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Original article

Effect of simultaneous variation of weight, density, temperature and O2 concentration on rainbow trout *(Oncorhynchus mykiss)* body composition

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Summary — The simultaneous effect of weight, initial density (kg/m^3, temperature and O_2 concentration on rainbow trout body composition (fat, protein, moisture and ash) has been studied. In 3 successive experimental phases fish were kept in different lots of varying initial weight (178–372 g), initial density (7.2–38.8 kg/m^3) and temperature (15–20 °C). Simple correlations were statistically significant for weight *vs* fat ($r = 0.56$; $P < 0.001$) and moisture ($r = -0.57$; $P < 0.001$); temperature *vs* fat ($r = 0.73$; $P < 0.001$) moisture ($r = -0.73$; $P < 0.001$) and ash ($r = -0.26$; $P < 0.02$); and O_2 concentration *vs* fat ($r = 0.22$; $P < 0.05$). Multivariant equations for the different compounds were obtained. Only fat and moisture percentages showed significant differences ($r_m = 0.75$; $P < 0.00005$); an inverse relation existing between them ($r = -0.94$; $P < 0.001$). Temperature is the factor which has the strongest influence on fat and moisture when it varies simultaneously with weight, initial density and O_2 concentration, which is shown by its equation coefficients ($P < 0.00005$).

rainbow trout / body composition / temperature / weight / density / O_2 concentration

Résumé — Effet de différents facteurs sur la composition corporelle de la truite arc-en-ciel. *L'effet simultané du poids, de la densité de population initiale (kg/m^3), de la température et de la concentration d'oxygène a été étudié sur la composition corporelle de la truite arc-en-ciel (graisses, protéines, humidité et cendres). Les poissons ont été maintenus pendant 3 périodes expérimentales successives en lots séparés différant par le poids initial (178–372 g), la densité initiale (7,2–38,8 kg/m^3) et la température (15–20 °C).*
Les corrélations simples sont statistiquement significatives entre le poids des animaux et le pourcentage des graisses ($r = 0,56$; $P < 0,001$) *et celui de l'humidité* ($r = -0,57$; $P < 0,001$), *et entre la température et le pourcentage des graisses* ($r = 0,73$, $P < 0,001$), *de l'humidité* ($r = -0,73$; $P < 0,001$) *et des cendres* ($r = -0,26$, $P < 0,02$).
Des équations multivariantes ont été obtenues pour les différentes composantes. Seuls les pourcentages de graisse et d'humidité changent significativement en relation inverse ($r = -0,94$, $P < 0,001$), *sous l'effet des facteurs considérés* ($r_m = 0,75$, $P < 0,00005$).

Quand les facteurs changent tous ensemble, la température est le facteur qui influence le plus les pourcentages de graisses et d'humidité, ce qu'on peut observer par ses coefficients des équations (P < 0,000 05).

truite arc-en-ciel / composition corporelle / température / poids / densité de population / concentration d'oxygène

INTRODUCTION

The chemical composition of the whole body is often used as an index of quality in fish (Reinitz, 1983).

A great number of factors has been shown to influence body composition (Love, 1957; Weatherley and Gill, 1983a, 1983b). Parker and Varstone (1966) have described changes in composition according to the size, age, diet and particular life stages of young pink salmon *(Oncorhynchus gorbuscha)*.

Steffens (1979) has shown that the edible portion (muscle + tegument) of older rainbow trout contains a greater proportion of lipids and dry substance than their younger counterparts.

As fish increase in weight, their water and protein percentages decrease and those of fat increase (Papoutsoglou and Papaparaskeva-Papoutsoglou, 1978), a negative correlation between relative water content and lipid content thus being established (Brett *et al,* 1969; Love, 1970; Kausch and Ballion-Cusmano, 1976). Denton and Yousef (1976) and Staples and Nomura (1976), have also demonstrated a tendency in older and heavier examples of rainbow trout for fat depot.

