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Seasonal variations in white muscle and kidney carbohydrate metabolism in domesticated rainbow trout (*Oncorhynchus mykiss* Walbaum)

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Abstract

SOENGAS, J.L., ANDRÉS, M.D. & ALDEGUNDE, M. (1992). Seasonal variations in white muscle and kidney carbohydrate metabolism in domesticated rainbow trout (*Oncorhynchus mykiss* Walbaum). *Nova Acta Científica Compostelana (Bioloxía)*, **3**: 169-179

An assessment of levels of glycogen, glucose and protein contents and the activities of glycogen phosphorylase (GPase), glycogen synthetase (GSase), fructose 1,6-bisphosphatase (FBPase) and hexokinase (HK) was carried out during 1990 in the white muscle and kidneys of domesticated rainbow trout (*Oncorhynchus mykiss*), reared in freshwater, to determine metabolic changes related to variations in the activity of gill Na^+/K^+-ATPase. Glycogen in white muscle and kidney appears not to be involved in metabolic changes related to ATPase activity since no noticeable changes in levels were detected. Activities of GPase and GSase activity and levels of protein in both white muscle and kidney did not show any variations related to the activity of the ATPase. HK activity and levels of free glucose in both white muscle and kidney were lowest in spring and summer. Of all parameters analyzed, only the change in FBPase activity in the kidney seems to be related to the increase in ATPase activity since peak activity was detected when the increase in ATPase activity ocurred. In contrast to the results obtained in liver, these results suggest that changes in carbohydrate metabolism in kidney and white muscle are not related to the increase in gill Na^+/K^+-ATPase activity.

Key words: Rainbow trout, white muscle, kidney, glycogen, glucose, glycolysis, gluconeogenesis, seasonal changes, smoltification.

Resumen

SOENGAS, J.L., ANDRÉS, M.D. & ALDEGUNDE, M. (1992). Variaciones estacionales en el metabolismo de los carbohidratos de músculo blanco y riñón de trucha arco iris doméstica (*Oncorhynchus mykiss* Walbaum). *Nova Acta Científica Compostelana (Bioloxía)*, **3**: 169-179

Durante 1990 se realizó, cara a relacionarlas con las variaciones estacionales de la actividad Na^+/K^+-ATPasa branquial, el seguimiento en una población de trucha arco iris doméstica *(Oncorhynchus mykiss)* de los niveles de glucógeno, glucosa y proteína así como de las actividades de los enzimas glucógeno fosforilasa (GPase), glucógeno sintetasa (GSase), fructosa-1,6-bifosfatasa (FBPase) y hexoquinasa (HK) tanto en músculo blanco como en riñón. Ni en músculo blanco ni en riñón se observan variaciones en los niveles de glucógeno y en la actividad de los enzimas implicados en su metabolismo que pudieran relacionarse con las variaciones en la actividad ATPasa. Los niveles de glucosa y la actividad HK son menores en primavera y verano tanto en músculo como en riñón. De todos los parámetros analizados, únicamente los cambios en la actividad FBPase renal pueden tener relación con la actividad ATPasa dado que se observa un incremento a la par de actividad. En contraste con los resultados obtenidos en hígado parece que, en general, los cambios que existen en el metabolismo de carbohidratos de músculo y riñón no están relacionados con las variaciones en la actividad ATPasa.

Palabras clave: Trucha arco iris, músculo blanco, riñón, glucógeno, glucosa, glucolisis, gluconeogénesis, cambios estacionales, esmoltificación.

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INTRODUCTION

Smoltification in salmonids (BARRON, 1986; HOAR, 1988), has not been examined in detail in terms of the metabolic changes that occur during the process itself and the subsequent transfer to seawater (MCCORMICK & SAUNDERS, 1987). In addition, most studies have been performed on liver, with few studies dealing with other tissues (KIESSLING *et al.*, 1991a,b; SOENGAS *et al.*, 1993a).

With respect to tissues other than the liver, the muscle has been the most frequently used for assessment of metabolic changes that occur in various physiological processes in fish, for example, smoltification. There is disagreement in the literature about muscle glycogen and its relationship to smoltification. SWEETING & MCKEOWN (1989) detected a decrease in muscle levels of glycogen in smoltificant coho salmon, whereas WOO *et al.* (1978) did not find differences in muscle levels of glycogen between parrs and smolts of the same species. By contrast, a depletion of muscle lipids is clearly evident during smoltification of several species (Sheridan *et al.*, 1985; SHERIDAN, 1988). The fish kidney is an important gluconeogenic tissue (MOMMSEN *et al.*, 1985; SUÁREZ & MOMMSEN, 1987), as well as one of the more important osmoregulatory organs (RANKIN *et al.*, 1983), and there is also little available information about metabolic changes in the kidney during smoltification and adaptation to seawater. Only the activity of respiratory enzymes in kidneys of coho salmon smolts (MCCORMICK *et al.*, 1989a) has been evaluated.

