was attributed to cannibalism.

As illustrated in Fig. 2, respiration rates of control lobsters increased slightly during the molt cycle of each larval stage (molt stage B–D_1) and with the molt to the subsequent stage with the exception of the molt to the first postlarval form (stage V). Weight-specific respiration rates increased with each molt stage and were highest among stage IV larvae, then decreased just prior to the molt to stage V (D_3, 80 h, Fig. 3). Ammonia excretion rates of control lobsters followed similar patterns as respiration

Table 1. Size, age, photoperiod and feeding effects on plasma parameters and gill Na^+, K^+-ATPase. Sample size (N), range, mean, standard deviation (SD), and slope (b), y-intercept (y-int.), coefficient of determination (r^2), and significance of regression slope (p), are given for each physiological variable and their regression on length and age. Brook trout were 6.0 and 30.5 cm fork length and 180 to 700 d old. Feeding and photoperiod effect were determined using two-way ANOVA (Yes, $P < 0.05$; No, $P > 0.05$). Yes (H) indicates that photoperiod effect was only significant in high (H) feeding group

					Linear regression						
	N	Range	Mean	SD	Independent variable	b	y-int.	r^2	P	Feeding effect	Photoperiod effect
Osmolarity	793	272–362	307	12.4	Length	0.64	296	0.12	<0.01	YES	NO
					Age	0.022	298	0.07	<0.01		
$[Na^+]$	535	126–186	152	8.0	Length	0.33	146	0.06	<0.01	NO	NO
					Age	0.013	147	0.06	<0.01		
$[K^+]$	607	0.10–7.12	1.99	1.24	Length	−0.037	2.68	0.03	<0.01	YES	NO
					Age	0.0009	2.42	0.01	<0.01		
$[Mg^{2+}]$	605	0.64–2.23	1.04	0.22	Length	—	—	—	0.47	NO	YES(H)
					Age	—	—	—	0.12		
$[Cl^-]$ (100 l)	352	96–133	108	5.5	Length	0.13	105	0.02	0.02	—	—
					Age	0.012	102	0.08	<0.01		
$[Cl^-]$ (1000 l)	311	103–142	124	6.6	Length	−0.15	127	0.02	0.02	—	—
					Age	−0.008	128	0.03	<0.01		
Hematocrit	940	30–72	48	7.2	Length	0.47	40	0.18	<0.01	—	—
					Age	0.01	44	0.04	< 0.01	—	—
Na^+, K^+-ATPase	687	1.3–21.2	7.9	3.4	Length	—	—	—	0.52	NO	NO
					Age	—	—	—	0.60		
Thyroxine	728	0.0–10.1	3.01	1.87	Length	0.14	0.48	0.20	< 0.01	YES	YES(H)
					Age	0.0045	1.05	0.10	< 0.01		

al. (1974) found that plasma free fatty acids of juvenile sockeye salmon peaked and declined during daylight. Other nutrients and waste products may cycle in a similar fashion and result in the observed "afternoon" peak in plasma osmolarity.

Diel cycles of thyroxine, which in the present study peaked during daylight, have been observed in other teleosts. White and Henderson (1977) reported levels of thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) in brook trout that were higher at midday and evening than at dawn. Similar diel patterns in T_4, and possibly T_3, have been reported for rainbow trout and goldfish (Eales *et al.*, 1981; Spieler and Noeske, 1979). In contrast, Osborn *et al.* (1978) described diel cycling of T_4 and T_3 in rainbow trout, in which lowest values were observed during daylight and highest values at night. Since other investigators have failed to find diurnal variations in plasma thyroxine in rainbow trout (Leatherland *et al.*, 1977; Brown *et al.*, 1978), it seems clear that experimental conditions are involved in diel variations. Eales *et al.* (1981) have shown that starvation for 72 hr eliminates diel variations in T_4. They also demonstrate that it is not the time of feeding which determines the timing of T_4 and T_3 peaks. From their results it appears that feeding stimulates the diel thyroxine cycle, while some other factor (possibly the light–dark period, or the animals locomotor response to it) acts to synchronize it .

Plasma thyroxine concentrations varied 40% over 24 hr, and approximately 31% over the daytime period in which our sampling for annual cycles occurred. These variations probably did not affect our ability to detect seasonal cycles since sampling occurred during the day when thyroxine levels were highest, and because the seasonal variability (ranging over an order of magnitude) was 3–4 times greater than that of the diel cycle. Changes in the magnitude and timing of diel cycling of thyroxine, however, could vary with season. Meier's (1975) review of circadian prolactin and cortisol rhythms in birds has shown that seasonal changes in diel cycles exist and possess a regulatory function. The role of diel thyroxine cycles in teleosts has yet to be established.

