DAYLENGTH PERCEPTION AND THE PHOTOPERIODISM OF SMOLTING IN COHO SALMON (*Oncorhynchus kisutch*).

by

Helgi Þór Thorarensen

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Name: **Helgi Thorarensen**

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Examining Committee:

Chairman: Dr. L. Druehl, Professor

Dr. A.P. Farrell, Associate Professor, Senior Supervisor

Dr. W.C. Clarke, Pacific Biological Station, Nanaimo

Dr. B.A. McKeown, Professor

Dr. A.H. Burt, Associate Professor, Dept. of Biological Sciences, Simon Fraser University, Public Examiner

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Daylength perception and photoperiodism of smolting in coho salmon (Oncorhynchus kisutch)

Author:

(signature)

Helgi Thorarensen

(name)

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(date)

Abstract

The long or increasing daylengths of spring set the time for smolting in many salmonids. Under natural conditions coho smolt as yearlings but under controlled photoperiods underyearlings will also smolt in response to longer days after a priming period of short days. Although it is known which photoperiods will induce smolting, little is known about the underlying mechanism of daylength perception. The current study examined different aspects of this mechanism. The fish were exposed to different combinations of photoperiods and their smolting response monitored as growth rate and seawater adaptability.

The results suggest that coho salmon perceive daylength through a circadian rhythm of photoinducibility with a light sensitive phase occurring at a specific time during the day. The induction of smolting by photoperiods depends on whether the fish experience light during this sensitive phase, while the total exposure to light is less important. The photoinducible phase appears to occur from 12 to 19 hours after dawn, while the fish are most receptive to stimulation between hours 14 and 16. During winter the phase would fall in darkness but as daylength increases in spring the fish are exposed to light when the sensitive phase occurs, leading to stimulation of smolting.

To smolt successfully in response to inductive daylengths the fish must be exposed to short days for a two month priming period, the critical daylength being between 10 and 12 hours. Exposure to longer daylengths during the priming period inhibited smolting. It has been suggested that this prevents underyearling coho from smolting as they emerge from the bottom gravel around and after the time of the vernal equinox.

Night illumination over 10^{-4} lx during the priming period interferes with smolting. This may represent the threshold level of sensitivity for the eyes and the pineal organ.

The sensitivity to night illumination indicates that moonlight can inhibit smolting of underyearling coho in their natural habitat. However, it is suggested that presmolts are less sensitive to night illumination.

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INTRODUCTION

1.1. Overview of the problem.

The diurnal light cycle and the seasonal cycle of daylength (photoperiod) produce a predictable variation in the environment of organisms. The ability to anticipate these changes would clearly be an adaptive advantage, allowing preparations for future challenges or opportunities. Phenomena such as flowering, hibernation, migration, and reproduction are regulated by photoperiods, the most reliable and "noise-free" environmental cue for making such predictions. Seasonal responses to daylength are referred to as photoperiodism.

To measure daylength an organism must measure time, and not surprisingly some form of time-keeping may be common to all eukaryotes (Brady 1982a). Some models of photoperiodism assume the involvement of a circadian (period of approximately 24 h) rhythm in measuring daylength. Although the existence of circadian rhythms for measurements of daylength remains hypothetical, the presence of internal time-keeping is manifested by circadian rhythms at all levels of animal organization, examples being cycles of cell divisions, hormone release and at the organismal level, behavioural patterns (cfr. Brady, 1982b). These rhythms may be endogenous and proceed in the absence of external stimuli, although they are entrained or reset by photoperiods which synchronize them to the diurnal light cycle (Daan 1982).

The driving mechanism or the 'clock-work' of these circadian rhythms is still unknown, but it has been suggested that cycles of protein synthesis (Ehret & Trucco 1967; Schröder-Lorenz & Rensig 1986) or membrane permeability (McMahon & Block 1987a,b) may be involved.

All members of the subfamily Salmoninae spawn in fresh water although many species spend a substantial part of their life cycle in seawater (Randall *et al.* 1987). In most populations of coho salmon (*Oncorhynchus kisutch*) the fry emerge from the bottom gravel between March and July, spend one or two years in fresh water before migrating to sea in spring, and return back to the river to spawn during the autumn of the same year (jacks) or the following year (Scott & Crossman 1973). The transformation of the fresh water resident parr to the migrating smolt is referred to as smolting.

Many links in the chain of events leading from perception of light to smolting are still not understood. The sensory pathways through which daylength is perceived could involve the eyes, the pineal organ, which has been reported to mediate effects of photoperiods both on circadian and seasonal rhythms (Kavaliers 1981a; De Vlaming & Olcese 1981), and diencephalic photoreceptors (van Veen *et al.* 1976; Kavaliers 1981b). From the receptors, the information may be passed on to a mechanism that perceives the daylength and upon the right photoperiodic stimulation this mechanism in turn initiates the smolting process perhaps by stimulating the release of various hormones (Hoar 1965). The location and the mode of operation of the daylength measuring mechanism for smolting still remains unknown. The present study focused on the mechanism behind daylength perception in coho parr by examining the effect of photoperiods on smolting.

1.2. Smolting.

Salmonids migrate from fresh water to exploit the more abundant food resources and favorable temperatures of the ocean (Thorpe 1982, 1987; Gross 1987). Consequently, smolting involves a wide array of physiological, biochemical, morphological, and behavioural changes (Hoar 1976; Wedemeyer *et al.* 1980; McCormick & Saunders 1987) in preparation for the higher growth rate at sea, hypoosmoregulation, and the migration. The fish will only grow well in seawater if they successfully complete the smolting process. There is no single measure of smolting but a number of parameters can be used to predict the future performance of the fish in seawater.

Growth rate of smolting coho and Atlantic salmon (*Salmo salar*) increases (Saunders & Henderson 1970; Clarke & Shelbourn 1986) and is higher than for non-smolting fish of comparable size under identical daylengths (Clarke & Shelbourn 1986; Björnsson *et al.* 1989). Even though long days may promote growth of both parrs and smolts (Brett 1979; Higgins 1985), the growth rate of smolts will be comparatively higher. Hence growth rate can be used as an indicator of smolting in studies where smolting and non-smolting fish of similar size are compared. Comparisons of growth rate of different sized fish is complicated by the inherent decrease in growth rate with size (Brett & Shelbourn 1975). That problem can be solved by appropriate transformation of the data (Jobling 1983).

During smolting, condition factor ($K = \text{weight} \times 100 / \text{length}^3$) tends to decrease (Wedemeyer *et al.* 1980). This formula, which is commonly used, suggests that weight increases as a function of the cube of length. This is an approximation for most species and the true value for coho parr is slightly above the power of three (Vanstone & Markert 1968). For a qualitative measure of weigth-length relationship, the exact power is irrelevant and for the sake of consistency a value of three was

used here. The decrease may be due to loss of energy reserves from increased catabolism during smolting (McCormick & Saunders 1987) and also from changes in the shape of the fish by elongation of the caudal penducle proportional to other parts of the body (Winans & Nishioka 1987). Condition factor has been used as an indicator of smolting for Atlantic salmon, steelhead trout (*Salmo gairdneri*), and coho (Vanstone & Markert 1968; Saunders & Henderson 1970; Wagner 1974). In coho the decrease may be masked by high feeding rates (W.C. Clarke pers. com.) and therefore not always observed.

During smolting the hypoosmoregulatory ability of salmon increases as the gill epithelial Na^+/K^+-ATPase activity is elevated and other changes occur (cfr. reviews Folmar & Dickhoff 1980; McCormick & Saunders 1987). The 24-h seawater challenge test gives a measure of the development of the osmoregulatory ability of coho salmon (Clarke 1982). After a challenge with 30 ppt seawater smolts will maintain low plasma sodium ion concentration (165-170 meq/l) while unsmolted fish will have higher plasma sodium concentrations (over 175 meq/l) after seawater challenge.

The most visible changes during smolting are the increased silvering of the fish and darkening of fin margins. The silvering results from deposition of purines (guanine and hypoxanthine) beneath scales and in a second deep dermal layer (Johnston & Eales 1967). These changes may be more dependent on size (Johnston & Eales 1968) and temperature (Johnston & Eales 1970) than photoperiod. Pigmentation may be of use as an additional indicator of the parr-smolt transformation, although by itself it is of limited value (Gorbman *et al.* 1982).

The indices for smolting used in this study were growth rate, performance on 24-h seawater challenges, condition factor and the silvering index (measure of silvering). The two former indices may be more reliable than the latter two. Smolting consists of a number of distinct processes whose endocrine mediators need not to be functionally linked (Simpson 1985) and therefore some of these processes may

not take place concurrently resulting in incomplete smolting if the fish are not ready to smolt or receive insufficient stimulation (McCormick & Saunders 1987). This emphasizes the need for different indices of smolting pertaining to the different processes. The indicators used in the present study were chosen to give a measure of different aspects of smolting.

Hoar (1965) describes the endocrine system as an important link between environmental changes and physiological changes in fish. Smolting is largely under the control of hormones such as thyroid hormones, growth hormone (GH), prolactin, and corticosteroids (Folmar & Dickhoff 1980; Barron 1986; Hoar 1988). The release of both thyroid hormones and growth hormone in salmonids appears to be affected by photoperiods (Clarke *et al.* 1978a; Grau *et al.* 1982; Sweeting *et al.* 1985; Björnsson *et al.* 1989).

1.3 Photoperiods and smolting.

Photoperiods appear to be the most important environmental cue for inducing and synchronizing the smolting process in salmonids, such as spring chinook salmon (*O. tshawytscha*), coho salmon, Atlantic salmon, steelhead trout (Hoar 1976; Wedemeyer *et al.* 1980; Clarke *et al.* 1989). Parr of these species smolt under increasing natural daylengths following short days provided that they have reached a certain developmental stage (Saunders & Henderson 1970; Wagner 1974; Knutsson & Grav 1976; Brauer 1982). Natural photoperiods are not a prerequisite and smolt characteristics will also develop after an abrupt increase in daylength (Clarke *et al.* 1989; Björnsson *et al.* 1989). The phase of the smolting cycle can be altered by accelerated (period less than 365 days), decelerated, and phase shifted photoperiod cycles (Wagner 1974; Brauer 1982; Clarke *et* al. 1985).

The development of smolting appears to be driven by an endogenous cycle, which is entrained by photoperiod. Although photoperiod affects the time of smolting there are results indicating that smolting will proceed in the absence of photoperiodic cues. Steelhead trout held under continuous darkness develop some smolt characteristics (Wagner 1974). Both Atlantic (Eriksson & Lundqvist 1982) and coho salmon (Lundqvist & Clarke 1989) held under constant 12 hour daylengths and constant temperature show seasonal cycles in condition factor and silvering. The duration of the cycles in Atlantic salmon was close to 10 months.

The photosensitivity of the daylength measuring mechanism which induces smolting is not known. Teleost fish may show nocturnal behaviour with an illumination as high as 0.1 lx (Byrne 1971; Eriksson 1978) although their visual threshold is much lower. The threshold level for visual feeding activity and schooling in Pacific salmon is between 10^{-4} and 10^{-3} lx (Ali 1959), which may represent the limits for scotopic vision. The threshold levels for neural responses in the pineal of the rainbow trout and the ayu (*Plecoglossus altivelis*) are between 10^{-5} and 10^{-4} lx (Morita 1966; Hanyu *et al.* 1978) but due to the absorption of the overlying tissues the actual sensitivity of the pineal is somewhat less.

Salmonids reside in fresh water for periods ranging from the completion of embryonic development to the entire life cycle (Randall *et al.* 1987). Only some species go through a marked parr-smolt transformation and the development of seawater tolerance in species that migrate as underyearlings such as pink (*O. gorbuscha*), chum (*O. keta*), and fall chinook (*O. tshawytscha*) salmon does not appear to be affected by photoperiods (Wagner *et al.* 1969; Hoar 1976; Clarke *et al.* 1989).

1.4. Models to explain photoperiodism.

Although photoperiodism has been demonstrated in many organisms, the details of the underlying mechanism remain to be elucidated. Most experiments describe secondary responses such as hibernation or reproductive stages rather than the actual mechanism for daylength perception.

Most models that have been proposed to account for the effects of photoperiods on seasonal cycles can be classified into three categories; "hour-glass", "external coincidence", and "internal coincidence" models.

"Hour-glass" models suggest that the organisms measure directly the duration of day or night through a mechanism that in principle resembles a hour-glass. It is envisaged as a reaction product that accumulates during one phase of the light cycle, but is broken down in the other. The "glass is turned" at the beginning of the phase being measured and the accumulated amount of the substance will depend on the length of that phase. The responses of the organisms in turn depend on the concentration of the substance. The hour-glass model has mainly been used to describe photoperiodism in terrestrial arthropods (Pittendrigh 1972; Lees 1973; Vaz Nunes & Veerman 1982, 1986) and lizards (Underwood 1981). In arthropods the duration of the night is generally measured (Lees 1973), while the lizards appear to measure daylength (Underwood 1981).

The "external coincidence" model was initially proposed by Bünning (1936) but later refined by Pittendrigh & Minis (1964; Pittendrigh 1972). According to this model daylength is measured through a light sensitive phase (ϕ_i) occurring daily. Stimulation by photoperiod depends on whether the organism is exposed to light during the sensitive phase. Under short days ϕ_i occurs during night, but during long days the ϕ_i is exposed to light. In this model the daily light cycle assumes two roles. First it may stimulate photoperiodic responses under the appropriate daylength and secondly, it

entrains the circadian cycle of ϕ_i to a period of 24 h. This kind of model is suggested for photoperiodism in the flesh fly (*Sarcophaga argyrostoma*) (Saunders 1981) and, as will be discussed later, it may also describe photoperiodism in fish.

The presence and the temporal location of ϕ_i can be demonstrated through skeleton photoperiods. These photoperiods consist of two light pulses separated by periods of darkness. In asymmetrical skeleton photoperiods the initial or "morning" pulse is longer (6-9 h.) while the later pulse generally ranges from 15 minutes to one hour. Skeleton photoperiods should be as effective as complete photoperiods of equal duration if exposure to light during ϕ_i is sufficient to stimulate photoperiodic responses.

One of the assumptions of the model is that ϕ_i occurs as an endogenous circadian rhythm and will therefore reappear for at least some cycles if the organism is in continuous light or darkness. There is an circadian component in external coincidence but not in hour-glass models and that distinguishes the two. The endogenous circadian rhythm means that responses do not depend on the total duration of either the light or dark phases. Exposure to light during every second or third occurrence of ϕ_i may be sufficient to induce responses but the total duration of the light or dark phases is crucial to hour-glass mechanisms. Experimental protocols to distinguish the two involve for example skeleton photoperiods with light cycles of 48 or 72 hours (Hamner 1964) and pulses occurring when ϕ_i is expected to occur.

An expansion of the external coincidence model has been proposed (Lewis & Saunders 1987), where the circadian rhythm of ϕ_i is described as a damped oscillation rather than of fixed amplitude. This model may accommodate cases that previously had been described by hour-glass mechanism by assuming that the circadian rhythm of ϕ_i declines within the first oscillation. Saunders and Lewis (1987a,b) propose this model as a unified hypothesis to account for photoperiodism.

The internal coincidence model also assumes the involvement of circadian rhythms but in a different way (Tyshchenko 1966; Pittendrigh 1972). In its simplest form it suggests that daylength measurements are accomplished through two circadian oscillators one of which is entrained by dawn and the other by dusk. Responses depend on the phase angle of the two oscillators, which is shifted by seasonal daylengths.

A more advanced version of the model has been proposed (Pittendrigh 1981). It was spurred by the increasing evidence that the vertebrate circadian system is composed of a hierarchy of circadian oscillators residing within different organs (Pittendrigh & Daan 1976). These oscillators are synchronized by a master phase maker (Pittendrigh *et al.* 1984) and the phase maker is in turn composed of two oscillators that are entrained by dawn and dusk (Pittendrigh 1981; Pittendrigh *et al.* 1984). Induction by photoperiods depends on the mutual phase relationship of all the oscillators, which may be altered through the pacemaker system. This model has been used to describe photoperiodism in some higher vertebrates (Boulos & Rusak 1982; Gwinner 1986) and a similar model has been proposed to explain seasonal cycles in killifish (Meier 1984).

