EFFECTS OF BODY SIZE AND TEMPERATURE ON PERIODICITES IN FEEDING AND GROWTH OF BROOK TROUT (SALVELINUS FONTINALIS) AND IN AMMONIA CONCENTRATION OF WATER

D Harper, H Martins, D Boisclair, P Dutilleul

VIE ET MILIEU, 1999, 49 (4) : 261-273

EFFECTS OF BODY SIZE AND TEMPERATURE ON PERIODICITIES IN FEEDING AND GROWTH OF BROOK TROUT *(SALVELINUS FONTINALIS)* AND IN AMMONIA CONCENTRATION OF WATER

D. HARPER, H. MARTINS*, D. BOISCLAIR*, P. DUTILLEUL***

** Département de sciences biologiques, Université de Montréal, C.P. 6128 Succursale Centre-Ville, Montréal, Québec, Canada, H3C 3J7*
*** Department of Plant Science, Macdonald Campus of McGill University, 21,111 Lakeshore, Ste-Anne-de-Bellevue, Québec, Canada H9X 3V9*
To whom correspondence should be addressed, E-mail Daniel.Boisclair@UMontreal.ca

DAILY FOOD INTAKE
GROWTH
AMMONIA CONCENTRATION
PERIODICITIES
MULTI-FREQUENTIAL PERIODOGRAM
BODY SIZE
TEMPERATURE
PISCICULTURE

ABSTRACT. – Periodicities in daily food intake, growth, and ammonia concentration of water were estimated and compared for two size classes of brook trout (~ 5 g and > 30 g) held at two temperatures (10 and 15 °C) under laboratory conditions. Periodicities in ammonia concentration of water were also estimated for a basin containing 37,000 juvenile brook trout of 3-6 g at 8 °C under farming conditions and compared to those of the laboratory setting. A multi-frequential periodogram analysis identified short-to-intermediate periodicities in daily food intake, growth, and ammonia concentration under laboratory conditions. The predominant periods in daily food intake ranged from 7 to 12 d (R^2 = 82-95 %). Prevailing periodicities in the temporal trajectory of mean fish weight (7 and 10 d ; R^2 = 82-93 %) coincided with those estimated for daily food intake. Ammonia concentration in the experimental tanks had predominant periods of 7-8, and 12 d (R^2 = 86-99 %), coinciding with both the daily food intake and the trajectory of mean fish weight. Periods were similar at both temperatures for both size classes, illustrating the absence of an effect of body size and temperature on periodicities in daily food intake, growth, and ammonia concentration. In the fish farm, periodic components of the ammonia concentration in basin jointly explained 96 % of the total variation of the data. The predominant period was 7-8 d, supporting the results obtained in laboratory.

INGESTION JOURNALIÈRE
CROISSANCE
CONCENTRATION D'AMMONIAQUE
PÉRIODICITÉS
PÉRIODOGRAMME MULTI-FRÉQUENTIEL
TAILLE
TEMPÉRATURE
PISCICULTURE

RÉSUMÉ. – Les périodicités dans l'ingestion journalière, la croissance, et la concentration en ammoniaque de l'eau ont été estimées et comparées pour deux classes de taille de Truites mouchetées (~ 5 g et > 30 g), à deux températures (de 10 et 15 °C). De plus, les périodicités dans la concentration en ammoniaque de l'eau d'un bassin de pisciculture contenant 37 000 Truites mouchetées de 3 à 6 g à 8 °C ont été estimées et comparées à celles du laboratoire. Les résultats de l'analyse du périodogramme multi-fréquentiel ont confirmé la présence de périodicités courtes à intermédiaires dans l'ingestion, la croissance, et la concentration en ammoniaque. Les périodicités prédominantes dans les séries d'ingestion varient de 7 à 12 jours (R^2 = 82-95 %). Les périodicités de 7 et 10 jours, prévalant dans les séries dę poids moyen (R^2 = 82-93 %), coïncident avec celles d'ingestion. Les séries de la concentration en ammoniaque montrent des périodicités prédominantes de 7-8 et 12 jours (R^2 = 86-99 %), coïncidant avec les séries d'ingestion et de poids moyen. Les périodes sont similaires aux deux températures dans les deux classes de taille, illustrant l'absence d'un effet de taille et de température sur les périodicités dans l'ingestion journalière, la croissance, et l'excrétion en ammoniaque. Les composantes périodiques dans la série d'excrétion en ammoniaque à la pisciculture expliquent conjointement 96 % de la variation totale de la série, et la périodicité prédominante est de 7-8 jours, confortant ainsi les résultats obtenus en laboratoire.

INTRODUCTION

The presence of rhythms in fishes has long been established (see Ali 1992 for historical account), and circannual rhythms of growth have often been reported (Brown 1946, Swift 1955, Eriksson & Lundqvist 1982, Griffiths & Kirkwood 1995, Jensen & Berg 1995, Saether *et al.* 1996). Circasemilunar and circalunar rhythms in fish growth have also been characterized (Brown 1946, Panella 1971, Campana 1984, Wagner & McKeown 1985, Farbridge & Leatherland 1987a,b). The analysis of calcareous deposits on otoliths has also demonstrated the presence of circadian growth rhythms in fish (Panella 1971, Taubert & Coble 1977, Campana & Neilson 1982). Recently, Aboul-Hosn *et al.* 1997 elucidated non circa rhythms in the growth rate of brook trout, *Salvelinus fontinalis*. Thus, it would appear that fish have the propensity to exhibit both circa and non circa growth rhythms.

Since the growth rate of a fish under laboratory conditions is primarily determined by the rate at which food is consumed (Brett 1979), periodicities in growth should coincide with periodicities in food intake, as demonstrated by Brown 1946, Campana 1984, Farbridge & Leatherland 1987a, and Saether *et al.* 1996. Feeding practices used in fish farming contrast with the rhythmic nature of physiological processes in that the quantity of food distributed on a daily basis generally represents a constant proportion of fish biomass. The existence of rhythms suggests that fish will consume a large proportion of the feed allotted to them on some days, but on others, a fraction of the feed distributed may not be ingested. This situation may lead to a net loss of feed investment for culturists. Self-feeders or interactive feeding systems may minimize this situation. However, these systems are not always used in intensive farming and they may also be partly controlled by dominant fish (Tackett *et al.* 1988; see also Trudel & Boisclair 1993). The study of fish rhythms should not be perceived as a means to supplant demand-feeders, but more as a way to complement or guide their use.

Based on the established occurrence of rhythms in growth and food intake in fish, it can be inferred that rhythms in excretion may also exist. Ammonia, the predominant end-product of protein metabolism in fish (Fromm 1963), is excreted passively through the gills and can account for over 80 % of the nitrogenous wastes in freshwater fish (Jobling 1994). Ammonia excretion is most affected by the daily rate of protein intake (Beamish & Thomas 1984) and increases substantially after feeding (Brett & Zala 1975, Kaushik 1980, Lied & Braaten 1984). It is therefore conceivable that ammonia excretion rhythms exist and coincide with rhythms in food intake and/or growth. Indeed, if ammonia excretion rhythms exist and coincide with rhythms in food intake and/or growth, a culturist could use variations in ammonia concentrations in rearing facilities as an effective tool to determine what phase of a food intake and/or growth rhythm fish are situated. Culturists could adjust feeding strategies accordingly and increase production by allotting more food in periods of increased food intake and/or growth rates and allotting less food in periods of decreased food intake and/or growth rates. Thus, a chronobiological approach to aquaculture could be used to increase the efficiency in the production of fish (Spieler 1977, Parker 1984).

