

SOME PHYSIOLOGICAL CHANGES IN COHO SALMON (ONCORHYNCHUS KISUTCH)
DURING SMOLTIFICATION AND SEAWATER ADAPTATION

by

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Abstract

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Changes in gill $\text{Na}^+\text{-K}^+$ ATPase specific activities and plasma concentrations of T_4 , T_3 , Na^+ , K^+ and Cl^- were monitored during the periods of smoltification and seawater adaptation, and then at 30 and 180 days after seawater entry in 10 groups of yearling hatchery-reared coho salmon. During the period of smoltification (April-May), dramatic increases in both plasma T_4 concentrations and gill $\text{Na}^+\text{-K}^+$ ATPase activities occurred; these changes were found to be significantly correlated in their timing. Plasma T_3 levels slightly increased during this period and were significantly correlated with both plasma T_4 concentrations and gill $\text{Na}^+\text{-K}^+$ ATPase activities. Plasma concentrations of T_4 and T_3 were significantly correlated. Some of the hatchery groups demonstrated a significant decrease in plasma Na^+ and Cl^- concentrations during the same period. Plasma concentrations of Na^+ and Cl^- were found to be significantly related, but were both unrelated to gill $\text{Na}^+\text{-K}^+$ ATPase activities or plasma T_4 and T_3 concentrations. Plasma K^+ concentrations changed significantly in some groups, but not in others.

Transportation from hatcheries and transfer of yearling coho salmon from freshwater to seawater resulted in significant increases in gill $\text{Na}^+\text{-K}^+$ ATPase activity during the first eight days of seawater residence. In contrast, plasma T_4 concentrations exhibited a variety of responses which included increases, decreases and no change. Plasma T_3 concentrations showed similar variable responses to seawater transfer. Plasma T_4 and T_3 concentrations showed a significant correlation, but neither T_4 nor T_3 were related to gill $\text{Na}^+\text{-K}^+$ ATPase activities. All of the hatchery groups showed an initial increase in both plasma Na^+ and Cl^- during the first or second day of residence in seawater. By the eighth day in seawater Na^+ and Cl^- levels were stabilized in some of the groups, but continued to rise through 180 days in seawater in others. Plasma Na^+ and Cl^- concentrations were significantly related, as they were in freshwater. Plasma K^+ concentrations increased slightly sometime during the first six days in seawater, but generally remained uniform throughout the seawater sampling period. There were no significant relationships between any of the plasma electrolyte concentrations and gill $\text{Na}^+\text{-K}^+$ ATPase activities or plasma concentrations of T_4 or T_3 .

The success of smoltification for the various stocks was assessed by determining the proportions of surviving fish and smolted fish after six months in seawater. The percentage of surviving fish ranged from 27-80%. The percentages of smolted fish among the survivors ranged

from 48-90%. Percent smolts and percent survival were not significantly related.

The peak of plasma T_4 activity in freshwater coho salmon was comparable to that of anuran amphibians undergoing metamorphosis which suggests that thyroxine may play a similar role in the parr-smolt transformation.

The concurrent increases in plasma T_4 concentrations and gill Na^+-K^+ ATPase activities in freshwater fish suggested that ATPase activity may be regulated through the hypophyseal-thyroid axis. In contrast to the situation in freshwater fish, the absence of a statistical relationship between T_4 and ATPase in seawater fish suggested that the increases in freshwater fish were developmental events preparing the fish for seawater entry. The absence of a statistically significant relationship between the plasma electrolytes and gill Na^+-K^+ ATPase activities suggested that there are other components, in addition to ATPase, which are involved in salmonid ionoregulation. The relationships between gill Na^+-K^+ ATPase activities and plasma thyroid hormones and electrolytes and the possibility that smoltification is regulated by neuroendocrine mechanisms was discussed. The absence of a significant relationship between percent smolts and percent survival after six months in seawater suggested that smoltification and seawater adaptation were not interdependent events.

Additionally, all of the physiological measurement collected in both freshwater and seawater were compared with the number of surviving and smolted fish after six months in seawater to determine if any of the physiological measurements could be used to predict seawater success. Only one relationship, a component of the plasma T_4 curve in freshwater fish and percent survival, was found to be significant. The usefulness of this comparison in predicting hatchery releases was discussed.

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INTRODUCTION

The salmon and steelhead hatcheries of the Pacific Northwest are among the most highly developed and technically sophisticated aquatic-culture systems in the world. The hatcheries on the Columbia River have developed in large part to enhance declining natural runs resulting from dam construction and have enjoyed unparelled success in providing fish for a large segment of the United States Pacific salmon fishery. Releases now average 110-million fish annually (Wahle et al. 1975). Nearly one-half of all anadromous salmonid releases from Pacific coast artificial rearing facilities (2.5 million pounds annually) are now released from Columbia River hatcheries. These massive releases of juvenile salmon and steelhead are presently justified by favorable benefit-cost comparisons, but additional large-scale releases may not be the most cost-effective means for further improving the contribution to the fishery. Recently, it has been recognized by fishery agencies that the release of smolts better prepared for marine survival may produce the same results at far lower cost than that required to construct and operate new hatcheries. The value of the fish from the 28 Columbia River Fish Development Program-funded facilities to the Pacific coast sport and commercial fisheries has been estimated at over \$33 million annually (Wahle et al. 1975). A doubling of survival of these fish could potentially yield \$66 million annually. A doubling of survival does not seem unreasonable, considering some British Columbia and Washington State

hatcheries have previously achieved coho salmon survivals of 25 to 30 percent average versus the six to seven percent survival obtained at Columbia River facilities (Wahle et al. 1975).

Little is known about the fate of hatchery fish during the critical period of downstream migration and seawater entry. Success during this critical period depends largely on the status of smoltification of the released juveniles and their ability to pass rapidly downstream, through the estuary and into the sea. Readiness for migration and adaptability to seawater are two of the most critical factors in determining early survival of hatchery migrants. Their subsequent survival in seawater depends upon a diversity of controlling factors such as; stage of development, age, nutritional condition, disease status, food availability, predation and environmental variables such as water temperature. The importance of rapid and early downstream migration was emphasized in the spring of 1977 when unseasonably warm river temperatures and reduced spill at the dams hindered the passage of downstream migrants.

Determining release times for hatchery stocks of salmonids is not an easy matter. Quite often these dates are selected to fit best with water releases at dams, to avoid public sport fishing seasons, to adjust for space requirements at hatcheries, to utilize available feed and money, and to meet with a host of special considerations. Some releases are made when certain behavioral or morphological characteristics are observed (i.e., migratory restlessness, reduced

feeding behavior, silvering, fin coloration or changes in condition index). However, these criteria are not always accurate, do not always coincide with the peak of smoltification, and cannot be used to quantitate the status of the smoltification process.

Therefore, the purpose of this study was to establish patterns of some basic quantifiable physiological changes (i.e., gill $\text{Na}^+\text{-K}^+$ ATPase activities, and plasma thyroxine, triiodothyronine, sodium, potassium and chloride ion concentrations) which occur during the periods of smoltification in freshwater, seawater adaptation and seawater residence for ten groups of Columbia River coho salmon. Secondly, all of the physiological measurements accumulated during the course of this investigation were compared with the numbers of surviving and smolted fish through six months of seawater residence to determine whether a statistically significant relationships existed, and if such relationships could be used as an index to predict optimal hatchery release dates.

LITERATURE REVIEW

The coho salmon (Oncorhynchus kisutch) is an anadromous species, and as such, spends its early life history in the coastal freshwater rivers and streams of the Pacific Northwest. Generally, eggs are deposited in October or November, hatch in April or May, with the fry emerging from the stream bed gravel in May or June. These young salmon, referred to as parr, remain in their parent stream for periods of two months to two years depending upon the latitude (Hart 1973; Scott and Crossman 1973). In general, California fish have the shortest freshwater residence period, while Alaskan fish have the longest. In the Columbia River drainage, the young salmon typically begin their seaward migration during the spring of their second year. Prior to, or accompanying seaward migration, a period of development known as smoltification occurs. The phenomenon consists of a spectrum of simultaneous or consecutive morphological, behavioral and biochemical changes that transform the darkly pigmented, bottom-dwelling parr into a pelagic, silvery smolt prepared for the transition into seawater (Hoar 1963). The onset and synchronization of smoltification and migration appear to be regulated by environmental factors; primarily increasing daylength and water temperature (Foerster 1937; White 1939; Keenleyside and Hoar 1954; Malikova 1957; Elson 1962; Eales 1963, 1964, 1965; Conte and Wagner 1965; Hoar 1965, 1976; Conte et al. 1966; Hartman et al. 1967; Johnston and Eales 1968, 1970; Pinder and Eales 1969; Saunders and Henderson 1970; Zaugg et al. 1972; Adams et

al. 1973, 1975; Withey and Saunders 1973; Zaugg and Wagner 1973; Richardson and McLeave 1974; Wagner 1974a, b; Jessop 1975; Solomon 1975, 1978; Murphy and Houston 1977; Clarke et al. 1978, Fried et al. 1978). There is also evidence for an endogenous factor, manifested as a critical size, which may be important in the timing of seaward migration (Huntsman and Hoar 1939; Black 1951; Maher and Larkin 1954; Shapovalov and Taft 1954; Elson 1957; Chapman 1958; Parry 1960a; Houston 1961; Meehan and Siniff 1962; Wagner et al. 1963; Church 1963; Church and Nelson 1963; Conte and Wagner 1965; Wagner 1968; Folmar et al. 1979a, b). It should also be noted that the characteristic changes associated with smoltification and migration are freely reversible in coho salmon, i.e., fish that are prevented access to seawater during this period will again assume the appearance and physiological characteristics of the freshwater parr (Hoar 1976).

The remainder of this review will be devoted to describing the specific changes associated with smoltification and their relationships with development and survival in the marine environment.

Two morphological characteristics, coloration and body shape, have been observed to change during the parr-smolt transformation. The freshwater parr can be identified most readily by darkly pigmented (melanin) bars (parr marks) on the lateral surface, perpendicular to the lateral line. During smoltification, the parr marks, which are located deep in the dermis, become visually obstructed by the accumulation of purines, specifically guanine and hypoxanthine, in the

scales and superficial dermal layers of the skin (Robertson 1948; Vanstone and Markert 1968; Johnston and Eales 1967, 1968, 1970; Hayashi 1970). The purine layers produce the characteristic silver appearance of the transformed seawater smolt. Characteristic changes in body form and condition index accompany this color change and result in a more slender, streamlined fish (Hoar 1939b; Martin 1949; Houston and Threadgold 1963; Vanstone and Markert 1968; Fessler and Wagner 1969).

At least two behavioral changes, salinity preference and migration to seawater, are associated with the smoltification process. Differences in the salinity preference have been attributed to phylogeny as well as the state of physiological development. Parry (1960, 1961) has shown that juvenile Atlantic salmon (Salmo salar) and steelhead trout (Salmo gairdneri) transferred to various salinities did not become homeo-osmotic with their environment, and that the ability to osmoregulate at different salinities depended upon size and age of the fish as well as the species. McInerney (1964) reported that five species of Oncorhynchus showed seasonal patterns of saltwater preference, but not all of the patterns were identical. Pink (O. gorbuscha) and chum (O. keta) salmon regulated serum chloride to prevent accumulation of toxic levels, while at the other extreme, chinook salmon (O. tshawytscha) regulated plasma chloride ions with less precision. However, they tolerated much higher plasma chloride levels than the other species tested. This same pattern of adaptive tolerance was also observed by Weisbart (1968). Other studies (Black

1951; Houston 1959, 1961; Baggerman 1960a, b; Conte et al. 1966; Leatherland and McKeown 1974) have also shown a phylogenetic relationship in salinity preference for under-yearling fish. These authors have found that Oncorhynchid species showed salinity preference at an earlier age and showed greater seawater tolerance as zero-age fish than did similar fish of the genus Salmo. This salinity preference response has also been associated with the physiological development of the individual fish, and preceded the parr-smolt transition in coho salmon by a period of six to seven months (Baggerman 1960a; Conte et al. 1966). However, due to the method of examination, i.e., using only two salinity levels rather than a continuous salinity gradient (Otto and McInerney 1970), the authors were unable to determine whether a gradual increase in salinity, such as those encountered in downstream migration, would enhance adaptation to full strength seawater. The preference for a salinity gradient may also serve as an orientation mechanism during seaward migration (Fried et al. 1978; LaBar et al. 1978).

Prior to smoltification, Atlantic and coho salmon spend approximately one year in freshwater residence. During this period the parr establish and maintain bottom feeding territories (Kalleberg 1958; Chapman 1962; Keenleyside and Yamamoto 1962; Hartman 1965; Dill 1978). This demersal behavior allows these species to maintain their positions in the stream, rather than being transported downstream during their first year as in pink and chum salmon. At smoltification, there are

abrupt changes in behavior; the fish abandon their bottom feeding territories, decrease aggressive behavior and tend to form aggregates or schools. A marked reduction in swimming ability has also been observed during this period. Atlantic and coho salmon parr swam at speeds of 7.0 (Wankowski 1977) and 7.3 (Glova and McInerney 1977) body lengths per second (BLs^{-1}) respectively, while smolts were limited to speeds of 2.0-2.5 (McCleave and Stred 1975; Thorp and Morgan 1978) and 5.5 (Glova and McInerney 1977) BLs^{-1} , respectively. Using a swimming chamber, Kutty and Saunders (1973) demonstrated that Atlantic salmon parr swam to exhaustion and collapsed, while smolted animals ceased swimming but exhibited no signs of fatigue. They postulated that the refusal to swim was a behavioral component characteristic of migration.

There have been observations that downstream migrations are passive, undirected events dependent upon riverine and tidal currents (Huntsman 1939, 1962, 1973; Jones 1959; Stuart 1962; Mills 1964; Fried et al. 1978; LaBar et al. 1978). Although the fish are dependent upon currents for movement, the speed at which they travel in these currents can be effectively regulated by their position in the water column (Harden Jones 1968; Arnold 1974). There have also been observations that migrating smolts will actively swim downstream to reach seawater (White and Huntsman 1938; Kalleberg 1958; Stasko et al. 1973; Solomon 1978). Most references to diel timing of migration have shown that salmonids prefer nocturnal movements (Berry 1931; Alexander et al. 1935; White 1939; Hayes 1953; Neave 1955; Hoar 1956; Osterdahl 1969;

Jessop 1975); however, daylight migrations have also been observed (Solomon 1975; Bakstansky et al. 1976). It is possible that the observed differences could have been influenced by species difference or other characteristics such as schooling behavior (Hoar 1956, 1958a) or stream depth (Coburn and McCart 1967). There are many incongruities associated with the behavioral aspects of smoltification, however, as Hoar (1976) adroitly points out "There has sometimes been a tendency to consider one behavior more correct than another, but perhaps it is more accurate to argue that all of the activities are appropriate and normal to the particular situation where the fish is being observed, and most of them have a real meaning in nature."

There have been numerous investigations concerned with the physiological aspects of seawater adaptation and more recently, their relation to smoltification. Unfortunately, most of the studies have been concerned with isolated events, and have been performed on a variety of species and at different life stages. Therefore, in order to evaluate completely the physiological or biochemical changes associated with smoltification and establish causal relationships, it has been necessary to consider reports of similar changes in other diadromous and euryhaline species as well.

In freshwater, diadromous and euryhaline fish are hyperosmoregulators (body fluids have a higher osmolality than the surrounding medium) and consequently acquire water passively and lose electrolytes through the gill membrane. This diffusive loss of ions and uptake of

water is balanced, in part, by the uptake of ions through the gills or diet and by the production of hypo-osmotic urine. Freshwater adapted rainbow trout excrete urine in a volume equivalent to the osmotic uptake of water (Fromm 1963). The undesirable influx of water through swallowing is apparently not a problem in freshwater salmonids (Shehadeh and Gordon 1968) or other freshwater teleosts (Smith 1930; Keys 1933; Maetz and Skadhauge 1968; Oide and Utida 1968).

A number of studies have reported plasma electrolyte concentrations [sodium (Na^+), potassium (K^+) and chloride (Cl^-)] in freshwater salmonid fishes. The ranges of electrolyte concentrations reported for both Salmo and Oncorhynchus were: Na^+ , 133-155 meq/l; K^+ , 3-6 meq/l; and Cl^- , 111-135 meq/l (Fontaine and Hatey 1950; Kubo 1955; Phillips and Brockway 1958; Gordon 1959; Houston 1959; Conte and Wagner 1965; Conte et al. 1966; Holmes and Stainer 1966; Miles and Smith 1968; Milne 1974; Clarke and Blackburn 1977; Newcomb 1978; Folmar et al. 1979a). The plasma concentration of Cl^- has been reported to decrease just prior to migration in Atlantic (Houston 1959; Fontaine 1960) and masu (O. masu) salmon (Kubo 1955), however, this observation has not been corroborated⁰ in the coho salmon (Conte et al. 1966; Miles and Smith 1968). Kubo (1953) also reported an increase in plasma osmotic pressure during this period. Koch and Evans (1959) and Parry (1960) obtained the same results with smolts and post-smolts of S. salar maintained in freshwater, while Saunders and Henderson (1970) found the osmotic pressure of S. salar to remain constant prior to seawater

entry. Miles and Smith (1968) reported no changes in plasma calcium (Ca^{++}) or magnesium (Mg^{++}) ion concentrations in pre-migratory O. kisutch. During the freshwater residence period, the young fish maintain a stable osmolality of their internal body fluids, although marked changes occur during the first 36-100 hours of seawater residence. This period has been termed the "adjustive phase" and varies among the different salmonid species (Houston 1959; Conte and Wagner 1965; Conte et al. 1966; Miles and Smith 1968). Upon entering seawater, the fish must prevent dehydration by ingesting seawater, reducing urine flow and actively excreting the excess salts via an extrarenal mechanism (Smith 1930, 1932). This maintenance of internal electrolyte balance has been attributed to the production of a hypertonic rectal fluid in the hindgut (divalent ions) and the active transport of monovalent ions across the gill membrane (Parry 1966; Conte 1969). The impetus to commence "drinking" in seawater adapted eels has been attributed to increased tissue osmolality (Sharret et al. 1964), increased plasma Cl^- levels and decreased blood volume (Hirano et al. 1972; Hirano 1974). For freshwater to be gained in seawater-adapted fish, they must excrete hypertonic solutions as waste products (Philpott and Copeland 1963) and initiate a Na^+ and Cl^- ion transport system across the gut wall, which in turn, produces a concentration gradient allowing water to be passively transported from the gut into the body fluids (House and Green 1963; Skadhauge 1969; Maetz 1970; Oide 1970; Utida et al. 1972). Holmes (1961) found the seawater maintenance levels of urine flow were reduced to one to five percent of

the freshwater excretion rate and that these maintenance levels were reached within 18 hours after transfer to seawater.

In seawater, the gill membrane also becomes less permeable and reduces the influx of monovalent ions from the external medium. This change has been related to decreased pH at the membrane surface (Busnel 1943; Shanes 1960; Houston 1964) and the interaction of Ca^{++} with the superficial membrane sites associated with monovalent ion transport (Houston 1959, 1964; Shanes 1960). Other studies have also reported that prolactin reduces turnover rates of water and ions at the gill membrane (Pickford and Phillips 1959; Potts et al. 1967, Chan et al. 1968; Ball 1969; Evans 1969a, b; Lam 1969; Lahlou and Sawyer 1969; Motais et al. 1969; Pickford et al. 1970a; Potts and Fleming 1970; Pang et al. 1971; Maetz 1972; Ogawa et al. 1973; Pang 1973; Ogawa 1974; Pic and Maetz 1975; Cameron 1976; Wendelaar Bonga 1978).

During the seawater adjustment phase in salmonids there are rapid, transient changes in plasma Na^+ and Cl^- levels (Fontaine 1954; Kubo 1953; Koch et al. 1959; Houston and Threadgold 1963; Miles and Smith 1968; Komourdjian et al. 1976a; Clarke and Blackburn 1977; Folmar et al. 1979a). Although these ions have been recently shown to move together (Murphy and Houston 1977; Folmar et al. 1979a) some investigators have reported decreases in the Na^+/Cl^- ratio during this period (Gordon 1959; Parry 1966). Miles and Smith (1968) reported a transient rise in plasma Mg^{++} levels 30 hours after transfer to seawater, which could represent the time required for the initiation of divalent ion

control by the hindgut (Sharratt et al. 1964). Regardless of the composition and osmolality of the external medium, plasma K^+ tends to remain relatively constant. The more stringent regulation of this ion may be associated with its importance in DNA, RNA and general protein synthesis (Lubin 1964; Bygrave 1967; Orr et al. 1972). Two mechanisms that have been reported to reduce the rate and magnitude of plasma ion increases at seawater entry include the redistribution of tissue water (Fontaine and Hatey 1950; Fontaine 1951; Black 1951; Gordon 1959; Houston 1959, 1964; Chartier-Baraduc 1960; Houston and Threadgold 1963) and the uptake of ions by the soft tissue and bone (Black 1951; Gordon 1959). Although there is a phase shift in the distribution of body water upon entry into seawater, total body water remains constant (Gordon 1959; Parry 1966). The ability to regulate plasma electrolytes upon entering seawater has been related to the status of smoltification and has been used as a quantitative index to determine release dates for hatchery reared salmon (Komourdjian et al. 1976a; Clarke and Blackburn 1977). Other studies have shown that this test should not be influenced by changes in photoperiod (Saunders and Henderson 1970; Wagner 1974a; Clarke et al. 1978); however, this test may not be valid for fish maintained at low temperatures (Houston 1973; Murphy and Houston 1977). Burton (1973) has suggested that teleosts maintain plasma osmolalities in the range of 300-400 mOsm. A study by Parry (1961) showed that S. salar maintained their plasma osmolalities at 328 and 344 mOsm in freshwater and seawater, respectively; however, Gordon (1957) found that the char, Salvelinus alpinus had freshwater plasma

osmolalities of 328 mOsm, but when transferred to seawater, the plasma osmolalities increased to 431 mOsm. The increases in plasma osmolalities were presumably a reflection of the higher electrolyte levels generally found in salmonids after acclimation to seawater (Fontaine and Hatey 1950; Kubo 1955; Phillips and Brockway 1958; Gordon 1959; Houston 1959; Conte and Wagner 1965; Holmes and Stainer 1966; Miles and Smith 1968; Milne 1974; Clarke and Blackburn 1977; Newcomb 1978; Folmar et al. 1979a).

The mechanisms by which plasma electrolytes are maintained at relatively constant levels has been a somewhat controversial topic. In freshwater, there has been some agreement that diffusive ion losses are balanced, in part, by active Na^+ uptake coupled to H^+ , and/or NH_4^+ efflux across the gill (Maetz and Garcia Romeu 1964; Kerstetter et al. 1970). Kerstetter et al. (1970) and Maetz (1972) have shown that the $\text{Na}^+/\text{NH}_4^+$ exchange was not obligatory in trout or goldfish since removal of Na^+ from the external medium did not alter NH_4^+ excretion. When the diadromous eel (Anguilla anguilla) or euryhaline flounder (Plactichthyes flesus) were transferred to seawater, the Na^+ gradient reversed, Na^+ turnover rates increased, but no chronic Na^+ loading occurred (Motais and Maetz 1964; Motais et al. 1969; Maetz et al. 1967; Motais 1967). In contrast, Wood and Randall (1973) using S. gairdneri stated that Na^+/H^+ exchange was probably not stimulated by seawater transfer and did not contribute to increased Na^+ influx. This observation was further confirmed by Milne (1974) and Milne and Randall

(1976) who found no change in plasma pH and no significant change in H^+ excretion in S. gairdneri transferred to seawater. Greenwald et al. (1974), Kirschner et al. (1974), Shuttleworth et al. (1974) and Pic and Maetz (1975) have suggested that Na^+ loading was prevented in seawater by the reversal of the electrogenic potential (transepithelial potential or TEP) across the gill membrane which prevented the net diffusion of Na^+ into the fish. Transfer into seawater results in the external surface of the gill becoming negatively charged in relation to the blood (Shuttleworth et al. 1974; Pic and Maetz 1975; Silva et al. 1977) and establishes an electrogenic gradient which favors active Cl^- transport. This would require that Na^+ regulation be passive (Kerstetter and Kirschner 1972; Potts and Eddy 1973; House and Maetz 1974; Kirschner et al. 1974); however, if molar ratios of Na^+ and Cl^- remain constant (Murphy and Houston 1977; Folmar et al. 1979a), Na^+ loading would be prevented in seawater. There is also evidence for a carbonic anhydrase-dependent HCO_3^-/Cl^- exchange system in salmonids (Kerstetter and Kirschner 1972, 1974; McCarty and Houston 1974, 1977; Solomon et al. 1975; Houston and McCarty 1978). The process of linking HCO_3^- efflux with Cl^- influx would be beneficial in freshwater, but would cause Cl^- loading in seawater (Milne and Randall 1976). Although the foregoing literature has been concerned primarily with Na^+ and Cl^- balance, the ion exchange system also serves a broader function. The excretion of NH_4^+ serves as a regulatory mechanism for blood pH and the removal of nitrogenous wastes, while the HCO_3^- exchange accounts for

the net loss of respiratory CO₂ (deRenzis and Maetz 1973; Evans 1975a, b; Maetz 1974).

Two other changes which have been associated with ion regulation during entry into seawater are increases in gill and gut Na-K⁺ ATPase and a proliferation of chloride cells in the gill and subopercular epithelium. Since the initial description of the chloride cell in 1932 (Keys and Willmer) there have been many histochemical (Threadgold and Houston 1964; Conte 1969; Degnan et al. 1977; Karnacky and Kinter 1977; Karnacky et al. 1977) and biochemical (Maetz and Bornancin 1975) studies which strongly suggest that these mitochondria-rich cells are engaged in active transport of ions. The number of chloride cells in eels have been shown to proliferate upon seawater transfer and decrease when the animals are returned to fresh water (Olivereau 1970; Shirai and Utida 1970; Utida et al. 1971; Doyle and Epstein 1972). The number of chloride cells has been found to be directly proportional to the external salinity in Fundulus heteroclitus and Cyprinodon variegatus (Karnacky et al. 1976; Karnacky and Kinter 1977) and A. japonica (Shirai and Utida 1970; Utida et al. 1971). No relationship has been found between the number of chloride cells and external salinity in the coho salmon (H. A. Bern, Univ. Calif., Berkeley, personal communication). There are numerous reports which have shown biochemical and histological localization of ATPase in the gill chloride cells of F. heteroclitus (Karnacky et al. 1976), Lagodon rhomboides (Dendy et al. 1973a, b) and both European and Japanese eels

(Mizuhira et al. 1970; Shirai and Utida 1970; Utida et al. 1971; Kamiya et al. 1972; Sargent and Thomson 1974; Sargent et al. 1975; Thomson and Sargent 1977). Thomson and Sargent (1977) found, however, that gill $\text{Na}^+ - \text{K}^+$ ATPase activities did not show a linear correlation with the number of chloride cells.

Since the first report of increased gill $\text{Na}^+ - \text{K}^+$ ATPase activity in Japanese eels after transfer to seawater (Utida et al. 1966), there have been numerous reports which have demonstrated similar changes in gill (Epstein et al. 1967, 1971; Kamiya and Utida 1968, 1969; Maetz 1969, 1971, 1974; Zaugg and McLain 1969, 1970, 1971, 1972; Jampol and Epstein 1970; Pickford et al. 1970a, b; Butler and Carmichael 1972; Zaugg et al. 1972; Adams et al. 1973, 1975; Towle and Gilman 1973; Zaugg and Wagner 1973; Sargent and Thomson 1974; Evans and Mallery 1975; Giles and Vanstone 1976; McCarty 1976; Thomson and Sargent 1977; Dickoff et al. 1977, 1978a; Towle et al. 1977; Boeuf et al. 1978; Clarke et al. 1978; Folmar and Dickhoff 1978, 1979; Lasserre et al. 1978; Saunders and Henderson 1978; Folmar et al. 1979a, b) and gut (Oide 1967; Jampol and Epstein 1970; Pickford et al. 1970a) $\text{Na}^+ - \text{K}^+$ ATPase of several other diadromous and euryhaline fishes transferred from freshwater to seawater. In all cases the fish adapted to seawater had enzyme activities two to five times greater than fish adapted to freshwater. However, Kirschner (1969) was unable to show an effect of transfer to seawater on gill $\text{Na}^+ - \text{K}^+$ ATPase activity in either A. anguilla or P. flesus. Kirschner's (1969) inability to demonstrate

differences between fish in freshwater and seawater was attributed to the absence of the detergent desoxycholate in his homogenizing medium (Zaugg and McLain 1970; Kamiya 1972). Kamiya and Utida (1968) and Maetz (1969, 1971) have suggested that the increase in ATPase at seawater transfer was due to the increase in environmental K^+ . Increased environmental K^+ was apparently not required for the elevation of ATPase in migrating salmon smolts (Zaugg and McLain 1970, 1972; Boeuf et al. 1978, Lasserre et al. 1978, Folmar et al. 1979a). However, increased environmental K^+ may be necessary to maintain elevated enzyme activities, since the ATPase activities of migrating smolts regress to presmolt freshwater levels if the fish are prevented access to seawater. Fluctuations in gill Na^+-K^+ ATPase have also been attributed to changes in environmental temperature and photoperiod (Zaugg et al. 1972; Adams et al. 1973, 1975; Zaugg and Wagner 1973; Giles and Vanstone 1976; Clarke et al. 1978). Giles and Vanstone (1976) found that coho salmon gill Na^+-K^+ ATPase was extremely cold sensitive, which prompted McCarty and Houston (1977) and Houston and McCarty (1978) to suggest that carbonic anhydrase, which they found to be insensitive to reduced temperatures, was responsible for providing the H^+ and HCO_3^- needed to fuel the Na^+/H^+ and Cl^-/HCO_3^- exchanges at low environmental temperatures.

Although there have been a number of demonstrations that gill Na^+-K^+ ATPase was stimulated by entry into seawater, the biochemical impetus for these changes remains unresolved. There are at least three

possible mechanisms which have been investigated: 1) the presence of an isozyme system with two functional alleles, one for freshwater and one for seawater, 2) the unmasking of currently existing enzyme sites by conformational changes in the gill epithelium, and 3) an increase in the number of pumping sites via the synthesis of new enzyme protein. The first possibility has not been supported by recent investigations by Towle et al. (1976). Using polyacrylamide gel electrophoresis, they were unable to discern any isozyme-like protein bands from the gills of the euryhaline blue crab (Callinectes sapidus). There has been evidence published for the second hypothesis in euryhaline fish. Towle et al. (1977) measured the binding of tritiated ouabain (a specific inhibitor of $\text{Na}^+\text{-K}^+$ ATPase) in the gills of F. heteroclitus and found that the number of binding sites was a reflection of the external salinity. These observations resulted in a model (Towle 1977) which demonstrated rapid conformational changes in the epithelial membrane, allowing more receptor sites to be exposed with increased salinity. This mechanism appears logical for euryhaline fish which must quickly and constantly adjust to different salinities. However, it does not fit the three to five days adjustment period observed in salmonids (Giles and Vanstone 1976; Dickhoff et al. 1977; Folmar and Dickhoff 1978, 1979; Folmar et al. 1979a). The third hypothesis, an increase in protein synthesis probably best explains the changes observed in diadromous fishes. Conte and Lin (1967) measured DNA turnover rates in gill filaments of chinook salmon before and after entry into seawater. Their results indicated that de novo synthesis of new gill epithelial

protein required four to six days, which coincided with the time required for gill $\text{Na}^+\text{-K}^+$ ATPase activities to increase in coho salmon transferred from freshwater to seawater (Giles and Vanstone 1976; Dickhoff et al. 1977; Folmar and Dickhoff 1978, 1979; Folmar et al. 1979a). The preceding information cannot be considered definitive proof, but it appears that euryhaline and diadromous fishes differ in their method of ATPase regulation.

Regulatory differences that may exist between euryhaline and diadromous fishes are probably attributable to different endocrine influences, since there has been evidence presented that prolactin (Pickford et al. 1970a; Utida et al. 1972; Hirano et al. 1973) cortisol (Epstein et al. 1967, 1971; Pickford et al. 1970b; Hirano and Utida 1971; Butler and Carmichael 1972; Butler 1973) and thyroid hormones are capable of regulating $\text{Na}^+\text{-K}^+$ ATPase activity in teleosts.

