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CHANGES IN GILL Na^+,K^+-ATPase, THYROXINE AND TRIIODOTHYRONINE OF COHO SALMON HELD IN TWO DIFFERENT REARING DENSITIES DURING SMOLTIFICATION

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Abstract—1. The effects of rearing density on the parr-smolt transformation in coho salmon were assessed by measurement of gill Na^+,K^+-ATPase activity and circulating levels of plasma thyroxine and triiodothyronine in two experiments.

2. There was an inverse relationship between rearing density and gill Na^+,K^+-ATPase activity between salmon reared at the different densities in both experiments. Gill Na^+,K^+-ATPase activity peaked in April of fish held at low densities in both experiments.

3. There was no consistent trend of differences in thyroid hormone levels between juvenile salmon at different rearing densities in the two experiments.

4. Peak elevations of thyroxine occurred in May in fish in both experiments concurrent with increases in water temperature.

INTRODUCTION

Coho salmon (*Oncorhynchus kisutch*) are anadromous and undergo two migrations during the life cycle. During the first migration, the juvenile fish undergo morphological and physiological changes referred to as smoltification prior to seaward migration (Hoar, 1976). Elevated plasma thyroxine (Dickhoff *et al.*, 1978, 1982; Nishikawa *et al.*, 1979) and gill Na^+,K^+-ATPase activity (Zaugg and McLain, 1970; Zaugg and Wagner, 1973; McCormick *et al.*, 1987) in salmon undergoing parr–smolt transformation have been well documented. Additionally, plasma cortisol has been reported to increase during this smoltification period (Specker and Schreck, 1982; Barton *et al.*, 1985; Patino and Schreck, 1986). The second migration occurs after 18 to 24 months at sea when the salmon return as adults to freshwater to complete their final maturation and spawning, after which they die.

Intensive fish culture of salmon is often aimed at optimizing production in terms of weight or number of fish that can be reared in a facility. Recent studies in the north west of North America have indicated that maximization of salmon production by high rearing densities may be deleterious to fish health and performance capacities, thus ultimately reducing the survival of juvenile salmon to adulthood. Research has demonstrated that high rearing densities can affect performance, growth rates (Sandercock and Stone, 1982; Fagerlund *et al.*, 1983, 1984, 1987), and normal physiological development of juvenile salmonids (Fagerlund *et al.*, 1981; Schreck *et al.*, 1985; Leatherland and Cho, 1985; Patino *et al.*, 1986).

Our objective was to determine the effects of rearing densities on coho salmon by measurement of gill Na^+,K^+-ATPase activity and plasma thyroxine and triiodothyronine in two different experiments in cold water hatcheries in the north east.

MATERIALS AND METHODS

Experimental Animals

Juvenile coho salmon were maintained under natural photoperiod in well water ranging from 5 to 16°C throughout the sampling period at New Hampshire Fish and Game Milford Hatchery, New Hampshire, U.S.A. Water flow rates were maintained under operating hatchery conditions and varied during the experimental period. Fish were fed commercial diets several times a day at a total ration of 0.5–3.1% of body weight per day, depending on body size and temperature.

Experiment 1 (*year* 1)

Juvenile coho salmon were reared at Milford Hatchery in fresh water for 15 months. Outdoor circular pools (8 m in diameter) were randomly stocked with a total of 45,000 salmon in May. The initial numbers of fish at the time of stocking were 15,000 fish/pond (low density) and 30,000 fish/pond (high density). In September, half of the fish from each pond were placed in two separate ponds replicating the treatments. The final rearing densities were as follows: high, 24.62 kg/m^3 (1.54 lbs/ft^3) and low, 13.91 kg/m^3 (0.87 lbs/ft^3). The high rearing density had been the normal rearing density at Milford Hatchery for coho salmon during the past several years.

Experiment 2 (*year* 2)

The juvenile salmon were reared for 15 months. Outdoor circular ponds were randomly stocked with 30,000 fish/pond

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Table 1. Experiment 1. Mean condition factor ± SE of coho salmon reared at high or low density. Condition factor was calculated as $w(g)/l^3$ (cm^3) × 100

Date	Density	Condition factor
Feb 15	High	1.10 ± 0.01
	Low	1.10 ± 0.01
Feb 29	High	1.07 ± 0.01
	Low	1.08 ± 0.01
Mar 14	High	1.05 ± 0.01
	Low	1.07 ± 0.01
Mar 28	High	1.03 ± 0.01
	Low	1.00 ± 0.01
Apr 11	High	0.97 ± 0.02
	Low	0.96 ± 0.01
Apr 25	High	0.92 ± 0.01
	Low	0.92 ± 0.01
May 9	High	0.92 ± 0.01
	Low	0.91 ± 0.02
May 22	High	0.94 ± 0.01
	Low	0.91 ± 0.01
June 6	High	0.95 ± 0.01
	Low	0.91 ± 0.01

or 15,000 fish/pond in May. In September, half of the fish from each pond were placed into two separate ponds replicating the treatments. The final rearing densities were as follows: high, 26.86 kg/m^3 (1.68 lbs/ft^3) or low, 13.75 kg/m^3 (0.86 lbs/ft^3).