There is no way to discriminate experimentally between the internal and external coincidence models (Follet *et al.* 1981; Saunders 1982; Pittendrigh 1984) and most cases that fit one model can also be described by the other, in fact they are not necessarily seen as mutually exclusive (Pittendrigh 1981, Pittendrigh *et al.* 1984). The external coincidence model focuses mainly on the daylength measuring mechanism, while the internal coincidence model attempts a more holistic approach by emphasizing the importance of mutual phase relations of a number of peripheral cycles.

1.5. Photoperiodism in fish.

The research on mechanisms behind photoperiodism in teleosts has mainly been on sexual maturation. Maturation in many species is affected by photoperiods, although it is an endogenous rhythm that will proceed in the absence of photoperiodic stimulation (Sundararaj *et al.* 1973; Day & Taylor 1984; Bromage *et* al. 1984).

Daylength measurements in teleosts appear to be accomplished through an endogenous rhythm of light sensitivity (ϕ_i). Skeleton photoperiods have induced maturation comparable to the corresponding complete photoperiods in sticklebacks (*Gasterosteus aculeatus*), catfish (*Heteropneustes fossilis*), medaka (*Oryzias latipes)*, mummichog (*Fundulus heteroclitus*), and rainbow trout (*Salmo gairdneri*) (Baggerman 1972; Sundararaj & Vasal 1976; Chan 1976; Duston & Bromage 1986) indicating a distinct period when the fish can be stimulated. Light cycles with a period longer than 24 h are stimulatory if the photophase (light on) is properly placed even after a scotophase (lights off) of 54 h (Baggerman 1972; Duston & Bromage 1986) indicating that ϕ_i is endogenous and can be stimulated even if the fish are not exposed to light during every cycle. Appropriately placed one hour pulses every 24 h also produce long-day responses (Sundararaj & Vasal 1976). All these responses may be explained by the external coincidence model.

The above results could also be explained by the internal coincidence model if it is assumed that instead of ϕ_i there is a specific stimulatory phase relationship between dawn and dusk oscillators. Some observations on the teleost circadian system may be difficoult to accomodate within the external coincidence model. There is evidence to indicate that the teleost circadian system consists of several oscillators that may be synchronized by the pineal (Kavaliers 1980). The threshold daylengths for stimulation of maturation in sticklebacks changes during winter (Baggerman

1980), indicating that more than one rhythm may be involved in daylength measurements. These observations may suggest that the internal coincidence can be as good working model for research on photoperiodism in fish as the external coincidence, although the two are not mutually exclusive.

1.5. Objectives.

The objective of this project was to study functional aspects of the mechanism for daylength measurement which induces smolting. The questions posed in the study focused on how daylengths are measured by coho parr and how that affects the photoperiodism of smolting. Do the same general principles apply as for the induction of sexual maturation in some other teleosts or do alternative explanations have to be sought? The questions and hypotheses posed are discussed further in the next chapter.

II. METHODS.

All experiments were performed at the Pacific Biological Station (PBS) in Nanaimo except measurements of optical density which were made at Simon Fraser University. The study included three experiments. Exp. 1 commenced on February 9, 1987 and ended on July 10, 1987. Exp. 2 and 3 ran concurrently, beginning May 4, 1987 and terminated September 24, 1987.

2.1. Experimental design.

2.1.1. Exp. 1.

A 3 x 3 factorial design provided nine combinations of initial and final photoperiodic treatments. During the priming period from February 9 to April 26 the fish were exposed to photoperiods of 6L:8D, 10L:14D, or 14L:10D (light:dark). During the inductive period from April 27 to July 10 the fish were exposed to 10L:14D, 16L:8D, or 9L:6D:1L:8D. For each treatment there were duplicates with 150 - 200 fish in each tank. In addition, 15 - 20 individually tagged fish were held with the untagged fish under each treatment.

Three hypotheses were tested with this experimental design:

1) Coho parr measure daylength by accumulating the total daily exposure to light.

2) Coho parr measure daylength through a phase of sensitivity to light occurring at specific time during the day.

3) Responses of coho parr to long inductive daylengths are independent of previous photoperiod exposure.

The first two hypotheses are juxtaposed in the experimental design by comparing the responses to the final photoperiods. The skeleton photoperiod 9L:6D:1L:8D has the same number of hours of light every day as 10L:14D, but the light is spread over the same period as in 16L:8D. The first hypothesis would be refuted if fish exposed to 9L:6D:1L:8D responded similarly to those on 16L:8D and better than those on 10L:14D. The second hypothesis would be refuted if only the groups exposed to 16L:8D smolted.

The third hypothesis is addressed by comparing the responses to each final photoperiod with reference to the initial treatment. If responses to a final photoperiod are the same regardless of which initial photoperiod the fish were exposed to, then the responses are independent of the initial treatment. If, however, the fish have to be exposed initially to daylengths that are under some threshold level in order to smolt, then the responses of groups which had different initial treatments will not be the same when exposed to identical final photoperiods.

At the end of the experiment, blood was sampled from the tagged fish for growth hormone analysis (See Appendix A).

2.1.2. Exp. 2.

The results of Exp. 1. suggested the existence of a photosensitive phase during which exposure to light will induce smolting. This experiment was conducted to establish the temporal location of the sensitive phase. The skeleton photoperiods of 9L:2D:1L:12D, 9L:5D:1L:9D, 9L:9D:1L:5D, and 9L:12D:1L:2D were used to stimulate smolting. They represent analogs to daylengths of 12, 15, 19, and 22 hours. Further, they form two pairs of photoperiods that are composed of identical periods of light and dark but arranged in different ways. Thus, 9L:2D:1L:12D and 9L:12D:1L:2D could be interpreted by the fish in the same way and so could 9L:5D:1L:9D and

9L:9D:1L:5D depending which of the light periods (9L or 1L) is perceived as the 'morning' pulse.

During the priming period from May 4 the fish were exposed to 10L:14D. The inductive period began on July 16 the fish were transferred to the skeleton photoperiods. For each treatment there were duplicates with 250 fish in each tank and 20-25 tagged fish were kept in separate tanks.

2.1.3. Exp. 3.

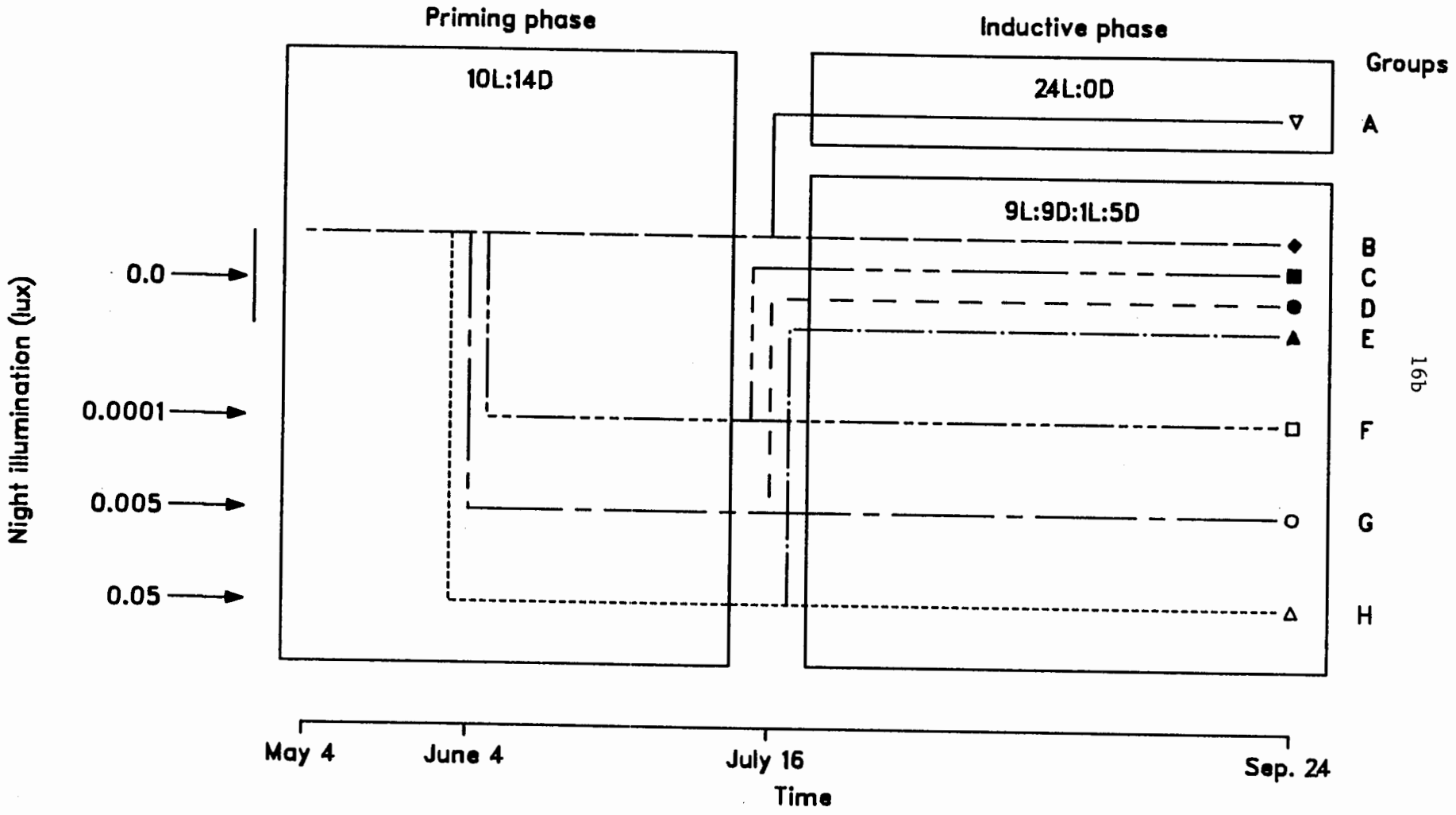
This experiment was conducted to answer the question: Does night illumination interfere with smolting and if so does it render the fishes incapable of smolting or does it interfere with their ability to smolt in response to longer daylengths?

The priming period began on May 4 when the fry were exposed to 10L:14D as their first regular photoperiod. On June 4 (Fig. 1) the fish were distributed among four rooms. The photoperiod remained 10L:14D but, in addition, some of the groups were exposed to low intensity light during the 'night'. The night illumination was: 0.0001 ± 0.00005 lx (groups C and F), 0.005 ± 0.001 lx (D and G), 0.05 ± 0.01 (E and H). A control (B) group and group A were held under no night illumination. On July 16 the photoperiods were changed and all groups except A were transferred to 9L:9D:1L:5D for the inductive phase. The night illumination for groups C - E was discontinued while F - H remained under their previous level of night illumination. Group A was transferred to continuous light (24L:0D) of 150 -200 lx.

If the night illumination during the priming period had made the fish incapable of smolting then the responses of groups (C - H) should be inferior to those of the control (B). However, if the night illumination interferes with the ability of the fish to smolt in response to longer daylengths, the responses of groups C - E should be similar to the control and better than those of groups F - H.

All groups except C, D and E were duplicate with 200 fish in each tank. Due to lack of tank space there were no replicate tanks for groups C - E. To make efficient use of tank space group B was shared between Exp. 2. and Exp. 3, thus skeleton photoperiods were used in this experiment rather than complete photoperiods.

Figure 1. Flow chart of the design of Exp. 3. Illumination was 150 lx during the 'lights on' (L) period and 0.05, 0.005, 0.0001 or 0.0 lx during the dark (D) periods. Lines within the same boxes denote groups on identical photoperiods (10L:14D; 9L:9D:1L:5D; 24L:0D). There were replicate tanks for each of groups A, B, F, G, and H, but single tanks for groups C, D, and E.


Priming phase
Inductive phase
10L:14D
24L:0D
9L:9D:1L:5D
Groups
A
B
C
D
E
F
G
H
Night illumination (lux)
0.0
0.0001
0.005
0.05
May 4
June 4
July 16
Sep. 24
Time

2.2. Experimental fish.

The fish that were used for the experiments were underyearling coho salmon (*Oncorhynchus kisutch*). All eggs were incubated at PBS - those used in Exp. 1 were obtained from Big Qualicum hatchery while eggs for Exp. 2 and 3 came from Inch Creek hatchery. Eggs for Exp. 1 were from an early spawning and their development was accelerated by incubating them at higher temperatures (10° C) than the eggs for Exp. 2 and 3. Hence the fry for Exp. 1 were ready for ponding in early February, whereas those used in Exp. 2 and 3 were not ready until May.

2.3. Rearing conditions.

2.3.1. Tanks.

At first feeding the fish were introduced to the experimental treatments and transferred to the rearing tanks. All tanks in Exp. 2 and 3 were 200 l oval tanks and so were half of the tanks used in Exp. 1. Replicate groups in Exp. 1 were kept in 500 l tanks. No effect of different tank volume was evident in Exp. 1. (see results) and therefore treatment groups kept in tanks of different volume were regarded as replicates. Every 2 to 3 days the tanks were cleaned and excess food removed.

2.3.2. Water flow and temperature.

A steady flow of 5-10 l/min of dechlorinated water was maintained through the tanks. In Exp. 1 the temperature was kept at 11° ± 0.4° C except during the last week when the temperature fluctuated ± 1.0° due to system failure. In Exp. 2 and 3 the temperature was maintained at 12.5° ± 0.5° C. All tanks were aerated.

2.3.3. Feed.

The fry were fed Oregon moist pellets® during the first few weeks and subsequently White Crest® dry feed. The amount of feed offered was in excess of satiation as indicated by accumulation of food remains on the bottom of the tanks. Feed was presented continuously from automatic feeders. In Exp. 1 all groups were fed for 6 hr every day, beginning as lights were turned on, but in Exp. 2 and 3 the daily feeding period was 9 hr.

2.3.4. Light and photoperiods.

Photoperiods were controlled by automatic timers that turned light on and off without a twilight period. Morning (lights on) was at 8 AM during both priming and inductive phases and the longer light period of the skeleton photoperiods began also at that time. Therefore daylengths were increased unidirectionally by turning lights off later at night. Each photoperiod was maintained in a separate light tight room. The light sources were daylight type Vita lite® fluorescent lamps that were suspended over the tanks and provided an illuminance of 100 - 200 lux at the water surface. In Exp. 3 some groups were exposed to low intensity light during the night. All these groups were kept in the same room where the night light was bounced of the ceiling and the illumination adjusted for each set of replicate tanks by screening them with black plastic covers.

2.4. Evaluation of responses.

The responses of the fish to different photoperiods were observed by monitoring their growth, performing 24-h seawater challenge tests, and by assessing their silvering index.

2.4.1. Growth.

At regular intervals the fish were measured and/or weighed. The fish were starved for at least 24 hr before they were measured or weighed. While measured the fish were anaesthetized in 500 ppm 2-phenoxy-ethanol. The length was measured to the nearest 0.1 cm and they were weighed to the nearest 0.1 g. The readings from the length measuring caliper and from the scale were fed directly to a computer. In Exp. 3 growth was monitored by weighing alone.

When both length and weight were measured condition factor (K) was calculated as:

$$K = \frac{\text{weight} \times 100}{\text{length}^3}$$

2.4.2. Individual growth rate.

In some groups individual growth rate was observed by tagging 20 - 30 fish with coded PIT-tags (Destron Identification Devices Inc.). The tags (length 10 mm, diam. 1.5 mm) were injected with a syringe into the abdomen of anaesthetized fish (size 2.6 - 8.7 g). The code of the tags was read with an electronic wand. Mortalities due to tagging were generally less than 5%.

Specific growth rate (SGR) for length and weight were calculated according to the formula of Brown (1946)

$$SGR = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \times 100$$

Where X_1 and X_2 are the length or the weight of the fish at time t_1 and t_2 respectively.

There is an inherent decrease in SGR with increasing size, but the above equation makes the approximation that over short intervals SGR remains constant. This has to be taken into account when the growth rate of fish of different sizes is compared. The natural logarithm (ln) of SGR for weight decreases linearily with the ln of weight and therefore a single slope can describe this decrease in fishes of different sizes (Brett & Shelbourn 1975; Jobling 1983). The effect of size can thus be resolved by comparing ln SGR for weight with ln weight as covariate and in EXP. 2 it was necessary to use this approach.