Ontogenetic changes in freshwater

Plasma osmotic and ionic concentrations. Range, mean and standard deviation of plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{2+}]$ are shown in Table 1. Brook trout used for this analysis were between 6.0–30.5 cm fork length, 6–30 months old and contained both mature and immature individuals. Plasma osmolarity, $[Na^+]$ and $[K^+]$ were significantly correlated with length and age (Table 1), but length and age explained little of the variation in these plasma constituents ($0.02 < r^2 < 0.12$, Table 1). Plasma $[Mg^{2+}]$ was not significantly correlated with length or age.

Plasma $[Cl^-]$ was significantly correlated with length and age, but in 100 l tanks the relationship was positive ($r^2 = 0.02$ and 0.08, respectively) while in 1,000 l tanks it was negative ($r^2 = 0.02$ and 0.03, respectively). Plasma $[Cl^-]$ of fish kept in 100 l and 1,000 l tanks were significantly different ($P < 0.01$, student's t-test). These differences were observed within 24 hr of transfer from 1,000 to 100 l tanks. pH of water was greater in smaller tanks (6.2–6.4) than in the larger tanks (5.8–6.1), and was possibly due to increased aeration in small tanks. Plasma $[K^+]$ showed inconsistent differences under the two culture conditions (being sometimes higher and sometimes lower in 100 l tanks), while plasma osmolarity, $[Na^+]$, $[Mg^{2+}]$, thyroxine concentration, hematocrit, and gill Na^+, K^+-ATPase activity showed no significant differences between tanks ($P > 0.05$, student's t-test).

Exposure of fish to lethal and sublethal acidic conditions results in decreased plasma $[Na^+]$ and $[Cl^-]$ (Packer and Dunson, 1970; Neville, 1979; McDonald *et al.*, 1980; Holeton *et al.*, 1983). Plasma $[Na^+]$ however, did not differ between 1000 l and 100 l tanks. The relatively small differences in pH may account for the absence of plasma $[Na^+]$ reduction. It is interesting to note that while plasma $[Cl^-]$

of fish in 100 l tanks decreased an average 16 mmole/l, no decrease in total osmolarity occurred, nor was there a substantial change in other plasma ions. An unmeasured plasma constituent, probably anionic, must substitute for decreased plasma $[\mathrm{Cl}^-]$.

Feeding regime had a significant effect on plasma osmolarity and $[\mathrm{K}^+]$, but not on other plasma ion concentrations (Table 1). When significant differences between high and low feeding groups were observed ($P < 0.05$, student's t-test), plasma osmolarity was always higher in the high feed group, and plasma $[\mathrm{K}^+]$, with one exception, was always higher in the low feed group. Significant differences in plasma osmolarity and $[\mathrm{K}^+]$ between high and low feed groups occurred even for the largest and oldest fish in the low feed group, indicating that meal size itself was exerting an influence.

Photoperiod treatment had a significant effect on plasma $[\mathrm{Mg}^{2+}]$ (in high feed group only, Table 1), but not on other plasma ions. No consistent difference in $[\mathrm{Mg}^{2+}]$ due to daylength was found.

Plasma ion and osmotic concentrations of brook trout in freshwater are typical of those reported for other freshwater teleosts (Holmes and Donaldson, 1969). Size and age related adjustments in plasma ion and osmotic concentrations can explain only a small amount of the variation of these parameters. Size related changes in blood ions may not be due to osmoregulatory changes *per se*, but rather to other impinging physiological responses that vary with size. For example, the degree of digestion of the previous days meal is size dependent (Jobling *et al.*, 1977) and could result in nutrient transport related changes in plasma ionic and osmotic concentrations. Despite these alternative explanations, size related changes in hyperosmoregulation may exist.

Significant seasonal changes in plasma ion levels that might signal preparatory physiological adaptations were not found in the present experiment. Seasonal changes in plasma ions have been found in rainbow trout (Lane, 1979; Houston and Smeda, 1979). We found seasonal changes in $[\mathrm{Mg}^{2+}]$ only, and this effect occurred only in the high feed group. There was no clear trend of variations in plasma $[\mathrm{Mg}^{2+}]$ with changing daylength in either photoperiod. Lack of photoperiod effect on plasma ions in our experiments, which were conducted at constant temperature, indicates that temperature (or synergy between photoperiod and temperature) may play a more important role than photoperiod alone in determining seasonal ion changes reported by other researchers. Alternatively, brook trout may not display seasonal cycles in ion concentration that are seen in other salmonids.