There have been a number of reviews concerned with thyroid function in teleosts (Goldsmith 1949; Fleischman 1951; Lynn and Wachowski 1951; Gorbman 1955, 1959, 1969; Berg et al. 1959; Hoar 1959; Leloup and Fontaine 1960; Roche 1960; Dodd and Matty 1964; Bern and Nandi 1964; Matty 1966; Barrington 1968; Barrington and Sage 1972; Sage 1973; Fontaine 1975; Woodhead 1975), but there are no recent reviews on the influence of thyroid hormones on smoltification and the accompanying changes in morphology, behavior, and hydromineral balance. Thyroid involvement in smoltification was originally suggested by Hoar (1939b) who observed activation (histological) of the thyroid tissue of

Atlantic salmon during the parr-smolt transformation. Since then, there has been an accumulation of information which has shown that thyroid hormones variously affect growth, morphogenesis, skin pigmentation, osmoregulatory properties, and behavior during the smoltification process. The characteristic melanin dispersal (Matty and Sheltaw 1967) and integumentary deposition of guanine (LaRoche and Leblonde 1952; Dales and Hoar 1954; Johnston and Eales 1967; Sage 1968) have been associated with increased thyroid activity. There are reports relating thyroid hormones to the behavioral changes associated with smoltification: salinity preference (Baggerman 1963), seaward migration (Hoar 1939a, 1952, 1959, 1976; Koch and Heuts 1942; Hoar and Bell 1950; LaRoche and Leblonde 1952; Dales and Hoar 1954; Fontaine 1958; Olivereau 1954; Baggerman 1957, 1960a, b, 1962; Fage and Fontaine 1958; Hickman 1959, 1962; Homma 1959; Woodhead 1959a, b, 1975; Honma and Tamura 1963; Koch 1968; Gorbman 1969; Honma et al. 1977) and changes in swimming activity (Hoar et al. 1956; Baggerman 1962; Woodhead 1970; Godin et al. 1974, Katz and Katz 1978). There have also been behavioral studies related to the influence of thyroxine (T_4) on orientation and the perception of environmental changes in photoperiod and temperature (Hoar et al. 1952; Hara et al. 1965, 1966; Oshima and Gorbman 1966; Hara and Gorbman 1967; Sage 1968; Oshima et al. 1972).

The continuation of growth following the period of smoltification has also been related to both T_4 and triiodothyronine (T_3). It has been suggested that the thyroid hormones play a permissive role in the

growth of teleosts, as in mammals, since both stimulation and inhibition of growth can be achieved by T_4 injections at different life stages (Gorbman 1969; Higgs et al. 1976). Recent evidence has indicated that this regulation may be achieved through a positive feedback relationship between T_4 and somatotrophic hormone (STH or GH) (Sage 1967; Pandey and Leatherland 1970; Leatherland and Hyder 1975; Chan and Eales 1976; Higgs et al. 1976; Milne and Leatherland 1978). A reduction in thyroid hormone production after seawater entry has been related to growth cessation in coho salmon (Clarke and Nagahama 1977; Bern 1978; Folmar et al. 1979b).

There appeared to be a seasonal cycle of thyroid hormone production in freshwater salmonids, with peaks occurring during the spring and fall of the year (Swift 1955, 1959, 1960; White and Henderson 1977; Osborn et al. 1978). The peak occurring in the fall has been associated with gonadal development and spawning in freshwater teleosts (Swift 1959; Gorbman 1959; Matty 1960; Dodd and Matty 1964; White and Henderson 1977; Osborn et al. 1978). The magnitude of this peak has also been related to the sex of the fish (Chan and Eales 1976). The springtime peak of T_4 production appeared to coincide with the period of smoltification in anadromous salmonids (Dickhoff et al. 1978b). These authors provided evidence that this peak occurred at a critical stage of development analogous to metamorphic climax in anuran amphibian metamorphosis (Leloup and Buscaglia 1977; Regard et al. 1978). However, there were major differences in the duration of

thyroid activation. In anurans, the T_4 surge occurred over a period of six days and was associated with rapid and distinct changes in morphological indices (Taylor and Kollros 1946), while the peak in coho salmon extended for 30-60 days and appeared to exert a gradual influence on changes in morphological characteristics (Dickhoff et al. 1978b). This springtime surge of thyroid hormone activity in coho salmon occurred concomitantly with the increased ATPase activity associated with smoltification (Folmar and Dickhoff 1978, 1979). Activation of ATPase has been suggested as the mechanism by which thyroid hormones mediate their metabolic effects (Ismail-Beigi 1977). Evidence has been presented for the activation of Na^+-K^+ ATPase by thyroid hormones in a variety of mammalian (Valcana and Timiras 1969; Ismail-Beigi and Edelman 1970, 1971, 1974; Ismail-Beigi 1977), amphibian (Kawada et al. 1969), chondrichthyan (Honn and Chavin 1977) and teleostean (Dickhoff et al. 1977; Folmar and Dickhoff 1978, 1979; Folmar et al. 1979a) tissues. However, Hoar (1968) found no increases in oxygen consumption after T_4 injections in goldfish, and concluded that T_4 did not induce a calorogenic effect in goldfish. Also, Ray et al. (1977) found that T_4 injection stimulated para-nitrophenyl-phosphatase (pNPPase), an alkaline phosphatase thought to play a role in active transport of ions in the teleost gut (Oide 1970). Increased circulating levels of T_4 have also been observed after transfer of coho salmon to seawater (Dickhoff et al. 1977; Folmar and Dickhoff 1978, 1979; Folmar et al. 1979a), probably as a result of the increased availability of environmental iodine (Gorbman and Berg 1955; Hickman

1959). The opposite effect, a reduction in thyroid hormone production, has been demonstrated with coho salmon exposed to low environmental iodine or xenobiotics such as mirex or polychlorinated biphenyls (Black and Simpson 1974; Drongowski et al. 1975; Sonstegard and Leatherland 1976; Moccia et al. 1977; Leatherland and Sonstegard 1978). Teleost thyroid hormones have also been shown to promote mobilization of lipids, stimulate protein synthesis, and influence general carbohydrate metabolism (Gorbman 1969, Narayansingh and Eales 1975a, b).

The springtime surge of thyroid hormones may be responsible, at least in part, for other changes observed at smoltification. These changes include decreased body lipids (Lovern 1934; Hoar 1939a; Kizevetter 1948; Fontaine and Hatey 1950; Evropeitsevia 1957; Malikova 1957; Parker and Vanstone 1966; Fessler and Wagner 1969; Vanstone and Markert 1968, 1969; Foda 1974; Wagner 1974a; Komourdjian et al. 1976a; Clarke et al. 1978; Farmer et al. 1978), alterations in carbohydrate metabolism (Fontaine and Hatey 1950; Fontaine 1960; Wendt and Saunders 1973; Withey and Saunders 1973) changes in the amino acid composition of muscle (Malikova 1957; Cowey et al. 1962) and increased buoyancy (Saunders 1965; Pinder and Eales 1969).

This review has been primarily concerned with the thyroid hormones which appear to play a major role in salmonid smoltification, but virtually all of the known teleost endocrine principles have been shown to play at least a minor role in salmonid smoltification (c.f. Bern 1978) and hydromineral balance in general (c.f. Johnson 1973).

METHODS AND MATERIALS

The yearling coho salmon used in this study were obtained from hatcheries on tributaries of the Columbia River: Klickitat, Kalama Falls, Rocky Beach, Toutle and Willard; Washington Department of Fisheries, and Big Creek and Sandy; Oregon Department of Fish and Wildlife. Two groups of Big Creek hatchery fish were tested in this study. The first was a Big Creek stock (Big Creek I), while the second was a group of Cowlitz hatchery (Washington Department of Fisheries) fish reared at the Big Creek hatchery (Big Creek II). There were also two groups of Sandy hatchery fish tested. The first group was reared on commercial Oregon Moist pellets (Sandy I), while the second group (Sandy II) was reared on a substituted soy oil diet. A serial release program at the Willard hatchery enabled us to collect fish at two different release times: May 6th (Willard I) and May 24th (Willard II).

Samples of gill filaments and plasma were collected from fish in freshwater at the individual hatcheries at two week intervals from February through June 1978. For the seawater portion of the study, approximately 650 fish were collected from each hatchery at the time of release and transported by tank truck to the National Marine Fisheries Service (NMFS) Aquaculture Research Station at Manchester, Washington. Table 1 lists the dates of transfer from the respective hatcheries to Manchester, hauling times, loading densities and transportation mortalities for each of the test groups. The loading

Table 1. Dates of transfer from the hatchery to Manchester, tank truck hauling times, densities and transportation mortalities for each experimental group of Columbia River coho salmon.

Stock	Date of transfer	Hauling time (hr:min)	Hauling density (lb/gal)	Hauling mortalities (No.)
Klickitat	4/28/79	7:30	0.16	0
Toutle	4/28/79	2:45	0.19	0
Rocky Reach	5/2/78	6:00	0.15	5
Sandy I	5/4/78	7:00	0.20	0
Sandy II	5/4/78	7:00	0.20	0
Kalama Falls	5/4/78	4:20	0.13	2
Willard I	5/6/78	6:30	0.15	1
Big Creek I	5/8/78	5:20	0.19	0
Big Creek II	5/8/78	5:20	0.18	0
Willard II	5/24/78	6:45	0.15	0

densities were well below the recommended level of 0.5 lb/gal. There were no significant mortalities in any of the test groups. Upon arrival at Manchester, the fish were placed in freshwater, weighed and measured, and then divided into two groups: one for destructive subsampling (350 fish) and the other for a six month growth and survival study (300 fish). The fish designated for the survival study were further divided into two groups of 150 fish each; one group was vaccinated with a bivalent Vibrio vaccine, and the other received no treatment. Vaccination was accomplished by a 0.15 ml intraperitoneal (i.p.) injection of heat killed Vibrio anguillarum (Biotype I and Biotype II in a 3:1 ratio, Schiewe et al. 1977) in a vehicle of 0.85% sodium chloride solution. At this point, all of the experimental fish were transferred to sea water net pens (1.2 m wide x 1.2 m deep x 2.1 m long. Nylon mesh with 5 mm aperture, Delta weave). During their six months in seawater, all fish were weighed to the nearest one-tenth gram and measured to the nearest millimeter every 30 days from May through November. Mortalities were removed from the net-pens twice daily. Gill filaments and plasma were collected from fish in freshwater just prior to their seawater transfer, daily for the first eight days in seawater, and then at 30 and 180 days after transfer to seawater.

Gill filaments were collected and analyzed for gill $\text{Na}^+ - \text{K}^+$ ATPase activity according to the methods of Folmar and Dickhoff (1979). To prepare the enzyme, gill filaments from three fish were excised and pooled to yield approximately 0.2 g of tissue. The filaments were then

homogenized in 3.0 ml of a medium consisting of 300 mM sucrose, 20 mM disodium ethylene-diaminetetraacetic acid (EDTA) and 100 mM imidazole in distilled water. The homogenate was decanted and the tube was then rinsed with an equal volume of distilled water which was added to the homogenate. The homogenate was centrifuged at 800 x g for five minutes in a clinical centrifuge. The supernatant was then decanted and the pellet resuspended in 2.0 ml of the homogenizing medium with the addition of 0.01% sodium desoxycholate. The resuspended preparation was again centrifuged at 800 x g for five minutes, and from the resulting supernatant a 1.0 ml sample was removed, quick frozen on dry ice, and stored at -70°C for future analysis. Determinations of $\text{Na}^{+}\text{-K}^{+}$ ATPase specific activities were made by the discontinuous inorganic phosphate estimation method of Fiske and Subbarow (1925). The reaction mixture for this assay contained 135 mM NaCl, 65 mM KCl, 20 mM MgCl_2 , 250 mM imidazole and 5 mM ATP at pH 6.8 (37°C). A duplicate tube containing 0.5 mM ouabain, a cardiac glycoside which specifically inhibits $\text{Na}^{+}\text{-K}^{+}$ ATPase activity (Skou 1957), was used to determine residual activity attributable to other ATPase systems. The $\text{Na}^{+}\text{-K}^{+}$ ATPase activity was then calculated by subtracting the activity of the second tube from the total activity of the first tube. Samples of the enzyme preparation were diluted with homogenizing medium to contain 20-30 μg protein per reaction volume. After a 20 minute incubation at 37°C the reaction was terminated by the addition of 100 μl of ice-cold 30% trichloroacetic acid (TCA). The reaction mixtures were then transferred to cuvettes and the absorbance was determined in a

spectrophotometer at 720 nm, 35 minutes after the addition of the TCA. The temperature and pH optima were 40°C and 6.8 respectively. The apparent K_m for ATP in this reaction mixture was 1.0 mM. The K_i for ouabain was 2.5×10^{-5} M.

Protein determinations were made for each enzyme preparation according to the method of Lowry et al. (1951). For this assay 0.1 ml of the gill homogenate was diluted to 0.5 ml with homogenizing medium. After dilution, 5.0 ml of the Lowry reagents and 0.5 ml of Folin's reagent were added. The tubes were then agitated in a vortex mixer and allowed to stand for 45 minutes, at which time spectrophotometric measurements were made at 760 nm.

Plasma thyroxine (T_4) and triiodothyronine (T_3) levels were determined by radioimmunoassay (RIA) using the methods of Dickhoff et al. (1978). For this assay, 10 μ l aliquots of plasma were added in duplicate to the assay tubes. After the addition of plasma, 250 μ l of the following mixture was added: 150 mg of bovine δ -globulin, 60 mg of 8-anilo-1-naphthelenesulfonic acid (sodium salt) and 12×10^6 cpm of 125 I radiolabelled T_4 in 100 ml of 0.11 M barbital buffer (pH 9.0). Also included in this mixture was a small quantity of antiserum which had been previously diluted to a concentration which would result in 50% labelled T_4 bound with no added unlabelled T_4 . The tubes were then capped and incubated for 30 minutes at 37°C followed by 15 minutes at 4°C. The antibody was then precipitated by the addition of 0.3 ml of cold (4°C) 20% (w/v) polyethylene glycol followed by thorough mixing in

a vortex mixer. The precipitate was centrifuged at 2000 x g for 15 minutes at 4°C. The supernatant was then aspirated, and the remaining pellet was counted in a gamma well counter for three minutes. A sequence of serially diluted standards were analyzed with each assay. Dilution of coho salmon plasma showed parallel crossreactivity with the T₄ standard. Consequently, coho plasma was treated with an equal volume of dextran coated charcoal (5 g/l of Norit A and 5 g/l of dextran) which removed all cross-reactivity. Addition of T₄ to dextran-coated charcoal treated or untreated plasma followed by RIA resulted in average recoveries of 108% and 98% of added T₄ respectively. The coefficients of variation for the RIA of coho plasma were 5% intraassay and 17% interassay. All procedures and reagents for the T₃, RIA were the same as that for T₄ with the exception of the T₃ antisera and T₃ label. Trade names may be found in the original publications.

Plasma ions were measured by the use of a flame photometer (Na⁺, K⁺) and chloridometer (Cl⁻). Samples for plasma ion determinations were prepared in accordance with the manufacturer's suggested methods for each instrument. For the flame photometer, 10.0 μl of plasma was added to 2.0 ml of lithium diluent (15 meq Li/l). For the chloridometer 50.0 μl of plasma were added to a solution of 4.0 ml of nitric acid reagent (0.1 N nitric acid and 10% glacial acetic acid) and four drops of gelatin reagent (gelatin, thymol blue, thymol; 60:1:1).

Normal clinical control serum of known composition was prepared and analyzed in parallel with every 20 samples.

The data resulting from these experiments were placed on punch cards and subjected to computerized statistical analysis. The statistical methods and Fortran programs used to calculate linear regressions, correlation coefficients and analyses of variance (ANOVA) were those of Sokal and Rohlf (1968).

RESULTS

To facilitate comparison of the experimental measurements, graphic representations have been separated into four groups.

Gill Na⁺-K⁺ ATPase Activities and
Plasma T₄ and T₃ Concentrations in Freshwater Fish

Graphic representations of the measurements of gill Na⁺-K⁺ ATPase specific activities and plasma T₄ and T₃ concentrations in freshwater fish are presented in Figs. 1-9. Peak values for both gill Na⁺-K⁺ ATPase specific activities and plasma T₄ concentrations occurred during April or May for all of the test groups. Within each test group, the peaks for both ATPase and T₄ occurred concurrently in some groups (Figs. 3, 5, 8), while in the other groups they were separated by two weeks (Figs. 2, 4, 6), three weeks (Figs. 1, 9) or four weeks (Fig. 7). The T₃ concentrations remained relatively constant throughout the sampling period, with the exception of the Klickitat (Fig. 3) and Kalama Falls (Fig. 4) groups which showed increases. The standard errors of the means for ATPase and T₄ showed a tendency to increase during the peaks of activity, suggesting that these parameters did not change uniformly within the sampled population.

Figure 1. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Rocky Reach Hatchery (Brackets indicate \pm SEM, N = 10 samples of three fish each except where parenthetically noted, arrow indicates hatchery release date).

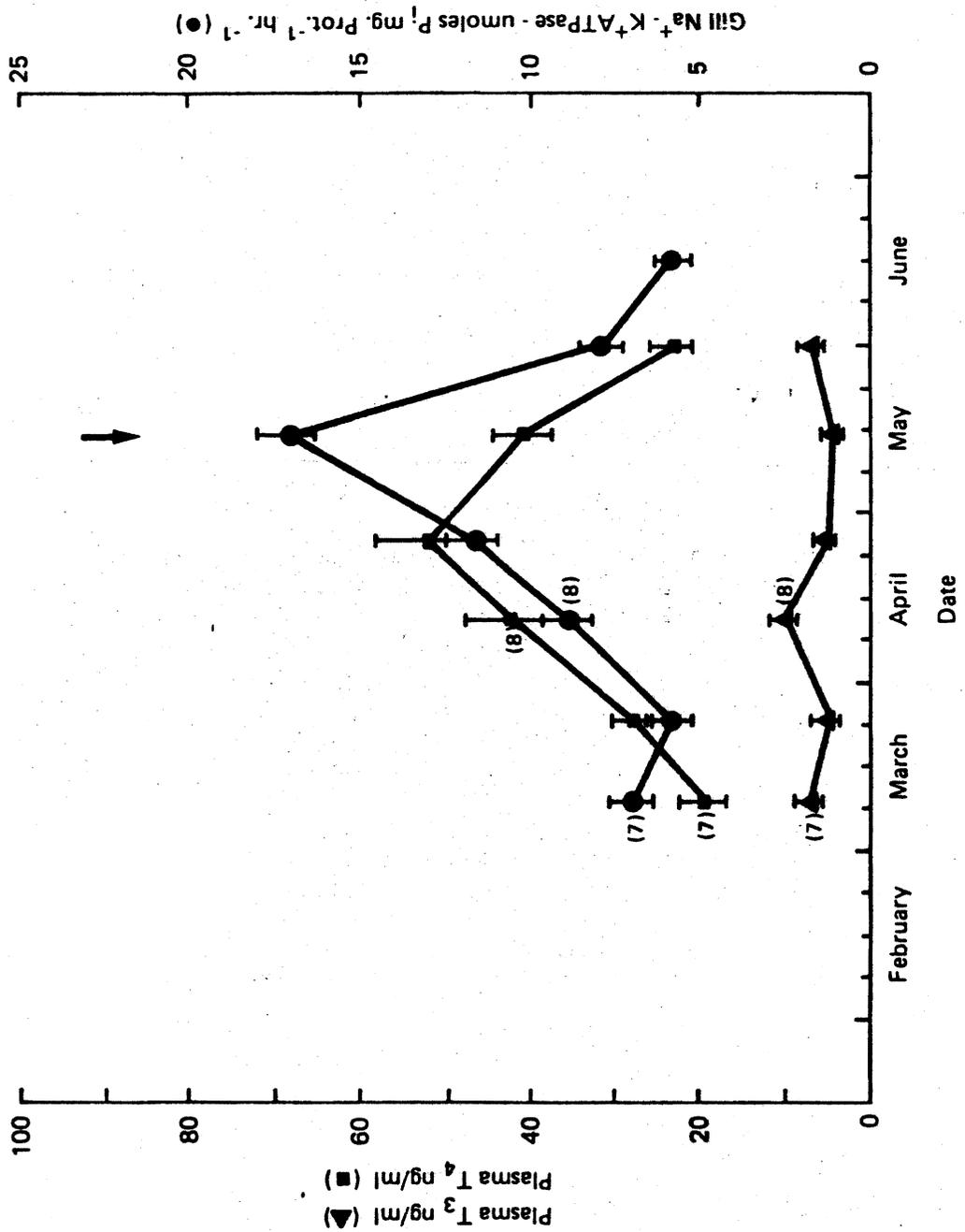


Figure 2. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Toutle hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted, arrow indicates hatchery release date).

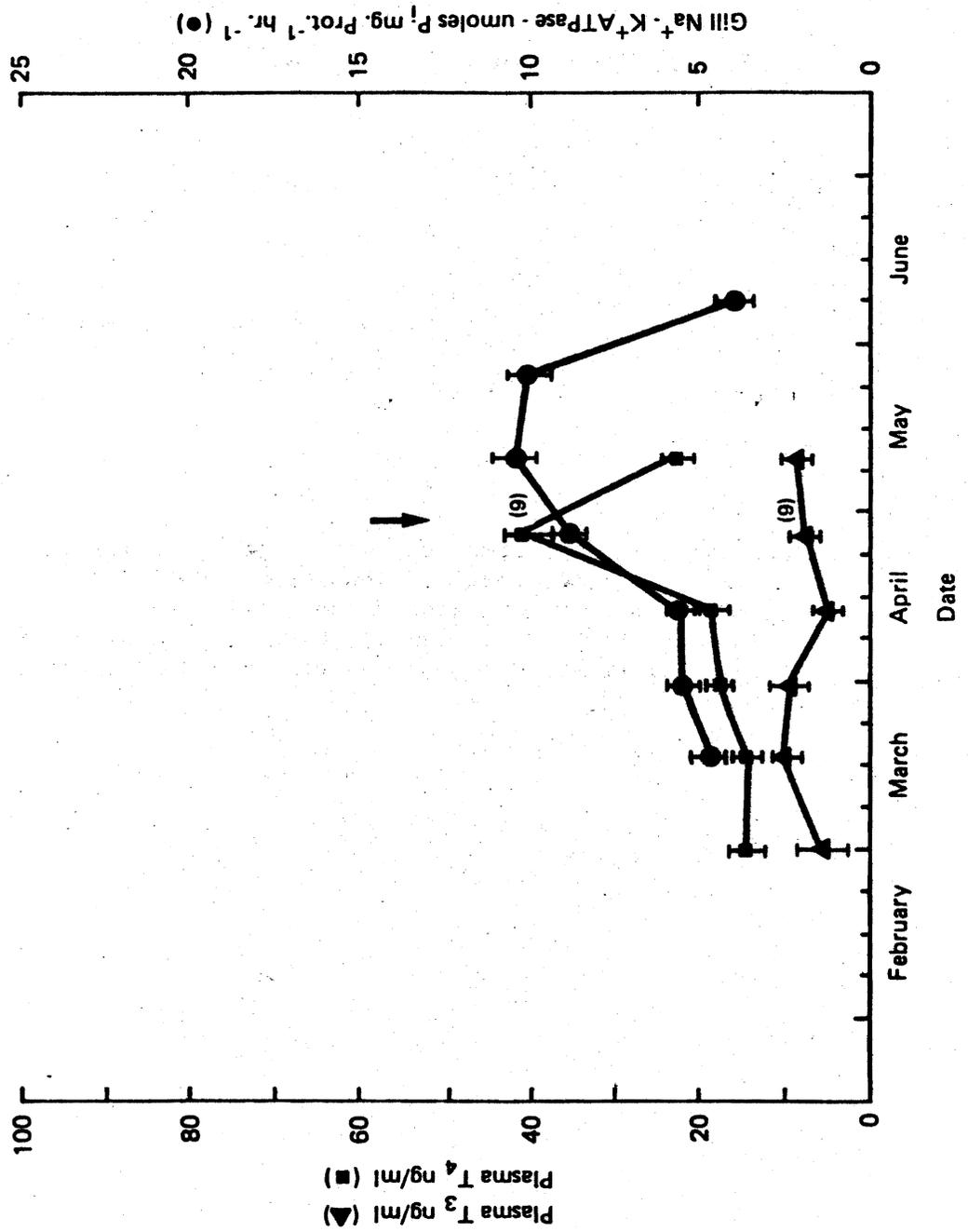


Figure 3. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Klickitat Hatchery (Brackets indicated \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted, arrow indicates hatchery release date).

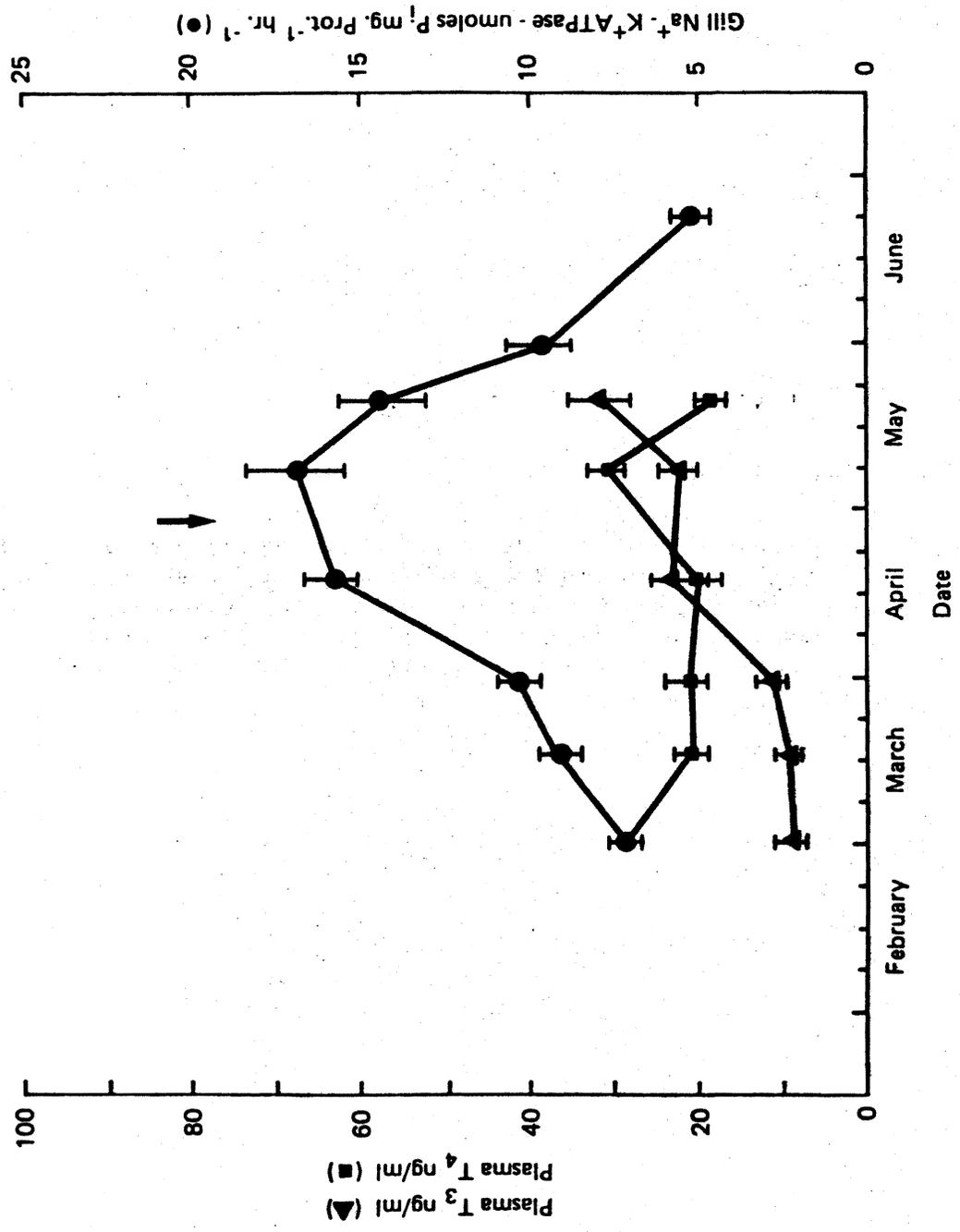


Figure 4. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Kalama Falls Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted, arrow indicates hatchery release date).

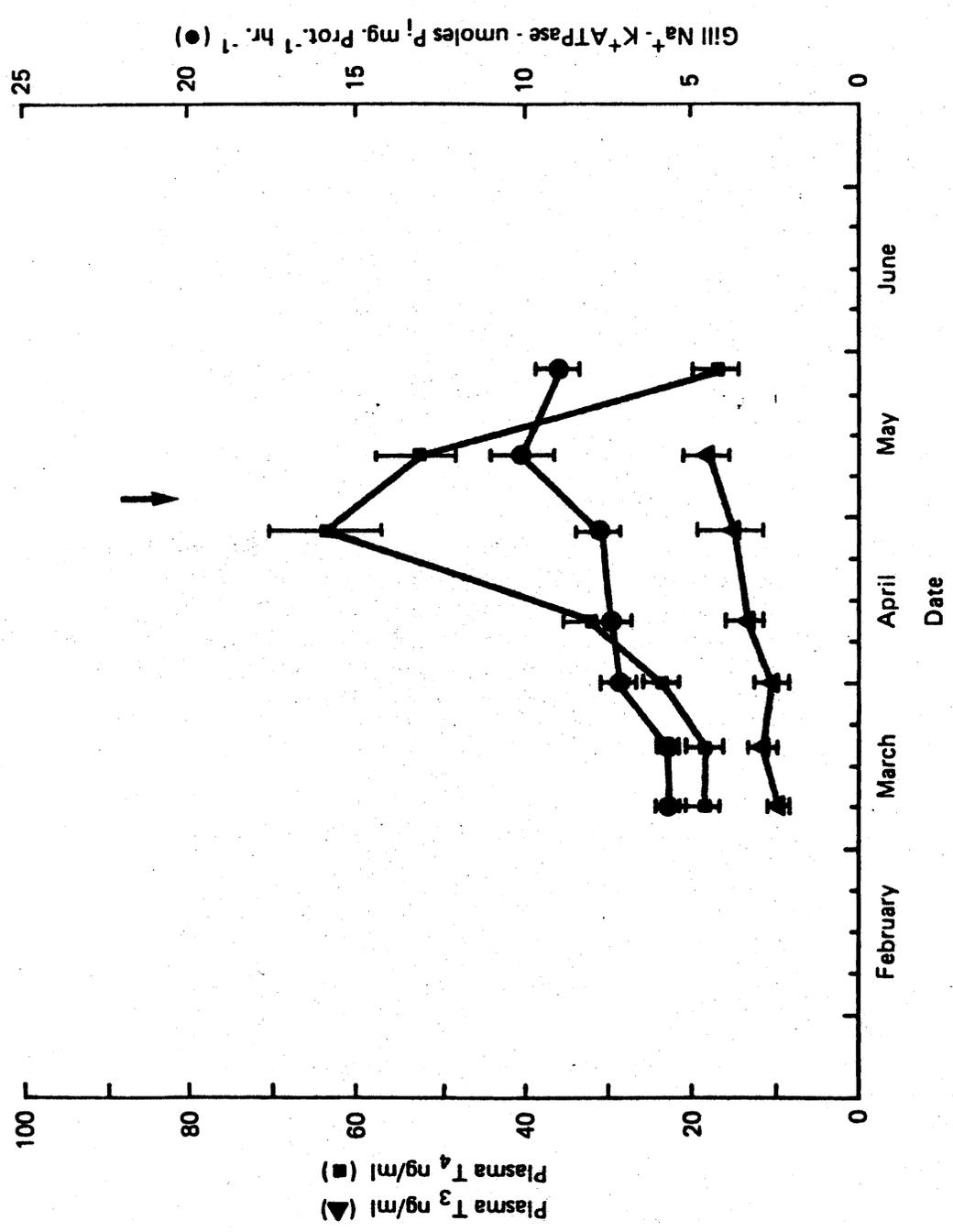


Figure 5. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Big Creek Hatchery (BC I--Big Creek stock) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted, arrow indicates hatchery release date).

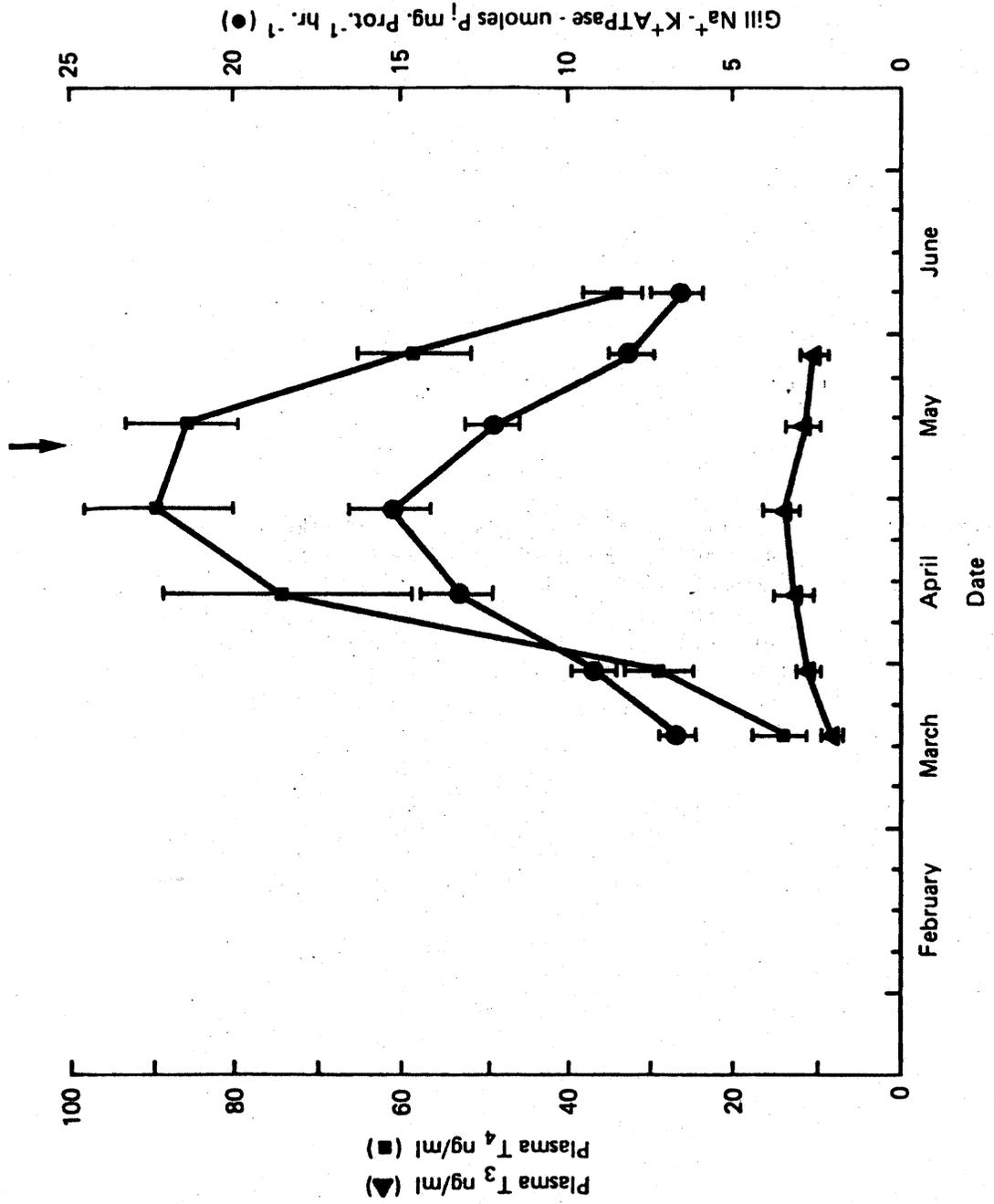


Figure 6. Mean gill Na^+-K^+ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Big Creek Hatchery (BC II--Cowlitz stock)(Brackets indicate \pm SECM, N = 10 samples of 3 fish each except where parenthetically noted, arrow indicates hatchery release date).