It was the purpose of this study to estimate and compare short-to-intermediate periodicities in the daily food intake and growth of brook trout and ammonia concentration in the water and determine the effect of body size (weight) and temperature on these periodicities. Periodicities in ammonia concentration estimated for a large group of brook trout at a fish farm were compared to those obtained in a laboratory setting.

2. METHODS AND MATERIALS

We compared short-to-intermediate periodicities in daily food intake, growth and ammonia concentration, and determined the effects of body size (weight) and temperature on these periodicities by monitoring daily food intake, mean weight, and ammonia concentration in the water for two size classes of brook trout for approximately one month in a laboratory setting. All experiments were replicated. The duration of our experiments was selected to correspond to the average time between two successive operations of sorting by size as practiced in fish farms (in fish farms, brook trout are sorted by size categories and restocked in different basin on a monthly basis). The length of our experiments was also selected to insure that rhythms with short to intermediate periodicities observed in previous studies (periodicities of 5 to 10 days; Aboul-Hosn *et al.* 1997) could be adequately discriminated in our work. Fish were fed once each day. Daily food intake of trout and ammonia concentration was estimated every two days on odd days of experiments (day 1, 3, 5, ...). Fish weight was estimated every two days on even days of experiments (day 2, 4, 6, ...) few hours before feeding. This strategy was adopted to avoid handling fish on days when feeding and ammonia concentration were estimated and to minimize the quantity of food from previous meal in the digestive tract of fish when estimating fish weight (maximum of 0.5 % of fish wet weight 24 h after a meal). In the fish farm, fish were also fed on a daily basis but only ammonia concentrations in the basin could be estimated over 19 days and compared to variations observed in the laboratory. Periodicities were assessed using time series analysis.

Table I. – Mean weight and weight range of replicates used in Series I & II. Comparison of the mean weights between replicates was performed using *t*-tests ($n = 46$ for each replicate).

Size Class	Replicate	Series I (15°C) Mean Weight (g)	Range (g)	*t*-test	Series II (10°C) Mean Weight (g)	Range (g)	*t*-test
I	1	4.4	3.5-5.2	$p = 0.83$	5.3	4.0-7.4	$p = 0.94$
	2	4.3	3.5-5.1		5.3	4.1-7.6	
II	1	39.2	34.1-44.8	$p = 0.84$	31.3	26.6-37.5	$p = 0.96$
	2	39.3	34.1-45.2		31.3	26.7-37.5	

2.1. Experimental tanks and operation : Two experimental environments were required to provide two different water temperatures. Experiments conducted at 15 °C (± 0.5 °C) were performed at the Départment de sciences biologiques of Université de Montréal and will be referred to as Series I. For Series I, trout were housed in 510-L flow through tanks (0.91 × 0.91 × 0.66 m). Tap water was dechlorinated using a Momentum CA1248 carbon filter before entering the tanks. Observations at 10 °C (± 0.5 °C) were performed at the Station de Biologie des Laurentides of Université de Montréal and will be referred to as Series II. The trout used in Series II were housed in 510-L flow through tanks (2.2 × 0.5 × 0.5 m). In this case, water from a nearby lake was pumped and filtered. For Series I and II, observations were made on four tanks (2 size classes × 2 replicates). A third time series, limited to the survey of ammonia concentrations in one basin of brook trout held at 8.1 °C (± 0.1 °C), was conducted at Pisciculture Simdar (Saint-Alexis-des-Monts, Québec), and will be referred to as Series III.

The rate of flow of water into all tanks of Series I and II was set at 2 $L \cdot min^{-1}$ using adjustable valves on the inlets of the tanks. Thus, the entire volume of water within the tanks was replaced about every 4 hours. All tanks were 100 % saturated with oxygen. The photoperiod was held constant for all experiments and consisted of 8-h light and 16-h dark (fluorescent light, ~ 45 lx at water surface). Fish were acclimated to the experimental conditions for two weeks. For Series III, fish were housed in a concrete raceway (3 m wide × 1 m deep × 20 m). The rate of water flow into the raceway was 660 $L \cdot s^{-1}$. The basin was 75 % saturated with oxygen, and the photoperiod was 9-h light and 15-h dark.

2.2 Experimental animals : For Series I and II, brook trout were obtained from genetically homogeneous stocks (Pisciculture Mont-Tremblant) to minimize the potential effect of genotype on ammonia excretion (Kaushik *et al.* 1984). Each experimental tank was stocked with 46 brook trout (Table I). Each group of fish was normally distributed with respect to body weight, and there was no significant difference between the mean weight of groups of fish used as replicates (*t*-test ; $p > 0.9$). For Series III, the basin contained approximately 37,100 juveniles aged 0+ (mass : 3-6 g wet).

2.3. Feeding and food composition : For Series I and II, the amount of food fed to the trout was dependent on water temperature and fish biomass. Food was always given in excess to insure that the variability of the daily food intake of the fish was independent of the food given to the fish. Allotted rations for both size classes ranged from 4.0 to 7.0 % body weight per day of Martin Starter Pellets. Replicates were always allotted the same amount of feed on any given day. Fish were fed once a day at 0930 and were given 30 minutes to ingest the given amount (fish would stop eating approximately 20 min after being fed). Feeding regime (1 meal per day) was selected to correspond to that frequently employed by fish farmers for brook trout of the sizes used in our study at the experimental temperatures. Excess food was then retrieved with a fine-meshed fish net and dried in an oven at 60 °C for 24 h. Preliminary experiments showed that the mass of the dried excess obtained in this manner should be multiplied by a correction factor of 1.3 to obtain the wet weight (i.e. the weight of the food when taken out of the feed bag). Thus, the daily food intake (wet $g \cdot day^{-1}$) by any group of fish was defined as the amount given subtracted by the amount leftover after 30 min (after drying and corrections). Daily food intake is presented as a two-day average by combining the intake the day prior to and the intake of the day of ammonia determinations. Daily food intake was calculated in this fashion because parallel experiments demonstrated that food intake on the day prior to an ammonia determination had a significant effect on the ammonia concentration in the water the following day (Harper 1997). The brook trout of Series III were fed Moore Clark feed provided at uneven intervals for eight hours each day by an automatic feeder (1.5 % of fish biomass).

2.4. Weight determination : For Series I and II, trout were weighed prior to the commencement of a series and after the completion of a series. Estimates of the mean weights of the replicates were obtained every two days within a series by sampling 20 fish from each tank. Estimates were obtained on days opposite to those where ammonia concentrations were determined. Every sixth weighing period within a series, all the fish of a given size class were weighed, providing the mean weight of each replicate. A computer simulation indicated that sampling 20 of the 46 fish would be sufficient to attain a mean weight for a given group that is within 1 SD of the "real" mean about 80 % of the time. The average intial weight of the brook trout of Series III was approximately 3 g and the average final weight was 6 g.