Domesticated rainbow trout (*Oncorhynchus mykiss*), reared in freshwater, although do not naturally migrate to seawater, exhibit changes in activity of osmoregulatory enzymes (REY *et al.*, 1990, 1991; FUENTES *et al.*, 1991) and hormone levels (REY *et al.* 1992) during the year, as well as metabolic changes during adaptation not only to diluted seawater (MORGAN & IWAMA, 1991; SOENGAS *et al.*, 1991) but also to seawater (KIESSLING *et al.*, 1991a,b; SOENGAS *et al.*,1993a,b), which are quite similar to those observed in anadromous salmonids (WOO *et al.*, 1978; HOAR, 1988). In addition, our previous study of domesticated rainbow trout (SOENGAS *et al.*, 1992a) demonstrated changes in liver carbohydrate metabolism that were related to the activity of gill Na^+/K^+-ATPase. In the present study we examined changes in carbohydrate metabolism in white muscle and kidney of domesticated rainbow trout reared in freshwater, in order to assess a possible relationship to changes in the activity of gill Na^+/K^+ -ATPase, which is used as an index of smoltification in anadromous salmonids (ZAUGG & MCLAIN, 1972; HOAR, 1988).

MATERIALS AND METHODS

Animals and experimental design

The experimental design, the source of animals and the sampling conditions were the same as those previously described (SOENGAS *et al.*, 1992a). Briefly, domesticated rainbow trout *(O. mykiss)* âged more than one year and weighing 150 g at the beginning of the year and about 300 g at the end of sampling period were biweekly sampled at the same time of day (11 a.m.), in order to avoid interferences in the measured parameters, in a commercial hatchery in Soutorredondo (Noia, Galicia). Fish were fed three times daily with manufactured dry pellets (1.5-2% of body wt/day) and maintained under natural conditions of temperature (from 8 °C in January to 20 °C in August) and photoperiod. On each sampling date, 10 fish were captured and killed by a sharp blow on the head. The kidney and a portion of white muscle, taken from the thick part of the muscle in front of the dorsal fin and just dorsal to the lateral line, in order to avoid differences among sample sites (KIESSLING *et al.*, 1990), were removed quickly, weighed, frozen on dry ice and stored at -80°C until further assay.

Analytical procedures

The assessment of parameters in white muscle and kidney was performed as described previously (SOENGAS *et al.*, 1991, 1992a). Methods were used after slight modifications of methods described in literature such as: KEPPLER & DECKER (1974) for tissue glycogen and glucose, LOWRY *et al.* (1951) for protein levels, MORATA *et al.* (1982a) for GPase activity, SHERIDAN *et al.* (1985) for GSase activity, VILLANUEVA & MARCUS

(1974) for FBPase activity and FIDEU *et al.* (1983) for HK activity. Enzyme assays were always carried out at maximun rates, being the reactions mixture and the protein concentration of crude homogenates established in preliminary experiments.

Tissue levels of glucose, glycogen and protein are expressed as mg/g wet weight whereas enzyme activities are expressed as μmol (substrate used or product formed)·min^{-1}·g wet weight^{-1}.

Data analyses

Differences between means, among sampling dates, for each parameter measured were assessed by one-way Anova followed by Student-Newman Keuls' multiple-range test. Group variance homogeneity was assesed using the and Bartlett-Box test. All analysis were performed with the statistical package SPSS/PC+.

RESULTS

The results are shown in figures 1 through 7. The activity of gill Na+/K+-ATPase was higher between July 20 and September 17 with a maximum on August 20 (SOENGAS *et al.*, 1992a). The correlations between gill Na^+/K^+-ATPase activity and the remaining parameters gave the following results: positive for kidney glycogen ($r= 0.235$, $P<0.001$), muscle protein ($r= 0.324$, $P<0.001$), kidney FBPase activity ($r= 0.404$, $P<0.001$) and negative for muscle levels of glucose ($r=0.323$, $P<0.001$), kidney levels of glucose ($r= -0.273$, $P<0.001$) and kidney HK activity ($r= -0.222$, $P<0.01$).

