# Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*)

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**Abstract**: The effects of water temperature (5, 8, 11, and 14°C) on the fractional rate (percent per day) of protein consumption ($k_r$) and on white muscle and whole-body fractional rates of protein synthesis ($k_s$), protein growth ($k_g$), and growth efficiency (PPV, growth/consumption; $k_g/k_s$, growth/synthesis) of juvenile Atlantic wolffish (*Anarhichas lupus*) (initial body weight 26 g) were studied. Rates of protein consumption and white muscle and whole-body protein synthesis increased in a linear fashion between 5 and 14°C. In contrast, the relationships between temperature and white muscle and whole-body protein growth, protein growth efficiency (PPV) and protein synthesis retention efficiency ($k_g/k_s$) were parabolic. The results indicated that the optimum water temperatures for growth ($T_{opt.G}$) and growth efficiency ($T_{opt.GE}$) were 10–11 and 9–10°C, respectively. The maximum white muscle and whole-body protein growth rates recorded at $T_{opt.G}$ were 0.9 and 0.7 %·day$^{-1}$, respectively. At $T_{opt.GE}$, the maximum white muscle and whole-body PPV values were 28 and 34%, respectively, and $k_g/k_s$ values were 92 and 51%, respectively. The growth performance data for juvenile Atlantic wolffish in comparison with published data for salmonids (rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*)) at 9–11°C further highlight its suitability as an alternative species for cold-water aquaculture in northern Europe and Atlantic Canada.

**Résumé** : Nous avons étudié les effets de la température de l'eau (5, 8, 11 et 14°C) sur le taux fractionnel (pour cent par jour) de la consommation de protéines ($k_r$) et sur les taux fractionnels de la synthèse de protéines ($k_s$), de la croissance protéinique ($k_g$) et de l'efficacité de la croissance (PPV, croissance/consommation; $k_g/k_s$, croissance/synthèse) dans les muscles blancs et le corps entier de loups atlantiques (*Anarhichas lupus*) (poids corporel initial de 26 g) juvéniles. Les taux de consommation de protéines et la synthèse de protéines dans les muscles blancs et le corps entier se sont accrus linéairement entre 5 et 14°C. Par ailleurs, les relations entre la température et la croissance protéinique dans les muscles blancs et le corps entier, l'efficacité de la croissance protéinique (PPV) et l'efficacité du maintien de la synthèse de protéines ($k_g/k_s$) étaient paraboliques. Les résultats ont indiqué que les températures de l'eau optimales pour la croissance ($T_{opt.G}$) et l'efficacité de la croissance ($T_{opt.GE}$) étaient de 10–11 et de 9–10°C, respectivement. Les taux de croissance protéinique maximaux dans les muscles blancs et le corps entier enregistrés à $T_{opt.G}$ étaient de 0,9 et 0,7%·jour$^{-1}$, respectivement. À $T_{opt.GE}$, les valeurs maximales de PPV dans les muscles blancs et le corps entier étaient de 28 et 34%, respectivement, et les valeurs de $k_g/k_s$ étaient de 92 et 51%, respectivement. Les données sur la performance de la croissance chez les loups atlantiques juvéniles en comparaison des données publiées pour les salmonidés (truite arc-en-ciel (*Oncorhynchus mykiss*), saumon atlantique (*Salmo salar*)) à 9–11°C témoignent de la valeur de cette espèce à titre d'espèce de remplacement pour l'aquaculture en eau froide dans le nord de l'Europe et au Canada atlantique.

[Traduit par la Rédaction]

## Introduction

In all animals, there is a continual cycle of synthesis and breakdown of protein with growth occurring under conditions where the rate of protein synthesis exceeds protein breakdown (Sugden and Fuller 1991; Houlihan et al. 1995*a*). In fish, the effects of various abiotic and nutritional influences on protein synthesis and protein growth have been studied for over 20 years and have been the subject of several reviews (Haschemeyer 1978; Fauconneau 1985; Houlihan 1991; Houlihan et al. 1993, 1995*a*, 1995*b*; McCarthy and Houlihan 1997). Water temperature has been identified as the major abiotic factor affecting the physiology and growth of fish (Brett 1979; Jobling 1997). However, although the effects of water temperature on rates of protein synthesis in fish have been well studied (reviewed by Haschemeyer 1978; McCarthy and Houlihan 1997), there are very few examples where rates of protein synthesis and growth have both been measured for the same animals (e.g., Fauconneau and Arnal 1985; Mathers et al. 1993; Reid et al. 1995,

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1997). However, these measurements are of value, as they allow the effects of water temperature on these two fundamental processes (synthesis and growth) to be modelled and also allow the effects of water temperature on the efficiency with which fish retain synthesised proteins as growth to be examined.

Our knowledge of the temperature response of protein synthesis and protein growth in fish is still limited in a number of respects. Any fish species exhibits thermal tolerance over a range of water temperatures where feeding and growth will occur and where growth performance (in terms of both rate and efficiency) can vary according to the water temperature (Jobling 1997). However, previous studies have reared groups of fish at a limited number of water temperatures within the thermal tolerance range for that species, 2°C (Reid et al. 1995, 1997) to 10°C (Fauconneau and Arnal 1985; Mathers et al. 1993) apart. To our knowledge, rates of protein synthesis and growth have not been measured over a range of water temperatures within the range of thermal tolerance for a species enabling the temperature response of these processes to be modelled. Secondly, most of the previous studies on protein synthesis and growth in fish have been carried out using salmonid fish (e.g., Fauconneau and Arnal 1985; Houlihan et al. 1986; Foster et al. 1991; Carter et al. 1993; McCarthy et al. 1994; Owen et al. 1999), most likely due to their commercial importance and the ease with which salmonids can be reared in the laboratory. It has been suggested that the pattern of protein turnover and the retention of synthesised protein as growth may vary between fish species that adopt different lifestyles (Houlihan et al. 1995*a*). For example, recent work has suggested that the proportion of synthesised proteins retained as protein growth may be higher in more sedentary fish (e.g., Atlantic halibut (*Hippoglossus hippoglossus*): Fraser et al. 1998) compared with salmonids. However, little data are available to examine this hypothesis.

The Atlantic wolffish (*Anarhichas lupus*) provides an ideal alternative model species for the study of protein metabolism in fish. It is a benthic marine fish whose habits differ from those of salmonid fish in that it has a sedentary nature and exhibits little aggression between conspecifics, factors that may promote increased growth efficiency through reduced energy expenditure. The Atlantic wolffish has also been identified as a possible candidate species for cold-water aquaculture in northern Europe (Tilseth 1990) and in Atlantic Canada (Brown et al. 1995). The progress made in the development of Atlantic wolffish aquaculture has been reviewed recently (Moksness and Pavlov 1996); however, there are no protein metabolism data available for this species. The study of the protein metabolism (rates of protein consumption, synthesis, and growth) of any potential aquaculture species will be of value in providing a better understanding of the growth performance and food conversion efficiency for that species and in allowing more meaningful comparisons to be made with the growth performance of existing culture species.

