

Smolting of Hatchery-Reared Steelhead Transferred as Eyed Eggs from the Northern to the Southern Hemisphere

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Abstract.—The effects of a Northern-to-Southern Hemisphere shift on the growth and smolting of steelhead (*Oncorhynchus mykiss*) were monitored by reference to physiological criteria. Eyed eggs were imported from the Alsea River Trout Hatchery, Oregon, USA (latitude 44°N), to the Lake Rupanco Experimental Hatchery in Chile (latitude 41°S). This transfer of eggs resulted in a notable variation in the age and size at which fish smolted. A minimum smolting size of about 10 cm was observed in the first southern spring of life, when fish were 7–8 months old. Fish that did not reach this size smolted in the second spring of life at age 20 months. Based on our current understanding of the effects of environmental variations on smolt physiology, we offer guidelines for increasing the efficiency of smolt production for this anadromous salmonid in the Southern Hemisphere.

Currently in South America, programs for the introduction and enhancement of salmonids depend, to a great extent, on eggs imported from the Northern Hemisphere (Méndez 1987; Claverie and Méndez 1990). In the Southern Hemisphere, these fish are exposed to inverted photoperiod and temperature conditions during juvenile development. Because smolting by anadromous salmonids is influenced by photoperiod and water temperature regimes (Wedemeyer et al. 1980, 1981; Zaugg 1981; Boeuf 1987; Johnsson and Clarke 1988; Clarke et al. 1989), this shift in environmental conditions should influence the smolting process. However, there is no information available regarding the effects of this natural inversion of temperature and photoperiod on the physiology of smolting.

Steelhead (*Oncorhynchus mykiss*), an anadromous variety of rainbow trout, spends one to several years in freshwater before migrating seaward (Scott and Crossman 1973). In southern Chile, growth of imported steelhead in freshwater is rapid. In their first southern spring of life, at age 8 months, steelhead imported as eggs usually reach sizes of 10–14 cm (Uribe 1988). We investigated their gill Na^+, K^+ -ATPase activity levels (Folmar and Dickhoff 1981; Zaugg 1981), condition factors (Wedemeyer et al. 1981; Virtanen and Soivio

1985), and seawater adaptability (Clarke and Blackburn 1978; Blackburn and Clarke 1990) in order to determine the degree of smolting during their first and the second springs of life (ages 8 and 20 months, respectively).

Methods

Eyed steelhead eggs were imported in March from the Alsea River Trout Hatchery, Oregon, USA (44°25'N, 123°30'W). Eggs were incubated in the Lake Rupanco Experimental Hatchery, Chile (40°45'S, 72°39'W), in water from the Huillin stream, a tributary of Lake Rupanco. Juveniles were reared in raceway tanks at a density of 20 kg/m³ and fed commercial dry pellets (J. C. Uribe and colleagues, unpublished). Photoperiod and temperature regimes were measured daily. Fish size (fork length) and weight were measured monthly on 250 individuals taken at random. Gill Na^+, K^+ -ATPase activity was measured once a week in spring and once a month between December and October. Fish were killed by a blow to the head. Two pools of gill filaments, each obtained from 3–5 specimens, were randomly collected for the determination of ATPase activity. Both gill pools were analyzed separately for ATPase specific activity. Individual values were also obtained to analyze the relationship between ATPase activity and fish size. The samples were washed in a 0.25 M sucrose solution and stored at –12°C for 2–3 d before being analyzed (Zaugg 1982). The enzymatic Na^+, K^+ -ATPase activities were measured as described by Zaugg (1982). The Ernster method was used for the determination of inorganic phosphate (P_i) (Ernster et al. 1959) and the Lowry method for protein concentration (Lowry et al. 1951). The ATPase specific activity (U/mg) was expressed as $\mu\text{mol } \text{P}_i \cdot (\text{mg protein})^{-1} \cdot \text{h}^{-1}$. One unit (U) of ATPase activity corresponded to the hydrolysis of 1 $\mu\text{mol ATP/h}$ at 37°C. The seawater challenge test was performed as described in Clarke and Blackburn (1978); a Corning flame photometer (model EEI) was used for plasma Na^+ determinations.