Without actually determining the influential factors Papoutsoglou *et al* (1979) have shown that stock density not only affects food consumption and growth but also body composition. Protein content fluctuates within normal values, although an increase in this parameter can be noted at maximum fish density while the opposite seems to occur at minimum density. Similar fluctuations appear for total lipid percentages (Papoutsoglou *et al,* 1980).

The component most influenced by temperature seems to be lipids. In general, fish fat deposits develop in parallel to temperature for the same level of fat in the diet (Murray *et al,* 1977; and Leatherland *et al* (1977) and Reinitz (1980) agree as to the smaller amount of body fat and the greater water content found in trout cultivated in colder temperatures.

Several studies on the factors that influence body composition have been performed but not when there is simultaneous variation in diverse factors. This leads to a specific approach.

A series of studies on the multifactorial effect of simultaneous variation in weight, density, temperature and O_2 concentration (within ranges considered as normoxia) on several aspects of rainbow trout *(Oncorhynchus mykiss)* were carried out and the influence on body composition reported.

MATERIAL AND METHODS

Rainbow trout *(Oncorhynchus mykiss)* from the Navarra fish farm, Riofrio (Granada) were used.

The adaptation period lasted 1 month (February). Fish were kept in 200-l fibre cement tanks, with a freely flowing water circuit, which was dechlorinated by an active carbon filter with a renewal rate of approximately 10%/h. Air was pumped into the water and the concentration of O_2 measured twice a day 30 min after feeding by means of a Simplair electrode (Syland), maintained at approximately 7 ± 2 ppm. The temperature was measured at similar intervals. Photoperiod was kept constant at 10:14 (L:D). The experimental period lasted 3 months (from March to May), and under the same conditions. The water temperature varied following the natural variation from 15 °C in February to 20 °C in May.

The fish were fed 2% of their initial weight (split into 2 feeds at 10.00 and 19.00) using a commercial trout feed (Cipasa) of the following composition: 40% protein; 7.75% fat; 9.5% moisture; 14% ash and 28.75% NFEM.

The experimental period was divided into 3 phases of 20, 38 and 26 days. At the beginning, 150 trout were weighed and distributed into 6 lots according to their size. After the first phase, fish were weighed to obtain their final weight. In the second phase, the trout were assigned to 6 new lots according to their new size. Food intake was also adapted to the new initial weight (2%). After this phase fish were weighed again. The same procedure was carried out in the third phase, obtaining a total of 19 lots, with an average initial weight which varied between 178 and 372 g. The lowest density was 7.2 kg/m^3 and the highest 38.8 kg/m^3. The characteristics of each lot appear in table I. Before each weighing, fish were starved for 20–24 h.

To determine body composition, 4 or 5 individuals were taken from each lot at the end of each experimental phase. Fish were triturated, homogenized and analyzed individually as follows:

Protein : determined by the Kjeldahl method using a mixture of potassium sulphate, copper sulphate and selenium as catalyzer. The factor 6.25 was used for the transformation of nitrogen into protein.

Fat : determined by extraction with ethyl ether in a Soxtec system HTC extractor.

Moisture : determined after dessication at 105 ± 1 °C until constant weight was reached.

Ash : determined by incineration in a muffle oven at 450 ± 2 °C until constant weight was reached.

Table I. Description of lots and experimental conditions.