White muscle levels of glycogen (Fig. 1) did not show significant changes among samplings, although the lowest levels were found between May 25 and June 8 and coincided with the highest liver levels of glycogen (SOENGAS *et al.*, 1992a). No significant changes were detected in

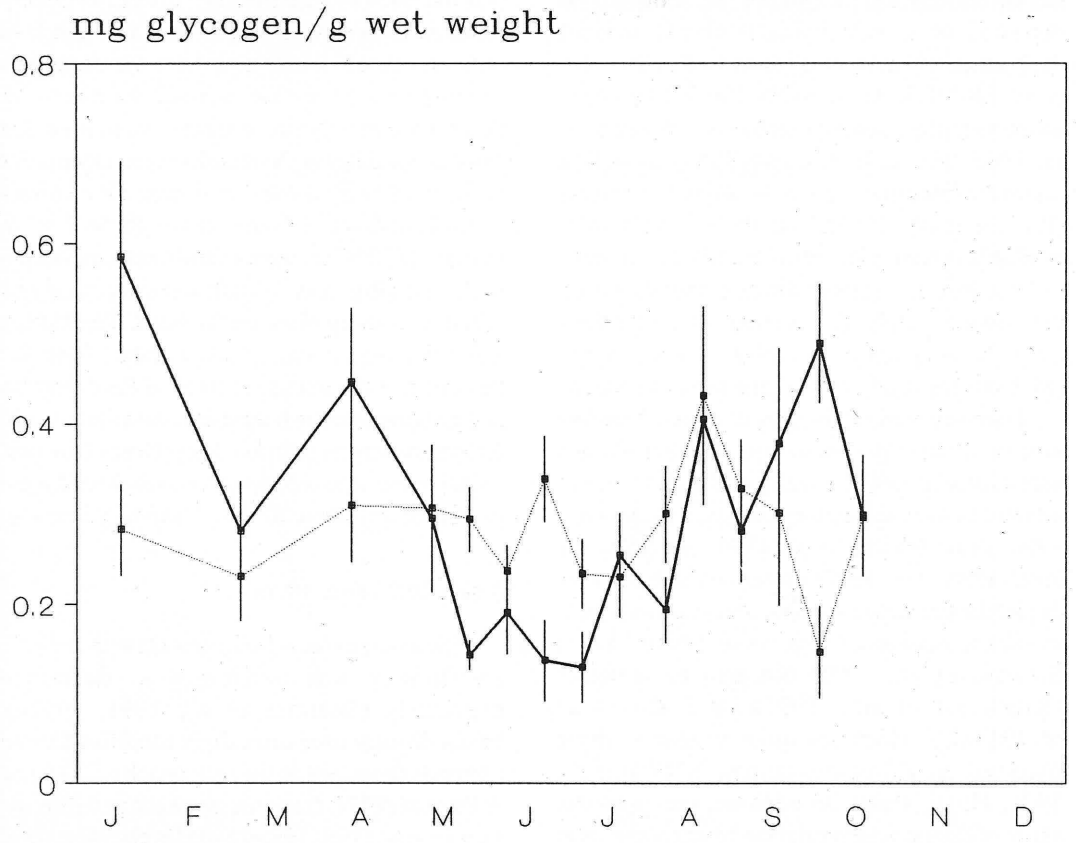

Fig. 1. Seasonal variations in levels of glycogen in white muscle (bold line) and kidney (dotted line) during 1990. Data presented are the means ± S.E.M. of results from 10 fishes.

kidney levels of glycogen (Fig. 1). Tissue levels of glucose displayed a similar pattern in both white muscle and kidney (Fig. 2) with higher values in fall and winter than in spring and summer. Thus, muscle and kidney levels of glucose were positively correlated ($r= 0.745$, $P<0.001$). Although muscle levels of protein (Fig. 3) tended to be higher between August 6 and September 17, the variations observed were not significant. With regard to kidney levels of protein (Fig. 3), the lower levels were found in June-July and October, but the differences from values at other samplings dates were small.

An increase in the muscle activity of GPase a (Fig. 4) was detected during spring and summer coinciding with the lower levels of muscle glycogen. Moreover, the activity of muscle GPase a+b displayed a slight decline over the same time period as that associated with an increase in the amount of GPase a as a percentage of the total GPase activity (data not showed). In contrast to the results obtained for GPase activity in muscle, no significant changes among samplings were obtained for activity of kidney GPase a (Fig. 4). Most strikingly, the activity of muscle GSase (Fig. 5) was higher when muscle levels of glycogen seemed to be lower, as observed in the case of kidney GSase activity (Fig. 5), although in GSase activity the observed differences were small for the kidney.