The range of thermal tolerance for the Atlantic wolffish is unknown; however, Atlantic wolffish have been recorded at water temperatures ranging between 0 and 16°C (reviewed in Moksness and Pavlov 1996). Recently, by rearing fish at a range of water temperatures between 5 and 14°C, we have shown that the optimum water temperatures for wet weight growth and growth efficiency of juvenile Atlantic wolffish are 11 and 9.6°C, respectively (McCarthy et al. 1998). At the end of that study, we had the opportunity to measure fractional rates of protein synthesis in fish sampled from each of the temperature groups, using the flooding dose method of Garlick et al. (1980) (for reviews of this technique, see Houlihan et al. 1995*a*, 1995*b*). Thus, the aims of this paper are to (*i*) examine the effect of water temperature on white muscle and whole-body rates of protein synthesis and protein growth in juvenile Atlantic wolffish, (*ii*) determine the water temperature(s) at which white muscle and whole-body protein growth rates and protein synthesis retention efficiencies are maximal, and (*iii*) compare the protein metabolism of juvenile Atlantic wolffish with that of salmonid fish (rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*)), the most commonly studied species in temperature–growth studies in fish and also the dominant fish species in cold-water aquaculture.

## Materials and methods

### Animal husbandry

The experiment was carried out at the Institute of Marine Research, Flødevigen Marine Research Station, Norway, between February and July 1995 using juvenile Atlantic wolffish reared from eggs obtained from adult broodstock held at the station. The origin of the broodstock and the methods of artificial fertilisation and egg incubation are described in the review of Moksness and Pavlov (1996). The fish used in this experiment were hatched and weaned onto food during May and June 1994. The holding conditions and water quality are as previously described in McCarthy et al. (1998). The thermal history experienced by these fish prior to the experiment was 6°C in May 1994, rising to about 12°C in September–October and then declining to 5°C by February 1995. This experiment was started on 20 February 1995 and lasted for 98 days. A total of 400 juvenile Atlantic wolffish were divided into four treatments, with two replicate tanks of 50 fish per treatment. Fifteen fish in each tank were individually tagged using a coding system of visible implanted fluorescent elastomer (Northwest Marine Technology, Shaw Island, WA 98286, U.S.A.) injected into their dorsal fins. A control group of 10 fish (mean weight 25.1 ± 0.7 g, $n$ = 10) from the same stock were killed on 20 February 1995 in order to estimate the initial white muscle and whole-body protein content and initial white muscle fillet weight (expressed as a percentage of initial body weight) of the experimental fish. These data were used to calculate the white muscle and whole-body growth rates of the experimental fish (see below).

All groups of fish were kept in green-walled tanks (1 × 1 × 0.3 m, 260–280 L) and exposed to a 16 h light : 8 h dark photoperiod. Salinity varied between 33.6 and 34.8‰, the average oxygen saturation was about 115%, and the dissolved ammonium concentration varied between 1.0 and 6.5 μmol $NH_4 \cdot L^{-1}$. Desired experimental water temperatures were obtained using three sources: (*i*) water pumped directly from the sea channel, which fluctuated between 5 and 10°C during the study, and water from the same source that had been (*ii*) cooled to between 2 and 5°C or (*iii*) heated to between 15 and 16°C. Water from two of the three sources was mixed to provide the desired temperature of water for each tank. The water temperature in each tank was checked daily and carefully regulated to minimise day-to-day fluctuations. Nominal water temperatures of the four treatments were 5, 8, 11, and 14°C, respectively. Although the measured water temperatures varied slightly from those expected, they were not significantly differ-

ent from 5, 8, 11, and 14°C ($\chi^2$ = 1.32–3.51, 94–97 df, $p \leq 1.00$) (Table 1). The experimental groups were designated as groups 5.1, 5.2, 8.1, 8.2, 11.1, 11.2, 14.1, and 14.2, respectively.

The fish were fed a fish meal diet (NorSeaMink; Elite Plus, Skretting, Stavanger, Norway) by hand to apparent satiation two to six times per day (feeding motivation dependent on water temperature). At each feeding, the fish were fed slowly and care was taken to ensure that food offered was eaten before more was presented. The fish usually fed quickly, and uneaten food on the bottom of the tank after 5 min was taken as indicating satiation. The amount of food presented to each group was calculated by weighing the amount of food in each group's feeding jar at the beginning and end of each week.

## Measurement of protein synthesis

At the end of the experiment, fractional rates of protein synthesis were measured in the surviving tagged fish in each group. On the day of injection, 24 h after their last meal, the fish were quickly weighed in a pretared beaker of water to estimate their wet weight and then injected into the peritoneum without anaesthesia with a solution containing 135 mM L-phenylalanine and L-[2,6-$^3$H]phenylalanine (Amersham International, 37 × $10^6$ Bq·mL$^{-1}$). The injection volume administered was 1 mL·100 g body weight$^{-1}$, and the specific activity of the injection solution was 2682 (±117, $n$ = 4) disintegrations per minute (dpm) per nanomole of phenylalanine. The fish were injected over 4 days in the morning (a.m.) and afternoon (p.m.) in the following order: day 95, groups 14.2 (a.m.) and 5.1 (p.m.); day 96, groups 8.1 (a.m.) and 11.1 (p.m.); day 97, groups 5.2 (a.m.) and 14.1 (p.m.); day 98, groups 11.2 (a.m.) and 8.2 (p.m.)

Following an incorporation period of about 3 h, each fish was killed by an overdose of the anaesthetic MS 222 (Sigma, Poole, Dorset, U.K), the time of death noted, and the fish weighed. The peritoneum was opened up and rinsed with water in order to remove any excess unabsorbed injection solution. For each fish, one white muscle fillet was quickly removed and weighed. The fillet and remaining carcass were frozen together on dry ice. Prior to analysis, the samples were stored at –70°C in Fløtevigen and were transported from Fløtevigen to Aberdeen on dry ice (total transport time <24 h). The subsequent treatment of triplicate white muscle and whole-body samples to measure the white muscle and whole-body free-pool phenylalanine-specific radioactivity ($S_a$), protein content, protein-bound phenylalanine-specific radioactivity ($S_b$), and RNA concentrations was as described in Houlihan et al. (1995*a*, 1995*b*). Values of $S_a$ and $S_b$ were measured using the methodologies of Suzuki and Yagi (1976) and Garlick et al. (1980). Protein contents were measured using the method of Lowry et al. (1951) as modified by Schacterle and Pollock (1973). RNA concentrations were measured using the orcinol method of Mejbaum (1939).

## Calculations

The average daily fractional protein consumption rates ($k_r$, percent per day, expressed as a percentage of the final body protein mass) for the tagged fish were estimated as follows. The average daily protein consumption rate for each group ($Con_p$, grams protein per fish per day) was derived using the equation

$$Con_p = (FD \cdot 0.435)/(N_x \cdot W_{mid} \cdot t)$$

where FD is the amount of feed offered to the group during the experiment, 0.435 is the proportion of protein in the diet, $N_x$ is the average number of fish in the group ((initial + final)/2) during the experiment, $W_{mid}$ is the average body weight of the group ((initial + final)/2), and $t$ is the length of the experiment in days. These data are presented in table 2 of McCarthy et al. (1998). Following this, fractional rates of protein consumption ($k_r$, percent per day) were estimated for the tagged fish as

$$k_r = (Con_p \cdot 100)/W_{prot}$$

where $Con_p$ is the average daily protein consumption rate (grams protein per fish per day) and $W_{prot}$ is the average final protein mass (i.e., wet weight × percent protein content) of the tagged fish in each group.