In both experiments, from February until June, 20 fish from each pond were randomly sampled twice a month by use of a cast net. The fish were then sampled for fork length, weight, blood and gills. The fish were released into the Lamprey River for ocean migration in April. A few hundred fish from each of the four ponds were held back and placed into smaller tanks at similar densities.

Assays

Fish were not fed during the 24 hr period before sampling. Fish were stunned by a blow to the head and blood was collected immediately from the severed caudal blood vessels into lithium-heparinized tubes. Blood was centrifuged and plasma collected and stored at −20°C until analysed for thyroxine and triiodothyronine. Plasma thyroxine (T4) and triiodothyronine (T3) were measured by the method of Dickhoff *et al.* (1978) as modified by McCormick *et al.* (1987). Gill Na^+,K^+-ATPase activity was determined according to Zaugg (1982).

Data for hormone concentrations were analysed by a Student Newman-Keuls test after preliminary analysis of variance. In all tests, the level of significance for differing groups was $P < 0.05$.

Table 2. Experiment 2. Mean condition factor ± SE of coho salmon reared at high or low density. Condition factor was calculated as $w(g)/l^3$ (cm^3) × 100

Date	Density	Condition factor
Feb 13	High	1.09 ± 0.01
	Low	1.13 ± 0.01
Feb 27	High	1.13 ± 0.01
	Low	1.15 ± 0.01
Mar 13	High	1.05 + 0.01
	Low	1.08 ± 0.01
Mar 27	High	1.00 ± 0.03
	Low	1.05 ± 0.01
Apr 10	High	1.00 + 0.02
	Low	1.00 + 0.01
Apr 24	High	0.95 ± 0.02
	Low	0.91 ± 0.01
May 8	High	0.98 ± 0.01
	Low	0.96 ± 0.01
May 22	High	0.95 ± 0.01
	Low	0.94 ± 0.01

RESULTS

Condition factor

The data for replicate treatments were analyzed together since the data were not significantly different. Condition factor was calculated as $(g)/(cm)^3 \times 100$. There were no significant differences in condition factors between the fish reared at different densities in Experiments 1 or 2 (Tables 1 and 2). With respect to condition factor and morphological criteria (Sower *et al.*, 1984), stages of early smoltification were evident in April with most of the fish smolts by June. Temperatures (°C) generally were very low during January through April with increasing temperatures occurring in early May in Experiment 1 and in late April in Experiment 2 (Fig. 1).

Gill Na^+,K^+-ATPase: Experiments 1 and 2

Gill Na^+,K^+-ATPase activity peaked in April in fish held at low densities in both experiments. In contrast, fish reared at high density in Experiment 1 had a less pronounced, more gradual increase in gill Na^+,K^+-ATPase activity and did not decrease in late spring (Figs 2 and 3). Gill Na^+,K^+-ATPase activity levels were inversely related to rearing density in fish in both experiments during April when the fish were undergoing smoltification; gill Na^+,K^+-ATPase activity was significantly higher at this time in fish held at low densities. Gill Na^+,K^+-ATPase activity increased markedly and significantly between 15 February–25 April in Experiment 1 (2.5-fold increase) and between 13 February–8 May in Experiment 2 (1.8-fold increase).

Thyroxine, triiodothyronine: Experiment 1

Plasma thyroxine changed significantly ($P < 0.05$) through time in fish reared at low and high densities (Fig. 2). Plasma triiodothyronine also varied significantly ($P < 0.05$) through time in fish reared at low densities (Fig. 2). Thyroid hormone levels were significantly higher in fish reared at low densities

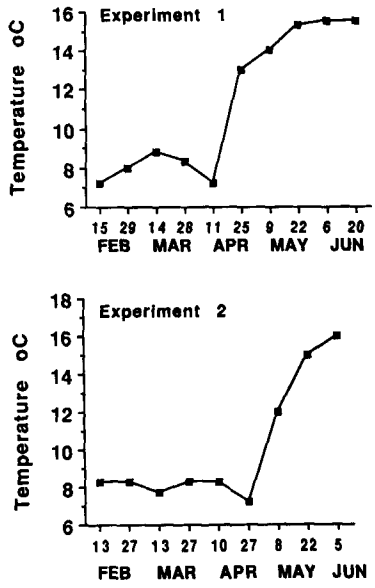

Fig. 1. Water temperature profile (°C) in Experiment 1 and Experiment 2.