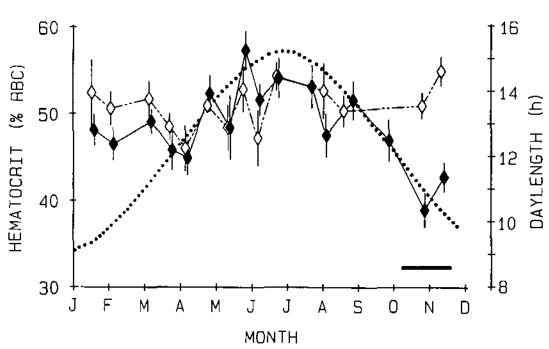


Fig. 4. Annual variation in hematocrit (% red blood cells) for males (open diamonds) and females (closed diamonds) under normal photoperiod conditions. Mean value of six to ten fish per sample ± 1 standard error of the mean. Daylength (˙) and spawning time (horizontal bar) are also shown. Fish in this experiment were from high feed group, all of which became mature at age 1+ yr in their second autumn (November 1982). Mean female hematocrit in autumn in significantly lower than the non-autumn mean ($P < 0.05$, Student's t-test).

Hematocrit. Hematocrit increases with increasing length which can explain a portion of the variation in hematocrit ($r^2 = 0.18$, Table 1), while age can explain little of the variation ($r^2 = 0.04$). This relationship held true when mature fish were excluded ($r^2 = 0.13$ and 0.09 for length and age, respectively). Adult male and female hematocrits of 1+ year fish are the same for much of the year (Fig. 4) and do not differ until autumn when male hematocrit rises slightly and female hematocrit drops significantly from the spring and summer average ($P < 0.01$, Student's t-test). Changes in hematocrit occur simultaneously with final maturation, when sperm is running freely and egg diameters are at a maximum. Significant male–female differences in hematocrit, however, were also observed in immature fish; immature males and females in autumn photoperiod (11.5–9.5 hr daylength) had mean hematocrits of 50% ($n = 15$) and 44%, respectively ($n = 13$; $P < 0.01$, Student's t-test). Mean hematocrits of immature males and females during winter photoperiod (9.1–10.2 hr daylength) were not significantly different (44% and 46%, $N = 15$ and 11, respectively, $P > 0.25$).

Hematocrit levels and their variability as reported here are typical of those reported elsewhere for brook trout and other salmonids (Sniezko, 1960; Sano, 1960). Since size explains a greater portion of the variation in hematocrit than age, age is probably significant only to the extent that it covaries with size. Adult male rainbow trout, pike (*Esox lucius*) and largemouth bass (*Micropterus salmoides*) have higher hematocrit than females (Sano, 1960; Mulcahy, 1970; Steuke and Atherton, 1965) indicating that sexual differences in teleost hematocrit are not rare. Sano (1960) also reported a sharp reduction in hematocrit of adult rainbow trout of both sexes that was correlated with gonadal development. In brook trout, only the female hematocrit declines during the onset of spawning. Further work is necessary to determine the mechanistic control of hematocrit and how this control may be related to sex, spawning and photoperiod cycles.

Gill Na^+, K^+-ATPase. Individual gill Na^+, K^+-ATPase activities ranged from 1.3 to 21.2 $\mu\mathrm{MP_i} \times \mathrm{mg\,prot.}^{-1} \cdot \mathrm{hr}^{-1}$, with a mean value of 7.9 (Table 1). Gill Na^+,K^+-ATPase activity (and its log transformation) were not significantly correlated with size or age. Mean value of gill Na^+,K^+-ATPase did not rise above 13.0 $\mu\mathrm{MP_i} \cdot \mathrm{mg\,prot.}^{-1} \cdot \mathrm{hr}^{-1}$ for any sampling period. Feeding treatment had no effect on gill Na^+,K^+-ATPase levels in either photoperiod (Table 1), nor did the two photoperiod treatments differ in their effect on gill Na^+,K^+-ATPase activity.

