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# Rythmes d'ingestion, de croissance et d'excrétion en ammoniaque de l'omble de fontaine (*Salvelinus fontinalis*)

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Rythmes d'ingestion, de croissance et d'excrétion en ammoniaque
de l'omble de fontaine (*Salvelinus fontinalis*)

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## Sommaire

Une meilleure compréhension de la nature rythmique de l'alimentation, de la croissance et de l'excrétion en ammoniaque de l'omble de fontaine (*Salvelinus fontinalis*), une espèce importante au niveau de l'industrie piscicole, peut potentiellement servir à augmenter l'efficacité de production de cette industrie tout en minimisant ses coûts d'exploitation. Les stratégies d'alimentation actuelles utilisées en pisciculture ignorent la rythmicité des processus physiologiques, menant ainsi à des pertes au niveau d'investissement en nourriture. Étant donné que ces dépenses constituent en moyenne 50 % des coûts d'opération d'une pisciculture, l'amélioration des stratégies d'alimentation par l'utilisation d'une approche chronobiologique pourrait avoir un impact positif sur l'efficacité de production de poissons en réduisant les coûts associés à cette production. Pour les pisciculteurs, une façon indirecte de déceler et de prédire les rythmes d'alimentation et de croissance des poissons serait de doser l'ammoniaque dans les bassins de culture. L'excrétion d'ammoniaque par les poissons est intrinsèquement reliée au métabolisme de l'azote et par le fait même à l'alimentation et à la croissance.

L'objectif général de cette étude a été d'évaluer l'utilisation des périodicités de l'excrétion en ammoniaque dans le but de prédire les variations rythmiques de l'ingestion et de la croissance. Pour atteindre cet objectif, il a été premièrement nécessaire d'effectuer des expériences sur les patrons journaliers d'excrétion en ammoniaque et d'évaluer l'effet de la température, de la consommation d'azote et de la masse sur ceux-ci. Ainsi, les objectifs du premier chapitre de ce mémoire ont été de: 1) déterminer l'effet de la température, de la consommation d'azote et de la masse des individus sur les patrons journaliers d'excrétion en ammoniaque; 2) d'examiner s'il existe des différences au niveau des patrons d'excrétion de la truite arc-en-ciel (*Oncorhynchus mykiss*) et de l'omble de fontaine, deux espèces d'importance dans l'industrie de l'aquaculture.

Pour le premier chapitre de ce mémoire, cinq expériences de 24 heures ont été effectuées dans le but de déterminer l'effet de la température, de l'ingestion d'azote et de

la masse sur les patrons journaliers d'excrétion en ammoniaque de la truite arc-en-ciel et de l'omble de fontaine. Chaque expérience a impliqué le dosage de l'ammoniaque à l'intérieur des bassins à toutes les heures, sur une période de 24 heures, et l'estimation de la consommation d'azote le jour précédant une expérience et le jour même d'une expérience pour chaque groupe de poissons. Des patrons d'excrétion réguliers ont été observés pour les deux espèces et ce, aux deux températures où les expériences ont été effectuées, suggérant que l'excrétion en ammoniaque est rythmique lorsque l'horaire d'alimentation est régulier et que les poissons sont nourris une seule fois par jour. La température a affecté et le temps de la production maximale en ammoniaque et le taux d'augmentation de la concentration en ammoniaque suite à l'ingestion de nourriture par les deux espèces. En moyenne, le taux d'augmentation de la concentration en ammoniaque à l'intérieur des bassins contenant des ombles de fontaine dont la masse moyenne était de 25 grammes a été 2 fois plus élevé que celui des ombles de fontaine de 4,4 grammes. La consommation d'azote a affecté la quantité d'ammoniaque produite par les poissons mais n'a pas affecté les patrons journaliers d'excrétion lorsque les poissons étaient nourris le jour de l'expérience. Les patrons d'excrétion pour les deux espèces étaient similaires. L'information obtenue dans ce premier chapitre a permis de définir la stratégie d'échantillonnage pour les expériences présentées dans le second chapitre.

Les objectifs spécifiques du deuxième chapitre de ce mémoire ont été 1) d'estimer les courtes périodicités au niveau de l'ingestion journalière, de la croissance et de l'excrétion d'ammoniaque de l'omble de fontaine; 2) de déterminer l'effet de la masse et de la température sur de potentielles périodicités dans l'ingestion, la croissance et l'excrétion; 3) de tester l'existence d'une relation entre les rythmes d'ingestion, de croissance et d'excrétion. Les périodicités au niveau de la consommation journalière, de la croissance et de l'excrétion en ammoniaque ont été estimées pour des ombles de fontaine de deux classes de taille (~ 5 et > 30 g) et ce, à deux températures (10 et 15° C) pour une période d'environ un mois. De plus, les périodicités de l'excrétion en

ammoniaque de l'omble de fontaine d'un bassin de pisciculture (3 à 6 g.; 8° C) ont été estimées et comparées à celles trouvées dans des conditions de laboratoire. L'analyse du périodogramme multi-fréquentiel a confirmé la présence de courtes périodicités et de périodicités de longueurs intermédiaires dans l'ingestion journalière, la croissance et l'excrétion en ammoniaque. Les périodes prédominantes pour les séries de consommation journalière ont varié entre 7 et 12 j ($R^2_{pred}$ = 82 - 95%). Celles estimées pour les séries présentant la masse des individus ont varié entre 7 et 10 j ($R^2_{pred}$ = 82 - 93%) et coïncident avec celles de l'ingestion journalière. Les séries de concentrations en ammoniaque ont présenté des périodes dominantes de 7, 8 et 12 j ($R^2_{pred}$ = 86 - 99%), coïncidant ainsi avec celles des séries d'ingestions et de masses moyennes. Les périodes trouvées à chacune des températures et pour chaque classe de taille ont été similaires, illustrant ainsi l'absence d'effet de la température et de la masse des individus sur les rythmes d'alimentation, de croissance et d'excrétion. Les composantes périodiques pour la série d'excrétion du bassin de pisciculture ont conjointement expliqué 96% de la variation totale et la période dominante a été de 7 - 8 j, supportant ainsi les résultats obtenus au laboratoire. Les résultats de ces expériences ont supporté le développement de modèles prédictifs pour l'ingestion journalière et la croissance, utilisant l'excrétion en ammoniaque comme variable descriptive et l'élaboration de stratégies d'alimentation pour les piscicultures basées sur une chronobiologique.

## Introduction

La croissance explosive de l'industrie piscicole au Canada durant les dernières décennies a mené à un accroissement simultané des recherches scientifiques dévouées à la production piscicole ainsi qu'à la santé, la nutrition, la physiologie, la génétique et la reproduction des poissons. Ces études tendent à se concentrer sur l'augmentation de la production piscicole tout en minimisant les coûts d'exploitation de cette industrie. L'achat de nourriture constituont en moyenne 50% des dépenses annuelles liées à la production de poissons (SORDAC, communication personnelle). Ainsi, l'amélioration des stratégies d'alimentation pourrait avoir un impact significatif sur l'efficacité de production. Une façon d'améliorer ces stratégies et de minimiser ainsi les coûts de production piscicole serait d'utiliser une approche chronobiologique au niveau des piscicultures (Spieler, 1977; Parker, 1984).

La présence de rythmes chez les poissons a depuis longtemps été établie (voir Ali 1992 pour historique). Plus particulièrement, il est reconnu que la croissance chez les poissons passe par une succession de périodes caractérisées par des taux de croissance allant de fort a faible. La croissance circannuelle des poissons a été mise en évidence dans un grand nombre d'études (Brown, 1946; Swift, 1955; Eriksson et Lundqvist, 1982; Griffiths et Kirkwood, 1995; Jensen et Berg, 1995; Saether *et al.*, 1996). Des rythmes circalunaire et semi-lunaire dans la croissance des poissons ont aussi été caractérisés (Brown, 1946; Panella, 1971; Campana, 1984; Wagner et McKeown, 1985; Farbridge et Leatherland, 1987a,b). L'analyse de dépôts calcaires sur les otolithes a aussi démontré la présence de rythmes circadiens au niveau de la croissance des poissons (Panella, 1971; Taubert et Coble, 1977; Campana et Neilson, 1982). Des rythmes de croissance endogènes non circadiens avec des périodes de 5 à 10 jours ont aussi été démontrés (Aboul-Hosn *et al.*, 1997). Ainsi, il apparaît que les poissons présentent des rythmes circadiens et non circadiens au niveau de leur croissance.

D'autres études ont fourni des évidences de rythmes circadien, circannuel, semilunaire et saisonnier au niveau de l'ingestion (circadien: Eriksson et van Veen, 1979; Boujard et Leatherland, 1992; Cuenca et de la Higuera, 1994; Boujard, 1995; Boujard *et al.*, 1996; circalunaire et semi-lunaire: Farbridge et Leatherland, 1987a; Luquet *et al.*, 1995; saisonnier: Villarreal et al., 1988; Pálsson *et al.*, 1992; Thorpe et Cho, 1995; Seather *et al.*, 1996). De plus, lorsque l'ingestion et la croissance ont été suivi simultanément, les patrons de croissance ont démontré une forte corrélation avec l'ingestion de nourriture (Brown, 1946; Campana, 1984; Farbridge et Leatherland, 1987a; Saether *et al.*, 1996). Aussi, il y a des raisons de croire qu'il existe une correspondance entre la croissance et les rythmes d'alimentation puisque, sous des conditions de laboratoire, les taux de croissance sont principalement déterminés par les taux d'ingestion (Brett, 1979).

Les pratiques communes d'alimentation utilisées en pisciculture contrastent avec la nature rythmique de certains processus physiologiques. La quantité journalière de nourriture distribuée aux poissons représente une fraction constante de la biomasse de ceux-ci. La présence de rythmes suggère que certains jours, les poissons peuvent consommer une large proportion de la nourriture qui leur est administrée. Par contre, ces rythmes suggèrent aussi qu'en d'autres jours, une importante fraction de cette nourriture ne sera pas ingérée. Non seulement cet événement signifie une perte nette au niveau de l'investissement en moulée pour les pisciculteurs, mais il implique aussi une hausse des nutriments dans les eaux rejetées par les piscicultures. Ainsi, une meilleure connaissance des rythmes chez les poissons allouerait une diminution des pertes de l'industrie piscicole et une baisse des impacts environnementaux négatifs des eaux de piscicultures.

Ainsi, le but général de ce mémoire est d'élaborer des stratégies d'alimentation basées sur les rythmes physiologiques. Ceci requiert le développement de modèles pour quantifier ces rythmes et des outils pour prédire l'appétit des poissons en pisciculture sur une échelle temporelle appropriée. Le développement des modèles et outils nécessaires est compliqué par deux problèmes pratiques. Premièrement, les rythmes de croissance ne peuvent être estimés dans les piscicultures puisque la pesée d'un nombre important de poissons sur une base journalière peut avoir un impact négatif sur le taux de croissance et de survie de ces derniers (Weatherley et Gill, 1987). Deuxièmement, les rythmes d'ingestion (défini par la différence entre la quantité donnée et la quantité non ingérée) ne peuvent être estimés en pisciculture à cause de la grandeur des bassins utilisés, de la turbulence à l'intérieur de ceux-ci résultant en une perte par désintégration de la moulée et du trop grand débit d'eau à la sortie qui empêche la filtration de la moulée restante.

Les problèmes pratiques rencontrés lors de l'estimation des rythmes de croissance et d'ingestion peuvent être résolus par l'estimation indirecte de ces rythmes. L'ammoniaque, le principal produit du métabolisme de l'azote chez les poissons (Fromm, 1963), est le produit énergétique azoté le plus réduit de l'oxydation des acides aminés et des protéines (19,5 $kj \cdot g$ $protéine^{-1}$; Smith et Rumsey, 1976). L'ammoniaque, qui constitue pour 80% des déchets azotés chez les poissons d'eau douce, est formée au niveau du foie et est excrétée passivement par les branchies mais peut aussi être éliminée par la peau et l'urine (Jobling, 1994). L'excrétion en ammoniaque est principalement affectée par l'ingestion de protéines (Beamish et Thomas, 1984) et augmente substantiellement après l'ingestion (Brett et Zala, 1975; Kaushik, 1980; Lied et Braaten, 1983). Étant donné la relation fonctionnelle entre l'ingestion et l'excrétion en ammoniaque ainsi qu'entre l'ingestion et la croissance, il est raisonnable de croire que des rythmes d'excrétion existent et qu'ils coïncident avec des rythmes d'ingestion et de croissance.