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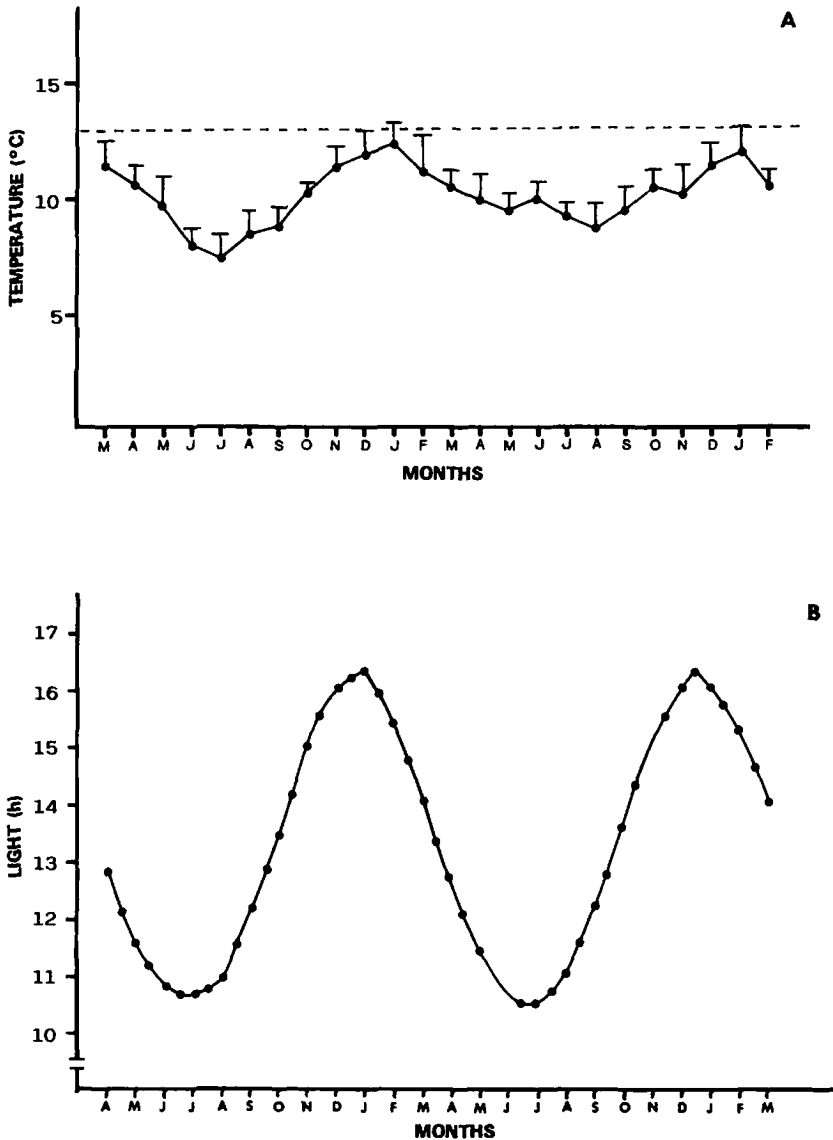


FIGURE 1.—(A) Water temperatures (mean \pm SD; $N = 3$) of the Huillin stream during the steelhead rearing period in Rupanco Experimental Hatchery. The dashed line corresponds to the reversion temperature of smolting (13 $^{\circ}$ C) for this species (Adams et al. 1975; Wedemeyer et al. 1980). (B) Photoperiod cycle during steelhead rearing in Lake Rupanco Experimental Hatchery (means of daily measurements). Monthly data start with March for temperature and with April for photoperiod.

Results

Figure 1 shows the temperature and photoperiod conditions to which fish were exposed during rearing. During the first 8 months of culture (into the first spring; October), fish achieved a mean size of 8.5 cm; in the second spring (October, age 20 months), they reached 18.9 cm (Figure 2).