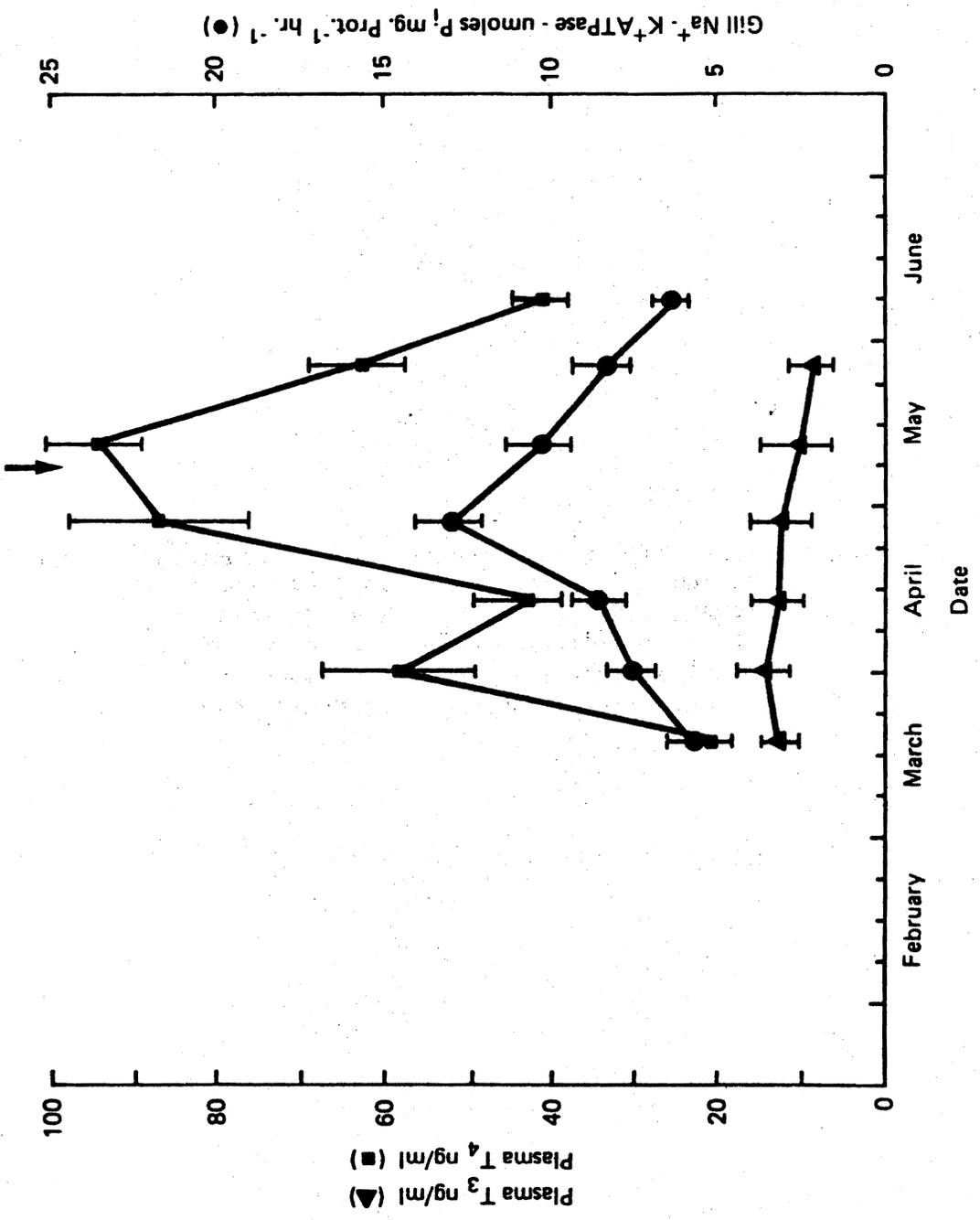


Figure 7. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Sandy Hatchery (Sandy I--normal production diet) (Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted, arrow indicates hatchery release date).

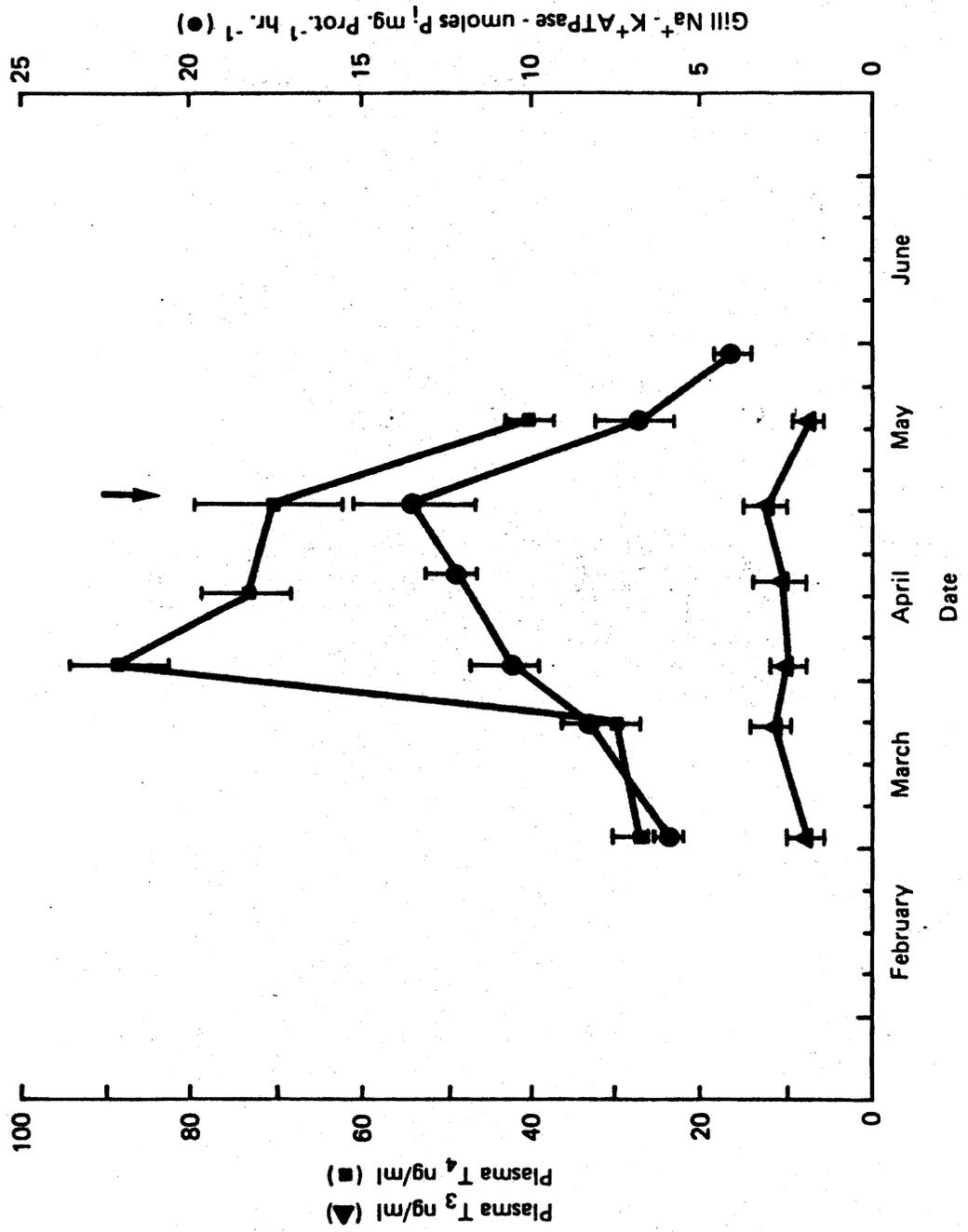


Figure 8. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Sandy Hatchery (Sandy II--soy oil substituted diet)(Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted, arrow indicates hatchery release date).

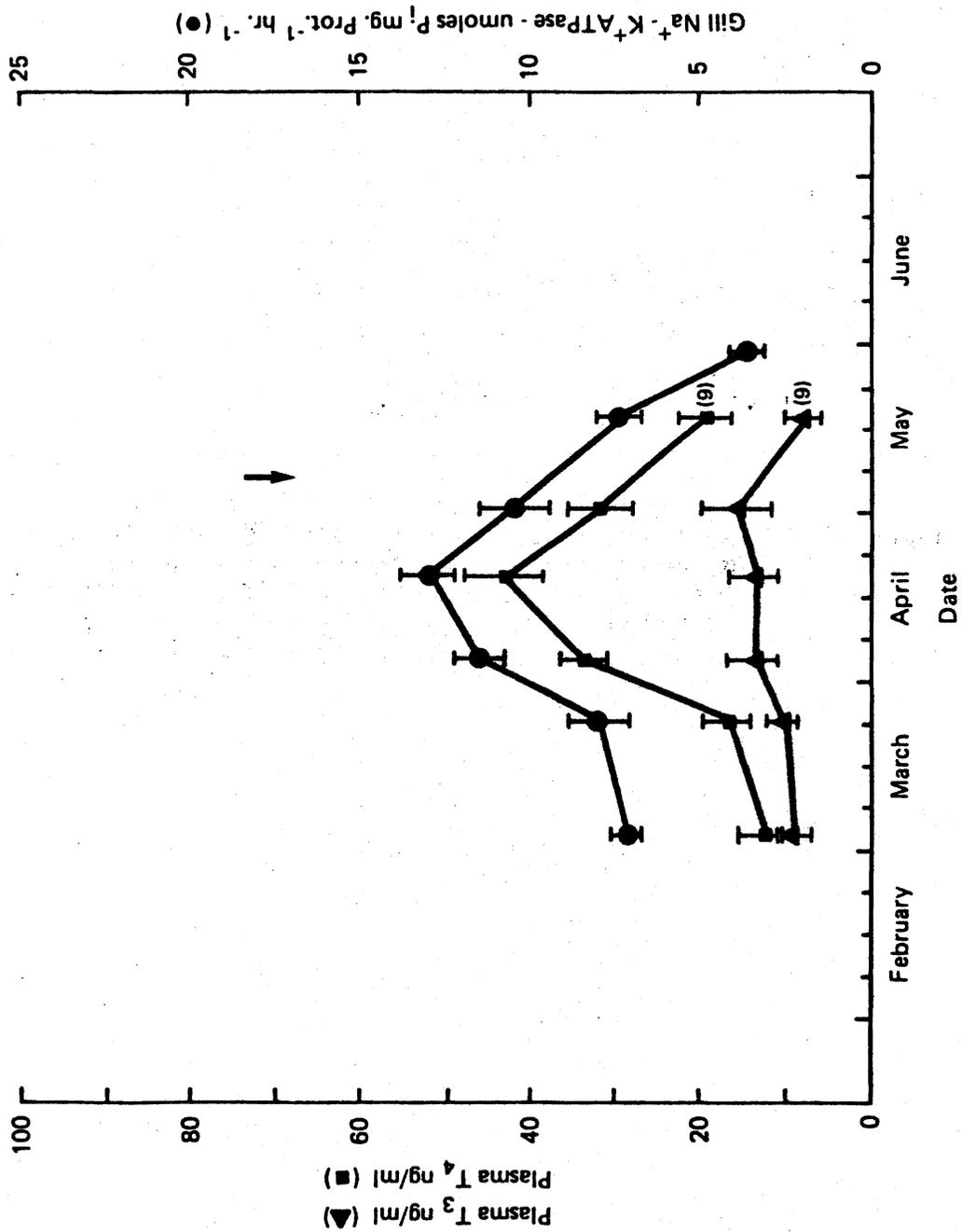
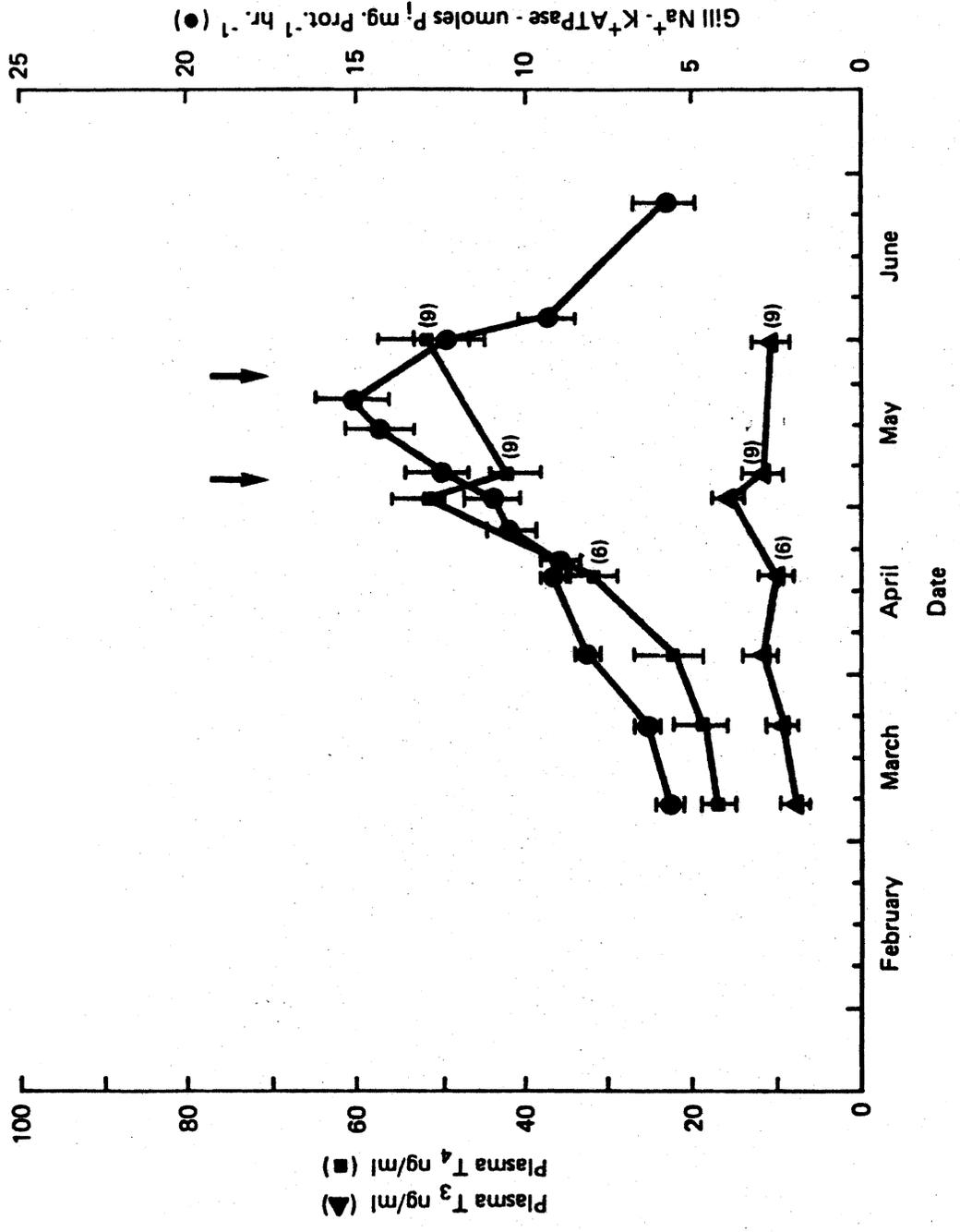


Figure 9. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations from fish in freshwater at the Willard Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted, arrow indicates hatchery release date).





Gill Na⁺-K⁺ ATPase Activities and
Plasma T₄ and T₃ Concentrations in Seawater Fish

Measurements of gill Na⁺-K⁺ ATPase specific activities and plasma T₄ and T₃ concentrations of fish in seawater are graphically presented in Figs. 10-19. Transfer of yearling coho salmon from freshwater to seawater resulted in significant ($P \leq 0.001$) increases in gill Na⁺-K⁺ ATPase activity during the first eight days of seawater residence in all of the test groups. In contrast, changes in plasma T₄ concentrations varied among the test groups. The initial changes after one day in seawater included increased (Figs. 11, 16, 17), decreased (Figs. 10, 12, 14, 15, 18, 19) or no change (Fig. 13) in plasma levels. After these initial changes all groups showed a gradual decline through the remaining sampling periods. Plasma T₃ concentrations showed similar, variable changes after seawater transfer.

Plasma Electrolytes in Freshwater Fish

Figures 20-28 show the plasma electrolytes (sodium, potassium, and chloride ions) for coho salmon in freshwater. The blood electrolyte data were collected from the same fish used for the ATPase, T₄ and T₃ analyses in Figs. 1-9. Insufficient amounts of plasma prevented measurement of chloride ions for the blood samples presented in Figs. 20 and 21. Plasma Na⁺ and Cl⁻ concentrations tended to decrease in late April or early May then returned to their

Figure 10. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Rocky Reach Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted).

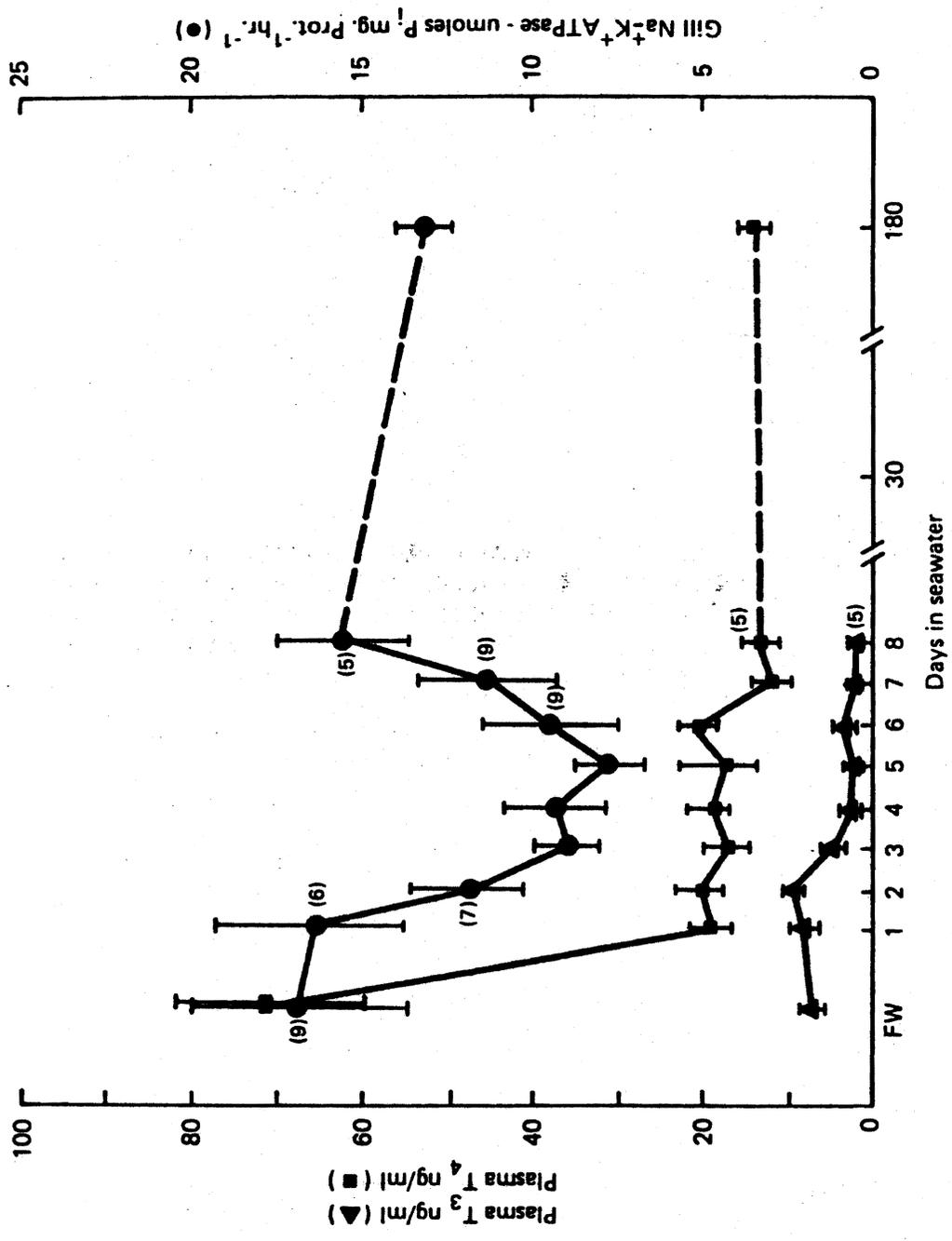


Figure 11. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase (●) activities and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Toulle Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted).

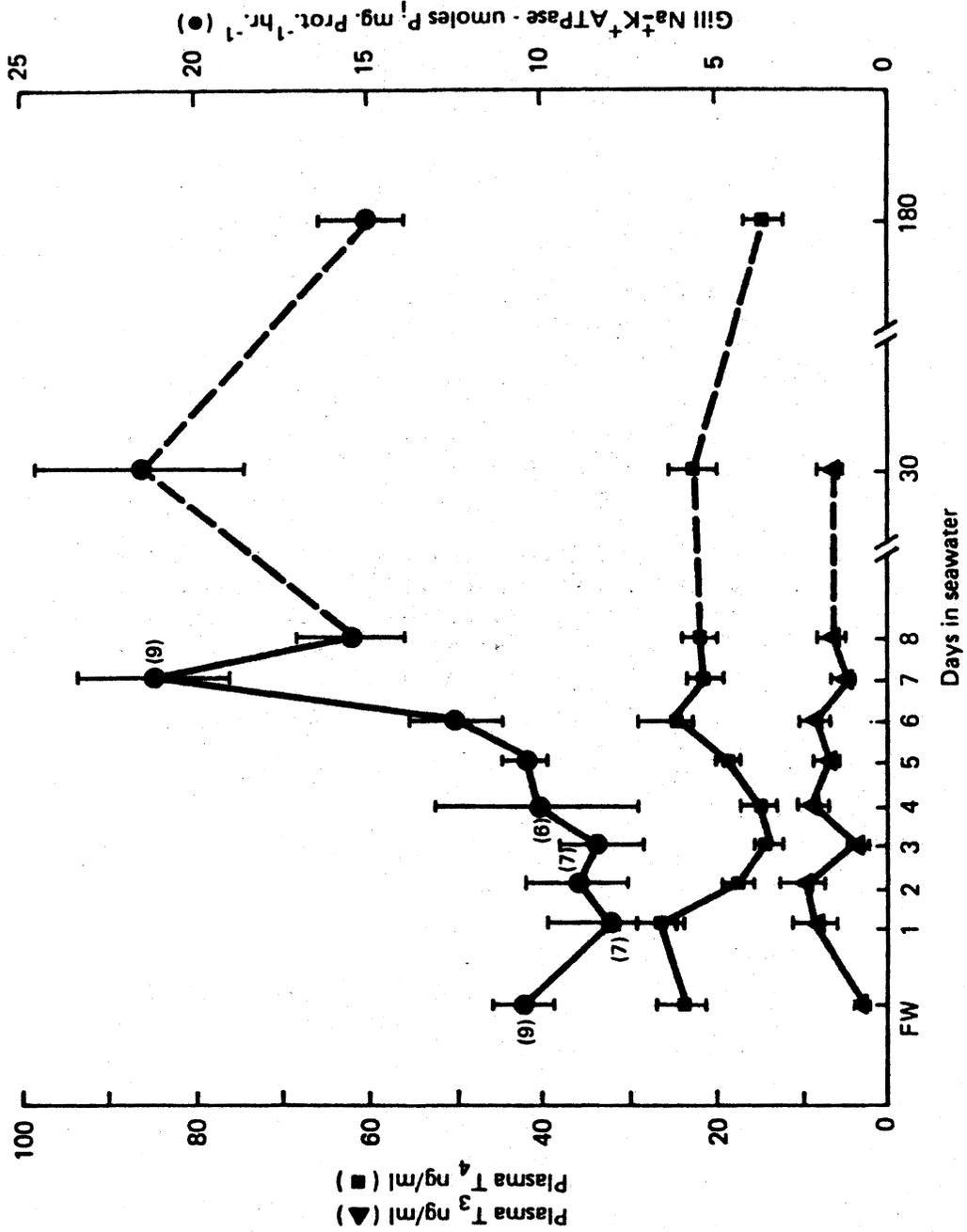


Figure 12. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Klickitat Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish each except where parenthetically noted).

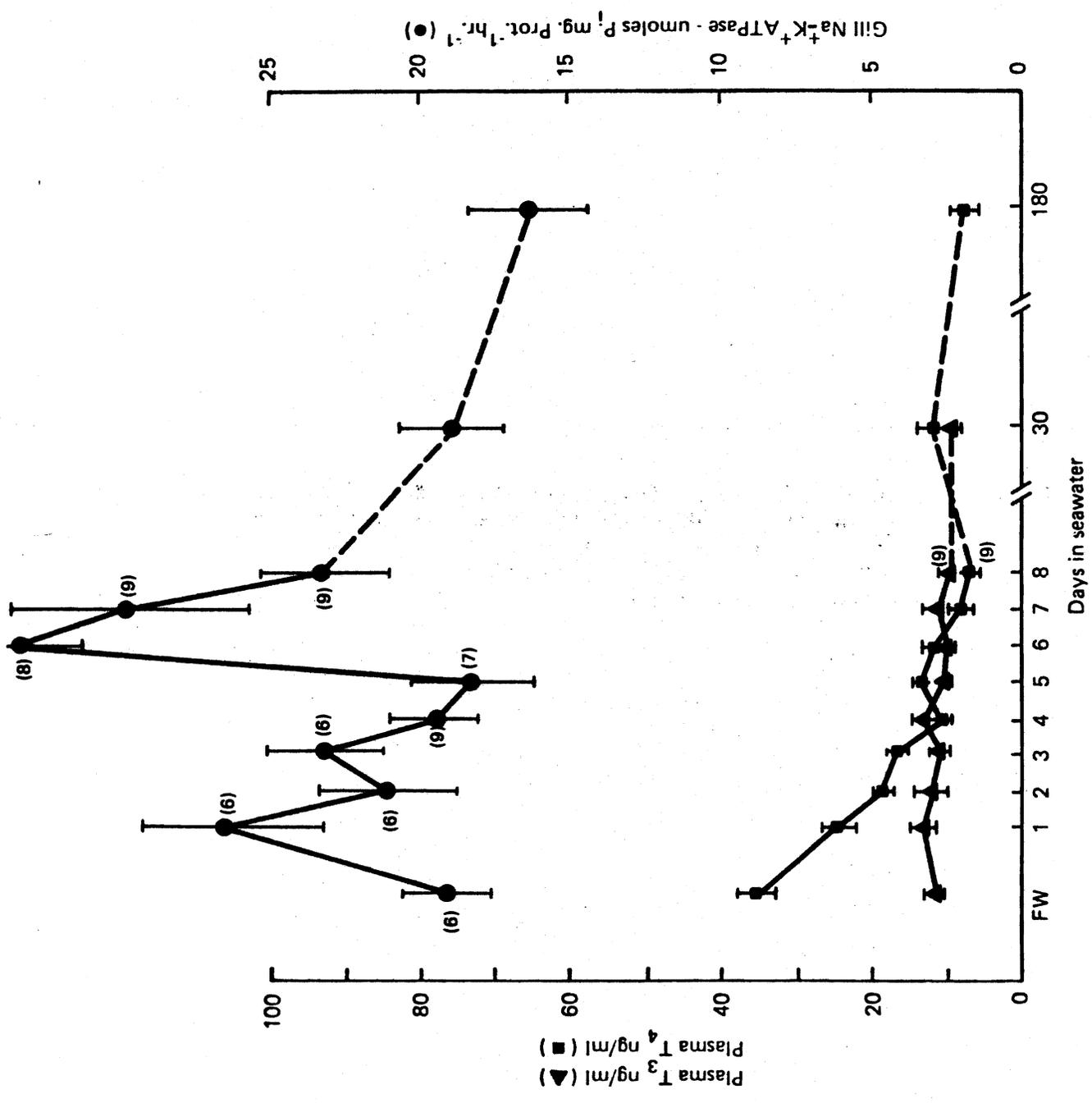


Figure 13. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Kalāma Falls Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

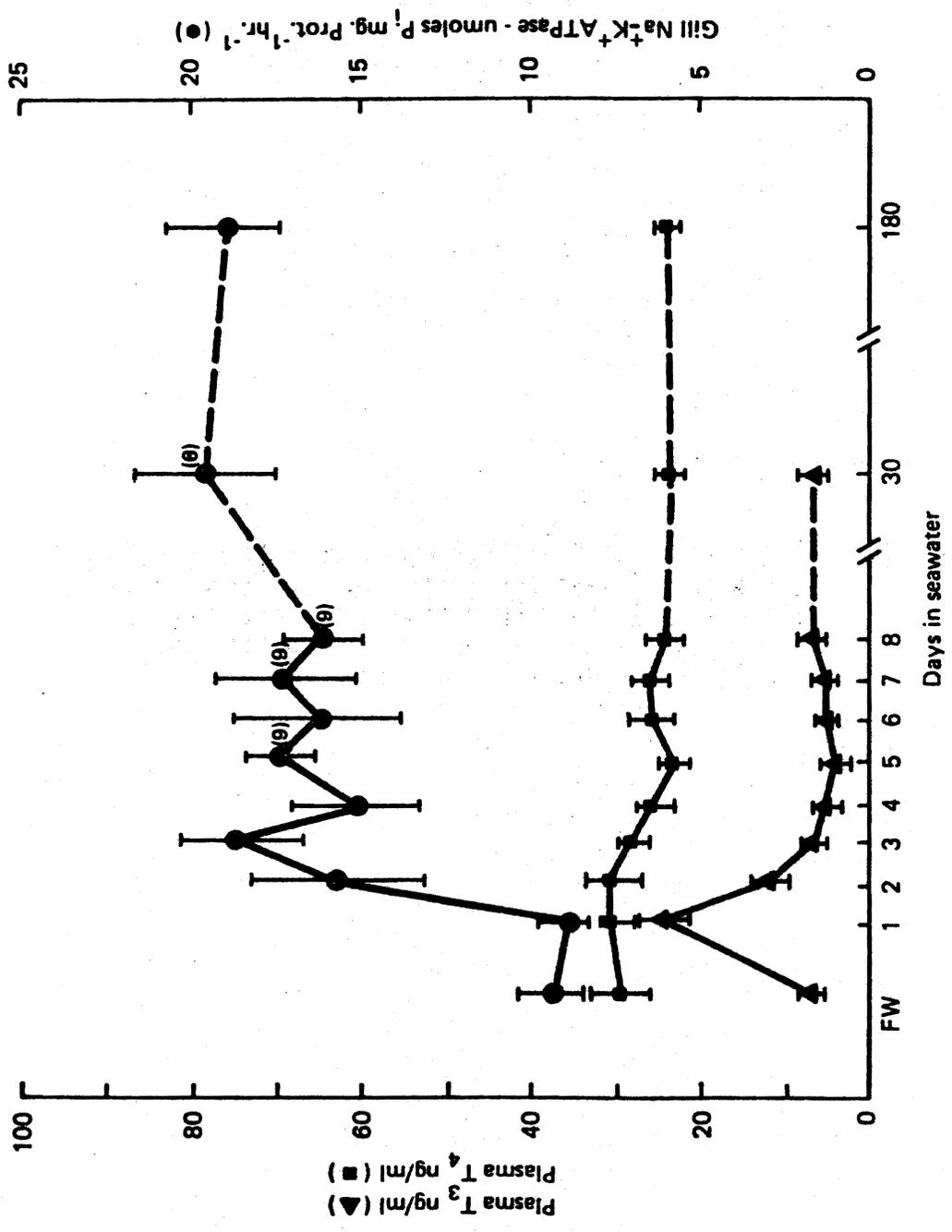


Figure 14. Mean gill Na^+-K^+ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Big Creek Hatchery (BC I--Big Creek stock) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