2.4.3. Seawater challenge.

Seawater adaptability was tested with 24 hr seawater challenge tests following procedures developed at PBS (Clarke 1982; Blackburn & Clarke 1987). Prior to challenge the fish were starved for 24 hr. Ten fish were sampled with a dipnet from each tank and transferred to 30 ppt seawater at the same temperature as the fish had been reared in (i.e. 11° in Exp. 1 and 12.5° in Exp. 2. and 3). After 24 hr the caudal penducle was severed and blood was collected into heparinized capillary tubes. Within 20 minutes the blood was spun down for 5 minutes in an Eppendorf centrifuge. The plasma section of the tubes was cut off, sealed, and kept refrigerated for one or two days until analyzed. The blood collecting procedure was always carried out between 8:30 and 11:30 AM. Only a half of the groups could be challenged each time so replicate groups were challenged on consecutive days.

Plasma sodium and potassium levels were analyzed in a flame photometer. Five-μl of plasma were diluted from each sample. To secure accuracy and to reduce interassay variance standards of 160 meq/l Na^+ were analyzed parallel to the

samples and sodium levels adjusted accordingly. To check the linearity of the photometer three series of standards containing 0, 50, 100, 150, 200, and 250 meq/l of Na^+ were included each time samples were analyzed.

Hemolysis decreases the concentration of plasma sodium. Therefore the sodium levels were corrected according to the following equation if high potassium concentration (>10 meq/l) indicated hemolysis.

$$[Na^+]_{corr} = [Na^+]_{obs} + 1.33 \times ([K^+] - 6)$$

This formula was derived empirically by Blackburn and Clarke (1987), by inducing hemolysis in blood samples with known sodium concentration.

2.4.4. Optical densitiy.

The results of Exp. 3 raised the question: Can the light transmission of the tissues over the pineal account for difference in sensitivity of zero-aged (0+) fry and older presmolts to night illumination? The optical density of the 'pineal window' (pw) was measured in yearling and underyearling coho. The optical density of patches of skin from the back of the fish was also measured to see if 1+ fish accumulated comparatively more pigment over the pineal area than underyearlings. Transmission (T) is the proportion of the incident light that is transmitted through a sample and optical density is $-\log_{10}(T)$. Optical density is a linear function of both concentration and thickness of a sample.

Ten 0+ fry (from PBS) and 14 presmolts (1+) from Capilano hatchery were used for these measurements. The fish were anaesthetized in 2-phenoxy-ethanol beyond recovery. Patches of skin and skull from between and posterior to the eyes were excised in one piece. When these are observed from below a circular area more translucent than the surrounding tissues is evident. The optical density of that area,

the pw, was measured. Another patch of skin was taken from the back laterally to the midline and anterior to the dorsal fin.

To mount the samples in the spectrophotometer a thin metal sheet with a circular aperture (diam 3 mm) was placed inside a cuvette in the middle of the light path. The samples were placed over the aperture and covered with a glass plate. This allowed the cuvette to be placed in the spectrophotometer with the sample fixed perpendicular to the light path. All components were painted black to reduce errors due to reflection. A Cary 14 spectrophotometer with a special scattering tranmission acessory was used for the measurements. The samples were placed near the photomultiplier tube to minimice loss of scattered light. The optical density was measured in the range between 400 and 700 nm.

2.4.5. Statistical analysis.

One-, two- or three-way analysis of variances (anova) were performed on the data depending on the experimental design. Means of groups and means of the level of each factor in the factorial designs were compared in a Tukey's Studentized range test (Sokal & Rohlf 1981) setting the significance limits at $p<0.05$. In Exp. 3 some preplanned comparisons were made among different groups and those are included in the anova tables.

Analysis of covariance (ancova) was used to adjust for effects of continuous variables on the variable being analyzed such as the inherent decrease in growth rate with weight. The adjusted mean squares were compared with least mean square procedures (SAS 1985).

Most experimental designs had replicates for each treatment. If a significant variance existed among replicate tanks (tank effect), the significance of the main

effects and their interactions were tested over the tank mean square rather than the residual mean square.

The statistical analyses were made on the mainframe computers of SFU and PBS using SAS (SAS 1985).

III. Results

3.1. Exp. 1.

3.1.1. Growth.

The SGR for length and weight were affected by initial ($p<0.0001$) and final ($p<0.0001$) photoperiods. The SGR of the groups exposed initially to long-days (14L:10D) was significantly (Tukey's test $p<0.05$) lower than of the short-day groups (Table 1). The mean SGR for groups exposed to different final photoperiods were all significantly different (Tukey's test $p=0.05$) with the highest in the 16L:8D groups, then the 9:6D:1L:8D, with the 10L:14D having the lowest growth rate (Table 1).

These results are consistent with the pattern of growth of the untagged fish. At the end of the priming period on April 27 there was a significant difference ($p<0.0001$) in length and weight of the untagged fish with the groups exposed to 6L:18D significantly (Tukey's test $p<0.05$) shorter and smaller than the fish under the other two initial treatments. However, at the end of the experiment, the long-day groups were significantly shorter and lighter than the short-day groups (Table 1). The mean size of the groups exposed to different final photoperiods also reflects the SGR with the 16L:8D groups being largest (Table 1) and the 10L:14D smallest.

When the SGR over the the four growth intervals were studied in a three-way anova (Initial photoperiod; Final photoperiod; Intervals) there were various significant interactions among the main effects. These interactions can be eliminated if the data from the short-day groups and long-day groups are considered separately. This indicates that most of the interactions are due to the different pattern of responses of the short- and long-day groups.

The SGR of the short-day groups was the same regardless of the initial photoperiods, but responses to final photoperiods were significantly different

($p<0.0001$) (Table 2, Figs. 2a and 3a). The average growth rates for length of groups on each final photoperiod were significantly different (Tukey's test $p<0.05$) with the 16L:8D group growing fastest (0.76%day^{-1}), then the 9L:6D:1L:8D (0.67%day^{-1}), and finally the 10L:14D (0.52%day^{-1}). The SGR for weight showed similar trends (Fig. 3a) although the responses of the 9L:6D:1L:8D groups were not significantly different from the 10L:14D. However, if the SGR for weight is adjusted for weight the SGR of the skeleton photoperiod groups, becomes significantly higher than for the 10L:14D, as the former were significantly heavier.

The SGR for length of the long-day groups was significantly different ($p<0.0001$) with the groups on 10L:14D growing significantly (Tukey's test $p<0.05$) slower than the other two (Fig. 2b). The SGR for weight however was not significantly different (Fig 3b).

There was no indication of bimodality in responses as the responses of the long-day groups were only slightly more variable (C.V. around 40%) than those of the short-day groups (C.V. 23-30%).

A transient decrease of condition factor after the photoperiods had been changed was evident for the short-day groups exposed to the final photoperiod of 16L:8D while other groups only showed minor changes (Fig. 4). On May 23 the mean condition factor of the groups was significantly different ($p<0.0001$) with 16L:8D significantly lower (Tukey's test $p<0.05$) than of the other two (Table 1) and the average condition factor of the long-day groups was significantly higher than of the short-day groups (Fig. 4, Table 1).

3.1.2. Seawater adaptability.

After the photoperiods were changed on April 27, the seawater adaptability of the short-day groups that were exposed to either 16L:8D or 9L:6D:1L:8D increased gradually as indicated by the lower plasma sodium levels after seawater challenge (Fig. 5). At some time all these groups reached sodium levels lower than 167 meq/l, while the concentration remained higher in the plasma of fish exposed initially to long-days or to 10L:14D as final photoperiods (Fig. 5). Seawater adaptability was affected both by initial ($P<0.0001$) and final ($p<0.0001$) photoperiods. The short-day groups had significantly lower plasma sodium levels (Tukey's test $p<0.05$) than the long-day groups (Table 3). The groups exposed to the final photoperiods of 16L:8D and 9L:6D:1L:8D had significantly lower sodium levels than the 10L:14D groups (Table 3). There was no significant tank effect ($p<0.15$) and therefore replicate tanks were pooled.

As for the growth data, the plasma sodium data for the short-day groups were analyzed separately to eliminate interactions arising from different patterns of responses of long- and short-day groups. The effects of the two initial short-day photoperiods were not significantly different (Table 3 and 4). The average responses to the final photoperiods were all significantly different (Tables 3 and 4) with the 16L:8D having lowest sodium levels, the 9L:6D:1L:8D intermediate, and the 10L:14D highest.

The rate of response of the short-day groups under 9L:6D:1L:8D appears to have been slower than for the 16L:8D groups (Fig. 5a,b). This may be partly reflected by the significant interactions (Table 3) between time of challenge and final photoperiod. There were no indications of bimodality in the responses as C.V. for all groups was close to 5%.

There were significant differences among the responses of all the long-day groups to the final photoperiods ($P<0.0001$). The groups on the skeleton photoperiod had the lowest and the 16L:8D the highest average sodium levels (Table 3b, Fig. 5c).

3.1.3. Silvering index.

The fish exposed initially to short-day treatment and then to 16L:8D showed the greatest change in appearance and assumed the coloration typical of smolts (Table 1). Some of the fish that were pretreated with short-day and then exposed to the skeleton photoperiods were classified as having smolt appearance (silvering index 3) but most of them remained in an intermediate state (silvering index 2). The silvering index of the fish pretreated with 14L:10D was significantly lower than that of the other groups (Table 1).

Figure 2 a,b. SGR for length in Exp. 1. a) Short-day groups (initial photoperiods 6L:18D or 10L:14D). b) Long-day groups (initial photoperiod 14L:10D). Groups identified with the same letter are not significantly different.

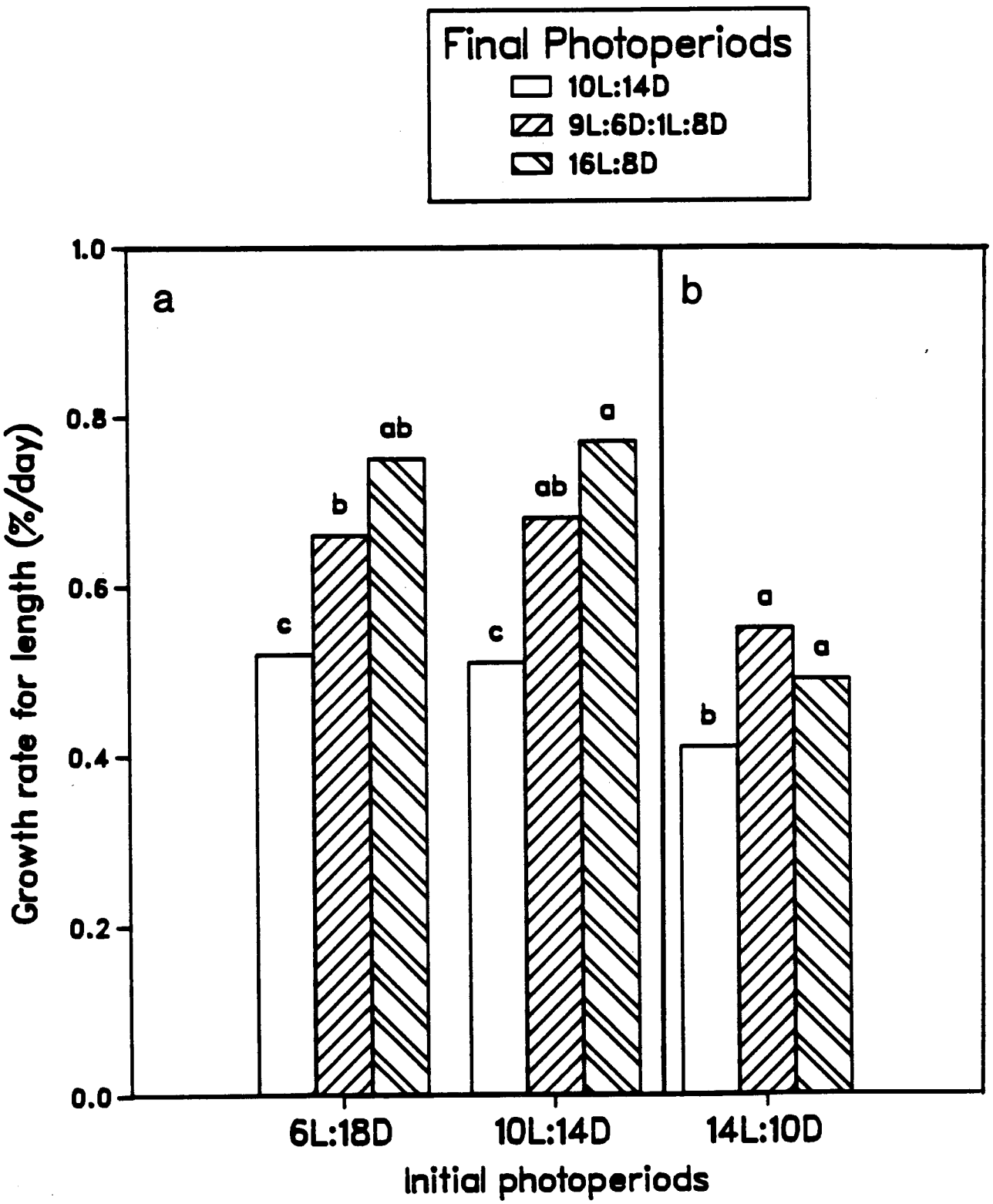

Final Photoperiods
10L:14D
9L:6D:1L:8D
16L:8D
1.0
0.8
0.6
0.4
0.2
0.0
a
b
c
b
ab
c
ab
a
b
a
a
Growth rate for length (%/day)
6L:18D
10L:14D
14L:10D
Initial photoperiods

Figure 3 a,b. SGR for weight in Exp. 1. a) Short-day groups (initial photoperiods 6L:18D or 10L:14D). b) Long-day groups (initial photoperiod 14L:10D). Groups identified with the same letter are not significantly different.

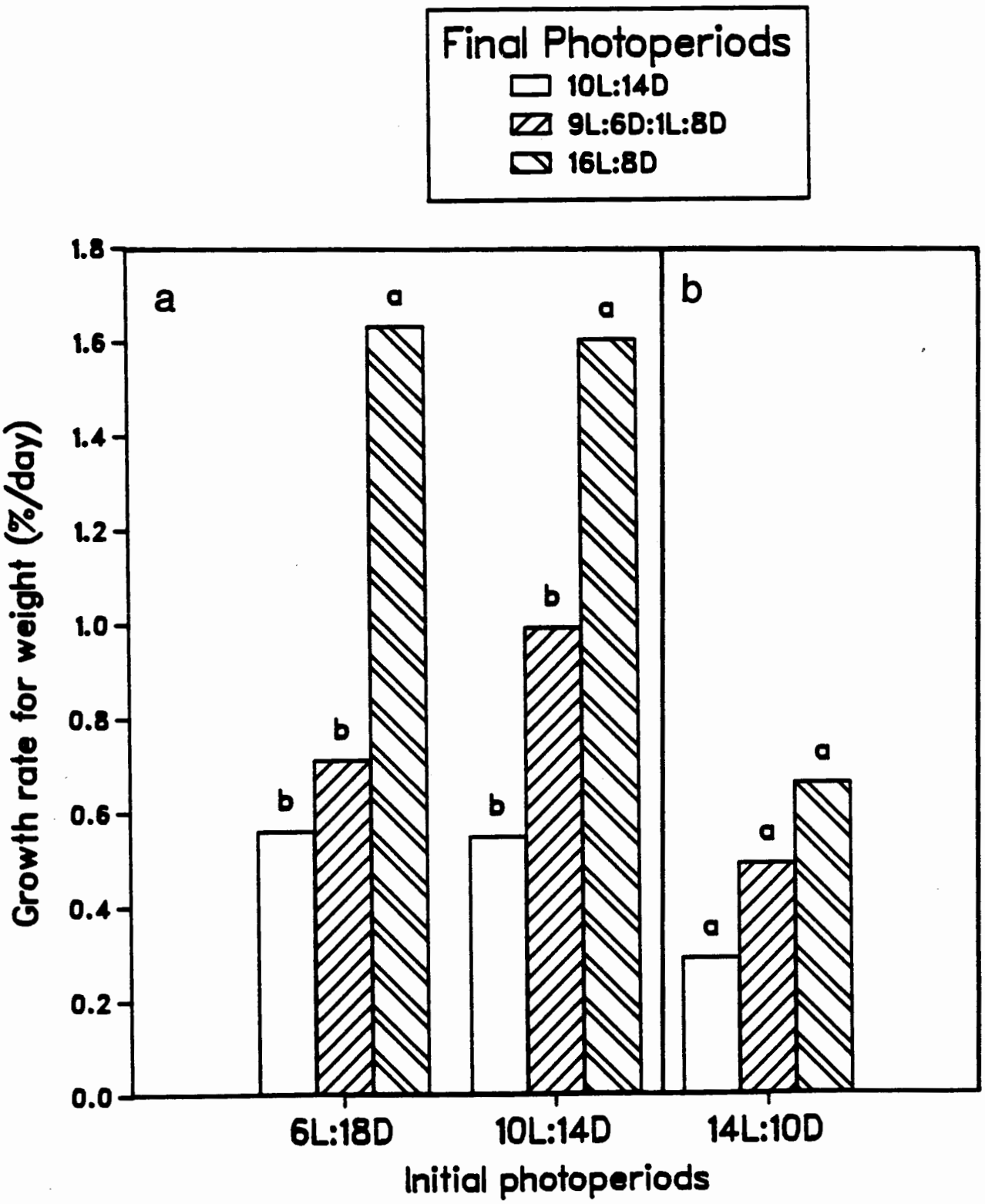

Final Photoperiods
10L:14D
9L:6D:1L:8D
16L:8D
Growth rate for weight (%/day)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
b
a
a
b
b
b
b
a
a
a
6L:18D
10L:14D
14L:10D
Initial photoperiods

Figure 4 a-c. Condition factor of fish in Exp. 1. a) Initial photoperiod 6L:18D. b) Initial photoperiod 10L:14D. c) Initial photoperiod 14L:10D. Triangles: final photoperiod 10L:14D; circles: final photoperiod 9L:6D:1L:8D; squares: final photoperiod 16L:8D.