2.5. Ammonia determination : To establish an appropriate sampling time to estimate ammonia concentration, we performed 24-h experiments where ammonia concentrations were sampled every hour subsequent to feeding in order to elucidate a possible peak in ammonia concentration. Peak ammonia concentrations were recorded approximately 6 hours after feeding at 15 °C and approximately 8 h after feeding at 8-10 °C. Sampling at the time of a peak in ammonia concentration within a tank ensures consistency in relating ammonia to food intake from tank to tank. Every two days within Series I and II, four 500 ml water samples were removed from each aquarium at 1600 for Series I and at 1800 for Series II. For Series III, six 500 ml water samples were removed daily from the basin at 1800. One 50 ml subsample from each 500 ml sample was then analyzed for ammonia using the phenol-hypochlorite method (Soloranzo 1969). For all series, a preliminary experiment was performed to test intra-tank variation in ammonia concentrations ; ten water samples per rearing facility were removed at the appropriate sampling time and analyzed for ammonia. The results demonstrated that four water samples per tank in Series I and II and six water samples for the basin of Series III would be sufficient to account for the intra-tank variation of ammonia concentrations (CV of 3.0 %). Moreover, water samples were obtained from different parts of the tanks to account for potential intra-tank variation.

2.6. Statistical analysis : Dutilleul's multi-frequential periodogram and the related stepwise procedure (Dutilleul 1990, Legendre & Dutilleul 1992, Dutilleul 1998, Legendre & Legendre 1998) were used to detect the number and value of periodicities in daily food intake, mean weight, and ammonia concentration. The multi-frequential periodogram analysis can reveal several periods in a given trajectory ; however, these periods may not correspond to distinct rhythms. In fact, several periodic components may compose one true rhythm. For example, if periods of 6, 8, and 12 d are revealed for the replicate 1 series of a given variable in a given size class, the periods of 8 and 12 d will be interpreted as the true periods, and the 6 d period will be interpreted as a component of the 12-d period (Fig. 6a in the appendix). Moreover, if periods of 10.78 d and 13.54 d are revealed for the replicate 2 series of the same variable in the same size class, they will be interpreted as a pseudo-periodic signal with an approximate 12-d period because of the predominant 12-d period of replicate 1 (Fig. 6b in the appendix).

3. RESULTS

3.1. Series I

The daily food intake, mean weight, and ammonia concentration trajectories of the replicates of Size Class I all displayed rhythmic patterns (Fig. 1). The multi-frequential periodogram analysis revealed a range of periodicities in daily food intake, from 4 to 13 d, and, on average, the frequency components in daily food intake of the replicates of Size Class I jointly explained 83 % of the total dispersion of the time series (Table II). The predominant period for both replicates was about 12 d.

A range in periodicities of about 4 to 10 d was observed for the mean weight series of Size Class I (Table II). The frequency components, on average, jointly explained 86 % of the total variation in the time series. The predominant periods that emerged from the mean weight rhythms of the replicates were about 9 and 10 d, slightly shorter periodicities than the 12 d period observed for the daily food intake series. The average daily growth rate for the replicates of Size Class I was 4.6 % per day. Although the rhythmicity in the mean weight series is not particularly evident in Fig. 1, peak growth rates were, on average, 4 times those observed in the trough of a cycle.

A range in periodicities of about of about 6 to 13 d was observed in the ammonia concentration series (Table II). The frequency components in ammonia concentration jointly explained 99 % of the total variation of the time series for both replicates. Periods 8 and 12 d were prevalent in the ammonia concentration series.

The mean weight and ammonia concentration trajectories of the replicates of Size Class II displayed rhythmic patterns. However, the daily food intake trajectory did not exhibit a rhythmic pattern (Fig. 2). Mean weight displayed a range in periodicities of about 4 to 14 d, and, on average, the frequency components in mean mass of the replicates of Size Class II jointly explained 86 % of the total dispersion of the time series (Table II). The predominant period for both replicates was about 9-10 d. A longer periodicity of 14 d emerged in the mean weight series of replicate 2. The average daily growth rate for the replicates of Size Class II was 2.5 % per day. Peaks in growth rates for the replicates were, on average, 3.6 times those recorded in troughs.

A range of periodicities in the ammonia concentration series of about 4 to 12 d was observed (Table II). On average, the frequency components in the ammonia concentration series of the replicates jointly explained 88 % of the total variation of the time series. Periods of about 8 and 12 d once again emerged for the ammonia concentration rhythms.

Comparing the periodic components in the mean weight and the ammonia concentration series of Size Class I and Size Class II, it is evident that their respective rhythms are similar, suggesting that body size did not have an effect on the rhythmic behaviour of the variables. For instance, the mean weight series of both size classes demonstrated a period of about 9-10 d. Moreover, periods of about 7-8 and 12 d were found for the

Table II. – Periodic components estimated at the end of the stepwise procedure involving the analysis of Dutilleul's (1990) multi-frequential periodogram for the daily food intake, mean weight, and ammonia concentration series of two size classes of brook trout. ΔR^2 in parentheses. Water temperature was 15 °C.

Size class	Variable	Replicate	Step 1	Step 2	Step 3	R^2	Range of periodicities
I	Daily food intake	1	12.46 (0.64)	36.19 N.S.*‡ (0.13)	4.91 N.S. (0.08)	0.85	4 - 13
		2	13.41 (0.46)	10.06 N.S.* (0.21)	33.22 N.S.‡ (0.15)	0.82	
I	Mean weight	1	10.55 (0.40)	4.26 (0.24)	19.29 N.S.*‡ (0.15)	0.79	4 - 10
		2	4.75 (0.58)	8.91 (0.20)	27.06 ‡ (0.14)	0.92	
I	Ammonia concentration	1	8.37 (0.66)	6.19 (0.22)	12.08 (0.11)	0.99	6 - 13
		2†	10.88 (0.47)	13.64 (0.29)	6.36 (0.23)	0.99	
II	Daily food intake	1	19.80 ‡ (0.45)	3.34 N.S. (0.19)	2.00 N.S. (0.12)	0.76	2 - 5
		2	15.02 ‡ (0.44)	5.09 N.S.* (0.24)	4.33 (0.21)	0.89	
II	Mean weight	1	9.67 (0.50)	5.51 (0.31)	4.34 (0.10)	0.91	4 - 14
		2	10.12 (0.50)	14.35 (0.31)	--	0.81	
II	Ammonia concentration	1	7.25 (0.69)	9.42 N.S.* (0.15)	28.45 N.S.*‡ (0.07)	0.91	4 - 12
		2	7.96 (0.44)	4.48 N.S.* (0.21)	12.58 (0.21)	0.86	

N.S. Non significant periods
N.S.* Significance probability $0.05 \leq P < 0.15$
† Fourth significant period not shown
‡ Frequency components corresponding to autocorrelation rather than rhythms

ammonia concentration series of both size classes. While the daily food intake trajectory of Size Class I displayed a rhythmic pattern with a period of about 12 d, the daily food intake series of Size Class II was not rhythmic. Series I, in particular Size Class I, illustrates that periods in the daily food intake, mean weight, and ammonia concentration series do, indeed, coincide.