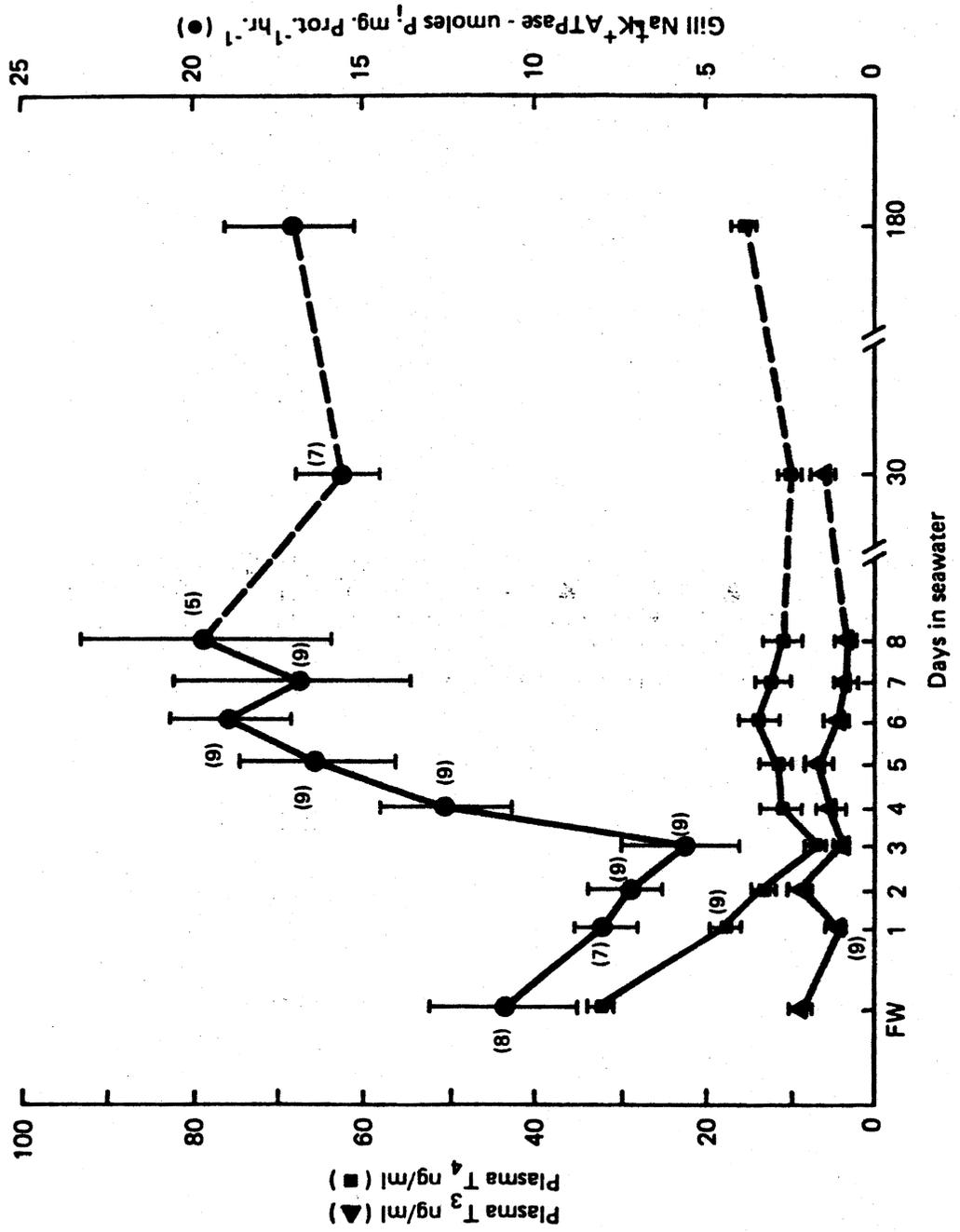


Figure 15. Mean gill Na^+-K^+ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Big³ Creek Hatchery (BC II--Cowlitz stock) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

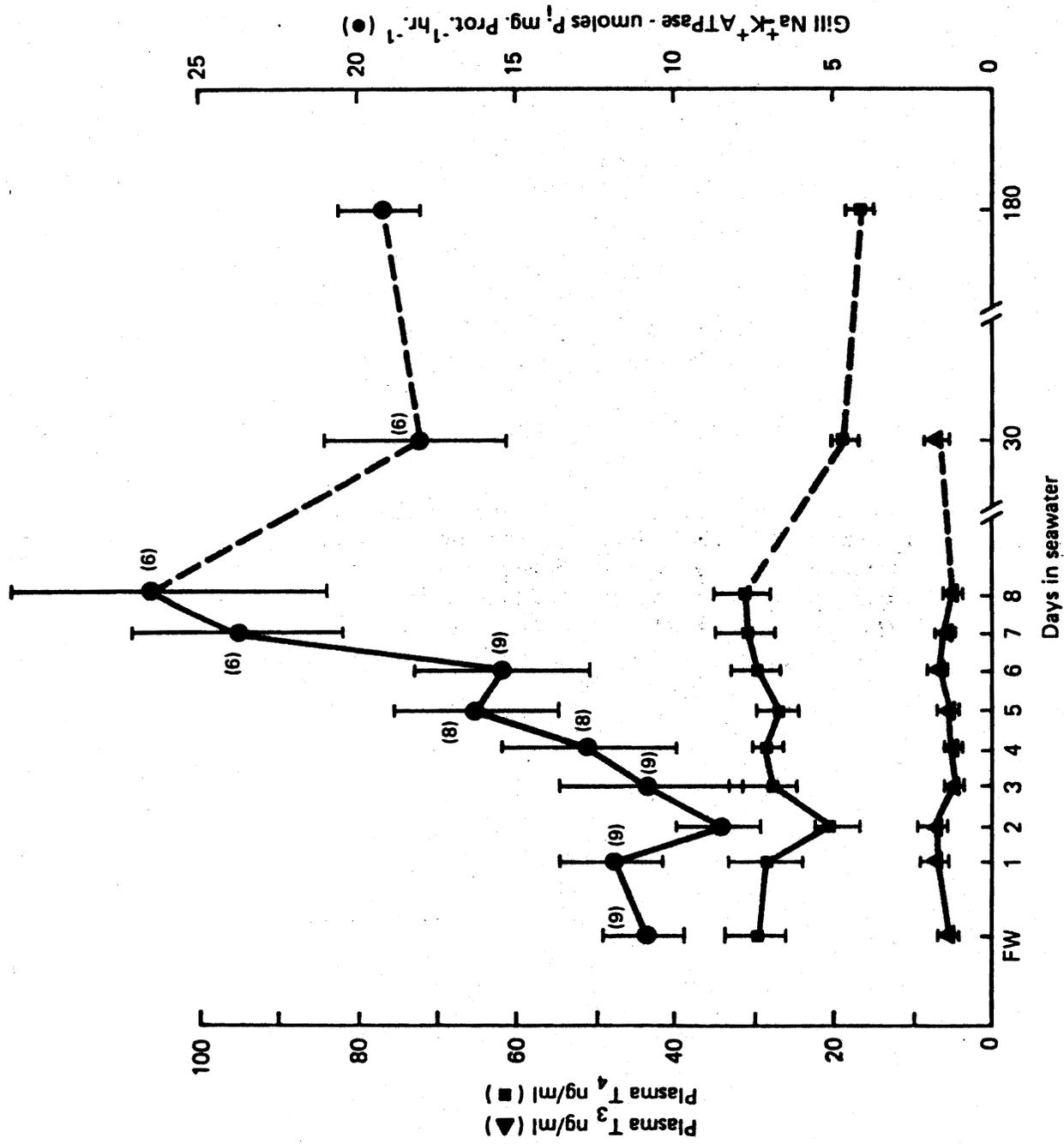


Figure 16. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (●) and T_3 (▲) concentrations in seawater fish from the Sandy Hatchery (Sandy I--normal production diet) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

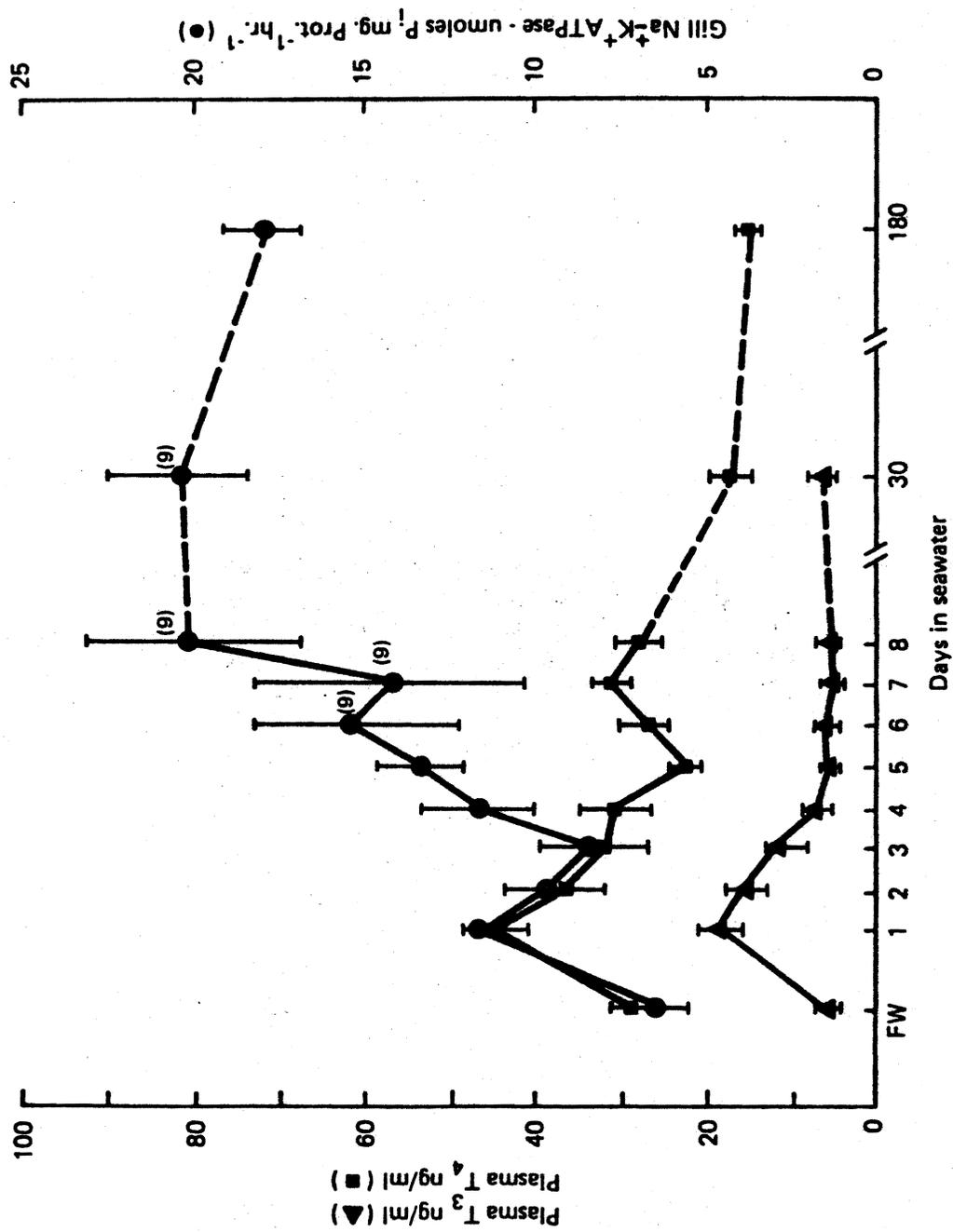


Figure 17. Mean gill Na^+-K^+ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Sandy Hatchery (Sandy II--soy oil substituted diet)(Brackets indicate \pm SEM, N = samples of 3 fish except where parenthetically noted).

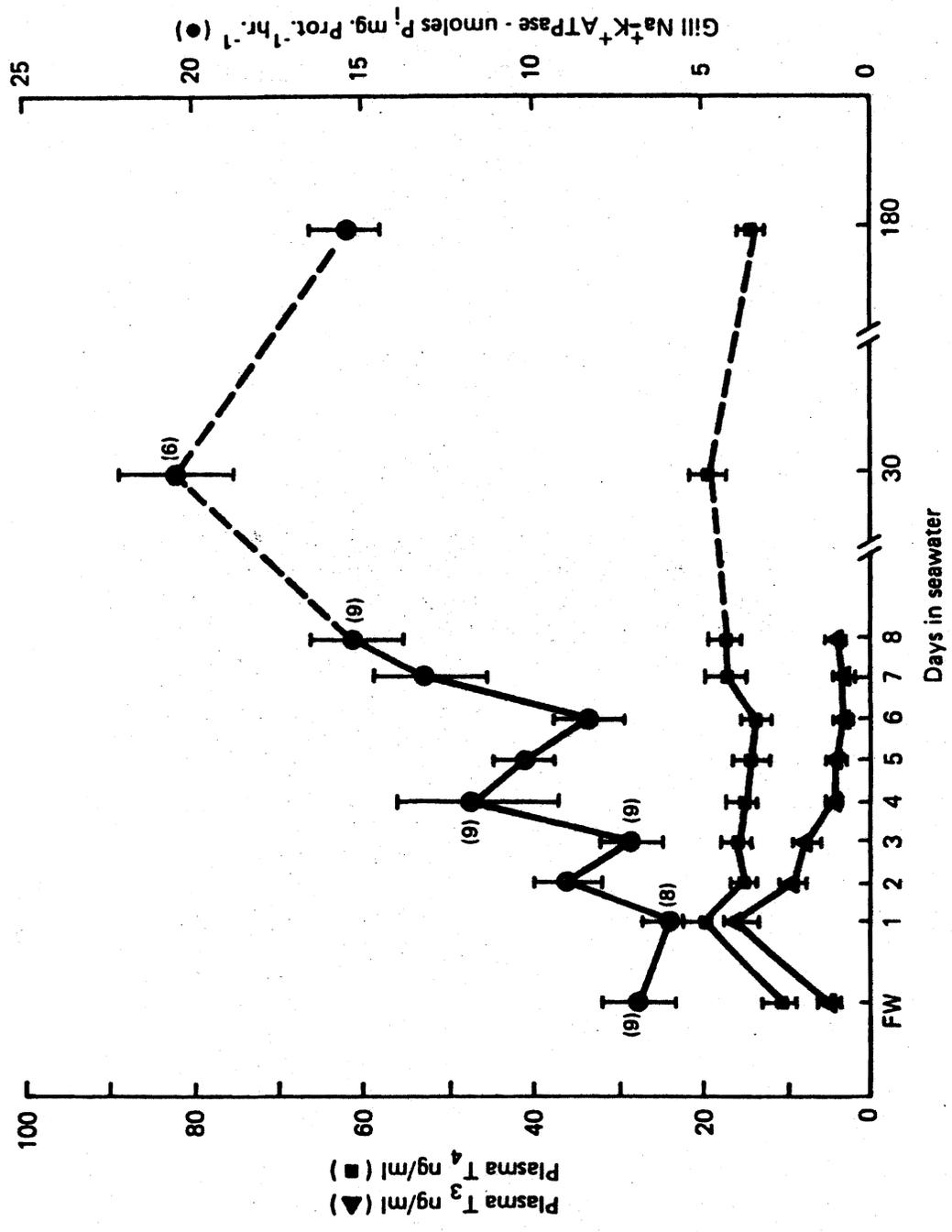


Figure 18. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Willard Hatchery (Willard I--first serial release)(Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

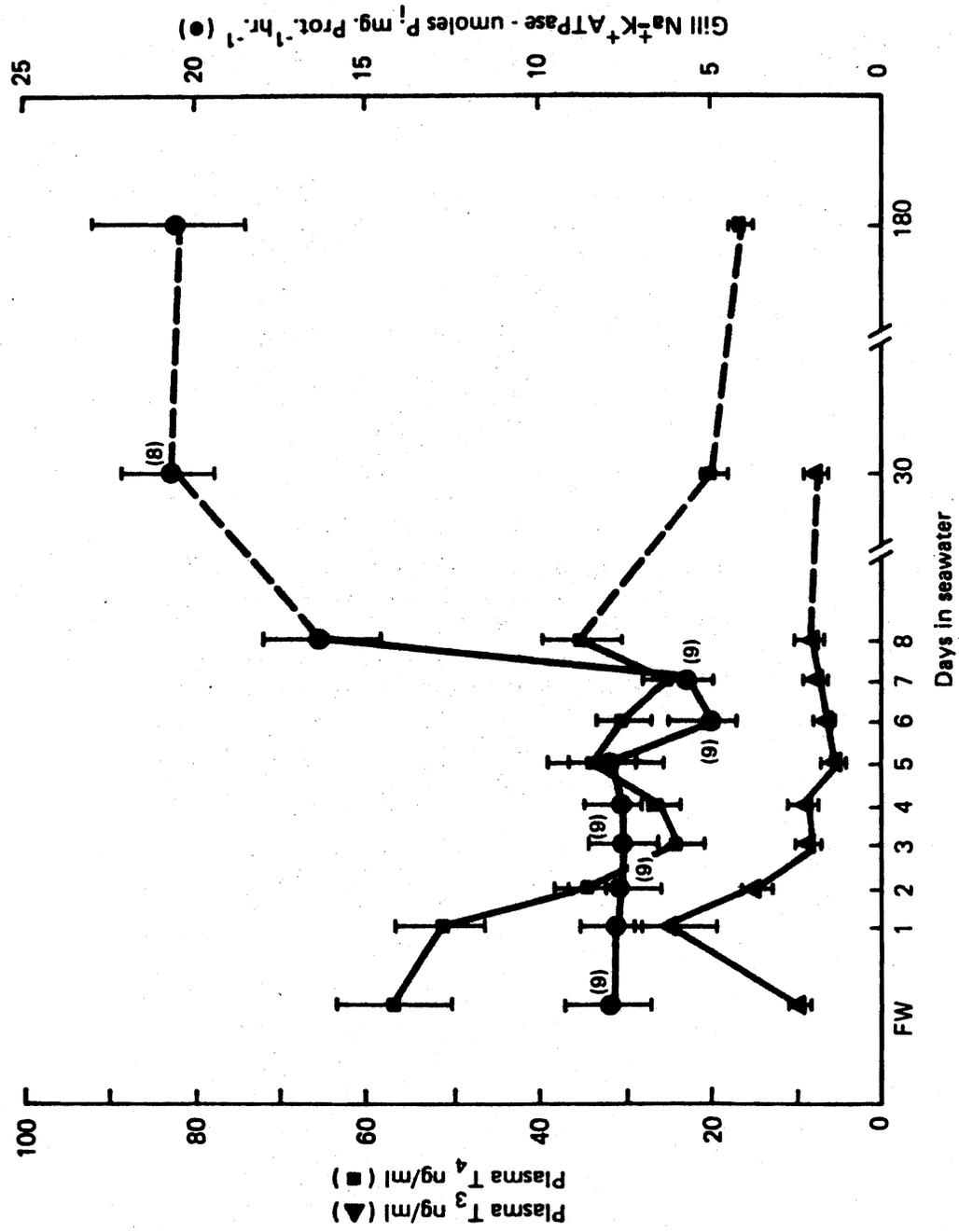


Figure 19. Mean gill $\text{Na}^+ - \text{K}^+$ ATPase activities (●) and plasma T_4 (■) and T_3 (▲) concentrations in seawater fish from the Willard Hatchery (Willard II--second serial release) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

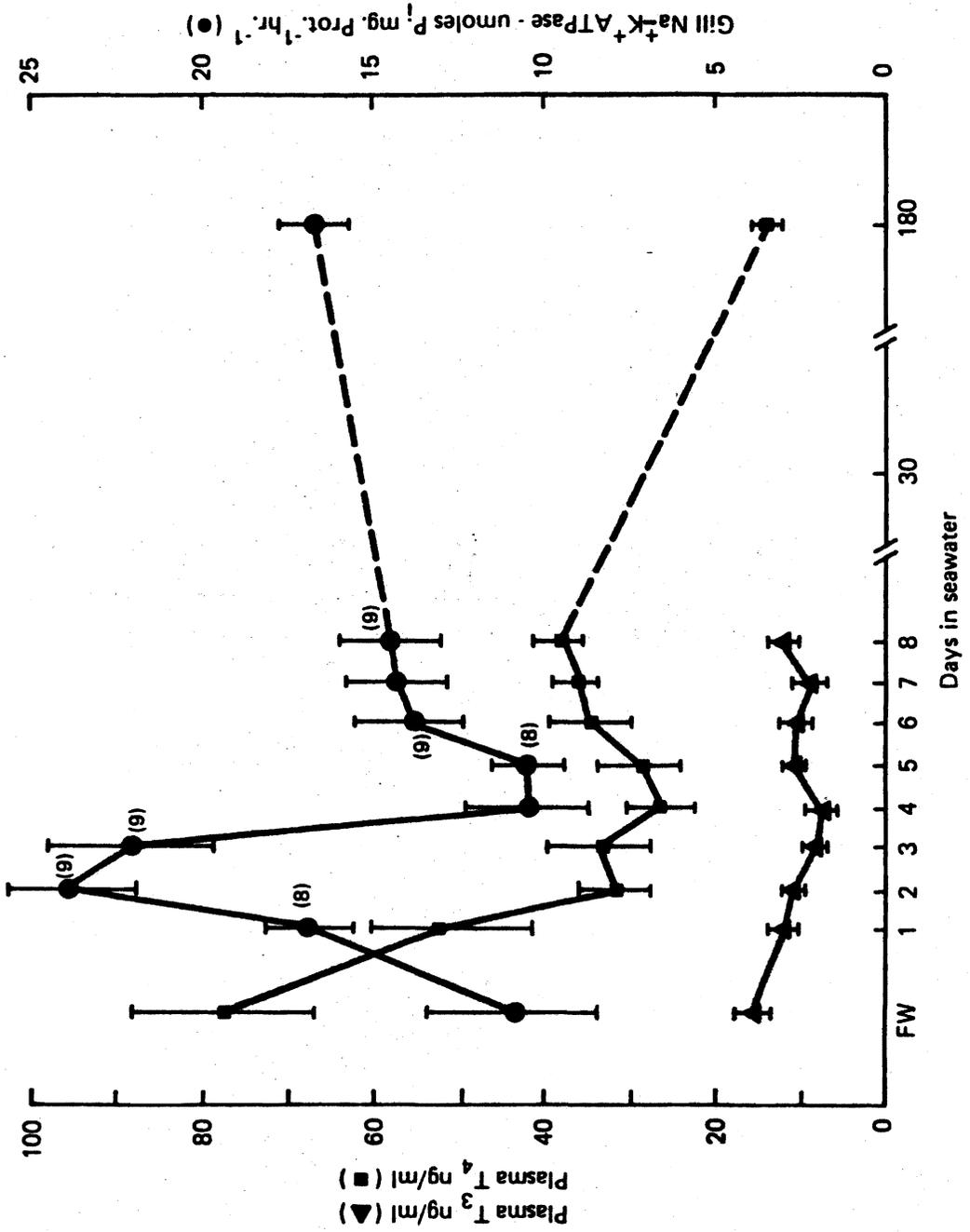


Figure 20. Mean plasma sodium (●) and potassium (■) ion concentrations from fish in freshwater at the Rocky Reach Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

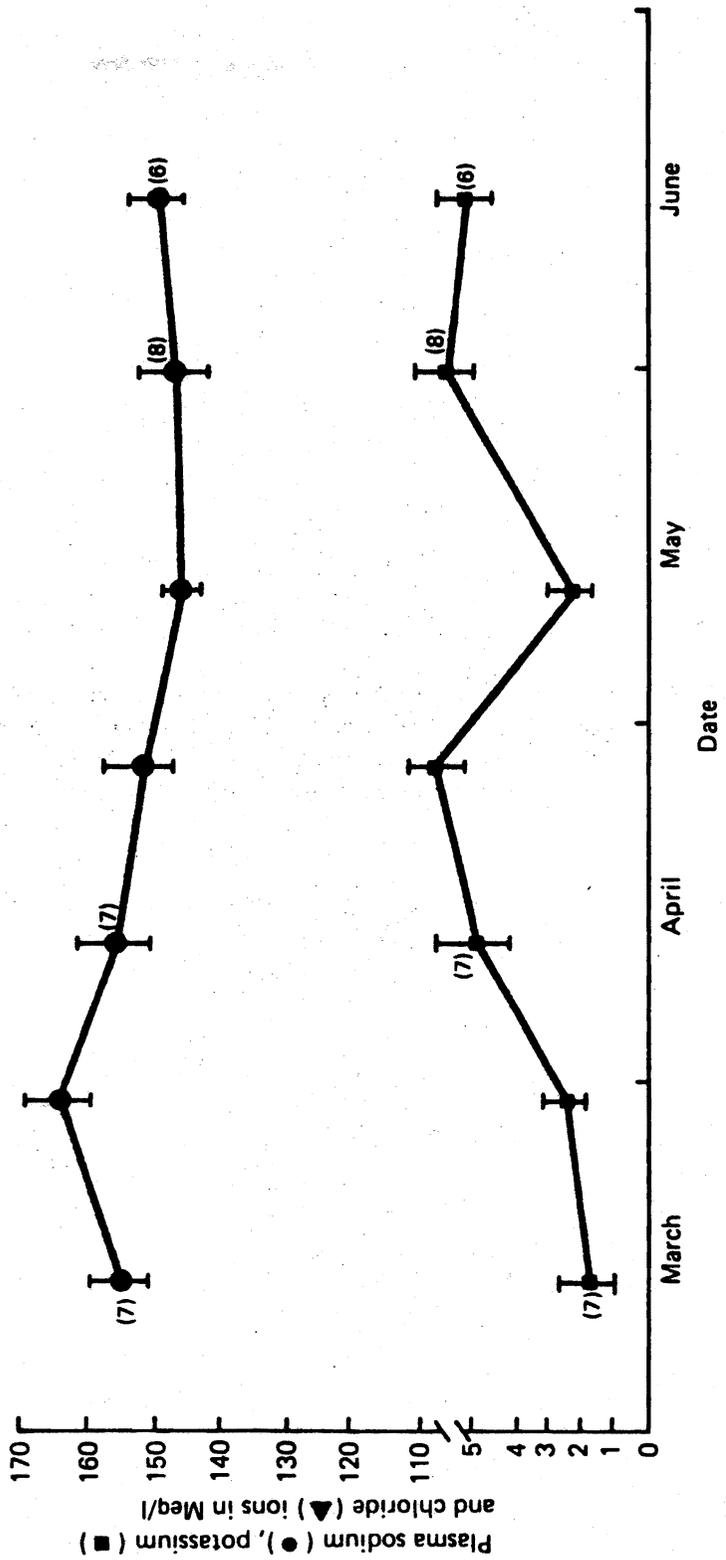


Figure 21. Mean plasma sodium (●) and potassium (■) ion concentrations from fish in freshwater at the Toutle Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

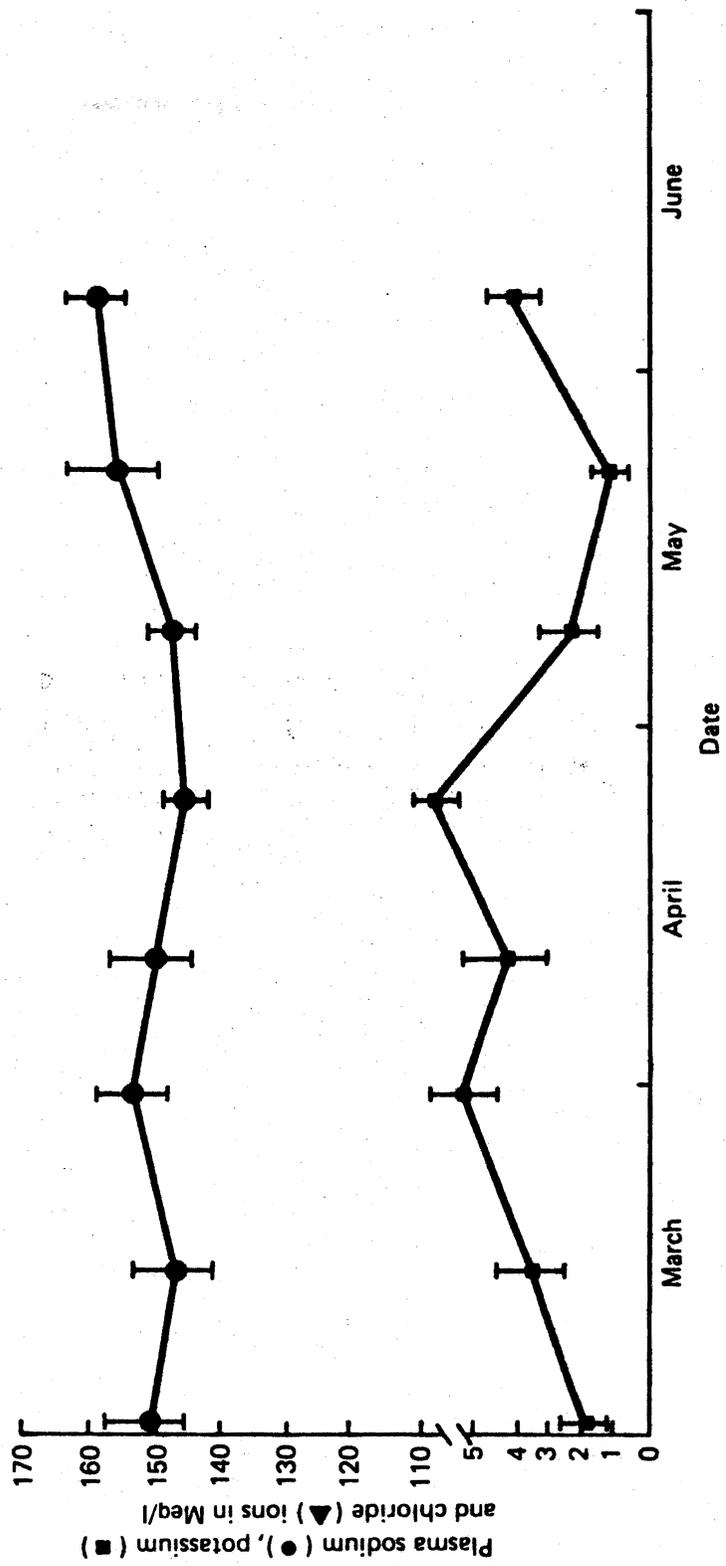


Figure 22. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations from fish in freshwater at the Klickitat Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

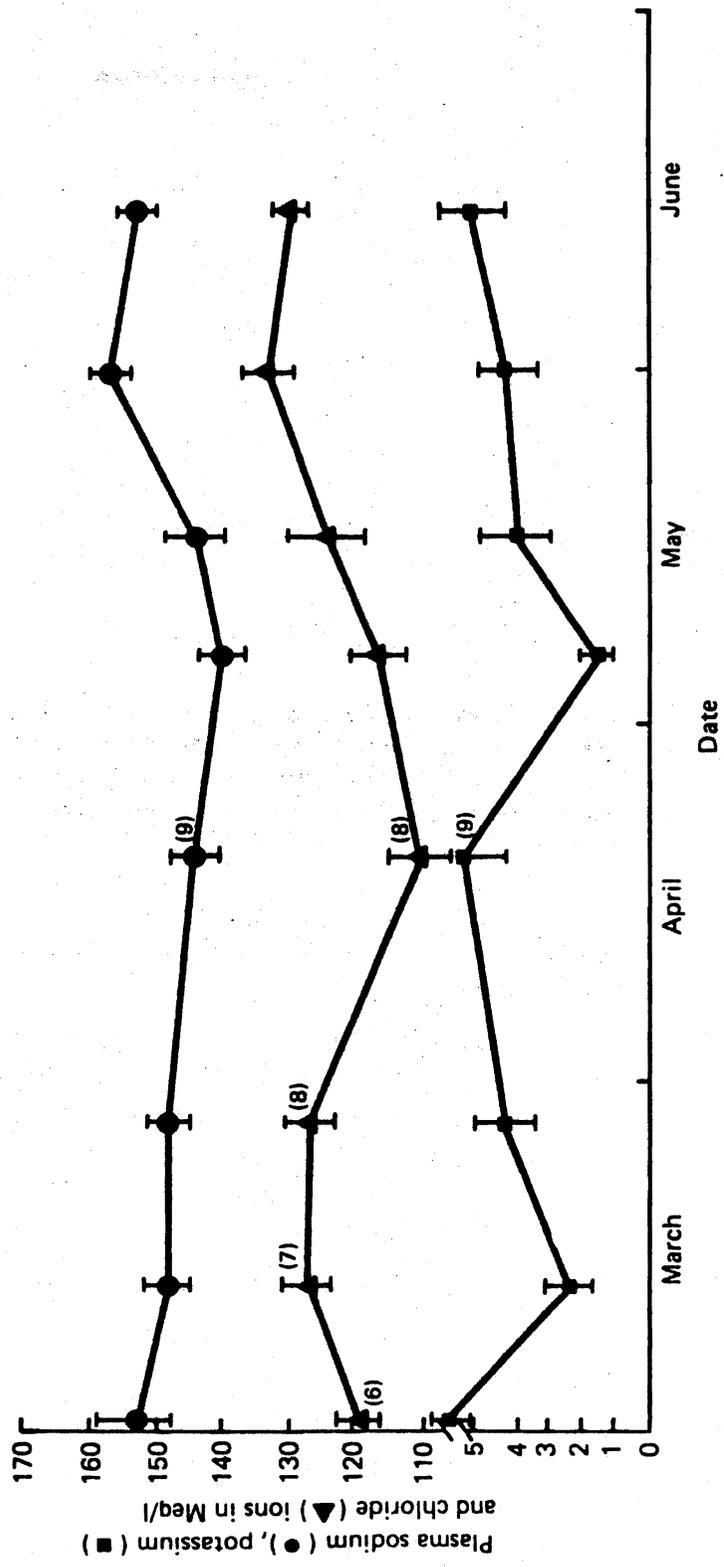


Figure 23. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations from fish in freshwater at the Kalama Falls Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