La description des rythmes d'excrétion requiert un échantillonnage temporel adéquat de l'eau des bassins de culture, et par conséquent, une connaissance des patrons journaliers d'excrétion en ammoniaque des poissons. L'excrétion en ammoniaque est connue pour augmenter et atteindre un plateau dans les heures suivant l'alimentation (Kaushik, 1980). Aussi, l'échantillonnage de l'eau des bassins au temps de production maximale assure une certaine consistance dans l'élaboration des relations entre l'excrétion et les taux d'ingestion et de croissance. Plusieurs études ont mesuré les variations d'excrétion en ammoniaque par les poissons à l'intérieur d'une journée (Brett et Zala, 1975; Rychly et Marina, 1977; Kaushik 1980; Lied et Braaten, 1984; Forsberg et Summerfelt, 1992; Wagner *et al.*, 1995). Par contre, les conclusions tirées de ces différentes études sont inconsistantes. Ainsi, des patrons d'excrétion journaliers réguliers n'ont pas été observés lorsque les poissons étaient nourris plusieurs fois par jour. Par exemple, Forsberg et Summerfelt (1992) n'ont pas observé de patrons d'excrétion pour la plie (*Stizostedion vitreum vitreum*) nourrie toutes les dix minutes durant une période de 18 h à 20 et 25°C. De même, Wagner *et al.* (1995) n'ont pas observé de patrons constants pour la truite arc-en-ciel nourrie trois fois par jour à 17°C. Même si des pics d'excrétion ont été observés pour ces deux études, ceux-ci ne pouvaient être prédits par l'heure d'ingestion ou par l'heure de la journée. Alors que le moment de production maximale est plus prévisible lorsque les poissons sont nourris moins fréquemment, i.e., 1 à 2 fois par jour, les patrons d'excrétion journaliers, eux, se sont avérés très variables. Brett et Zala (1975) ont nourri le saumon sockeye (*Oncorhynchus nerka*) une seule fois par jour à 15°C et ont observé un pic d'excrétion 4 à 4,5 h après le début de l'ingestion (0830) et des taux d'excrétion de base (i.e. niveau d'excrétion d'azote endogène) de 0200 à 0800 avant l'ingestion du lendemain. Rychly et Marina (1977) ont observé un pic dans le taux d'excrétion 6 heures après l'ingestion pour la truite arc-en-ciel à 14°C, mais contrairement à Brett et Zala (1975), ils ont observé une hausse de l'excrétion dans les heures précédant l'ingestion du lendemain. Kaushik (1980) a observé un pic d'excrétion en ammoniaque 4 à 6 heures après ingestion pour la truite arc-en-ciel et 8 à 10 heures après ingestion pour la carpe (*Cyprinus carpio*) à 15°C. Kaushik (1980) a lui aussi observé des taux d'excrétion plus élevés que ceux de base pour chacune des espèces dans les heures précédant l'alimentation. Lied et Braaten (1983) ont observé un pic dans le taux d'excrétion de la morue (*Gadus morhua*) 5 à 6 heures suivant l'unique ingestion à 8°C et une diminution des taux d'excrétion débutant 17 à 18 heures après ingestion. Les différences observées dans les études précédentes peuvent être reliées à des disparités au niveau de la température, de la consommation en azote, de la masse des poissons ou des espèces.

Ainsi, les objectifs du premier chapitre de ce mémoire ont été les suivants:

- mieux comprendre la dynamique des patrons journaliers d'excrétion en déterminant l'effet de la température, de la consommation d'azote et de la masse des poissons sur ceux-ci;

- déterminer s'il existe des différences entre les patrons d'excrétion de la truite arc-en-ciel (*Oncorhynchus mykiss*) et ceux de l'omble de fontaine (*Salvelinus fontinalis*), deux espèces de grande importance dans l'industrie piscicole.

Les information recueillies dans le premier chapitre de ce mémoire ont été utilisées dans le but de définir la stratégie d'échantillonnage des expériences présentées dans le second chapitre. Les objectifs spécifiques de ce second chapitre ont été:

- d'estimer les courtes périodicités au niveau de l'ingestion journalière, de la croissance et de l'excrétion en ammoniaque de l'omble de fontaine;

- de déterminer l'effet de la masse et de la température sur les périodicités de l'ingestion, de la croissance et de l'excrétion.

- de tester l'existence d'une relation entre les rythmes d'ingestion, de croissance et d'excrétion;

- d'évaluer l'utilisation potentielle des périodicités de l'excrétion en ammoniaque dans le but de prédire l'ingestion et la croissance.

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To Silke, mom, and dad

Listen:
you can hear soft wind blowing
among tall fir trees on Vancouver Island
it is the same wind we knew
whispering along Cote des Neiges
on the island of Montreal
when we were lovers and had no money
Once flying in a little Cessna 180
above the great spine of mountains
where a continent attempts the sky
I wondered who owns this land
and knew that no one does
for we are tenants only

Al Purdy
" A Handful of Earth (to René Lévesque)"

# Chapitre 1

# The influence of temperature, nitrogen consumption, and body weight on the diel ammonia excretion patterns of two salmonid species

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## Abstract

Five 24 h experiments were performed to determine the effects of temperature, nitrogen consumption, and body weight on the diel ammonia excretion patterns of brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*). Each experiment involved the measurement of ammonia excretion on an hourly basis for 24 hours and the determination of nitrogen consumption, both the day of an experiment and the day before an experiment, for each group of fish. Consistent diel ammonia excretion patterns were evidenced at both temperatures for both species, suggesting ammonia excretion is rhythmic if fish are placed on a consistent feeding schedule where fish are fed once daily. Temperature affected both the time of maximal production of ammonia and the rate of increase of ammonia excretion subsequent to feeding for both brook trout and rainbow trout. On average, the rate of increase in the ammonia excretion rates of the large brook trout (mean: ~ 25.0 g) subsequent to feeding was twice that of the small brook trout (mean: ~ 4.4 g). Varying levels of nitrogen intake the day of an experiment and the feeding regime the day before an experiment only affected the amount of ammonia excreted by the fish and not the diel ammonia excretion patterns when fish were fed the day of an experiment. The excretion patterns of the brook trout and the rainbow trout were similar.

## Introduction

The explosive growth of the aquaculture industry in North America these past decades has led to a concomitant increase in scientific research devoted to fish farming practices and techniques as well as fish health. Of great interest to both researchers and aquaculturists alike has been the study of ammonia excretion in fish. Ammonia, the predominant end-product of nitrogen metabolism in fish (Fromm, 1963), can become toxic to fish at high concentrations, causing neurological and cytological failure, kidney damage, and suffocation (see Meade, 1985 for review). Since culturists rear fish at high densities, ammonia production of cultured fish is a primary concern; ammonia concentration increases with the density of fish, and if ammonia concentrations in rearing facilities become too high, the health of the cultured fish will be jeopardized. Thus, several scientific works have investigated the factors that influence ammonia production in fish.

Considering that ammonia production is intrinsically related to nitrogen metabolism, studies on the effects of temperature, body weight, and nitrogen consumption on ammonia excretion are ubiquitous (Savitz, 1969; Savitz, 1971; Guerin-Ancey, 1976; Paulson, 1980; Jobling, 1981; Tatrai, 1986; Forsberg and Summerfelt, 1992; Cai and Summerfelt, 1992). Generally, ammonia excretion ($mg \cdot kg^{-1} \cdot h^{-1}$) increases with an increase in nitrogen consumption and temperature and decreases as individual fish mass increases. While knowledge of these relationships has assisted culturists in maintaining water quality, diel fluctuations in ammonia production can complicate the monitoring of ammonia concentrations and the optimization of water quality. Optimization of water quality in high-density ponds or water recirculation systems would be facilitated if diel excretion patterns of cultured fish could be elucidated.

Several studies have measured diel variation of ammonia excretion in fish (Brett and Zala, 1975; Rychly and Marina, 1977; Kaushik 1980; Lied and Braaten, 1984; Forsberg and Summerfelt, 1992; Wagner et al., 1995); however, evidence of diel excretion patterns has been inconsistent. In experiments where fish were fed several times daily, diel ammonia excretion patterns were not observed; for example, Forsberg and Summerfelt (1992) did not observe consistent diel ammonia excretion patterns for walleye (*Stizostedion vitreum vitreum*) fed every 10 min for 18 h at 20 and 25°C. Similarly, Wagner et al. (1995) did not observe consistent diel excretion patterns for rainbow trout (*Oncorhynchus mykiss*) fed three times daily at 17°C. While ammonia excretion peaks were observed in both studies on several occasions, these peaks could not be accurately predicted by feeding time or time of day.

While ammonia excretion peaks have occurred at more predictable times in experiments where fish have been fed less frequently (i.e. 1 to 2 times daily), the observed diel ammonia excretion patterns in these studies have varied considerably. Brett and Zala (1975) fed sockeye salmon (*Oncorhynchus nerka*) once daily at 15°C and recorded peak ammonia excretion rates 4-4.5 h after the start of feeding (0830) and baseline excretion levels (i.e. endogenous nitrogen excretion levels) from 0200 to 0800, prior to the next morning's feeding. Rychly and Marina (1977) observed a peak in excretion rates 6 hours after feeding rainbow trout (*Oncorhynchus mykiss*) once at 14°C, but in contrast to Brett and Zala (1975), noticed an increase in excretion levels in the hours prior to the next day's feeding. Kaushik (1980) observed peaks in ammonia excretion 4-6 and 8-10 hours after feeding at 15°C for rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*), respectively. Kaushik (1980) also noticed that pre-feeding excretion levels for both species were higher than the average baseline levels in the same fish. Lied and Braaten (1983) recorded a peak in the excretion rate of cod (*Gadus morhua*) 5-6 h after a single feeding at 8°C and an apparent die-off of excretion rates commencing 17-18 hours after feeding. Differences between the observed diel ammonia excretion patterns in the aforementioned studies may have been a consequence of disparities in temperatures, nitrogen consumption, body weights, or species.

To better understand the dynamics of diel ammonia excretion patterns in fish, it was the purpose of this work to determine the effects of temperature, nitrogen consumption, and body weight on daily patterns of ammonia excretion. Furthermore, we examined whether or not differences exist between the excretion patterns of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*).

## Methods and Materials

The effects of nitrogen consumption, body size, and temperature on diel ammonia excretion patterns of rainbow trout and brook trout were investigated by performing five experiments. Each experiment involved the measurement of ammonia excretion on an hourly basis for 24 hours and the determination of nitrogen consumption the day of an experiment and the day before an experiment for groups of rainbow trout and brook trout. Experiments 1-4, conducted at 5°C, were used to investigate the effects of nitrogen consumption on diel ammonia excretion patterns of similar-sized brook trout and rainbow trout. Experiment 5, conducted at 15°C, utilized two size classes of brook trout to examine the effects of body size on diel ammonia excretion patterns. Two groups of fish utilized in Experiment 5 were of similar size to those of Experiments 1-4, allowing for a comparison of diel ammonia excretion patterns at different temperatures for both species.

### *Experimental tanks and operation*

Rainbow trout and brook trout were obtained from genetically homogenous stocks (Pisciculture Mont-Tremblant, Québec) as to minimize the potential effect of genotype on ammonia excretion patterns (Kaushik *et al.*, 1984). Trout were housed in 510-L tanks (0.91 × 0.91 × 0.66 m). Tap water was flushed through a Momentum CA1248 carbon filter before entering the tanks, and the rate of flow of water into the tanks was set at 2 $L \cdot min^{-1}$ using an adjustable valve on the inlet. A 61 cm overflow tube (2.54 cm diameter) fitted to the bottom of the tank allowed for an open system, continuously draining excess water from the surface. Thus, the entire volume of water within the tank was replaced about every 4 hours. This rate of flow was chosen as it closely reflects the flow rates used by aquaculturists in open-system fish basins.

For the duration of the experiments, the temperature of the water within (and entering) the tanks was constant: 5 ± 0.5°C for Experiments 1-4 and 15 ± 0.5°C for Experiment 5. All the tanks were 100% saturated with oxygen. The photoperiod within the laboratory 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 laboratory conditions for three weeks.