3.2. *Series II*

The daily food intake, mean weight, and ammonia concentration trajectories of the replicates of Size Class I all displayed rhythmic patterns (Fig. 3). A range of periodicities in daily food intake of about 5 to 10 d was recorded (Table III). On average, the frequency components in daily food intake of the replicates of size class I jointly explained approximately 91 % of the total dispersion of the time series. The prevalent period for both replicates was about 7-8 d, but a period of about 10 d emerged in the series of replicate 2.

A range in periodicities of about 4 to 10 d was observed for the mean weight series of Size Class I. On average, the frequency components jointly explained about 81 % of the total variation in the time series. The predominant periods found from the mean weight rhythms of the replicates were about 7 and 10 d, similar to the periodicities observed for the daily food intake series. The average daily growth rate for the replicates of Size Class I was 3.3 % per day. Maximum growth rates for the replicates were, on average, 10 times greater than the minimum growth rates recorded.

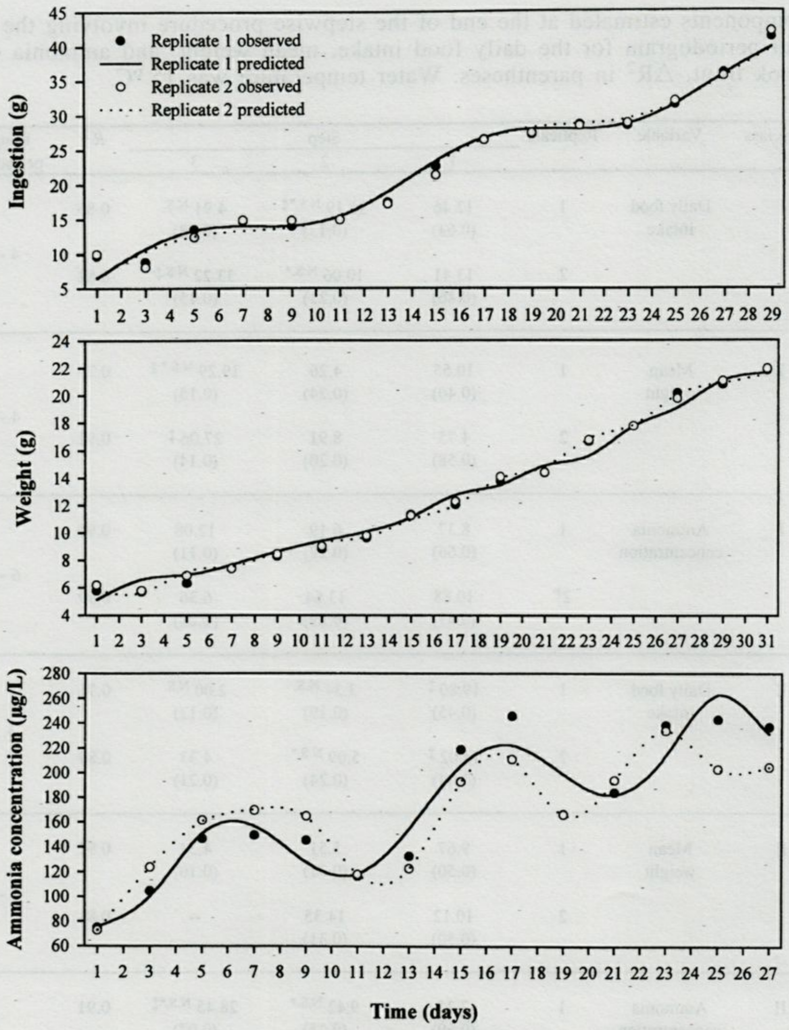

Fig. 1. – Profiles of Size class I (mean weight ≈ 4.4 g) of Series I: daily food intake (top), mean weight (middle), and ammonia concentration (bottom).

Ammonia concentration demonstrated periodicities of about 4 to 13 d (Table III), and, on average, the frequency components in the ammonia concentration series of the replicates of size class I jointly explained 88 % of the total variation of the time series. A period of 5 d emerged for both replicates. Periods of about 8 d and 13 d were also observed.

The daily food intake, mean weight, and ammonia concentration trajectories of the replicates of Size Class II all displayed rhythmic patterns (Fig. 4). Periods of about 7 and 10 d emerged in the daily food intake rhythms of replicates 1 and 2 (Table III). The periodic components accounted for about 86 % of the variation of the time series, and ranged from about 4 to 10 d. The frequency components of the daily food intake series were similar to those of Size Class I for the same variable.

Mean weight displayed a range in periodicities of about 5 to 10 d, similar to the daily food intake series, and, on average, the frequency components in mean mass of the replicates of Size Class II jointly explained 91 % of the total dispersion of the time series. The predominant periods were about 7-8 and 10 d. The average daily growth rate for the replicates of Size Class II was 1.9 % per day. Maximum growth rates for the replicates were, on average, 3.8 times greater than the minimum growth rates recorded.

Finally, a range of periodicities in the ammonia concentration series of about 6 to 12 d was recorded (Table III). The frequency components of the ammonia concentration rhythms of the replicates 1 and 2 explained, on average, 90 % of the total variation in the time series. Periods of about 8 and 12 d were once again prevalent in the ammonia concentration series.

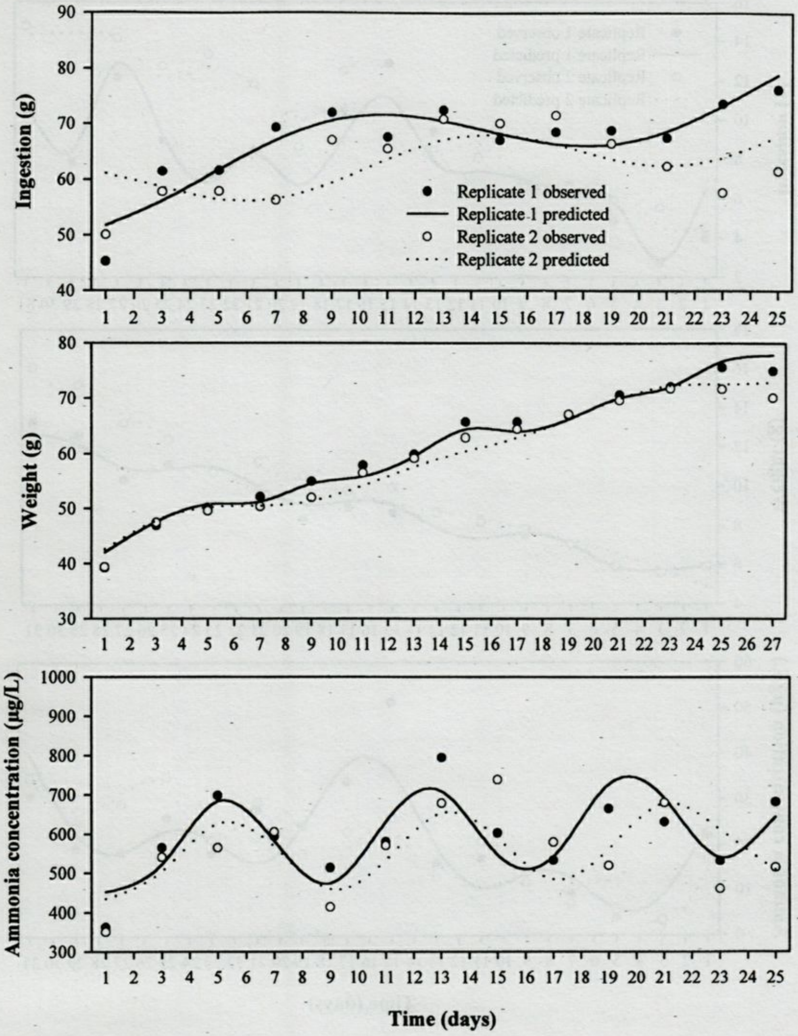

Fig. 2. – Profiles of Size class II (mean weight ≈ 39.0 g) of Series I : daily food intake (top), mean weight (middle), and ammonia concentration (bottom).