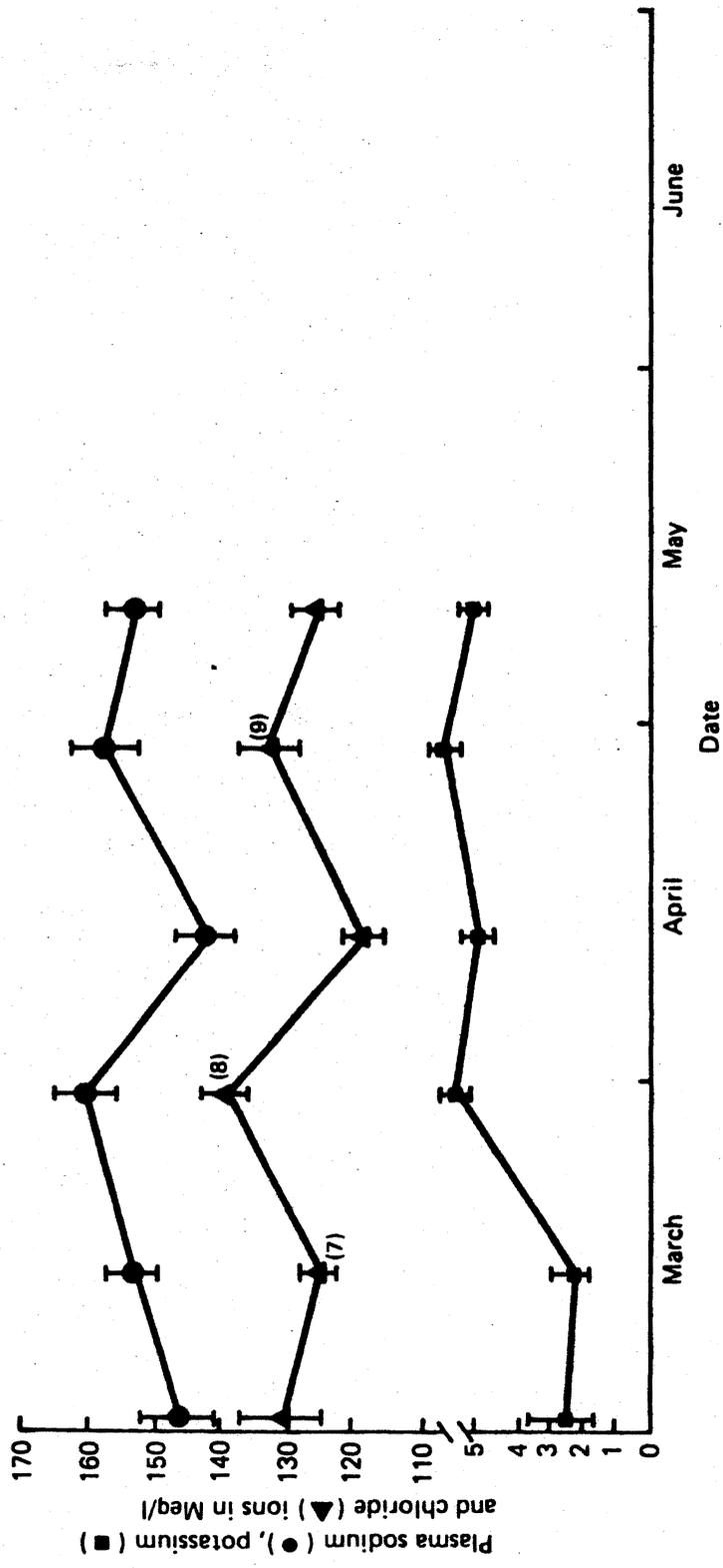


Figure 24. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations from fish in freshwater at the Big Creek Hatchery (BC I--Big Creek stock) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

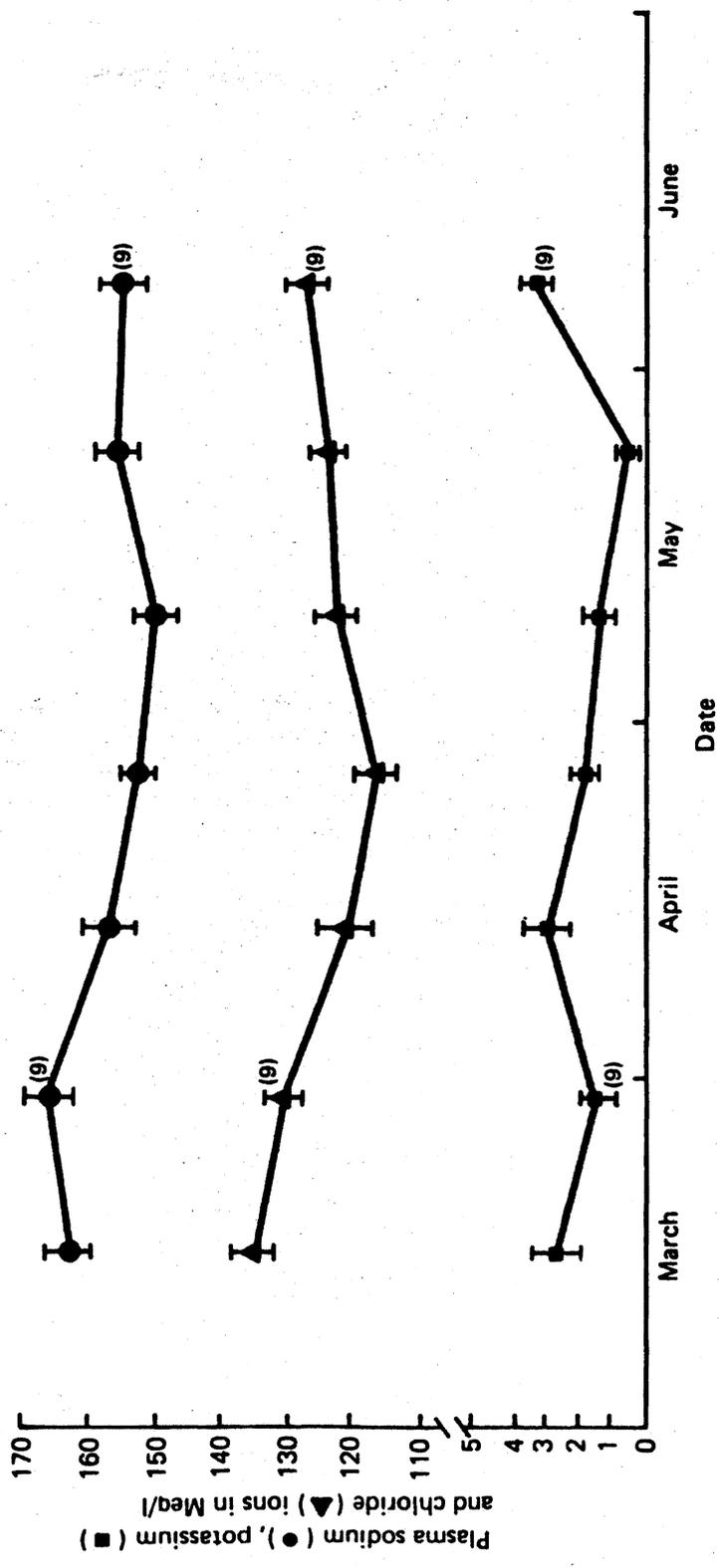


Figure 25. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations from fish in freshwater at the Big Creek Hatchery (BC II--Cowlitz stock) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

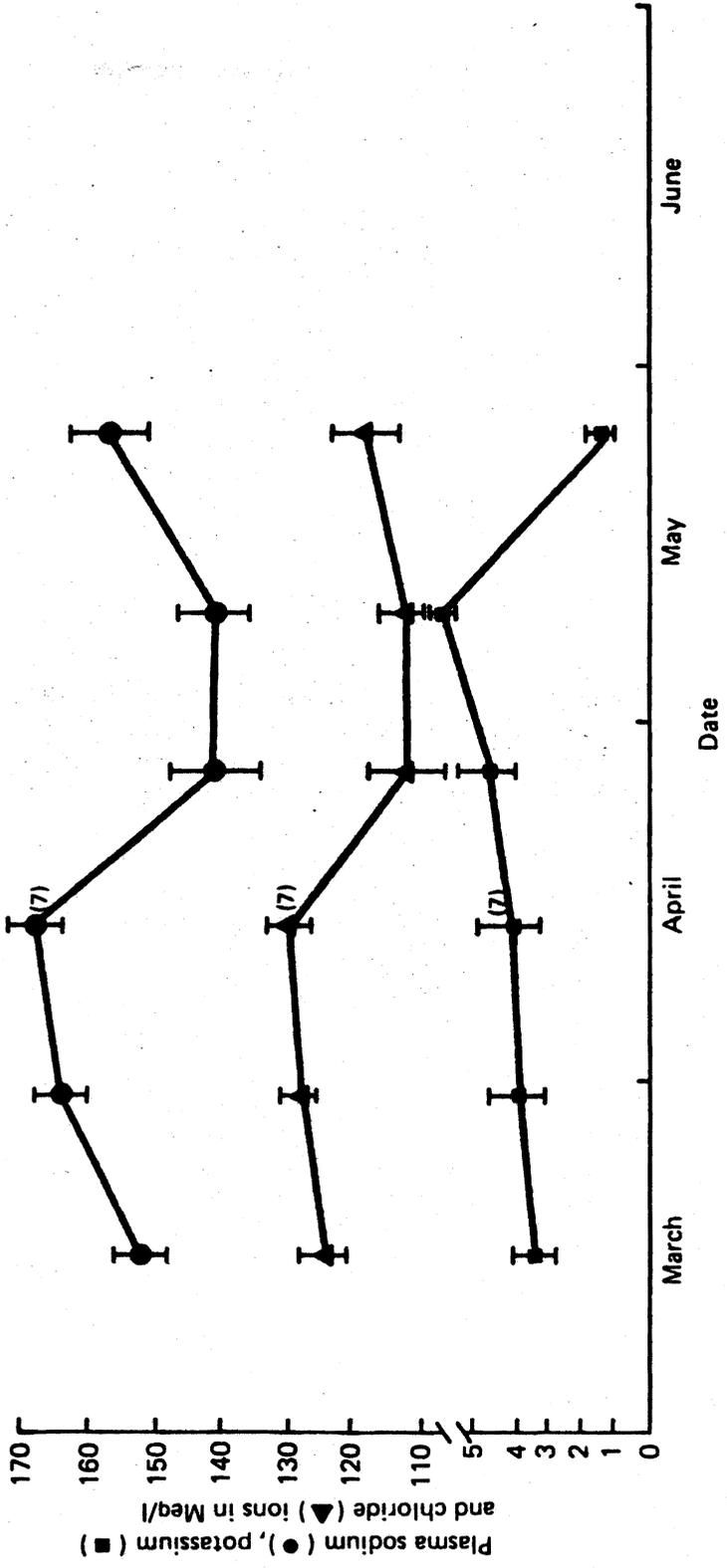


Figure 26. Mean plasma sodium (●), potassium (■), and chloride (▲) concentrations from fish in freshwater at the Sandy Hatchery (Sandy I--normal production diet)(Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

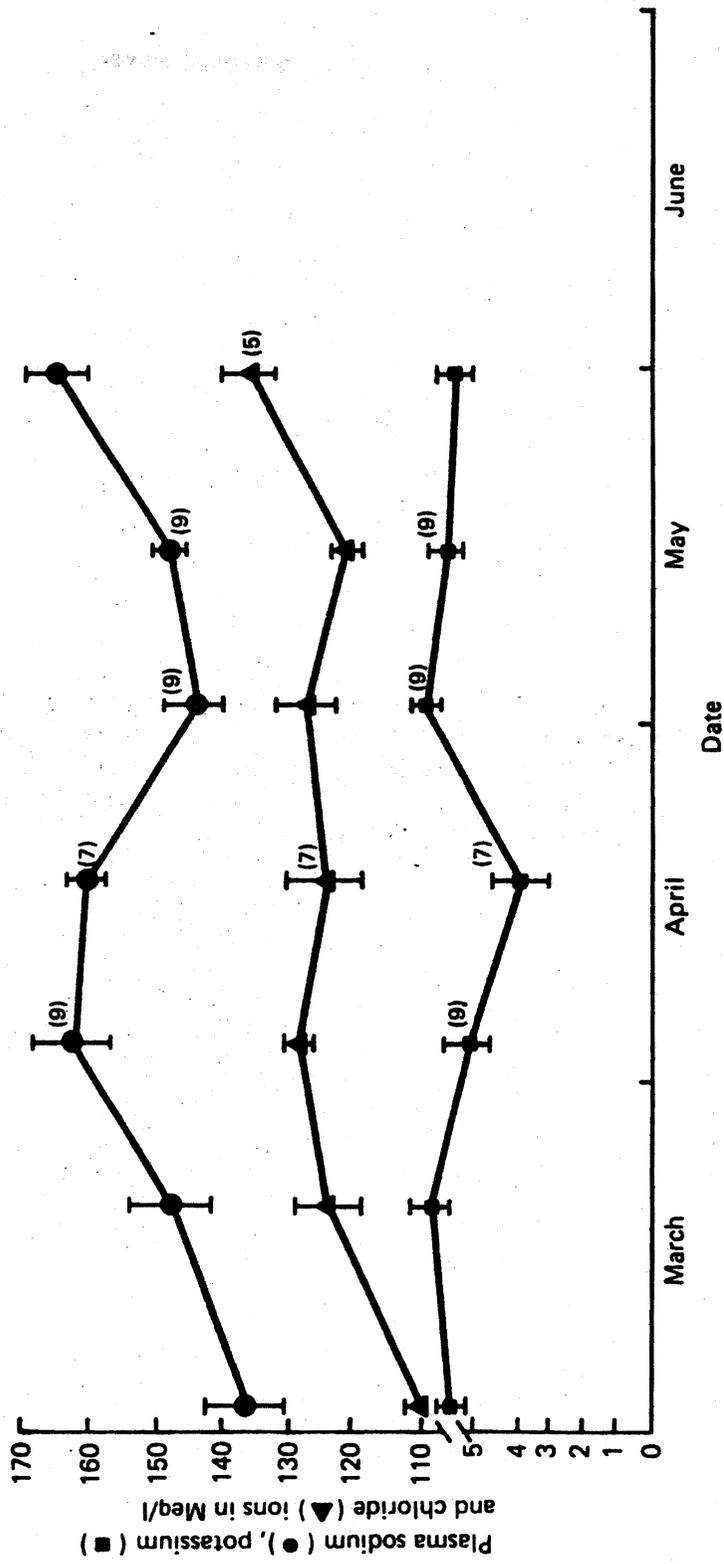


Figure 27. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations from fish in freshwater at the Sandy Hatchery (Sand II--soy or substituted diet)(Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

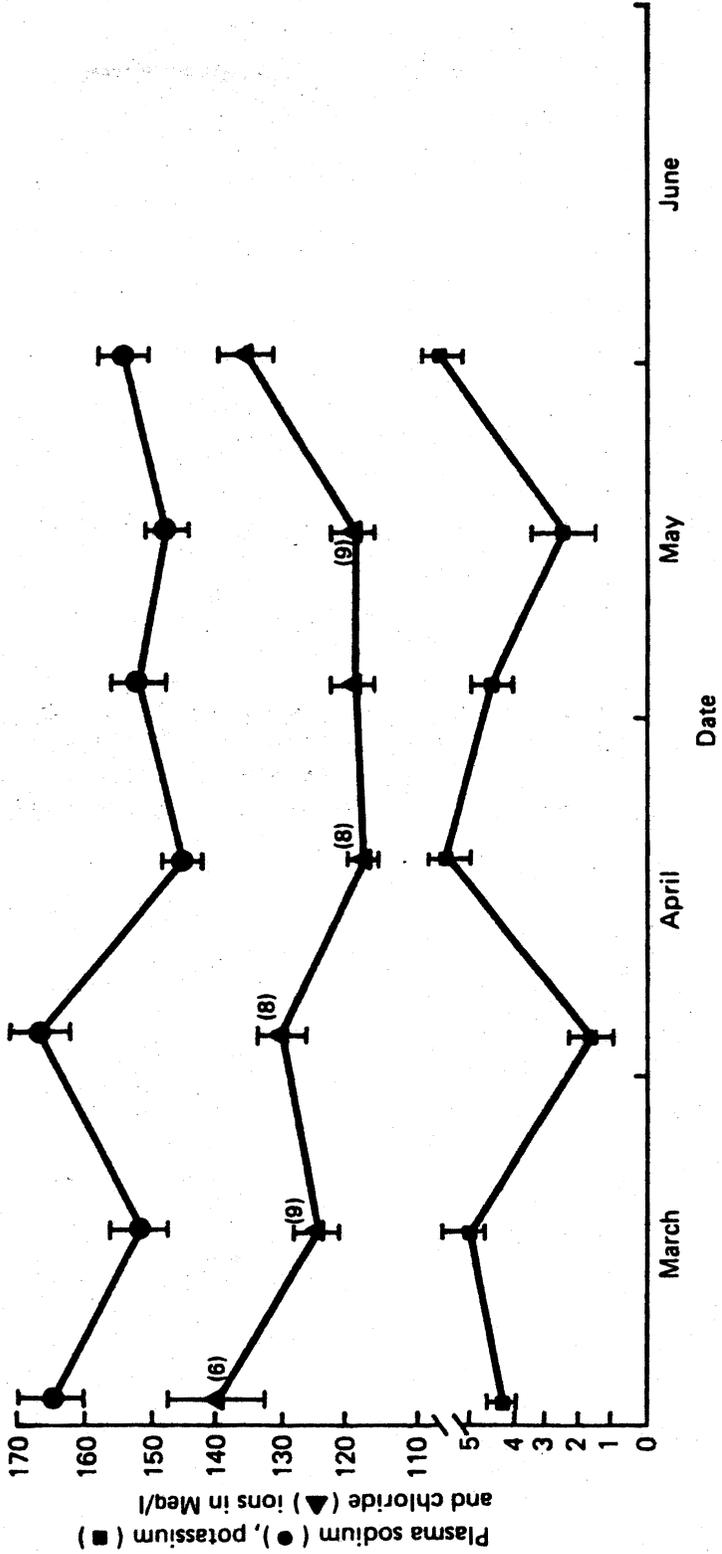
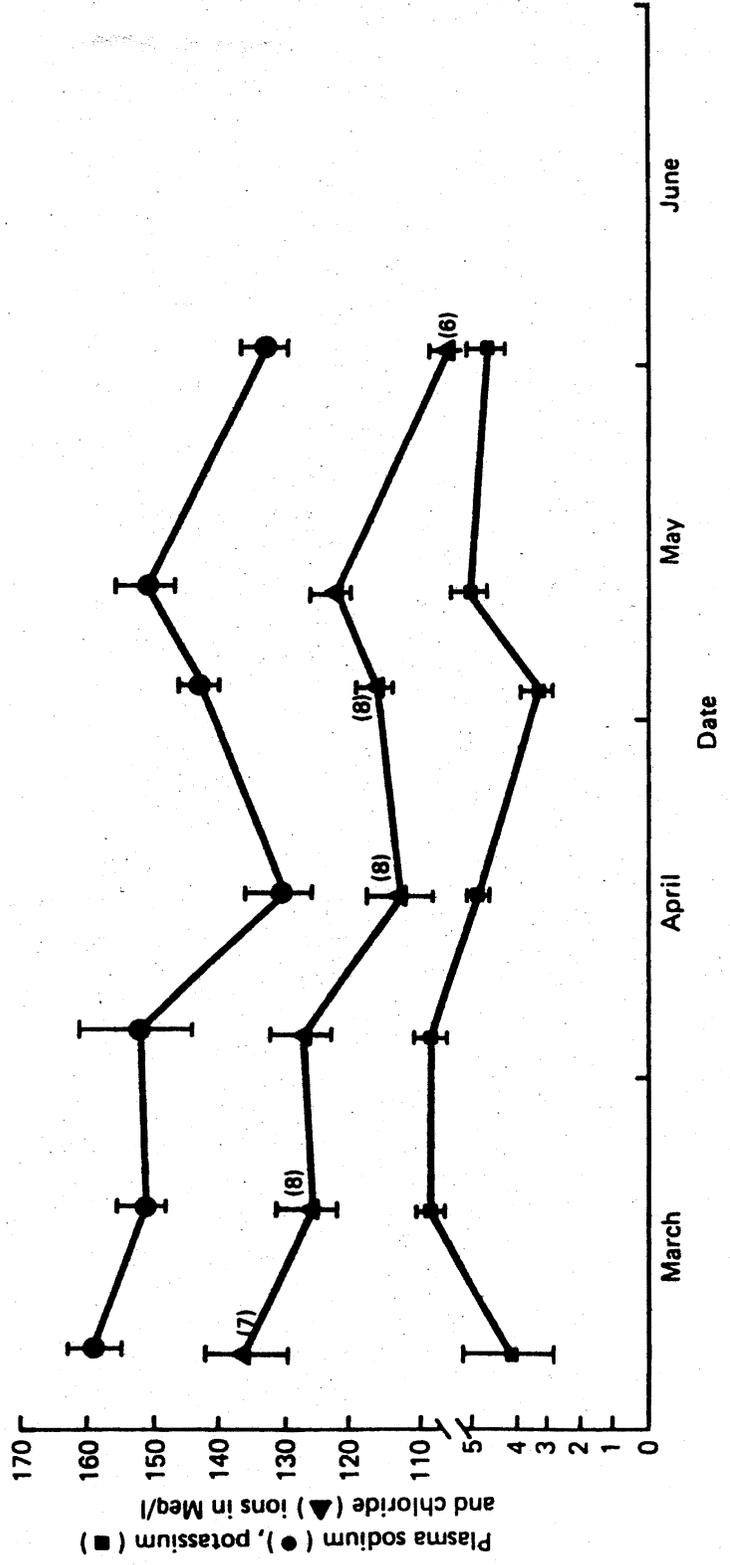


Figure 28. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations from fish in freshwater at the Willard Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).



previous levels by late May or early June. Plasma K^+ levels were inconsistent, showing patterns that included both increases and decreases throughout the sampling period. The absolute levels of freshwater plasma electrolytes tended to be lower than the same measurements from fish in seawater (see below).

Plasma Electrolytes in Seawater Fish

Measurements of the plasma electrolytes from fish in seawater (sodium, potassium, and chloride ions) are graphically presented in Figs. 29-38. The blood electrolyte data were collected from the same fish used for the ATPase, T_4 and T_3 analyses in Figs. 10-19. All test groups showed an initial increase in both plasma Na^+ and Cl^- during the first or second days of residence in seawater. By the eighth day of residence in seawater, Na^+ and Cl^- levels were stabilized in some of the test groups (Figs. 29, 39, 31, 35, 36, 38). In others (Figs. 32, 33, 34, 37) the values continued to increase through 180 days in seawater. Plasma K^+ concentrations for all groups increased slightly at some time during the first six days in seawater, but generally remained uniform throughout the sampling period.

An analysis of the relationship between plasma Na^+ and Cl^- concentrations in both freshwater and seawater fish indicated that circulating levels of the two ions were significantly related ($P \leq 0.001$) (Fig. 39), suggesting that Na^+ and Cl^- transport may be

Figure 29. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Rocky Reach Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

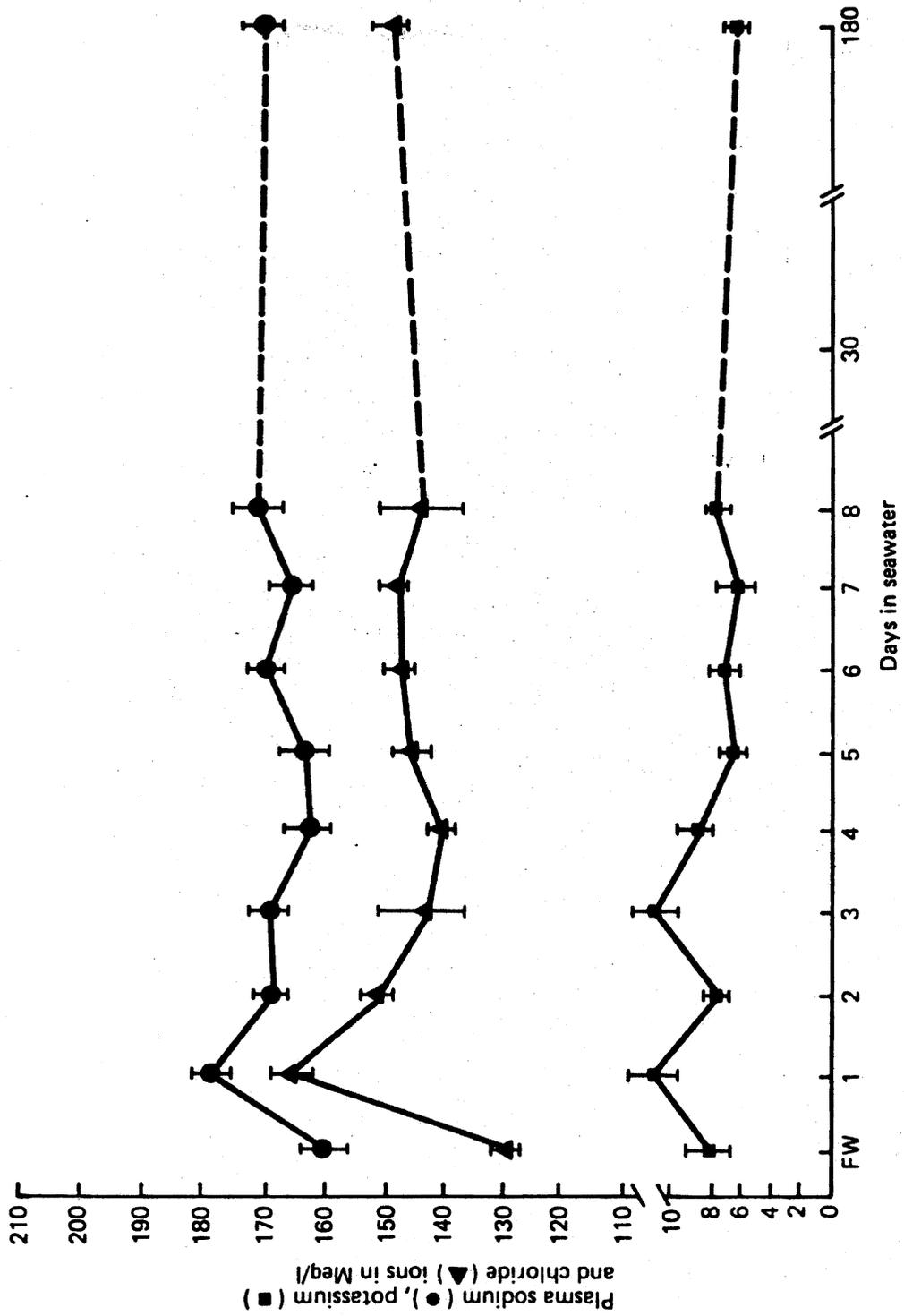


Figure 30. Mean plasma, sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Toutle Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

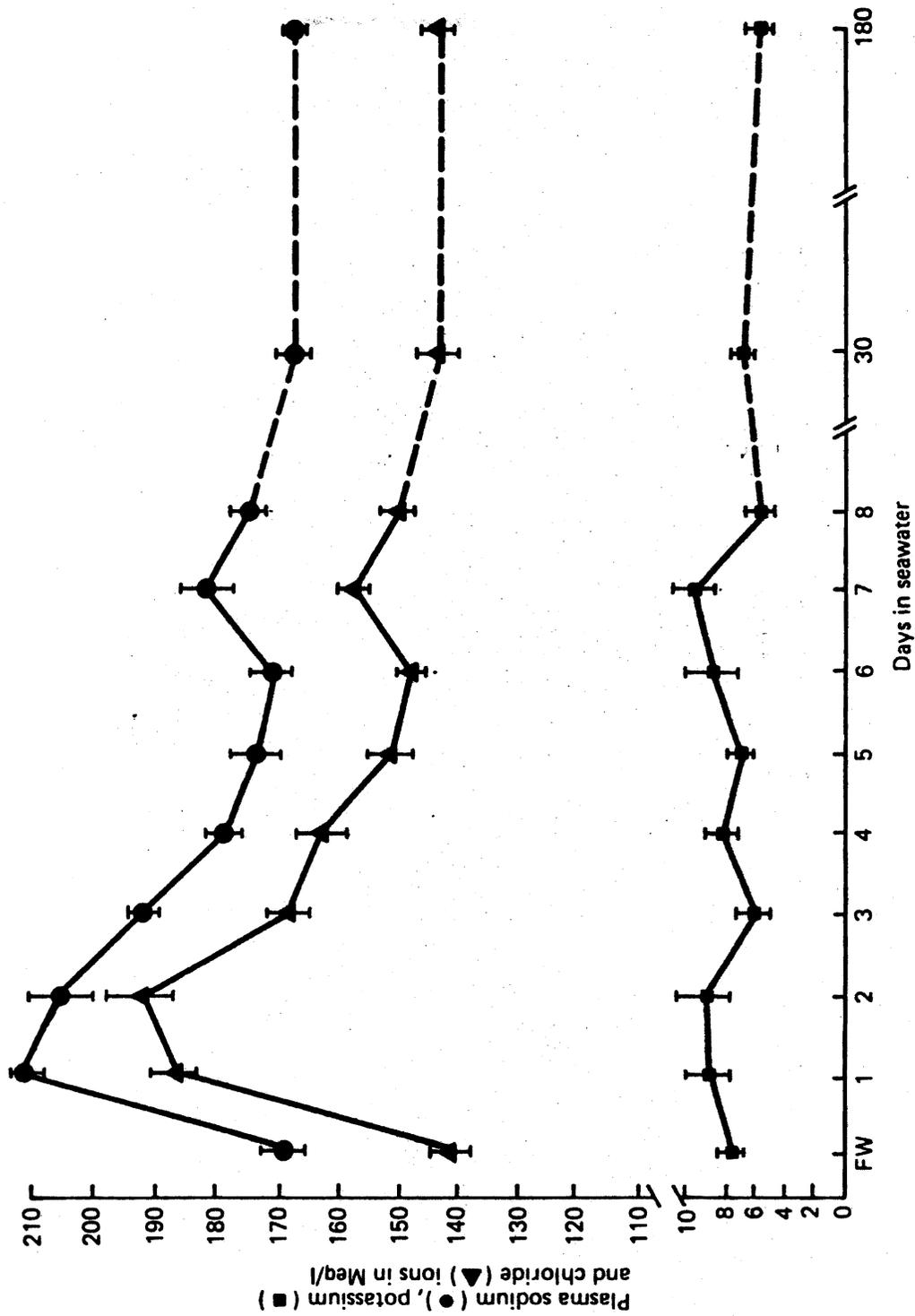


Figure 31. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Klickitat Hatchery (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