The white muscle and whole-body specific growth rates (SGR, percent per day) of the tagged fish were calculated according to Ricker (1979):

$$SGR = [\ln(W_f) - \ln(W_0)] \cdot 100/t$$

where $W_0$ and $W_f$ are the initial and final white muscle or whole-body weights of the fish, respectively, and $t$ is the length of the experiment in days for each experimental group. A value of 38.3% (±0.5, $n$ = 10) of the body weight, derived from the initial control group, was used to estimate the initial white muscle weight of the tagged fish. The value of $t$ varied between experimental groups according to the days on which fish were killed: day 95 (groups 5.1 and 14.2), day 96 (groups 8.1 and 11.1), day 97 (groups 5.2 and 14.1), and day 98 (groups 8.2 and 11.2), respectively.

White muscle and whole-body fractional rates of protein synthesis ($k_s$, percent per day, expressed as a percentage of the protein mass synthesised per day) were calculated using the equation of Garlick et al. (1983):

$$k_s = (S_b/S_a) \cdot (1440/t) \cdot 100$$

where $S_b$ and $S_a$ are the protein-bound and free-pool phenylalanine-specific radioactivities and $t$ is the incorporation time (from injection to death) in minutes. Incorporation times ranged between 169 and 208 min for individual fish with an overall average of 190 min (±8, $n$ = 110). The mean white muscle and whole-body $S_b$ values were 1648 dpm·nmol$^{-1}$ (±25, $n$ = 110) and 1615 dpm·nmol$^{-1}$ (±39, $n$ = 110), respectively, and attained values of 61.5% (±0.9, $n$ = 110) and 60.2% (±1.2, $n$ = 110) of the phenylalanine-specific radioactivity of the injection solution, respectively. White muscle and whole-body fractional protein growth rates ($k_g$, percent per day, expressed as a percentage of the final protein mass) were calculated using the equation of Ricker (1979) above where $W_0$ and $W_f$ are the initial and final white muscle or whole-body protein masses, respectively. The initial white muscle and whole-body protein contents were estimated using the values obtained from the initial control fish (white muscle, 214.1 mg protein·g$^{-1}$ (±7.1, $n$ = 10); whole body, 229.8 mg protein·g$^{-1}$ (±8.5, $n$ = 10)). White muscle and whole-body RNA concentrations were expressed using the RNA:protein ratio, which has been termed the capacity for protein synthesis ($C_s$, milligrams RNA per gram protein) (Sugden and Fuller 1991). White muscle and whole-body RNA activity ($k_{RNA}$, grams protein synthesised per gram RNA per day) gives an approximate indication of the translational efficiency of the ribosomes and was calculated by dividing $k_s$ by the appropriate $C_s$ value (Houlihan et al. 1995*a*). The protein growth efficiency (PPV, percent), the efficiency with which ingested protein was retained as growth, was calculated by dividing the fractional protein growth rate by the fractional protein consumption rate. The efficiency with which synthesised proteins were retained as growth ($k_g/k_s$, percent) was calculated by dividing the fractional rate of protein growth by the synthesis rate.

## Statistical analysis

All data are presented as mean values ± 1 SE. Two-way analysis of variance was used to (*i*) examine for differences between replicate groups at each of the four temperature treatments and (*ii*) examine the effect of water temperature on various indices of growth performance. Where the two-way analysis of variance indicated a significant temperature effect, this was further analysed using both linear (method of least squares) and quadratic regression analysis

and the model of best fit (as indicated by the highest coefficient of determination, $R^2$) presented. Where a linear model was the best fit, analysis of covariance was used to compare the slopes of the regression lines. All statistical tests are as described in Zar (1984) and differences present at the 5% level were considered significant.

## Results

The average initial body weight of the tagged fish in each of the experimental groups varied between 25.0 and 26.3 g (Table 1). Two-way analysis of variance indicated that there were no differences in the average initial body weight of the tagged fish of the two replicate groups at each temperature ($F_{(1,28)} = 1.95$, $p > 0.05$) and among the four temperature treatments ($F_{(3,84)} = 0.36$, $p > 0.05$). Likewise, at the end of the experiment, the average final body weight of the tagged fish in the two replicate groups at each water temperature were not significantly different ($F_{(1,23)} = 1.02$, $p > 0.05$). However, there was a significant temperature effect on final body weight ($F_{(3,69)} = 11.54$, $p < 0.001$). The average final body weight increased between 5 and 11°C, was highest at 11°C, and declined at 14°C (Table 1).

The effects of water temperature on white muscle specific growth rate ($SGR_{WM}$) and whole-body specific growth rate ($SGR_{WB}$) of the tagged fish in each group are presented in Table 1. Two-way analysis of variance indicated that for both $SGR_{WM}$ and $SGR_{WB}$, the growth rates of the tagged fish in the two replicate groups at each water temperature were similar ($SGR_{WM}$: $F_{(1,23)} = 0.51$, $p > 0.05$; $SGR_{WB}$: $F_{(1,23)} = 0.97$, $p > 0.05$) and there was a significant temperature effect ($SGR_{WM}$: $F_{(3,69)} = 12.87$, $p < 0.001$; $SGR_{WB}$: $F_{(3,69)} = 7.76$, $p < 0.001$). Both $SGR_{WM}$ and $SGR_{WB}$ exhibited a similar parabolic relationship to water temperature, increasing between 5 and 11°C and declining at 14°C with growth rates being highest at 11°C (Table 1), and were best described by the following equations:

$$SGR_{WM} = -0.826 + 0.330 \cdot T - 0.016 \cdot T^2 \quad (R^2 = 0.733,\ n = 8,\ p < 0.02)$$

$$SGR_{WB} = -0.690 + 0.293 \cdot T - 0.014 \cdot T^2 \quad (R^2 = 0.761,\ n = 8,\ p < 0.01)$$

where $T$ is the water temperature (degrees Celsius).

Differentiation of the regression coefficients indicated that maximum $SGR_{WM}$ and $SGR_{WB}$ values were at 10.3 and 10.5°C, respectively. As expected, the fish exhibited isometric growth: least squares regression analysis of the relationships between $SGR_{WB}$ and $SGR_{WM}$ at the four water temperatures indicated slope coefficients ranging between 1.04 and 1.07 (data not shown).