It did not appear that body size had a significant effect on the rhythmic behaviour of the variables. For both size classes, periods of 7 and 10 d emerged in both the daily food intake and mean mass series and periods of 7-8 and 12 d emerged in the ammonia concentration series. The results of Series II confirm the results of Series I; periodicities in the daily food intake, mean weight, and ammonia concentration series coincide.

3.3. A comparison of temperatures

Similar periodicities in daily food intake, mean weight, and ammonia concentration emerged at both temperatures. Periodicities of 7 and 10 d were common in the daily food intake and mean mass series at both temperatures, and in most cases, periodicities of about 8 and 12 d in the ammonia concentration series emerged at both temperatures. These results suggest that temperature did not affect the rhythms of daily food intake, growth, and ammonia concentration.

3.4. Comparison with a fish farm

Periodicities in the ammonia concentration series observed at a fish farm (Pisciculture SIMDAR; Series III) ranged from about 2 to 8 d (Fig. 5). The periodicites were 7.50, 3.67, and 2.48 d, and accounted for 96 % of the variation in the series. The first periodicity of 7-8 d accounted for 70 % of the variation in the series. The other two periodicities can be considered as components of the 7-8 d period. The results of the fish farm experiment support the laboratory experiments as similar 7-8 d periods were observed in the ammonia concentration series of Series I and Series II. Furthermore, the fact that Series I, II and III were conducted at temperatures of

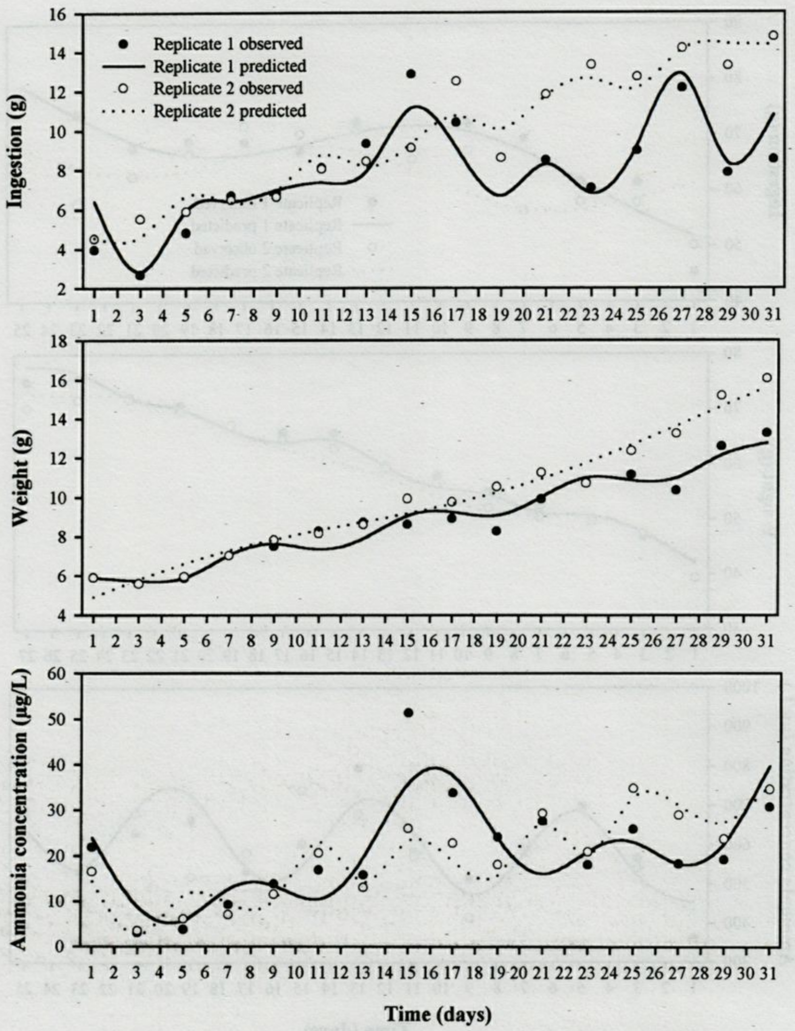

Fig. 3. – Profiles of Size class I (mean weight ≈ 5.3 g) of Series II : daily food intake (top), mean weight (middle), and ammonia concentration (bottom).

approximately 8, 10, and 15 °C, respectively, and that similar periods were observed further supports the notion that temperature did not affect the periodicities in the ammonia concentration series of the brook trout.

4. DISCUSSION

The purpose of our study was to estimate short-to-intermediate periodicities in the daily food intake, growth, and ammonia concentration in the water, and to determine the effect of body size and temperature on these periodicities. Our work demonstrates that short-to-intermediate periodicities in daily food intake, growth, and ammonia concentration exist, that they coincide with each other, and that variations in body size and temperature do not appear to affect the periodicities of the measured variables.

Our study suggests the existence of predominant short-to-intermediate periodicities of 7 and 10 d in the growth of brook trout. Short-to-intermediate periodicities in growth rate reported in other studies appear to correspond to the periodicites in growth observed in our work. Farbridge & Leatherland (1987b) reported 10 and 13-14 d periods in growth rate for the coho salmon. Yet, upon re-analysis of these published data using the multifrequential periodogram used in our study, Aboul-Hosn *et al.* (1997) further elucidated two superimposed periodicites of 8 and 10 d (R^2 = 0.70), similar to those found for brook trout in our work. We also observed short-to-intermediate periodicites in daily food intake, with predominant periods ranging from about 7 to 12 d. Others studies have elucidated the existence of intermediate periodicities in feeding. For instance, Brown

Table III. – Periodic components estimated at the end of the stepwise procedure involving the analysis of Dutilleul's (1990) multi-frequential periodogram for the daily food intake, mean weight, and ammonia concentration series of two size classes of brook trout. $\triangle R^2$ in parentheses. Water temperature was 10 °C.