L	W_1	*WR*	d	D_1	O_2	T
1	178.8 ± 1.22	165–185	20	24.1	6.1 ± 1	15.3 ± 0.5
2	206.5 ± 0.43	205–210	20	29.9	5.9 ± 0.5	15.2 ± 0.5
3	217.6 ± 0.96	215–225	20	20.6	6.6 ± 1	15.1 ± 0.5
4	220 ± 0.70	215–225	20	27.5	6.1 ± 1	15.3 ± 0.5
5	231.5 ± 0.52	230–235	20	23.1	6.9 ± 1	15 ± 0.5
6	242 ± 0.93	235–250	20	29.0	6.7 ± 1	15 ± 0.3
7	232.5 ± 2.41	205–250	38	25.5	7 ± 2	16.8 ± 2
8	256.4 ± 1.45	245–275	38	30.7	6.8 ± 1.5	16.7 ± 1.5
9	266 ± 2.46	250–285	38	18.6	7.5 ± 1.5	16.5 ± 1.5
10	272.7 ± 1.59	255–290	38	27.2	6.7 ± 1.5	16.7 ± 1.5
11	281 ± 2.01	260–290	38	21.0	7.5 ± 2	16.5 ± 1.5
12	295.7 ± 2.62	275–320	38	28.1	7.4 ± 1.5	16.5 ± 1.5
13	248.3 ± 8.26	200–280	26	11.1	7.5 ± 2	19.7 ± 2
14	295.5 ± 4.47	260–320	26	25.1	6.3 ± 2	20.7 ± 2
15	317.1 ± 4.72	295–330	26	11.1	7.8 ± 2	20.7 ± 2.5
16	342.6 ± 3.67	315–355	26	25.7	6.7 ± 2	20.6 ± 2
17	353.1 ± 4.49	310–380	26	38.8	5.8 ± 2	20.6 ± 2.5
18	363.7 ± 2.33	360–370	26	7.2	8.1 ± 2	20.6 ± 2.5
19	372.2 ± 14.7	295–430	26	16.7	6.7 ± 1.5	20.7 ± 2

L = No lot; W_1 = initial average weight ± SE in g; WR = weight range in g; *d* = No of days; D_1 = Initial density kg/m^3; O_2 = oxygen concentration ± range (ppm); *T* = temperature ± range °C.

To study the interrelationship of protein, fat, moisture, and ash percentages and their dependence on weight, density, temperature, and O_2 concentration – when these factors varied simultaneously –, a simple and a multiple correlation and regression analysis were made by means of a BMDP4M program (Dixon and Brown, 1982) in a terminal connected to the Murcia University Data Processing Centre.

RESULTS

Table II shows the results of mean body composition corresponding to trout taken from each lot. The most notable variations are in body fat and moisture content.

Fat had a significant negative correlation with moisture content (table III). The simple correlation of protein with fat was similar, but with a smaller determination coefficient (r^2). Protein had a significant positive correlation with moisture content, with the smallest determination coefficient.

Temperature had a significant positive correlation with fat and a negative correlation with moisture content (table IV). The simple correlation of weight with both variables was similar but with a smaller determination coefficient and thus a smaller dependence. There was also a negative simple correlation between temperature and total mineral percentage, but with a determination coefficient of 6.74%.

Table II. Results for body composition. These data correspond to the average values calculated in each lot by the analysis of a trout sample.

L	ΔW	W_a	*Protein (%)*	*Fat (%)*	*Moisture (%)*	*Ash (%)*
1	49.1	210 ± 6.08	18.03 ± 0.16	6.92 ± 0.70	72.27 ± 0.63	2.72 ± 0.20
2	47.6	237.4 ± 2.41	17.86 ± 0.73	7.78 ± 0.65	72.18 ± 0.46	2.49 ± 0.15
3	45.5	252 ± 4.56	18.29 ± 0.15	7.15 ± 0.41	72.67 ± 0.36	2.66 ± 0.13
4	49.4	249.4 ± 3.93	17.90 ± 0.36	8.54 ± 0.88	70.92 ± 0.84	2.42 ± 0.11
5	48	275.4 ± 8.94	18.32 ± 0.53	7.52 ± 0.52	71.97 ± 0.49	2.59 ± 0.32
6	50.9	285 ± 3.30	18.32 ± 0.34	7.65 ± 0.84	72.61 ± 0.78	2.65 ± 1.05
7	48.8	266.2 ± 5.54	17.91 ± 0.13	9.28 ± 0.54	69.76 ± 0.49	2.86 ± 0.29
8	69.5	327.5 ± 10.3	17.95 ± 0.26	8.84 ± 0.52	70.90 ± 0.58	2.55 ± 0.13
9	81.5	335 ± 7.01	18.14 ± 0.64	8.06 ± 0.58	71.76 ± 0.35	3.06 ± 0.31
10	80	330 ± 4.07	17.90 ± 0.37	9.85 ± 0.95	69.98 ± 0.84	2.39 ± 0.14
11	97.3	341.2 ± 6.58	18.25 ± 0.07	9.62 ± 0.91	70.17 ± 0.68	2.49 ± 0.13
12	93.7	375 ± 7.90	18.01 ± 0.22	9.94 ± 0.81	69.73 ± 0.77	2.34 ± 0.19
13	31.1	281.2 ± 10.0	17.78 ± 0.18	10.79 ± 1.22	70.05 ± 0.81	1.91 ± 0.16
14	30	330 ± 16.1	17.87 ± 0.22	11.11 ± 0.45	69.00 ± 0.43	2.16 ± 0.31
15	54.3	348.7 ± 12.9	18.44 ± 0.37	12.50 ± 0.63	67.50 + 0.85	2.39 ± 0.19
16	45	361.2 ± 17.0	17.89 ± 0.38	12.49 ± 1.49	67.65 ± 0.84	2.51 ± 0.10
17	26.9	368.7 ± 11.6	18.07 ± 0.22	11.51 ± 0.32	68.43 ± 0.32	2.37 ± 0.19
18	38.8	375 ± 13.2	18.28 ± 0.13	10.83 ± 1.46	69.27 ± 0.94	2.59 ± 0.28
19	47.8	367.5 ± 24.1	17.43 ± 0.18	13.97 ± 0.97	66.45 ± 0.76	2.28 ± 0.19