The effects of water temperature on the fractional rate of protein consumption ($k_r$) and whole-body and white muscle fractional rates of protein synthesis (WB $k_s$ and WM $k_s$) and protein growth (WB $k_g$ and WM $k_g$) are presented in Fig. 1. Values of $k_r$ increased with increasing water temperature between 5 and 14°C (Fig. 1*a*), the relationship of which was best described by a linear model (see caption to Fig. 1*a*). For each of WB $k_s$, WM $k_s$, WB $k_g$, and WM $k_g$, two-way analysis of variance indicated that there were no differences between the average fractional rates of the tagged fish in the two replicate groups at each water temperature ($F_{(1,23)} = 0.89$ (WB $k_s$), 0.18 (WM $k_s$), 1.68 (WB $k_g$), and 1.72 (WM $k_g$), respectively, all $p > 0.05$), but there was a significant temperature effect (WB $k_s$: $F_{(3,69)} = 2.71$, $p < 0.05$; WM $k_s$: $F_{(3,69)} = 2.95$, $p < 0.05$; WB $k_g$: $F_{(3,69)} = 18.70$, $p < 0.001$; WM $k_g$: $F_{(3,69)} = 9.55$, $p < 0.001$). WB $k_s$ (Fig. 1*b*) and WM $k_s$ (Fig. 1*c*) increased in a linear fashion with increasing water temperature. Analysis of covariance indicated that there was no difference between the slopes of the two regression lines ($t = 1.09$, 12 df, $p > 0.05$), indicating a common temperature response in the fractional rates of protein synthesis in the whole-body and white muscle (Figs. 1*b* and 1*c*). The relationships between water temperature and WB $k_g$ and WM $k_g$ were parabolic; fractional protein growth rates increased between 5 and 11°C, were highest at 11°C, and decreased at 14°C (Figs. 1*d* and 1*e*). Differentiation of the regression coefficients for these relationships (see captions to Figs. 1*d* and 1*e*, respectively) indicated that maximum WB $k_g$ and WM $k_g$ values were at 11.2 and 10.2°C, respectively. The percent protein contents of the white muscle ($PRO_{WM}$) and whole body ($PRO_{WB}$) are shown in Table 1. Two-way analysis of variance indicated that for both $PRO_{WM}$ and $PRO_{WB}$, the percent protein contents of the tagged fish in the two replicate groups at each water temperature were similar ($PRO_{WM}$: $F_{(1,23)} = 3.35$, $p > 0.05$; $PRO_{WB}$: $F_{(1,23)} = 2.73$, $p > 0.05$). $PRO_{WM}$ increased with increasing water temperature ($F_{(3,69)} = 3.08$, $p < 0.05$), although no temperature effect was observed with $PRO_{WB}$ ($F_{(3,69)} = 2.39$, $p > 0.05$).

The relationships between water temperature and protein growth efficiency in the whole body ($PPV_{WB}$) and white muscle ($PPV_{WM}$) and between water temperature and the efficiency of retention of synthesised proteins in the whole body (WB $k_g/k_s$) and white muscle (WM $k_g/k_s$) were parabolic and are shown in Fig. 2. $PPV_{WB}$ values increased between 5 and 11°C, were maximal at 11°C, and decreased at 14°C (Fig. 2*a*). $PPV_{WM}$ values also appeared to be exhibit a parabolic relationship to water temperature (Fig. 2*b*), although this relationship was not significant at the 5% level ($p < 0.07$). Differentiation of the regression coefficients for these relationships (see captions to Figs. 2*a* and 2*b*, respectively) indicated that maximum $PPV_{WB}$ and $PPV_{WM}$ values were at 10.0 and 9.5°C, respectively. Two-way analysis of variance indicated that for both the WB $k_g/k_s$ and WM $k_g/k_s$ values, there were no differences between the replicate groups at each water temperature (WB $k_g/k_s$: $F_{(1,23)} = 0.92$, $p > 0.05$; WM $k_g/k_s$: $F_{(1,23)} = 1.42$, $p > 0.05$), but there was a significant temperature effect (WB $k_g/k_s$: $F_{(3,69)} = 4.72$, $p < 0.01$; WM $k_g/k_s$: $F_{(3,69)} = 2.86$, $p < 0.05$). Both WB $k_g/k_s$ and WM $k_g/k_s$ values increased between 5 and 11°C, were maximal at 11°C, and decreased at 14°C (Figs. 2*c* and 2*d*). Differentiation of the regression coefficients for these relationships (see captions to Figs. 2*c* and 2*d*, respectively) indicated that maximum WB $k_g/k_s$ and WM $k_g/k_s$ values were at 10.4 and 8.6°C, respectively.

The effects of water temperature on the whole-body and white muscle capacity for protein synthesis (WB $C_s$ and WM $C_s$) and ribosomal translational efficiency (WB $k_{RNA}$ and WM $k_{RNA}$) are shown in Fig. 3. For each of WB $C_s$, WM $C_s$, WB $k_{RNA}$, and WM $k_{RNA}$, two-way analysis of variance indicated that there were no differences between the average values of the tagged fish in the two replicate groups

**Table 1.** Initial and final numbers ($N_0$ and $N_f$), initial and final body weights ($W_0$ and $W_f$, g), white muscle ($SGR_{WM}$) and whole-body ($SGR_{WB}$) specific growth rates (%·day$^{-1}$), and white muscle ($PRO_{WM}$) and whole-body ($PRO_{WB}$) protein contents (% wet weight) of juvenile Atlantic wolffish (*Anarhichas lupus*) at 5, 8, 11, and 14°C.

| Group | Temperature (°C) | $N_0$ | $W_0$ | $N_f$ | $W_f$ | $SGR_{WM}$ | $SGR_{WB}$ | $PRO_{WM}$ | $PRO_{WB}$ |
|---|---|---|---|---|---|---|---|---|---|
| 5.1 | 4.9 (0.04) | 15 | 26.3 (0.6) | 15 | 46.0 (2.0) | 0.39 (0.06) | 0.43 (0.05) | 21.1 (1.0) | 21.4 (0.8) |
| 5.2 | 4.8 (0.04) | 15 | 25.3 (0.8) | 15 | 40.8 (1.9) | 0.54 (0.05) | 0.48 (0.04) | 18.9 (0.9) | 19.5 (0.9) |
| 8.1 | 7.7 (0.08) | 15 | 26.7 (0.6) | 11 | 48.8 (3.8) | 0.68 (0.09) | 0.62 (0.08) | 18.4 (0.7) | 19.7 (0.9) |
| 8.2 | 7.8 (0.05) | 15 | 25.8 (0.7) | 14 | 56.3 (3.6) | 0.74 (0.09) | 0.73 (0.07) | 21.4 (1.0) | 22.3 (1.0) |
| 11.1 | 10.9 (0.08) | 15 | 25.5. (0.7) | 14 | 64.6 (5.7) | 0.98 (0.10) | 0.94 (0.09) | 19.2 (1.1) | 19.8 (0.6) |
| 11.2 | 11.0 (0.07) | 15 | 25.8 (0.5) | 14 | 64.9 (2.9) | 1.03 (0.05) | 0.93 (0.05) | 22.9 (1.2) | 22.7 (0.8) |
| 14.1 | 14.0 (0.07) | 15 | 26.3 (1.0) | 12 | 52.5 (3.9) | 0.70 (0.12) | 0.65 (0.10) | 22.7 (1.3) | 21.0 (0.9) |
| 14.2 | 13.9 (0.05) | 15 | 25.0 (0.5) | 15 | 50.1 (5.1) | 0.67 (0.12) | 0.66 (0.10) | 22.1 (0.9) | 21.9 (0.5) |

**Note**: Values are presented as mean ± 1 SE.

at each water temperature ($F_{(1,23)}$ = 0.31 (WB $C_s$), 0.10 (WM $C_s$), 0.76 (WB $k_{RNA}$), and 0.22 (WM $k_{RNA}$), respectively, all $p > 0.05$), but there was a significant temperature effect (WB $C_s$: $F_{(3,69)} = 12.09$, $p < 0.001$; WM $C_s$: $F_{(3,69)} = 14.63$, $p < 0.001$; WB $k_{RNA}$: $F_{(3,69)} = 16.23$, $p < 0.001$; WM $k_{RNA}$, $F_{(3,69)} = 15.02$, $p < 0.001$). The relationships between water temperature and WB $C_s$ and WM $C_s$ were linear with the capacity for protein synthesis decreasing with increasing water temperature (Fig. 3*a*). WB $k_{RNA}$ and WM $k_{RNA}$ values exhibited an exponential increase with increasing water temperature (Fig. 3*b*) and, following log transformation of the $k_{RNA}$ values, were best described by a linear regression model (see caption to Fig. 3*b*). Analysis of covariance indicated that there was no difference between the slopes of the two regression lines ($t$ = 1.27, 12 df, $p > 0.05$), indicating a common temperature response in RNA activity in the whole body and white muscle.