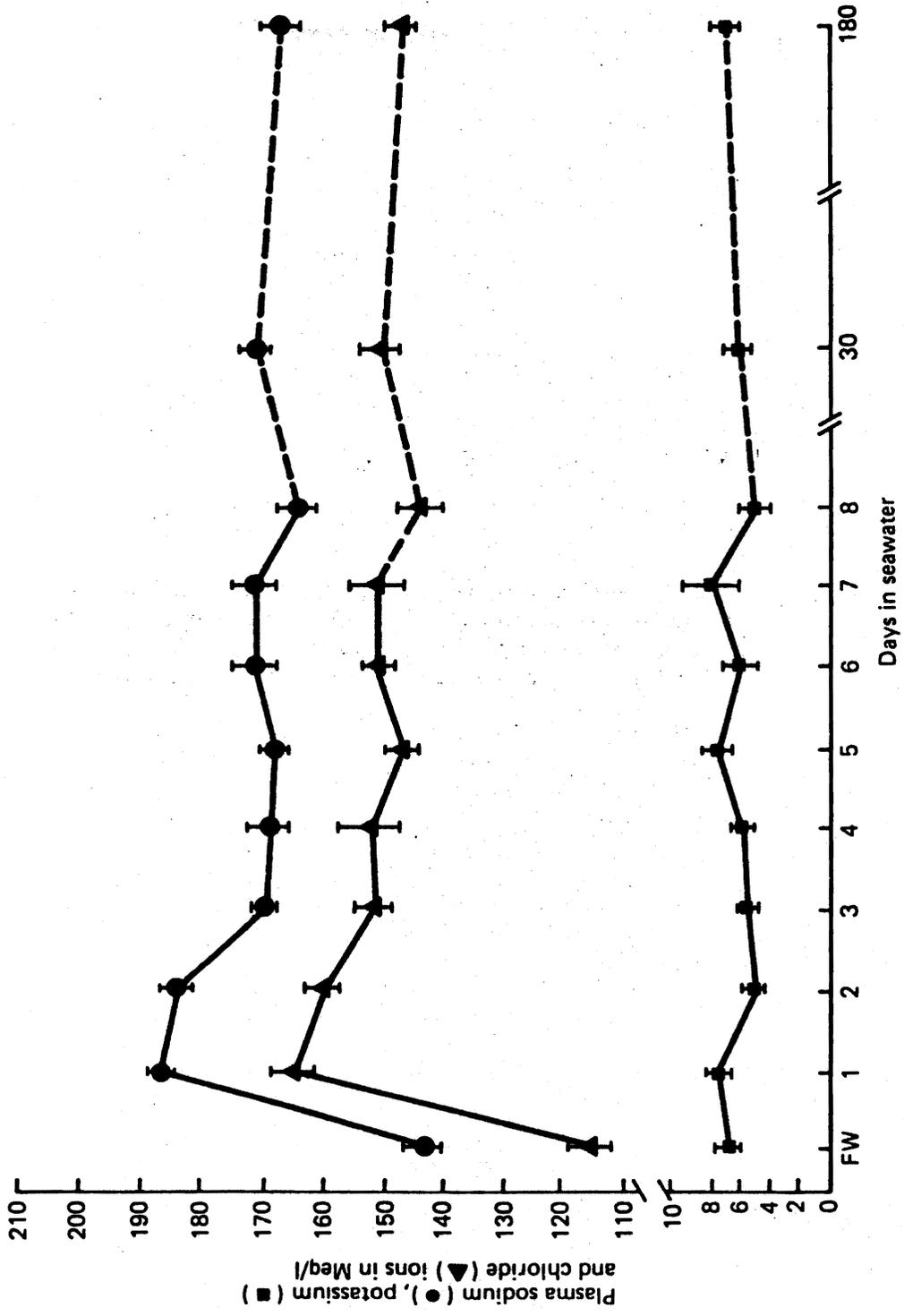


Figure 32. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Kalama Falls Hatchery (Brackets indicated \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

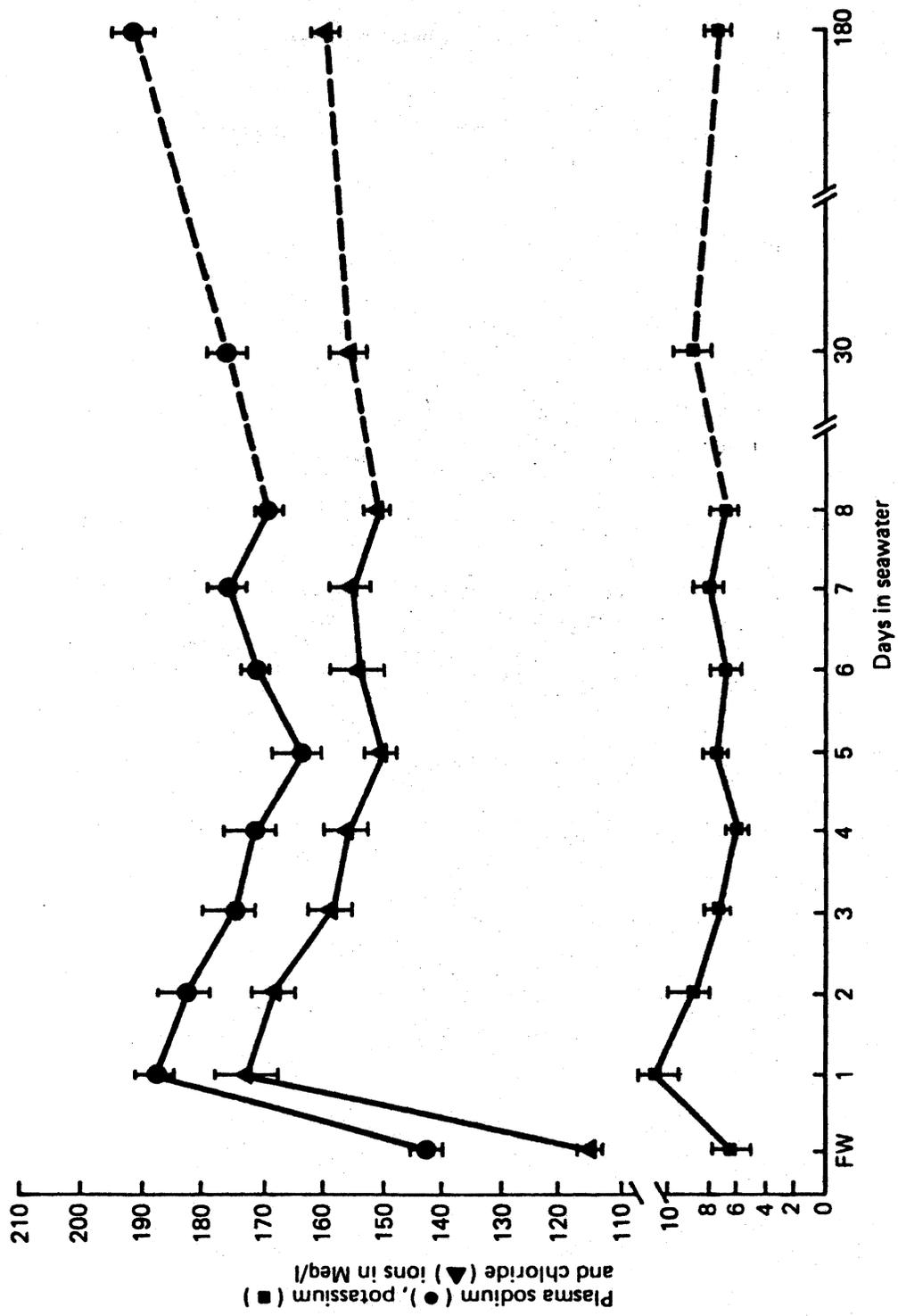


Figure 33. Mean plasma sodium (●), potassium, (●), and chloride (▲) ion concentrations in seawater fish from the Big Creek Hatchery (BC I--Big Creek stock) (Brackets indicatd \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

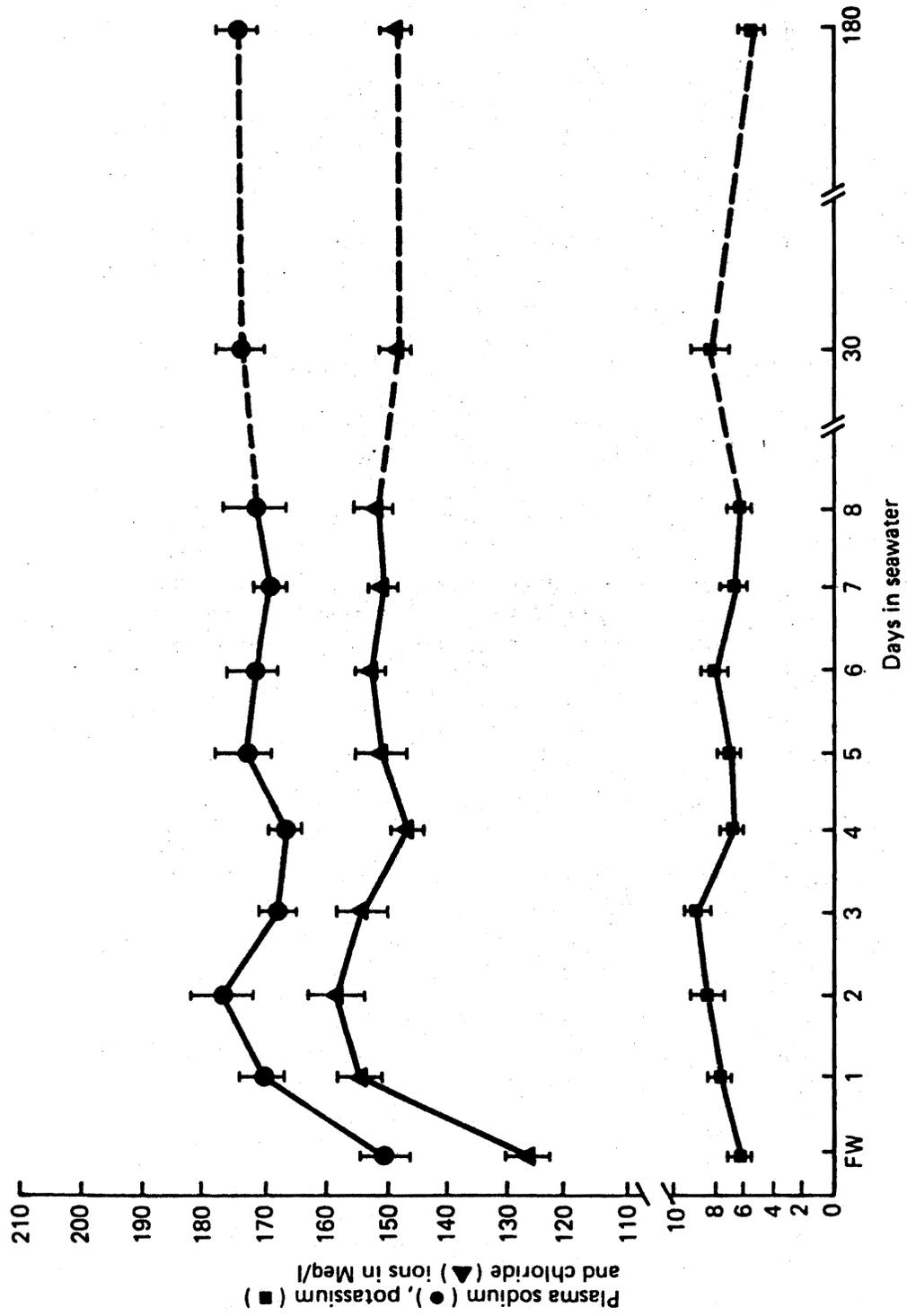


Figure 34. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Big Creek Hatchery (BC II--Cowlitz stock) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

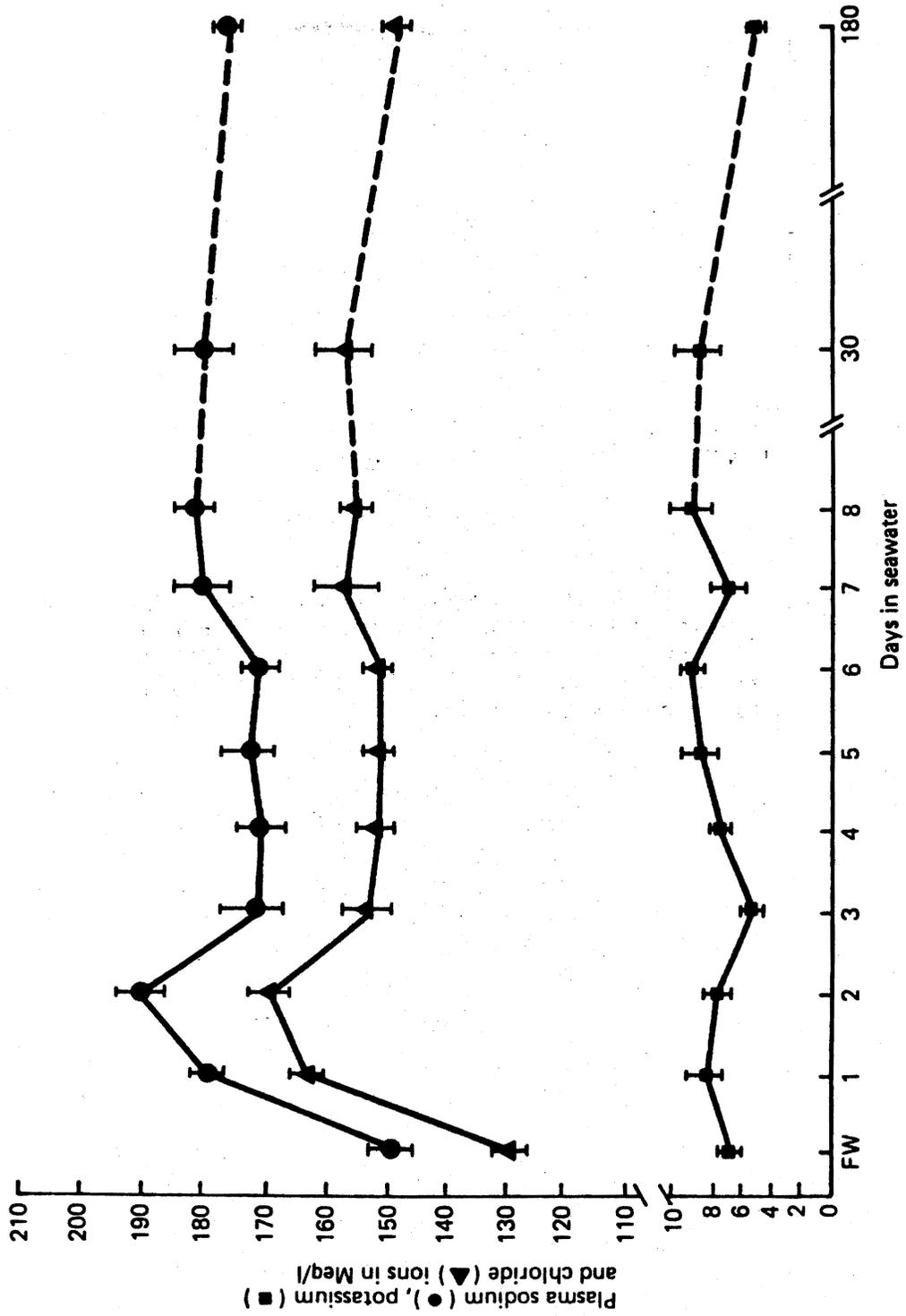


Figure 35. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Sandy Hatchery (Sandy I--normal production diet) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

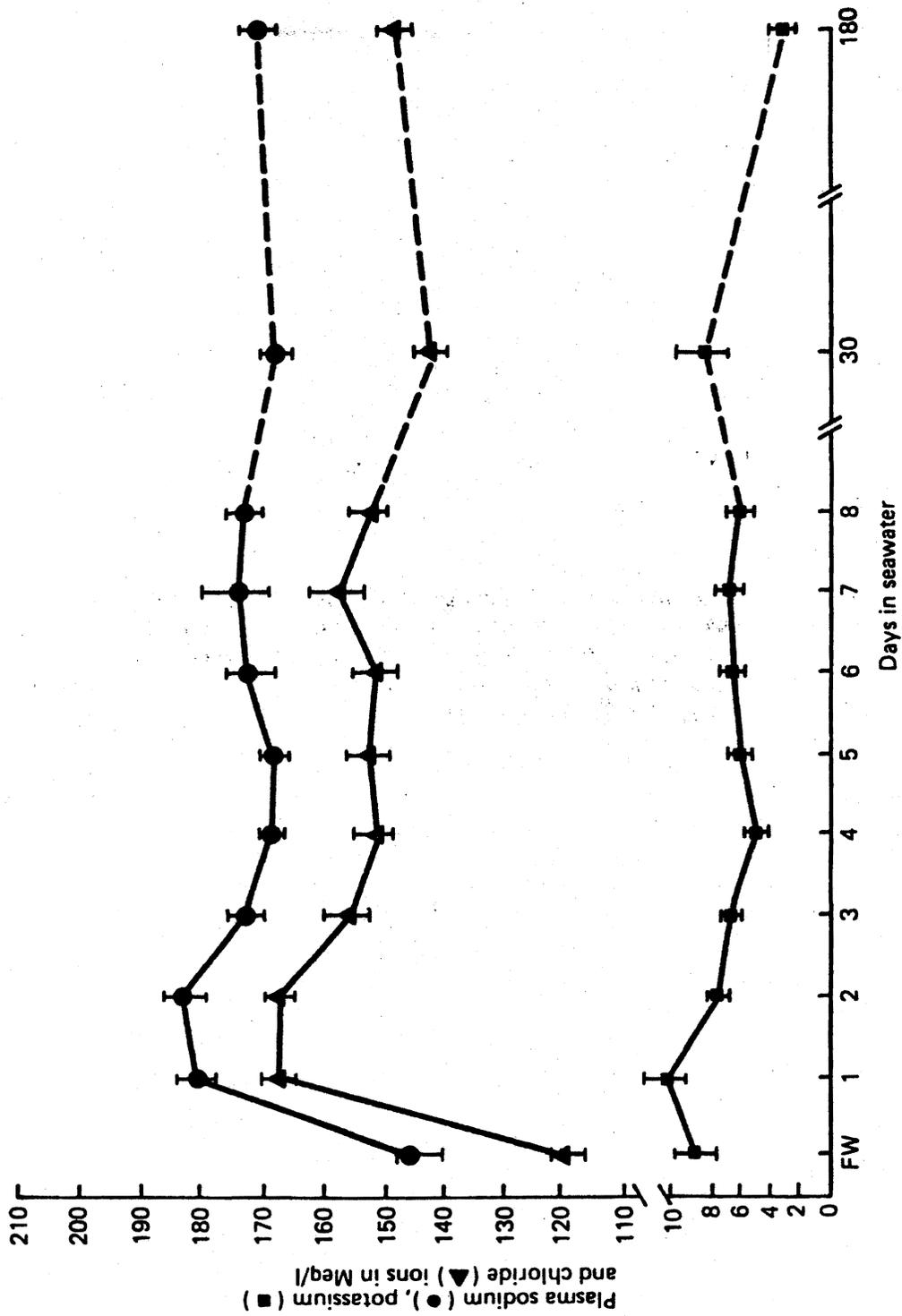


Figure 36. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Sandy Hatchery (Sandy II--soy oil substituted diet)(Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

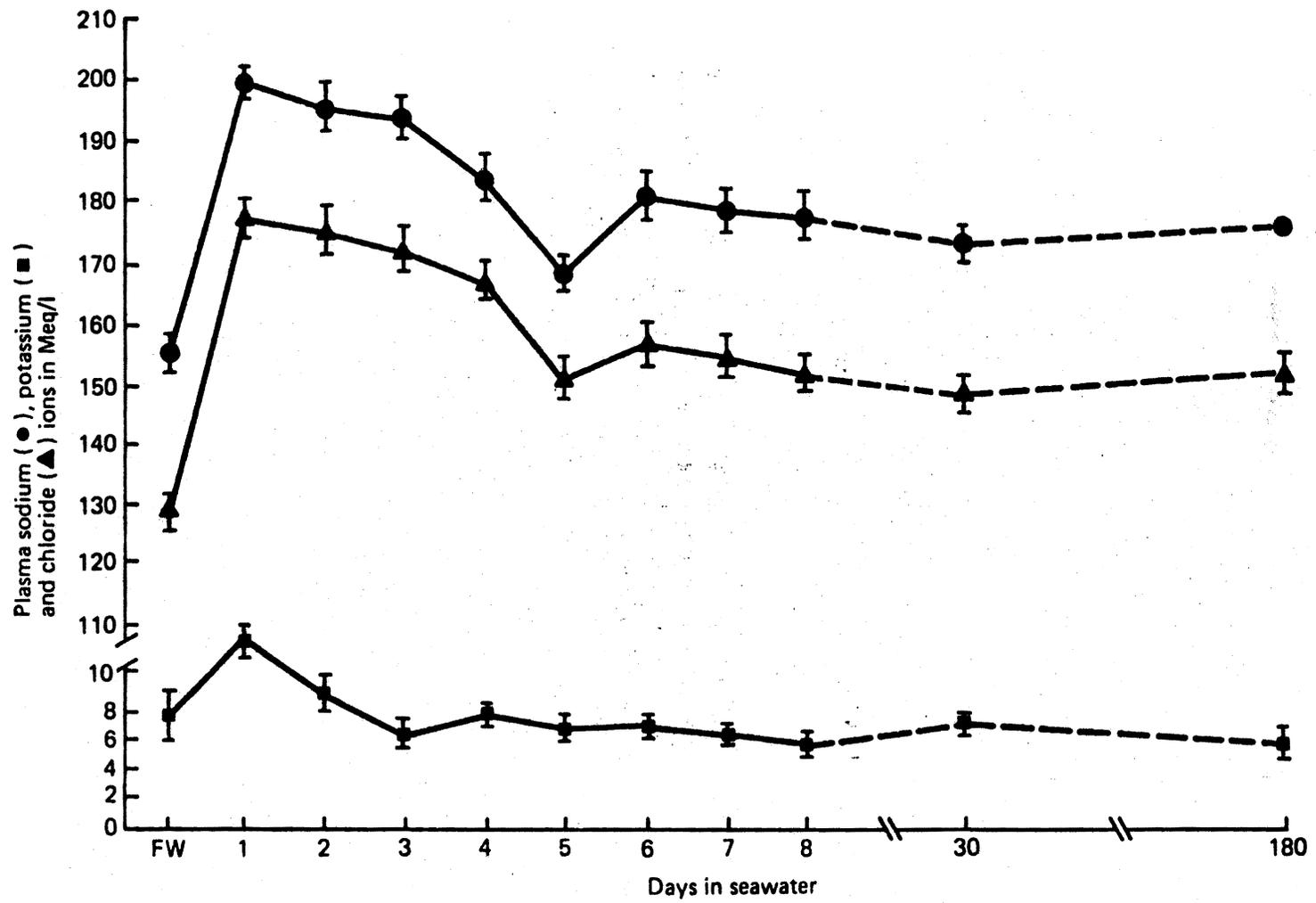


Figure 37. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Willard Hatchery (Willard I--first serial release) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

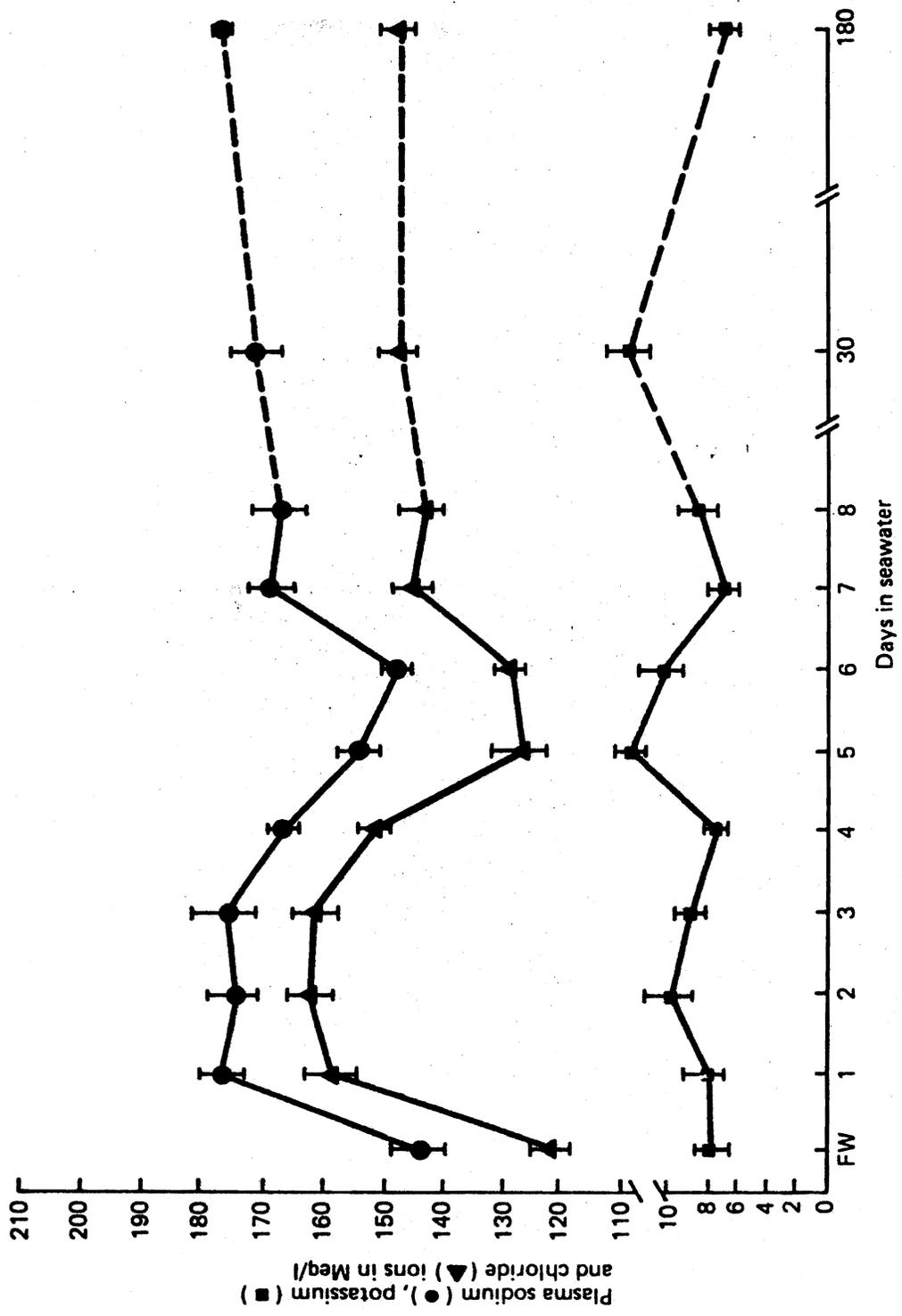


Figure 38. Mean plasma sodium (●), potassium (■), and chloride (▲) ion concentrations in seawater fish from the Willard Hatchery (Willard II--second serial release) (Brackets indicate \pm SEM, N = 10 samples of 3 fish except where parenthetically noted).

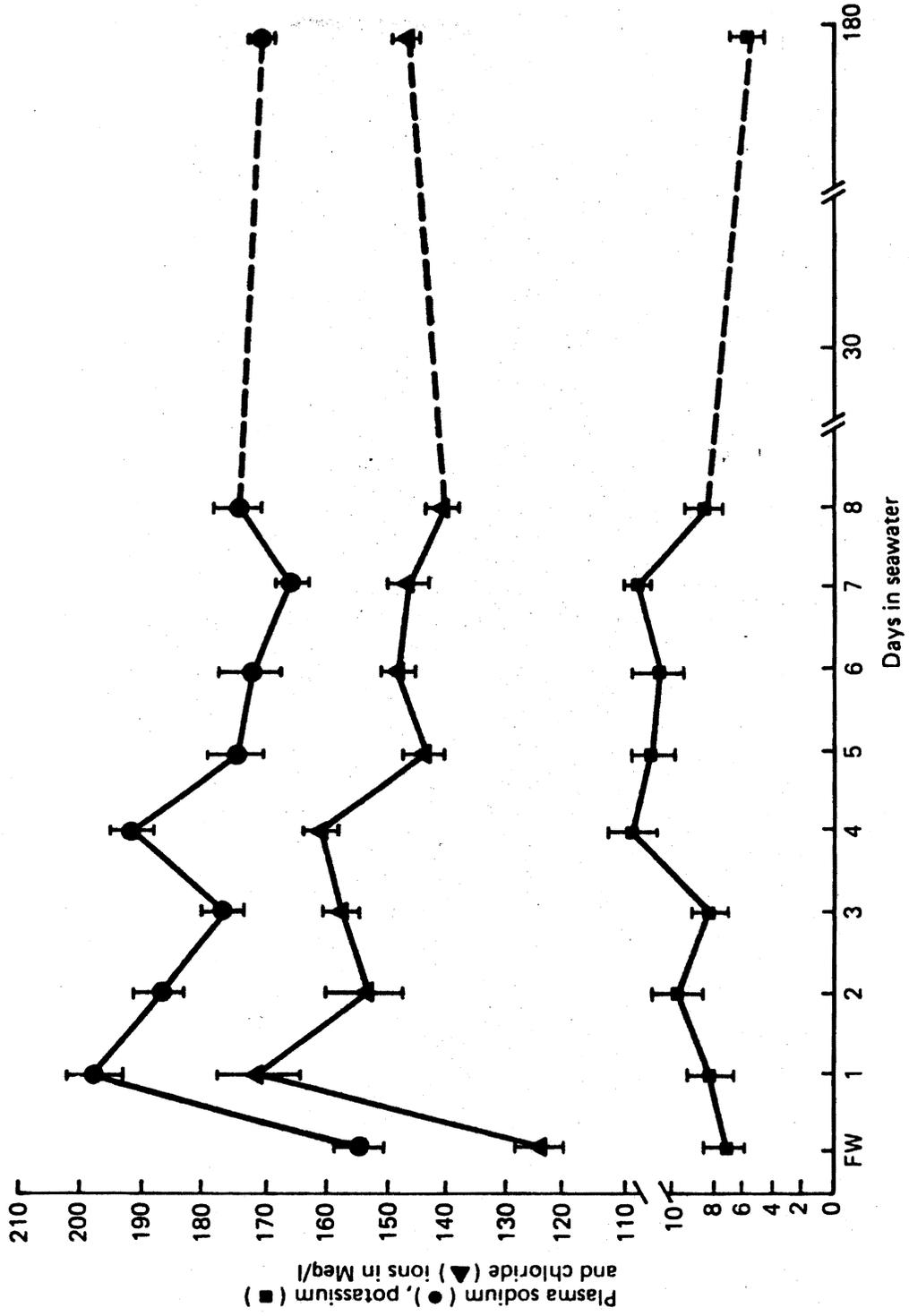
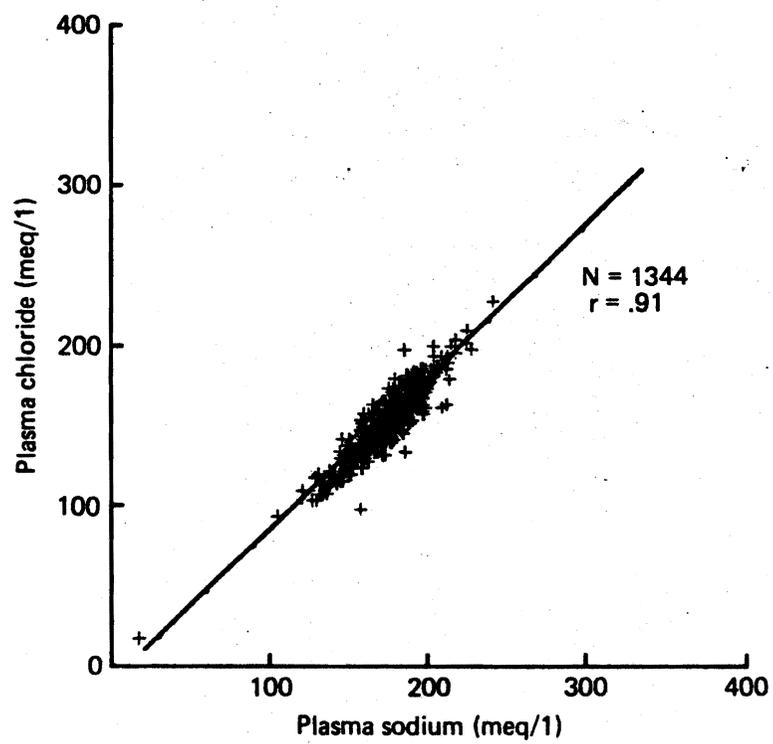


Figure 39. Linear regression analysis of gill $\text{Na}^+ - \text{K}^+$ ATPase specific activities vs plasma sodium ion concentrations for all experimental fish.



coupled. Although the gill enzyme, $\text{Na}^+\text{-K}^+$ ATPase, has been implicated in this transport, ATPase activities showed no statistically significant relationship to plasma Na^+ levels (Fig. 40).

The success of smoltification for the various stocks was assessed by determining the proportions of surviving fish and smolted fish after six months in seawater. Figure 41 represents the percent of smolted fish after six months in seawater for each of the groups. The percentage of smolted fish ranged from 48% (Willard II) to 90% (Sandy I) with the other groups being intermediate to those values. Figure 42 represents the percent of fish surviving after six months in seawater in each of the groups. The percentage of surviving fish ranged from 27% (Kalama Falls) to 80% (Sandy I) with the other groups being intermediate to those values.

While the fish were in freshwater, gill $\text{Na}^+\text{-K}^+$ ATPase activities and plasma T_4 concentrations appeared cyclical and related in time. To evaluate this possible interrelationship statistically, and compare each of the individual curves with other events occurring in both freshwater and seawater, it was necessary to separate the measurable components in each curve (Figs. 43 and 44). Figure 43 shows a hypothetical representation of either gill $\text{Na}^+\text{-K}^+$ ATPase specific activities or plasma T_4 concentrations in the coho salmon groups in freshwater, indicating those aspects of the

Figure 40. Linear regression analysis of plasma sodium vs plasma chloride ion values for all experimental fish.