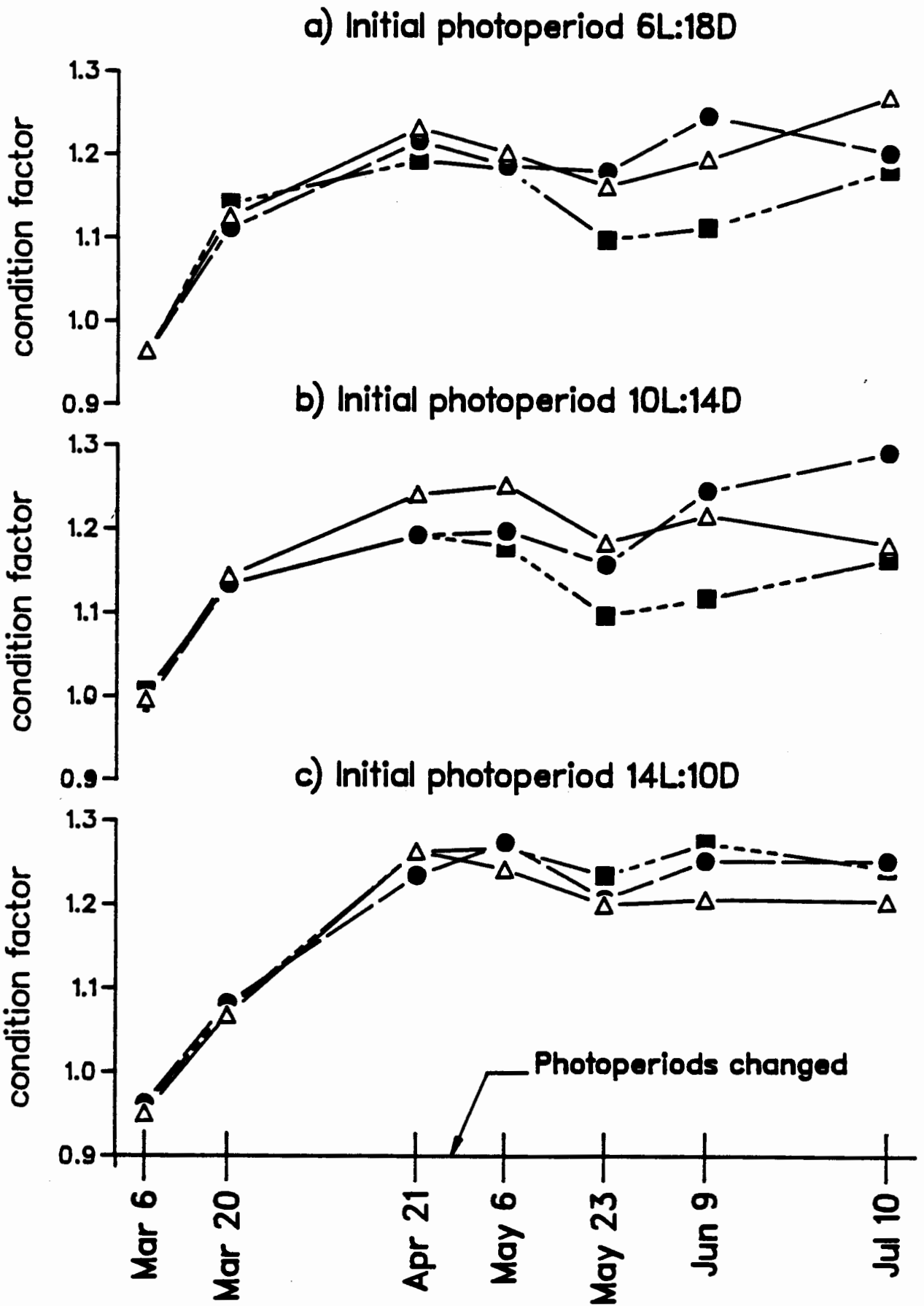
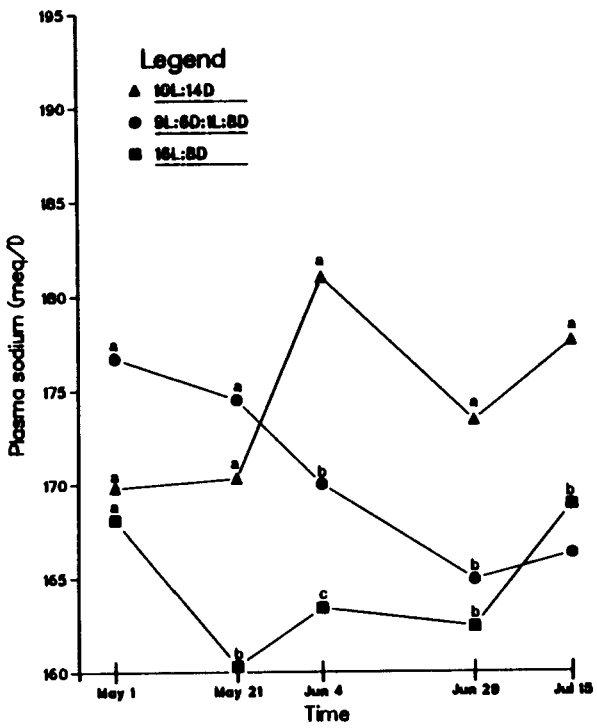
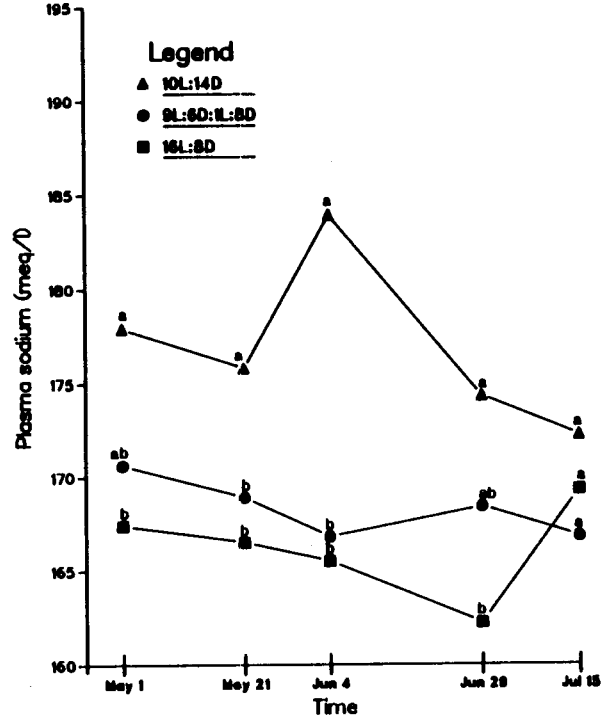

a) Initial photoperiod 6L:18D
b) Initial photoperiod 10L:14D
c) Initial photoperiod 14L:10D
condition factor
1.3
1.2
1.1
1.0
0.9
Photoperiods changed
Mar 6
Mar 20
Apr 21
May 6
May 23
Jun 9
Jul 10

Figure 5 a-c. Plasma sodium concentration after 24-h seawater challenge in Exp. 1. a) Initial photoperiod 6L:18D. b) Initial photoperiod 10:14D. c) Initial photoperiod 14L:10D. Points on each date for each initial photoperiod that are identified with the same letter are not significantly different.

a) Initial photoperiod 6L:18D



b) Initial photoperiod 10L:14D



c) Initial photoperiod 14L:10D

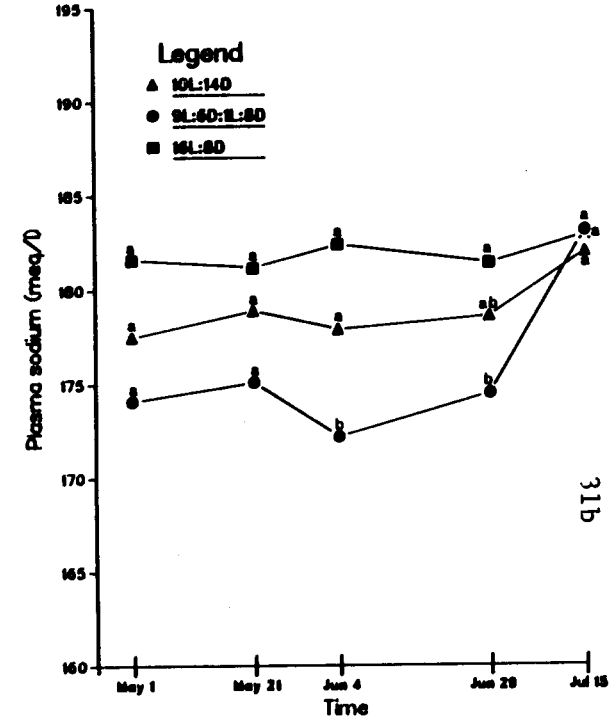


Table 1. Final size, weight, and silvering index, condition factor on May 23 of untagged fish and SGR for length and weight of tagged fish in Exp. 1.

a) Mean values for groups exposed to different initial photoperiods.

	6L:18D	10L:14D	14L:10D
N	108	161	74
Length (cm)	10.5^{y}	10.9^{x}	9.6^{z}
Weight (g)	14.4^{y}	16.6^{x}	11.1^{z}
Condition factor	1.19^{x}	1.19^{x}	1.24^{y}
Silvering index	2.3^{x}	2.4^{x}	2.0^{y}
SGR length (%day^{-1})	0.66^{x}	0.66^{x}	0.48^{y}
SGR weight (%day^{-1})	1.02^{x}	1.08^{x}	0.47^{y}

b) Mean values for groups exposed to different final photoperiods.

	16L:8D	9L:6D:1L:8D	10L:14D
N	117	136	90
Length (cm)	11.0^{x}	10.5^{y}	9.8^{z}
Weight (g)	16.5^{x}	14.9^{y}	12.1^{z}
Condition factor	1.16^{x}	1.25^{z}	1.21^{y}
Silvering index	2.5^{x}	2.2^{y}	2.0^{z}
SGR length (%day^{-1})	0.68^{x}	0.63^{x}	0.48^{y}
SGR weight (%day^{-1})	1.29^{x}	0.73^{y}	0.44^{z}

* Groups identified with different superscripts are significantly different by Tukeys-test ($p = 0.05$).

Table 2. Anova for specific growth rate for length of short-day (initial photoperiod 6L:18D or 10L:14D) in Exp. 1.

Source of variation	df	SS	F	Pr>F
Intervals	3	0.00101857	111.6	0.0001
Initial photoperiod	1	0.00000001	0.0	0.9585
Final photoperiod	2	0.00030263	49.7	0.0001
Int. x In.p.	3	0.00001569	1.7	0.1632
Int. x Fi.p.	6	0.00000437	0.2	0.9633
In.p. x Fi.p.	2	0.00000032	0.1	0.9482
Int. x In.p. x Fi.p.	6	0.00001384	0.8	0.6035
Error	306	0.00093127		
Total	329	0.00228669		

* Interactions are indicated by 'x'.

Table 3. Mean plasma sodium in meq/l (SE) from five 24h seawater challenge tests.

a) Short-day groups (initial photoperiods 6L:18D or 10L:14D).

Initial	Final photoperiod			
php.	16L:8D	9L:6D:1L:8D	10L:14D	Average
6L:18D	165.2(0.7)	170.8(0.9)	174.5(0.9)	170.2^{x}
N	101	108	99	
10L:14D	167.3(0.7)	168.9(0.7)	177.1(0.7)	171.1^{x}
N	102	101	100	
Average	166.2^{a}	169.9^{b}	175.8^{c}	

b) Long-day groups (initial photoperiod 14L:10D).

14L:10D	182.3^{a}(0.7)	175.8^{b}(0.8)	179.1^{c}(0.7)	179.1^{y}
N	105	99	98	

* Average values with identical superscripts are not significantly different by Tukey's Studentized range test at the 0.05 level.

Table 4. Anova for seawater challenge in of short-day (initial photoperiod 6L:18D or 10L:14D) groups in Exp. 1.

Source of variation	df	SS	F	Pr>F
Time of challenge(T.o.c.)	4	1339.4	6.4	0.0001
Initial photoperiod(In.p.)	1	123.6	2.4	0.1255
Final photoperiod(Fi.p.)	2	9458.8	90.1	0.0001
T.o.c. x In.p.	4	277.1	1.3	0.2613
T.o.c. x Fi.p.	8	4111.2	9.8	0.0001
In.p. x Fi.p.	2	580.0	5.5	0.0042
T.o.c x In.p. x Fi.p	8	1696.8	4.0	0.0001
Tank effect	6	592.9	1.9	0.0817
Error	575	30182.2		
Total	610	48361.9		

* Interactions are indicated by 'x'.

3.2. Exp. 2.

3.2.1. Growth.

The tagged fish under 9L:5D:1L:9D grew faster than any other group. (Fig. 6 and 7). A two-way anova of growth rate over the three intervals showed no significant difference in SGR. To adjust for decreased growth rate with increasing size the natural based (ln) logarithm of growth rate was analyzed with ln weight as the covariate (Brett & Shelbourn 1975; Jobling 1983). The adjusted SGR of the 9L:5D:1L:9D was significantly higher than the growth rate of any other group ($p<0.001$), while other groups were not significantly different.

A common slope of -0.34 described how ln SGR for weight was reduced as a function of ln weight. There was not a significant difference among the slopes of individual groups.

The growth rate of all the untagged groups except the 9L:5D:1L:9D appears to have been similar to the SGR of the tagged fish (Fig. 8). The weight of the untagged groups under 9L:2D:1L:12D and 9L:5D:1L:9D was significantly higher than that of the other two groups. No depression of condition factor was associated with the change in photoperiod.

3.2.2. Seawater adaptability.

Apart from the 9L:12D:1L:2D fish all groups showed increased seawater adaptability after the photoperiods were changed (Fig. 9, Table 5). The plasma sodium concentration of the 9L:12D:1L:2D fish remained around 175 meq/l while the minimum levels in the other groups were around 165 meq/l. When the sodium concentrations after all challenges were compared in an anova (Table 6) the mean values were significantly different with that of the 9L:12D:1L:2D (Tukey's test $p<0.05$) higher than 9L:2D:1L:12D (Table 5).

There was a significant tank effect and therefore the significance of the main effects were tested over the nested mean square rather than the residual mean square.

3.2.3 Silvering index.

There was a significant ($p<0.03$) difference among the silvering indexes of groups on different photoperiods (Table 5). The silvering index of the group on 9L:2D:1L:12D was highest and significantly higher than the silvering index of the group on 9L:12D:1L:2D.

Figure 6. SGR for length in Exp. 2. Groups identified with the same letter on each growth interval were not significantly different.