## Discussion

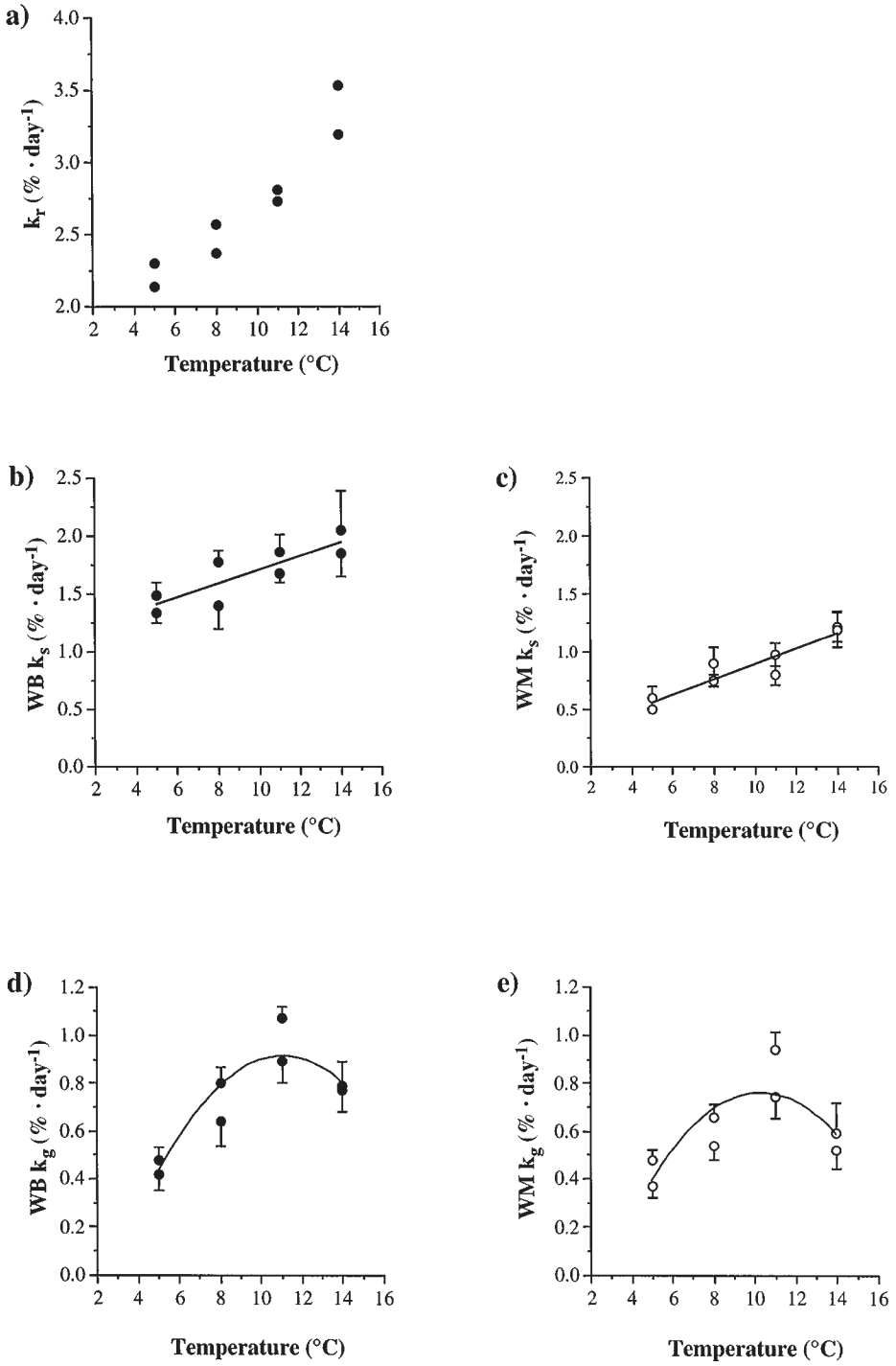
For ectotherms, the relationship between the rate of many biochemical or physiological processes and water temperature is asymmetrical. Providing that resources are not limiting and the temperature increase is within the thermal limits for the species, the rate of any biochemical or physiological process will increase with increasing water temperature to a maximum, above which the rate declines near the upper limit for the species (Huey and Kingsolver 1989; Atkinson 1994). Since growth performance is the net expression of physiological performance, these parameters would also be expected to follow an asymmetrical relationship with temperature. In fish, consumption rates, growth rates, and growth efficiency (growth/consumption) all follow this temperature-dependent increase to an optimum followed by a decline with any further increase in water temperature. Previous studies have shown that the optimum water temperature for consumption ($T_{opt.R}$) is higher than the optimum water temperature for growth ($T_{opt.G}$), which in turn is higher than the optimum water temperature for growth efficiency ($T_{opt.GE}$) (Woiwode and Adelman 1991; Imsland et al. 1995; Björnsson and Tryggvadóttir 1996; Jobling 1997). In this study, the protein consumption rates, growth rates, and growth efficiency of juvenile Atlantic wolffish were examined at 5, 8, 11, and 14°C. Protein consumption rates increased over this range of water temperatures, and it was not possible to identify $T_{opt.R}$ (Fig. 1*a*) (also see McCarthy et al. 1998). However, the growth rate and growth efficiency data of the tagged juvenile Atlantic wolffish did exhibit a temperature-dependent pattern, and it was possible to determine $T_{opt.G}$ and $T_{opt.GE}$. The results of this study indicated that $T_{opt.G}$ for whole-body and white muscle specific growth rates and fractional protein growth rates in juvenile Atlantic wolffish was 10–11°C (Table 1; Fig. 1) and that $T_{opt.GE}$ for protein growth efficiency and protein synthesis retention efficiency in both the whole body and white muscle of juvenile Atlantic wolffish was 9–10°C (Fig. 2). As expected, these values are in agreement with the $T_{opt.G}$ and $T_{opt.GE}$ values for whole-body specific growth and growth efficiency (wet weight gain/dry weight of food offered) obtained using the group growth rate data from this experiment (McCarthy et al. 1998).