Changes in FBPase activity were different in muscle and kidney (Fig. 6). The activity of muscle FBPase did not show significant changes during the year, whereas that in the kidney tended to be higher in August and September. This increase coincides not only with a similar result obtained for the activity of liver FBPase but also with the increase observed in the activity of the gill Na^+/K^+-ATPase (Soengas *et al.*, 1992a). Levels of HK activity showed similar variations in muscle and kidney (Fig. 7) with higher activity recorded at the beginning and the end of the sampling period.

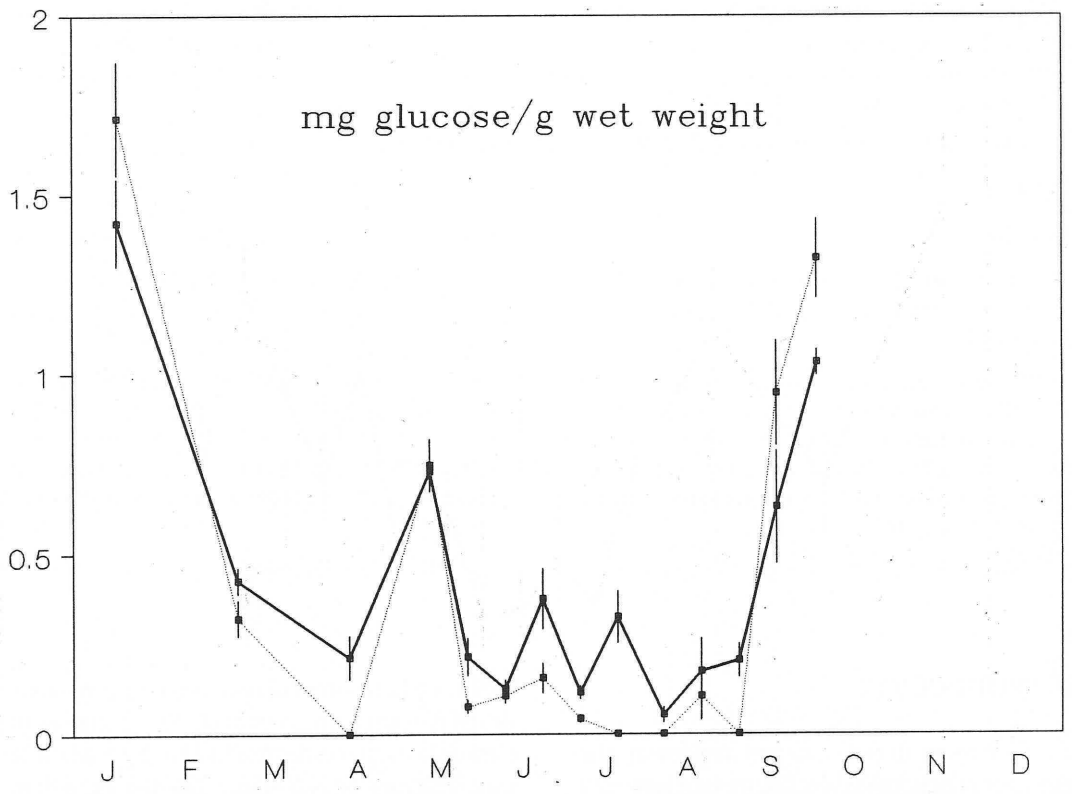

Fig. 2. Seasonal variations in levels of glucose in white muscle (bold line) and kidney (dotted line). The time axis is the same as in Fig. 1. Data presented are the means ± S.E.M. of results from 10 fishes.

