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Alimentation et Nutrition des juvéniles de *Heterotis niloticus* (Arapaimidae, Teleostei)

Premières estimations des besoins nutritionnels et valorisation des sous-produits végétaux

Faculté des Sciences

DEPARTEMENT DE BIOLOGIE

Dissertation présentée par
Serge Eric MONENTCHAM MONENTCHAM
en vue de l'obtention du grade
de Docteur en Sciences

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FACULTES UNIVERSITAIRES NOTRE-DAME DE LA PAIX

Faculté des Sciences
Département de Biologie
Unité de Recherche en Biologie des Organismes

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Couverture : *Heterotis adulte, Fleuve Nyong et étangs en terre au Cameroun*

A

Mon épouse Nadine Patricia

et à nos enfants

A

la mémoire de mes parents

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Alimentation et nutrition des juvéniles de *Heterotis niloticus* (Arapaimidae, Teleostei) : premières estimations des besoins nutritionnels et valorisation des sous-produits végétaux

Par Serge Eric Monentcham Monentcham

Résumé

L'Arapaimidae *Heterotis niloticus* est une espèce africaine à fort potentiel aquacole en raison de sa forte croissance, sa double respiration, la bonne qualité gustative et ferme de sa chair, et sa relative haute valeur commerciale. Toutefois, son utilisation à des fins piscicoles est limitée par la difficulté d'approvisionnement en alevins de qualité et par l'absence de connaissances sur ses besoins nutritionnels indispensables à l'optimisation de formules alimentaires pour l'espèce. En vue de sa contribution efficace dans l'augmentation de la production aquacole du Cameroun et des autres pays africains, la détermination entre autres de ses besoins alimentaires et nutritionnels à divers stades ontogénétiques s'avère nécessaire et incontournable. La présente recherche, inscrite dans cette perspective de domestication plus avancée de l'Heterotis du Nil, s'est proposée d'établir certaines variables nutritionnelles de base relatives à son pré-grossissement et d'évaluer ses capacités de valorisation des sous-produits oléagineux.

Entre mai 2005 et août 2008, une série d'expériences en triplicat sur la nutrition des juvéniles de *H. niloticus* ont été réalisées dans des hapas (0,5 m^3 de volume en eau) installés en étangs de dérivation (300-600 m^2) à la station aquacole de Melen (Yaoundé, Cameroun). Les analyses ont été effectuées au sein de l'Unité de Recherche en Biologie des Organismes des Facultés Universitaires Notre-Dame de la Paix (Namur, Belgique) et de l'Unité de Chimie Biologique Industrielle de la Faculté Universitaire des Sciences Agronomiques (Gembloux, Belgique).

Sur le plan protéique, les résultats ont révélé que cette espèce nécessite 310 et 345 g protéines kg^{-1} d'aliment respectivement pour la croissance optimale et maximale des sujets pesant entre 3 et 62 g et que ses besoins spécifiques en acides aminés indispensables, excepté pour le tryptophane et l'histidine, sont similaires à ceux d'autres poissons tropicaux omnivores. Ensuite, le phénomène d'épargne des protéines par les lipides alimentaires a été clairement mis en évidence lorsque l'énergie a été augmentée de 17 à 19,6 MJ kg^{-1} d'aliment contenant 28% de protéines, ce qui nous a permis de

suggérer que les besoins protéiques des juvéniles de *H. niloticus* peuvent être réduits de 310 à 280 g protéines kg^{-1} d'aliment dans les conditions expérimentales de notre étude. Enfin, la dernière approche de notre investigation a montré que les tourteaux de soja et de coton peuvent remplacer jusqu'à hauteur de 50% la farine de poisson dans l'alimentation des juvéniles de cette espèce. Ces différents acquis constituent en soi une avancée certaine dans la connaissance des besoins nutritionnels et alimentaires des Heterotis.

Le parcours de la domestication de cette espèce reste néanmoins encore difficile, notamment avec l'épineux problème de la production massive de juvéniles de qualité. Les perspectives de recherche-développement envisagées au terme de cette thèse laissent entrevoir de réelles chances de surmonter cet autre handicap-clé. Nous restons donc optimistes quant à l'élevage durable et rentable de ce poisson en Afrique sub-saharienne.