This is the first study to measure fractional rates of protein synthesis in juvenile Atlantic wolffish. Therefore, it is desirable to examine whether the protein synthesis rates measured at 5, 8, 11, and 14°C in this study are within the range of values to be expected for an ectotherm at these water temperatures. In a recent review on the effects of water temperature on protein synthesis in fish, McCarthy and Houlihan (1997) summarised the general effect of water temperature on the process of muscle protein synthesis using the available ectotherm and endotherm data. Figure 4 summarises the relationship between temperature and muscle protein synthesis derived from these data (references are cited in McCarthy and Houlihan (1997) or are available from the corresponding author). The fractional rate of muscle protein synthesis ($k_s$) increased exponentially with increasing temperature ($T$°C) and, following logarithmic transformation of the protein synthesis values, was best described by the following linear regression equation:

$$\log_{10} k_s = 0.035T°C - 0.529 \quad (R^2 = 0.738,\ n = 34,\ p < 0.001).$$

The average fractional rates of white muscle protein synthesis for juvenile Atlantic wolffish at 5, 8, 11, and 14°C measured in this study (0.55, 0.82, 0.88, and 1.20%·day$^{-1}$, respectively) are also shown in Fig. 4. These average values were significantly higher than the muscle protein synthesis

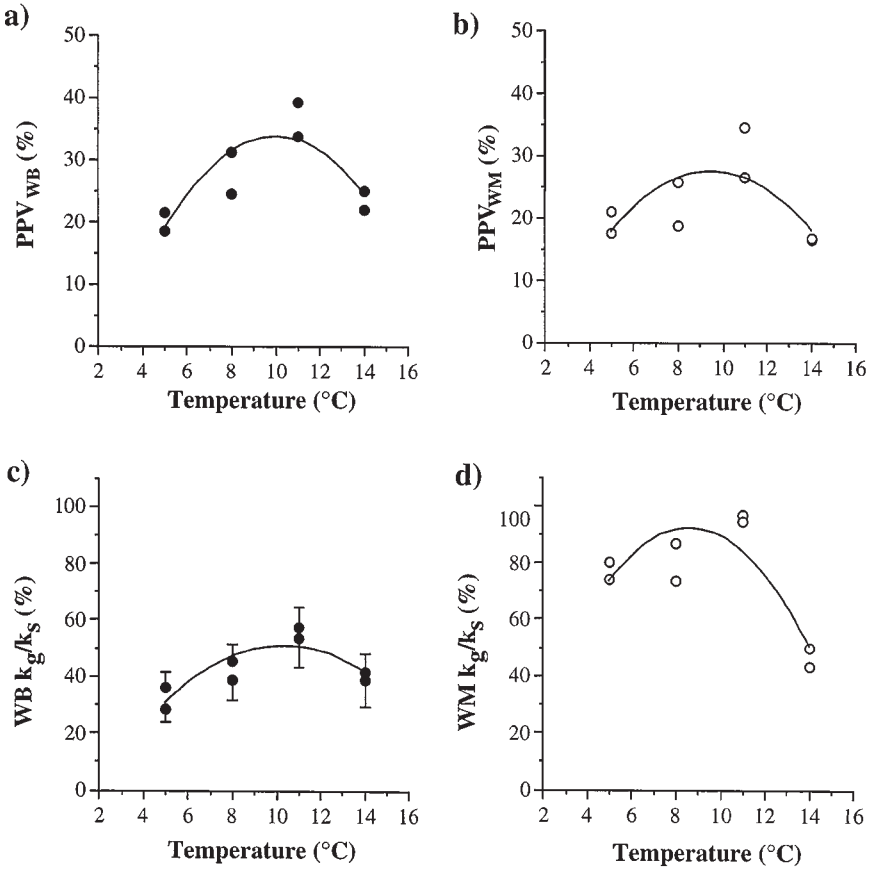
**Fig. 1.** Relationships between water temperature ($T$) and ($a$) the fractional rate of protein consumption, $k_r$ ($k_r = 1.527 + 0.124 \cdot T$; $R^2 = 0.881$, $n = 8$, $p < 0.001$), ($b$) the fractional rate of whole-body protein synthesis, WB $k_s$ (WB $k_s = 1.105 + 0.060 \cdot T$; $R^2 = 0.693$, $n = 8$, $p < 0.006$), ($c$) the fractional rate of white muscle protein synthesis, WM $k_s$ (WM $k_s = 0.230 + 0.067 \cdot T$; $R^2 = 0.854$, $n = 8$, $p < 0.001$), ($d$) the fractional rate of whole-body protein growth, WB $k_g$ (WB $k_g = -0.695 + 0.290 \cdot T - 0.013 \cdot T^2$; $R^2 = 0.774$, $n = 8$, $p < 0.01$), and ($e$) the fractional rate of white muscle protein growth, WM $k_g$ (WM $k_g = -0.604 + 0.264 \cdot T - 0.013 \cdot T^2$; $R^2 = 0.652$, $n = 8$, $p < 0.03$), for juvenile Atlantic wolffish (*Anarhichas lupus*). Values are presented as mean ± 1 SEM.




rates predicted from the regression analysis (0.44, 0.56, 0.72, and 0.91%·day$^{-1}$, respectively) (paired $t$ test, $t = 4.87$, 3 df, $p < 0.02$) but were not significantly different from the muscle protein synthesis rates predicted at the upper 95% confidence limit for the above regression line at 5, 8, 11, and 14°C (0.59, 0.74, 0.93, and 1.18%·day$^{-1}$, respectively) (paired $t$ test, $t = 0.08$, 3 df, $p > 0.05$).

In this study, under satiation feeding conditions, white muscle and whole-body fractional rates of protein synthesis increased with water temperature between 5 and 14°C

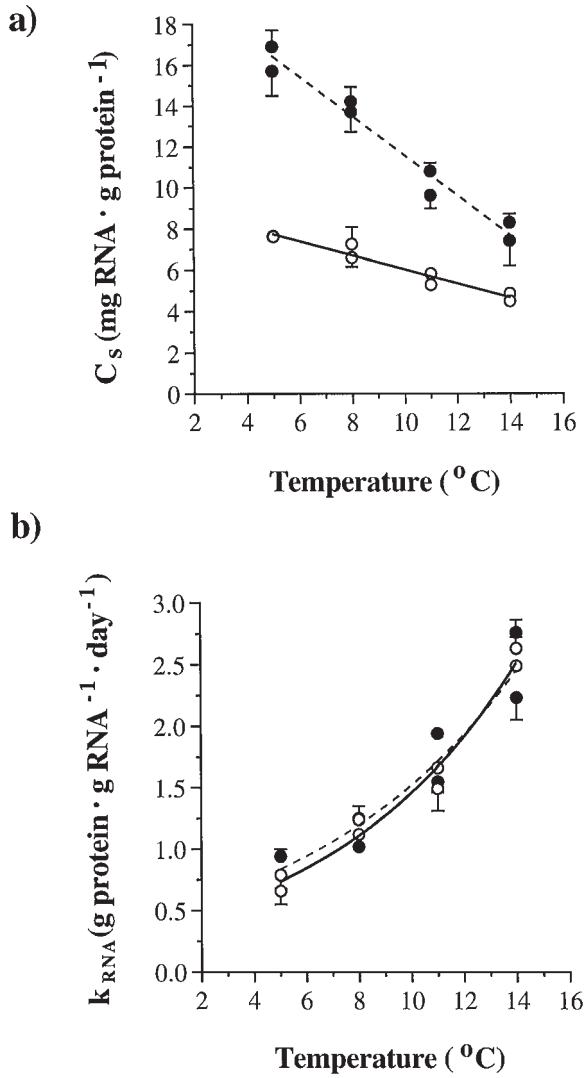
**Fig. 2.** Relationships between water temperature ($T$) and (*a*) whole-body protein growth efficiency, $PPV_{WB}$ ($PPV_{WB} = -25.314 + 11.720 \cdot T - 0.583 \cdot T^2$; $R^2 = 0.609$, $n = 8$, $p < 0.04$), (*b*) white muscle protein growth efficiency, $PPV_{WM}$ ($PPV_{WM} = -15.191 + 8.933 \cdot T - 0.469 \cdot T^2$; $R^2 = 0.503$, $n = 8$, $p < 0.07$), (*c*) whole-body protein synthesis retention efficiency, WB $k_g/k_s$ (WB $k_g/k_s = -24.110 + 14.410 \cdot T - 0.693 \cdot T^2$; $R^2 = 0.614$, $n = 8$, $p < 0.04$), and (*d*) white muscle protein synthesis retention efficiency, WM $k_g/k_s$ (WM $k_g/k_s = -14.792 + 24.778 \cdot T - 1.438 \cdot T^2$; $R^2 = 0.617$, $n = 8$, $p < 0.04$), for juvenile Atlantic wolffish (*Anarhichas lupus*). Values are presented as mean ± 1 SEM.