### *Experimental animals*

The five 24 h experiments were conducted using 8 different groups of 46 fish (see Table 1). Each group of fish was normally distributed with respect to body weight. There was no significant difference between the mean weight of the group of brook trout and the group of rainbow trout used in Experiments 1-4 nor between the mean weight of the replicates of fish used in Experiment 5 (see Table 1). Furthermore, there was no significant difference (ANOVA; $p > 0.50$) between the replicates of the brook trout (mean ~ 25.0 g) and the replicates of the rainbow trout (mean ~ 25.0 g) in Experiment 5.

Table 1. Number, mean weight, and weight range of the groups used in experiments 1-5. Comparison of mean weights between groups or replicates was performed using *t*-tests.

| Species | *n* | Mean Weight (g) | Range (g) | *t*-test |
|---|---|---|---|---|
| Experiment 1-4 (5°C) | | | | |
| Rainbow trout | 46 | 20.8 | 16.7-24.8 | $p = 0.46$ |
| Brook trout | 46 | 20.5 | 16.5-24.3 | |
| Experiment 5 (15°C) | | | | |
| Brook trout (rep. 1) | 46 | 4.4 | 3.5-5.2 | $p = 0.83$ |
| Brook trout (rep. 2) | 46 | 4.3 | 3.5-5.1 | |
| Brook trout (rep. 1) | 46 | 25.2 | 20.0-30.4 | $p = 0.66$ |
| Brook trout (rep. 2) | 46 | 24.9 | 19.7-29.8 | |
| Rainbow trout (rep. 1) | 46 | 25.8 | 19.6-30.6 | $p = 0.83$ |
| Rainbow trout (rep. 2) | 46 | 25.7 | 19.9-30.1 | |

### *Feeding and food composition*

Nitrogen ingestion was measured on the day of a 24 h sampling period as well as the day prior to a 24 h sampling period. For Experiments 1-4 at 4°C, the trout were given rations of 2.0% body weight per day of Martin Starter Pellets (wet g; 3 Gr.) when fed. Fish were not fed the day prior to Experiment 2 nor the day of Experiment 4 to further explore the effects of nitrogen consumption on diel ammonia excretion patterns. For Experiment 5 at 15°C, the rations were increased to 4.0% body weight per day. The day-to-day variation in nitrogen consumption by each group of fish allowed for the effects of nitrogen consumption on diel ammonia excretion patterns to be examined. 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). Excess food was then retrieved with a fine-meshed fish net and dried in an oven at 60°C for 24-h. Excess food did not dissolve into the water and was always recoverable. The mass of the dried excess was 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 consumption (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). Nitrogen intake was obtained by multiplying the amount ingested by 0.074, assuming that 90% of the protein content of the feed was digestible and that nitrogen makes up approximately 16% of a mixture of proteins (Jobling, 1994). The Martin Starter Pellets have a protein content of 51.7%, a lipid content of 15.1%, and a fibre content of 1.7% (Chavez, E., McGill University, MacDonald Campus, Ste. Anne de Bellevue, QC, Canada). The total digestible energy per gram wet feed is 4.9 kcal.

### *Ammonia determination*

For each 24 h experiment, four 500 ml water samples were removed from each aquarium at 0830 (1 hour before feeding), at 1030 (1 hour after feeding), and each subsequent hour until 0730 the following day. The water samples were obtained from different parts of the aquarium to account for potential intra-tank variation. Four subsamples of 50 ml were immediately analyzed for ammonia using the phenol-hypochlorite method (Soloranzo, 1969).

## Results

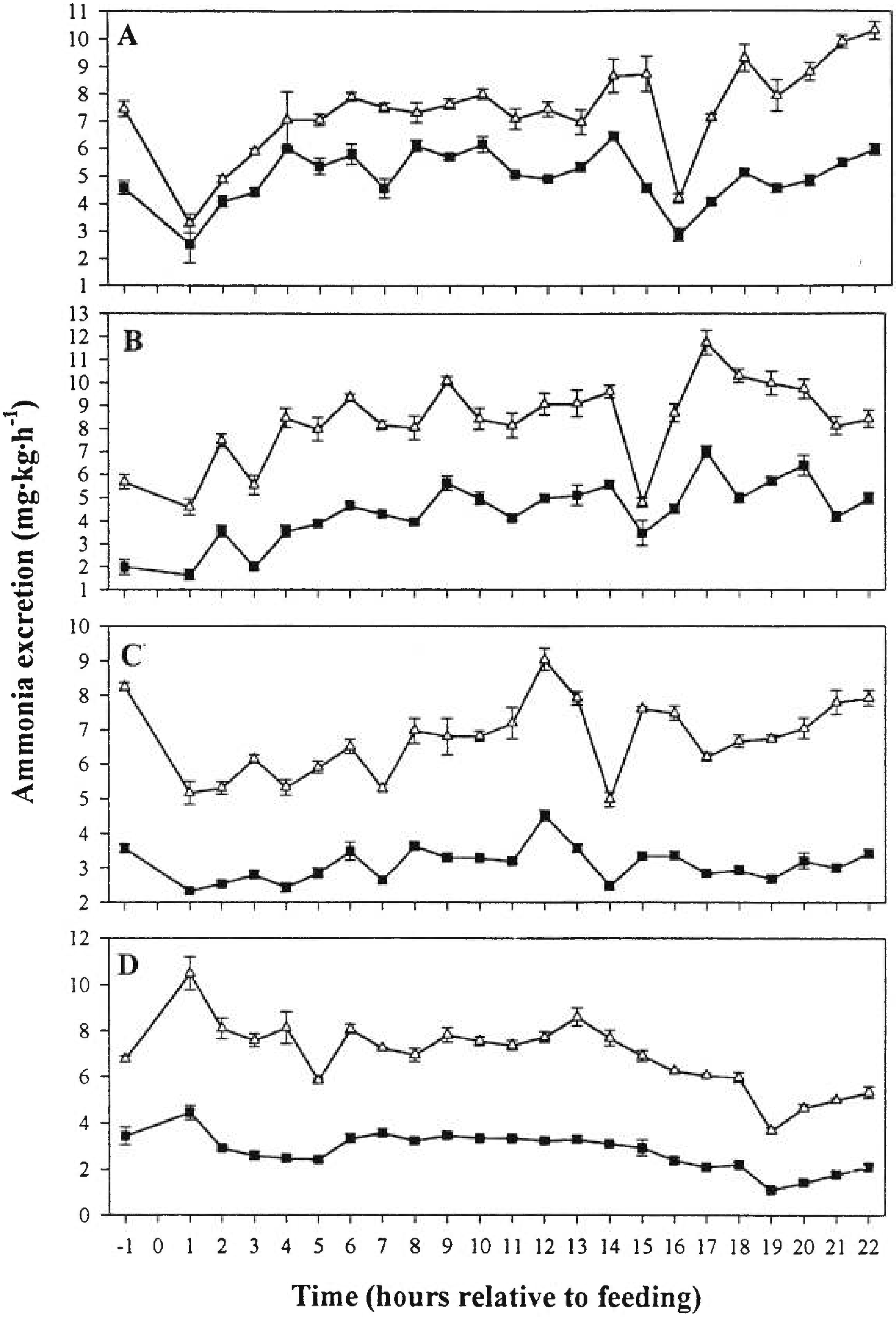
### *N-consumption and species effects at 5°C*

A consistent diel ammonia excretion pattern was exhibited by both the rainbow trout and the brook trout at 5°C when fed the day of an experiment (i.e. Experiments 1-3 -- see Fig. 1). On these occasions, the lowest ammonia excretion rates for both the rainbow trout and brook trout occurred 1 hour after feeding and a marked drop in excretion rates occurred in the middle of the night, approximately 14-16 hours after feeding. Although no defined diurnal pulse (an excretion rate approximately twice that of the 24 h mean excretion rate) occurred in Experiments 1-3, gradual increases in ammonia production beginning 2 hours after feeding and ending 10-12 hours later were observed. Furthermore, increasing or elevated rates of ammonia production were observed prior to the next morning's feeding.

Although fish were not fed the day before Experiment 2, their diel excretion patterns remained similar to those of Experiments 1 and 3, where fish were fed the day of the experiment as well as the day before the experiment; however, the average pre-feeding excretion rates (1 h before feeding) of Experiments 1 and 3 were 92% and 34% greater than those of Experiment 2 for the brook trout and the rainbow trout, respectively. Moreover, the final excretion rates recorded in Experiment 4 (brook 2.1 mg/kg/h; rainbow trout 5.3 mg/kg/h), where fish were not fed the day of the experiment, were similar to the pre-feeding excretion rates of Experiment 2 (brook 2.0 mg/kg/h; rainbow 5.6 mg/kg/h). Thus, the pre-feeding excretion rates appear to be lower when fish have not been fed the day before.

When fish were not fed the day of an experiment (i.e. Experiment 4), the diel ammonia excretion pattern of Experiments 1-3 was not observed. In this case, the highest levels of ammonia production were recorded 1 hour after the usual feeding time and then gradually decreased until the lowest production levels were reached 19 hours

Figure 1. Excrétion en ammoniaque (moyenne ± 1ET) sur une période de 24 h débutant à 0830 (1 heure avant ingestion pour les Expériences 1 à 3) par 46 truites arc-en-ciel (triangles vides) et par 46 ombles de fontaine (carrés pleins) variant de 16,5 à 24,8 g (moyenne: ~ 20 g): (A) Expérience 1, (B) Expérience 2, (C) Expérience 3, (D) Expérience 4. Température de l'eau = 5°C


A
B
C
D
Ammonia excretion (mg·kg·h$^{-1}$)
Time (hours relative to feeding)
1 2 3 4 5 6 7 8 9 10 11
1 2 3 4 5 6 7 8 9 10 11 12 13
2 3 4 5 6 7 8 9 10
0 2 4 6 8 10 12
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

later; however, similar to Experiments 1-3, increasing or elevated rates of ammonia production were observed prior to the next morning's feeding.

For Experiments 1-4, an analysis of the crosscorrelation between the excretion patterns of the brook trout and the rainbow trout (PROC ARIMA -- SAS Institute, 1988) confirmed the similarity of the diel excretion patterns of the two species; for each experiment, the excretion patterns of the two species were significantly crosscorrelated, with r-values ranging from 0.78 to 0.88 (lag=0; $p<0.05$). In each experiment, however, the group of rainbow trout excreted more ammonia and, when fed, consumed more nitrogen than the group of brook trout (see Table 2).

Table 2. Nitrogen intake (day before and day of experiment) and ammonia excretion (24 h mean) of 46 juvenile rainbow trout (mean: 20.8 g) and 46 juvenile brook trout (mean: 20.5 g).

| | N-Intake (g) previous day | | N-Intake (g) day of | | Excretion ($mg \cdot kg^{-1} \cdot h^{-1}$) | |
|---|---|---|---|---|---|---|
| | Rainbow | Brook | Rainbow | Brook | Rainbow | Brook |
| Experiment 1 | 0.804 | 0.335 | 0.581 | 0.208 | 7.4 | 5.0 |
| Experiment 2 | 0.000 | 0.000 | 0.849 | 0.603 | 8.3 | 4.4 |
| Experiment 3 | 0.558 | 0.313 | 0.521 | 0.320 | 6.7 | 3.1 |
| Experiment 4 | 0.581 | 0.313 | 0.000 | 0.000 | 6.9 | 2.8 |