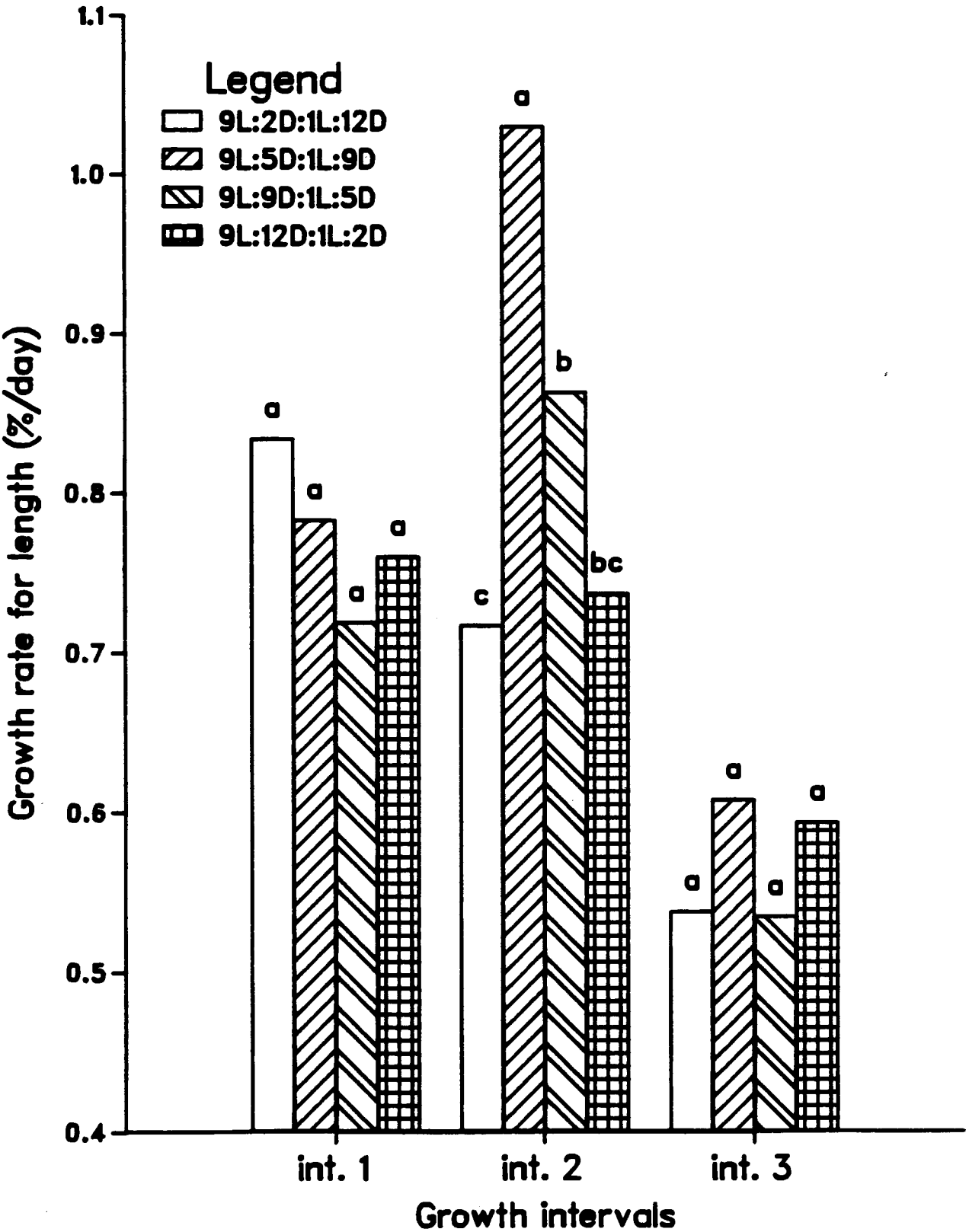

Legend
9L:2D:1L:12D
9L:5D:1L:9D
9L:9D:1L:5D
9L:12D:1L:2D
Growth rate for length (%/day)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
a
a
a
a
c
a
b
bc
a
a
a
int. 1
int. 2
int. 3
Growth intervals

Figure 7. SGR for weight in Exp. 2. Groups identified with the same letter on each growth interval were not significantly different.

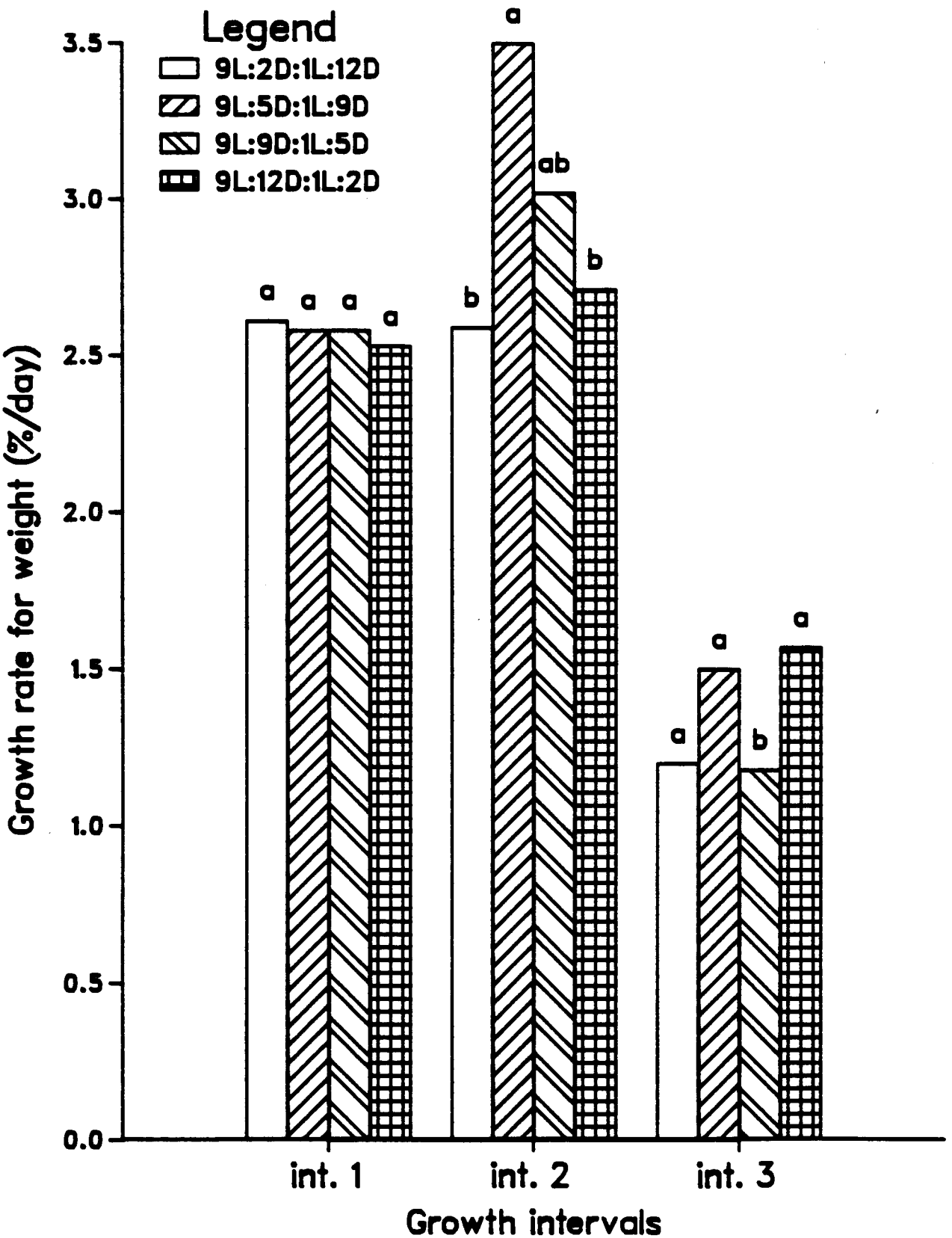

Legend
9L:2D:1L:12D
9L:5D:1L:9D
9L:9D:1L:5D
9L:12D:1L:2D
Growth rate for weight (%/day)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
a a a
b
a
ab
b
a
a
b
a
int. 1
int. 2
int. 3
Growth intervals

Figure 8. Weight of untagged fish in Exp. 2 (open symbols) and final weights of tagged fish (closed symbols). Means on each date that are identified with the same letter are not significantly different.

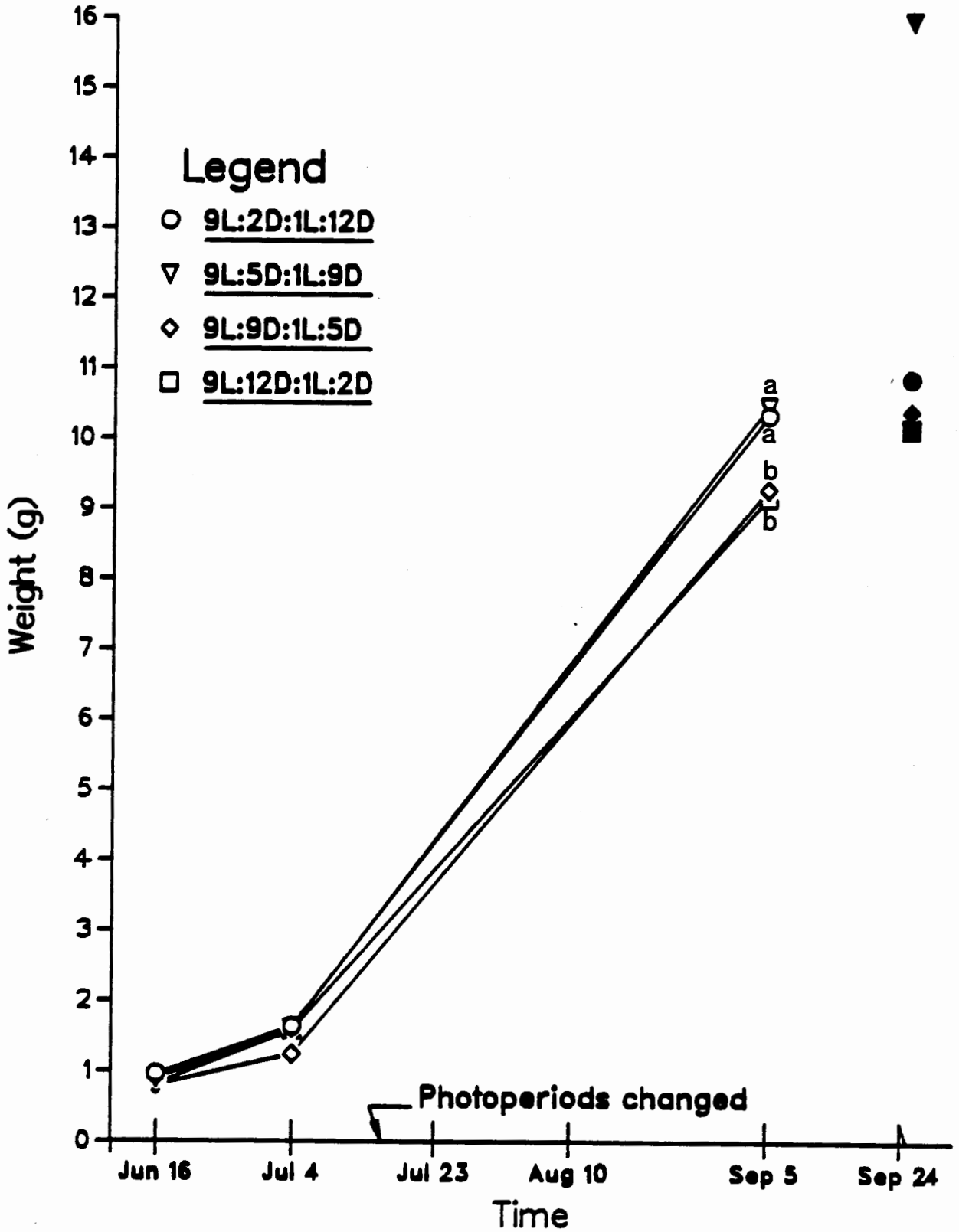

Legend
9L:2D:1L:12D
9L:5D:1L:9D
9L:9D:1L:5D
9L:12D:1L:2D
Weight (g)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
a
a
b
b
Photoperiods changed
Jun 16
Jul 4
Jul 23
Aug 10
Sep 5
Sep 24
Time

Figure 9. Plasma sodium concentration after 24-h seawater challenge in Exp. 2. Means on each date that are identified with the same letter are not significantly different.

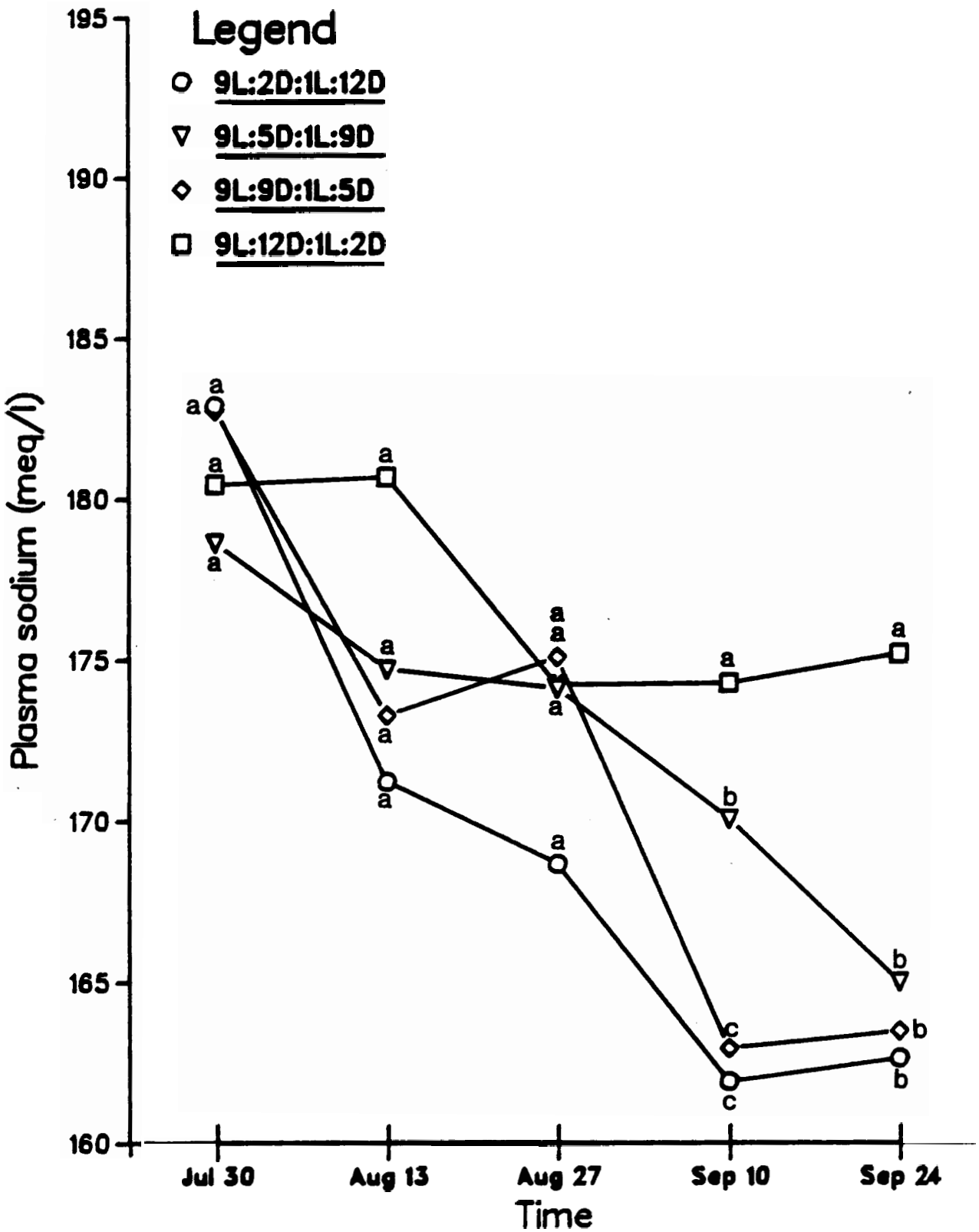

Legend
9L:2D:1L:12D
9L:5D:1L:9D
9L:9D:1L:5D
9L:12D:1L:2D
Plasma sodium (meq/l)
195
190
185
180
175
170
165
160
Jul 30
Aug 13
Aug 27
Sep 10
Sep 24
Time

Table 5. Mean plasma sodium ion concentration after seawater challenges, and final silvering index in Exp 2.

Final photoperiod	Sodium (meq/l)	Silvering index
9L:2D:1L:12D	169.5[a]	2.75[a]
9L:5D:1L:9D	171.7[ab]	2.4[ab]
9L:9D:1L:5D	171.3[ab]	2.5[ab]
9L:12D:1L:2D	176.9[b]	2.3[b]

* Means identified with same superscript are not significantly different.