Size class	Variable	Replicate	Step 1	Step 2	Step 3	R^2	Range of periodicities
I	Daily food intake	1†	16.18 ‡ (0.54)	8.47 (0.28)	6.54 (0.13)	0.95	5 - 10
		2	5.62 (0.44)	7.64 (0.25)	10.68 (0.17)	0.86	
I	Mean weight	1	7.35 (0.36)	20.77 N.S.*‡ (0.19)	10.17 (0.24)	0.79	4 - 10
		2	30.20 ‡ (0.53)	4.72 N.S.* (0.15)	6.13 N.S.* (0.14)	0.82	
I	Ammonia concentration	1	17.15 ‡ (0.49)	8.30 (0.23)	4.86 (0.15)	0.87	4 - 13
		2	5.00 (0.63)	13.45 (0.17)	21.65 ‡ (0.09)	0.89	
II	Daily food intake	1	4.85 (0.42)	6.83 (0.28)	17.42 ‡ (0.17)	0.87	4 - 10
		2	4.23 (0.36)	15.72 ‡ (0.26)	10.06 (0.22)	0.84	
II	Mean weight	1†	31.08 ‡ (0.50)	10.76 (0.28)	4.63 (0.14)	0.92	5-10
		2	31.07 ‡ (0.44)	8.38 (0.29)	6.99 (0.20)	0.93	
II	Ammonia concentration	1	7.82 (0.58)	6.00 (0.21)	12.23 (0.10)	0.89	6 - 12
		2	7.54 (0.51)	6.10 (0.26)	20.92 ‡ (0.14)	0.91	

N.S. Non significant periods
N.S.* Significance probability $0.05 \leq P < 0.15$
† Fourth significant period not shown
‡ Frequency components corresponding to autocorrelation rather than rhythms

(1946) reported biweekly patterns in the food intake of brown trout. Shorter periodicities of about 3.5 (circasemiseptan) to 7 (circaseptan) d in the food intake and growth of rats have been reported (Mercer *et al.* 1993, Temur'Yants *et al.* 1995). However, our work is the first study, to our knowledge, that has reported periodicities of about one week in the food consumption of fish. The fact that periodicities in growth coincide with periodicites in feeding is not surprising since, under laboratory conditions, growth rate is primarily determined by food intake (Brett 1979). Findings similar to ours regarding the coincidence of growth and feeding rhythms were obtained by Farbridge & Leatherland (1987a). Predominant short-to-intermediate periodicities of 7-8 and 12 d in the ammonia concentration were also demonstrated in this study. These periodicities coincide with those of daily food intake and growth. Ammonia production is intrinsically related to nitrogen metabolism (Savitz 1971, Brett and Zala 1975, Paulson 1980, Jobling 1981, Lied & Braaten 1984, Tatrai 1986). While body weight and temperature can affect ammonia excretion (Savitz 1969, Guerin-Ancey 1976, Paulson 1980, Jobling 1981, Cai & Summerfelt 1992, Forsberg & Summerfelt 1992), sampling ammonia concentrations at the peak of ammonia concentration generally gives a good indication of the amount of nitrogen consumed within a 48 hour period (Harper 1997). This is confirmed by the coincidence of periods of daily food intake and ammonia concentration.

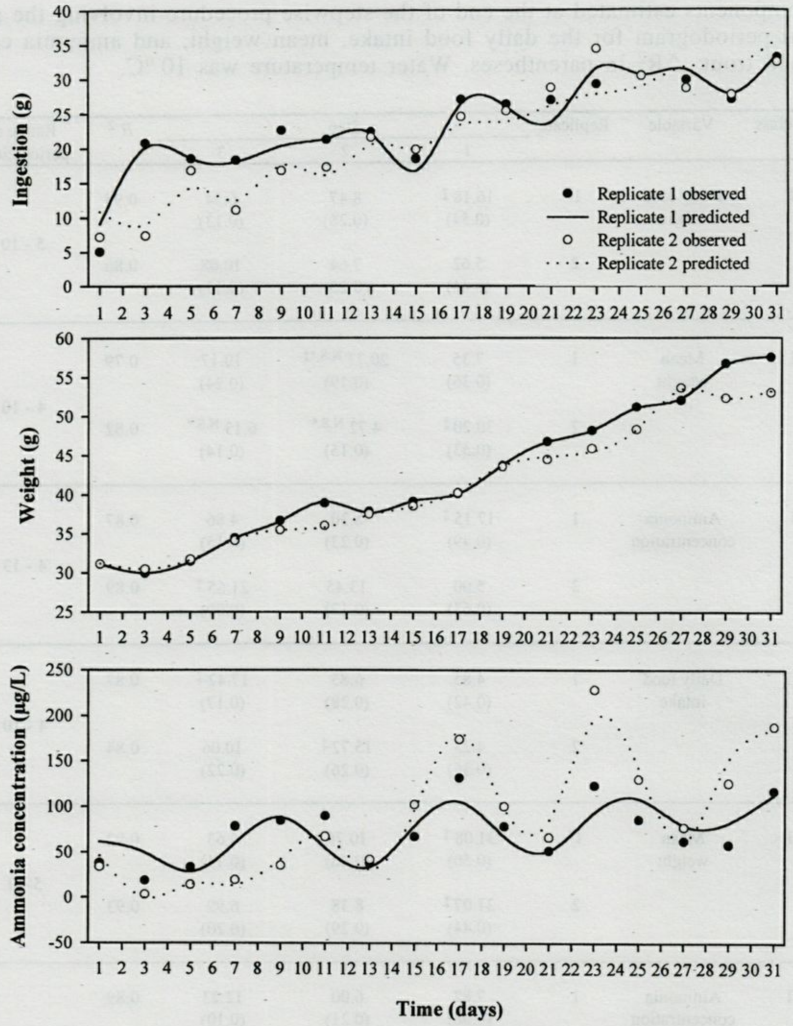

Fig. 4. – Profiles of Size class II (mean weight ≈ 31.3 g) of Series II : daily food intake (top), mean weight (middle), and ammonia concentration (bottom).

All studies that examine the interactions between growth and feeding rhythms are subjected to problems associated to the manipulation of fish that must be weighed on a regular basis. Our study is no exception. In addition, the sampling regime we adopted to weigh 50 % of the fish at two day intervals only exacerbates this situation, and further increases the probability that the rhythms we observed have nothing to do with rhythms of fish that would not be manipulated. However, we speculate that the fact that the periodicities in ammonia concentration found in the laboratory are undistinguishable from those found in a fish farm (where fish were not handled during the experiment) suggests that the rhythms we observed may reflect those of fish unaffected by manipulations. Furthermore, similarities between the rhythms in ammonia concentration in the laboratory and the pisciculture setting suggest that short-term forecasting of rhythms in food intake and growth using ammonia concentrations as a surrogate descriptor may be possible for large groups of fish in a pisciculture setting. The detection of periodicities in groups of fish homogenous in body size, similar to those found at piscicultures, may be less complicated since rates of nitrogen consumption and utilization by the fish should then also be homogenous. In the present study, groups of fish were normally distributed with respect to size to satisfy statistical analyses. However, the resulting range of body sizes may have confounded the measurements of the chosen variables since rates of ammonia excretion, nitrogen consumption, and growth for the large fish in a given group would be different from the smaller fish of the same group (Jobling 1981).