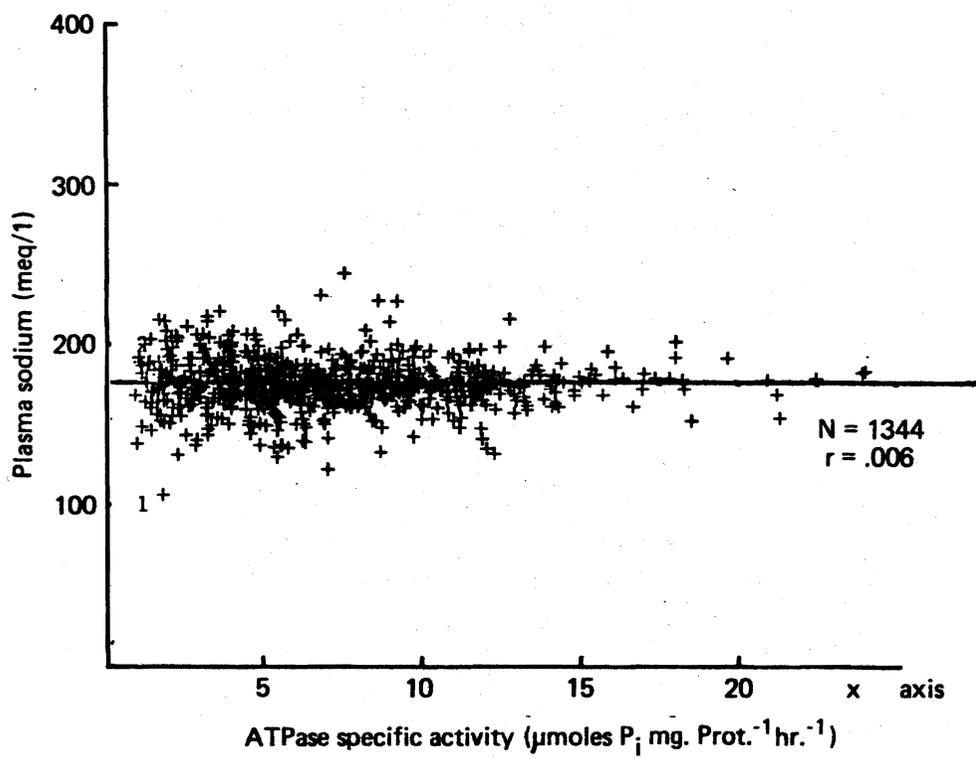


Figure 41. Success of smoltification for each of the Columbia River test groups after 6 months in seawater.

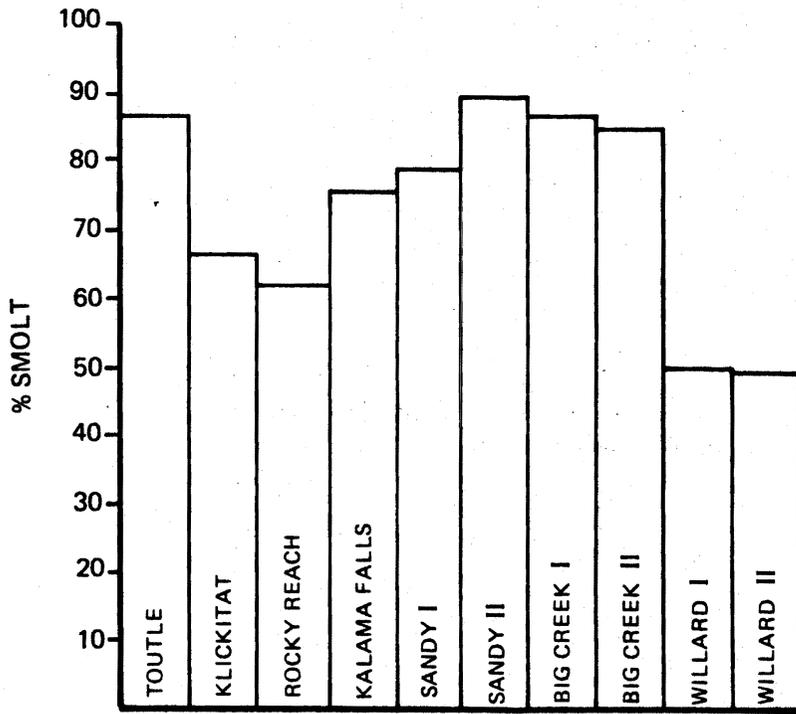


Figure 42. Survival of the Columbia River test groups after 6 months in seawater.

% SURVIVAL

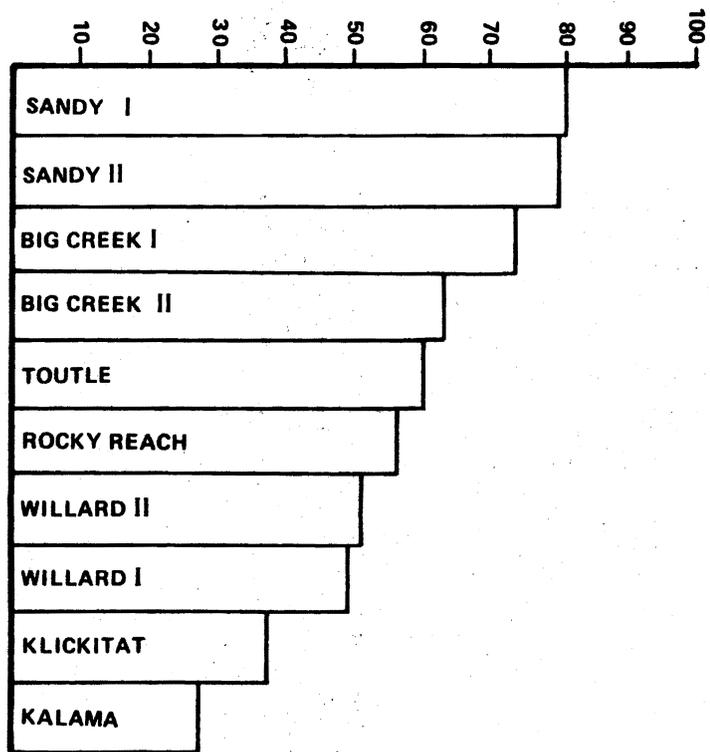


Figure 43. A representative graph of Columbia River coho salmon plasma T_4 concentrations or gill $Na^+ - K^+$ ATPase activities in freshwater, indicating those aspects of the curve which were quantitated for statistical analysis.

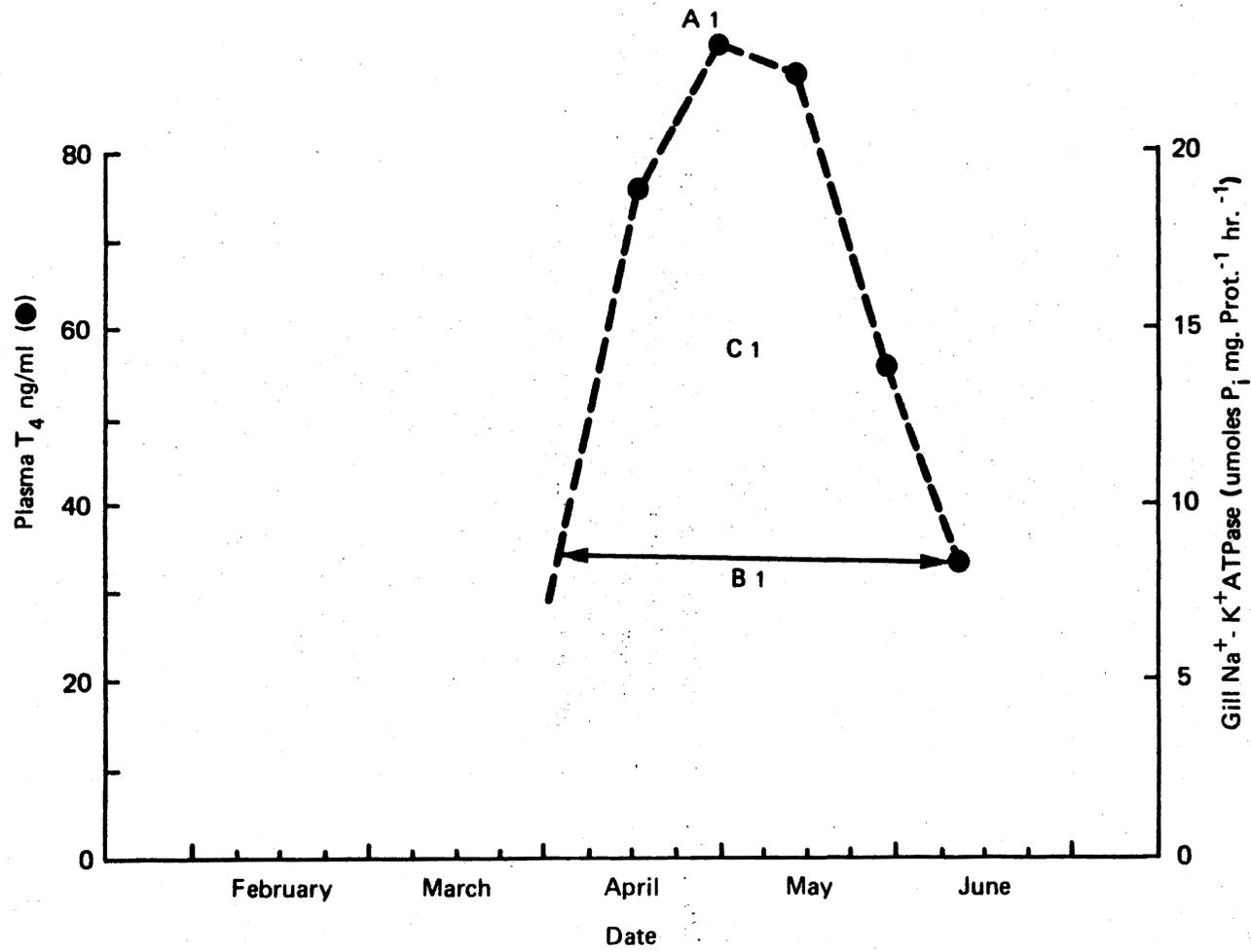
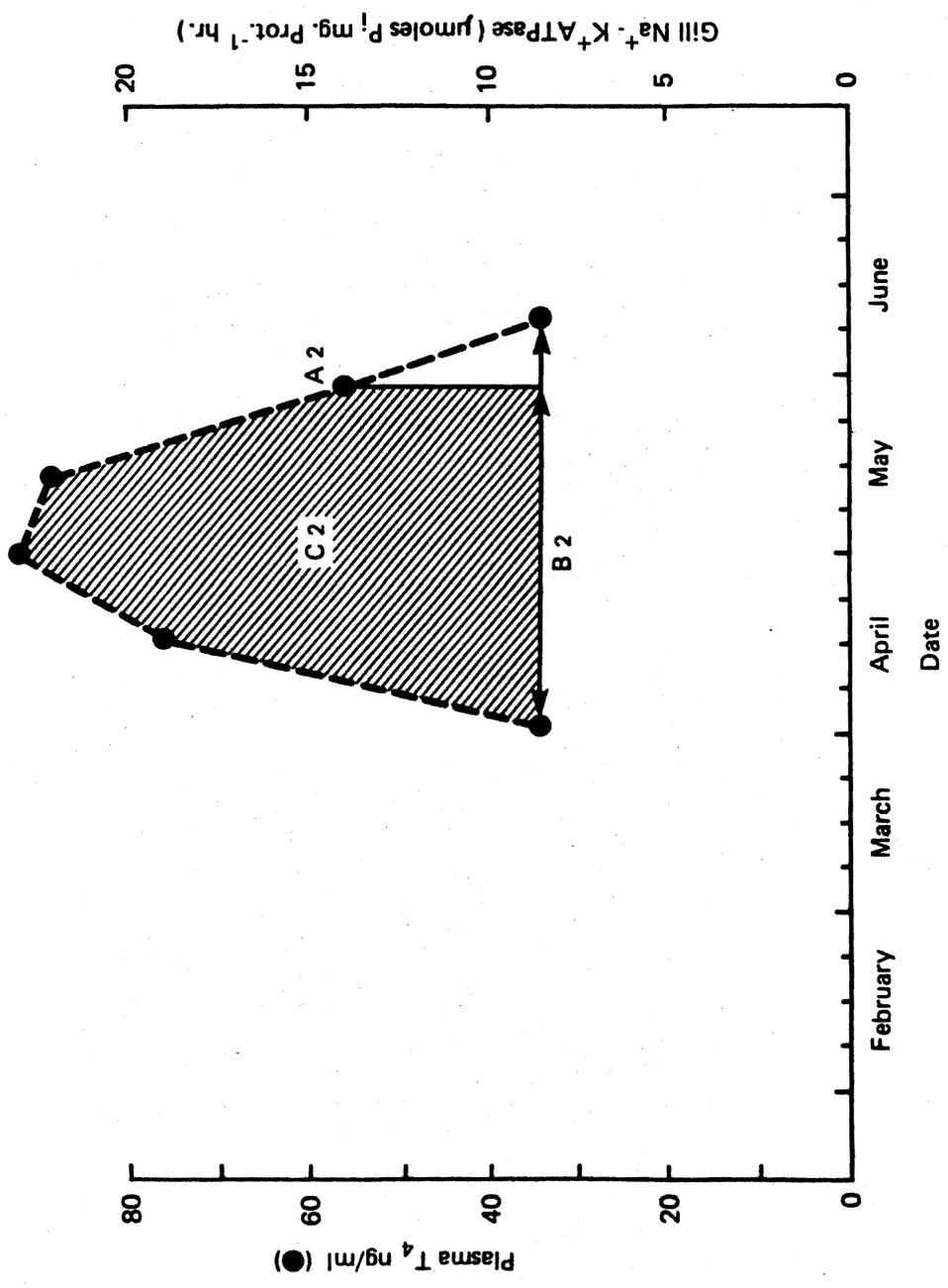


Figure 44. A representative graph of Columbia River plasma T_4 concentrations or gill $Na^+ - K^+$ ATPase activities in freshwater, indicating the influence of the hatchery release date upon those aspects of the curve which were quantitated for statistical analysis.



curve which were quantitated for statistical analysis. Figure 44 shows a hypothetical representation of either gill Na^+-K^+ ATPase specific activities or plasma T_4 concentrations in the coho salmon groups in freshwater, indicating the influence of the hatchery release upon those aspects of the curve which were quantitated for statistical analysis. At the time of hatchery release (indicated by an arrow) the peak height, duration of the curve, and area beneath the curve were all reduced.

Statistical evaluations of the data shown in Figs. 1-42 are presented in Tables 2 (non-significant relationships) and 3 (significant relationships). The size of the fish (length) was significantly related to gill Na^+-K^+ ATPase activities in both freshwater and seawater, plasma T_4 concentrations of fish in both freshwater and seawater, plasma T_3 concentrations in both freshwater and seawater, plasma Na^+ and Cl^- in freshwater and Plasma K^+ (negative) in seawater (Table 3: Items 9, 14, 10, 15, 11, 16, 12, 13, and 17, respectively). However, no significant relationship existed between length and plasma Na^+ and Cl^- in seawater fish or plasma K^+ in freshwater fish (Table 2: Items 102, 103, 104). In addition to length, gill Na^+-K^+ ATPase activities significantly correlated with both plasma T_4 and T_3 concentrations in freshwater fish (Table 3: Items 1 and 2), but not in seawater fish (Table 2: Items 99 and 100). Gill Na^+-K^+ ATPase and plasma electrolytes showed no correlations in either freshwater or seawater fish

Table 2. Statistical evaluations of the Columbia River coho salmon data. I. Non-significant relationships.

Test Parameters ^{1/2/3/}	Number of Samples
1. Gill Na ⁺ -K ⁺ ATPase activities vs plasma Na ⁺ concentrations (FW)	534
2. Gill Na ⁺ -K ⁺ ATPase activities vs plasma K ⁺ concentrations (FW)	534
3. Gill Na ⁺ -K ⁺ ATPase activities vs plasma Cl ⁻ concentrations (FW)	406
4. Plasma T ₄ concentrations vs plasma Na ⁺ concentrations (FW)	534
5. Plasma T ₄ concentrations vs plasma K ⁺ concentrations (FW)	534
6. Plasma T ₄ concentrations vs plasma Cl ⁻ concentrations (FW)	406
7. Plasma T ₃ concentrations vs plasma Na ⁺ concentrations (FW)	503
8. Plasma T ₃ concentrations vs plasma K ⁺ concentrations (FW)	503
9. Plasma T ₃ concentrations vs plasma Cl ⁻ concentrations (FW)	373
10. Changes in plasma Na ⁺ during the FW sampling period (Rocky Reach, Toutle, Klickitat, Kalama Falls, Sandy I, Sandy II)	534
11. Changes in plasma K ⁺ during the FW sampling period (Sandy I, Willard)	534
12. Changes in plasma Cl ⁻ during the FW sampling period (Kalama Falls, Sandy I, Sandy II)	414
13. Peak gill Na ⁺ -K ⁺ ATPase activity (A ₁) (FW) vs % survival (SW)	10
14. Duration of gill Na ⁺ -K ⁺ ATPase peak (B ₁) (FW) vs % survival (SW)	10
15. Integrated area beneath gill Na ⁺ -K ⁺ ATPase peak (C ₁) (FW) vs % survival (SW)	10

Table 2. Continued

Test Parameters	Number of Samples
16. Percent of gill Na^+-K^+ ATPase peak value at hatchery release (A_2/A_1)(FW) vs % survival (SW)	10
17. Percent of duration of gill Na^+-K^+ ATPase peak transpired at hatchery release (B_2/B_1)(FW) vs % survival (SW)	10
18. Percent of integrated area beneath gill Na^+-K^+ ATPase peak transpired at hatchery release (C_2/C_1) vs % survival (SW)	10
19. Peak plasma T_4 level (A_1)(FW) vs % survival (SW)	10
20. Duration of plasma T_4 peak (B_1)(FW) vs % survival (SW)	10
21. Integrated area beneath plasma T_4 peak (C_1)(FW) vs % survival (SW)	10
22. Percent of plasma T_4 peak value at release (A_2/A_1)(FW) vs % survival (SW)	10
23. Percent of duration of plasma T_4 peak transpired at hatchery release (B_2/B_1)(FW) vs % survival	10
24. Peak gill Na^+-K^+ ATPase activity (A_1)(FW) vs % smolt (SW)	10
25. Duration of gill Na^+-K^+ ATPase peak (B_1)(FW) vs % smolt (SW)	10
26. Integrated area beneath gill Na^+-K^+ ATPase peak (C_1)(FW) vs % smolt (SW)	10
27. Percent of gill Na^+-K^+ ATPase peak value at hatchery release (A_2/A_1)(FW) vs % smolt (SW)	10
28. Percent of duration of gill Na^+-K^+ ATPase peak transpired at hatchery release (B_2/B_1)(FW) vs % smolt (SW)	10

Table 2. Continued

Test Parameters	Number of Samples
29. Percent of integrated area beneath gill $\text{Na}^+ - \text{K}^+$ ATPase peak transpired at hatchery release (C_2/C_1)(FW) vs % smolt (SW)	10
30. Peak plasma T_4 level (A_1)(FW) vs % smolt (SW)	10
31. Duration of plasma T_4 peak (B_1)(FW) vs % smolt (SW)	10
32. Integrated area beneath plasma T_4 peak (C_1)(FW) vs % smolt (SW)	10
33. Percent of plasma T_4 peak value at hatchery release (A_2/A_1)(FW) vs % smolt (SW)	10
34. Percent of duration of plasma T_4 peak transpired at hatchery release (B_2/B_1)(FW) vs % smolt (SW)	10
35. Percent of integrated area beneath plasma T_4 peak transpired at hatchery release (C_2/C_1)(FW) vs % smolt	10
36. FW plasma Na^+ 1 day prior to SW entry vs % survival (SW)	10
37. FW plasma Na^+ 1 day prior to SW entry vs % smolt (SW)	10
38. Peak SW plasma Na^+ vs % survival (SW)	10
39. Peak SW plasma Na^+ vs % smolt (SW)	10
40. Plasma Na^+ after 8 days in SW vs % survival (SW)	10
41. Plasma Na^+ after 8 days in SW vs. % smolt (SW)	10
42. Plasma Na^+ after 30 days in SW vs % smolt (SW)	10
43. Plasma Na^+ after 30 days in SW vs % smolt (SW)	10

Table 2. Continued

Test Parameters	Number of Samples
44. Plasma Na ⁺ after 180 days in SW vs % survival (SW)	10
45. Plasma Na ⁺ after 180 days in SW vs % smolt (SW)	10
46. FW plasma K ⁺ 1 day prior to SW entry vs % survival (SW)	10
47. FW plasma K ⁺ 1 day prior to SW entry vs % smolt (SW)	10
48. Peak SW plasma K ⁺ vs % survival (SW)	10
49. Peak SW plasma K ⁺ vs % smolt (SW)	10
50. Plasma K ⁺ after 8 days in SW vs % survival (SW)	10
51. Plasma K ⁺ after 8 days in SW vs % smolt (SW)	10
52. Plasma K ⁺ after 30 days in SW vs % survival (SW)	10
53. Plasma K ⁺ after 30 days in SW vs % smolt (SW)	10
54. Plasma K ⁺ after 180 days in SW vs % survival (SW)	10
55. Plasma K ⁺ after 180 days in SW vs % smolt (SW)	10
56. Plasma Cl ⁻ 1 day prior to SW entry vs % survival (SW)	10
57. Plasma Cl ⁻ 1 day prior to SW entry vs % smolt (SW)	10
58. Peak SW plasma Cl ⁻ vs % survival (SW)	10
59. Peak SW plasma Cl ⁻ vs % smolt (SW)	10
60. Plasma Cl ⁻ after 8 days in SW vs % survival (SW)	10

Table 2. Continued

Test Parameters	Number of Samples
61. Plasma Cl^- after 8 days in SW vs % smolt (SW)	10
62. Plasma Cl^- after 30 days in SW vs % survival (SW)	10
63. Plasma Cl^- after 30 days in SW vs % smolt (SW)	10
64. Plasma Cl^- after 180 days in SW vs % survival (SW)	10
65. Plasma Cl^- after 180 days in SW vs % smolt (SW)	10
66. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity at SW entry vs % survival (SW)	10
67. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity at SW entry vs % smolt (SW)	10
68. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity after 8 days in SW vs % survival (SW)	10
69. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity after 8 days in SW vs % smolt (SW)	10
70. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity after 30 days in SW vs % survival (SW)	10
71. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity after 30 days in SW vs % smolt (SW)	10
72. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity after 180 days in SW vs % survival (SW)	10
73. Gill $\text{Na}^+ - \text{K}^+$ ATPase activity after 180 days in SW vs % smolt (SW)	10
74. Plasma T_4 concentration at SW entry vs % survival (SW)	10
75. Plasma T_4 concentration at SW entry vs % smolt (SW)	10
76. Plasma T_4 concentration after 8 days in SW vs % survival (SW)	10

Table 2. Continued

Test Parameters	Number of Samples
77. Plasma T_4 concentration after 8 days in SW vs % smolt (SW)	10
78. Plasma T_4 concentration after 30 days in SW vs % survival (SW)	10
79. Plasma T_4 concentration after 30 days in SW vs % smolt (SW)	10
80. Plasma T_4 concentration after 180 days in SW vs % survival (SW)	10
81. Plasma T_4 concentration after 180 days in SW vs % smolt (SW)	10
82. Plasma T_3 concentration at SW entry vs % survival (SW)	10
83. Plasma T_3 concentration at SW entry vs % smolt (SW)	10
84. Plasma T_3 concentration after 8 days in SW vs % survival (SW)	10
85. Plasma T_3 concentration after 8 days in SW vs % smolt (SW)	10
86. Plasma T_3 concentration after 30 days in SW vs % survival (SW)	10
87. Plasma T_3 concentration after 30 days in SW vs % smolt (SW)	10
88. Plasma T_3 concentration after 180 days in SW vs % survival	10
89. Plasma T_3 concentration after 180 days in SW vs % smolt	10
90. Gill Na^+-K^+ ATPase activity vs plasma Na^+ concentration (SW)	954
91. Gill Na^+-K^+ ATPase activity vs plasma K^+ concentration (SW)	954
92. Gill Na^+-K^+ ATPase activity vs plasma Cl^- concentration (SW)	824

Table 2. Continued

Test Parameters	Number of Samples
93. Plasma T_4 concentration vs plasma Na^+ concentration (SW)	935
94. Plasma T_4 concentration vs plasma K^+ concentration (SW)	935
95. Plasma T_4 concentration vs plasma Cl^- concentration (SW)	803
96. Plasma T_3 concentration vs plasma Na^+ concentration (SW)	815
97. Plasma T_3 concentration vs plasma K^+ concentration (SW)	815
98. Plasma T_3 concentration vs plasma Cl^- concentration (SW)	683
99. Gill Na^+-K^+ ATPase activity vs plasma T_4 concentration (SW)	935
100. Gill Na^+-K^+ ATPase activity vs plasma T_3 concentration (SW)	815
101. Plasma Na^+ concentration vs plasma K^+ concentration (FW)	534
102. Length vs plasma Na^+ concentration (SW)	954
103. Length vs. plasma Cl^- concentration (SW)	824
104. Length vs plasma K^+ concentration (FW)	534
105. Percent smolt vs % survival	10

87

1/ FW = freshwater, SW = seawater

2/ Items 10-12 were tested by single classification, Analysis of Variance, while the remainder were tested by Pearson's Product Moment Test of Correlation

Table 2. Continued

3/ Items 13-35 refer to the ATPase and T_4 curves shown in Figures 43 and 44, and the histograms for % smolt (Figure 41) and % survival (Figure 42).

Table 3. Statistical evaluations of the Columbia River coho salmon data. II. Significant relationships.

Test Parameters ^{1/}	Number of Samples	Level of Significance (α) ^{2/}
1. Gill $\text{Na}^+ - \text{K}^+$ ATPase activities vs plasma T_4 concentrations (FW)	534	.01
2. Gill $\text{Na}^+ - \text{K}^+$ ATPase activities vs plasma T_3 concentrations (FW)	523	.01
3. Plasma T_4 concentrations vs plasma T_3 concentrations (FW)	503	.01
4. Percent of integrated area beneath plasma T_4 peak before hatchery release (C_1/C_2) FW vx % survival (SW)	10	.05
5. Plasma Na^+ concentrations vs plasma Cl^- concentrations (FW)	286	.01
6. Changes in plasma Na^+ during the FW sampling period (BC I, BC II, Willard)	534	.01
7. Changes in plasma K^+ during the FW sampling period (Rocky Reach, Toutle, Klickitat, Kalama Falls, BC I, BC II, Sandy II)	534	.01
8. Changes in plasma Cl^- during the FW sampling period (Klickitat, BC I, BC II, Willard)	414	.01
9. Length vs gill $\text{Na}^+ - \text{K}^+$ ATPase activities (FW)	534	.01
10. Length vs plasma T_4 concentrations (FW)	534	.01
11. Length vs plasma T_3 concentrations (FW)	523	.05
12. Length vs plasma Na^+ concentrations (FW)	534	.01
13. Length vs plasma Cl^- concentrations (FW)	534	.01
14. Length vs gill $\text{Na}^+ - \text{K}^+$ ATPase activities (SW)	954	.01

Table 3. Continued.

Test Parameters ^{1/}	Number of Samples	Level of Significance (α) ^{2/}
15. Length vs plasma T ₄ concentrations (SW)	934	.01
16. Length vs plasma T ₃ concentrations (SW)	815	.01
17. Length vs plasma K ⁺ concentrations (SW)	954	.01 (-)
18. Plasma Na ⁺ concentrations vs plasma K ⁺ concentrations (SW)	954	.01
19. Plasma Na ⁺ concentrations vs plasma Cl ⁻ concentrations (SW)	934	.01
20. Plasma T ₄ concentrations vs plasma T ₃ concentrations (SW)	844	.01
21. Two-way interactions between T ₄ concentrations at 8, 30, and 180 days in SW and the 10 groups	275	.001
a. Interaction between days 8, 30, and 180 in SW	-	.001
b. Interaction among the 10 hatchery groups	-	.001
22. Two-way interactions between gill Na ⁺ -K ⁺ ATPase activities at 8, 30, and 180 days in SW and the 10 groups	238	.001
a. Interaction between days 8, 30, and 180 in SW	-	.005
b. Interaction among the 10 hatchery groups	-	.01

06

^{1/} FW = freshwater, SW = seawater

^{2/} Items 1-5 and 9-20 were tested by the Pearson's Product Moment Test of Correlation. Items 6-8 were tested by single classification ANOVA. Items 21 and 22 were tested by two-way ANOVA.

(Table 2: Items 1, 2, 3, 90, 91, 92). There were no statistically significant relationships between the parameters measured for the gill $\text{Na}^+\text{-K}^+$ ATPase curves in freshwater fish (Figs. 43 and 44) and the number of surviving (Fig. 42) or smolted (Fig. 41) fish after six months in seawater (Table 2: Items 13-18 and 24-29). There were also no statistically significant relationships between the gill $\text{Na}^+\text{-K}^+$ ATPase measurements taken at entry into seawater, or at 8, 30, and 180 days afterward with percent survival (Fig. 42) or percent smolts (Fig. 41) (Table 2: Items 66-73). The gill $\text{Na}^+\text{-K}^+$ ATPase measurements taken at 8, 30, and 180 days after entry into seawater were found significantly different both by day and hatchery (Table 3: Item 22). Plasma T_4 and T_3 concentrations were significantly related in both freshwater and seawater fish (Table 3: Items 3 and 20). There were no statistically significant relationships between plasma T_4 or T_3 concentrations and any of the electrolytes in either freshwater or seawater (Table 2: Items 4-9 and 93-98). The percent of the integrated area beneath the plasma T_4 curve before hatchery release (C_2/C_1) (Figs. 43 and 44) was significantly related with the percent of surviving fish after six months of seawater residence (Fig. 42) (Table 3: Item 4), however, there was no significant relationships between any of the other T_4 curve parameters and percent survival (Fig. 42) or percent smolts (Fig. 41) (Table 2: Items 19-23 and 30-35). This was true for both the vaccinated and unvaccinated replicates. There were no

significant relationships between the T_4 or T_3 measurements taken upon entering seawater, or at 8, 30, or 180 days after entering seawater were significantly different both by day and by hatchery (Table 3: Item 21). There were no significant relationships between any of the plasma electrolyte measurements in freshwater fish at their peak two days after entering seawater, or at 8, 30, or 180 days later with percent survival (Fig. 42) or percent smolts (Fig. 41) (Table 2: Items 36-65). The decrease in Na^+ and Cl^- while in freshwater was significant in some of the test groups (Table 3: Items 6 and 8), but not in others (Table 2: Items 10 and 12). Also in freshwater fish significant changes in plasma K^+ were observed in most of the test groups (Table 3: Item 7), but not all (Table 2: Item 11). Plasma Na^+ levels were significantly correlated with plasma Cl^- levels in fish in either freshwater or seawater (Table 3: Items 5 and 19). Plasma Na^+ concentrations were also significantly related to plasma K^+ concentrations in fish in seawater (Table 3: Item 18), but not those in freshwater (Table 2: Item 101). There was no statistical relationship between percent smolts (Fig. 41) and percent survival after six months in seawater (Fig. 42) (Table 2: Item 105).

DISCUSSION

Previous reports have demonstrated that coho salmon were capable of tolerating seawater transfer both six to seven months prior to (Baggerman 1960a; Conte et al. 1966) and five to seven months after (Folmar et al. 1979b) the period they normally enter seawater. These results have suggested that smoltification and seawater adaptation may not reflect identical physiological processes, at least quantitatively. Therefore, these phenomena will be discussed separately. In addition to smoltification and seawater adaptation a third aspect of the present study, predictive indices, will be addressed in this section. Predictive indices relate to physiological measurements which may be applicable in determining the optimal time for hatchery release or direct transfer to seawater of yearling coho salmon.

Smoltification

In his review, Hoar (1976) noted that many of the physiological changes associated with smoltification of some salmonid species were reversible if the fish were prevented access to seawater. The present study has demonstrated that both gill $\text{Na}^+\text{-K}^+$ ATPase activities and plasma T_4 concentrations exhibit reversible changes during the period of smoltification (Figs. 1-9). At the onset of smoltification both ATPase activities and plasma T_4 concentrations increased, peaked, and in those fish that were not released from the

hatchery, decreased to presmoltification levels. These concomitant changes in ATPase activities and plasma T_4 concentrations were found to be significantly related in their timing (Table 3, Item 1). The basis for this relationship has yet to be established. Evidence has been presented for T_4 activation of Na^+-K^+ ATPase in a variety of mammalian (Valcana and Timiras 1969; Ismail-Beigi and Edelman 1970, 1971, 1974; Ismail-Beigi 1977), amphibian (Kawada *et al.* 1969) and chondrichthyan (Honn and Chavin 1977) tissues. However, in the present study gill Na^+-K^+ ATPase activity and plasma T_4 concentration peaks were separated by as much as three to four weeks (Figs. 1, 7, 9), and increases in ATPase activity were not always preceded by increases in plasma T_4 concentrations (Figs. 3, 8, 9). These results suggest that the increases in coho salmon gill Na^+-K^+ ATPase activities and plasma T_4 concentrations were correlative in their timing, but that gill Na^+-K^+ ATPase activities and plasma T_4 concentrations may be independent developmental events responding simultaneously to environmental changes presumably mediated through the hypothalamo-hypophyseal axis. Plasma T_3 concentrations were significantly related to both plasma T_4 concentrations and to gill Na^+-K^+ ATPase activities (Table 3: Items 2 and 3). The relationship between plasma T_4 and T_3 concentrations could have been related to simultaneous synthesis of T_4 and T_3 by the thyroid or conversion of T_4 to T_3 by the peripheral tissue (Ramsden 1978). The T_4 to T_3 ratio remained relatively uniform in all of the groups except

Klickitat (Fig. 3) which had much higher plasma T_3 concentrations than any of the other groups tested. ~~The significant correlations of both plasma T_4 and T_3 concentrations with gill Na^+K^+ ATPase activities suggests that the enzymatic activity may be related to the general activity of the thyroid.~~

The size of the fish (length) was found to be positively correlated with gill Na^+K^+ ATPase activities and plasma T_4 and T_3 concentrations (Table 3: Items 14-16). This size relationship probably explains the large standard errors around the means of the increasing ATPase and T_4 values during the period of smoltification. Prior to smoltification the standard errors were small, at which time the fish were also relatively uniform in length. However, at the onset of smoltification the variation in ATPase and T_4 levels in the population, as indicated by standard errors, began to increase. This suggested that smoltification was not a uniform event among the members of each group.

