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INTRODUCTION

Suite à la surexploitation des ressources maritimes et à l'effondrement de l'industrie de la pêche, le Québec se voit dans l'obligation de mettre en place une solution de rechange. L'aquaculture constitue selon plusieurs organismes de développement économique une réelle opportunité qui permet, lorsque bien établie, de réduire les pressions exercées sur les populations naturelles et d'autre part de relancer et de maintenir des emplois reliés au domaine des pêches tout en développant d'autres créneaux de l'économie des régions. L'aquaculture est le secteur de production alimentaire connaissant la plus forte croissance au monde (BCDA, 2002), pourtant le Québec accuse un retard considérable à participer à ce développement. Il présente un très grand potentiel en possédant 1200 km de côtes propices à l'implantation de sites maricoles (Le François et al., 2002). Pourtant, très peu d'entreprises sont en opération. De plus, les activités d'élevage sont très limitées: en 2001, 97% du volume de production se constituait de moules bleues et de pétoncles géants (MAPAQ, 2002). Le Québec aurait grand avantage à diversifier sa production maricole car le nombre le restreint d'espèces sur lequel l'industrie repose lui confère un niveau de vulnérabilité très élevé (maladies dévastatrices, concurrence, effondrement des marchés, perte d'intérêt du consommateur) (BCDA, 2002).

Dans cette optique, une étude portant sur le potentiel aquacole de diverses espèces a été réalisée (Le François et al., 2002). Cette étude révèle que le loup Atlantique (*Anarhichas lupus*) et le loup Tacheté (*Anarhicas minor*) se sont vu attribuer les premier

et deuxième rangs sous les conditions d'élevage prévalant au Québec (Le François et al., 2002). L'intérêt de l'élevage de ces poissons réside d'abord en leurs adaptations à des eaux relativement froides. De plus, les juvéniles peuvent être nourris avec des aliments formulés dès l'éclosion. À l'éclosion les larves sont à un stade relativement avancé; le stade juvénile est d'ailleurs atteint après quelques jours (Pavlov et Moksness, 1994). Ceci présente un grand avantage pour l'aquaculture car chez d'autres espèces le stade larvaire est souvent fragile et sujet à de forts taux de mortalité.

Un atelier international sur le développement de l'élevage du loup de mer s'est tenu à Rimouski en 2002. Cet atelier a permis d'identifier les priorités de recherche nécessaires au développement d'un élevage commercial de ces espèces. Il a été déterminé qu'un des axes à privilégier est l'établissement de conditions nutritionnelles et d'élevage optimales nécessaires à la stabilisation des performances lors des jeunes stades. Le loup tacheté présente effectivement des taux de survie très variables durant la période d'incubation (0-78%) et lors de la première alimentation (18-50%) (Falk-Petersen et al., 1999). Ceci peut être attribué à la formulation des aliments, à la qualité des œufs et des juvéniles ou au développement des capacités digestives. Une meilleure compréhension de la biologie de cette espèce et des facteurs pouvant affecter sa croissance ou sa survie est donc souhaitable.

Concernant la formulation des aliments, l'ajout d'hydrolysats de protéines à la nourriture permet d'améliorer les performances des juvéniles et la digestibilité de l'aliment (Hardy, 2000). En effet, chez plusieurs espèces de poissons l'alimentation

exogène débute avant que le système digestif ne soit complètement fonctionnel. La digestion des protéines intactes est donc plus difficile (Hardy, 2000). Les hydrolysats présentent une plus grande biodisponibilité (Kristinsson et Rasco, 2000) et améliorent la croissance en stimulant la prise alimentaire et les fonctions digestives (plus forte activité de la trypsine et de la pepsine; de la Higuera, 2001). Plusieurs études ont montré que des hydrolysats ajoutés en diverses proportions à la nourriture augmentaient la survie et la croissance des larves de carpe (Szlaminska et al., 1993; Carvalho et al., 1997) et de bar commun (Cahu et Zambonino Infante, 1995; Zambonino Infante et al., 1997).

Par ailleurs, deux hypothèses ont été proposées pour expliquer les limites physiologiques à la croissance chez les jeunes stades de poissons: premièrement la capacité aérobie, car l'activité des enzymes métaboliques est faible chez les jeunes stades (Blier et al., 1997) et deuxièmement la capacité digestive, car le système digestif n'est pas complètement développé et la production d'enzymes digestives est faible (Kiorboe et al., 1987, Kiorboe, 1989). Houlihan et al., (1988) ont émis l'hypothèse que les capacités de croissance seraient limitées par la digestion et le transport des nutriments. L'activité de la trypsine a été corrélée au taux de croissance chez les larves de carpe (*Cyprinus carpio*) (Sharma et Chakrabarti, 1999). Les travaux de Falk-Petersen et Hansen (2001) montrent que la variabilité de survie et de croissance entre les juvéniles de loup atlantique (*Anarchichas lupus*) est reliée à une faible activité sécrétrice du pancréas lors de la première alimentation. D'autres études portant sur la morue (*Gadus morhua*) montrent de fortes corrélations entre le taux de croissance et l'activité de certaines enzymes

glycolytiques (Pelletier et al., 1993a), mitochondriales (Pelletier et al., 1993b) et digestives (Lemieux et al., 1999).

Objectifs

Le présent projet propose d'évaluer l'impact de la diète et de la température sur les performances de croissance et de survie chez des loups tachetés dont le taux de croissance a été modulé en par l'utilisation de différentes températures. Le premier objectif vise à estimer l'impact de l'incorporation d'hydrolysats de protéine (protéines pré-digérées en polypeptides) à la nourriture sur la croissance et la survie. Afin d'augmenter les chances de détecter un effet bénéfique des hydrolysats sur les performances post-éclosion, nous avons modulé le taux de croissance en soumettant les individus à différents régimes de températures. Les poissons nouvellement éclos ont donc été soumis à trois régimes de température (5, 8, et 12 °C) et à trois différentes diètes (0,10 et 20% d'hydrolysats de protéines) pour chaque température. Les individus à 4 °C devraient avoir un taux de croissance diminué alors que ceux à 12 °C devraient croître plus vite mais subir de plus fortes mortalités. Le premier article intitulé : " DO PROTEIN HYDROLYSATES IMPROVE SURVIVAL AND GROWTH OF NEWLY-HATCHED SPOTTED WOLFISH (*ANARHICHAS MINOR*), A NON-METAMORPHIC AQUACULTURE FISH SPECIES?" porte sur les taux de croissance et de survie obtenus en fonction de la diète et de la température.

Le second volet vise à évaluer les capacités métaboliques et digestives chez les jeunes stades de loup tacheté, plus spécifiquement leur impact sur les capacités du loup tacheté.

Les capacités métaboliques (activité de la citrate synthase (CS), lactate déshydogénase (LDH), pyruvate kinase (PK), et aspartate amino transférase (AAT)) et les capacités digestives (activité de la trypsine) ont été mesurées chez les mêmes individus soumis à différentes temperatures. Nous avons aussi tenté d'établir des relations avec le taux de croissance des individus aux différentes températures. Les principaux résultats sont présentés dans l'article intitulé « THE METABOLIC AND DIGESTIVE RESPONSE OF SPOTTED WOLFFISH (*ANARHICHAS MINOR*) TO VARIOUS GROWTH RATES INDUCED BY TEMPERATURE ».

CHAPITRE 1

DO PROTEIN HYDROLYSATES IMPROVE SURVIVAL AND GROWTH OF NEWLY-HATCHED SPOTTED WOLFISH (*ANARHICHAS MINOR*), A NON-METAMORPHIC AQUACULTURE FISH SPECIES?

1.1 RÉSUMÉ

Le loup tacheté (*Anarhichas minor*) présente un stade larvaire très robuste se rapprochant plus du stade juvénile et ne subit pas de métamorphose. Les « larves » débutent l'alimentation exogène dès l'éclosion et acceptent la nourriture formulée. Le taux de survie à la première alimentation est toutefois très variable ; une meilleure compréhension des facteurs pouvant influencer les performances de survie et de croissance chez les jeunes stades est donc grandement souhaitable. Nous proposons d'évaluer les impacts de l'ajout de trois concentrations d'hydrolysats de protéine (0, 10, 20%) à la nourriture et ce, à trois différentes températures (5, 8, et 12 °C). La température a eu un effet notable sur la survie et la croissance ; des poids finaux au jour 60 de 0.35, 1.19 et 2.02 g correspondant à un taux spécifique de croissance de 1.97, 4.01 et 4.88%/jour et des taux de survie de 49.8, 53.4 et 33.2% ont été obtenus à 5, 8, et 12 °C respectivement. Les hydrolysats de protéines n'ont eu aucun effet sur la croissance à aucune des températures testées mais une augmentation de la survie (quoique non-significative) a été observée avec la diète contenant 20% d'hydrolysats. L'ontogénie précoce du système digestif de cette espèce pourrait être le facteur explicatif de l'absence d'effet bénéfique des hydrolysats de protéines. Nos résultats sont comparés à ceux

obtenus pour des espèces de poissons marins métamorphiques, qui eux, présentent un développement incomplet de leur système digestif à l'éclosion.