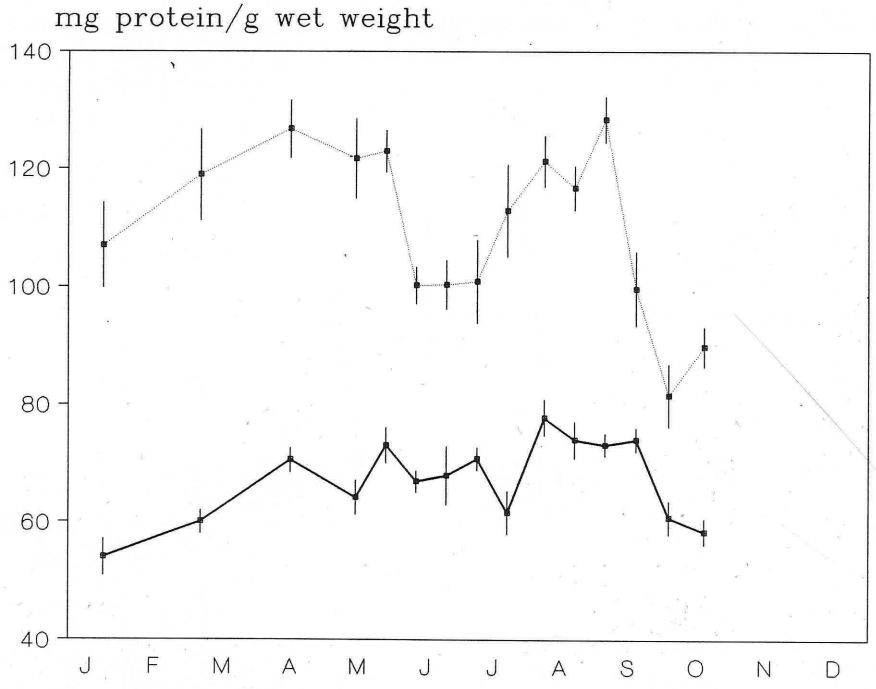

Fig. 3. Seasonal variations in levels of protein in white muscle (bold line) and kidney (dotted line). The time axis is the same as in Fig. 1. Data presented are the means ± S.E.M. of results from 10 fishes.

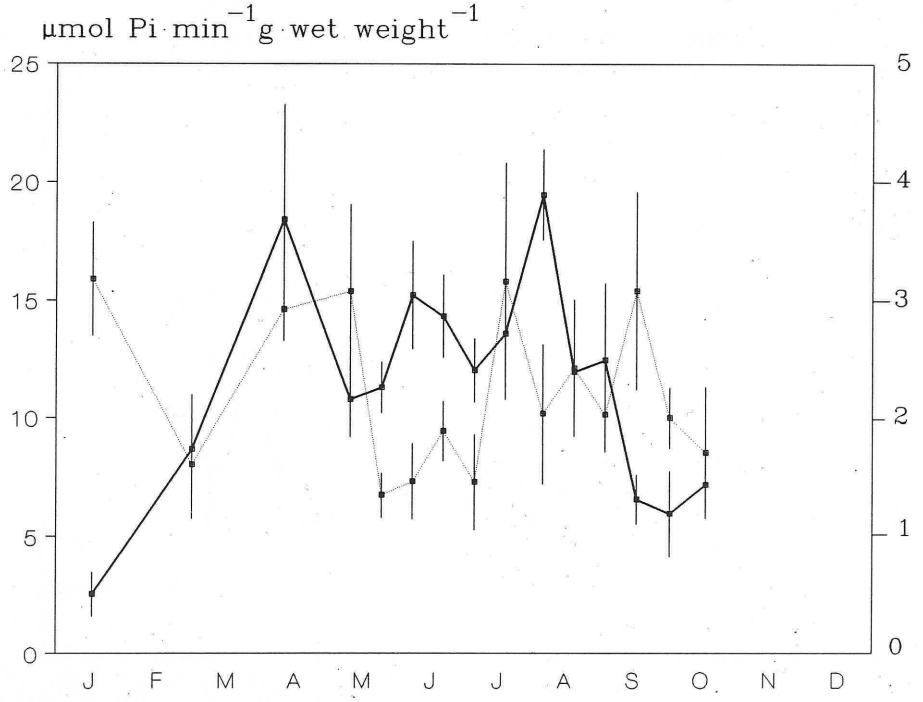

Fig. 4. Seasonal variations in glycogen phosphorylase activity in white muscle (bold line) and kidney (dotted line). Left axis is for activity in white muscle whereas right axis is for activity in kidney. The time axis is the same as in Fig. 1. Data presented are the means ± S.E.M. of results from 10 fishes.