Morphological changes associated with smolting (silvering and fin margin darkening) were observed in both springs. In the first spring, only the larger steelhead, 30% of the population, exhibited these morphological signs of smolting. Figure 3 shows the relationship between size and gill Na^+, K^+ -ATPase activity during the first spring

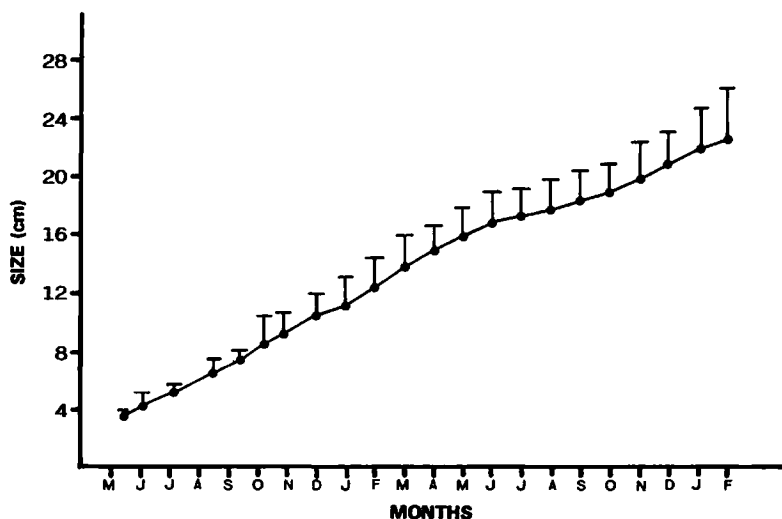


FIGURE 2.—Growth (mean fork length + SD; $N = 250$) of steelhead in Lake Rupanco Experimental Hatchery during the freshwater culture phase. Monthly data start with May.

(October 15–November 15). Only 29.6% of the fish reached 10 cm in their first October under the rearing conditions applied (Table 1).

The gill Na^+, K^+ -ATPase activity was measured during the entire rearing period in freshwater (Figure 4). In the first year (August–December; first

ATPase peak), only the larger specimens of the population (30%) showed elevated ATPase activities (Figure 3). During the following spring, an increase in the ATPase activity was observed again (October–November) for the entire fish population (second ATPase peak in Figure 4).

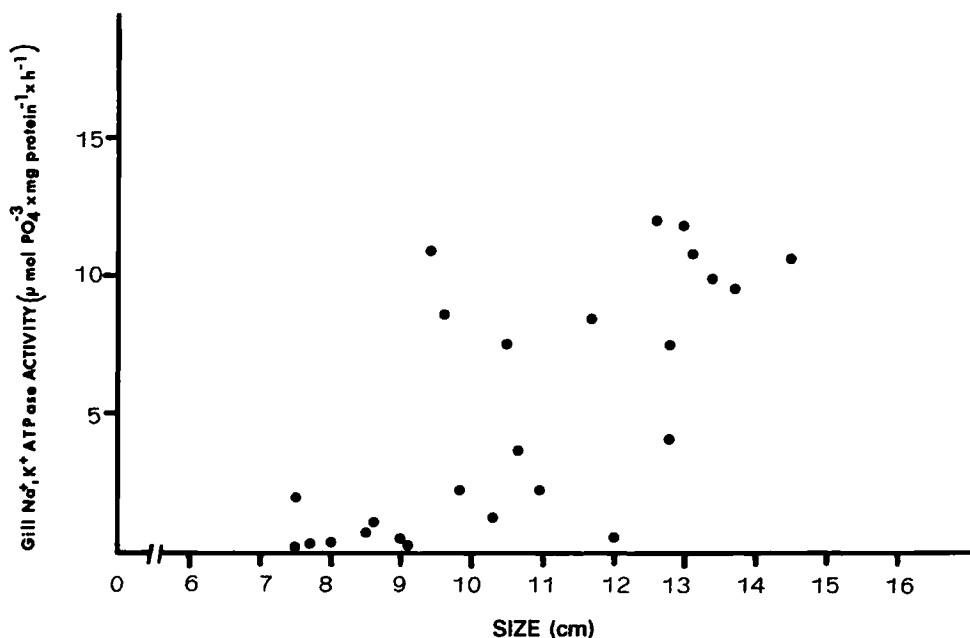


FIGURE 3.—Relation between steelhead size (fork length) and gill Na^+, K^+ -ATPase activity during the first spring of life (age, 8 months). The ATPase values were obtained for individual specimens between October 15 and November 15 (first-spring ATPase peak).