Do protein hydrolysates improve survival and growth of newly-hatched spotted wolfish (*Anarhichas minor*), a non-metamorphic aquaculture fish species?

A. Savoie[1], N. R. Le François[1-2§] , C. Cahu[3], P. U. Blier[2], and I. Andreassen[4]

[1] Laboratoire de biologie intégrative, Université du Québec à Rimouski, QC, Canada, G5L 3A1.

[2] Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Centre aquacole marin, Grande-Rivière, QC, Canada, G0C 1V0.

[3] Unité Mixte INRA IFREMER de Nutrition des Poissons, B.P. 70, 29280 Plouzané, France.

[4] Troms Steinbit, Rubbestad, Vangsvik, Norway.

§ Corresponding author: NR Le François, Université du Québec à Rimouski/MAPAQ, Centre Aquacole Marin, 6, rue du Parc, Grande-Rivière, QC, Canada G0C 1V0 Tel : 418-385-2251 (222), Fax : 418-385-3343, Email : Nathalie_Le-Francois@uqar.qc.ca

1.2 ABSTRACT

Despite larval robustness characterized by the absence of metamorphosis and readiness for exogenous feeding based on commercial feed, the spotted wolffish (Anarhichas minor) displays highly variable survival at first-feeding. In this study, we investigated the use of three dietary concentrations of protein hydrolysates (PH, pre-digested proteins) (0, 10 and 20%) when newly hatched juvenile wolfish were held at three different rearing temperatures namely, 5, 8 and 12°C to determine whether digestion of protein was a limiting factor for fish growth and survival. Final weights for fish at 5, 8,and 12 °C at day 60 were respectively 0.35, 1.19 and 2.02 g. Mean specific growth rates were 1.97, 4.01 and 4.88%/day and survival rates were 49.8, 53.4 and 33.2% respectively. No significant effects of PH were observed on growth or survival at any time during the experiment. However, as a general trend, fish survival was always higher when the diet contained 20% PH. We suggest that the degree of hydrolysis of the PH used may have been insufficient to induce specific digestive enzyme stimulation for promoting larval growth. Moreover, precocious ontogeny of the digestive system may have precluded any significant effect of using dietary protein hydrolysates. Our results are discussed in comparison with metamorphic species which characteristically display incomplete development of the digestive system.

Keywords

Anarhichas minor, spotted wolffish, protein hydrolysates, first feeding, growth, survival.

1.3 INTRODUCTION

Spotted wolffish is a marine fish species that is extremely well suited for culture in cold northern climates (Le François et al.; 2002, Foss et al., 2004). Research efforts can be qualified as recent and have mainly been conducted in northern Norway where one commercial facility is in operation. In the eastern part of Québec province, Canada, research on spotted wolffish culture in collaboration with Norway, Iceland and Newfoundland has been underway since 1999 and a project aimed at creating an experimental farm with a production of 10-20 metric tons) is actually under evaluation. The advantages of raising this species as part of a mariculture diversification strategy include 1) their high growth rate at cold water temperature, 2) the low complexity of the larval-juvenile period and 3) their farming-friendly behaviour, all of which should facilitate technological transfer to an aquaculture industry that is currently solely based on salmonid culture. However, despite larval robustness that is characterized by large egg and larval size, the absence of metamorphosis and readiness for exogenous feeding, high variability of survival at first-feeding is frequently reported: e.g. 18-32% (Falk-Petersen et al. (1999), 47-64% (Hansen and Falk-Petersen, 2002), 12-30% (Hansen and Falk-Petersen, 2001a), 88-96% (Hansen and Falk-Petersen, 2001b). The observed variation in fish survival at first-feeding can be attributed to variable egg quality, development of digestive capacities and/or differences in the efficacies of the feed formulation (Falk-Petersen et al., 1999; Lamarre et al., 2004).

Digestive capacity has been proposed as a factor limiting growth rate and survival in larval fish (Gisbert et al., 1999; Sharma and Chakrabarti, 1999; Gawlicka et al. , 2000).

Many authors hypothesize that growth capacity and survival rate could be limited by digestion and nutrient transport capacities (Houlihan et al., 1988; Blier et al., 1997; Lemieux et al., 1999). Trypsin activity, more specifically, a key-enzyme of protein digestion, has been correlated to growth rate (Cyprinus carpio: Sharma et Chakrabarti, 1999; Gadus morhua: Lemieux et al., 1999) and survival (Anarhichas lupus: Lamarre et al. 2004). If digestive capacity was a limiting factor at first-feeding of spotted wolffish, the provision of a more digestible diet to the newly hatched fish should improve both their growth and survival since amino acids from dietary proteins are absorbed mainly as peptides or amino acids (Silk et al., 1985; Rust, 1995; Roennestad et al., 2001). Protein hydrolysates (PH), are pre-digested proteins, that could enhance the larval performance of fish species based on the assumption that an "immature" digestive system limits nutrient absorption (Hardy, 2000). In several species, the inclusion of PH has been reported to have a positive effect on larval performance (Szlaminska et al., 1993; Cahu and Zambonino Infante, 1995; Carvalho et al., 1997; Zambonino Infante et al., 1997). Spotted wolffish, a non-metamorphic species, displays a well-advanced digestive system at hatching i.e. a twisted and differentiated digestive tract (Falk-Petersen and Hansen, 2001). However, due to high variability in their survival and growth at first-feeding, we investigated whether the early-life performance of this species might be limited by protein digestion capacities and whether the dietary inclusion of PH would improve the early-rearing performance of this novel aquaculture fish species.

In an attempt to increase the possibility of detecting beneficial effects of dietary PH supplementation, larval wolfish were maintained at each of the three water

temperatures: namely, 5, 8 and 12 °C. Based on a literature review, fish reared at 5 °C should display impaired growth whereas those reared at 12 °C should display higher growth compared to fish reared at 8 °C, which is a regarded as near the optimal temperature for growth of newly-hatched spotted wolffish during the first 60 days of culture (Hansen and Falk-Petersen, 2002). The nutritional requirements of wolffish have not been thoroughly investigated (Foss et al., 2004). To our knowledge, the effect of adding pre-digested proteins to the feed on growth or survival combined with the effect of varying water temperature have never been tested on any marine fish species. In addition, we selected spotted wolffish as a non-metamorphic aquaculture marine fish species in order to compare the growth and survival responses of this species compared to the more studied metamorphic species. We assumed at the outset of this study that if protein digestion capacities were a limiting factor for the optimal growth and survival of larval wolffish, dietary PH supplementation would have a limited positive effect on fish performances especially at optimal temperature (8 °C) and to a lesser extent, at 5 and 12 °C. Accordingly, three levels of dietary PH (0, 10 and 20%) were tested in fish held at each of the foregoing experimental temperatures.

1.4 MATERIAL AND METHODS

Experimental animals and rearing conditions

The study was carried out at the aquaculture facilities of the Centre Aquacole Marin, Grande-Rivière, Québec, Canada. Fertilized eggs from three different females provided by Troms Steinbit (Tromso, Norway) were sent by air transportation from northern Norway to Québec, Canada. The eggs were at about 380 degree-day upon

arrival. Periodic disinfections were performed regularly with glutaraldehyde (150 ppm) according to protocols established by Hansen and Falk-Petersen (2001b). Individual egg masses were incubated in upwelling incubators at 5 °C until hatching. The hatching success of the three egg masses was 53, 36, and 23% and hatching was completed in a week. Newly hatched fish (mean weight 0.10 ±0.010g and mean length 24.5 ±1.41 mm) from all families were evenly mixed and 40 fish were randomly placed in each of the 27 low-level rearing units (1.5 L volume) that were provided with running (0.5 L/min), oxygenated (oxygen saturation was always above 80%, mean 97 ± 5.5%) sea water (salinity: mean 30.4 ± 0.38 ‰). Water depth was adjusted to 2 cm in order to facilitate feed ingestion (Strand et al., 1995). Light intensity varied between 800 and 1000 lux and a 12/12-h light/dark cycle was adopted. Fish were reared at each of three different temperatures (5 °C: 5.2 ± 0.75 °C, 8 °C: 8 ± 0.25 °C, and 12 °C: 11.5 ± 0.82 °C) and each of the diets was assigned to triplicate groups of fish of each temperature (3 diets X 3 temperatures X 3 replicates = 27 units). The fish assigned to 8 °C and 12 °C were gradually acclimated to their respective temperature (1 °C per hour). Daily record of fish mortality was monitored, dead fish were removed and the rearing units were carefully cleaned with minimal fish disturbance. Initially, fish were fed on *Artemia* in combination with their prescribed experimental diet each hour for the first two weeks post-hatching to promote good feeding behaviour and secure the experiment. After this period, they were fed only on their respective formulated diet. Fish were hand-fed daily to excess (approximately 1 g per day in each tank) every hour from 0800 to 1700 hr and during the interval mentioned above, a concentration of 500 artemia /L was manually distributed eight times per day to each experimental unit. The compositions of the three test diets that

were designated as Control, $PH_{10\%}$ and $PH_{20\%}$, are provided in Table 1. Each was formulated and processed at INRA-IFREMER, Centre de Brest, France. In regards to the latter, the formulated feeds were processed as follows: ingredients were mechanically mixed with water, pelleted and then the pellets were dried at 50 °C for 20 min (Cahu et al., 1999). Thereafter, the pellets were sieved to obtain different ranges of particle sizes namely 200-400, 400-600, 600-800, 800-1000, and 1000-1200 μm. The different size ranges were given to the fish depending on their length using the following formulae: Particule size (μm) = Length of the larvae (mm)·25. Each new size range was gradually introduced in combination with the previous one over a few days. All diets were stored at 4 °C during the study.