Our study was not designed to assess the ultimate cause(s) of the rhythms we observed. While our study does not allow us to draw definitive conclusions in this respect, we can nevertheless make parallel between our results and published

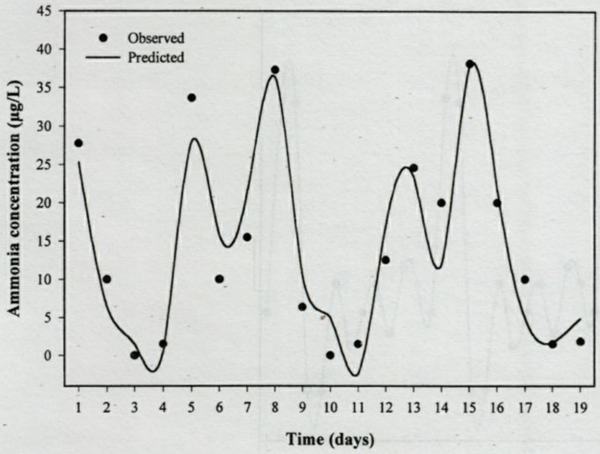

Fig. 5. – Observed (dots) and predicted (curve) ammonia concentrations in a brook trout basin in a fish farm (Pisciculture SIMDAR; Series III).

studies on the origin of the rhythms we observed. For instance, short-to-intermediate periodicities in growth rate may be related to pineal gland activity. Circasemiseptan and circaseptan periodicities in pineal gland activity have been demonstrated in rats (Mercer *et al.* 1993) and, more recently, in pike (Cornélissen *et al.* 1995). The pineal organ may play a role in mediating the effects of photoperiod which, in turn, may affect growth through variations in hormone secretions (Vodicnik *et al.* 1978, Weatherley & Gill 1987). Thus, it appears that short-to-intermediate periodicities in growth rate may be a result of controlling factors such as the endocrine system. In addition, since the periodicities we observed were apparently unaffected by water temperature, fish mass, the origin of the water (lake or aqueduct), fish density (difference between laboratory and fish farm settings), and whether or not fish were manipulated, our results are compatible with hypotheses suggesting that short to intermediate periodicities in growth and feeding may be driven by endogenous rather than exogenous factors.

ACKNOWLEDGMENTS. – This work was supported by Actions concertées FCAR-SORDAC (Fonds pour la Formation de Chercheurs et l'Aide à la Recherche, Société de Recherche et de Développement en Aquaculture Continentale Inc.). Special thanks go to Pisciculture SIMDAR for the use of their facilities and to our technicians V. Lapointe and M. Bellavance.

REFERENCES

Aboul-Hosn W, Dutilleul P, Boisclair D 1997. Use of spectral analysis to estimate short-term periodicities in growth rates of the brook trout *Salvelinus fontinalis. Can J Fish Aquat Sci* 54 : 1532-1541.

Ali MA (Editor) 1992. Rhythms in fishes. Plenum Press, New York, 348p.

Beamish FWH, Thomas E 1984. Effects of dietary protein and lipid on nitrogen losses in rainbow trout, *Salmo gairdneri. Aquaculture* 41 : 359-371.

Brett JR, Zala CA 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. *J Fish Res Board Can* 32 : 2479-2486.

Brett JR 1979. Environmental factors and growth. *In* WS Hoar DJ Randall, & JR Brett (Ed.), Academic Press, New York. *Fish Physiol* 8 : 599-677.

Brown ME 1946. The growth of brown trout (*Salmo trutta* Linn.). II. The growth of two-year trout at a constant temperature of 11.5 °C. *J Exp Biol* 22 : 130-144.

Cai YJ, Summerfelt RC1992. Effects of temperature and size on oxygen consumption and ammonia excretion by walleye. *Aquaculture* 104 : 127-138.

Campana SE 1984. Lunar cycles of otolith growth in the juvenile starry flounder *Platichthys stellatus. Mar Biol* 80 : 239-246.

Campana SE, Neilson JD 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influence of some environmental variables on their production. *Can J Fish Aquat Sci* 39 : 937-942.

Cornélissen G, Portel A, Halberg F, Billiet V, Falcón J 1995. Toward a chronome of superfused pike pineals : about-weekly (circaseptan) modulation of circadian melatonin release. *In Vivo*, 9 : 323-330.

Dutilleul P 1990. « Apport en analyse spectrale d'un périodogramme modifié et modélisation des séries chronobiologiques avec répétitions en vue de leur comparison en fréquence ». D.Sc. Dissert, Départ Mathématiques, Univ catholique, Louvain La-Neuve, Belgium.

Dutilleul P 1998. Incorporating scale in ecological experiments : Data analysis. *In* Ecological scale : Theory and applications, Peterson DL, & Parker VT (Editors), Columbia Univ Press, New York : 387-425.

Eriksson LO, Lundqvist H1982. Circannual rhythms and photoperiod regulation of growth and smolting in baltic salmon (*Salmo salar* L.). *Aquaculture* 28 : 113-121.

Farbridge KJ, Leatherland JF 1987a. Lunar cycles of the coho salmon, *Oncorhynchus kisutch*. I. Growth and Feeding. *J Exp Biol* 129 : 165-178.

Farbridge KJ 1987b. Lunar periodicity of growth cycles in rainbow trout (*Salmo gairdneri*) Richardson. *J Interdisc Cycle Res* 18 : 169-177.

Forsberg JA, Summerfelt RC 1992. Effect of temperature on diel ammonia excretion of fingerling walleye. *Aquaculture* 102 : 115-126.

Fromm PO 1963. Studies of renal and extra-renal excretion in a freshwater teleost, *Salmo gairdneri. Comp Biochem Physiol* 10 : 121-128.

Griffiths D, Kirkwood RC1995. Seasonal variation in growth, mortality and fat stores of roach and perch in Loch Neagh, Northern Ireland. *J Fish Biol* 47 : 537-554.

Guerin-Ancey O 1976. Etude expérimentale de l'excrétion azotée du bar (*Dicentrarchus labrax*) en cours de croissance. I. Effets de la température et du poids

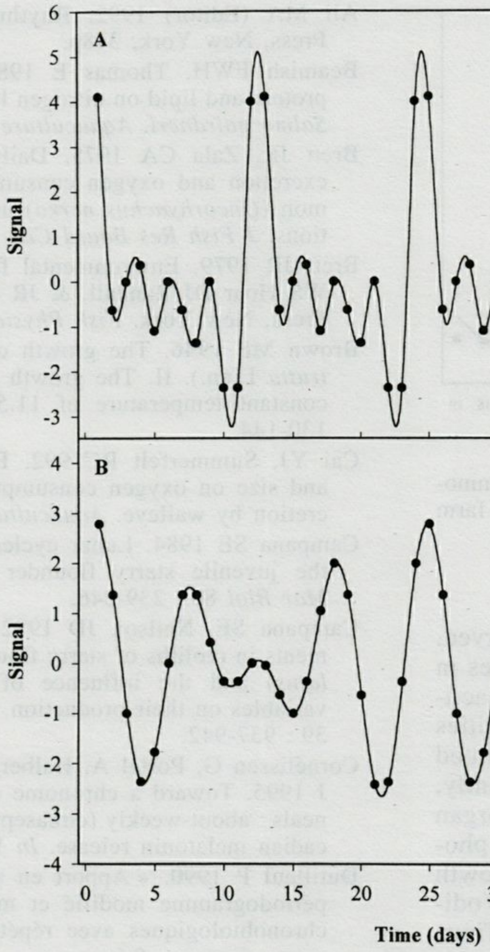

Fig. 6. – A, Periodic signal of period 12, made up as the sum of four cosine and four sine terms at periods 12, 6, 4 and 3 days. B, Pseudo-periodic signal with an average period of 7 and a non-stationary (non-constant) amplitude over time, made up as the sum of two cosine and two sine terms at periods of 8 and 6 days.

du corps sur l'excrétion d'ammoniaque et d'urée. *Aquaculture* 9 : 71-80.