TABLE 1.—Sizes and condition factors of 8-month old steelhead in the first spring (October 20) spent at the Lake Rupanco Experimental Hatchery.

Size range (cm)	Mean size ^a (cm)	% of population	Condition factor ^a (100-g/cm ³)
6.0–8.0	7.13 ± 0.62	32.4	1.226 ± 0.090
8.1–10.0	8.96 ± 0.54	37.2	1.134 ± 0.106
>10.0	11.36 ± 1.01	29.6	1.047 ± 0.096

^a Mean ± SD; N = 250.

Seawater challenge tests (Clarke and Blackburn 1978) during the two ATPase peak periods caused no mortality and revealed similar plasma Na⁺ concentrations (Table 2). Control values of plasma Na⁺ from steelhead not exposed to seawater averaged 160 meq/L, somewhat lower than those of Table 2. When the seawater challenge test was performed with nonsmolting steelhead between January and September, as well as before the first ATPase peak, high mortality resulted; plasma Na⁺ values were above 220 meq/L for the survivors.

Discussion

In the present study, smolting was monitored physiologically by the increase in gill Na⁺,K⁺-ATPase activity (Zaugg 1981, 1989), which showed that approximately 30% of the steelhead population—the larger fish—smolted after 8 months of rearing under the photoperiod and temperature conditions applied. This was also reflected by seawater challenge tests during the first-spring peak in ATPase activity, in which the fish presented a high capacity for survival in seawater and a basal plasma sodium level after 24 h in seawater (Clarke et al. 1981; Morgan and Iwama 1991). Challenge tests brought similar results during the second ATPase peak, when fish were 20 months old. The ATPase peaks were similar in shape during the first and second springs. During the first spring, a narrow ATPase peak was observed for the largest specimens; this was probably due to the higher water temperatures registered during December of that year (Figure 1). It has