### *Body weight and species effects at 15°C*

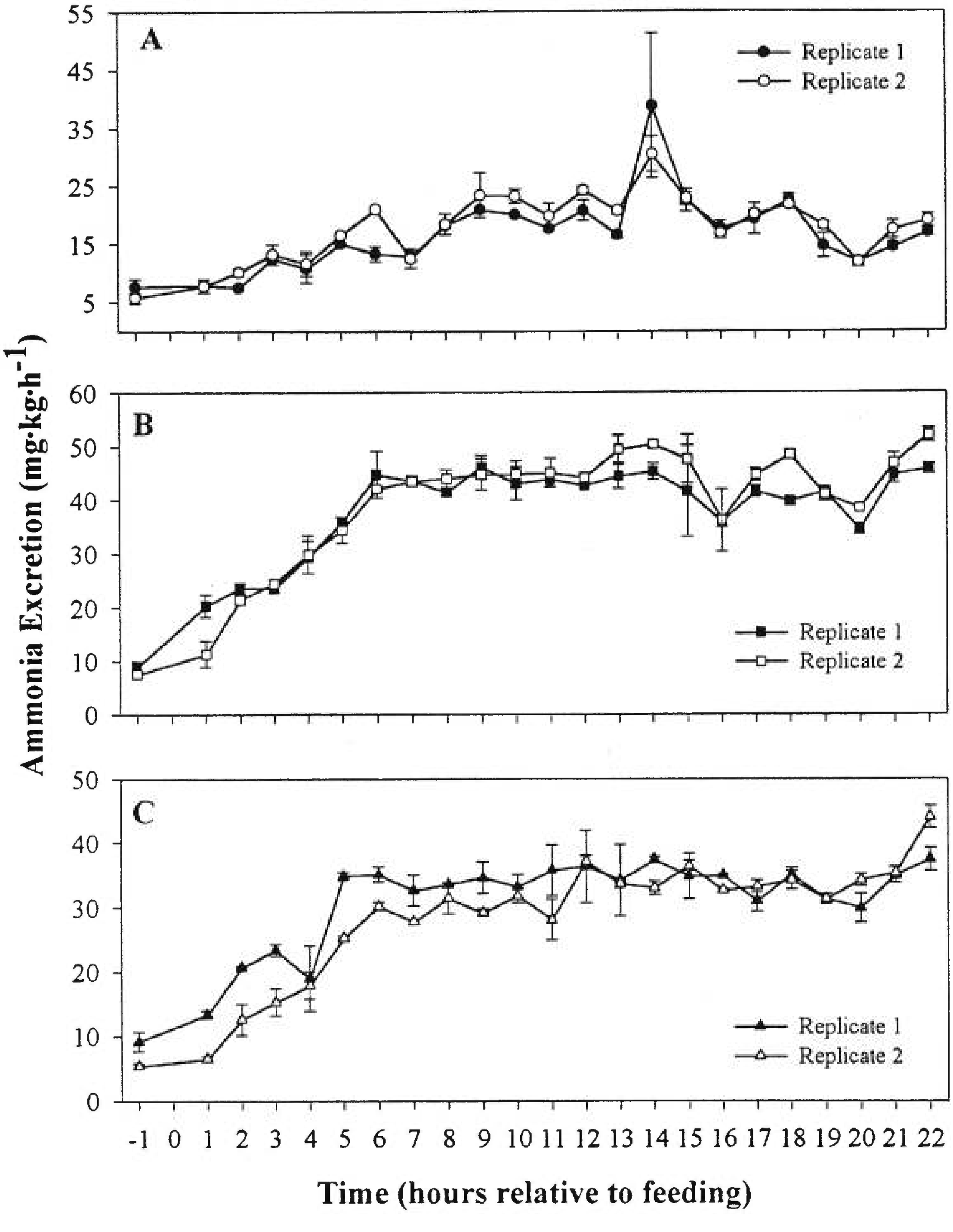
Experiment 5 permitted the examination of diel ammonia excretion patterns of two size classes of brook trout. Each size class of brook trout consumed similar quantities of nitrogen with respect to their body weights; replicates 1 and 2 of the small brook trout (mean: ~ 4.4 g) consumed 2.3 and 2.5% their body weights, respectively, and replicates 1 and 2 of the large brook trout (mean: ~ 25.0 g) consumed 2.3 and 2.1% their body weights, respectively. The average hourly ammonia excretion rates of the replicates of large brook trout, however, were significantly higher than the replicates of the smaller-sized brook trout, and, on average, the absolute nitrogen consumption of the large brook trout was approximately 5 times that of the small brook trout (see Table 3).

Table 3. Nitrogen intake (day before and day of experiment) and ammonia excretion (24 h mean) by rainbow trout and brook trout of experiment 5.

| | | N-Intake (g) | | Excretion |
|---|---|---|---|---|
| | Replicate | previous day | day of | ($mg \cdot kg^{-1} \cdot h^{-1}$) |
| Brook Trout | 1 | 0.499 | 0.462 | 16.6 |
| mean: ~ 4.4 g | 2 | 0.640 | 0.514 | 17.7 |
| Brook Trout | 1 | 3.328 | 2.695 | 37.5 |
| mean: ~ 25.0 g | 2 | 2.926 | 2.367 | 38.8 |
| Rainbow Trout | 1 | 3.328 | 1.980 | 30.5 |
| mean: ~ 25.0 g | 2 | 2.539 | 2.695 | 28.1 |

The excretion patterns of the small brook trout (Fig. 2a) were crosscorrelated (lag=0; r=0.77, $p<0.05$). For each replicate, the lowest ammonia production was recorded one hour before feeding (Replicate 1 = 7.6 mg/kg/h; Replicate 2 = 5.8 mg/kg/h). Thereafter, a gradual increase in excretion rates occurred until a pulse was observed 14 hours after feeding (Replicate 1 = 38.8 mg/kg/h; Replicate 2 = 30.5 mg/kg/h). Note, however, that there was a 361% and 262% increase in excretion rates in the first 6 hours following feeding for replicates 1 and 2, respectively. The pulses were followed by a sharp decline in ammonia output, reaching a level of 12.0 mg/kg/h for both tanks 20 hours after feeding. Finally, in the hours preceeding the next morning's feeding, an increase in excretion rates was observed.

Figure 2. Excrétion en ammoniaque (moyenne ± 1ET) sur une période de 24 h débutant à 0830 (1 heure avant l'ingestion) dans l'Expérience 5 pour (A) 2 réplicats de 46 ombles de fontaine de 3,5 à 5,2 g (moyenne: ~ 4,4 g); (B) 2 réplicats de 46 truites arc-en-ciel de 19,7 à 30,4 g (moyenne: ~ 25,0 g); (C) 2 réplicats de 46 truites arc-en-ciel de 19,6 à 30,6 g (moyenne: ~ 25,0 g). Température de l'eau = 15°C



A
B
C
Replicate 1
Replicate 2
Ammonia Excretion (mg·kg·h-1)
55
45
35
25
15
5
60
50
40
30
20
10
0
50
40
30
20
10
0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Time (hours relative to feeding)

The excretion patterns of the large brook trout (Fig. 2b) were akin, having a crosscorrelation coefficient of 0.87 with a lag of 0 ($p<0.05$). Similar to the small brook trout, the excretion rates of the large brook trout were also lowest one hour before feeding (Replicate 3 = 8.9 mg/kg/h, Replicate 4 = 7.5 mg/kg/h). Subsequent increases of 403% and 460% in the ammonia excretion rates of replicates 1 and 2, respectively, were then observed over the next seven hours until production reached a plateau 6 hours after feeding. The marked drops in ammonia production that occurred 16 hours after feeding and the gradual increase in excretion rates preceeding the next morning's feeding matched those of the small brook trout.

The diel ammonia excretion patterns for small and large brook trout were similar in that, for both size classes, the lowest ammonia production occurred one hour prior to feeding, a marked drop occurred 16 hours after feeding, and increasing exretion rates were observed prior to the next day's feeding. Body weight, however, appeared to affect the rate of increase in ammonia excretion rates in the hours subsequent to feeding. The excretion rates of the large brook trout 6 hours after feeding were, on average, 5.4 times greater than the pre-feeding excretion rates whereas the excretion rates of the small brook trout 6 hours after feeding were only, on average, 2.7 times greater than the pre-feeding excretion rates.

Experiment 5 also allowed for a comparison of the diel ammonia excretion patterns of similar size classes (mean: ~ 25.0 g) of rainbow trout and brook trout. The patterns exhibited by the rainbow trout (Fig. 2c) were crosscorrelated (lag 0; $r=0.63$, $p<0.05$). Similar to the excretion patterns of the large brook trout, the lowest rates of ammonia production occurred 1 hour before feeding (Replicate 1 = 9.2 mg/kg/h, Replicate 2 = 5.4 mg/kg/h); these rates were then followed by distinct increases in excretion over the next seven hours (281% for replicate 1 and 460% for replicate 2) until a plateau was reached 6 hours after feeding. Excretion rates remained constant until the hours preceeding the next day's feeding, when gradual increases in excretion rates occurred once again. No marked drop in excretion rates occurred subsequent to feeding. Aside from the marked drop in the excretion rates of the brook trout 16 hours after feeding, the excretion patterns of the two species were alike.

Although the diel excretion patterns of the large brook trout and the rainbow trout of Experiment 5 were similar, the 24 h mean excretion rates of the brook trout were significantly higher than those of the rainbow trout (Table 3). Even when replicate 1 of the large brook trout consumed the same quantity of nitrogen as replicate 2 of the rainbow trout on the day of the experiment (2.695 g N), the 24 h mean excretion rate of the brook trout was 37.5 mg/kg/h compared to 28.1 mg/kg/h for the rainbow trout. Note, however, that the nitrogen consumption of the brook trout the day before the experiment (3.328 g N) was significantly greater that of the rainbow trout (2.539 g N), again demonstrating the influence of the previous day's nitrogen consumption on excretion rates.

### *Comparison of temperature effects*

The ammonia excretion patterns of the brook trout and the rainbow trout of Experiment 5 at 15°C (mean weight 25 g -- Fig. 2a,b) exhibited similar characteristics to those of the brook trout and rainbow trout of Experiments 1-3 at 5°C (mean weight 20 g -- Fig. 1a,c).. At both temperatures, increases in ammonia excretion rates were observed subsequent to feeding, although the increases at 15°C were rapid, reaching a plateau 6 hours after feeding; the increases of ammonia production at 5°C were more gradual, ceasing 13 to 15 hours after feeding. Marked drops in excretion 14-16 hours after feeding were observed for both species at 5°C. At 15°C, marked drops were recorded for the brook trout 16 hours after feeding but not for the rainbow trout. Also observed at both temperatures were elevated or increasing rates of ammonia excretion in the hours preceeding the next day's feeding. Differences between the excretion patterns of the two temperatures occurred at the beginning of the of the day; at 5°C, the ammonia production

1 hour after feeding was lower than the ammonia production 1 hour prior to feeding. The converse was observed for the fish at 15°C.

## Discussion

It was the purpose of this work to determine the effects of temperature, nitrogen consumption, and body weight on the diel ammonia excretion patterns of two salmonid species. This study illustrates that disparities in diel ammonia excretion patterns do exist at different temperatures, levels of nitrogen intake, and body weights; however, similarities of certain characteristics in the excretion patterns -- low levels of ammonia production at the beginning of the day, post-prandial increases in ammonia excretion rates, marked drops in ammonia production 14 to 16 hours after feeding, and increasing or elevated rates prior to feeding -- suggest that ammonia excretion is rhythmic when fish are placed on a consistent diel feeding schedule where fish are fed once daily. Furthermore, there appears to be no difference between the excretion patterns of similar-sized rainbow trout and brook trout at the same temperature.

The rate of increase of ammonia excretion rates and the time of maximal production of ammonia subsequent to feeding were different depending on temperature. The ammonia excretion patterns of the brook trout and the rainbow trout (mean: 25.0 g) at 15°C were similar to those observed in other studies conducted at the same temperature; for instance, Kaushik (1980) and Rychly and Marina (1977) also observed peaks in ammonia excretion rates for rainbow trout approximately 6 hours after feeding. Paulson (1980) observed a peak in ammonia excretion 7-8 hours after feeding at 12°C for both brook trout and rainbow trout. In the present study, the ammonia production of trout at 5°C increased gradually until 12-14 hours after feeding. This suggests that peaks in the diel excretion ammonia patterns of the rainbow trout and the brook trout are dependent on temperature. Maximal rates of ammonia production subsequent to feeding will be attained more rapidly at higher temperatures due to a higher metabolic rate or a faster speed of digestion. Moreover, at 5°C, the ammonia production 1 hour after feeding was lower than the ammonia production 1 hour prior to feeding whereas the converse occurred for fish at 15°C. This suggests that increases in ammonia production subsequent to feeding will be evidenced sooner in fish at higher temperatures.