(Figs. 1*b* and 1*c*). These results are in agreement with earlier studies that have shown that protein synthesis rates in fish increase with increasing water temperature when food is not limiting (Fauconneau and Arnal 1985; Loughna and Goldspink 1985; Watt et al. 1988; Mathers et al. 1993; McCarthy and Houlihan 1997) compared with no increase in synthesis rates when food is restricted (Foster et al. 1992). In this study, between 5 and 14°C, a linear model was the best descriptor of the relationship between water temperature and protein synthesis in juvenile Atlantic wolffish (Figs. 1*b* and 1*c*). However, there is evidence to suggest that the relationship between protein synthesis and water temperature is asymmetrical with maximum rates of protein synthesis occurring at the optimum temperature for any single species. Pannevis and Houlihan (1992) measured in vivo rates of protein synthesis in isolated hepatocytes from rainbow trout at incubation temperatures of 5, 10, 14.5, 17.5, and 20°C and reported an asymmetrical relationship between protein synthesis and temperature (their fig. 2) with the highest rates occurring at 14.5 and 17.5°C, close to the $T_{opt.G}$ for rainbow trout of 17°C (Hokanson et al. 1977). The work of Loughna and Goldspink (1985) also suggests the possibility of maximum rates of protein synthesis occurring at the $T_{opt.G}$ for common carp (*Cyprinus carpio*) and rainbow trout. Their results suggest that the relationship between protein synthesis and water temperature may reach an asymptote at water temperatures of 25 and 15–20°C for common carp and rainbow trout, respectively (their fig. 2). These temperature values are close to the $T_{opt.G}$ values that have previously been reported for these species, 27°C for common carp (Goolish and Adelman 1984) and 17°C for rainbow trout (Hokanson et al. 1977), and would support the hypothesis that maximum rates of protein synthesis occur at water temperatures close to the $T_{opt.G}$ value for a particular species. To date, however, where the effect of water temperature on in vivo rates of protein synthesis has been measured on whole animals (as opposed to isolated cell cultures) over a range of water temperatures (Loughna and Goldspink 1985; this study), the range selected for study has not included a sufficient number of post-$T_{opt.G}$ temperature values to allow this hypothesis to be tested

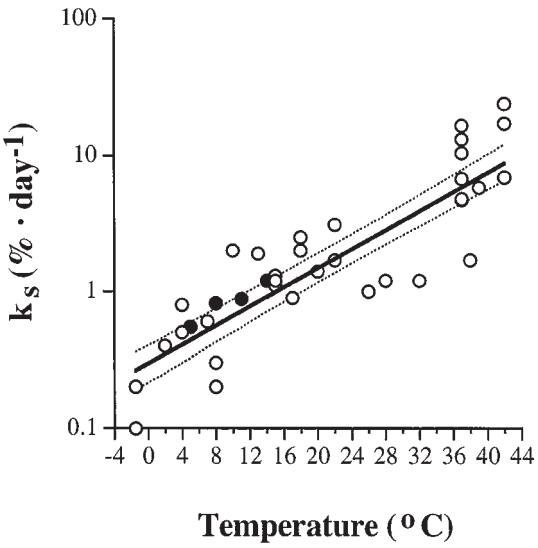
In this study, the capacity for protein synthesis ($C_s$, milligrams RNA per gram protein) in the white muscle and whole body increased with decreasing water temperature (Fig. 3*a*). This increase in RNA concentration with decreasing water temperature has been noted in previous studies (e.g., Goolish et al. 1984; Foster et al. 1992; Mathers et al. 1993) and is

**Fig. 3.** Relationships between water temperature ($T$) and (*a*) whole-body (WB, solid circles) and white muscle (WM, open circles) RNA to protein ratios, $C_s$ (WB $C_s = 21.292 - 0.970 \cdot T$; $R^2 = 0.963$, $n = 8$, $p < 0.001$; WM $C_s = 9.437 - 0.341 \cdot T$; $R^2 = 0.939$, $n = 8$, $p < 0.001$), and (*b*) whole-body (WB, solid circles) and white muscle (WM, open circles) $k_{RNA}$ values ($\log_{10}$(WB $k_{RNA}$) $= -0.340 + 0.052 \cdot T$; $R^2 = 0.922$, $n = 8$, $p < 0.001$; $\log_{10}$(WM $k_{RNA}$) $= -0.428 + 0.059 \cdot T$; $R^2 = 0.970$, $n = 8$, $p < 0.001$) for juvenile Atlantic wolffish (*Anarhichas lupus*). Values are presented as mean ± 1 SEM.




recognised as a thermal compensatory response by fish at colder water temperatures where the rate at which proteins are translated during the ribosome cycle is lower (McCarthy and Houlihan 1997). This decrease in translation rates at low water temperatures is also indicated by the decrease in white muscle and whole-body $k_{RNA}$ values (grams protein synthesised per gram RNA per day), also known as "RNA activity" or "RNA translational efficiency" (Sugden and Fuller 1991), observed in this study (Fig. 3*b*). This decrease in $k_{RNA}$ with decreasing temperature has been observed in both single-species studies (e.g., Foster et al. 1992; this study) and is also a general biological phenomenon observed across ectothermic and endothermic vertebrate species (McCarthy and Houlihan 1997, their fig. 3*b*).

**Fig. 4.** Relationship between temperature and muscle fractional rates of protein synthesis, $k_s$ (open circles), for ectotherms (fish, frog, lizard) and endotherms (birds, mammals) (note logarithmic $y$-axis). The regression line (solid line) and its 95% confidence limits (dotted lines) are also presented. The least squares linear regression analysis of the data, following logarithmic transformation of the protein synthesis rates, is presented in the text. The sources of these data are cited in McCarthy and Houlihan (1997) and are also available from the corresponding author. The average white muscle $k_s$ values at 5, 8, 11, and 14°C for the juvenile Atlantic wolffish in this study are indicated by the solid circles.