Table 6. Anova of plasma sodium ion levels after seawater challenge tests in Exp. 2.

Source of variation	df	SS	F	Pr>F
Time of challenge(T.o.c.)	4	9904.09	36.03	0.0001
Photoperiods(Ph)	3	2911.61	14.12	0.0001
(T.o.c x Ph.)	12	3217.29	3.90	0.0001
Replicate tanks**	20	4352.44	3.17	0.0001
Error	347	23846.69		
Total	38	44232.12		

* Interactions are indicated by 'x'.

** As the nested effect is significant the F for the main effects and the interaction are computed over the mean square of replicate tanks.

3.3. Exp. 3.

The results from this experiment were analyzed in two parts. To examine the effect of different levels of night illumination (Fig. 1) group B (no night illumination) and groups F - H (night illumination during both priming and inductive phase) were compared. To study whether night illumination affects smolting during the priming or inductive phase groups F - H were compared with groups C - E (night illumination only during priming phase). Group A was studied with reference to B to provide information on the effect of continuous light (24 h) after a priming phase with total darkness during the night period.

3.3.1. Growth.

During the first two months of the experiment, groups exposed to night illumination grew faster than the control (B) group (Fig. 10). On July 4 (Fig. 10) there was a significant difference in the weight of the groups ($p<0.0001$) with the control group significantly (Tukey's test $p<0.05$) smaller than F, G, or H.

After the fish were exposed to the inductive photoperiods the control group grew faster than those exposed previously to night illumination Fig. 10. On September 24 (Table 7a) the control groups was significantly ($p<0.0001$) larger than the other groups (Fig. 10).

Group A showed a similar increase in growth rate as group B after the photoperiods had been changed. There is no information on the weight of group A on September 24 but on September 5 there was no significant difference between those two groups (Fig. 10).

There was no significant difference among groups that were exposed to different levels of night illumination (F - H) or among the groups that received night illumination at different times (C-E vs. F-H) (Table 7b) .

There was no significant variation in weight between tanks within the treatment groups and consequently replicate tanks were pooled.

3.3.2. Seawater adaptability.

The seawater adaptability of both the control (B) and group A increased after the fish were exposed to the inductive photoperiods (Fig. 11a) as indicated by the low plasma sodium levels after seawater challenge. The responses of those groups were not significantly different. The groups that were exposed to night illumination did not perform as well in seawater challenges and had plasma sodium levels around 175 meq/l or higher (Fig. 11 a,b). The mean responses of the groups on night illumination (F - H) were significantly ($p<0.0001$) higher than those of the control group (Table 8a). The mean sodium levels of the group that experienced the lowest levels of night illumination (F) were significantly ($p<0.02$) lower than of the other two (G and H) (Table 8a). The mean sodium concentration of the groups exposed to night illumination only during the priming phase (C - E) was significantly lower than the mean levels of F - H (Table 8b).

There was a significant tank effect on sodium levels after seawater challenge tests ($p<0.0001$) and therefore the significance of the main effects were tested over the mean square of the nested (tank) effect (Table 8a,b).

3.3.3. Silvering index.

There was a significant difference among the silvering indexes of all the groups on September 24. Group A and the control group had significantly higher silvering index than G or H (Table 9). There was also a significant difference between the mean silvering index of groups C - E and F - H.

Figure 10. Weight of fish in Exp. 3. Only final weights are shown for groups C - E. Means of each date that are identified with the same symbol are not significantly different.

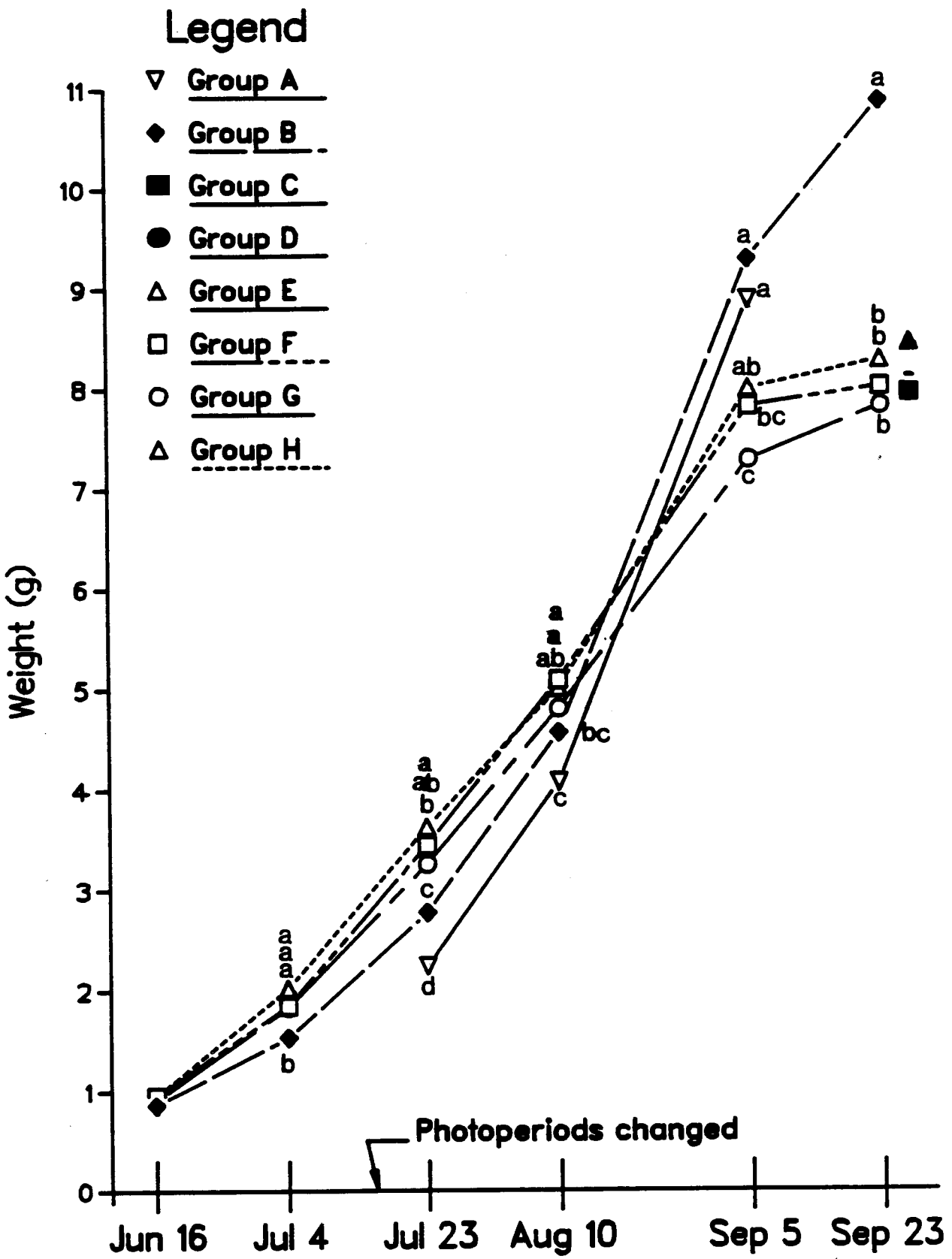
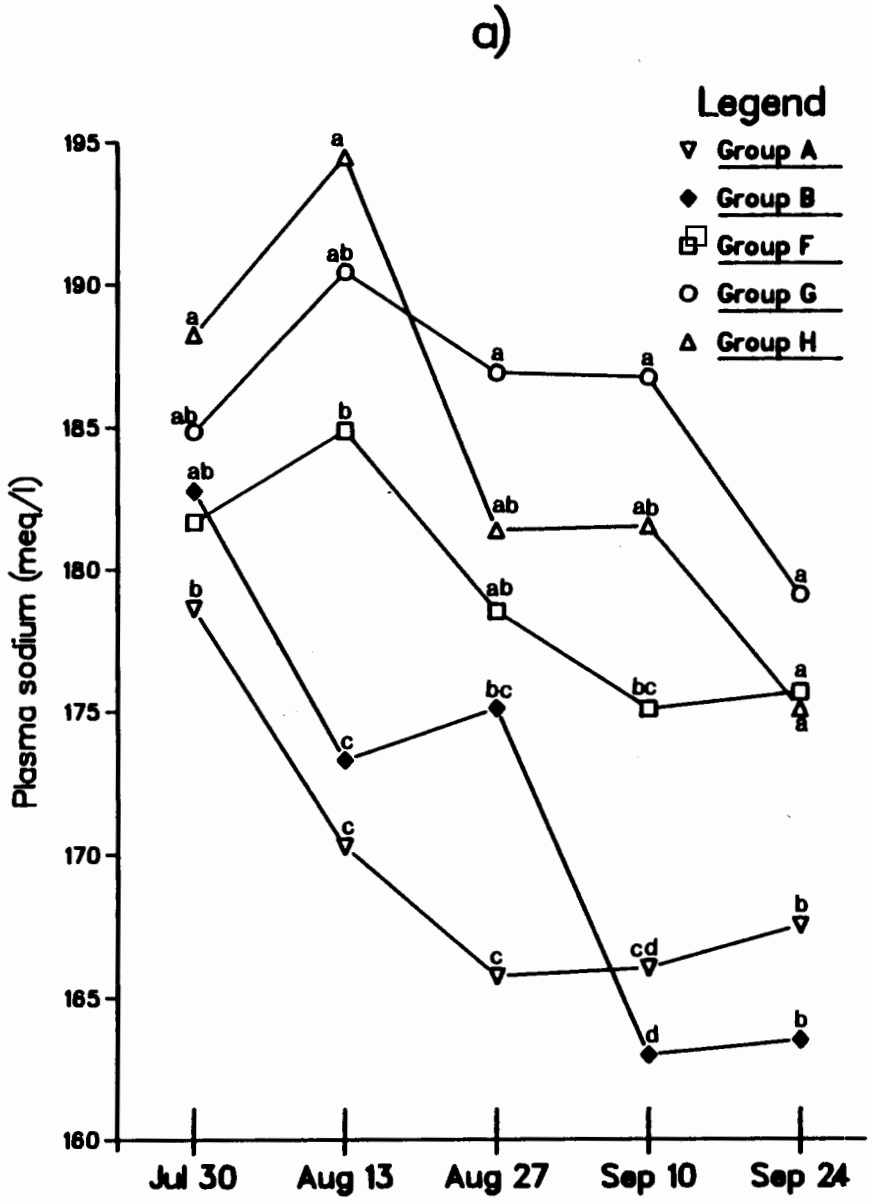

Legend
Group A
Group B
Group C
Group D
Group E
Group F
Group G
Group H
Weight (g)
0
1
2
3
4
5
6
7
8
9
10
11
Photoperiods changed
Jun 16
Jul 4
Jul 23
Aug 10
Sep 5
Sep 23

Figure 11 a,b. Plasma sodium concentration after 24-h seawater challenge in Exp. 3. a) Groups A, B, F, G, and H. b) Groups C, D, and E. Means on each date identified with the same letter were not significantly different.


a)
Legend
Group A
Group B
Group F
Group G
Group H
Plasma sodium (meq/l)
195
190
185
180
175
170
165
160
Jul 30
Aug 13
Aug 27
Sep 10
Sep 24

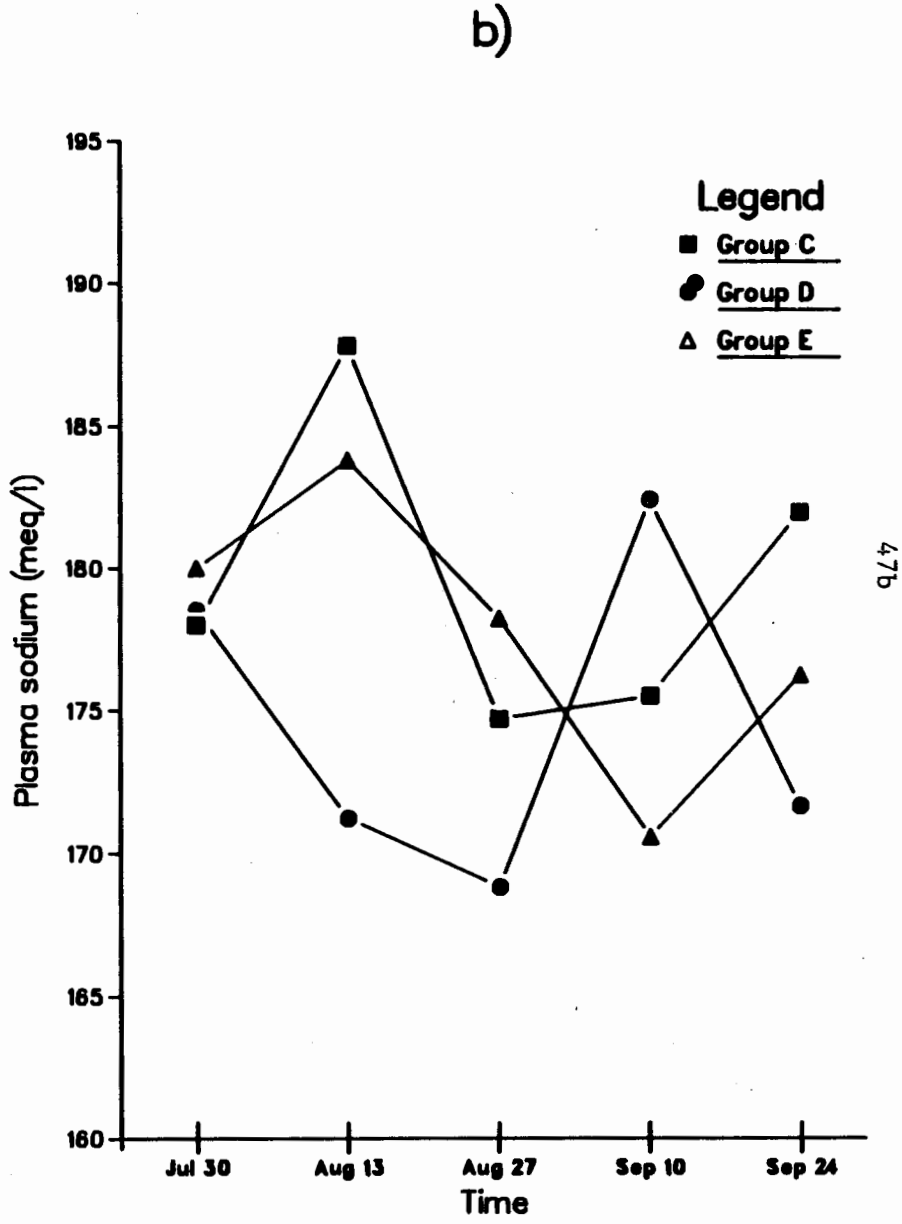

b)
Legend
Group C
Group D
Group E
Plasma sodium (meq/l)
195
190
185
180
175
170
165
160
Jul 30
Aug 13
Aug 27
Sep 10
Sep 24
Time

Table 7. Anova of final weight of fishes in Exp. 3.

a) Groups B, F, G, and H.

Source of variation	df	SS	F	Pr>F
Level of				
night illumination	3	986.02	25.26	0.0001
Planned comparisons				
B vs F - H	1	890.40	68.42	0.0001
F vs G - H	1	1.20	0.10	0.7557
G vs H	1	1.67	0.13	0.7215
Tank effect	4	86.01	1.65	0.1597
Error	541	7040.63		
Total	348	8112.66		

b) Groups C - H.

Source of variation	df	SS	F	Pr>F
Between rooms	3	98.31	1.29	0.2654
Planned comparisons				
C - E vs F - H	1	17.99	1.18	0.2772
C vs D - E	1	1.74	0.11	0.7351
D vs E	1	20.23	0.13	0.2492
Tank effect	3	77.83	1.71	0.1648
Error	447	6794.16		
Total	455	6970.31		

Table 8. Anova tables for plasma sodium levels after 24h seawater challenge in Exp. 3.

a) Groups B, F, G, and H.

Source of variation	df	SS	F	Pr>F
Time of challenge(T.o.c.)	4	8854.8	8.9	0.0003
Level of night illumination(L.o.n.i.)	3	12210.2	16.3	0.0001
Planned comparisons				
B vs F - H	1	9867.7	39.6	0.0001
F vs G - H	2	2348.5	4.7	0.0211
(T.o.c x L.o.n.i.)	12	4067.6	1.4	0.2624
Tank effect**	20	4984.1	2.8	0.0001
Error	369	33006.2		
Total	408	63101.0		

b) Groups C - H.