Similar to the excretion patterns of fish at both temperatures were increasing or elevated excretion rates in the hours preceeding the next feeding. Rychly and Marina (1977) also observed an increase in ammonia excretion rates for rainbow trout in the hours prior to feeding. This rise in ammonia excretion rates may coincide with a rise in metabolic rate prior to feeding. While Brett and Zala (1975) noticed a rise in the metabolic rate (oxygen consumption) of sockeye salmon prior to feeding, no corresponding rise in ammonia excretion was observed until feeding began. Brett and Zala (1975) recorded a drop in ammonia excretion rates at 0200 (15-16 hours after feeding) and baseline levels of ammonia production (i.e. endogenous nitrogen excretion levels) in the hours prior to feeding. Similarly, Lied and Braaten (1983) observed successive drops in ammonia production commencing 9-10 hours after feeding, reaching baseline levels 25-26 hours after feeding. Note, however, Lied and Braaten (1983) starved cod for 48 hours prior to their experiment, and in the case of Brett and Zala (1975), restricted rations (i.e. maintenance levels of nitrogen) were given to sockeye salmon. This may indicate that when fish are given restricted rations or are starved prior to a 24 h ammonia excretion rate sampling period, elevated or increasing rates may not be observed prior to feeding, even though a rise in metabolic rate occurs, because all exogenous sources of nitrogen may have been depleted. This suggests that a consistent feeding pattern, where nitrogen consumption is not restricted, is required for the observance of a consistent diel ammonia excretion pattern.

In this study, a consistent diel ammonia excretion pattern was evidenced at both temperatures when fish were fed the day of an experiment. When fish were not fed on the day of an experiment (i.e. Experiment 4 of this study), some characteristics of the diel ammonia excretion patterns when fish were fed the day of an experiment disappeared (i.e. low ammonia productions one hour after the feeding time and marked drops in excretion

14-16 hours after the feeding time); however, a rise in excretion rates prior to the next morning's feeding, similar to the other experiments, was observed, indicating a rise in metabolic rate occurred. This suggests that a feeding-entrained ammonia excretion rhythm was in place due to a consistent diel feeding schedule where fish were fed at 0930 every day for 3 weeks (Davis and Bardach, 1965; Spieler, 1992). This is further supported by the resumption of the diel excretion pattern evidenced in Experiment 2 where fish were not fed the day before the experiment. Kaushik (1980) found that about one week was needed for a daily nitrogen excretion pattern to stabilize after a feeding rhythm is changed. This knowledge benefits the culturist that feeds fish 6 days a week; a one day absence of feeding would not attenuate the underlying exogenous rhythm that leads to consistent diel ammonia excretion patterns, thus simplifying the task of monitoring ammonia concentrations and optimizing water quality in basins. The amount of nitrogen consumed by the fish does affect the level of ammonia excretion however, and this was observed throughout the experiments. Generally, the level of ammonia excretion rates increased with increasing quantities of nitrogen consumed; this relationship is supported by previous studies (Savitz *et al.*, 1977; Paulson, 1980; Jobling, 1981). This study also illustrated that nitrogen intake the day prior to the experiments had a significant effect on ammonia excretion the day of an experiment. This is in agreement with Savitz *et al.* (1977) who found that when largemouth bass were given large rations, several days were required before fish depleted the exogenous source of nitrogen and returned to maintenance nitrogen excretion.

As illustrated, it appears that body weight did affect the diel ammonia excretion pattern of the brook trout. Increases in the excretion rates of the large brook trout (mean ~ 25.0 g) subsequent to feeding were more rapid than those of the small brook trout (mean ~ 4.4 g). Moreover, the excretion rates of the large brook trout reached a plateau 6 hours after feeding whereas the excretion rates of the small brook trout continued to increase gradually, reaching a peak 14 hours after feeding. Similar to both size classes were the marked drops in excretion levels approximately 15 hours after feeding and the increases in ammonia production prior to the next day's feeding, again suggesting a feeding-entrained rhythm of ammonia excretion. The post-prandial difference in patterns of ammonia excretion of the two size classes may have been a result of the pellet size. By feeding the small trout a 3 Gr. pellet, instead of the recommended 2Gr. pellet, to obtain a symmetric experimental design, the small brook trout may have had difficulty digesting and absorbing the feed, leading to a less rapid rate of increase of ammonia production and a delayed time of maximal ammonia excretion subsequent to feeding.

There is no difference between the diel ammonia excretion patterns of similar-size rainbow trout and the brook trout under similar environmental conditions. At both temperatures, the excretion patterns of both species were similar. Paulson (1980) also evidenced no defined difference in the daily excretion patterns of the rainbow trout and brook trout at 12°C. At 5°C the rainbow trout consumed and excreted more ammonia than the brook trout, but at 15°C, the converse was true.

Taken together, rhythms of ammonia excretion seem to be elicited in both the rainbow trout and the brook trout when they are placed on a consistent diel feeding schedule where fish are fed once daily. This knowledge can greatly benefit the culturist wishing to optimize water quality in high-density ponds or water recirculation systems. The culturist can synchronize flow rates and aeration in basins with the rhythm of ammonia excretion in the fish. Because nitrogen consumption only affects the level of ammonia production and not the diel ammonia excretion pattern of fed fish, temperature and body weight should be the principle concerns of the culturist since increases of ammonia excretion rates and the time of maximal production of ammonia subsequent to feeding apppear to be dependent on temperature and body weight.

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# Chapitre 2

# Effects of body size and temperature on periodicities in feeding, growth, and ammonia excretion of the brook trout (*Salvelinus fontinalis*)

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## Abstract

Periodicities in daily food intake, growth, and ammonia excretion were estimated and compared for two size classes of brook trout (~ 5 and >30 g) held at two temperatures (10 and 15°C). Moreover, periodicities in ammonia excretion of brook trout at a pisciculture (3-6 g at 8°C) were estimated and compared to those of the laboratory setting. The results of the multi-frequential periodogram analysis confirmed the presence of short-to-intermediate periodicities in daily food intake, growth, and ammonia excretion. The predominant periods in the daily food intake series ranged from 7 to 12 d ($R^2$ = 82-95%). Prevailing periodicites in the mean weight series of 7 and 10 d ($R^2$ = 82-93%) coincided with daily food intake. The ammonia concentration series displayed predominant periods of 7-8 and 12 d ($R^2$ = 86-99%), coinciding with both the daily food intake and mean mass series. 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 excretion. Periodic components in the ammonia concentration series at the pisciculture jointly explained 96% of the total variation in the series, and the predominant period was 7-8 d, supporting the results obtained in the laboratory.

## Introduction

The presence of rhythms in fishes has long been established (see Ali 1992 for historical account). One physiological phenomenon among fish that has been categorized as both a circa rhythm and a non circa rhythm is growth. Circannual rhythms of fish growth have been evidenced in a number of studies (Brown, 1946; Swift, 1955; Eriksson and Lundqvist, 1982; Griffiths and Kirkwood, 1995; Jensen and Berg, 1995; Saether et al., 1996). Circasemilunar and circalunar rhythms in fish growth have also been characterized (Brown, 1946; Panella, 1971; Campana, 1984; Wagner and McKeown, 1985; Farbridge and Leatherland, 1987a,b). The analysis of calcareous deposits on otoliths has also demonstrated the presence of circadian growth rhythms in fish (Panella, 1971; Taubert and Coble, 1977; Campana and 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. Indeed, the studies of Brown (1946), Campana (1984), Farbridge and Leatherland (1987a), and Saether et al. (1996) have demonstrated that growth patterns coincide with patterns in food intake. Common feeding practices used in piscicultures contrast with the rhythmic nature of physiological processes. The quantity of food distributed to fish on a daily basis generally represents a constant proportion of fish biomass. The existence of rhythms suggests that fish, on some days, will consume a large proportion of the feed allotted to them while, on other days, a fraction of the feed distributed to them will not be ingested. This potential occurrence can lead to a net loss of feed investment for culturists. However, elaboration of feeding strategies based on feeding and/or growth rhythms would require frequent estimation of the ingestion and weight of fish -- an impracticality in a pisciculture setting.

The practical problems associated with the direct estimation of rhythms in a pisciculture setting may be circumvented by assessing growth and feeding rhythms indirectly. 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 and Thomas, 1984) and increases substantially after feeding (Brett and Zala, 1975; Kaushik, 1980; Lied and Braaten, 1984). Thus, it is 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. Thus, the culturist could adjust feeding strategies accordingly and increase production by alotting more food in periods of increased food intake and/or growth rates and alotting 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, growth, and ammonia excretion of brook trout and determine the effect of body size (weight) and temperature on these periodicities. Furthermore, periodicities in ammonia excretion estimated for a large group of brook trout at a pisciculture will be compared to periodicites in ammonia excretion of brook trout in a laboratory setting.

## Methods and Materials

To estimate and compare short-to-intermediate periodicities in daily food intake, growth and ammonia excretion and determine the effects of body size (weight) and temperature on these periodicities, daily food intake, mean weight and ammonia excretion of two size classes of brook trout were monitored for approximately one month at both 10°C and 15°C in a laboratory setting. In each time series, daily food intake of trout was determined daily while ammonia excretion was measured every two days. Indications of growth were also obtained by weighing a subsample of fish every two days. Measurements of ammonia excretion and growth were taken on opposite days within a time series. To estimate and compare periodicities in ammonia excretion of brook trout in a pisciculture to periodicities in ammonia excretion of brook trout in a laboratory setting, ammonia concentrations in a pisciculture basin containing brook trout were measured daily for approximately one month. Periodicities were assessed using time series analysis.

### *Experimental tanks and operation*

Two experimental environments were required to provide two different water temperatures. The time series conducted at 15°C was performed at the Département de Biologie de Université de Montréal and will be referred to as Series I. The time series conducted at 10°C was performed at the Station de Biologie des Laurentides de l'Université de Montréal and will be referred to as Series II. A third time series, limited to the survey of ammonia concentrations in one basin of brook trout held at 8.1°C, was also conducted at Pisciculture Simdar (Saint-Alexis-des-Monts, Québec) and will be referred to as Series III.

For Series I, trout were were housed in 510-L tanks (0.91 × 0.91 × 0.66 m). A 61 cm overflow tube (2.54 cm diameter) was fitted to the bottom of the tanks. Tap water was flushed through a Momentum CA1248 carbon filter before entering the tanks. The trout used two Series II were housed in 510-L tanks (2.2 × 0.5 × 0.5 m). A 43 cm overflow tube (2.54 cm diameter) was fitted to the bottom of the tanks. Lake water was pumped from a depth of 6 m directly into the tanks to obtain a water temperature of 10°C. In all experimental tanks, the overflow tubes allowed for an open system, continuously draining excess water from the surface. The rate of flow of water into the tanks was set at 2 $L{\cdot}min^{-1}$ using adjustable valves on the inlets. Thus, the entire volume of water within the tanks was replaced about every 4 hours.

For the duration of the experiments, the temperature of the water within (and entering) the tanks was constant: 15 ± 0.5°C for series I and 10 ± 0.5°C for series II. All the tanks were 100% saturated with oxygen. The photoperiod within the laboratory 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 laboratory conditions for two weeks.

For Series III, fish were housed large concrete raceways (3 × 1× 20 m). The rate of flow entering the basin was 660 L/s, and the temperature was constant at 8.1 ±0.1°C. The basin was 75% saturated with oxygen, and the photoperiod consisted of 9-h light and 15-h dark.

### *Experimental animals*

For Series I and II, brook trout were obtained from genetically homogenous stocks (Pisciculture Mont-Tremblant) to minimize the potential effect of genotype on ammonia excretion (Kaushik *et al.* 1984). Each series utilized four groups of 46 brook trout, two replicates per size class (the mean weight and range of Size Class I and Size Class II for Series I and Series II are shown in Table 1). Each group of fish was normally distributed with respect to body weight, and there was no significant difference between the mean weight of the replicates. For Series III, the basin contained approximately 37 100 juveniles aged 0+ (mass: 3-6 g).

Table 1. 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).

| | | Series I (15°C) | | | Series II (10°C) | | |
|---|---|---|---|---|---|---|---|
| Size Class | Replicate | Mean Weight (g) | Range (g) | *t*-test | 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 | |

### *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 and was adjusted continually throughout a given series. Moreover, the amount of food given always surpassed the food intake capacity of a given replicate, ensuring that the variability of the daily food intake of the fish was independent of the daily ration. Alotted rations for both size classes ranged from 4.0 to 7.0% body weight per day of Martin Starter Pellets (wet g; 3 Gr.). Replicates were always alotted 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 feeding approximately 20 min after being fed). Excess food was then retrieved with a fine-meshed fish net and dried in an oven at 60°C for 24-h. The mass of the dried excess was 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 the day of ammonia determinations. Daily food intake was calculated in this fashion because previous experiments demonstrated that food intake on the day prior to an ammonia determination had a significant effect on the excretion rate of fish the following day (Harper et al., submitted to *Aquaculture*). The brook trout of Series III were fed Moore Clark feed periodically for eight hours each day by an automatic feeder (1.5% of fish biomass).