Lots 1, 2, 3, 4, 5 and 6 (n = 5); lots 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 19 (n = 4); lot 18 (n = 3). L = No of lot; ΔW = weight increase in g; W_a = average weight of sample ± SE in g.

Table III. Simple correlation *(r)* and determination (r^2) coefficients between percentages of protein *(P)*, fat *(F)*, ash *(A)* and moisture *(M)*.

	P		F		A	
	r	r^2	r	r^2	r	r^2
F	−0.31 [b]	9.61	–	–	NS	–
A	NS	–	NS	–	–	–
M	0.23 [a]	5.29	−0.94 *	88.36	NS	–

[a] $P < 0.05$; [b] $P < 0.005$; * $P < 0.001$; NS : not significant; $n = 81$.

Table IV. Simple correlation (r) and determination (r^2) coefficients between dependent variables: percentages of protein (P), fat (F), ash (A) and moisture (M); and independent variables: weight (W), initial density (D_1), temperature (T) and O_2 concentration (O_2).

	W		D_1		O_2		T	
	r	r^2	r	r^2	r	r^2	r	r^2
P	NS	–	NS	–	NS	–	NS	–
F	0.56*	31.36	NS	–	0.22[a]	4.84	0.73	53.29
A	NS	–	NS	–	NS	–	−0.26b	6.76
M	−0.57*	32.49	NS	–	NS	–	−0.73*	53.29

[a] $P < 0.05$; [b] $P < 0.02$; * $P < 0.01$; NS : not significant.

Only body fat percentage was significantly correlated with O_2 concentration, although its determination coefficient indicated a very low dependence.

The multiple correlation table (table V) illustrates that only fat and moisture percentages were affected when all 4 factors were taken into account, although in both

Table V. Multiple correlation *(r_m)* and determination (r^2) coefficients between dependent variables: percentage of protein *(P)*, fat *(F)*, ash *(A)* and moisture *(M)*; and independent variables: weight *(W)*, initial density *(D_1)*, O_2 concentration *(O_2)* and temperature *(T)*; and corresponding regression coefficients next to their degree of significance *(P)*.

	r_m	r^2	b_0	b_1(W)	b_2(D_1)	b_3(O_2)	b_4(T)
P	0.16	2.56	16.67	$-0.74 \cdot 10^{-3}$	0.009	0.258	−0.025
F	0.75*	56.25	−2.33	0.009	−0.014	−0.306	0.651*
A	0.29	8.41	2.44	$-0.19 \cdot 10^{-3}$	0.005	0.129	−0.054
M	0.75*	56.25	82.8	−0.007	−0.114	0.060	−0.602*