Source of variation	df	SS	F	Pr>F
Time of measurement	4	4788.0	4.0	0.0003
Among groups	5	24201.0	16.3	0.0001
Planned comparisons				
C - E vs F - H	1	3290.0	11.1	0.0045
C vs D - E	2	664.0	1.1	0.3514
(Tom x Groups)	20	4920.0	0.8	0.6539
Tank effect	15	4446.0	2.8	0.0001
Error	413	34196.4		
Total	408	72551.4		

*Interaction is indicated with a 'x'.

**There was a significant variance between tanks within treatments so the mean square of the tank effect is used instead of the residual mean square for F-tests.

Table 9. Mean plasma sodium concentration after the five seawater challenges and the silvering index on September 24 in Exp. 3.

Groups (Night illumination)	Silvering index	Sodium (meq/l)
A***	2.5[a]	169.9
B (0.0 lx)	2.5[a]	171.6
C (0.0001 lx)****	2.1[ab]	179.4
D (0.005 lx)****	2.0[ab]	174.4
E (0.05 lx)****	2.0[b]	177.7
F (0.0001 lx)	2.1[ab]	179.1
G (0.005 lx)	1.9[b]	185.6
H (0.05 lx)	1.9[b]	184.2

*For comparisons of mean sodium values consult Table 8 a) and b).

**Silvering indexes that have identical superscripts were not significantly different by Tukey's test.

***After July 16 continuous (24L:0D) 150 lx.

****Night illumination only during priming phase.

3.4. Optical density of the pineal window and skin from the back of the fish.

At all wavelengths the optical density of both the the pineal window (pw) and the skin from the back was significantly higher in the 1+ fish than for the 0+ fish. (Fig. 12).

Regression of the optical density of the pw over fork length (Fig. 13) was significant and the optical density at 500 nm can be described as

$$A_{500} = 0.176 + 0.061 \times \text{length}$$

$$(R^2 = 0.70)$$

The optical density of the pw of the two age groups was compared in an analysis of covariance to see if the optical density in the 1+ fish was higher than expected from size differences alone (Table 10). There were no significant differences between the age groups when the optical density was adjusted for length.

To see if the optical density of the pw proportional to the patch from the back was higher in the 1+ fish, a two-way anova was conducted (Table 11) but there was no significant interaction between age and locations.

Figure 12. Optical density of the 'pineal window' area and a skin patch from the back of 0+ and 1+ coho salmon. Vertical bars show SEM.

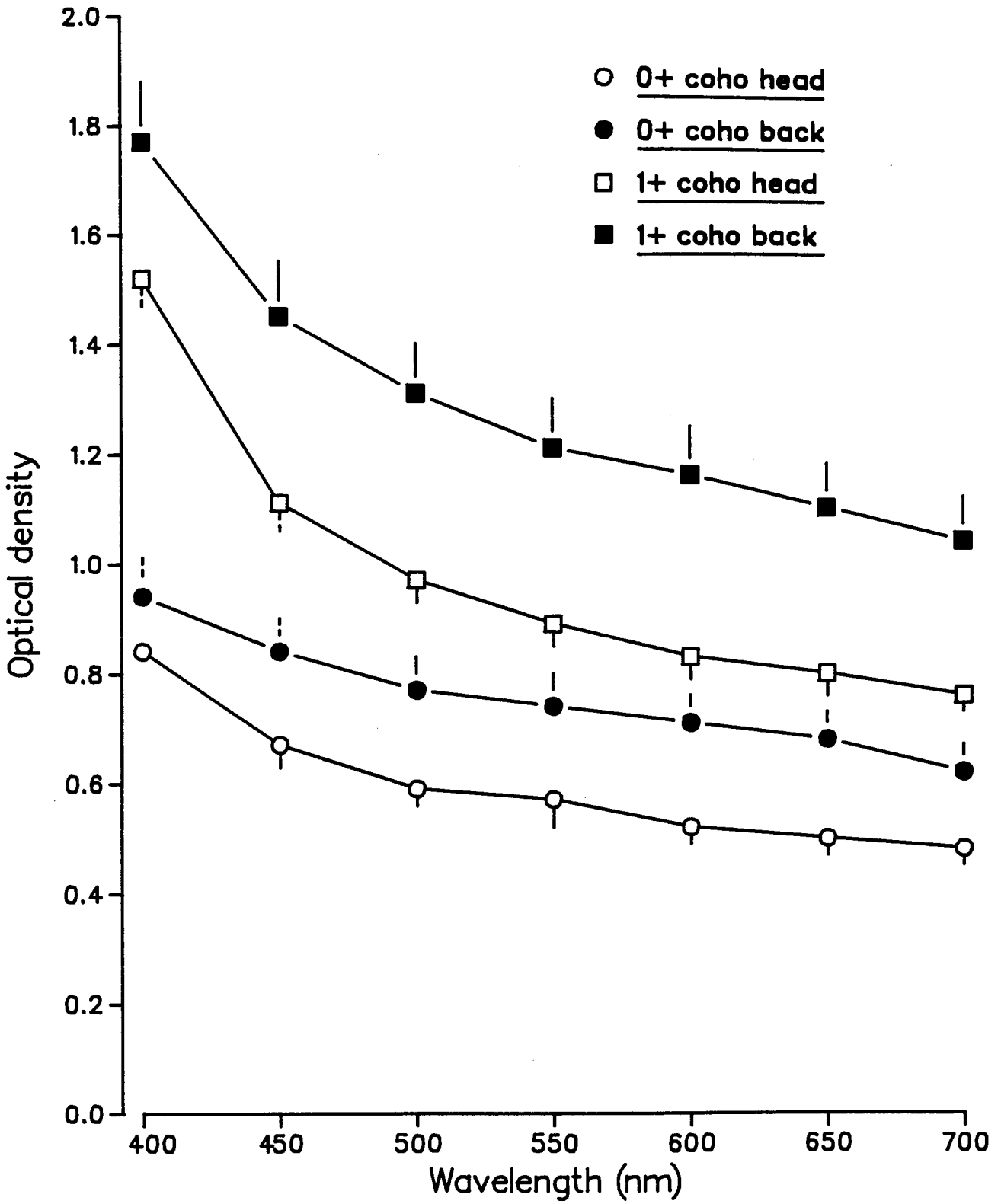

2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Optical density
400
450
500
550
600
650
700
Wavelength (nm)
0+ coho head
0+ coho back
1+ coho head
1+ coho back

Figure 13. Optical density of the 'pineal window' area of 0+ and 1+ coho over length.

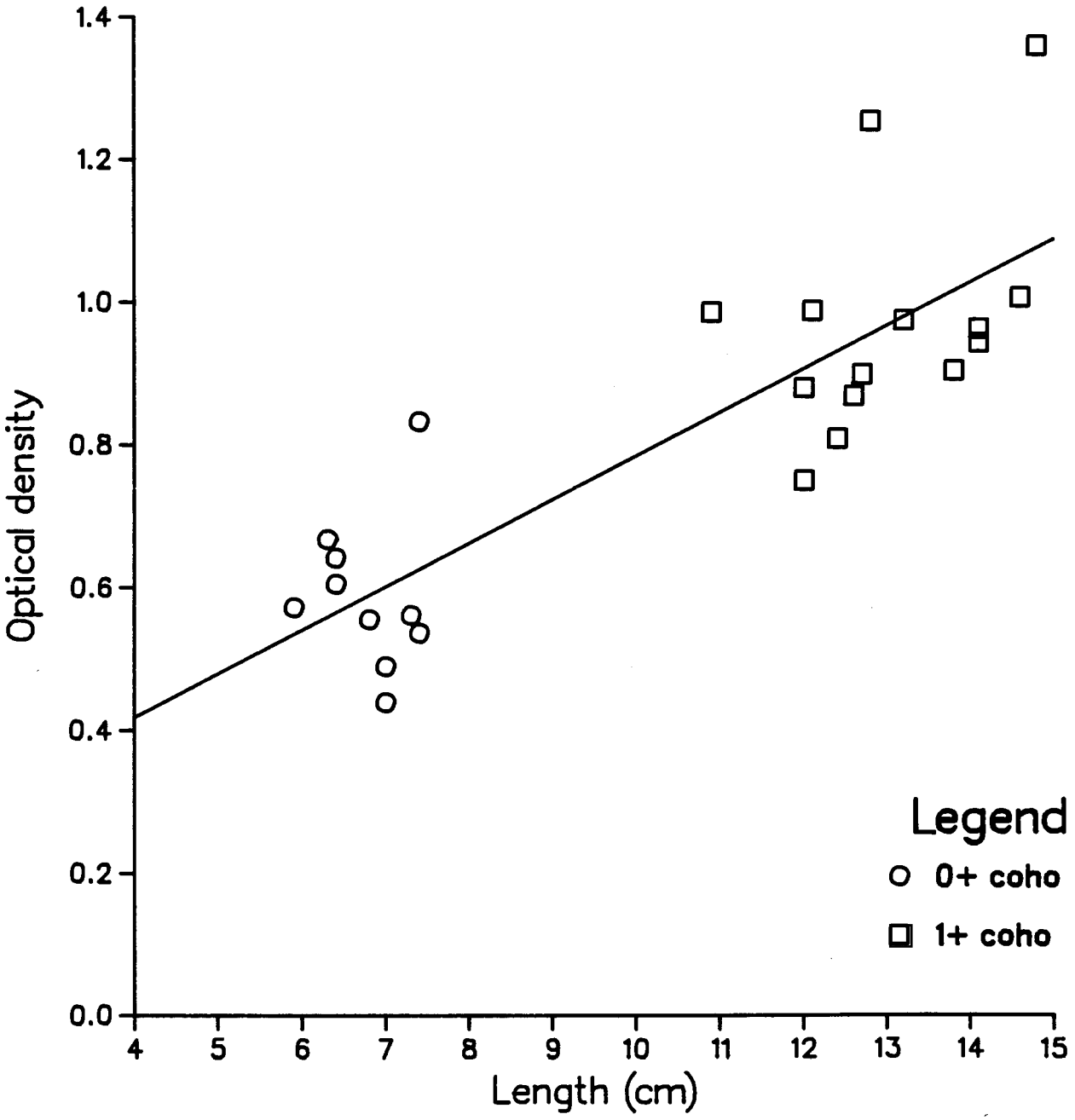

Optical density
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
4
5
6
7
8
9
10
11
12
13
14
15
Length (cm)
Legend
0+ coho
1+ coho

Table 10. Analysis of covariance for optical density at 500 nm over the pineal of 0+ and 1+ coho. Fork length of the fish is a covariate.

Source of variance	df	SS	F	Pr>F
Age	1	0.00097974	0.05	0.8207
Fork length	1	0.90828273	51.00	0.0001
Error	21	0.39082736		
Total	23	1.30008983		

Table 11. Anova of optical density at 500 nm of tissues over the pineal and from the back of 0+ and 1+ coho.

Source of variation	df	SS	F	Pr>F
Age groups(A.g.)	1	2.44	46.92	0.0001
Locations(L.)	1	0.89	17.19	0.0002
(A.g. x L.)	1	0.07	1.27	0.2665
Error	44	2.29		
Total	47	5.70		

* Interaction is indicated by 'x'.

IV. DISCUSSION

4.1. Exp. 1.

The groups that were exposed to the complete 16 L photoperiod after a short-day treatment appeared to acquire smolt status as indicated by their growth rate, seawater adaptability, condition factor, and silvering index. This is in accordance with the results of Clarke & Shelbourn (1986) that underyearling coho fry kept under short daylengths for two months will smolt and grow well in seawater.

The groups that were exposed to 10 L after a short-day treatment did not smolt. Their growth rate was reduced compared to the 16 L groups, their seawater adaptability was low, and their appearance remained parr-like.

The fish that experienced skeleton photoperiods after short-day treatment showed responses that were similar although not as pronounced as the 16 L groups, but superior to the 10L:14D group. The higher mean sodium levels of the skeleton photoperiod groups appears to result from slower development of seawater adaptability (Fig. 5a,b).

These results indicate that long daylengths do not induce smolting by virtue of the greater exposure of the fish to light but rather by providing light during period of the day which is critical for inducing smolting. This is consistent with the hypothesis that coho salmon measure daylength through an endogenous rhythm of sensitivity to light. This mechanism appears similar to those described in previous studies of regulation of maturation in teleosts (Baggerman 1972 1980; Chan 1976; Sundararaj & Vasal 1976; Day & Taylor 1984; Duston & Bromage 1986). The results of Clarke *et al.* (1978b) indicated that the stimulation of smolting might not depend on the total exposure of the fish to light, but this is the first time it has been demonstrated.

The responses of the fish to inductive daylengths depended on the photoperiods they had previously been exposed to. The short-day groups developed smolt characteristics upon exposure to either 16 L or skeleton photoperiods while the long-day groups did not. The long-day treatment appeared to make the fish incapable of smolting in response to inductive daylengths as they grew at similar rates as the 16 L groups during the priming period and significantly better than the 6 L groups. There was no difference between the responses of the short-day fish exposed initially to either 6L:18D or 10L:14D. This indicates that in order to smolt coho salmon have to be reared at daylengths under some threshold level that lies between 10 and 14 hours if they are subsequently to smolt when daylength increases. The results of Clarke and Shelbourn (1986) suggested that the threshold might be between 10 and 12 hours.

Apart from its regulatory role through photoperiod, light also appears to have effects on growth rate and seawater adaptability. Atlantic salmon, kept under continuous light, will grow faster and have better seawater adaptability than if kept under shorter daylengths (Björnsson *et al.* 1989), although they will not smolt. The results of the present study also suggest that to some extent growth and seawater adaptability depend on the total exposure to light. At the end of the inductive period the fish kept under 6L were smaller than those on either 10 L or 14 L. The responses of the skeleton photoperiod groups were also less pronounced than those of the 16 L groups. This effect of light could be indirect and brought about by higher activity levels of fish that are exposed to long days compared to those under short days. The increased activity could in turn stimulate release of e.g. growth hormone (Barrett & McKeown 1988) or thyroxin (Youngson *et al.* 1986), both of which could induce higher growth rate and seawater adaptability.

Smolting may not to be an 'all or none' response and it has been suggested that salmonids may undergo what has been called incomplete smolting (McCormick

& Saunders 1987; Saunders *et al.* 1989). Even though the long-day groups did not reach smolt status their responses were different and the skeleton photoperiod group had significantly higher seawater adaptability (Table 3b) than the other two groups. It could be suggested that the fish may have responded to the skeleton photoperiods by adopting the 6 hour dark period as subjective night rather than the 8 hour period. Having done that they would have perceived the skeleton photoperiod as 18L:6D. The results of Exp. 2, would predict that fish should respond better under 9L:6D:1L:8D than 1L:8D:9L:6D, which may indicate that the long-day treatment shifted the light inducible phase later into the night.

4.2. Exp 2.

The group that showed consistently the best smolting responses was the 9L:5D:1L:9D. The growth rate of both the tagged and the untagged fish in that group was highest and they developed good seawater adaptability. Other groups did not show as pronounced increases in growth rate. The 9L:2D:1L:12D and 9L:9D:1L:5D developed good seawater adaptability, while the 9L:12D:1L:2D showed little or no smolting responses.

These results suggest that a circadian light sensitive phase (ϕ_i) is used by the fish to measure daylength. The light pulses of 9L:2D:1L:12D and 9L:9D:1L:5D may mark the beginning and the end of ϕ_i respectively. The light pulses of the 9L:5D:1L:9D photoperiod and the 9L:6D:1L:8D that was used in Exp. 1 would however fall totally inside ϕ_i and hence give better stimulation.

The results indicate that the mechanism for daylength measurements for smolting and for sexual maturation in fish are similar (Chan 1976; Sundararaj & Vasal 1976; Baggerman 1980; Day & Taylor 1984; Duston & Bromage 1986). Both events can be stimulated by appropriately placed light pulses and they are not dependent on

the total exposure to light. All these results are compatible with the hypothesis that daylength measurements are based on an endogenous circadian rhythm. Maximum stimulation of maturation was accomplished 16 - 18 hours after the beginning of the initial long pulse, but pulses falling on either side were less effective. This is similar to the pattern of stimulation of smolting accomplished by the skeleton photoperiods.