The experimental diets contained equivalent protein, lipid and energy content. They differed only in the molecular form of a part of the protein fraction with the control diet containing only native (non-hydrolysed) protein, diets $PH_{10\%}$ and $PH_{20\%}$ containing 10 and 20% hydrolysed protein respectively in combination with native proteins (Table 1).

The PH used, Asta-Pep^{MC}, was made by enzymatic hydrolysis of shrimp (Pandalus borealis) waste, and was provided by ABK-Gaspésie Inc. (Matane, QC, Canada). The amino acid profile was determined using the Waters AccQ Tag method (liquid chromatography (HPLC) with fluorescence detector). Peptide molecular weight

Table 1. Percent composition of the experimental diets.

Ingredients[1] (in %)	**Control**	**PH_{10}**	**PH_{20}**
Fish meal LT	74	64	54
Protein hydrolysates[2]	-	10	20
Precooked potato starch	5	5	5
Cod liver oil	3	3	3
Soy lecithin	5	5	5
Vitamin Mixture[3]	8	8	8
Mineral Mixture[4]	4	4	4
Betaine	1	1	1
Moisture	7.8	8.8	7.4
Protein (N X6.25)/d.m.	58.2	58.0	57.2
Lipid/d.m.	16.4	16.6	16.8
Ash/d.m.	12.8	13.7	13.9

[1]Dietary ingredients were commercially obtained. Fish meal, and cod liver oil were from *La Lorientaise* (Lorient, France). The soy lecithin was from *Ets Louis François* (St Maur des Fossés, France). The precooked potato starch (Nutralys) was from *Roquette* (Lille, France). Asta-pep: ABK Gaspésie (Matane, QC, Canada).

[2]Asta-Pep: Total carotenoids 550 ±10ug/g, proteins 65.8 ± 1.5%/dry product, ash 6.9±0.2 %/dry product, total lipid 20.4 ± 1.5%/dry product.

[3]Per kg of diet: retinyl acetate 80mg; cholecalciferol 0.2mg; all-*rac*- □-tocopherol acetate 800mg; menadione 80mg; thiamin 80mg; riboflavin 22mg; D- calcium pantothenate 160mg; pyridoxine HCl 24mg; cyanocobalamin 80mg; niacin 80mg; choline chloride 16g; ascorbic acid 1.6g; folic acid 8mg; biotin 80mg; meso-inositol 2.4g.

[4]Per kg of diet: KCl 3.6g; KI 1.6mg; $CaHPO_4 \cdot 2H_2O$ 20g; NaCl 1.6g; $CuSO_4 \cdot 5H_2O$ 120mg; $ZnSO_4 \cdot 7H_2O$ 160mg; $CoSO_4 \cdot 7H_2O$ 0.8mg; $FeSO_4 \cdot 7H_2O$ 800mg; $MnSO_4 \cdot H_2O$ 120mg; $CaCO_3$ 8.6g; $MgSO_4 \cdot 7H_2O$ 4.96g; NaF 40mg.

distribution was determined using a Superdex peptide column 10/300GL (Amersham Biosciences), followed by HPLC (Waters 600) analysis. Peptides of known molecular weight were used to calibrate the column. The relative areas of each peak on the chromatograph were given in percent of the total area (Guerard et al., 2002).

Fish were sampled on day 15 (n=5 per replicate), 20 (n=3), 30 (n=3), 48 (n=3) and 60 (n=3)) after 18 hours of food deprivation. Individual weights and lengths of the sampled fish were measured and then the fish were quickly frozen at –80°C for use in a complementary experiment. Survival rate was calculated by withdrawing total number of dead individuals to the initial number of fish in each tank each day. The results are presented as cumulative fish mortality over 60 days.

Specific growth rate (SGR) and condition factor (CF)

SGR was calculated as follows:

$SGR\ (\%.day^{-1}) = 100[(\ln W_{t2} - \ln W_{t1}) \cdot days^{-1}]$

Where W_{t1} is mean weight (g) of the fish at hatching, W_{t2} is mean final weight of the fish.

Condition factor (CF) was calculated according to the formula of Bagenal and Tesch (1978) and Bolger and Connolly(1989):

$CF=100(W/L^n)$

where W= weight (g), L= total length (cm) and n= slope of the logarithmic length-weight equation. The value of "n" calculated for wolffish was 3.24 for the duration of the experiment.

Statistical analysis

Mean values of SGR, survival and CF were compared by two-way analysis of variance (temperature and diet) with the replicates as a nested factor using the general

linear model procedure in the Systat 10.2 computer software (SPSS Inc., 2002). When no significant difference was detected between the diet groups at a given temperature, groups were pooled and then compared to these of the other temperatures. When a significant difference was detected, a Tukey *post-hoc* test was used (Zar, 1984). Treatments were considered to be significantly different when $P< 0.05$. The data were tested for homogeneity of variance and normal distribution prior to the analyses.

1.5 RESULTS

The amino acid profile of the test protein hydrolysate showed that the essential amino acids equalled about 50% of the total. The sulphur amino acids methionine and cystine comprised 7 and 1.3% of total amino acids (Table 2). The Table 2 also shows that the composition in essential amino acids do not differ to a great extent between the LT fish meal and the protein hydrolysates. The molecular weight distribution showed that PH was composed of 25% peptides that had less than 50 amino acids, 65% peptides that had between 50 and 500 amino acids, and 10% peptides with more than 500 amino acids (Table 3).

Dietary PH concentration did not have any significant effect on survival or values for SGR and CF ($p>0.05$) (Table 4) regardless of rearing temperature. Temperature had a highly significant ($p<0.0001$) effect on SGR. In general, for a given temperature and diet, fish performance was highly variable both between and within replicates.

Table 2. Essential amino acid composition of the fish meal LT and protein hydrolysates utilized in the fabrication of the diets (in g per 100 g protein)

Indispensable amino acids (essentials)	Fish meal LT	Protein hydrolysates
Arginine	5.6	7.5
Histidine	1.8	2.9
Isoleucine	4.1	4.7
Leucine	6.7	7.7
Lysine	6.0	7.0
Méthionine	2.4	1.8
Phénylalanine	6.0	6.2
Thréonine	2.6	6.6
Tryptophan	0.8	1.2
Valine	4.4	4.8

Table 3 Molecular weight distribution (percent of total area) from size exclusion chromatography of protein hydrolysates

Molecular weight (Da)	Approximative area (%)
31	1.37
253	13.29
643	7.50
6492	45.20
9818	21.06
20199	9.63
> 20199	1.31

Growth trials that were conducted at different temperatures (pooled results) indicate that percent survival from hatching to day 60, was significantly lower for groups at 12 °C ($p<0.001$) relative to these at 8 and 5 °C (34 versus 52 and 54% respectively; Figure 1). Visual observations of the fish during the feeding trials showed that food ingestion behaviour was fully established by day 12. The observed mortalities always corresponded to non-feeding individuals and were apparent after day 13. Peak mortalities due to starvation occurred at day 16, 23 and 30 and stabilized at day 28, 42 and 53 for groups at 12, 8 and 5 °C respectively (Figure 1).

Values for SGR were directly related to rearing temperature (Figure 2). Overall values for SGR between day 0 and day 60 were 1.97, 4.01 and 4.88%·day^{-1} at 5, 8 and 12 °C respectively. Growth rates at different stages of the study were also calculated and the data have been presented in figure 2.

Table 4 Mean percent survival, specific growth rate (%/day) and condition factor (CF) of wolfish in relation to rearing temperature and diet treatment at day 60 post-hatch.