None of the experimental manipulations of the

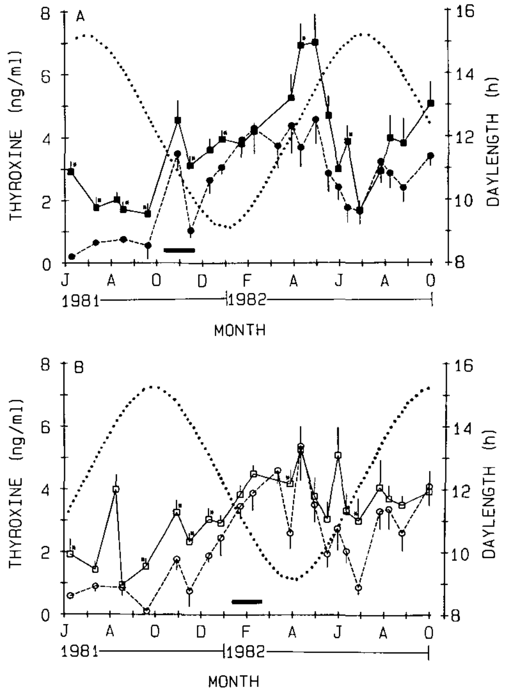


Fig. 5. Annual cycles of plasma thyroxine in high feed (squares) and low feed (circles) groups in normal photoperiod (A, solid symbols) and 3 month delayed photoperiod (B, open symbols), as a function of calendar date of sampling. Mean value of 5–16 fish per sample ± standard error of the mean. An asterisk (*) next to mean of high feed group indicates a significantly ($P < 0.05$, Student's t-test) higher mean plasma thyroxine levels than the low feed group at that sampling period. Each point represents samples taken on a single day except for three instances (June, July and August 1981) when samples taken within 2–4 days were combined. Daylength (·) and time of spawning (horizontal bar) are shown for each photoperiod. Feeding treatment had a significant effect on thyroxine levels in both photoperiods ($P < 0.01$, two-way ANOVA). Photoperiod treatment significantly affected thyroxine levels in the high feed group ($P < 0.01$, two-way ANOVA), but not the low feed group ($P = 0.43$).

present study affected gill Na^+,K^+-ATPase activity of brook trout. In contrast, gill Na^+,K^+-ATPase activity in migratory Pacific salmon (*Oncorhynchus* sp.), Atlantic Salmon (*Salmo salar*) and steelhead trout (*Salmo gairdneri*) exhibit a seasonal cycle (usually peaking in spring or autumn, and corresponding to the period of seaward migration) which is synchronized by photoperiod (Zaugg and McLain, 1970; Zaugg and Wagner, 1973; Saunders and Henderson, 1978). Ewing *et al.* (1979) found that although chinook salmon displayed a seasonal cycle of gill Na^+,K^+-ATPase activity under simulated normal photoperiod conditions, a 3 month advanced photoperiod did not significantly alter the cycle, indicating the controlling influence of endogenous rhythms. In the present study there was no daylength-related rhythm of gill Na^+,K^+-ATPase activity in either photoperiod treatment, and no evidence of endogenous rhythms.

Ewing *et al.* (1979) found that growth rate of chinook salmon (altered by changes in temperature) affected the cyclic annual change in gill Na^+,K^+-ATPase activity and that size was positively correlated with this activity. Size, growth and photoperiod did not alter gill Na^+,K^+-ATPase activity of brook trout in the present study, possibly due to the less specialized nature of its seaward migration. Brook trout, and charr in general, show less anadromy than other salmonids (Hoar, 1976), and there are more non-migratory than migratory populations of brook trout (Power, 1980). Anadromous brook trout spend long periods in estuaries (Montgomery *et al.*, unpublished data) where gill Na^+,K^+-ATPase activity increases (McCormick and Naiman, 1984b), indicating that adaptations for seawater entry have an important behavioral component.

It is possible that possession of preparatory changes in gill Na^+,K^+-ATPase activity is genetically determined in brook trout. However, the migratory pattern of the hatchery stock used in these experiments did not differ from that of natural populations (Mullan, 1958). Furthermore, gill Na^+,K^+-ATPase activities of an anadromous population of brook trout, which show external signs of smolting (silvering), were not significantly different from a nearby non-anadromous population (McCormick and Naiman, 1984b).

Plasma thyroxine. Plasma thyroxine concentration was significantly correlated with both size and age ($P < 0.01$) and explained 20% and 10% of the thyroxine variation, respectively (Table 1). The significance of these correlations may be explained, in part, by changes in growth rate. Within each photoperiod, feeding treatment resulted in significant differences in thyroxine levels (Table 1 and Fig. 5). Fish in high feed groups had significantly higher plasma thyroxine at most sampling times starting from the first sample period (June 1981) and continuing until December 1981. This is the same time that growth rates in the high feed group were much greater than in the low feed group (Fig. 2(D)). After this period, growth rates and thyroxine levels were similar for both feeding groups until April 1982, when high feed fish again attained higher plasma thyroxine levels. This pattern was similar for both photoperiods (Fig. 5(A, B)). Under normal photoperiod conditions the percent mean difference in plasma thyroxine (between the high and low feed group) and the mean difference in specific growth rate per unit body weight (a) 1–2 week later were significantly correlated ($r = 0.71$). These results indicate that higher growth rates in brook trout are associated with higher levels of plasma thyroxine.

Under normal photoperiod conditions (Fig. 5A) there was a strong pattern of high thyroxine levels in the "spring" (increasing photoperiod), low "summer" levels which rose to a secondary "autumn" peak. This pattern was consistent in both high and low feed fish, with the exception that the "spring" peak was attenuated in the low feed group.

Although a single "spring" peak occurred under the 3-month delayed photoperiod for the high feed fish, there was no rise in thyroxine levels in either group during the second "spring", and there was no subsequent "summer" decline (Fig. 5B). As such, there was no clearly discernible daylength pattern in thyroxine levels under 3-month delayed photoperiod.