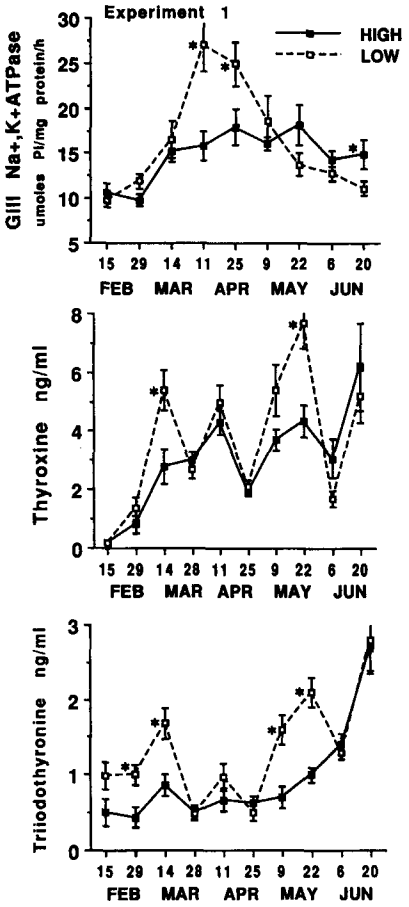

Fig. 2. Gill Na^+,K^+-ATPase activity (μmoles Pi/mg protein/hr), plasma thyroxine and triiodothyronine in coho salmon reared at high or low density in Experiment 1 through time. Each point represents the mean $\pm$ SE ($n = 10$). *Denotes significance ($P < 0.05$) between rearing densities at same date.

at certain times compared to those reared at high densities: plasma thyroxine levels were significantly higher in salmon reared at low densities than high densities on 14 March, 9 and 22 May (Fig. 2) and plasma triiodothyronine levels were significantly higher in salmon reared at low densities than high densities on 15 and 29 February, 14 March, 11 April, 9 and 22 May (Fig. 1).

Thyroxine, triiodothyronine: Experiment 2

Plasma thyroxine levels of fish reared at high and low densities fluctuated significantly ($P < 0.05$) through time with elevations occurring 10 and 24 April and 22 May (Fig. 2). Plasma triiodothyronine also fluctuated significantly ($P < 0.05$) through time in fish reared at low and high densities with elevations occurring on 27 February, 24 April and 5 June (Fig. 2). Except for two sampling times (13 February and 22 May) plasma thyroxine did not differ between fish reared at low or high densities (Fig. 2). Plasma triiodothyronine levels did not differ between fish reared at low or high densities (Fig. 2).

DISCUSSION

The results of this study show a clear inverse relationship between rearing density and increases in gill Na^+,K^+-ATPase activity during smoltification, a finding similar to that of Schreck *et al.* (1985). In other studies high rearing densities have been shown to affect performance and growth rates (Sandercock and Stone, 1982; Fagerlund *et al.*, 1983, 1984, 1987) and normal physiological development of juvenile salmonids (Fagerlund *et al.*, 1981; Schreck *et al.*, 1985; Leatherland and Cho, 1985; Patino *et al.*, 1986). Fagerlund *et al.* (1981) reported that at high population densities, smoltification of small fish may be retarded to a greater extent than that of large fish. In addition to finding an inverse relationship between rearing density and plasma thyroid hormone levels, Leatherland and Cho (1985) reported an inverse relationship between rearing density and plasma cortisol in rainbow trout, *Salmo gairdneri*. Patino *et al.* (1986) reported that rearing density affected plasma cortisol levels by altering the submaximal stimulation of interrenal cells in smolting coho