T °	5°C			8°C			12°C		
Diet	**Control**	**PH_{10}**	**PH_{20}**	**Control**	**PH_{10}**	**PH_{20}**	**Control**	**PH_{10}**	**PH_{20}**
Survival	51.6±1.8	51.2±7.6	53.7±17.0	49.7±16.3	53.3±13.5	59.4±9.6	33.9±6.3	32.3±6	37.0±3.2
SGR	2.1±0.3	1.7±0.6	2.1±0.6	4.1±0.4	3.7±0.4	4.2±0.5	5.1±0.1	4.4±0.5	5.0±0.7
CF	0.73±0.16	0.65±0.04	0.61±0.04	0.67±0.14	0.60±0.09	0.59±0.05	0.58±0.03	0.55±0.17	0.55±0.04

Expressed as mean ± standard deviation; initial fish weight: 103.0 ± 10.3 mg; n=40 individuals per replicate (3 replicates)

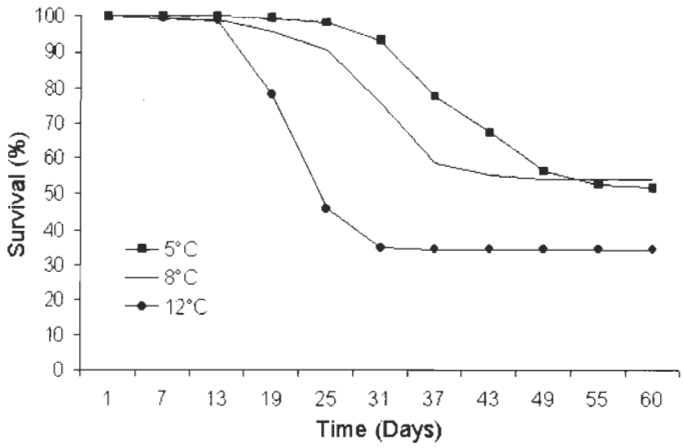

Figure 1 Survival of the wolffish reared at different temperatures

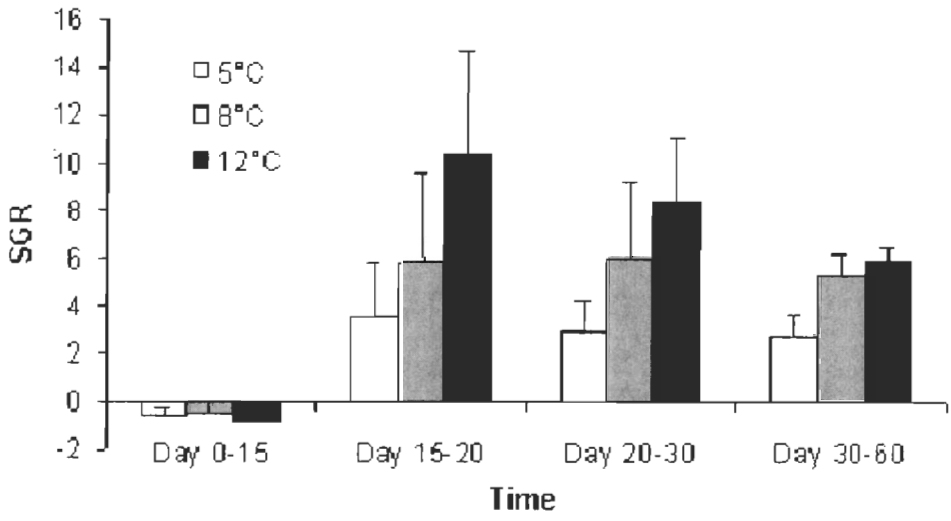

Figure 2 Specific growth rate of spotted wolffish reared at different temperature from hatching to day 60

1.6 DISCUSSION

The mean SGR values calculated for wolffish at the different temperatures in the present study were in accordance with those reported by others for first-feeding wolffish (Hansen and Falk-Petersen, 2002; Hansen and Falk-Petersen, 2001; Falk-Petersen et al., 1999). Hence, our experimental diets containing 58% protein and 16.5% lipid were likely suitable for culturing this species.

The negative SGR values obtained between day 0-15 probably reflected the large proportion of starved individuals in all groups in which a loss of weight was noticeable. Those individuals eventually died after day 13 and then the calculated SGR values were no longer influenced by the reduced weight of the non-feeding individuals. The larvae grew better at higher temperature from day 15-20 and day 20-30. However, between day 30 and 60, SGR values were almost the same when the fish were held at 8 and 12 °C. When calculated from day 48 to 60, SGR was slightly higher for the fish held at 8 °C (results not shown) suggesting a lowering of the optimal temperature. This is in accordance with the results of Hansen and Falk-Petersen (2002) who further reported better growth in spotted wolffish when water temperature was regulated to 8 °C compared with those at 10 or 12 °C from day 30 post-hatch. According to our results and previous research, it is therefore proposed that wolffish be reared at 8-10 °C for the entire juvenile period if we consider the high mortality rates that are encountered at 12 °C and the reduced growth rate of the fish at 5 °C.

During the first 12 days post-hatching, most larvae lay on the bottom of the tank and do not display active feeding behaviour. This was also observed by Strand et al. (1995) in a closely related species (A. lupus) during the first 6 days post-hatch. The mortalities observed always corresponded to the smallest non-feeding individuals that were lying on the bottom of the tank. This is an indication, as proposed by Strand et al. (1995), that the failure to initiate feeding may be the main cause of mortality and not the unsuitability of the diets. Falk-Petersen et al. (1999) observed through histological examinations that wolffish larvae that never start exogenous feeding often have abnormalities of the mucosa cells of the digestive tract. No feeding individuals died during the 60 days of the experience.

Statistical analyses of the growth and survival data did not reveal any significant effect of dietary PH concentration. However, we cannot conclude that dietary PH did not have any effect on the newly hatched wolffish. Indeed, we obtained highly variable results among replicates that likely precluded us from seeing significant diet differences. As a general trend, we observed that highest fish survival was always achieved by the groups given the PH20 diet at all temperature. Also, the use of *Artemia* in combination with the formulated diets during the first two weeks of culture might have masked the appearance of marked diet differences in fish survival rates by lessering the eventual differences induced by the diets.

Dietary protein hydrolysate supplementation has been reported to yield highly variable results among different studies even in closely related species. For instance,

some studies have shown that PH supplementation enhanced the growth of salmon fry (Salmo salar) (Berge and Storebakken, 1996), post-smolt Atlantic salmon (Refstie et al., 2004) and carp larvae (Cyprinus carpio) (Carvalho et al., 1997). Moreover, Cahu et al.(1999) observed improved survival and growth of Sea bass when a moderate amount of PH was included in their feed. Further, another study conducted on Atlantic halibut showed that inclusion of PH in the diet slightly improved growth and survival of the fish (not statistically significant) of Atlantic halibut larvae compared to those fed control diet (Kvâle et al., 2002). Positive effects of dietary protein hydrolysate supplementation on growth and survival have only been reported on larval stages of fish in the literature. By contrast, no or negative effects have been reported on juvenile rainbow trout (Hardy et al. 1983, Stone et al. 1989) and turbot (Oliva Teles et al., 1999). We tentatively suggest that the absence of a significant beneficial effect of dietary PH supplementation in this study may be linked to the relatively advanced stage of wolffish at hatching. Wolffishes hatch with a morphology more characteristic of juvenile fish i.e. they display external morphology and internal organs that are not typical of larval fish (Falk-Petersen and Hansen, 2001). The stomach and gastric glands are formed many weeks before hatching in wolffish (Falk-Petersen and Hansen, 2001). On the other hand, many fish species begin exogenous feeding before the onset of a fully functional digestive system and larval fish generally display poor assimilation efficiency for protein (Ronnestad et al., 2001). Furthermore, metamorphic species have an undifferentiated digestive system which appears as a straight tube at hatching (Kolkovski, 2001; Gisbert, 2004 ; Hamlin et al., 2000). Stomach development is achieved at day 15 post-hatch in sea bass (Walford and Lam, 1993) and day 22 post-hatch in Dover sole (Solea solea) (Bouhlic and Gabaudan,

1992), and gastric glands appear at day 25 post-hatch in European sea bass (Zambonino-Infante and Cahu, 2001) and at day 22 post-hatch in sole even though no pepsin activity has been observed after 5 weeks post-hatching (Bouhlic and Gabaudan, 1992).

Day et al. (1997) and Cahu and Zambonino-Infante, (2001) suggest that dietary PH supplementation is beneficial to fish larvae but may have no effect or even depress growth of juvenile fish. Pancreatic proteolytic enzyme (e.g. trypsin) is generally low at the onset of first-feeding in most fish larvae and is enhanced only at metamorphosis (Gawlicka et al., 2000, Cahu and Zambonino-Infante, 2001). Wolffish have well developed endo- and exocrine pancreatic tissue at hatching (Falk-Petersen and Hansen, 2001). Wolffish also have trypsin activity well before hatching (activities of up to 3.41 and 6.73 U/g $*10^{-4}$ protein at 340 and 900 degree-days, respectively) (Desrosiers et al., in preparation). Furthermore, Lamarre et al. (2004) suggested that trypsin activity levels at hatch could be used as an indicator of growth and survival in a closely related species (A. lupus).

The biochemical composition of PH was examined in this study to facilitate interpretation of the growth and survival results. In this regard, the amino acid composition of PH was found to be very close to that of fish meal (Zambonino Infante et al. 1997) and well suited to support larval growth. But the peptide sizes of PH were noted to be very large compared to those present in PHs used in other studies. Indeed, in our study, 65% of the peptides were composed of 50 to 500 amino acids, whereas an effect was seen in sea bass larvae ingesting di-and tri-peptides (Zambonino Infante et al., 1997)

and 20 amino acid peptides (Cahu et al., 1999). It is therefore possible that the degree of hydrolysis of the PH used in this study was insufficient to induce a specific digestive enzyme stimulation that would have promoted larval growth. This possibility should be examined in a future study.