Photoperiod treatment had a significant effect only on fish in the high feed group (Table 1). There was no clear pattern in the differences between normal and delayed photoperiods for the high feed group (Fig. 5(A, B)), and it was clear that a simple 3-month shift in the T_4 cycle did not occur as a result of treatment with a 3-month delayed photoperiod.

The significant effect of feeding level on circulating T_4 concentrations in brook trout is probably related to growth, since significant differences in T_4 levels in high and low feeding groups occurred when growth rates of the two groups were most different. It is unlikely that insufficient iodine in the low feeding group resulted in lower T_4 levels since under normal laboratory conditions less than 20% of the iodine needed by rainbow trout is obtained from the diet (Hunt and Eales, 1979), and only 5% of the total iodine is used by the thyroid. In addition, brook trout deprived of food for several weeks increase their plasma iodine (Higgs and Eales, 1971), while rainbow trout show no detectable change in T_4 after up to 40 days of starvation (Leatherland *et al.*, 1977; Milne *et al.*, 1979). More recent experiments indicate that drastically reduced feeding may decrease plasma T_4 of salmonids (Dickhoff, unpublished; Higgs *et al.*, 1982). Direct feeding effects cannot accurately describe our results, however, since feeding treatment lasted throughout the study while differences in T_4 between high and low feed groups occurred for a limited period. Thyroid hormones, particularly triiodothyronine, administered exogenously can stimulate growth in a variety of teleosts and most salmonids (Higgs *et al.*, 1982). T_4 may act in synergy with other anabolic hormones, particularly growth hormone, to stimulate somatic growth (Donaldson *et al.*, 1979). Our findings of increased circulating T_4 levels associated with higher growth rates support a model of thyroid influence on growth.

A seasonal pattern of circulating T_4, similar to that reported here was found in adult brook trout by White and Henderson (1977), with the one exception that a secondary fall peak was not found. This seasonal pattern in brook trout T_4 levels is similar to that found for smolting salmonids (Dickhoff *et al.*, 1982). The magnitude of the springtime peak, however, is generally greater in smolting salmonids. The lack of preparatory seawater-entry adaptations in brook trout (such as increases in gill Na^+,K^+-ATPase) suggests that spring thyroxine increases activate other physiological functions. The thyroxine cycle displayed in primitive salmonids such as brook trout has perhaps seen sequestered by specialized migrators to synchronize migration and smoltification.

Winter flounder (*Pseudopleuronectes americanus*) displayed peak thyroxine concentrations in spring and low concentrations in autumn (Eales and Fletcher, 1982), while high winter concentrations and low summer concentrations were observed in rainbow trout (Osborn *et al.*, 1978). Constant temperatures used in our experiments indicate that temperature changes are not necessary to elicit seasonal thyroxine cycles. Although feeding activity increased during spring photoperiod in our experiment (personal observation), we did not detect increased growth rates under increasing photoperiod and therefore cannot ascribe higher spring thyroxine levels to increased growth during this period.

The delayed photoperiod regime did not shift thyroxine cycles 3 months from the normal regime; in low feed fish photoperiod has no effect on plasma thyroxine, while in high feed fish the effect seemed to be a dampening of the normal cycle. These results raise the possibility that an endogenous cycle, or an exogenous cycle synchronized by an environmental factor other than photoperiod or temperature, exists in brook trout. Other photoperiod cued cycles, in particular maturation, did respond to the photoperiod treatment; final maturation under our experimental conditions was delayed 3 months in the delayed photoperiod (McCormick and Naiman, 1984c). These results are somewhat conflicting, especially in light of other evidence associating thyroid changes with maturation (see Leatherland, 1982, for review). Nonetheless, it appears that an annual cycle of thyroxine with a spring peak is not necessary to begin or synchronize the maturation cycle of brook trout.

SUMMARY

Significant diel cycles were observed in plasma osmolarity, $[Na^+]$ and thyroxine concentration, and were not detected in plasma $[Cl^-]$, $[K^+]$,$[Mg^{2+}]$ and hematocrit. Plasma osmolarity, $[Na^+]$ and thyroxine concentrations were highest during daylight and lowest at night. This cycle may be caused by feeding and locomotor activity which are highest during periods of light.

Plasma osmolarity, $[Na^+]$, $[K^+]$ and hematocrit increased with increasing size and/or age of brook trout, and can explain a small portion of their variation. Plasma osmolarity and $[K^+]$ were also influenced by feeding level. The effect of size on plasma ions may be explained by a more favorable surface area to volume ratio which, other things being equal, will result in lower net water influx, lower plasma water, higher plasma ions and higher hematocrit with increasing size. We cannot, however, rule out other factors which may also covary with size and/or age.