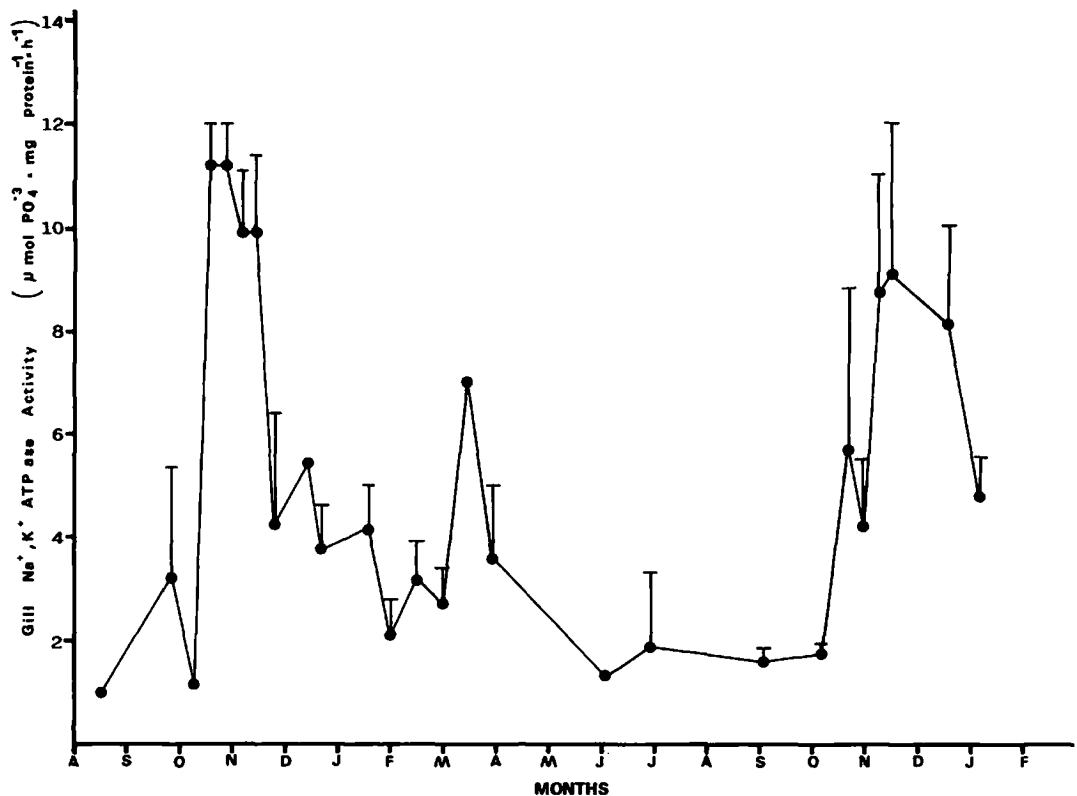


FIGURE 4.—Gill ATPase specific activity during 21 months (starting in August) of freshwater rearing of steelhead. Each value represents the mean + SE of two pools, each pool consisting of 3–5 fish.

TABLE 2.—Seawater challenge tests of steelhead.^a

Age of fish	Plasma Na ⁺ (meq/L) ^b	Na ⁺ ,K ⁺ -ATPase activity ^c	Mortality after 24-h sea- water test
8 months (first spring)	183.0 ± 1.1	11.2 ± 1.6	0
20 months (second spring)	175.0 ± 1.8	8.7 ± 4.6	0

^a The test was made at 10°C in 32‰ salinity, in November. Twenty specimens were placed in seawater for 24 h. After this period, plasma Na⁺ was measured individually for 10 fish selected at random. Steelhead 8 months old corresponded to larger fish of the cohort, measuring at least 10 cm.

^b Mean ± SE; N = 10.

^c Mean (±SE) ATPase activities on November 20 (Figure 4). Activity units are $\mu\text{mol PO}_4(\text{mg protein})^{-1}\cdot\text{h}^{-1}$.

been demonstrated that an increase in water temperature can reverse the smolting process in steelhead (Adams et al. 1975). In December, water reached temperatures above 13°C, which could explain the rapid decline in ATPase activity; thermal inhibition of smolting might have ended the second ATPase peak as well.

If fish did not reach 10 cm in their first spring, they did not smolt until the second spring (Figure 4). In addition, smaller fish in their first spring did not present morphological signs of smolting, such as silvering and decreased condition factor (Table 1; Johnsson and Clarke 1988). Thus, in order to reduce the freshwater rearing period in Chile by 6 months to attain rearing times common in the Northern Hemisphere, growth during the first 7 months of culture must be accelerated so that most of the population reach the minimum smolting size (10 cm). This is not difficult to do in southern Chile, where water temperatures are favorable for salmonid culture. Mean winter river temperatures are 8–10°C (Campos et al. 1986), and several lakes in this geographical area have winter temperatures 2–3°C higher than those of the rivers. Thus, it is possible to accelerate fish growth with an early transfer to a lake, allowing fish to smolt in the first spring. Otherwise, they will not smolt until the following spring, when they will have reached a fork length of about 20 cm and a weight of 100 g.

We suggest that lakes be used to accelerate steelhead growth, but not when water temperatures exceed 13°C, because smolting is reversed at this temperature threshold (Wedemeyer et al. 1980). Lakes in the south of Chile usually reach (or exceed) 13°C from November on (spring–summer).

For this reason, we propose the use of lakes for culture from April to September, after that steelhead must be returned to adjacent rivers or streams for smolting.

Chilean eggs (obtained from naturalized spawners) will be available in future years. Growth of naturalized fish should be delayed to obtain smolts of an appropriate size (40–50 g) in 12 months. Finally, it would be of interest to compare the culture of juveniles obtained from introduced and Chilean eggs to establish periods and sizes of smolting in relation to development in seawater, sizes at harvest, and occurrence of early maturity.