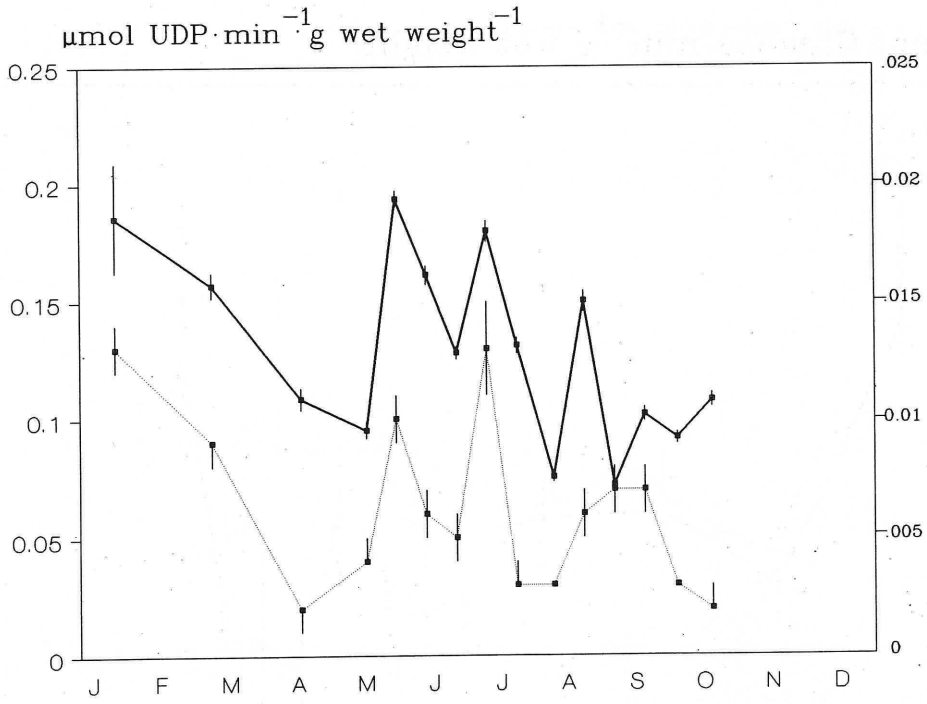

Fig. 5. Seasonal variations in glycogen synthetase activity in white muscle (bold line) and kidney (dotted line). Left axis is for activity in white muscle whereas right axis is for activity in kidney. The time axis is the same as in Fig. 1. Data presented are the means ± S.E.M. of results from 10 fishes.

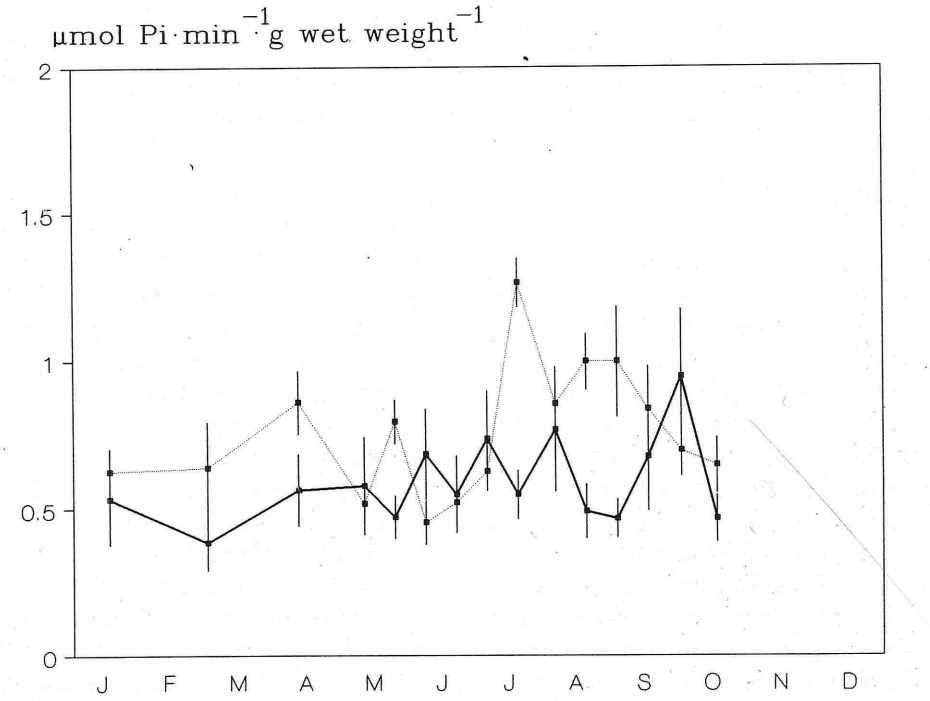

Fig. 6. Seasonal variations in fructose-1,6-biphosphatase activity in white muscle (bold line) and kidney (dotted line). The time axis is the same as in Fig. 1. Data presented are the means ± S.E.M. of results from 10 fishes.