Gill Na^+,K^+-ATPase activity in brook trout did not respond to feeding or photoperiod treatment, nor was there evidence of size, age or daylength related changes. Brook trout therefore do not possess preparatory physiological adaptations in gill Na^+,K^+-ATPase that are characteristic of other migratory salmonids. It would appear that the more variable and opportunistic nature of brook trout migrations has not resulted in sufficient selection pressure for the development of preparatory, photoperiod-controlled changes in gill Na^+,K^+-ATPase.

Plasma thyroxine concentrations were higher in high feed fish and were directly correlated with size. Significant differences in plasma thyroxine concentration between high and low feed groups occurred when differences in growth between the two groups were greatest. These results are best explained by an

interaction between growth rate and plasma thyroxine. Under normal photoperiod conditions, plasma thyroxine exhibited a seasonal cycle consisting of high levels in spring, low summer levels and a secondary peak in autumn. Three-month delayed photoperiod did not result in a shift of the thyroxine cycle. Since seasonal changes of brook trout gill Na^+,K^+-ATPase activity and hypoosmoregulatory ability did not occur (McCormick and Naiman, 1984a), the annual cycle of plasma thyroxine does not stimulate these physiological functions as it is presumed to in smolting salmonids. The seasonal thyroxine cycle which exists in the more primitive charrs must exert its influence through other seasonally occurring physiological functions such as growth, activity or appetite.

Acknowledgements—We thank the Massachusetts Division of Fisheries and Wildlife for providing brook trout eggs. M. Zaltas, M. Connor, E. Rosa-Molinar, R. Rheault and E. Montgomery ably provided technical assistance. V. Massard typed the first draft. J. M. Capuzzo, R. B. Gagosian, R. W. Griffith, J. J. Stegeman and G. C. Walker gave helpful advice during the conduct of our experiments and made many constructive comments in review. This project was supported by the U.S. Department of Commerce, NOAA, Office of Sea Grant NA80-AA-D-00077 (R/A-14). S. D. McC. was supported by a Tai-Ping Predoctoral Fellowship in Marine Biology and the Woods Hole Oceanographic Institution Education Office. This is Contribution No. 559 of the Woods Hole Oceanographic Institution and Contribution No. 85 of the Institution's Matamek Research Station.

REFERENCES

Brett J. R. (1979) Environmental factors and growth. In: *Fish Physiology, Vol. VIII* (edited by Hoar W. S., Randall D. J. and Brett J. R.), pp. 599–675. Academic Press; New York.

Brown J., Fedoruk K. and Eales J. G. (1978) Physical injury due to injections or blood removal causes elevations of plasma thyroxine in rainbow trout, *Salmo gairdneri. Can. J. Zool.* **56,** 1998–2003.

Castonguay M., Fitzgerald G. J. and Côté Y. (1982) Life history and movements of anadromous brook charr, *Salvelinus fontinalis*, in the St. Jean River, Gaspé, Québec. *Can. J. Zool.* **60,** 3084–3091.

Dickhoff W. W., Darling D. S. and Gorbman A. (1982) Thyroid function during smoltification of salmonid fish. In: *Gunma Symposia on Endocrinology, Vol. 19, Phylogenic Aspects of Thyroid Hormone Actions.* (edited by Institute of Endocrinology, Gunma Univ.), pp. 45–61. Center for Academic Publications Japan, Tokyo.

Dickhoff W. W., Folmar L. C. and Gorbman A. (1978) Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch. Gen. Comp. Endocrinol.* **36,** 229–232.

Donaldson E. M., Fagerlund U. H. M., Higgs D. A. and McBride, J. R. (1979) Hormonal enhancement of growth in fish. In: *Fish Physiology, Vol VIII.* (edited by Hoar W. S., Randall D. J. and Brett J. R.), pp. 456-597. Academic Press, New York.

Dutil J. D. and Power G. (1980) Coastal populations of brook trout, *Salvelinus fontinalis*, in Lac Guillaume-Delisle (Richmond Gulf) Quebéc. *Can. J. Zool.* **58**(10), 1828–1835.

Eales J. G. and Fletcher G. L. (1982) Circannual cycles of thyroid hormones in plasma of winter flounder (*Pseudopleuronectes americanus* Walbaum). *Can. J. Zool.* **60,** 304–309.

Eales J. G., Hughes M. and Uin L. (1981) Effect of food intake on diel variation in plasma thyroid hormone levels in rainbow trout, *Salmo gairdneri. Gen. Comp. Endocrinol.* **45,** 167–174.

Ewing R. D., Johnson S. L., Pribble H. J. and Lichatowich J. A. (1979) Temperature and photoperiod effects on gill Na^+, K^+-ATPase activity in chinook salmon (*Oncorhynchus tshawytscha*). *J. Fish. Res. Bd Can.* **36,** 1347–1353.