1.7 ACKNOWLEDGEMENTS

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CHAPITRE 2

THE METABOLIC AND DIGESTIVE RESPONSE OF SPOTTED WOLFFISH (*ANARHICHAS MINOR*) TO VARIOUS TEMPERATURE

2.1 RÉSUMÉ

Le loup tacheté étant une espèce menacée, une meilleure compréhension des facteurs pouvant influencer sa survie ou sa croissance est grandement souhaitable. Les juvéniles de loups tachetés ont été soumis à trois régimes différents de température (5, 8, 12 °C) afin de moduler le taux de croissance et ainsi déterminer l'impact sur des enzymes clé du métabolisme énergétique et des fonctions digestives. Les taux de croissance (SGR) obtenus étaient significativement différents entre les 3 temperatures (1.97, 4.01 et 4.88%/jour à 5, 8 et 12 °C respectivement). Le contenu en protéines ainsi que l'activité de la trypsine et de la LDH étaient corrélées positivement avec le taux de croissance à toutes les températures. Il semble que la capacité aérobie (représentée pas la CS) soit suffisante pour pallier à la forte demande énergétique engendrée par le fort taux de croissance chez les jeunes stades. La trypsine présente une compensation positive liée à la température alors que les enzymes glycolytiques présentent une compensation négative.

THE METABOLIC AND DIGESTIVE RESPONSE OF SPOTTED WOLFFISH (*ANARHICHAS MINOR*) TO VARIOUS TEMPERATURE

Arianne Savoie[1], Nathalie R. Le François[1-2§] , Chantal Cahu[3], Inger Andreassen[4] and Pierre U. Blier[1]

§ Corresponding author: NR Le François, Université du Québec à Rimouski/MAPAQ, Centre Aquacole Marin, 6, rue du Parc, Grande-Rivière, QC, Canada G0C 1V0 Tel : 418-385-2251 (222), Fax : 418-385-3343, Email : Nathalie_Le-Francois@uqar.qc.ca

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[1] Laboratoire de biologie animale intégrative, Université du Québec à Rimouski, QC, Canada, G5L 3A1. [2] Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Centre aquacole marin, Grande-Rivière, QC, Canada, G0C 1V0. [3] Unité Mixte INRA IFREMER de Nutrition des Poissons, B.P. 70, 29280 Plouzané, France. [4] Troms Steinbit, Rubbestad, Vangsvik, Norway.

2.2 ABSTRACT

Three groups of newly hatched spotted wolffish (*Anarhichas minor*) were held at different temperature in order to modulate growth rate and establish relationships between metabolic and digestive response in rapidly developing larvae. Growth rates were successfully modulated by temperature (1.97, 4.01 and 4.88%/day 60 days post-hatch at 5, 8 and 12°C respectively). Levels of activity of trypsin, pyruvate kinase and lactate dehydrogenase were positively linked to specific growth rate and at all temperatures. Citrate synthase was not affected by growth rate. The level of aerobic capacity was adequate in sustaining the high energetic demand associated with high growth rate early in the life of the spotted wolffish. Trypsin activity displayed positive temperature compensation whereas glycolytic enzymes displayed negative temperature compensation. Trypsin and LDH activities were strongly related to growth rate. The higher LDH activity of faster growing fish would be a consequence of high growth rate rather that a cause. Our results suggest that trypsin activity could be the factor limiting growth rate in newly hatched spotted wolffish.

2.3 INTRODUCTION

The spotted wolffish (*Anarhichas minor* Olafsen) is a marine fish species extremely well suited for cultivation under cold northern climates (Le François et al., 2002; Foss et al., 2004). The advantages of raising this novel species resides in 1) their high growth rate at low water temperature, 2) the low complexity of the larval-juvenile period and 3) their farming-friendly behaviour. Furthermore, the Committee on the Status of Endangered Wildlife (COSEWIC: www.cosewic.gc.ca) in Canada has recently identified the spotted wolffish as a threatened species in Canadian habitats. Newly hatched fish are at the most vulnerable stage of development and it is also at this critical period that they will experience the highest growth rate they will achieve in their entire lifetime. Consequently, a better understanding of the factors influencing early growth of this species in relation with energy metabolism and digestive capacities is of primary importance.

Metabolic and digestive capacities are two physiological processes proposed to be involved in restraining fish growth (Houlihan et al., 1988; Lemieux et al., 2003). Growth is an energetically costly process that is achieved by protein deposition in the muscle (Houlihan et al., 1995). Energy-rich molecules processed by the digestive system are made available to the different metabolic pathways in order to produce sufficient ATP to sustain the high protein turnover rate associated with high growth rate. Studies on Atlantic cod (*Gadus morhua,* L.) report strong relationships between glycolytic (Pelletier et al., 1993a), mitochondrial (Pelletier et al., 1993b), and digestive (Lemieux et al., 1999) enzyme activities and growth.

Recently, Lamarre et al. (2004), working on larval quality indicators, found indications that lactate dehydrogenase (LDH), pyruvate kinase (PK), trypsin (TRYP) and aspartate aminotransferase (AAT) activity levels were linked to growth performances and survival of newly hatched Atlantic wolffish (*Anarhichas lupus* L.), a closely related species. However, failure to directly relate enzyme activity levels to growth rate was reported by these authors. In order to evaluate more clearly the relationship between growth rate and key enzymes of metabolic and digestive enzymes activity levels in newly hatched wolffishes, we suggest the use of temperature modulation in order to generate different growth rates. Temperature is one of the most important environmental factor influencing the physiological processes in fish. For example, a 10 °C variation of temperature can lead to two to three-fold changes in metabolic rate (Schmidt-Nielsen, 1997; Clarke and Johnston, 1999). However, diverse teleosts have evolved the capacity to offset the acute response to temperature through metabolic compensation (Guderley, 1990; Johnson, 1993).

In our study, growth trials were conducted on the spotted wolffish, a species that displays a growth rate three to four times higher than the Atlantic wolffish and is accordingly considered a better candidate for cold-water mariculture activities (Le François et al. 2002). At hatching, wolffishes are very large in size (20-24 mm) and well-developed compared to most marine fish species of aquaculture interest: Atlantic cod (*Gadus morhua*): 3.5-4.5 mm or Atlantic halibut (*Hippoglossoides hippoglossoides)*: 6.5-7.0 mm). This enables the measurement of numerous enzymes in a single fish.

Wolffishes also display a precocious ontogeny of their digestive system, relying at hatching day on exogenous feeding for their growth and survival (Falk-Petersen and Hansen, 2001; Lamarre et al. 2004).

Metabolic capacities will be evaluated through the measurement of citrate synthase (CS: aerobic capacity), LDH (anaerobic glycolysis), PK (aerobic glycolysis) and AAT (protein oxidation and amino acid transamination). Digestive capacities will be assessed by measuring TRYP activity (protein digestion). Trypsin has been chosen because it is the only digestive enzyme showing correlations with growth rate and conversion efficiency in a provious study on cod (*Gadus morhua;* Lemieux et al., 1999).

2.4 MATERIAL AND METHODS

Experimental fish and rearing conditions

The study was carried out at the facilities of the Centre Aquacole Marin de Grande-Rivière, Québec, Canada. Spotted wolffish eggs from three different females were provided by Troms Steinbit (Tromso, Norway). The eggs were approximately 550 degree-days at their arrival in Québec, Canada. Egg masses were incubated separately in serial upwelling incubators at 5 °C. Periodic disinfections were performed as prescribed by Hansen & Falk-Petersen (2001). Hatching began on November 9th 2004 and in order to obtain a high number of larvae simultaneously to initiate the growth trials, hatching was provoked by gentle pressure on the eggs. Forty newly hatched spotted wolffish (mean weight 0.103 ±0.104g and mean length 24.5 ±1.4 mm) from the three families were randomly placed in each of the 36 low-level (2 cm) rearing units containing 1.5

litres with a water supply of 0.5 litres/minute. During the growth trials mean salinity was 30.4 ± 0.4‰ and oxygen saturation always above 80% (mean 97.0 ± 5.5%). Luminosity was between 150-200 lux and a 12/12-h light/dark cycle was adopted. Fish were reared at three temperatures (5.2 ±0.8 °C, 8.0 ±0.3 °C, and 11.5 ±0.8°C, 12 tanks per temperature). The fish transferred at 8 °C and 12 °C were gradually acclimated to their respective temperature (1 degree per hour). Mortality assessment, dead fish removal and careful cleaning of the rearing units were realized daily.

Fish were fed *Artemia* nauplii in combination with a formulated fish feed (formulated and processed at Ifremer, Centre de Brest, France) for the first two weeks post-hatch. During this period, the feed was offered every hour from 8am to 5pm. The fish were weaned after 2 week and hand-fed in excess. The formulated feed was processed as follows: ingredients were mechanically mixed with water, pelletized and dried at 50°C for 20 min (Cahu et al., 1999). The pellets were sieved to obtain different particle size of 200-400, 400-600, 600-800, 800-1000, and 1000-1200 µm. Different sizes were given to the fish depending on their length using this formulae : Particule size (µm)= Length (mm) * 25. Two sizes of particles were given in combination if a group was heterogenous.