Harper D 1997. Rythmes d'ingestion, de croissance et d'excrétion d'ammoniaque de l'omble de fontaine (*Salvelinus fontinalis*). M. Sc. Thesis. Univ Montréal, Montréal, 64 p.

Jensen JW, Berg T 1995. The annual growth rate cycle of brown trout. *The Progressive Fish-Culturist* 57 : 231-237.

Jobling M 1981. Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice *Pleuronectes platessa* L. *J Fish Biol* 18 : 87-96.

Jobling M 1994. Fish bioenergetics. Chapman & Hall, New York, 309 p.

Kaushik SJ 1980. Influence of nutritional status on the daily patterns of nitrogen excretion in the carp (*Cyprinus carpio* L.) and the rainbow trout (*Salmo gairdneri* R.). *Reprod Nutr Dév* 20 : 1751-1765.

Kaushik SJ, Fauconneau B, Blanc JM1984. A study of nitrogen excretion and oxygen consumption in five half-sib families of rainbow trout. *Reprod Nutr Dév* 24 : 431-438.

Legendre P, Legendre L 1998. Numerical ecology, second English edition. Elsevier, Amsterdam. 853 p.

Legendre P, Dutilleul P 1992. Introduction to the analysis of periodic phenomena. *In* Rhythms in fishes, Ali, MA (Editor), NATO ASI Series, Vol. A-236, Plenum Press, New York : 11-25.

Lied E, Braaten B 1984. The effect of feeding and starving and different ratios of protein-energy to total-energy in the feed on the excretion of ammonia in the Atlantic cod (*Gadus morhua*). *Comp Biochem Physiol* 78(A) : 49-52.

Mercer LP, Haijazi H, Hidvegi M 1993. Weanling rats display bioperiodicity of growth and food intake rates. *J Nut* 123 : 1356-62.

Panella G 1971. Fish otoliths : Daily growth layers and periodical patterns. *Science* 173 : 1124-1127.

Parker NC 1984. Chronobiologic approach to aquaculture. *Trans Am Fish Soc* 113 : 545-552.

Paulson LJ 1980. Models of ammonia excretion for brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). *Can J Fish Aquat Sci* 37 : 1421-1425.

Sæther BS, Johnsen HK, Jobling M 1996. Seasonal changes in food consumption and growth of Arctic charr exposed to either simulated natural or a 12 : 12 LD photoperiod at constant water temperature. *J Fish Biol* 48 : 1113-1122.

Savitz J 1969. Effects of temperature and body weight on endogenous nitrogen excretion in the bluegill sunfish (*Lepomis macrochirus*). *J Fish Res Bd Can* 26 : 1813-1821.

Savitz J 1971. Nitrogen excretion and protein consumption of the bluegill sunfish (*Lepomis macrochirus*). *J Fish Res Bd Can* 28 : 449-451.

Soloranzo L 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol Oceanogr* 19 : 984-989.

Spieler RE 1977. Diel and seasonal changes in response to stimuli : a plague and a promise for mariculture. *Proc World Maricult Soc* 8 : 865-873.

Swift DR 1955. Seasonal variations in the growth rate, thyroid gland activity and food reserves of brown trout (*Salmo trutta* Linn.). *J Exp Biol* 32 : 751-764.

Tackett DL, Carter RR, Allen KO 1988. Daily variation in feed consumption by channel catfish. *Prog Fish Cult* 50 : 107-110.

Tatrai I 1986. Influence of temperature, rate of feeding and body weight on nitrogen metabolism of bream, *Abramis brama* L. *Comp Biochem Physiol* 83(A) : 543-547.

Taubert BD, Coble DW 1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. *J Fish Res Bd Can* 34 : 332-340.

Temur'Yants NA, Chuyan YN, Shekhotkin AV 1995. Infradian rhythmics of the functional state of neutrophils and lymphocytes of rats with different constitutional features. *Biofizika* 40 : 1131-1135.

Trudel M, Boisclair D 1993. An in situ evaluation of the day-to-day variation in the quantity of food consumed by fish. *Can J Fish Aquat Sci* 50 : 2157-2165.

Vodicnik MJ, Kral RE, Crim LW, de Vlaming VL 1978. The effects of pinealectomy on pituitary and plasma gonadotropin levels in *Carassius auratus* exposed to various photoperiod-temperature regimes. *J Fish Biol* 12 : 187-196.

Wagner GF, McKeown BA 1985. Cyclical growth in juvenile rainbow trout, *Salmo gairdneri*. *Can J Zool* 63 : 2473-2474.

Weatherley AH, Gill HS 1987. The biology of fish growth. Academic Press, London, 443 p.

Reçu le 28 mai 1999 ; received May 28, 1999
Accepté le 9 août 1999 : accepted August 9, 1999

Appendix
Periodic Signal and Pseudo-Periodicity

The objective of this appendix is to set up the guidelines that we followed in the interpretation and discussion of our results, by using two simulated examples inspired from results of Table II (ammonia concentration series of Size Class I, Series I). These results were the detected periodic components with periods of 8, 6, and 12 days. The question that we address hereafter is whether such periodic components correspond to three distinct rhythms, or two rhythms, a "true" and a "false" one, or only one rhythm. In the first of our two examples, we show that several (i.e., we chose to work with four) periodic components may compose a periodic signal. In other words, there is one rhythm to be seen in Fig. 6A, but this rhythm has four components. This type of signal is "truly" periodic because the time interval separating successive main peaks is exactly 12. Of course, because of noise, the time interval separating peaks in the observations (where noise is superimposed to the signal) may vary, depending on the signal-to-noise ratio. To generate the signal in Fig. 6A, we used the equation :

$$S_t = \cos(2t/12) + \sin(2t/12) + \cos(1t/6) + \sin(2t/6) + \cos(2t/4) + \sin(2t/4) + \cos(2t/3) + \sin(2t/3) \quad \text{for } t = 1, \ldots, 50.$$

The period of 12 is called the fundamental period and the periods of 12/2 (6 days), 12/3 (4 days), and 12/4 (3 days) are the first, second and third harmonics, respectively. The components of a periodic signal thus correspond to periods of which the fundamental period is a multiple.

For the second example (Fig. 6B), the equation used was :

$$S_t = \cos(2t/8) + \sin(2t/8) + \cos(1t/6) + \sin(2t/6) \quad \text{for } t = 1, \ldots, 50.$$

The objective here is to show that two cosine-sine waves with periods close to each other generate a pseudo-periodic signal. "Pseudo-periodic" because 1) the amplitude of the signal is not constant over time, the signal tending to disappear when the two cosine-sine waves are out of phase, and 2) the time interval separating two successive peaks is not constant any more, but is about 7 on average in the example. Only the pseudo-periodic signal and the approximate 7 d period are biologically meaningful ; the periodic components generating it are not, or at the least, much less.