Gorbman A., Dickhoff W. W., Mighell J. L., Prentice E. F. and Waknitz F. W. (1982) Morphological indices of developmental progress in the parr-smolt coho salmon, *Oncorhynchus kisutch. Aquaculture* **28,** 1–19.

Grau E. G., Specker J. L., Nishioka R. S. and Bern H. A. (1982) Factors determining the occurence of the surge in thyroid activity during smoltification. *Aquaculture* **28,** 49–57.

Hannah S. G. and Pickford G. E. (1981) Diurnal peaks in hematocrit and in serum sodium in the killifish, *Fundulus heteroclitus*, and their absence in serum potassium and chloride: a lack of correlation. *Comp. Biochem. Physiol.* **70A,** 157–159.

Higgs D. A. and Eales J. G. (1971) Iodide and thyroxine metabolism in the brook trout, *Salvelinus fontinalis* (Mitchill) during sustained exercise. *Can. J. Zool.* **49,** 1255–1269.

Higgs D. A., Fagerlund U. H. M., Eales J. G. and McBride J. R. (1982) Application of thyroid and steroid hormones as anabolic agents in fish culture. *Comp. Biochem. Physiol.* **73B,** 143–176.

Hoar W. S. (1976) Smolt transformation: evolution, behavior and physiology. *J. Fish Res. Bd Can.* **33,** 1234–1252.

Holeton G. F., Neumann P. and Heisler N. (1983) Branchial ion exchange and acid-base regulation after strenuous exercise in rainbow trout (*Salmo gairdneri*). *Resp. Physiol.* **51,** 303–318.

Holmes W. N. and Donaldson E. M. (1969) The body compartments and the distributions of electrolytes. In: *Fish Physiology, Vol. I* (edited by Hoar W. S. and Randall, D. J.), pp. 1–90. Academic Press, New York.

Houston A. H. and Smeda J. S. (1979) Thermoacclimatory changes in the ionic microenvironment of hemoglobin in the stenothermal rainbow trout (*Salmo gairdneri*) and eurythermal carp (*Cyprinus carpio*). *J. exp. Biol.* **80,** 317–340.

Hunt D. W. C. and Eales J. G. (1979) Iodide balance in rainbow trout, *Salmo gairdneri* and effects of testosterone proprionate. *J. Fish. Res. Bd Can.* **36,** 282–285.

Jobling M. (1983) Growth studies with fish—overcoming the problems of size variation. *J. Fish Biol.* **22,** 153–157.

Jobling M., Gwyther D. and Grove D. J. (1977) Some effects of temperature, meal size and body weight on gastric evacuation time in the dab, *Limanda limanda* (L.). *J. Fish Biol.* **10,** 291–298.

Lane H. C. (1979) Progressive changes in hematology and tissue water of sexually mature trout (*Salmo gairdneri*) Richardson, during autumn and winter. *J. Fish Biol.* **15,** 425–436.

Leatherland J. F. (1982) Environmental physiology of the teleostean thyroid gland: a review. *Env. Biol. Fish.* **7,** 83–110.

Leatherland J. F., McKeown B. A. and John T. M. (1974) Circadian rhythm of plasma prolactin, growth hormone, glucose and free fatty acid in juvenile kokannee salmon, *Oncorhynchus nerka. Comp. Biochem. Physiol.* **74A,** 821–828.

Leatherland J. F., Cho C. Y. and Slinger S. J. (1977) Effects of diet, ambient temperature and holding conditions on plasma thyroxine levels in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd Can.* **34,** 677–682.

Leitritz E. and Lewis R. C. (1976) Trout and salmon culture. *Calif. Fish Game Bull. 164.* pp. 220.

Lowry O. H., Roseborough N. J., Farr A. L. and Randall

R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

McCormick S. D. and Naiman R. J. (1984a) Osmoregulation in the brook trout, *Salvelinus fontinalis.* II. Effects of size, age and photoperiod on seawater survival and ionic regulation. *Comp. Biochem. Physiol.* **79A.**

McCormick S. D. and Naiman R. J. (1984b) The Physiology of Smoltification in Anadromous and Non-anadromous Brook Trout (*Salvelinus fontinalis*) and Atlantic Salmon (*Salmo salar*) from the Matamek River and Rivière à la Truite, Québec. *Can. J. Fish. Aq. Sci.* (in press).

McCormick S. D. and Naiman R. J. (1984c) Some determinants of maturation in the brook trout, *Salvelinus fontinalis. Aquaculture* (in press).

McDonald D. G., Hobe H. and Wood C. M. (1980) The influence of calcium on physiological responses of the rainbow trout, *Salmo gairdneri,* to low environmental pH. *J. exp. Biol.* **88,** 259–267.