The temporal pattern of changes in gill Na^+K^+ ATPase activities and plasma T_4 and T_3 concentrations in freshwater fish (Figs. 1-9) were unique for each group of fish tested. At the Big Creek hatchery, two groups of fish were reared under identical conditions, but were of different genetic stocks (Big Creek I, Big Creek stock; Big Creek II, Cowlitz stock). The differences observed in enzyme activities and plasma hormone concentrations between the

two groups suggested that there may be a genetic component involved in the onset, duration, and magnitude of the physiological changes associated with smoltification. Previous studies (Black 1951; Houston 1959, 1961; Baggerman 1960a; Parry 1960, 1961; McInerney 1964; Conte et al. 1966; Weisbart 1968; Leatherland and McKeown 1974) have shown interspecific differences in patterns of salmonid salinity preference and seawater acclimation. The present results suggest there was also an intraspecific genetic variability in the smoltification patterns of O. kisutch. At the Sandy hatchery, two groups of the same genetic stock were reared under identical conditions, but were fed different diets (Sandy I, production OMP diet; Sandy II, soy oil substituted diet). The differences observed in enzyme activities and plasma hormone concentrations suggests that diet also influences some of the physiological changes associated with smoltification. Leatherland et al. (1977) also found that diet could significantly alter circulating T₄ levels in rainbow trout. There were no significant differences, however, in the number of smolts (Fig. 41) or surviving fish (Fig. 42) after six months in seawater between the two groups at the Sandy hatchery. These relationships suggest that although genetic strain and diet may influence the onset and/or duration of smoltification they do not ultimately control successful seawater adaptation. Therefore, the significant differences ($P \leq 0.05$) observed in the number of smolts and surviving fish as shown in Figs. 41 and 42 were probably due to

freshwater environmental variables such as photoperiod, temperature, iodine availability, and other water quality parameters (Foerster 1937; White 1939; Keenleyside and Hoar 1954; Gorbman and Berg 1955); Malikova 1957; Elson 1962; Eales 1963, 1964, 1965; Conte and Wagner 1965; Hoar 1965, 1976; Conte et al 1966; Hartman et al. 1967; Johnston and Eales 1968, 1970; Pinder and Eales 1969; Saunders and Henderson 1970; Zaugg et al 1972; Adams et al 1973, 1975; Withey and Saunders 1973; Zaugg and Wagner 1973; Richardson and McLeave 1974; Wagner 1974a, b; Jessop 1975; Solomon 1975, 1978; Murphy and Houston 1977; Clarke et al. 1978; Fried et al. 1978).

The first observation of salmonid gill $\text{Na}^+\text{-K}^+$ ATPase activation by transfer into seawater was reported in steelhead trout by Zaugg and McLain (1969). Since then, there have been numerous reports demonstrating the same phenomenon in other salmonid species as well (Zaugg and McLain 1970, 1971, 1972; Zaugg et al. 1972; Adams et al 1973, 1975; Zaugg and Wagner 1973; Giles and Vanstone 1976; McCarty 1976; Dickhoff et al. 1977; Boeuf et al. 1978; Clarke et al. 1978; Lasserre et al. 1978; Saunders and Henderson 1978). Two of these reports (Zaugg and McLain 1972; Adams et al. 1973) also presented information regarding changes in salmonid gill $\text{Na}^+\text{-K}^+$ ATPase activities during the period of smoltification in freshwater. The time-course changes in gill $\text{Na}^+\text{-K}^+$ ATPase activities found in the present study (Figs. 1-9) occurred in the same time period as those previously found in coho salmon by Adams et al. (1973). The

configuration of the curves and the magnitude of the peaks were different, probably as a result of the different methods (different cellular fractions) used to determine enzyme activity.

To statistically evaluate the relationship of the gill $\text{Na}^+\text{-K}^+$ ATPase curve in freshwater fish with other events occurring in both freshwater and seawater, it was necessary to separate the measurable components of each curve (Figs. 43 and 44). There were no statistically significant relationships between any of the components of the ATPase curve and percent smolts (Fig. 41) or percent survival (Fig. 42) (Table 2: Items 13-18). Also, there were no statistically significant relationships between gill $\text{Na}^+\text{-K}^+$ ATPase specific activities and the concentrations of the plasma electrolytes in freshwater or seawater (Table 2: Items 1-3 and 90-92). In freshwater, gill $\text{Na}^+\text{-K}^+$ ATPase activities increased in all of the groups tested, which suggests that an increase in this enzyme may be a developmental requirement for entry into seawater. However, the absence of significant relationships with the electrolytes or percent survival in seawater further suggests that $\text{Na}^+\text{-K}^+$ ATPase was not the sole mechanism of ionoregulation and was not solely responsible for mortalities due to osmoregulatory failure.

The principal causal factors for the increase in gill $\text{Na}^+\text{-K}^+$ ATPase activities have yet to be determined. Previous studies have

shown that both increased daylength and elevated water temperatures can significantly increase the activity of this enzyme system (Zaugg et al. 1972; Adams et al. 1973, 1975; Zaugg and Wagner 1973; Giles and Vanstone 1976; Clarke et al. 1978). Other studies have shown that the hormones prolactin and cortisol can also affect the activity of gill $\text{Na}^+\text{-K}^+$ ATPase (Pickford et al. 1970a, b; Epstein et al. 1971). Thyroxine has also been shown to activate this enzyme system in other vertebrates (Kawada et al. 1969; Honn and Chavin 1977; Ismail-Beigi 1977). Although the timing of the cyclic increases in plasma T_4 and gill $\text{Na}^+\text{-K}^+$ ATPase appeared to be independent in this study, direct T_4 activation of gill $\text{Na}^+\text{-K}^+$ ATPase, or activation of gill $\text{Na}^+\text{-K}^+$ ATPase through feedback relationships with other hormones cannot be discounted.

Thyroid involvement with smoltification was originally suggested by Hoar (1939b) who observed histological activation of thyroid tissue of the Atlantic salmon during the period associated with the parr-smolt transformation. Radiothyroidectomy, goitrogen treatment, and thyroid hormone administration have been used since then to show a relationship between thyroid gland function and development of juvenile salmonids (LaRoche et al. 1950; LaRoche and Leblond 1954; Dales and Hoar 1954). More recent studies have shown, however, that these methods may be unreliable. Drury and Eales (1968) were able to show discrepancies between histological and radio-chemical methods of determining thyroid activation. Thyroid

tissue which appears active by histological methods may be symptomatic of a hyperthyroid or goiterous condition if environmental levels of iodine are low. Similarly, rapid incorporation of radioactive iodine could erroneously suggest a hyperactive thyroid condition (Sonstegard and Leatherland 1976). Also, these methods do not provide information relative to the circulating levels of the thyroid hormones which are available to the tissue receptor sites. The recent development of RIA procedures for thyroid hormones now permits the determination of plasma concentrations of both T_4 and T_3 . Recently, this procedure was used to demonstrate a surge of thyroid hormone secretion just prior to the "metamorphic climax" in metamorphosing anuran amphibians (Leloup and Buscaglia 1977; Regard et al. 1978). Applying this approach to coho salmon during the period of parr-smolt transformation resulted in similar findings (Figures 1-9). In anurans, the thyroid surge occurred just prior to the transition from an aquatic to terrestrial environment, while in coho the thyroid surge preceded the transition from freshwater to seawater. The thyroid surge in anurans was approximately six days in duration and was associated with rapid and distinct morphological changes. In contrast, the longer T_4 peak (30-60 days) in young salmon was associated with gradual changes in behavior, coloration and condition index.

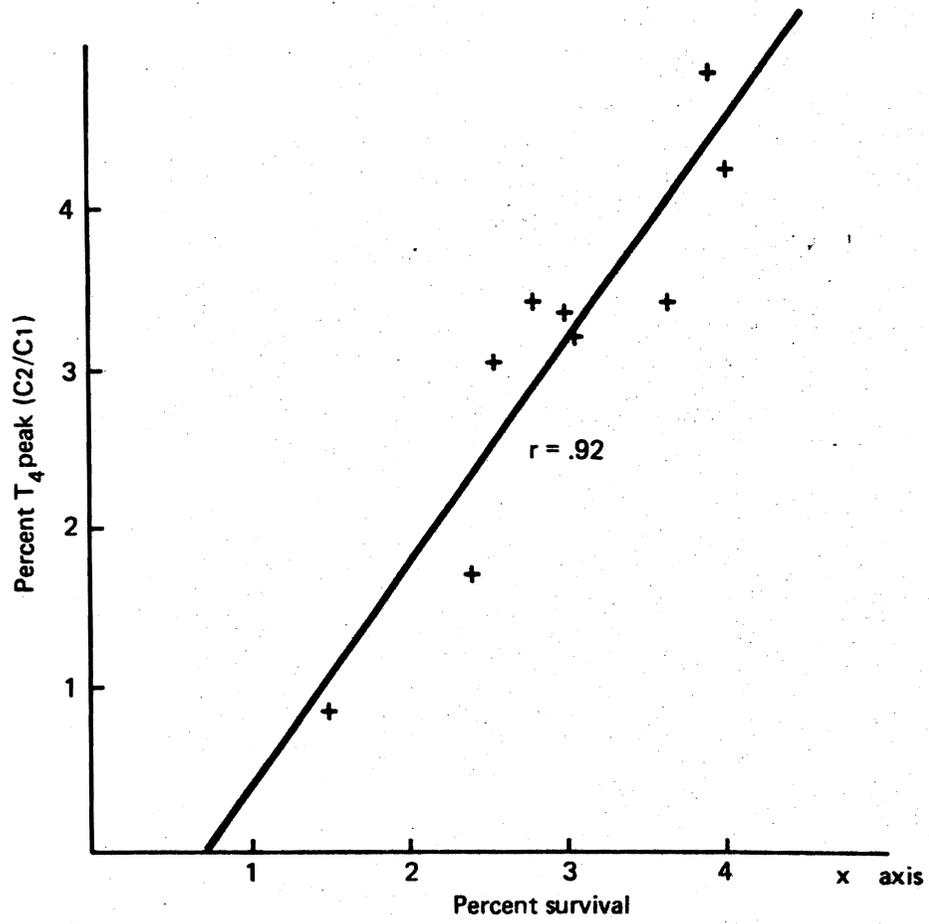
In late March or April, depending upon the stock, plasma T_4 concentrations began increasing until a peak was reached in late

April or May, then decreased until basal levels were reestablished at some time prior to summer solstice. Annual variations in circulating T_4 and T_3 concentrations have been previously reported for another salmonid, the brook trout (Salvelinus fontinalis) (White and Henderson 1977; Osborn et al. 1978). These authors observed a gradual elevation of both T_4 and T_3 during the spring and speculated that those changes were related to lengthening daylight and were perhaps involved in the regulation of growth. The springtime elevation of T_4 concentrations in the young coho salmon observed in this study were of much greater magnitude than those observed in brook trout. It is, therefore, probably that this springtime surge in T_4 is the impetus for a number of thyroid-related changes associated with smoltification. These changes include melanin dispersal (Matty and Sheltaw 1967), integumentary deposition of guanine (LaRoche and Leblond 1952; Dales and Hoar 1954; Woodhead 1966; Johnston and Eales 1967; Sage 1968), skeletal growth (Qureshi 1976), preference for seawater (Baggerman 1963), seaward migration (Hoar 1939, 1952, 1959, 1976; Koch and Heuts 1942; Hoar and Bell 1950; LaRoche and Leblond 1952; Dales and Hoar 1954; Fontaine 1954; Olivereau 1954; Baggerman 1957, 1960a, b, 1962; Fage and Fontaine 1958; Hickman 1959, 1962; Honma 1959; Woodhead 1959, 1975; Honma and Tamura 1963; Koch 1968; Gorbman 1969; Honma et al. 1977), increased swimming activity (Hoar et al. 1955; Baggerman 1962), decreased body lipids (Lovern 1934; Hoar 1939b; Kizevetter 1948; Fontaine and Hately

1950; Evropeitsevia 1957; Malikova 1957; Parker and Vanstone 1966; Fessler and Wagner 1969; Vanstone and Market 1968; Foda 1974; Wagner 1974a; Komourdjian et al. 1976; Clarke et al. 1978; Farmer et al. 1978), increased carbohydrate metabolism (Fontaine and Hatey 1950; Fontaine 1960; Wendt and Saunders 1973; Withey and Saunders 1973), increased amino acid concentrations in plasma (Cowey et al. 1963) and increased buoyancy (Saunders 1965; Pinder and Eales 1969).

To statistically evaluate the relationship of the plasma T_4 curve in freshwater fish with other events occurring in both freshwater and seawater it was necessary to separate the measurable components of each curve (Figures 43 and 44). When these measurements were compared with the two measures of seawater success, percent smolts (Figure 41) and percent survival (Figure 42), only the area beneath the T_4 curve prior to transfer into seawater (C_2/C_1 , Figure 44) and percent survival (Figure 42) were found to be statistically significantly related (Table 3, Item 4 and Figure 45). This relationship suggests that fish entering seawater near the completion of the T_4 surge have an increased chance for survival. In unpublished studies, Dickhoff and Folmar have observed that coho salmon transferred to seawater prior to the peak of the T_4 curve showed precipitous decreases in their plasma T_4 levels. These fish exhibited poor growth and a high rate of mortality. Also, Clarke and Nagahama (1977) found that premature transfer of coho salmon resulted in a high proportion of stunted fish in the test

Figure 45. Linear regression analysis of % survival vs % of the T_4 peak (C_2/C_1) which had transpired at the time of release.



population. After histological examination, these authors stated that the stunted coho appeared hypothyroid and were accumulating growth hormone (STH) in the somatotropic cells of the pituitary. The results of the present study, along with the unpublished observations of Dickhoff and Folmar and the report of Clarke and Nagahama (1977) tend to corroborate previous reports (Sage 1967; Pandy and Leatherland 1970; Leatherland and Hyder 1975; Chan and Eales 1976; Higgs et al. 1976; Milne and Leatherland 1978) that have suggested a positive feedback relationship between T_4 and STH in teleosts.

The exact function of the T_4 surge during smoltification has yet to be established. In mammals, thyroid hormones are required by the developing brain for growth and biochemical and morphological development (Geel and Timiras 1970; Balazs et al. 1971; Sokoloff and Kennedy 1973; Valcana and Timiras 1978). Therefore, the increased T_4 concentration in coho salmon may be responsible for the proper development of the brain and perhaps peripheral tissue as well (Qureshi 1976). If the T_4 surge is interrupted, as in the case of premature transfer into seawater, the decreased levels of circulating T_4 may be insufficient for continued development, resulting in the cessation of growth. Although the aforementioned relationships are conjectural, it does appear from the present study that the completion or near completion of the T_4 surge in freshwater is a developmental requirement for successful seawater survival and

growth. The cause for the sudden increase in plasma T_4 concentrations was also not determined, however, previous reports have shown that elevated temperatures and lengthened periods of daylight increased thyro^{id}~~id~~ stimulating hormone (TSH) secretion (Fontaine and Leloup 1964; Singh 1967; Jorgensen and Larsen 1967), at circulating T_4 levels (Leatherland et al. 1977) in fish. These findings suggest that environmental information is processed through hypothalamo-hypophyseal channels to activate the thyroid axis. However, such alternate mechanisms as change in thyroid sensitivity to TSH or hormone metabolic clearance rates may contribute to the observed T_4 elevations.

Changes in plasma Na^+ , K^+ , and Cl^- concentrations of freshwater fish during the period of smoltification were shown in Figures 20-28. There were no distinct patterns of change as observed with gill Na^+-K^+ ATPase activities and plasma T_4 concentrations. All of the groups showed a tendency for their plasma Na^+ and Cl^- concentrations to decrease sometime during the freshwater sampling period; however, the decreases were significant in some groups (Table 3, Items 6 and 8), but not in others (Table 2, Items 10 and 12). The reasons for the discrepancies in the electrolyte changes among the groups remain unknown, but it would not be unreasonable to assume that genetic or environmental factors were involved. Previous studies with coho salmon showed no change in plasma Cl^- during migration (Conte et al. 1966; Miles and Smith 1968), while in

O. masu plasma Cl^- levels were found to decrease significantly during this period (Kubo 1955). Also, Houston et al. (1968), Houston (1973) and Murphy and Houston (1977) found that low environmental temperatures effectively reduced the ionoregulatory ability of Atlantic salmon. Regardless of the nature of the plasma electrolyte profiles in the freshwater fish, there were no significant relationships between plasma electrolyte concentrations prior to seawater entry and seawater performance (percent smolts and percent survival).

Plasma K^+ concentrations also showed significant changes in some of the hatchery groups (Table 3, Item 7), but the direction of change was inconsistent, with some of the groups showing significant increases as well as decreases.

The reasons for the disparity in plasma electrolyte fluctuations between the hatchery groups could not be determined from the experimental data. The period of decrease in plasma Na^+ and Cl^- concentrations occurred at the same time as elevation in gill Na^+-K^+ ATPase activities; however, there was no statistically significant relationship between gill Na^+-K^+ ATPase activities and the plasma concentrations of any of the electrolytes (Table 2, Items 90-92). These results suggest that the premigratory decreases in plasma Na^+ and Cl^- concentrations and the activation of gill Na^+-K^+ ATPase were not closely dependent events. Changes in plasma

electrolytes were also not related to plasma concentrations of either T_4 or T_3 (Table 2, Items 93-98).

Plasma concentrations of Na^+ and Cl^- were significantly correlated with the length of the fish in all of the hatchery groups (Table 3, Items 12 and 13). Plasma K^+ concentrations, however, were found unrelated to the length of the fish. Previous reports (Lubin 1964; Bygrave 1967; Orr et al. 1972) have suggested that plasma K^+ concentrations may reflect growth rates; therefore, the absence of a correlation between plasma K^+ and length in this study may have been the result of different growth rates among the hatchery groups.

Plasma concentrations of Na^+ and Cl^- were significantly related (Table 3, Item 5), but Na^+ and K^+ were not (Table 2, Item 101). The absence of a relationship between plasma Na^+ and K^+ concentrations may have resulted from differential rates of intercellular uptake from the plasma, depending upon the growth rates of the different groups. The significant relationship between plasma Na^+ and Cl^- concentrations suggested that their plasma concentrations were regulated by the same mechanism. A possible model for this mechanism is discussed in the following section on seawater adaptation.

Seawater Adaptation

Seawater adaptation by young salmon requires them to make a transition from a low salinity (5 mOsm) to a high salinity (1000 mOsm) environment, while maintaining a relatively constant internal salt balance (300-400 mOsm) (Burton 1973). During this adjustive phase immediately following seawater entry, plasma Na^+ and Cl^- concentrations of all of the Columbia River coho salmon rapidly increased, peaked between 24 and 48 hours, decreased, and stabilized at levels appropriate for seawater acclimated salmonids (Fontaine et al. 1950; Phillips and Brockway 1958; Gordon 1959; Houston 1959; Conte and Wagner 1965; Conte et al. 1966; Holmes and Stainer 1966; Miles and Smith 1968; Milne 1974; Newcomb 1978).

The rapid initial increases in plasma Na^+ and Cl^- concentrations after seawater entry were probably caused by the concentration gradient differences between seawater and the internal fluids of the fish (Parry 1966). The 24-48 hour period of elevation would then represent the period of time required for the fish to establish the required changes in gill permeability and the activation of the Na^+/Cl^- pump(s) to a level commensurate with seawater residence. The exact mechanism by which these events are regulated remains to be established; however, recent evidence suggests that the endocrine system (hypothalamo-hypophyseal-interrenal axis) may be involved. In freshwater, prolactin reduces gill permeability

(Ball 1969, Utida et al. 1972) and depresses the activity of gill Na^+-K^+ ATPase (Utida et al. 1969, 1972; Pickford et al. 1970a). Cortisol, presumably acting through a stimulation of protein synthesis, stimulates increases in gill Na^+-K^+ ATPase activity (Epstein et al. 1971). Therefore continued prolactin influence after seawater entry combined with the time required for new enzyme synthesis under the influence of cortisol may account for the 24-48 hr period of elevated electrolyte concentrations observed in this study.

Alternatively, this increase could have been artificial, a result of dehydration and hemoconcentration which would indicate higher ion concentrations per unit volume of plasma. In this situation, the 24-48 hr period represents the time period for the fish to commence drinking seawater and activate the monovalent ion transport system in the gut (Oide and Utida 1968; Kirsh and Mayer-Gostan 1973). The initial transport of Na^+ and Cl^- from the gut into the blood causes a concentration gradient which then allows the transport of freshwater from the gut into the body tissues (House and Green 1963; Sharret et al. 1964; Shehadeh and Gordon 1969). This uptake of freshwater then increases plasma volume and thereby gives the appearance of decreasing plasma ion concentrations.

Some of the groups (Figs. 32, 33, 34, 37) showed significant increases in plasma Na^+ and Cl^- concentrations at 30 and/or 180 days

after seawater transfer; however, the measured values are still within the normal range of values for seawater-acclimated salmonids.

The duration of the adjustive phase in the Columbia River coho salmon was comparable with acclimation periods previously reported for coho salmon (Conte and Wagner 1965; Miles and Smith 1968; Clarke and Blackburn 1977). The absolute values of plasma Na^+ and Cl^- concentrations were higher in this study than those reported by Clarke and Blackburn (1977) and Miles and Smith (1968). Clarke and Blackburn (1977) stated that juvenile coho salmon were not considered smolts if their plasma Na^+ values exceeded 170 meq/l after 24 hr in seawater. However, these authors did not relate their test values to long-term growth or survival. The lowest plasma Na^+ concentration after 24 hr in seawater in any of the Columbia River salmon was 178 meq/l (Rocky Reach, Fig. 29) and ranged as high as 215 meq/l (Toutle, Fig. 30). There were no significant relationships between peak plasma Na^+ or Cl^- concentrations and seawater survival or smoltification in this study. These differences in the responses of plasma Na^+ concentrations after seawater transfer suggests that some caution should be exercised in determining hatchery release criteria from evaluations made at different geographic locations.

Plasma K^+ concentrations showed a tendency to increase slightly during the first six days after seawater transfer (Figs. 29-38); the

changes were not significant. Although plasma Na^+ and Cl^- concentrations were unrelated to the size of the fish (Table 2, Items 102, 103), plasma K^+ concentrations were found to be significantly correlated (negatively) with the length of the fish (Table 3, Item 17). Therefore, plasma concentrations were lowest in the larger, faster growing fish, probably the result of cellular K^+ uptake. This agrees with the opinions of Lubin (1964), Bygrave (1967), and Orr et al. (1972), who suggested that the cellular uptake of K^+ from plasma was responsible for increased growth mediated through K^+ -stimulated nucleic acid and protein synthesis.

Plasma electrolyte concentrations measured in freshwater, at their peak in seawater, and at 8, 30, and 180 days after seawater transfer were not significantly related to either the number of smolted (Fig. 41) or surviving (Fig. 42) fish after six months of seawater residence (Table 2, Items 36-65). The lack of a significant relationship between plasma electrolyte concentrations and the number of surviving fish could have been due to the ability of salmonid species to tolerate wide variations in plasma electrolyte levels (Fontaine et al. 1950; Kubo 1955; Phillips and Brockway 1958; Gordon 1959; Houston 1959; Conte and Wagner 1965; Conte et al. 1966; Holmes and Stainer 1966; Miles and Smith 1968; Milne 1974; Clarke and Blackburn 1977; Newcomb 1978). The lack of a significant relationship between plasma electrolytes and the number of smolted

fish could be further evidence that smoltification and seawater adaptation are separate events.

During the seawater adjustive phase, both gill Na^+-K^+ ATPase activities and plasma T_4 concentrations fluctuated, showing different patterns of acclimation. The differences in gill Na^+-K^+ ATPase acclimation patterns may have been caused by differential seawater activation of existing enzyme sites (Towle et al. 1977), or changes in the number of enzyme sites (Conte and Lin 1967). The increases in gill Na^+-K^+ ATPase activity upon seawater entry were within the range (2-5x) reported for other salmonids and diadromous fishes (Epstein et al. 1967, 1971; Kamiya and Utida 1968, 1969; Maetz 1969, 1971, 1974; Zaugg and McLain 1969, 1970, 1971, 1972; Jampol and Epstein 1970; Pickford et al. 1970a, b; Butler and Carmichael 1972; Zaugg et al. 1972; Adams et al. 1973, 1975; Towle and Gilman 1973; Zaugg and Wagner 1973; Sargent and Thomson 1974; Evans and Mallery 1975; Giles and Vanstone 1976; McCarty^{ne} 1976; Thomson and Sargent 1976; Dickhoff et al. 1977, 1978; Towle and Gilman 1977; Boef^u et al. 1978; Clarke et al. 1978; Folmar and Dickhoff 1978, 1979; Lasserre et al. 1978; Saunders and Henderson 1978). The changes in plasma T_4 concentrations could have resulted from hemoconcentration, hemo-dilution or actual fluctuations in thyroïdal T_4 production due to changes in available iodine (Gorbman and Berg 1955; Hickman 1959). As in freshwater, lengths of the fish were significantly related to both gill enzyme activities and plasma

concentrations of both T_4 and T_3 (Table 3, Items 14-16). The large standard errors around the means of these parameters were related to the random sampling procedure used to collect the samples. Within each sample group, particularly during the eight-day period immediately following seawater entry, there were wide ranges in fish lengths and consequently wide ranges of enzyme activities and plasma hormone concentrations. The seawater acclimation patterns for gill Na^+-K^+ ATPase activities and plasma T_4 concentrations were unique to each group, as they were during ^hte period of smoltification, again suggesting the expression of certain differential environmental and/or genetic components.

Analyses of variance of gill Na^+-K^+ ATPase activities and plasma T_4 concentrations at 8, 30, and 180 days after seawater entry showed that significant differences existed both between the hatchery groups and time for both ATPase ($P \leq 0.001$) and T_4 ($P \leq 0.001$) (Table 3, Items 21, 22). These results suggest that fish must continually adjust both gill Na^+-K^+ ATPase or plasma T_4 measurements while in seawater. None of the gill Na^+-K^+ ATPase or plasma T_4 measurements taken during the seawater sampling period were significantly related to the number of smolted or surviving fish after six months of seawater residence (Table 2, Items 66-81). Also, there were no significant relationships between any of the plasma T_3 measurements in seawater fish and the number of smolted or surviving fish after six months of seawater residence (Table 2,

Items 82-89). due to the number of variables in this study, the reasons for a lack of correlation between plasma thyroid hormone concentrations or gill $\text{Na}^+\text{-K}^+$ ATPase activities from seawater-adapted fish and the number of smolts or surviving fish in seawater cannot be completely resolved.

In freshwater, plasma T_4 and T_3 concentrations were significantly related to gill $\text{Na}^+\text{-K}^+$ ATPase activities (Table 3, Items 1 and 2). However, this relationship ended after the fish were transferred to seawater (Table 2, Items 99 and 100). The lack of a significant relationship between plasma T_4 concentrations and gill $\text{Na}^+\text{-K}^+$ ATPase in seawater further suggests that they may be freshwater developmental phenomena which prepare the fish for seawater entry rather than serving as regulatory mechanisms during seawater acclimation. Plasma T_4 and T_3 concentrations were significantly related ($P \leq 0.01$) in seawater (Table 3, Item 3) as they were in freshwater, indicating that peripheral conversion of T_4 to T_3 was not influenced by seawater transfer. Neither plasma T_4 or T_3 concentrations were related to plasma electrolyte concentrations during the sampling period in seawater.

There was also a notable absence of a statistically significant relationship between gill $\text{Na}^+\text{-K}^+$ ATPase specific activities and plasma electrolyte concentrations in both freshwater (Table 2, Items 1-3) and seawater (Table 2, Items 90-92). The relationship between

gill Na^+ - K^+ ATPase and plasma Na^+ was graphically presented in Fig. 40. Plasma Na^+ concentrations were, however, closely linked with plasma Cl^- concentrations both in freshwater (Table 3, Item 5) and seawater (Table 3, Item 19). This relationship was graphically presented in Fig. 39. Monovalent ion regulation has been attributed to both a putative Na^+ pump operated by gill Na^+ - K^+ ATPase (c.f. Maetz 1974) or an electrogenic Cl^- pump operated by changes in the trans-epithelial potentials across the gill membrane (Greenwald et al 1974; Kirschner et al. 1974; Shuttleworth et al. 1974; Pic and Maetz 1975). In this study, gill Na^+ - K^+ ATPase specific activities were shown to increase significantly when the fish were transferred from freshwater to seawater, but these increases did not have a marked effect of [^] plasma concentrations of either Na^+ or Cl^- . The increased ATPase activities of seawater-adapted fish suggested that this enzyme was involved in ion regulation, however, the lack of correlation with either plasma Na^+ or Cl^- concentrations suggested that an additional component, such as an electrogenic pump was also required.

The above results suggest that the Na^+ and Cl^- pumps are related, perhaps in the following manner. In seawater, the transport of Na^+ across the serosal membrane of the gill epithelium may be controlled by Na^+ - K^+ ATPase (Cl^- would be passively transported, linked to Na^+), then Cl^- is actively transported across the mucosal membrane of the gill epithelium, expelled by a favorable

transepithelial potential (Na^+ would be passively transported, linked to Cl^-). The process would be reversed in freshwater, since the fish then becomes positive to the environment.

There was no statistically significant correlation between percent survival and percent smolts in the experimental fish after six months of seawater residence (Table 2, Item 105). This further suggests that smoltification and seawater adaptation are distinct events. This relationship was not significant because of the variability in the parr-to-smolt ratios among the hatchery groups. It has been previously demonstrated that there are two possible sources of these seawater parr. Clarke and Nagahama (1977) have shown that fish in the parr state at seawater entry will remain in that state. Folmar *et al.* (1979b) have also shown that it was possible for fish entering seawater in the "transitional" state to revert to the parr state anytime during the summer or early fall.

Predictive Indices

All of the physiological measurements made during the course of this study were statistically compared with the number of surviving and successfully smolted fish after six months of residence in seawater net pens. The six month time period was selected for three reasons: 1) During this time period fish were subjected to both natural seawater disease challenges and to possible manifestations of latent freshwater disease problems: such problems would most

likely not be seen during a 30-day holding period, 2) Any mortalities attributable to osmoregulatory failure generally occur during this time period, 3) At seawater entry, the fish could be divided into three morphologically distinct phases of development; parr, smolt, and an intermediate or "transitional" form. After six months of seawater residence virtually all of the "transitional" fish had either smolted or reverted back to the parr state. However, it can be argued that the data obtained from net pen studies are invalid for hatchery fish since the riverine and estuarine aspects of their migration were ignored.

There were 127 statistical evaluations made during the course of this study; however, only one was statistically related to either the percentage of surviving or smolted fish after six months residence in seawater (Table 3, Item 4). That measurement was the percent of the area beneath the plasma T_4 curve prior to seawater transfer compared to percent survival (Fig. 45). The relationship shown in Fig. 45 suggests that survival was related to the proportion of the T_4 cycle that was completed prior to seawater entry. Another study (Folmar et al. 1979b) has shown that this relationship may be limited to coho salmon entering seawater prior to the summer solstice. Fish entering seawater after that time exhibited poor growth and high rates of parr-reversion, regardless of their age, size, or state of development at the time of seawater entry. This study also suggests that the successful transition of

coho salmon into seawater is related to the successful completion of an endogenous thyroid hormone surge prior to seawater entry. This information could ^{be} directly applicable to commercial fish-farming operations employing net pen culture of coho salmon. The six-month seawater holding period used in this study is comparable with the time required by operations to rear pan-sized coho salmon in the Puget Sound area. The results of this study have also shown that the six month seawater survival of net pen cultured coho salmon can vary by as much as 57 percent (Fig. 42) depending upon when they were transferred to seawater (Fig. 45). This relationship suggests that commercial growers could potentially increase their yield by monitoring the plasma T_4 concentrations of their freshwater fish to determine the optimal time to transfer them to seawater.

In net pen culture, fish can be transferred to seawater on short notice (24-48 hr); however, many of the fish released from the Columbia River hatcheries require 15-30 days to migrate to the estuary. Although plasma T_4 profiles may prove useful in predicting the appropriate time to transfer fish into seawater net pens, additional information regarding the relationship between the thyroid cycle and migratory behavior will be required prior to incorporating this method to predict Columbia River hatchery releases.

Despite a lack of correlation with either percent survival or percent smolts, gill $\text{Na}^+\text{-K}^+$ ATPase activities in freshwater and plasma Na^+ and Cl^- at seawater entry showed specific patterns of change which may be correlated with other events pertaining to smoltification and seawater adaptation, such as growth, disease incidence, migration, and adult returns. To this end, the measurements made during this study are currently being compared with disease, growth, and migration data collected by other investigators on the same groups of fish. These data will also be evaluated for statistical relationships with the 1981 hatchery returns of adult salmon for all of the groups tested.

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