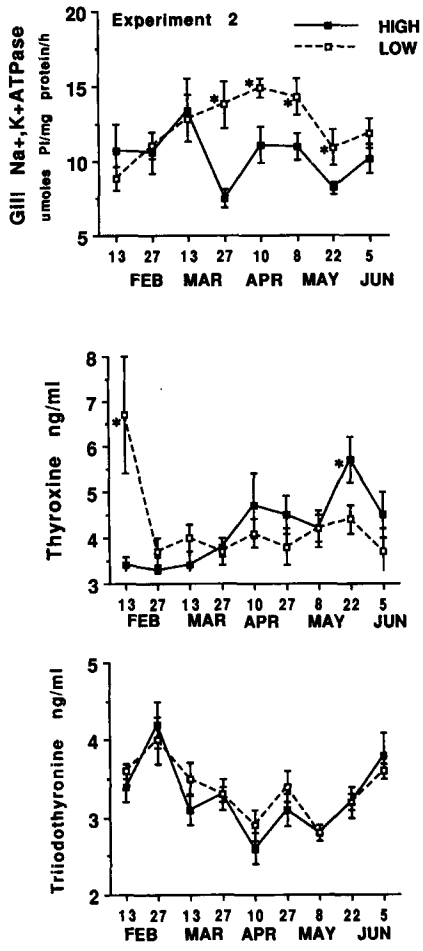

Fig. 3. Gill Na^+,K^+-ATPase activity (μmoles Pi/mg protein/hr), plasma thyroxine and triiodothyronine in coho salmon reared at high or low density in Experiment 2 through time. Each point represents the mean $\pm$ SE ($n = 10$). *Denotes significance ($P < 0.05$) between rearing densities at same date.

salmon. However, in Fagerlund *et al.* (1983) and Schreck *et al.* (1985) coho salmon held under varying densities did not effect plasma cortisol concentrations or interrenal cell nuclear diameter.

Increases in thyroid hormones (Dickhoff *et al.*, 1978, 1982; Nishikawa *et al.*, 1979; McCormick *et al.*, 1987; Young *et al.*, 1989) and gill Na^+,K^+-ATPase activity (Zaugg and McLain, 1970; Zaugg and Wagner, 1973; McCormick *et al.*, 1987) in salmon undergoing the parr–smolt transformation have been well documented. Leatherland and Cho (1985) and Schreck *et al.* (1985) have demonstrated in rainbow trout and coho salmon, respectively, higher thyroxine levels associated with fish reared at lower densities (135 g/l and 0.86 lbs/ft^3, respectively) compared to fish reared at high densities (277 g/l and 2.95 lbs/ft^3, respectively). Our studies, which include more sampling periods over a longer period of time than previous studies, did not demonstrate consistent differences in plasma thyroid hormone levels between juvenile salmon at different rearing densities. Although, in the present study, the high density used averaged 1.61 lbs/ft^3 which was not as high as the densities used in Schreck *et al.* and Leatherland and Cho's studies. The reason for the lack of effect of density on thyroid hormone levels in this study may be due to different high densities. However, in Schreck *et al.* (1985), plasma thyroxine was only measured once, at the time of release in the spring. Similar to the study of Schreck *et al.* (1985), plasma thyroxine was significantly elevated in fish reared at low density compared to fish reared at high density in early May. In the first experiment, plasma thyroxine and triiodothyronine were generally higher in fish reared at low density compared to high density although this pattern was not repeated in the second experiment. In both experiments, noted increases of plasma thyroxine were associated with increases in water temperature in May.

In this study there were no significant differences in condition factor in coho salmon reared at the high versus the low density. In all fish, condition factor decreased during smoltification which is a common occurrence noted in other salmonids during this period (Fagerlund *et al.*, 1987; McCormick and Saunders, 1987). There was a noted variation in plasma hormone levels and weights in the salmon between the two experiments. This is similar to other studies which have shown a marked variation in thyroid hormone levels reported by the same author as well as different authors in studies on salmonids (Dickhoff *et al.*, 1978, 1982; Folmar and Dickhoff, 1981; Milne and Leatherland, 1980). In only one of the two years of experiments of Fagerlund *et al.* (1983), weight and length were inversely correlated with rearing density in coho salmon which is similar to the present study where only weights were significantly higher in fish reared at low density compared to fish reared at high density in Experiment 1 but not in Experiment 2.

There are many factors other than rearing densities that may also influence physiological responses including water flow, water quality, feeding regimes and nutrition. In the present study, coho salmon were fed fish diets based on a per cent body weight compared to studies of Schreck *et al.* (1985) and Leatherland and Cho (1983) whose fish were fed to satiation. Limiting feed in salmonids has been shown to reduce plasma thyroid hormone levels (Leatherland *et al.*, 1977; Eales, 1979; McCormick and Saunders, in press). Systematic studies using similar feeding regimes and diets, rearing densities, and carrying capacity under similar photoperiod and temperature conditions are necessary to fully evaluate the influence of rearing density on physiological parameters in salmonids.

In summary, a clear inverse relationship between rearing density and gill Na^+,K^+-ATPase activity was demonstrated in both experiments suggesting that certain developmental processes during smoltification are influenced by rearing densities.

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