The results are less compatible with internal coincidence, where one oscillator is stimulated by dawn (lights on) another by dusk (lights off) and stimulation under some given phase angle between the two cycles. If such mechanism would apply, the same level of stimulation could be expected from the 9L:2D:1L:12D and 9L:12D:1L:2D on one hand and the 9L:5D:1L:9D and 9L:9D:1L:5D on the other as each pair would create identical phase angles between the cycles. This did not occur as pulses falling early in the night were more effective than pulses late in the night.

The onset of the longer pulses of the skeleton photoperiods were at the same time as lights were turned on during the priming phase and consequently the daylength was increased unidirectionally but not bidirectionally as under natural conditions. As the pulses early in the night are more effective the 9L:2D:1L:12D photoperiod may have created responses similar to natural photoperiods of 14 hours. This complicates the predictions of threshold daylengths for induction of smolting under natural daylight, but they may exceed 12 h.

The SGR for weight decreased at the same rate with increasing weight in all groups. This is in aggreement with the results of Brett & Shelbourn (1975) and Jobling (1983). The slope of -0.34 is precisely the same value as has been reported earlier for coho (see Brett 1979). Although the weight differences among the tagged fish were not great, they were enough to require adjustment to reduce the bias generated by difference in size.

The responses of the fish in this experiment were less consistent than they were in Exp. 1. This may be expected in experiments where 'graded' smolting

responses are under study and some of the groups may be undergoing incomplete smolting. If the smolting response consists of several different processes they may not all be stimulated to the same degree when the stimulus applied is only of marginal efficiency.

The responses of the fish may have been affected by the different density in the tanks. The density of the untagged fish was about ten times higher than in the tanks where the tagged fish were kept. This may explain the inconsistency between the growth rate of tagged fish and untagged (Fig. 7). In Exp. 1 where the responses were more consistent the tagged and untagged fish were kept in the same tanks.

4.3. Exp. 3.

Juvenile coho which did not experience night illumination during the priming period showed increased growth rate and seawater adaptability typical of smolting coho after being exposed to inductive photoperiods. The skeleton photoperiod of 9L:9D:1L:5D was equally effective as the constant light regime (24L:0D) in inducing development of smolt characteristics.

Night illumination interfered with smolting at all the levels of intensity that were tested. The groups exposed to night illumination (F-H) showed reduced growth rate, poorer seawater adaptability, and a lower final silvering index compared to group B that never was exposed to night illumination. Throughout the priming period groups F-H grew faster than group B and reached greater size. Consequently their responses cannot have been limited by a size threshold but must have been caused by some direct inhibitory action of the night illumination.

There were some indications of intensity dependent responses to the night illumination. Group F, which had the lowest night illumination, had lower plasma sodium levels and higher silvering index than the groups that were exposed to higher

illumination during the night. The threshold level for the inhibitory action of night illumination may therefore be close to 10^{-4} lx.

The inhibitory action of the night illumination appears to have been exerted during the priming phase while the fish were being exposed to the 10L:14D photoperiod. None of the groups that were exposed to night illumination responded as well as groups B or A, which had no night illumination during the priming phase. Applying the night illumination during only part of the priming period (for 1½ months out of 2½ total) was sufficient to suppress the smolting responses. The night illumination may also have some disturbing effect during the inductive period as groups C-E, which only had night illumination during the priming period had significantly lower plasma sodium ion concentration than groups F-H. Light at night does however appear not to be disturbing if the fish have been primed properly, as group A, which was exposed to continuous light after July 16, responded as well as group B and better than groups C-H. These results on effects of night illumination agree with those of Björnsson *et al.* (1989) which indicated that night illumination disturbed the process of smolting in Atlantic salmon.

The illumination from a clear sky under a new moon is in the range of 10^{-4} to 10^{-3} lx and under a full moon it may be as high as 3.71×10^{-1} lx (Thorington 1985). The light levels that the fish are exposed to would be reduced by clouds, surrounding trees, and light absorption by water. Cloud cover, in the most extreme cases, may reduce the intensity by a factor of 10 (Thorington 1985). During their first summer coho fry are mainly found in shallow coastal rivers or tributaries, but by the first fall they move into deeper pools and can often be found around or under log jams or in shades of banks (Hartman 1965). It is difficult to postulate an average photic environment of coho fry and parr without direct measurements and quite likely it will not be the same in different rivers. Even if we assume that only 1 to 10% of the incident moonlight reaches the fish they would still be exposed to light in the range of

10^{-3} to 10^{-2} lx over extended periods of time. This level of illumination is at least ten times higher than the level found to be inhibitory to smolting. The age at smolting in coho salmon varies between 0+ and 4+ although the most common age groups are 1+ and 2+ (Randall et al. 1987). It is therefore suggested that newly emerged fry are more sensitive to night illumination than one- or two-summer old parr and that this may result in delay of smolting for one or more years. The sensitivity to night illumination should also be a concern for hatcheries where the surroundings of raceways and ponds are illuminated during the night.

Both long daylengths (Clarke & Shelbourn 1986) and night illumination during the first two months will suppress smolting of underyearling coho in response to longer days. Whether the fish perceive the night illumination as long or continuous daylight cannot be deduced from the present results. Other species of salmonids maintain nocturnal behaviour patterns under higher levels (0.1 to 1.0 lx) of night illumination (Byrne 1971; Eriksson 1978). The night illumination may therefore not create the perception of continuous daylength although it may be affecting the same processes that are affected by long days.

4.4. Optical density.

The peak intensity of moonlight is between 500 and 550 nm (Munz & McFarland 1977). The greatest sensitivity of both the lateral eyes (Bridges 1972) and the pineal (Tamura & Hanyu 1980; Meissl *et al.* 1986) may be in a similar range, therefore the optical density at 500 nm was analysed in detail.

The measurements were performed to see if transmission qualities of the pineal window of underyearlings and yearling parrs could explain the difference in susceptibility to night illumination of the two age groups. In the previous section (4.3) it was argued that the fish might be exposed to an illumination of 10^{-3} to 10^{-2} lx

during the night and the threshold level for inhibition of smolting by night illumination is close to or slightly lower than 10^{-4} lx. If transmission by the pineal window was to account for the different sensitivity of the two age groups one would assume that it would have to be at least 10 to 100-times higher in yealings than in the underyearlings. The optical density at 500 nm of the pineal window of underyearling coho corresponded to a light transmission of 25% and for the 1+ presmolt it was 10% and consequently it cannot account for the different sensitivity to night illumination.

The fact that the optical density of the pineal window increased as a function of the length of the fish suggests that it increases passively as the thickness of the skin and the skull increases and that presmolts have proportionately the same amount of pigment as do underyearlings. This is also suggested by the lack of interaction between location and age (Table 11), which would be expected if there was an increased accumulation of pigment above the pineal of the presmolts.

If the threshold for inhibition by night illumination was close to 10^{-4} lx it may be in a similar range as the threshold level for electrophysiological responses from the pineal of salmonids, 10^{-5} to 10^{-4} lx (Morita 1966; Hanyu *et al.* 1978) when the optical density of the pineal window has been accounted for. This may suggest that the pineal organ is the mediator of the photoperiodic responses that entrain smolting.

The transmission of the pineal window of the coho is comparable to what Morita (1966) reports for the rainbow trout after size differences have been accounted for but lower than the 50% transmission reported for the ayu of mean length 15 cm (Hanyu *et al.* 1978).

The optical density of the skin and the skull of the coho was higher towards the shorter wavelengths. Hartwig and van Veen (1979) consider the short wavelength peak to result from optical density by melanin and because short wavelengths are scattered more in tissues than longer ones. The spectral composition of the light

transmitted to the brain of coho is similar to what has been reported for the ayu (Hanyu *et al.* 1978), the eel (*Anquilla anquilla*), and the catfish (*Ictalurus nebulosus*) (Hartwig & van Veen 1979).

4.5. Final conclusions.

The results stress the role of photoperiod in the regulation of the life history of coho - the best demonstration of this being that smolting can be induced in underyearlings through manipulations of photoperiod. The apparent sensitivity of the underyearlings to night illumination may suggest a further involvement of light in regulating the life history of coho salmon. The release of thyroid hormone (T_4) during smolting may have lunar cycles (Grau *et al.* 1982; Nishioka *et al.* 1983; Yamauchi *et al.* 1985). The results of Exp. 3 show that coho fry possess a sufficient photosensitivity for moonlight to be the entraining factor of these cycles.

Photoperiod appears to have at least a dual role in the regulation of smolting. The process of smolting is initiated under short daylengths (10-12 h) while long days (>12 h) and increasing daylengths induce and synchronize the various processes involved in smolting.

A similar mechanism of daylength perception appears to operate in all teleosts that have been investigated. The smolting responses of the fish in Exp. 1 and Exp. 2 resemble the maturation responses in various other teleosts, suggesting that the same mechanism is at least partly involved in both processes.

Evidently there is a circadian component in daylength perception by fish. Hence unlike lizards teleosts do not appear to rely on an hour-glass mechanism. The light sensitive phase during which coho can be induced to smolt is between 12 and 19 hours after dawn, with maximal stimulation between 14 and 16 hours after dawn. These results can be described both in terms of the external (ECM) or the internal

coincidence models (ICM). What is called light sensitive phase under external coincidence translates into "the phase angle of maximum induction" in terms of the internal coincidence model.

The results of Exp. 2 may favor ECM over internal coincidence. It is simpler and more explicit than the ICM. This may make the ECM a more attractive working model for future experiments. It has also been suggested that the behaviour of the light sensitive phase of the EMC could correspond to the nightly peak of melatonin secretion (Saunders & Lewis 1988). The pineal and its secretory hormone melatonin are considered likely links between photoperiods and the endocrine system (Lewinski 1986). Further, the sensitivity of coho to night illumination observed in Exp. 3 corresponds to the sensitivity of the pineal to light.

The internal coincidence model for photoperiod control of smolting has merit because it fits well with the concept of smolting being composed of separate components that do not need to be functionally linked (Simpson 1985). Each component of smolting could be regulated by a separate oscillator, which is in turn synchronized by photoperiod through some master phase maker. On the other hand accepting ECM does not deny the existence of multiple oscillators.

Even though models predict responses they do not necessarily describe the underlying mechanism. Eventually the neuroendocrine links between photoperiods and the release of the hormones involved have to be worked out, in order to understand the mechanism.

V. Appendixes

Appendix A.

Plasma growth hormone levels in smolting and nonsmolting underyearling coho salmon (*Oncorhynchus kisutch*).

1. Introduction.

At the end of Exp. 1 blood was sampled from the tagged fish for analysis of growth hormone (GH). This was done to compare levels in smolting and nonsmolting fish and to see if GH levels correlated with growth rate of individual fish.

2. Methods

The fish were anaesthetized in 2-phenoxy ethanol and the blood was sampled into heparinized capillary tubes. The plasma was separated and frozen at -20° C. The analyses were performed in the laboratory of Dr. Brian McKeown (Wagner & McKeown 1986). Aliquots 20-μl of plasma were analyzed in duplicates if sufficient plasma was available. Only part of the samples were successfully analyzed, but smolting (6L:18D/9L:6D:1L:8D; 10L:14D/16L:8D; 10L:14D/9L:6D:1L:8D) and nonsmolting (14L:10D/9L:6D:1L:8D; 14L:10D/16L:8D) groups were represented (cfr. section 4.1.). A model two anova indicated that of the total variation 3.7% were explained by variation between groups, 2.3% by duplicate samples, and 94% by variation among fish within groups.

3. Results

There was no significant difference among smolting and nonsmolting groups in mean levels of GH (Table 12). There was no significant correlation between GH levels and final length, final weight, SGR for length and weight for all or the last interval.

4. Discussion

GH levels at the time when the samples were taken were not affected by photoperiod. The samples that were analyzed came both from smolting and nonsmolting groups. This conforms with the results of Clarke *et al.* (1989), who found no significant rise in GH in underyearling coho. However, GH levels in Atlantic salmon increase during smolting in response to inductive photoperiods (Björnsson *et al.* 1989) and GH levels of yearling coho salmon are elevated in spring (Sweeting et al. 1985). This may suggest a difference in the pattern of smolting in yearling and underyearling salmon, but it should be noted that in the present experiment the blood samples were taken two months after the fish were exposed to the final photoperiods and any previous responses might have been missed.

Table 12. Growth hormone (ng/ml) in plasma samples from tagged fish at the end of Exp. 1.

Photoperiod	GH	N
6L:18D/9L:6D:1L:8D	58.3	22
10L:14D/16L:8D	49.7	16
10L:14D:/9L:6D:1L:8D	65.6	10
14L:10D/16L:8D	57.9	13
14L:10D/9L:6D:1L:8D	65.2	5

*No significant difference was found between the mean GH values.

Appendix B.

Cycles of growth rate in coho salmon (*Oncorhynchus kisutch*).

1. Introduction

A systematic variation in SGR of all groups was noted in Exp. 1. The subject will be expanded further below.

2. Results

The SGR for length and weight during each of the four intervals varied in all groups ($p<0.0001$) (Table 2 and 13). The SGR for length was higher during short than long intervals. This occurred concomitantly in all groups regardless of photperiodic treatment (Table 13). The growth rate for weight showed the opposite relation to interval length. The duration of the growth intervals varied from 7 to 21 days (Table 13).

3. Discussion

The variation of growth rate observed in Exp. 1 may suggest regular cycles of growth rate. The highest SGR for length were in all groups during the first and the last intervals but did not decrease progressively as the fish grew. The SGR for weight behaved in exactly the opposite way which also suggest that this was not incidental. Cycles of growth rate have been reported in other salmonids. Brown (1946) reported synchronized 4 - 6 week cycles of growth rate in brown trout (*Salmo trutta*) with

growth rate of weight and length out of phase as in the present experiment. Wagner and McKeown (1985) reported slightly shorter cycles of 3 - 4 weeks in rainbow trout. Farbridge and Leatherland (1987) reported semilunar cycles, but they did not measure individual growth rate which complicates the interpretation of their results. These results show that fish within the same tank can maintain synchronous cycles of growth rate without any apparent external synchronizers or `zeitgebers`. Fish in the present experiment kept in different tanks and separate rooms were also synchronized.

It is suggested that the measurements or the stress involved acted as zeitgeber. It appears to have been the only external stimulus with a period which might match the growth cycles and could have acted in all rooms simultaneously. The measurements could initiate cycles of high initial growth rate for length with transient decrease over time. Thus growth rate measured over short intervals would tend to be high and progressively lower the longer the duration. The growth in length may be at the expense of growth in weight and therefore they would tend to be out of phase. The growth intervals in Exp. 2 were all of approximately the same duration and therefore no cycles may have been observed there. This could also explain the results of Brown (1946) and Wagner and McKeown (1985) if we assume that there is a refractory period which makes the SGR cycles less frequent than their measurements. Whether these cycles are only artifacts or actually occur under natural conditions remains speculatory.

Table 13. Specific growth rate for length and weight during different intervals of Exp. 1.

Initial photop.	Final photoperiod	Intervals									
		1		2		3		4		Means	
		L	W	L	W	L	W	L	W	L	W
6L:18D	16L:8D	0.87	1.54	0.74	2.01	0.48	1.94	0.98	0.98	0.74	1.63
-	9L:6D:1L:8D	0.86	-1.60	0.60	2.82	0.40	1.20	0.77	0.47	0.66	0.71
-	10L:14D	0.67	-0.36	0.51	1.52	0.22	0.84	0.66	0.41	0.52	0.52
10L:14D	16L:8D	0.87	0.99	0.72	2.22	0.49	1.94	0.98	1.47	0.77	1.60
-	9L:6D:1L:8D	0.75	0.09	0.68	2.08	0.34	1.46	0.88	0.67	0.68	0.99
-	10L:14D	0.67	-0.03	0.48	1.12	0.22	0.94	0.71	0.21	0.51	0.55
14L:10D	16L:8D	0.60	0.15	0.48	1.46	0.23	0.91	0.66	0.10	0.49	0.66
-	9L:6D:1L:8D	0.73	0.02	0.46	1.37	0.18	0.86	0.68	-0.05	0.55	0.49
-	10L:14D	0.54	-0.20	0.33	0.62	0.14	0.79	0.64	0.01	0.42	0.29
Means of intervals		0.73^{a}	0.08^{d}	0.55^{b}	1.69^{a}	0.30^{c}	1.21^{b}	0.77^{a}	0.49^{c}		
Duration (days)		7		19		25		10			

*Means identified with same superscript are not significantly different.

VI. References

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