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References

- Adams, B. L., W. S. Zaugg, and L. R. McLain. 1975. Inhibition of salt water survival and Na-K-ATPase elevation in steelhead trout (*Salmo gairdneri*) by moderate water temperatures. Transactions of the American Fisheries Society 104:766–769.
- Blackburn, J., and W. C. Clarke. 1990. Lack of density effect on growth and smolt quality in zero-age coho salmon. Aquacultural Engineering 9:121–130.
- Boeuf, G. 1987. Bases physiologiques de la salmoniculture: le phénomène de la smoltification. Pisciculture Française 88:5–18.
- Campos, H., J. Arenas, W. Steffen, G. Agüero, L. Villalobos, and G. González. 1986. Investigación de la capacidad de cultivo de salmonídeos de las principales hoyas hidrográficas del país. Instituto de Fomento Pesquero, Corporación de Fomento de la Producción, Santiago, Chile.
- Clarke, W. C., and J. Blackburn. 1978. Seawater challenge tests performed on hatchery stock of chinook and coho salmon in 1977. Canada Fisheries and Marine Service Technical Report 761.
- Clarke, W. C., J. E. Shelbourn, and J. R. Brett. 1981. Effect of artificial photoperiod cycles, temperature, and salinity on growth smolting in underyearling coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*), and sockeye (*O. nerka*) salmon. Aquaculture 22:105–116.
- Clarke, W. C., J. E. Shelbourn, T. Ogasawara, and T. Hirano. 1989. Effect of initial daylength on growth, seawater adaptability and plasma growth hormone levels in underyearling coho, chinook, and chum salmon. Aquaculture 82:51–62.
- Claverie, I., and R. Méndez. 1990. Ovas de salmonídeos importadas: pasajeros VIP vuelan desde el hemisferio norte para el desarrollo salmónico chileno. Aquanoticias Internacional 2:20–25.

- Ernster, L., L. Zetter, R. Strom, and O. Lindberg. 1959. Method for the determination of trace phosphates in biological material. *Acta Chemica Scandinavica* 4:942-947.
- Folmar, L. C., and W. W. Dickhoff. 1981. Evaluation of some physiological parameters as predictive indices of smoltification. *Aquaculture* 23:309-324.
- Johnsson, J., and W. C. Clarke. 1988. Development of seawater adaptation in juvenile steelhead trout (*Salmo gairdneri*) and domesticated rainbow trout (*Salmo gairdneri*)—effects of size, temperature and photoperiod. *Aquaculture* 71:247-263.
- Lowry, O., N. Rosenbrough, A. Farr, and P. Randall. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193:265-275.
- Méndez, R. 1987. Desarrollo y estado de la situación actual de la salmonicultura en Chile. Pages 1-41 in *Perspectivas del cultivo del salmón en Chile*. Fundación Chile, Santiago.
- Morgan, J. D., and G. K. Iwama. 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 48:2083-2094.
- Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184.
- Uribe, J. C. 1988. Producción de alevines de salmón. Pages 67-93 in E. Uribe, editor. *Producción de larvas y juveniles de especies marinas*. Universidad del Norte, Coquimbo, Chile.
- Virtanen, E., and A. Soivio. 1985. The patterns of T3, T4, cortisol and Na⁺/K⁺-ATPase during smoltification of hatchery-reared *Salmo salar* and comparison with wild smolts. *Aquaculture* 45:97-109.
- Wedemeyer, G. A., R. L. Saunders, and W. C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. *Marine Fisheries Review* 42(6):1-14.
- Wedemeyer, G. A., R. L. Saunders, and W. C. Clarke. 1981. The hatchery environment required to optimize smoltification in the artificial propagation of anadromous salmonids. Pages 6-20 in L. J. Allen and E. C. Kinney, editors. *Proceedings of the bio-engineering symposium for fish culture*. American Fisheries Society, Fish Culture Section, Bethesda, Maryland.
- Zaugg, W. 1981. Advanced photoperiod and water temperature effects on gill Na⁺-K⁺ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 38:758-764.
- Zaugg, W. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 215-217.
- Zaugg, W. 1989. Migratory behaviour of underyearling *Oncorhynchus tshawytscha* and survival to adulthood as related to prerelease gill (Na⁺-K⁺)-ATPase development. *Aquaculture* 82:339-353.