MOTS-CLES : *Heterotis niloticus*, nutrition, croissance, énergie, protéine, acide aminé, substitution farine poisson, lipide.

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Feeding and nutrition of *Heterotis niloticus* fingerlings (Arapaimidae, Teleostei) : first estimations of the nutritional requirements and the use of local plant by-products

By Serge Eric Monentcham Monentcham

Abstract

Heterotis niloticus is an African species with a great potential for fish farming as a result of its remarkably rapid growth, air-breathing characteristic, omnivorous diet, good meat quality and relative high commercial value. However, its use for aquaculture is limited due to the difficulty in the massive production of fingerlings and the lack of knowledge on its artificial feeding. Therefore, the estimations of its nutritional requirements at different ontogenetic stages are necessary in view of its effective contribution to the aquaculture production in Cameroon and other African countries. The present research aims to establish some basic nutritional variables relative to its fingerlings rearing and to examine the suitability of plant oil cakes as a partial substitute of fish meal in its compounded diets.

Between May 2005 and August 2008, several experiments were conducted in rectangular hapas (0.5 m^3 useful capacity) placed in two earthen pond (300 and 600 m^2) at the Melen Aquaculture Station (Yaounde, Cameroon). Replicate groups of juveniles were handfed twice daily to apparent satiation all the time. Analyses were performed at the laboratory of the Research Unit in Organismal Biology (University of Namur, Belgium) and the Unit of Industrial Chemistry Biology (Gembloux Agricultural University, Belgium).

On the protein and amino acids requirements, the results revealed that the dietary protein requirements are 310 and 345 g protein per kg diet for optimal and maximal growth, respectively for fish size ranging from 3 to 62 g. Based on whole body or muscle tissue indispensable amino acids to A/E ratios, its IAA requirement profile is similar to those of other omnivorous tropical fish species with the exception of tryptophan and histidine.

The dietary protein-sparing effect has been clearly demonstrated when the dietary energy of lipid increases from 17 to 19.6 kJ g^{-1} at 28% crude protein.

Therefore, this result indicates that the optimum protein and lipid levels in its diet are 280 and 130 g kg^{-1} diet, respectively. Finally, the soybean and cottonseed oilcakes meals could partially replace up to 50% the dietary fish meal in its practical fingerlings diets without negative effect on maximal growth. This study contributes to improve our knowledge on basic nutrients requirements for the juveniles of a new aquaculture species for Africa.

However, massive production of quality fingerlings of this species remains a major obstacle to overcome on the road of the domestication of *H. Niloticus* for aquaculture use. Our work reveals that even with that constraint, optimism is permitted for a sustainable and rentable culture of this species in sub-Saharan Africa.

KEY WORDS: *Heterotis niloticus*, nutrition, growth, energy, protein, amino acid, fish meal substitution, lipids.

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Liste des abréviations

AA	Amino acid
ADC	Apparent digestibility coefficient
AG	Acide gras
AGE	Acide gras essentiel
ANOVA	Analyse de la variance
AOAC	Association of Official Analytical Chemists
°C	Degré Celsius
CAR	Central African Republic
CEA	Coefficient d'efficacité alimentaire
CEP	Coefficient d'efficacité protéique
CEFRA	Centre de Formation et de Recherches en Aquaculture
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
cm	centimètre
CMC	Carboxymethylcellulose
CMR	Republic of Cameroon
CSM	Cottonseed oilcake meal
CUD	Commission Universitaire pour le Développement
DE	Digestible energy
DP	Digestible protein
DRC	Democratic Republic of Congo
E	Est
ERE	Energy retention efficiency
F	Fecundity
FAO	Food and Agriculture Organization of the United Nations
FCFA	franc de la Coopération Financière d'Afrique centrale
FE	Feed efficiency
Fig	Figure
FP	Farine de poissons
FUNDP	Facultés Universitaires Notre-Dame de la Paix
FSAGx	Faculté Universitaire des Sciences Agronomiques de Gembloux
g	gramme
g N	gramme d'azote
GE	Gross energy
GP	Gain de poids
GPQ	Gain de poids quotidien
h	heure
ha	hectare
HPLC	High-performance liquid chromatography
IAA	Indispensable amino acid