Meier A. H. (1975) Chronoendocrinology of vertebrates. In: *Hormonal Correlates of Behavior,* Vol. 2. (edited by Eleftherious, B. E. & Sprout, R. L.), pp. 469–549. Plenum Press, New York.

Miller G. L. (1959) Protein determination for large numbers of samples. *Anal. Chem.* **31,** 964.

Milne R. S., Leatherland J. F. and Holub B. J. (1979) Changes in plasma thyroxine, triiodothyronine and cortisol associated with starvation in rainbow trout (*Salmo gairdneri*). *Env. Biol. Fish* **4,** 185–190.

Mulcahy M. F. (1970) Blood values in the pike *Esox lucius* L. *J. Fish Biol.* **2,** 203–209.

Mullan J. W. (1958) The sea-run or "salter" brook trout (*Salvelinus fontinalis*) fishery of the coastal streams of Cape Cod, Massachusetts. *Mass. Div. Fish and Game. Bull. No. 17.*

Neville C. M. (1979) Influence of mild hypercapnia on the effects of environmental acidification on rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **83,** 345–349.

Osborn R. H., Simpson T. H. and Youngson A. F. (1978) Seasonal and diurnal rhythms of thyroidal status in the rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **12,** 531–540.

Packer R. K. and Dunson W. A. (1970) Effects of low environmental pH on blood pH and sodium in brook trout. *J. exp. Zool.* **174,** 65–71.

Power G. (1980) The brook charr, *Salvelinus fontinalis.* In: *Charrs* (edited by Balon, E. K.), pp. 141–203. Dr. W. Junk bv Publishers, The Hague.

Ricker W. E. (1979) Growth rates and models. In: *Fish Physiology, Vol. VIII* (edited by Hoar, W. S., Randall, D. J. & Brett, J. R.), pp. 678–737. Academic Press, New York.

Sano T. (1960) Haematological studies of the culture fishes in Japan. 3. Changes in blood constituents with growth of rainbow trout. *J. Tokyo Univ. Fisheries* **46,** 77–87.

Saunders R. L. and Henderson E. B. (1978) Changes in gill ATPase activity and smolt status of Atlantic salmon (*Salmo salar*). *J. Fish. Res. Bd. Can.* **35,** 1542–1546.

Snieszko S. F. (1960) Microhematocrit as a tool in fishery research and management. *U.S. Fish and Wildl. Serv. Spec. Rep. Fish.* **341,** 1–15.

Spieler R. E. and Noeske T. A. (1979) Diel variations in circulating levels of triiodothyronine and thyroxine in goldfish, *Carrassius auratus. Can. J. Zool.* **57,** 665–669.

Steucke E. E. and Atherton C. R. (1965) Use of microhaematocrit values to sex largemouth bass. *Prog. Fish Cult.* **27,** 87–89.

Sutterlin A. M., Harmon P. and Barchard H. (1976). The culture of brook trout in salt water. *Can. Fish. Mar. Serv. Tech. Rept. No. 636.*

Wedemeyer G. A., Saunders R. L. and Clarke W. C. (1980) Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Mar. Fish. Rev.* **46,** 1–14.

White H. C. (1940) Life-history of sea-running brook trout (*Salvelinus fontinalis*) of Moser river, Nova Scotia. *J. Fish. Res. Bd. Can.* **5,** 176–186.

White B. A. and Henderson H. E. (1977). Annual variation in the circulating levels of thyroid hormones in the brook trout, *Salvelinus fontinalis,* as measured by radioimmunosassay. *Can. J. Zool.* **55,** 475–481.

Whoriskey F. G., Naiman R. J. and Montgomery W. L. (1981) Experimental sea ranching of brook trout, *Salvelinus fontinalis,* Mitchill. *J. Fish Biol.* **19,** 637–651.

Wilder D. G. (1952) A comparative study of anadromous and freshwater populations of brook trout (*Salvelinus fontinalis* (Mitchill)). *J. Fish. Res. Bd. Can.* **9,** 169–203.

Wood C. M. and Randall D. J. (1973) The influence of swimming activity on sodium balance in the rainbow trout (*Salmo gairdneri*). *J. Comp. Physiol.* **82,** 207–233.

Zaugg W. S. (1982) A simplified preparation for adenosine triphosphatase determination in gill tissue. *Can. J. Fish. Aq. Sci.* **39,** 215–217.

Zaugg W. S. and McLain L. R. (1970) Adenosine triphosphatase activity in gills of salmonids: seasonal variation and salt water influence in coho salmon, *Oncorhynchus kisutch. Comp. Biochem. Physiol.* **35,** 587–596.

Zaugg W. S. and Wagner H. H. (1973) Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. *Comp. Biochem. Physiol.* **45B,** 955–965.