### *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 ~80% of the time. Fish were weighed prior to the scotophase. The average intial weight of the brook trout of Series III was approximately 3 g and the average final weight was 6 g.

### *Ammonia determination*

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 (c.v. 3.0%). Moreover, water samples were obtained from different parts of the rearing facilities to account for potential intra-tank variation. To establish an appropriate sampling time, 24 h experiments were performed where ammonia concentrations were sampled every hour subsequent to feeding in order to elucidate a possible peak in excretion; peak ammonia excretions were recorded approximately 6 hours after feeding at 15°C and approximately 8 hours after feeding at 8-10°C. Sampling at the time of a peak in ammonia production within a tank ensures consistency in relating ammonia excretion to food intake from tank to tank.

### *Statistical analysis*

Dutilleul's multi-frequential periodogram and the related stepwise procedure was used to detect the number and value of periodicities in daily food intake, mean weight, and ammonia concentration, (Dutilleul, 1990; Dutilleul, 1998; Aboul Hosn et al., 1997). The multi-frequential periodogram analysis can reveal several periods close to each other in a given time series; 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 a given time series of a replicate of 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 periodicity (see Fig. 6a in the appendix). Moreover, if periods of 10.78 d and 13.54 d are revealed for another time series of a replicate of a given size class, they will be interpreted as a pseudo-periodic signal with an approximate 12 d period (see Fig. 6b in the appendix).

## Results

### *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 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 (average autocorrelation $\Delta R^2 = 0.14$ -- Table 2). 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 2). The frequency components, on average, jointly explained 86% of the total variation in the time series (average autocorrelation $\Delta R^2 = 0.15$). 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 Figure 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 2). 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 weight of the replicates of Size Class II jointly explained 86% of the total dispersion of the time series

(Table 2). The predominant period for both replicates was about 9-10 d, however 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 2). On average, the frequency components in the ammonia concentration series of the replicates jointly explained 88% of the total variation of the time series (replicate 1 autocorrelation $\Delta R^2 = 0.07$). 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 evidenced for the 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.

Table 2. 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 concentraton series of two size classes of brook trout. $\Delta R^2$ in parentheses. Water temperature was 15°C.

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

Figure 1. Profils de la classe de taille I (masse moyenne: ~ 4,4 g) de la Série I:  ingestion journalière (haut), masse moyenne (milieu), concentration d'ammoniaque (bas)

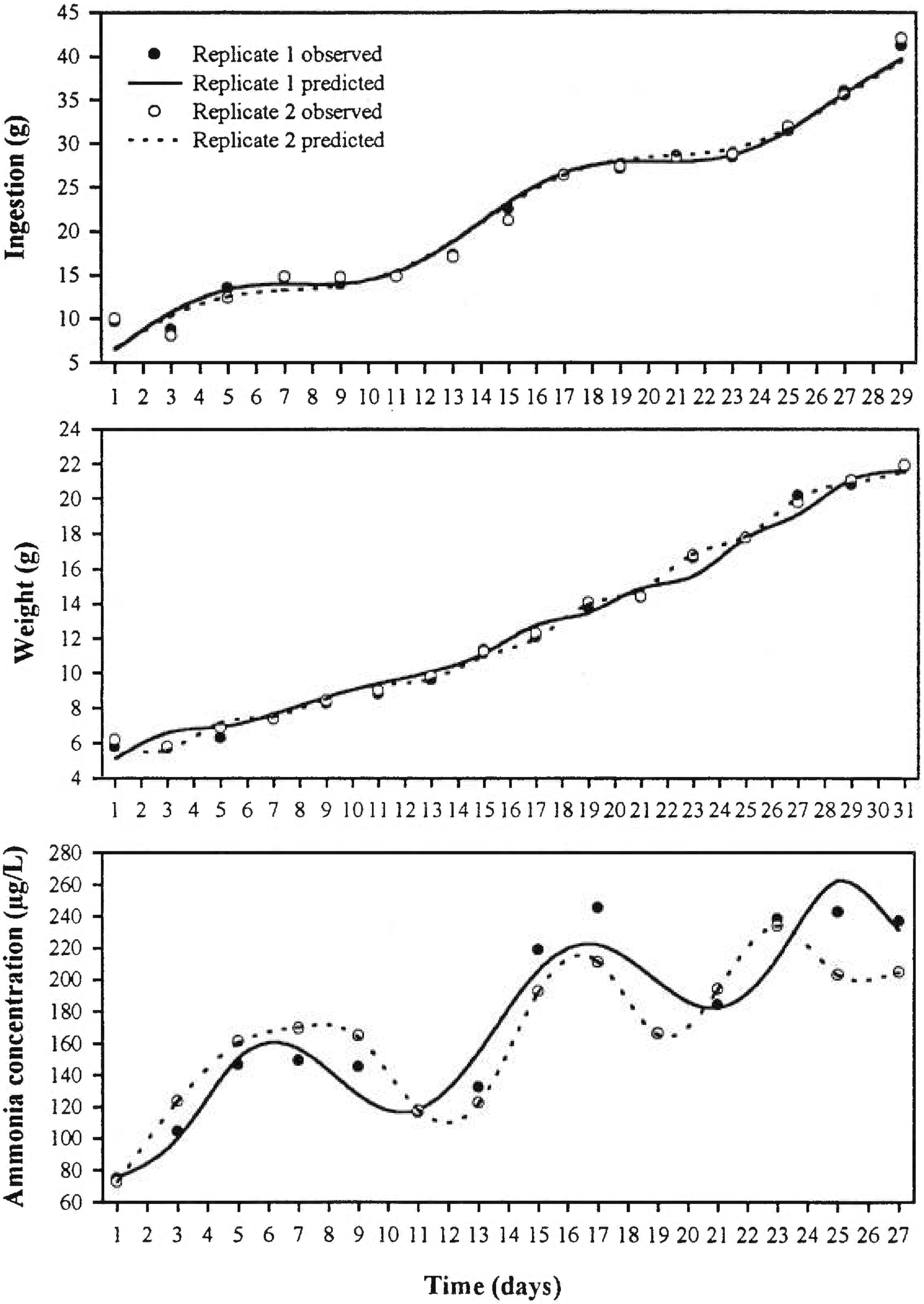
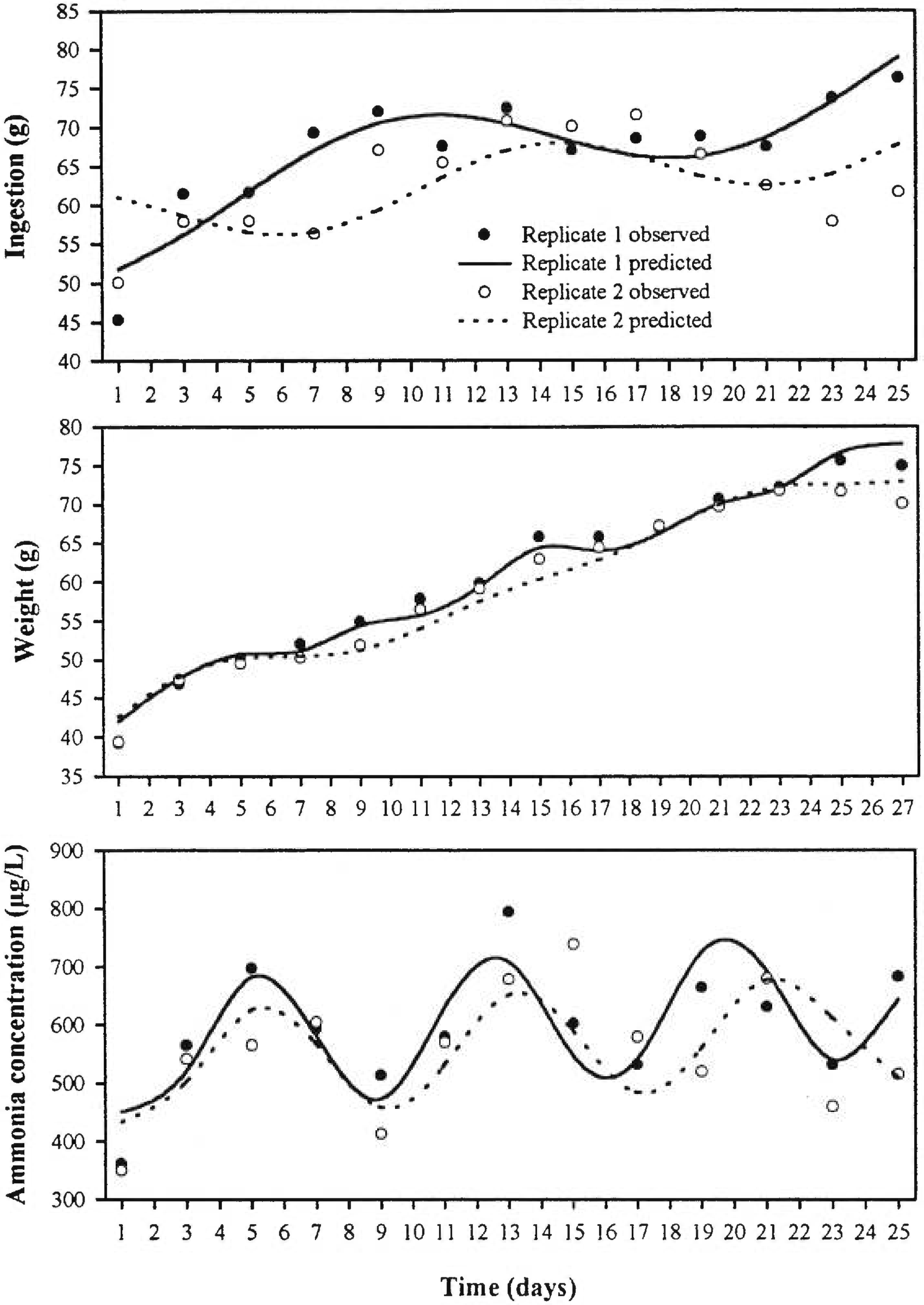

Replicate 1 observed
Replicate 1 predicted
Replicate 2 observed
Replicate 2 predicted
Ingestion (g)
45
40
35
30
25
20
15
10
5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Weight (g)
24
22
20
18
16
14
12
10
8
6
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Ammonia concentration (µg/L)
280
260
240
220
200
180
160
140
120
100
80
60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Time (days)

Figure 2. Profils de la classe de taille II (masse moyenne: ~ 39,0 g) de la Série I: ingestion journalière (haut), masse moyenne (milieu), concentration d'ammoniaque (bas)


Ingestion (g)
85
80
75
70
65
60
55
50
45
40
● Replicate 1 observed
Replicate 1 predicted
○ Replicate 2 observed
Replicate 2 predicted
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Weight (g)
80
75
70
65
60
55
50
45
40
35
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Ammonia concentration (µg/L)
900
800
700
600
500
400
300
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Time (days)

*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 3). 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 (replicate 1 autocorrelation $\Delta R^2 = 0.54$). 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 (replicate 1 autocorrelation $\Delta R^2 = 0.19$; replicate 2 autocorrelation $\Delta R^2 = 0.53$). The predominant periods that emerged 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.

Ammonia concentration demonstrated a range in periodicities of about 4 to 13 d (Table 3), 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 (replicate 1 autocorrelation $\Delta R^2 = 0.49$; replicate 2 autocorrelation $\Delta R^2 = 0.09$). 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 3). The periodic components accounted for about 86% of the variation of the time series (replicate 1 autocorrelation $\Delta R^2 = 0.17$; replicate 2 autocorrelation $\Delta R^2 = 0.26$), 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 (replicate 1 autocorrelation $\Delta R^2 = 0.50$; replicate 2 autocorrelation $\Delta R^2 = 0.44$). 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 3). 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 (replicate 2 autocorrelation $\Delta R^2 = 0.14$). Periods of about 8 and 12 d were once again prevalent in the ammonia concentration series.

Again, 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.

Table 3. 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. $\Delta R^2$ in parentheses. Water temperature was 10°C.