In this study, although whole-body fractional rates of protein synthesis were higher than those in the white muscle (Figs. 1*b* and 1*c*), both exhibited a similar response to water temperature as indicated by the similar slope coefficients (see captions to Figs. 1*b* and 1*c*, respectively). McCarthy and Houlihan (1997) have also shown a common temperature response when comparing the general effect of temperature on protein synthesis in the liver and muscle of ectotherms and endotherms (their fig. 3*a*). Despite significant differences in relative rates at any given temperature, protein synthesis rates being higher in the liver compared with the muscle, similar slope coefficients were observed for the two tissues. However, in contrast with the protein synthesis results, liver and muscle $k_{RNA}$ values did not differ and there was a single temperature response (McCarthy and Houlihan 1997, their fig. 3*b*): this single temperature response was also seen in the whole-body and white muscle $k_{RNA}$ values calculated in this study (Fig. 3*b*). At any given temperature, RNA concentrations are known to vary significantly between different tissues, when expressed on a weight-specific basis (milligrams RNA per gram) or as the capacity for protein synthesis ($C_s$, milligrams RNA per gram protein), in both ectotherms (e.g., Fauconneau and Arnal 1985; Foster et al. 1991; Martin et al. 1993) and endotherms (e.g., Garlick et al. 1980, 1983; Sugden and Fuller 1991). Therefore, it appears that tissue-specific differences in RNA concentrations, rather than tissue-specific differences in RNA translational efficiency, are responsible for the differences in relative synthesis rates observed between tissues. However, both tissue and whole-body fractional protein synthesis rates and tissue and

**Table 2.** Comparison between the fractional protein growth rate ($k_g$, %·day$^{-1}$) and protein synthesis retention efficiency ($k_g/k_s$, %) in the white muscle (WM) and whole body (WB) of juvenile Atlantic wolffish and salmonid fish.

| Species | Body weight (g) | Temperature (°C) | | $k_g$ | $k_g/k_s$ | Reference |
|---|---|---|---|---|---|---|
| Atlantic wolffish (*Anarhichas lupus*) | 65 | 9–11 | WB | 0.7 | 51 | This study |
| | | | WM | 0.9 | 92 | This study |
| Rainbow trout (*Oncorhynchus mykiss*) | 65 | 10 | WB | 0.9 | 35 | McCarthy et al. (1994) |
| | | | WM | 1.0 | 67 | I.D. McCarthy et al. (unpublished) |
| | 65 | 12 | WM | 0.4[a] | 73[a] | Houlihan et al. (1986) |
| | 75 | 12 | WB | 2.0 | 45 | Foster et al. (1991) |
| | 108 | 10 | WB | 0.9 | 30 | Fauconneau and Arnal (1985) |
| | | | WM | 1.3 | 52 | Fauconneau and Arnal (1985) |
| Atlantic salmon (*Salmo salar*) | 37 | 12 | WB | 1.6 | 53 | Owen et al. (1999) |

**Note**: The body weight and temperature for each study are also presented.
[a]Calculated from equations presented in table 2 of Houlihan et al. (1986).

whole-body $k_{RNA}$ values appear to follow a common temperature response as indicated by similar slope coefficients. Also, for both protein synthesis and $k_{RNA}$, a single line for both ectotherms and endotherms indicates a commonality in these processes controlled simply by temperature.

The study of protein synthesis and protein growth is of particular relevance to aquaculture of finfish species, where the aim in culture is essentially to maximise white muscle protein deposition. As a result, much of the work on protein synthesis and growth in fish has been carried out on salmonids (e.g., Fauconneau and Arnal 1985; Houlihan et al. 1986; Carter et al. 1993; McCarthy et al. 1994; Owen et al. 1999), as these are the dominant culture species in cold-water aquaculture. The suitability of any new finfish species for cold-water aquaculture will be, in part, dependent on its growth performance, in terms of growth rate and growth efficiency, in comparison with salmonids. Therefore, the results obtained for Atlantic wolffish in this study were compared with those of salmonid fish of a comparable size at a similar water temperature. Table 2 provides a comparison of the whole-body and white muscle fractional protein growth rates and protein synthesis retention efficiencies for juvenile Atlantic wolffish at 9–11°C (i.e., the range covering their $T_{opt.G}$ and $T_{opt.GE}$ values) with values obtained for salmonid fish of a comparable size (40–100 g) at a similar water temperature (10–12°C). The whole-body and white muscle fractional protein growth rates of juvenile Atlantic wolffish recorded in this study were lower than most of the growth rate values for salmonid fish of a comparable size presented in Table 2. The growth rate of juvenile Atlantic wolffish is significantly influenced by factors such as feed composition, dietary protein source, and the physical characteristics of the pellets (floating or sinking) (reviewed in Moksness and Pavlov 1996; McCarthy et al. 1998). However, increased growth rates will be possible as the optimal dietary formulation and pellet design for juvenile Atlantic wolffish become known and rearing conditions are optimised (McCarthy et al. 1998). The protein synthesis retention efficiencies for juvenile Atlantic wolffish recorded in this study were higher for both the whole body (51% cf. 35–53%) and white muscle (92% cf. 52–73%) compared with salmonid fish at a similar water temperature. This increased growth efficiency, particularly in the white muscle, may be due to a reduction in energy expenditure as a result of the behaviour of wolffish under culture conditions. In comparison with salmonid fish, Atlantic wolffish exhibit much less swimming activity with both juveniles and adults spending long periods of time inactive on the tank bottom (reviewed in Moksness and Pavlov 1996). Also, the only phase of the life cycle where any overt aggression has been observed is as small juveniles, 50–100 mm in length. However, in comparison with salmonid fish, the frequency of aggression is much lower (reviewed in Moksness and Pavlov 1996) and no consequent body injuries have been reported to date. This reduction in aggression will both lower the energy costs associated with activity and reduce the negative effects of stress on intermediary metabolism (Van der Boon et al. 1991) in Atlantic wolffish. The growth performance results of this study further highlight the potential of Atlantic wolffish as an alternative species for cold-water aquaculture in northern Europe and Atlantic Canada.

Houlihan et al. (1995*a*) have suggested that the pattern of protein turnover and protein retention efficiency may vary between fish species that differ in their lifestyle. For example, herbivorous fish appear to be able to better utilise a wider range of diets, exhibiting higher rates of protein synthesis and protein growth and increased protein retention efficiencies, compared with carnivorous fish (Houlihan et al. 1995*a*). The results of this study also suggest that differences in protein metabolism may exist between sedentary and active fish species. Whole-body protein synthesis retention efficiencies ($k_g/k_s$) of 51–77% have been recorded for sedentary fish species such as Atlantic cod (*Gadus morhua*) (53%, Houlihan et al. 1995*a*), Atlantic wolffish (51%, this study), and Atlantic halibut (77%, Fraser et al. 1998). In comparison, whole-body protein synthesis retention efficiencies of 30–53% have been reported for the more active salmonid fish (Table 2). Also, this study has shown a higher white muscle protein synthesis retention efficiency in a sedentary species, 92% for Atlantic wolffish, compared with values of 52–73% for more active salmonids. This increased efficiency may be a result of lower turnover rates of muscle myofibrillar proteins in a less active fish. However, to test this hypothesis, a comparative study of myofibrillar growth

and turnover rates in a sedentary and active fish species is necessary.

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