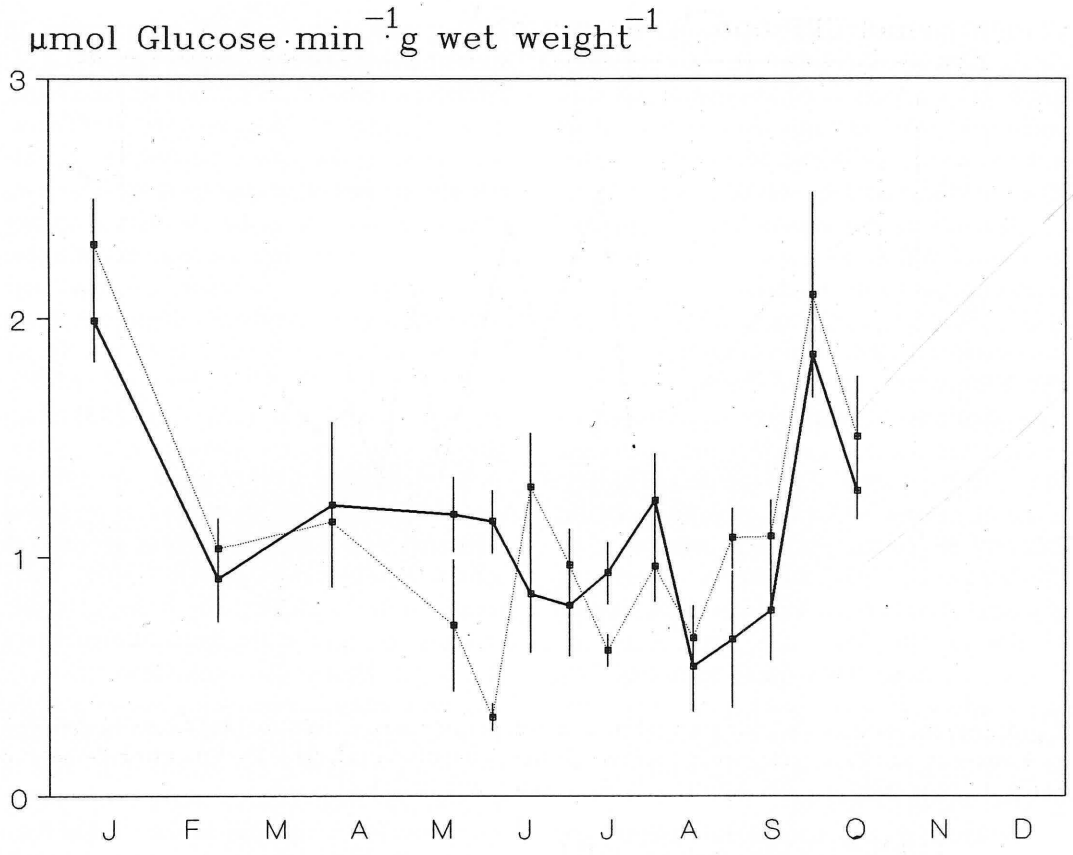

Fig. 7. Seasonal variations in hexokinase activity in white muscle (bold line) and kidney (dotted line). The time axis is the same as in Fig. 1. Data presented are the means ± S.E.M. of results from 10 fishes.

DISCUSSION

There are few available and updated studies in the literature about seasonal changes in white muscle levels of glycogen in fish (Ottolenghi *et al.*, 1981; Kiessling *et al.*, 1991a). We found a slight decrease in white muscle levels of glycogen prior to the increase in the activity of gill Na^+/K^+-ATPase (Soengas *et al.*, 1992a). This result seems to indicate that, in contrast to liver glycogen (Soengas *et al.*, 1992a), muscle glycogen is not stored for use when ATPase activity is increased. Likewise, this result disagrees with the decline reported in muscle glycogen by Sweeting & McKeown (1989) during the smoltification of coho salmon, but it coincides with data from Woo *et al.* (1978) who did not find differences in muscle levels of glycogen between coho salmon parrs and smolts. The variations in muscle levels of glycogen might be explained by the observed increase in GPase activity, which in the muscles of rainbow trout is responsible for breakdown of glycogen (Morata *et al.*, 1982a). However, the observed decrease disagrees with the high GSase activity detected over the same time period.