* $P < 0.00005$; $n = 81$.

cases only the regression equation coefficient corresponding to temperature was significant. The equations obtained for the multiple regression analysis are :

$$\% F = -2.33 + 0.009 \times W - 0.014 \times D_I - 0.306 \times O_2 + 0.651 \times T$$

$$\%M = 82.8 - 0.007 \times W - 0.114 \times D_I + 0.06 \times O_2 - 0.602 \times T$$

DISCUSSION

In general, tissue final composition is influenced by a variety of factors, the most important of them being the type of food and the environmental characteristics. This composition determines the nutritive value and palatability of the fish.

The results obtained in our experiment are comparable, at least when the experimental characteristics (temperature, initial density and O_2 concentration) are similar to those obtained by other authors (Reinitz, 1983; Weatherley and Gill, 1983b). It should be noted that experimental conditions in this study were within a very narrow range. Thus, neither weight, O_2 concentration ranges nor duration of the experiment permit the results for rainbow trout to be extrapolated to all situations.

In our study, only weight and duration of the experiment were controlled factors, while temperature, density and O_2 concentration were not. This design permitted an approximation to the practical conditions occurring in fish farming.

Of all the components analyzed, fat and moisture clearly varied between the different lots (table II), and their variations were observed to present an inverse correlation (table III), as described by other authors (Brett *et al,* 1969; Love, 1970; Kausch and Ballion-Cusmano, 1976; Kim *et al,* 1989). Protein percentage decreased as lipids increased, and showed little correlation with water content, as also observed by other authors (Weatherley and Gill, 1983b).

These changes were more pronounced as temperature rose (as noted in previous studies; Murray *et al,* 1977), and as weight increased (table IV), and as noted by other authors (Storebakken and Austreng, 1987a,b; Hepher, 1988). However, when all factors were taken into consideration (table V), the main factor responsible for the increase in fat and decrease in moisture was the rise in temperature. The influence of weight was overshadowed by the strong effect of temperature.

As is already known, the storage of fat in the tissues is accompanied by water decrease, which explains the inverse relation between both percentages obtained here. Our results do not permit a conclusion to be made on whether the effect of temperature concerns the fat or the moisture. It should also be noted that the influence of temperature results from its joint effect with the other factors: weight, density and O_2 concentration.

There is evidence that thermal tolerance at high temperatures is related to a reduction in free body water (Fry, 1957); in this manner, in ectotherms temperature acclimation and tolerance are closely associated with factors which govern their osmoregulation. An increase in elimination of urine results from an increase in cardiorespiratory activity (Houston, 1973). If the effect of temperature was only on moisture, all components would consequently increase, but our results show a decrease in protein percentage as moisture content falls.

Stomachal evacuation rate increases with temperature have been observed (Jobling and Spencer, 1979). This could result in lower diet digestibility; apparent digestibility coefficient (ADC), however, is not significantly influenced by water temperature (9–18 °C) in rainbow trout (Cho and Kaushik, 1990).

We have shown that the experimental conditions reported in this paper result in a lower growth and diet utilization efficiency (Martinez *et al*, 1992a, b), temperature being the most influential factor. This is in agreement with the findings of other authors (Creach *et al*, 1981–1982; Vellas *et al*, 1982; Zanuy and Carrillo, 1985). This fact requires a reduction in feeding level. To exclude other variables, maintaining a constant feeding rate was preferred in our study.

Jackim and La Roche (1973) found in *Fundulus heteroclitus* that protein synthesis increased with increase in temperature up to a critical point, beyond which it decreased sharply. In our study we consider that protein synthesis is depressed in fish subject to higher temperatures.

This "excess" energy could be converted into triglycerides which would give rise to fat deposits. The increase in fat (mainly peri-intestinal) observed in our study could be accounted for by that excess energy.

This work supports the view that the complexity of interaction of environmental factors renders necessary many more multifactorial experiments incorporating physical and biological factors (Hutchinson, 1976) to "improve predictive powers of assessing ecological consequences of altered thermal environments" (Brett, 1969).

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