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

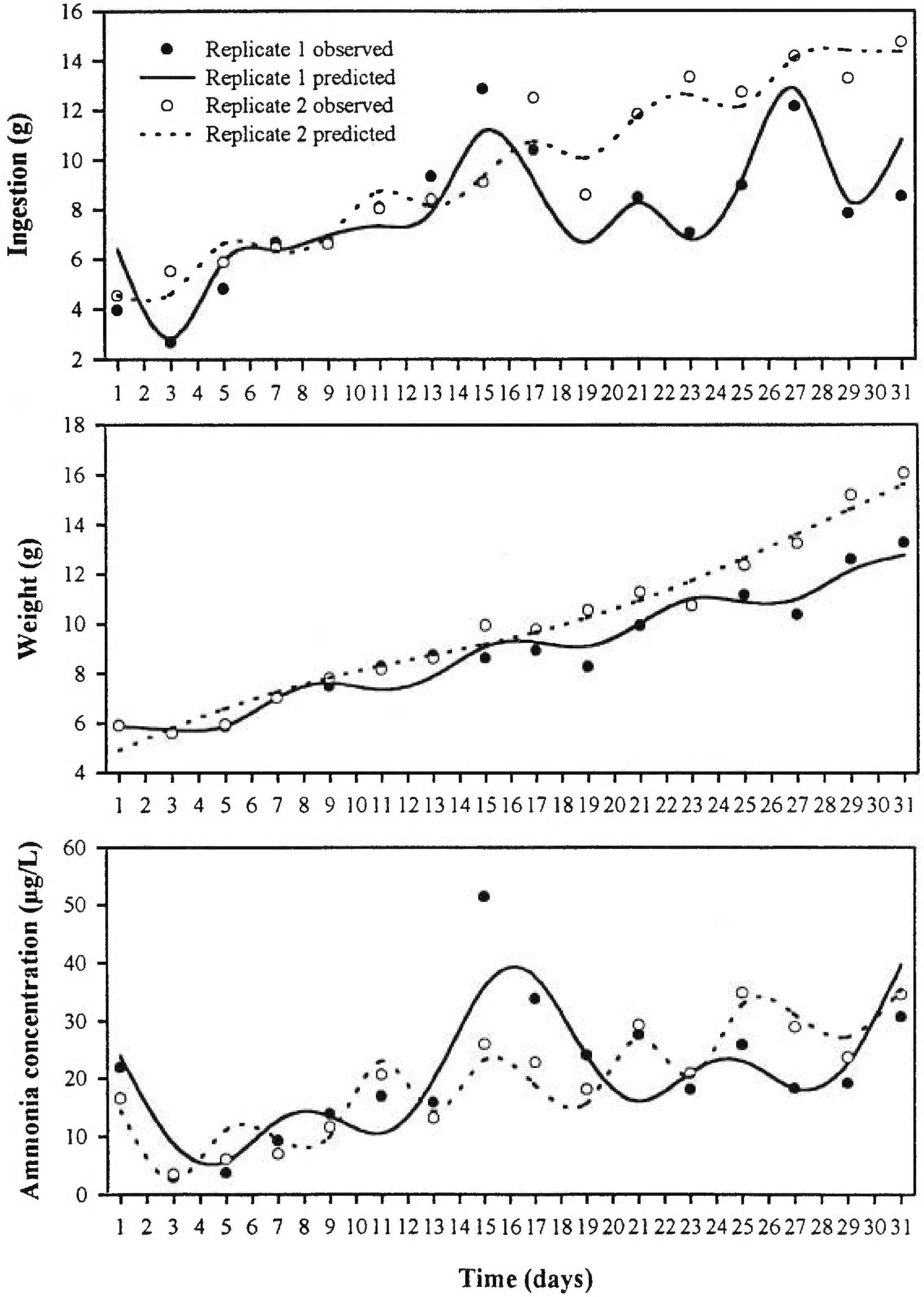
N.S. Non significant periods

N.S.* Significance probability $0.05 \le P < 0.15$

† Fourth significant period not shown

‡ Frequency components corresponding to autocorrelation rather than rhythms

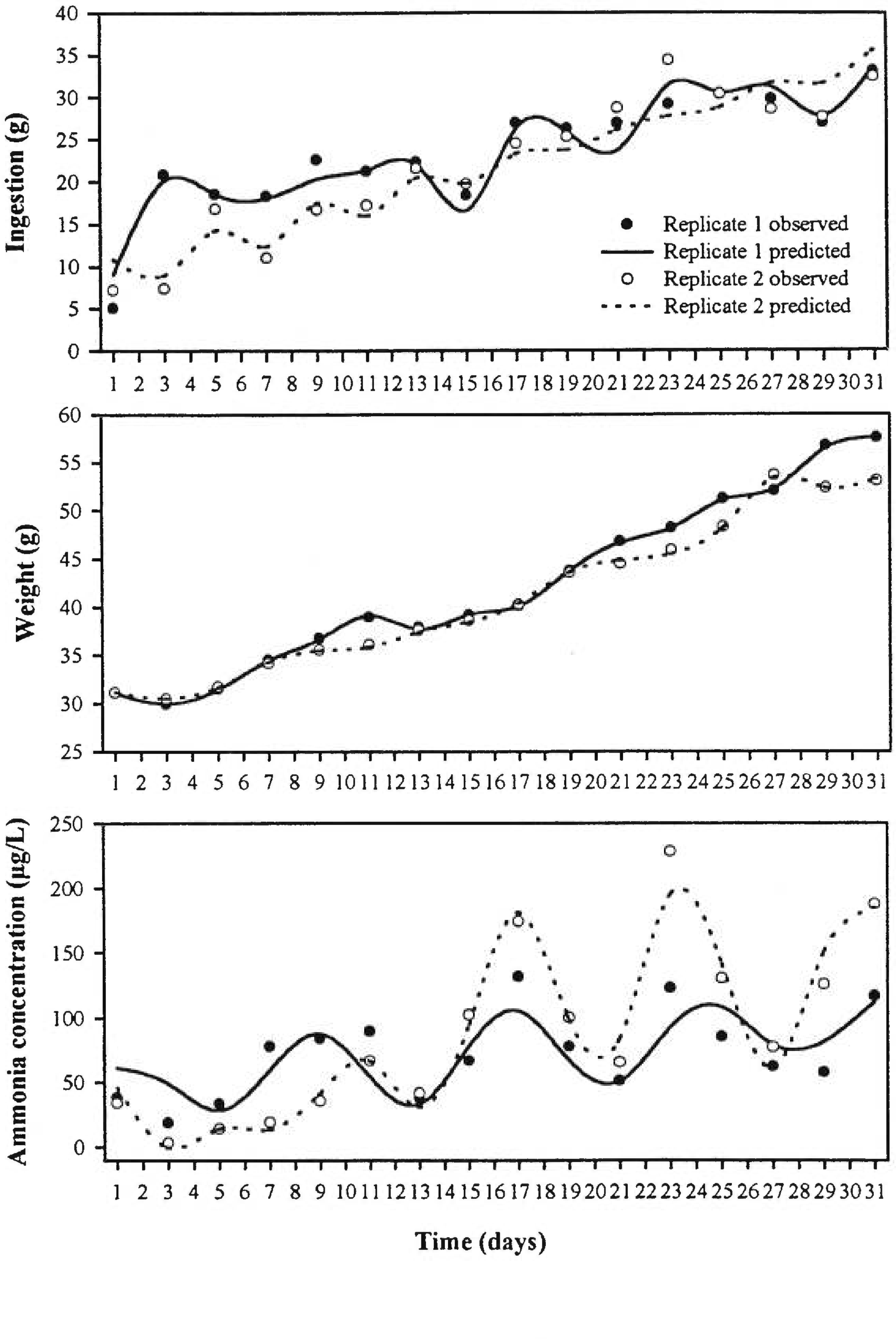
Figure 3. Profils de la classe de taille I (masse moyenne: ~ 5,3 g) de la Série II: ingestion journalière (haut), masse moyenne (milieu), concentration d'ammoniaque (bas)



Replicate 1 observed
Replicate 1 predicted
Replicate 2 observed
Replicate 2 predicted
Ingestion (g)
16
14
12
10
8
6
4
2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Weight (g)
18
16
14
12
10
8
6
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Ammonia concentration (μg/L)
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Time (days)

Figure 4. Profils de la classe de taille II (masse moyenne: ~ 31,3 g) de la Série II: ingestion journalière (haut), masse moyenne (milieu), concentration d'ammoniaque (bas)

J


Ingestion (g)
0
5
10
15
20
25
30
35
40
Replicate 1 observed
Replicate 1 predicted
Replicate 2 observed
Replicate 2 predicted
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Weight (g)
25
30
35
40
45
50
55
60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Ammonia concentration (µg/L)
0
50
100
150
200
250
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Time (days)

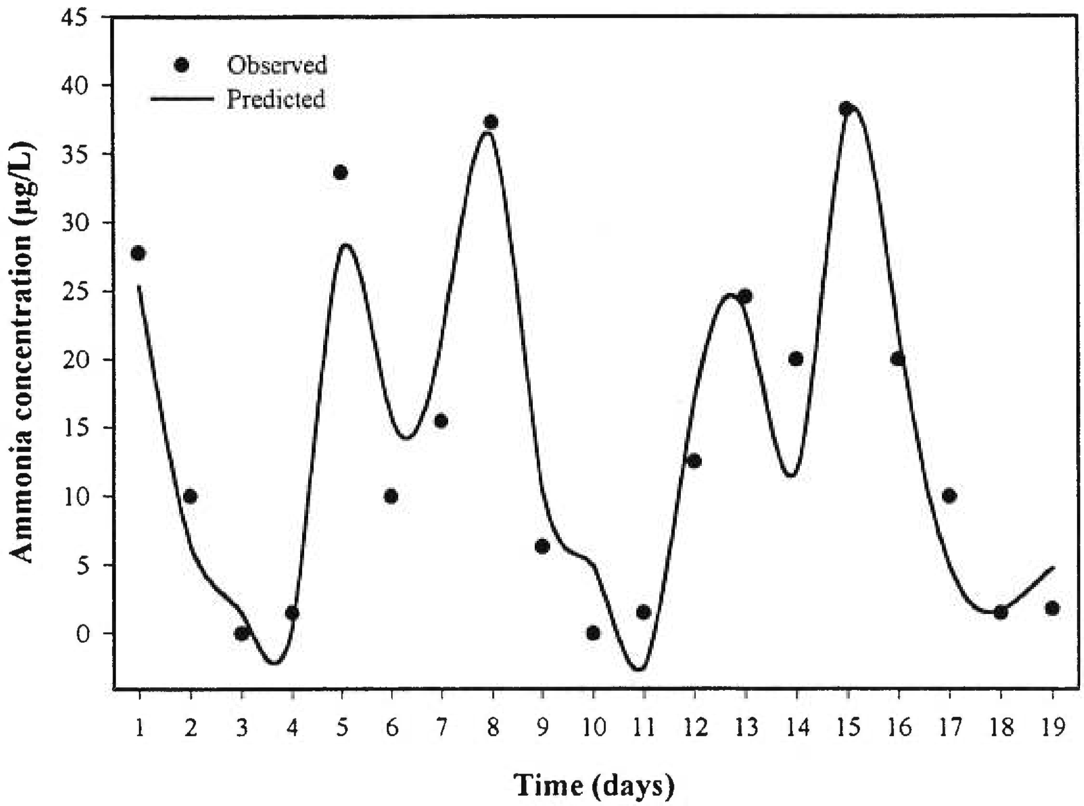
### *A comparison of temperatures*

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 excretion series emerged at both temperatures. These results suggest that temperature did not affect the rhythms of daily food intake, growth, and ammonia excretion.

### *Pisciculture comparison*

Periodicities in the ammonia concentration series observed at Pisciculture SIMDAR (Series III) ranged from about 2 to 8 d. 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 approximately 8 d accounted for 70% of the variation in the series. The other two periodicities can be consideried as components of the 7-8 d period. The results of the pisciculture 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, Series II, and Series III were conducted at temperatures of 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.

Figure 5. Concentrations d'ammoniaque observées (points) et prédites (courbe) dans un bassin d'ombles de fontaine à la Pisciculture SIMDAR



Observed
Predicted
Ammonia concentration (µg/L)
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Time (days)

## Discussion

It was the purpose of this study to estimate short-to-intermediate periodicities in the daily food intake, growth, and ammonia excretion of the brook trout and determine the effect of body size and temperature on these periodicities. This work demonstrates that short-to-intermediate periodicities in daily food intake, growth, and ammonia excretion do, indeed, exist and coincide with each other and that variations in body size and temperature do not appear to affect the periodicities of the measured variables. Furthermore, similar periodicities in ammonia excretion were observed in both a laboratory setting and a pisciculture setting.