Sampling

Fish were sampled over 60 days at different intervals (on hatching day (n=14), day 15 (n=36 per temperature), 30 (n=36 per temperature), 60 (n=36 per temperature). Fish were fasted for 18 hours before sampling. Individual weight and length were

measured and whole fish quickly frozen at –80°C until enzymatic analysis were performed. Specific growth rate (SGR) was calculated as follows:

SGR (%.day^{-1})= (ln W_{t2})-(ln W_{t1})·(experimental time in days)$^{-1}$ x 100.

Where W_{t1} is mean weight (g) of the fish at hatching, W_{t2} is mean final weight of the fish.

Enzymatic assays

Whole individuals were thawed on ice and homogenised in 9 volumes of Tris-HCl buffer (100mM, pH 7.5) using an Ultra Turrax T25 (IKA Labortechnik) electrical homogeniser for three 10-s periods. Between each period, samples were kept on ice for 1 min. An aliquot of the homogenate was centrifuged (400 *g* for 1 minute) for aspartate-amino-transferase (AAT, EC 2.6.1.1), citrate synthase (CS, EC 2.3.3.1) pyruvate kinase (PK, EC 2.7.1.40), and lactate dehydrogenase (LDH, EC 1.1.1.27) assays. Another aliquot was centrifuged at 13000 *g* for 15 minutes for trypsin assays. Enzyme activities were measured using a Lambda 11 UV/VIS spectrophotometer equipped with a thermostated cell holder (Perkin Elmer) assay conditions are presented in Table 5.

Activities of AAT, CS, PK and LDH were measured according to the methods described by Lemieux et al., (2003) and TRYP was measured according to Preiser and al., (1975) with modifications. Total protein content was determined using the bicinchoninic acid method (Smith et al. 1985). Assays were conducted at 15 °C and protein analyses were carried out at room temperature. All assays were run in duplicate. Enzymatic activities were expressed as U·mg^{-1} protein to ensure that an increase in any particular enzyme did not reflect the general increase in protein content.

Table 5 Parameters used for the enzyme analysis (Lemieux et al. 2003, Prieser et al., 1975, with modifications)

Enzyme	Substrate	Buffer solution	pH
AAT	Aspartate (22mM)	Phosphate (100mM)	7.4
CS	Oxaloacetate (0.2mM)	Imidazole-Hcl (100 mM)	8.0
Trypsin	Benzoyl-L-arginine-p-nitroanilide	Tris-HCl 0.2M, $CaCl_2$ 0.04M	8.0
PK	Phospho(enol)pyruvate(5 mM)	Imidazole-HCl (100mM)	7.5
LDH	Pyruvate (1mM)	Imidazole-HCl (50mM)	7.0

Statistical analysis

Mean SGR were compared between each temperature by analysis of variance using the general linear model procedure in the Systat 10.2 computer software (SPSS inc., 2002). When a significant difference was detected, a Tuckey *post-hoc* test was used (Zar, 1984). To detect a potential effect of growth rate, temperature and their potential interactive effect on enzymatic activities, analysis of variance was performed (temperature, SGR and temperature X SGR) were performed using the general linear model procedure. When a significant interaction was detected between temperature and growth rate, a linear regression of growth rate vs. enzymatic activity was performed for each temperature and slopes were compared and considered significantly different when the 95% confidence interval of the slope did not overlap between two treatments. Treatments were considered significantly different when $P< 0.05$.

2.5 RESULTS

Survival from hatching to day 60, was significantly lower for groups at 12 °C (52, 54, and 34% at 5, 8, and 12 °C respectively). Mortalities stabilized at day 28, 42 and 53 respectively for groups at 12, 8, 4 °C. Growth was successfully modulated by temperature (1.97, 4.01 and 4.88%·day^{-1} were achieved at 5, 8 and 12°C respectively, Figure 3).

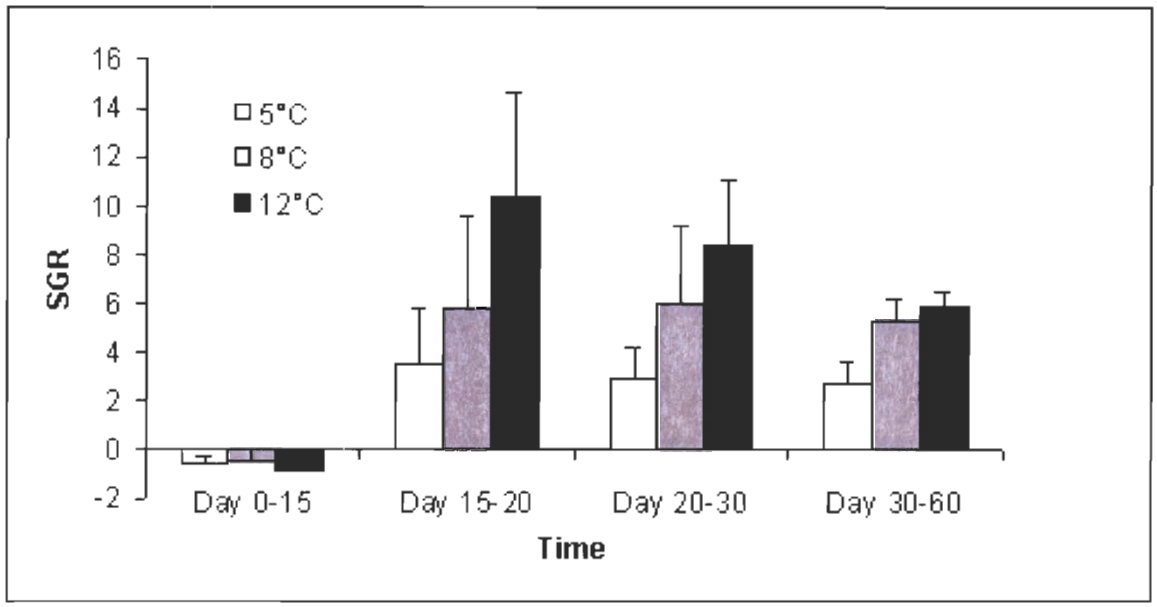

Figure 3 Specific growth rate of spotted wolffish reared at different temperature from hatching to day 60

Protein content

Protein content decreased at day 15 compared to day 0 but returned to comparable value from day 30. Protein content was significantly different between the different temperature treatments from day 30 (192.2, 213.2 and 218.3 mg protein·g of fish^{-1}at day 30 and 215.6, 201.6 and 196.2 mg protein·g of fish^{-1} at day 60, at 5, 8 and 12 °C, see figure 4a). A linear regression was made of protein content as function of SGR, there

was a significant effect of temperature (P=0.034) and SGR (P<0.001) and there was a significant interaction between those two factors (P=0.003, figure 5a). The correlation coefficient (R^2) was 0.59. The linear regressions showed that the slope at 4 °C was significantly steeper that at 12 °C.

Aspartate aminotransferase

There were significant differences in AAT activity between experimental temperatures from day 30 (0.251, 0.263 and 0.298 U·g protein^{-1} at 30 days post-hatching (DPH) and 0.300, 0.307 and 0.332 U·g protein^{-1} at 60 DPH at 4, 8 and 12 °C, Figure 4b). There were no relationships between AAT activity and SGR nor any interaction between temperature and SGR (Figure 5b).

Citrate Synthase

There was no differences in CS activity between the temperature treatments except for day 30 where the activity was lower at 12 °C (0.067, 0.060 and 0.049 U·g protein^{-1} at 4, 8 and 12 °C respectively, Figure 4c). Linear regression between CS activity and SGR was not significant and no differences were detected amongst temperature groups (Figure 5 c) were observed.