There are no reports available in which the role of kidney glycogen in the metabolic changes that occur during development of hypoosmoregulatory ability is examined. Our data show that kidney levels of glycogen, do not change significantly related to gill Na^+/K^+-ATPase activity. This result disagrees with the changes observed in the activity of kidney respiratory enzymes in *Salmo salar* during smoltification (McCormick *et al.*, 1989b), but it coincides with the lack of changes observed in the said enzyme activities during transfer to seawater of the same species (McCormick *et al.*, 1989a). In addition,

variations in the activities of kidney GPase and GSase did not exactly reflect changes in kidney levels of glycogen since this parameter was unchanged in June and July when a lower GPase activity, coincident with a recovery of higher levels of GSase activity, was detected.

Both kidney and muscle levels of glucose were lowest during spring and summer, in marked contrast to the result obtained in liver (SOENGAS *et al.*, 1992a) where maximum levels of glucose were found at that time. Unfortunately, we cannot compare our data with those of others since there are no data in the literature about seasonal changes in the levels of free glucose in tissues other than liver. The recovery of white muscle and kidney levels of glucose in October coincides with the recovery of plasma glucose in rainbow trout (SOENGAS *et al.*, 1992a), the increase observed in glycemia also occurred during smoltification of several species (VIRTANEN, 1987; AUDET & CLAIREAUX, 1992). Thus, the increase observed in HK activity in both white muscle and kidney could be due to a higher use of exogenous glucose in those tissues producing increased levels of glucose within the tissues.

Muscle levels of protein showed no significant changes related to gill Na^+/K^+-ATPase activity. The absence of changes is similar to other studies performed in coho salmon, which have not shown changes in muscle levels of protein during smoltification (WOO *et al.*, 1978; SWEETING & MCKEOWN, 1989). Thus, the mobilization of muscle protein appears to be unnecessary during the metabolic changes that occur during the increase in gill Na^+/K^+-ATPase activity, in contrast to other physiological processes, for example the spawning migration of anadromous salmonids (MOMMSEN *et al.*, 1980; ANDO *et al.*, 1986). In addition, kidney levels of protein displayed two clear decreases, the first prior to the increase in ATPase activity and the second inmediately after the decline in ATPase activity. The meaning of these results is unclear at this time.

Assessments of activities of key enzymes of glycolysis and/or gluconeogenesis in kidney and/or muscle have been mainly restricted to reproductive processes (MOMMSEN *et al.*, 1980; COUTURE & GUDERLEY, 1990; SOENGAS *et al.*, 1993c) and studies related to nutrition or starvation (MORATA *et al.*, 1982b; LUPIÁÑEZ *et al.*, 1989; KIESSLING *et al.*, 1990). In the present study, the activity of two key enzymes, HK and FBPase, was assessed. The pattern displayed by muscle HK activity paralleled changes in muscle levels of glucose. In contrast, muscle FBPase activity did not show any significant changes during the year. Therefore, the variations obtained here with respect to muscle glycolytic/gluconeogenic flux do not appear to be related to changes observed in gill Na^+/K^+-ATPase activity. The performance of the kidney, an important gluconeogenic tissue (MOMMSEN *et al.*, 1985; SUÁREZ & MOMMSEN, 1987), seems to be different since FBPase activity showed an increase that coincided with a similar increase obtained in activity in the liver (SOENGAS *et al.*, 1992a). These results seem to support the hypothesis that changes observed in the hypoosmoregulatory ability of domesticated rainbow trout are accompanied by an increase in gluconeogenesis. This increased gluconeogenesis had been previously proposed during smoltification in several species (GUILLAUME *et al.*, 1984; VIRTANEN, 1987) and may be due, as has been suggested by PLISETSKAYA *et al.* (1988) to a fall in plasma insulin levels once smoltification finished. Thus, in our case, a peak and a subsequent decline in plasma insulin levels could explain not only the changes observed in liver glycogenolysis and gluconeogenesis (SOENGAS *et al.*, 1992a) but also a possible increase in renal gluconeogenesis. However, some of the changes observed in metabolic parameters can be also atributted to changes in levels of those hormones involved in both carbohydrate metabolism and the control of hypoosmoregulatory ability in rainbow trout (MATTY, 1985; MOMMSEN & PLISETSKAYA, 1991; BERN & MADSEN, 1992; SOENGAS *et al.*, 1992b).

In summary, in contrast to the results obtained with liver (SOENGAS *et al.*, 1992a), white muscle and kidney do not appear to be mainly involved in the changes in carbohydrate metabolism that are related to the increase in gill Na^+/K^+-ATPase activity in domesticated rainbow trout. However, a possible role of carbohydrate metabolism in kidney needs further evaluation.

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