To begin with, this study has demonstrated that brook trout display 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; Farbridge and Leatherland (1987a) demonstrated that coho salmon display semi-lunar rhythms in food consumption, and Brown (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, this is the first study, to our knowledge, that has reported periodicities of about one week in the food consumption of fish. The infradian periodicities found in food intake appear to be endogenous rhythms with no counterpart in the environment, yet they also appear not to be characterized by allometric relationships since similar periodicities were observed in different size classes.

Periodicities in growth coincided with periodicites in daily food intake. Predominant short-to-intermediate periodicities of 7 and 10 d in the growth of brook trout were elucidated. The fact that periodicities in growth coincide with periodicites in food intake is not surprising since growth rate is primarly determined by food intake under laboratory conditions (Brett, 1979). Farbridge and Leatherland (1987a) reported a period of 13-14 d in the growth patterns of coho and found that growth patterns coincided with food intake. Short-to-intermediate periodicities in growth rate reported in other studies appear to correspond to the periodicites in growth observed in this study. Farbridge and Leatherland (1987b) reported 10 and 13-14 d periods in growth rate for the coho salmon, yet upon re-analysis of the published data using the multifrequential periodogram used in this study, Aboul Hosn et al. (1997) further elucidated two superimposed periodicites of 8 and 10 d ($R^2 = 0.70$) -- periods similar to those of the brook trout in this study. Moreover, the results of the present study confirm the results of Aboul Hosn et al. (unpublished) study which also reported similar periods of 6-7 and 9-10 d periods in the growth rate of brook trout. Short-to-intermediate periodicites 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 (Delahunty et al., 1978; Vodicnik et al., 1978; de Vlaming, 1980; Weatherley and 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.

Predominant short-to-intermediate periodicities of 7-8 and 12 d in the ammonia excretion of brook trout were also demonstrated in this study. These periodicites coincide with those of daily food intake and growth. Ammonia production is intrinsically related to nitrogen metabolism (Savitz, 1971; Brett and Zala, 1975; Savitz et al., 1977; Paulson, 1980; Jobling, 1981; Lied and Braaten, 1983; Tatrai, 1986). While body weight and temperature can affect ammonia excretion (Savitz, 1969; Guerin-Ancey, 1976; Paulson, 1980; Jobling, 1981; Forsberg and Summerfelt, 1992; Cai and Summerfelt, 1992), sampling ammonia concentrations at the peak of ammonia excretion generally gives a good indication of the amount of nitrogen consumed within a 48 hour period (Harper et al., unpublished). This is confirmed by the coincidence of periods of daily food intake and ammonia production.

Similarities between the rhythms in ammonia excretion 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. In fact, the detection of periodicities in groups of fish homogeneous 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 homogeneous. 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).

Taken together, this study supports the existence of statistically predictable patterns of daily food intake, growth, and ammonia excretion in fish and demonstrates that temperature and body size do not affect these patterns. Furthermore, the inter-relationship among the variables is further supported by the coincidence of their respective rhythms. The coincidence in the rhythmic behaviour of food intake, growth, and ammonia excretion could greatly benefit culturists wishing to optimize their feed investment. By monitoring ammonia concentrations in rearing facilities, a culturist could effectively predict what phase of a food intake and/or growth rhythm fish are situated. Then, in phases of high food intake and rapid growth, a culturist would apportion greater amounts of feed than in phases of low food intake and slow growth, thus, maximizing feed investment. Moreover, varying temperatures in rearing facilities should not concern the culturist since rhythms in daily food intake, growth, and ammonia excretion do not appear to be affected by temperature.

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# 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 2 (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:

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

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.

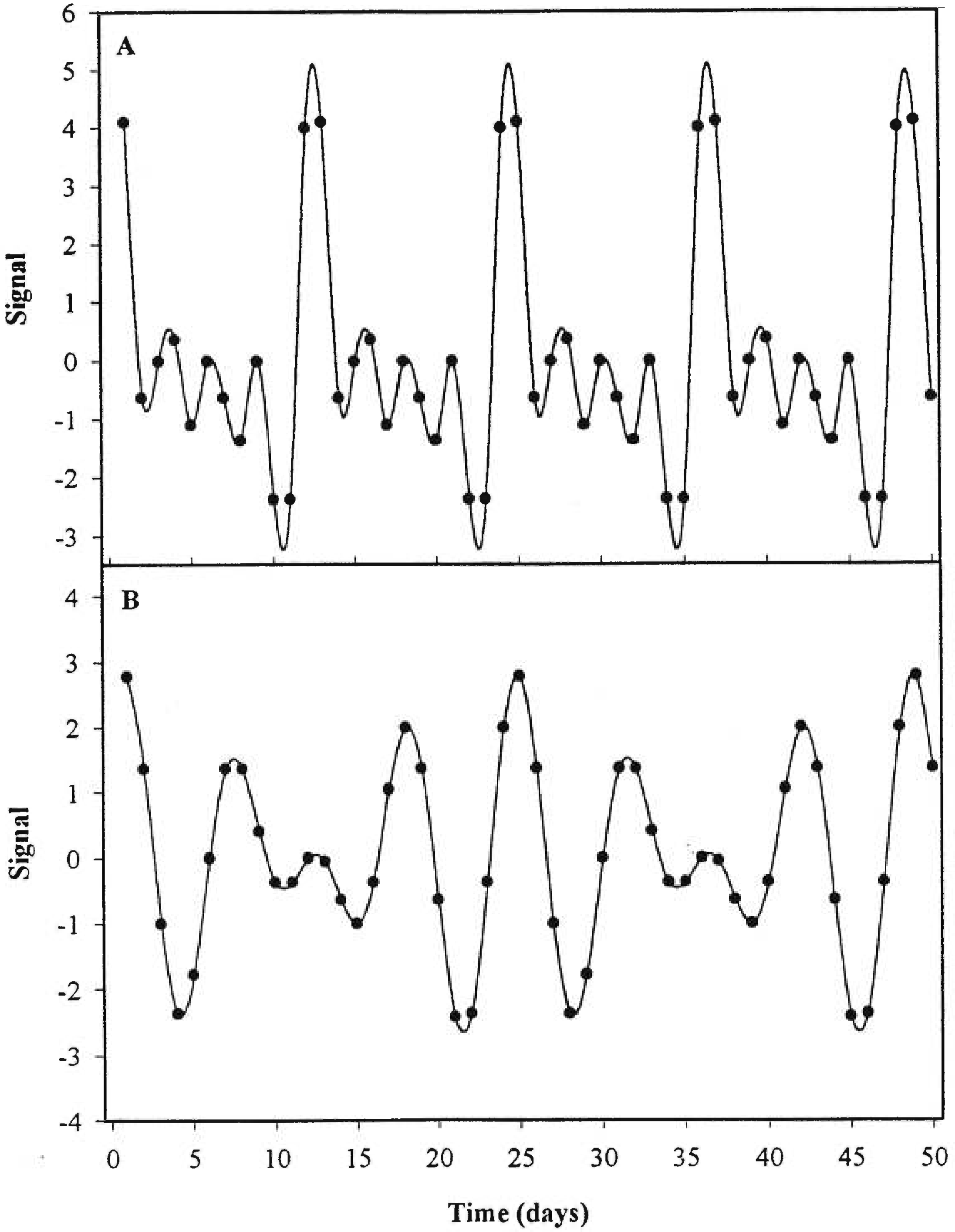
For the second example (Fig. 6b), the equation used was:

$$St = \cos(2\pi t/8) + \sin(2\pi t/8) + \cos(1\pi t/6) + \sin(2\pi t/6)$$

$$\text{for } t = 1, \ldots, 50.$$

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 d 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.

Figure 6. (A) Signal périodique de 12 jours, résultant de la surerposition de quatre cosinus et quatre sinus aux périodes de 12, 6, 4, et 3 jours. (B) Signal pseudo-périodique avec une moyenne de 7 jours et une amplitude non stationnaire (non constante) dans le temps, résultant de la superposition de deux cosinus et deux sinus aux périodes de 8 et 6 jours



A
B
Signal
Signal
6
5
4
3
2
1
0
-1
-2
-3
4
3
2
1
0
-1
-2
-3
-4
0
5
10
15
20
25
30
35
40
45
50
Time (days)

## Conclusion

Le premier chapitre de ce mémoire a illustré les disparités existant dans les patrons journaliers d'excrétion en ammoniaque selon la température, le niveau d'ingestion d'azote et la masse des poissons. Par contre, certaines similarités existent au niveau de ces patrons; une faible production d'ammoniaque au début de la journée, une augmentation de cette production suite à l'ingestion, des baisses marquées de celle-ci 14 à 16 heures après l'alimentation et une hausse de l'excrétion avant l'ingestion. Ceci suggère que l'excrétion en ammoniaque est rythmique lorsque les poissons sont nourris une fois par jour selon un horaire régulier. De plus, il ne semble pas y avoir de différence entre les patrons d'excrétion de la truite arc-en-ciel et de l'omble de fontaine à la même température.

Plus spécifiquement, le taux d'augmentation de l'excrétion et le moment de production maximale après l'ingestion diffèrent selon la température. Les niveaux de production maximale ont été atteint plus rapidement à des températures élevées dû à un taux métabolique plus élevé ou une digestion plus rapide. De plus, une augmentation de la production d'ammoniaque suite à l'alimentation a été observée plus tôt à des températures supérieures. La masse des poissons a semblé affectée le moment de production maximale, avec des pics d'excrétion atteints plus rapidement pour des poissons à masse plus élevée. Par contre, l'effet de la masse n'a été testé qu'à une seule température et on ne peut donc pas conclure avec certitude. La quantité d'azote ingérée a affecté les niveaux de production d'ammoniaque mais pas les patrons journaliers d'excrétion. Toutefois, le niveau d'ingestion d'azote le jour précédant les jours de dosage d'ammoniaque a affecté la quantité d'ammoniaque qui était produit durant ces jours là.

Les résultats de ce premier chapitre ont été utilisés pour la méthodologie de la principale étude: les effets de la température et de la masse sur les périodicités au niveau de l'ingestion, de la croissance et de l'excrétion de l'omble de fontaine (*Salvelinus fontinalis*). Le dosage de l'ammoniaque a été effectué deux heures plus tôt pour l'expérience à 15°C que celle à 10°C et ce à cause de l'effet de la température sur le moment de production maximale. Aussi, l'ingestion journalière a été traitée en prenant la moyenne de deux journées consécutives pour tenir compte de la durée de l'effet de l'ingestion d'azote sur la production d'ammoniaque.

Les résultats de ce second chapitre démontrent que de courtes périodicités existent au niveau de l'ingestion, de la croissance et de l'excrétion, qu'elles coïncident entre elles, et que ni la température, ni la masse ne semble affecter ces périodicités. De plus, les périodicités de l'excrétion en pisciculture ont été trouvées similaires à celles observées en laboratoire. Les périodicités prédominantes des séries d'ingestion ont varié entre 7 et 12 jours. Les périodicités prédominantes de 7 et 10 jours pour la croissance et de 7-8 jours pour l'excrétion coïncident avec celles de l'ingestion. Le fait que les périodicités sont similaires à différentes températures et pour des poissons de masses différentes et que celles-ci soient infradiennes suggère que les rythmes sont endogènes et sans relation avec l'environnement. Par contre, ces rythmes endogènes ne sont pas caractérisés par des relations d'allométrie, ceci suggérant que les rythmes peuvent être un résultat d'un contrôle du système endocrinien.

La coïncidence du comportement rythmique de l'ingestion, de la croissance et de l'excrétion pourrait grandement aider les pisciculteurs désirant optimiser leur investissement en nourriture. En dosant les concentrations d'ammoniaque dans les bassins, un pisciculteur pourrait efficacement prédire dans quelle phase d'ingestion et/ou de croissance les poissons se situent. Ainsi, lors des périodes d'ingestion accrue et de croissance rapide, une plus grande quantité de moulée pourrait être administrée et le contraire s'applique lorsque l'ingestion est faible et la croissance lente. Ceci assurera la maximisation de l'investissement en nourriture. De plus, les variations de température ne devraient pas concerner les producteurs puisque les rythmes d'alimentation, de croissance et d'excrétion ne semblent pas être affectés par celles-ci.

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