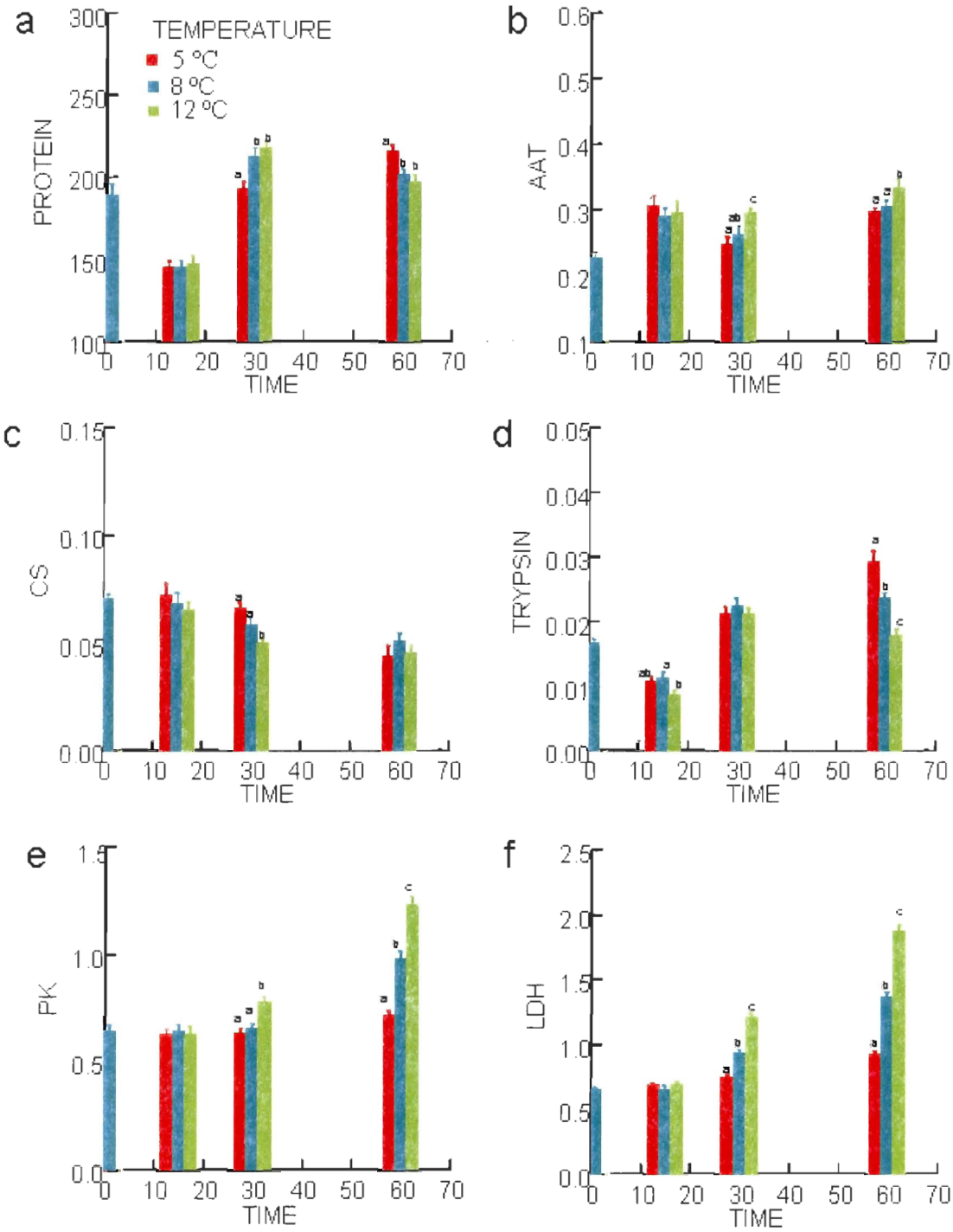

Figure 4. Protein content (a) and enzymatic activities of b) AAT, c) CS, d) trypsin, e) PK and f) LDH of spotted wolffish from 0-60 days post-hatching, at each temperature. Protein content expressed as mg protein/ g fish and enzymatic activities expressed as U·g protein^{-1}.

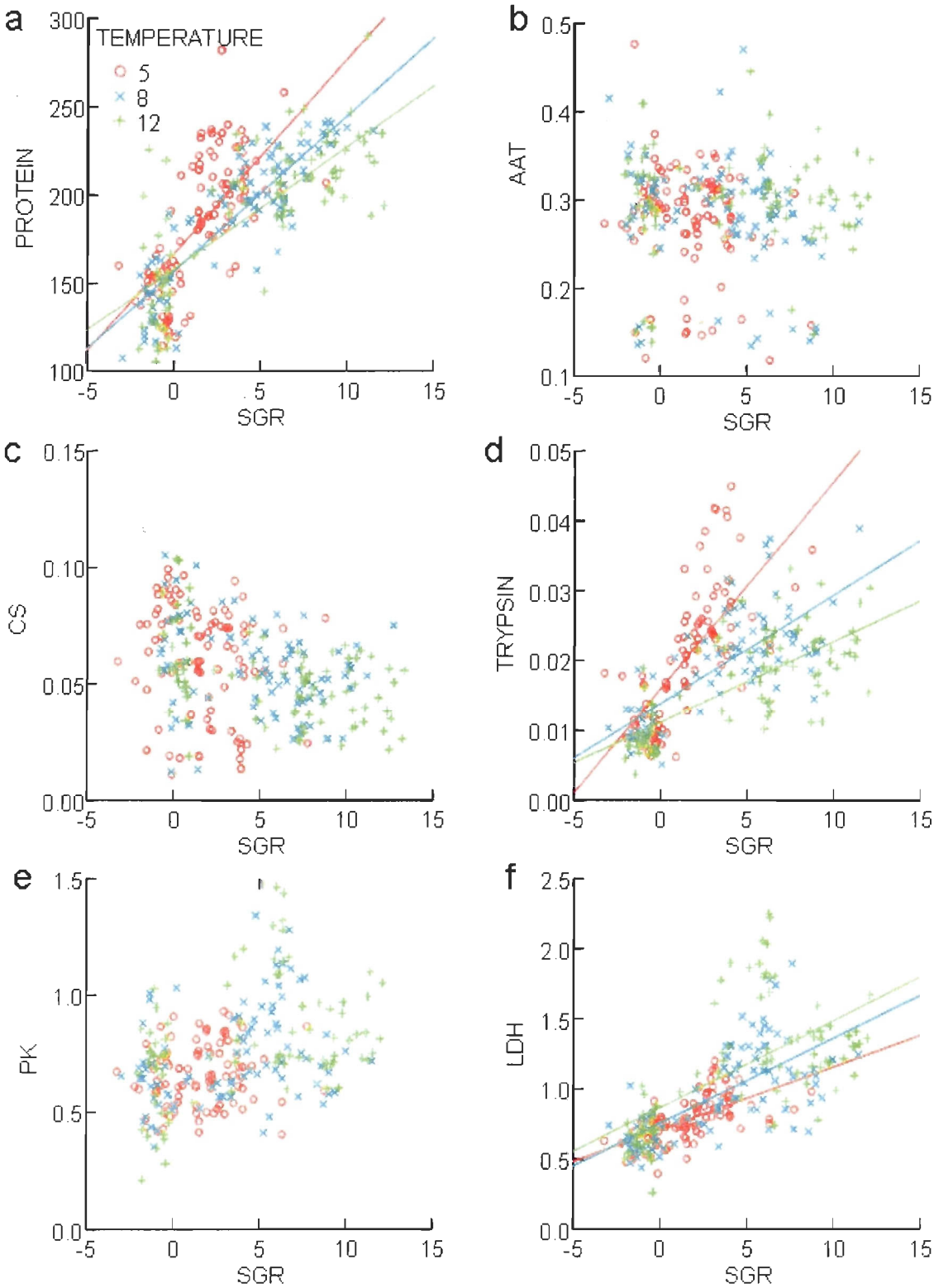

Figure 5. Linear regression of a) protein content and enzymatic activity of b) AAT, c) CS, d) trypsin, e) PK and f) LDH of newly-hatched spotted wolffish as function of SGR for each temperature. Protein content expressed as mg protein· g fish^{-1} and enzymatic activities expressed as U·g protein^{-1}.

Trypsin

Significant differences of TRYP activity levels between temperatures were observed at 15 DPH (0.011, 0.011 and 0.009 U·g protein^{-1} at 4, 8 and 12°C respectively, Figure 4d)and 60 DPH (0.030, 0.023 and 0.018 U·g protein^{-1} at 4, 8 and 12°C respectively, Figure 4d). The analysis of covariance revealed that TRYP activity was positively related to SGR at all temperature (R^2= 0.522) and that there was a significant interaction between SGR and temperature ($p<0.001$, Figure 5d). The slope was significantly steeper at 4°C compared to the two other temperatures (3 times lower at 12° compared to 4°).

Glycolytic enzymes

Pyruvate kinase and LDH activities were significantly different between the temperature treatments after 30 DPH. PK activity at day 30 was respectively of 0.640, 0.672 and 0.779 U·g protein^{-1} at 4, 8 and 12 °C and of 0.737, 0.979 and 1.248 U·g proteins^{-1} at 4, 8 and 12 °C respectively at 60 DPH (Figure 4e). Lactate dehydrogenase activities at day 30 were 0.749, 0.962 and 1.211 U·g protein^{-1} at 4, 8 and 12 °C and after 60 days 0.952, 1.375 and 1.890 U·g protein^{-1} at 4, 8 and 12 °C (Figure 4f). Activity of LDH was positively related to SGR (R^2= 0.502, p=0.006, Figures 6f), there was an effect of temperature (p=0.002) but no interaction between the two factors was detected (p=0.226) which mean that the slopes were parallel. The linear regression of SGR and PK activity was not significant (figure 5e).

2.6 DISCUSSION

Aspartate amino transferase activity is indicative of the capacity to oxydate amino acids for energy production or to mobilize amino acids for protein synthesis. At day 30 and 60, the higher AAT activity observed for fish at higher temperature is representative of the higher scope for growth or enhanced energy expenditure at that temperature. However, the failure to correlate AAT activity to SGR supports the latter hypothesis.

Aerobic capacity, represented here by CS activity, do not seem to adjust to the higher rate of protein synthesis and deposition encountered with higher growth rate even if this is an energetically costly process. The faster growing fish (at 12 °C) did not present a higher content of CS and the CS activity did not correlate to growth rate. This is indicative that newly hatched spotted wolffish have sufficient metabolic capacities to sustain elevated growth rates (up to 10% weight day^{-1}). Overnell and Batty (2000) did not observe any effect of temperature on CS activity of plaice (*Pleuronectes platessa*) and herring (*Clupea harengus*). They suggested that CS is produced at a constant level and that its concentration does not reflect oxidative capacity. Blier and Guderley (1988) showed that lake whitefish (*Coregonus clupeaformis*) showed little effect of temperature on CS activity. It has previously been suggested that the aerobic capacity of muscle of fish was sufficient to meet the cost of protein synthesis encountered with high growth rate (Blier et al., 1997).

In general, larval fish display poor assimilation efficiency for protein (Ronnestad et al., 2001) and do not present any tryptic activity at hatching (Gawlicka et al., 2000,

Cahu and Zambonino-Infante, 2001). This study showed that wolffish presented elevated tryptic activity at hatch which is in accordance with the findings of Lamarre et al. (2004, submitted). Desrosiers et al. (2005) also demonstrated that they even secrete trypsin early during embryogenesis.

Trypsin activity was higher at lower temperature (figure 5d) and higher for a given SGR at lower temperature (figure 5d). This may be an adaptive response to temperature or a result of the lower growth rate. Le François et al. (2000) and Lamarre et al. (submitted) observed the same relationship as function of growth in weight for newly hatched Atlantic wolffish in a study on the effect of food-restriction on development of enzymatic and digestive enzymes capacities. Heavily restricted-fish, experiencing slower growth than unrestricted fish, displayed activity levels significantly higher at a given weight. Therefore, we could see the higher trypsin content in slowly growing fish as an adaptation to an inappropriate environment (low temperature in this case). Protein is the major source of energy in carnivorous fish (Jobling, 1994) and trypsin is a key enzyme in acquisition of energy allowing the break-off of proteins in smaller peptides. In less favourable environments, such as sub-optimal growth temperature in this study, fish will indubitably benefit from an enhancement of their digestive capacity through increased synthesis of trypsin in an attempt to compensate for the reduced kinetic efficiency of trypsin at low temperature. The strong relationship and steepness of the slope at 4 °C give a clear indication that in order to achieve high growth rates encountered at larval stages, fish rely on trypsin activity. This is in accordance with the conclusions of Blier et

al. (1997). They suggest that the utilization of amino acids by the fish (for growth) could be limited by the rate of nutrient production by digestive enzymes or to their transport.

We propose, in the light of those results (pronounced trypsin enhanced activity at low temperature), that trypsin activitycould be an important factor permitting compensatory growth. Compensatory growth is defined as a sudden growth burst following a period of food restriction (Jobling, 2001). This growth acceleration can also be observed following periods of oxygen depletion (Foss and Imsland, 2002) or temperature reduction (Hurst and al., 2005; Treberg et al., 2005). Mechanisms through which growth recovery is achieved include improved efficiency of protein synthesis (Treberg et al., 2005), increased foraging activity (Hurst et al. 2005), increased pyloric caeca and intestine relative masses (Bélanger et al., 2002) and diversion of assimilated energy from lipid storage to growth (Hurst and al., 2005, Foss et Imsland 2002). Lemieux et al. (1999) linked increased trypsin activity to growth rate in Atlantic cod. Accordingly, our results suggest that the high levels of trypsin activity observed in fish held at low temperature could possibly be involved in compensatory growth. When the fish would be exposed to warmer temperature and adequate food supply, they would ''catch-up'' growth through increased protein digestion capacities. Bélanger et al., (2002), did not report any increase in trypsin activity levels in adult Atlantic cod during a period of compensatory growth. In the case of larval fish, there must be more pronounced digestive adjustments than in adult fish because growth rate is higher.

The inverse effect of temperature on trypsin activity is seen on glycolytic enzyme activity (PK and LDH) i.e. higher activity at higher temperature (negative temperature compensation). Furthermore, in the case of LDH, its activity scales positively with growth rate. Clarke et al. (1992) found that the LDH activity in entire lane snapper (*Lutjanus synagris*) larvae was higher in fish raised at 28 °C than at 25 °C, Vézina and Guderley (1991) also saw a negative temperature compensation of LDH activity in three-spined stickleback (*Gasterosteus aculeatus*). Mathers et al. (1992) and Pelletier et al. (1994) found a positive correlation between LDH activity and SGR. Lactate dehydrogenase is an anaerobic glycolytic enzyme and it is unlikely that an augmentation of its activity is required for growth; higher LDH activity would be a consequence of the higher growth rate rather than the cause (Pelletier et al., 1994).

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3. CONCLUSION GÉNÉRALE

Le premier objectif de cette étude était d'évaluer l'impact de l'ajout d'hydrolysats de protéines à la nourriture des loups tachetés lors de la première alimentation et de tester l'éventuel effet de cet ajout sur les fonctions métaboliques et digestives du loup tacheté et ce, à trois températures. À notre connaissance, cette étude était la première à tester l'effet des hydrolysats sur les performances de croissance et de survie des loups de mer ; elle était aussi la première à tester l'effet des hydrolysats combiné à l'effet de la température chez les poissons marins.

L'ajout d'hydrolysats n'a pas eu d'effet notable sur la croissance et la survie des loups. Par contre, la survie semblait plus élevée (non-significativement) chez les groupes ayant reçu la diète contenant 20% d'hydrolysats, bien que la différence n'était pas statistiquement significative. Deux hypothèses peuvent expliquer l'absence d'un effet bénéfique significatif: 1) Le stade avancé de développement des loups à l'éclosion ou 2) Les méthodes expérimentales utilisées dans la présente étude.

Selon la première hypothèse, les hydrolysats n'auraient pas d'effet bénéfique puisque le loup tacheté, dès l'éclosion, possède un système digestif bien développé et est en mesure de digérer correctement les protéines entières (les hydrolysats sont des polypeptides ou protéines pré-digérées). À notre connaissance, aucune étude ne s'était intéressé aux hydrolysats de protéines chez des poissons non-métamorphiques. En effet, contrairement aux espèces marines métamorphiques telles que le flétan ou la morue, le

loup a un estomac et un pancréas (glande responsable de la sécrétion de la trypsine, une enzyme clé de la digestion des protéines) complètement formés dès l'éclosion.

Selon la deuxième hypothèse, les méthodes expérimentales utilisées n'auraient pas permis de détecter les effets bénéfiques des hydrolysats. Les résultats obtenus étaient très variables entre les réplicats; l'échantillonnage d'un plus grand nombre de poissons par réplicat aurait peut-être permis la détection de différences statistiquement significatives entre les groupes. Les hydrolysats sont reconnus comme étant des "attractants gustatifs" pour diverses espèces de poissons. Ils pourraient donc faciliter la transition entre l'alimentation endogène (sac vitellin) et l'alimentation exogène. L'administration d'*Artémies* aux jeunes loups dans les deux premières semaines de la présente étude a peut-être occulté un effet potentiel des hydrolysats. Il serait intéressant de répéter cette expérience avec un nombre plus grand d'individus et ce, sans administrer d'artémies. Il serait aussi justifié de tester l'ajout d'une grande concentration d'hydrolysats (50-75%) afin de détecter un effet négatif potentiel sur le taux de croissance des juvéniles de loup de mer. Le système digestif est possiblement stimulé par les protéines entières et non les polypeptides.

Le deuxième objectif de ce travail était d'étudier le développement des capacités métaboliques et digestives et de tenter d'établir des corrélations avec le taux de croissance. Les principaux résultats indiquent que l'activité de la CS, une enzyme mitochondriale indicatrice de la capacité de production d'énergie sous forme aérobie n'était pas corrélée au taux de croissance. L'activité de cette dernière n'était pas

influencée par le SGR, indiquant qu'elle est présente en quantité largement suffisante pour répondre aux besoins énergétiques associés à la croissance.

L'activité de la trypsine semblait être fortement influencée par la température. Ainsi, les individus soumis à des conditions sous-optimales avaient un contenu en trypsine plus élevé par unité de masse. Nous émettons l'hypothèse que la trypsine pourrait ainsi être impliquée dans le processus de croissance compensatoire. Il serait intéressant de vérifier cette hypothèse chez le loup de mer.

Cette étude a permis une meilleure compréhension des facteurs régissant la survie et le taux de croissance chez les jeunes stades de loup tachetés. La validation de l'hypothèse selon laquelle un haut niveau de trypsine permettrait un taux de croissance plus élevé (croissance compensatoire), semble une avenue intéressante. Ceci permet une meilleure compréhension des facteurs physiologiques pouvant limiter la croissance. Il serait intéressant de refaire l'expérimentation portant sur les hydrolysats avec les conditions expérimentales mentionnées plus haut. En effet, si la trypsine est limitante pour la croissance, le fait de donner des protéines pré-digérées aux jeunes larves devrait nécessairement augmenter leur croissance.

En outre, il semble impératif d'identifier d'autres facteurs pouvant influer sur la survie ou la croissance des jeunes loups afin de permettre un apport constant de juvéniles dans un contexte où l'aquaculture commerciale de cette espèce est envisagée. Une meilleure connaissance des indicateurs de la qualité des œufs serait aussi grandement

souhaitable car ceux-ci nous permettraient une détection précoce des lots d'œufs produisant des juvéniles de qualité. Des recherches axées sur la mise en place d'un programme de sélection génétique des géniteurs pourraient aussi permettre d'obtenir une meilleure qualité de juvéniles.

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