IC	Ivory Coast
IFREMER	Institut Français de Recherche pour l'Exploitation de la Mer
INRA	Institut National de la Recherche Agronomique
IRAD	Institut de Recherche Agricole pour le Développement
IU	International Unit
kg	kilogramme
kJ	kilojoule
km	kilomètre
km²	kilomètre carré
L	litre
LSD	Least Significance Difference
µm	micromètre
m	mètre
m²	mètre carré
mg	milligramme
ml	millilitre
mm	millimètre
MINEPIA	Ministère de l'Elevage, des Pêches et des Industries Animales
MINRESI	Ministère de la Recherche Scientifique et de l'Innovation
MJ	Mégajoule
N	Nord
NFE	Nitrogen-free extract
NPK	Engrais ternaire (Azote, Phosphore, Potassium)
NRC	National Research Council
NS	not significant
ORSTOM	Institut Français de Recherche Scientifique pour le Développement en Coopération
P	Quantité de protéines alimentaires distribuée
P28L6	régime contenant 28% de protéines et 6% de lipides
P28L13	régime contenant 28% de protéines et 13% de lipides
P32L6	régime contenant 32% de protéines et 6% de lipides
P32L13	régime contenant 32% de protéines et 13% de lipides
P36L6	régime contenant 36% de protéines et 6% de lipides
P36L13	régime contenant 36% de protéines et 13% de lipides
P_f	Poids final
P_i	Poids initial
PA	Teneur protéique de l'aliment
PC_f	Teneur corporelle protéique finale
PC_i	Teneur corporelle protéique initiale
PD	Protein deposition
P/E	Protein/energy
PER	Protein efficiency ratio
PNSA	Programme National de Sécurité Alimentaire

PR	Protein retention
PRE	Protein retention efficiency
PVC	Polyvinyl chloride
Q	Quantité d'aliments distribuée
RP	Rétention protéique
S	significant
SBM	Soybean oilcake meal
SE	Standard error
SD	Standard deviation
SGR	Specific growth rate
TCP	Technical Cooperation Project
TCS	Taux de croissance spécifique
TL	Total length
UK	United Kingdom
ULg	Université de Liège
UNDP	United Nations Development Programme
URBO	Unité de Recherche en Biologie des Organismes
USD	United States Dollar
W	Body mass
WG	Weight gain
ZEE	Zone économique exclusive

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Biology and prospect for aquaculture of African bonytongue, *Heterotis niloticus* (Cuvier, 1829): A review. **Aquaculture**, 2009, in press.

Influence of dietary protein levels on growth performance and body composition of African bonytongue fingerlings, *Heterotis niloticus* (Cuvier, 1829). **Aquaculture Nutrition**, 2008, in press.

Egg and whole body amino acid profile of African bonytongue (*Heterotis niloticus*) with an estimation of their dietary indispensable amino acids requirements. Submitted to **Fish Physiology and Biochemistry**, 2009.

Growth, feed utilization and body composition of African bonytongue, *Heterotis niloticus* fingerlings fed diets containing various protein and lipid level. Submitted to **Aquaculture Research**, 2009.

Partial substitution of fish meal with soybean and cottonseed oilcakes meals in diets for African bonytongue, *Heterotis niloticus* (Cuvier, 1829) fingerlings: effects on growth, feed efficiency and body composition. Submitted to **Aquaculture Research**, 2009.

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INTRODUCTION GENERALE

1.1 Contexte et justification de l'étude

La production mondiale des pêches de capture et de l'aquaculture destinée à la consommation humaine a atteint en 2004 approximativement 106 millions de tonnes de poissons, dont 43% fourni par l'aquaculture (FAO 2006). Cette contribution du secteur aquacole aux approvisionnements mondiaux de poissons a connu un véritable essor durant les 50 dernières années. De 3,9% de la production pondérale totale en 1970, elle est passée successivement à 27,1, 30,2 et à 32,4% respectivement en 2000, 2002 et 2004 (FAO 2006). Ainsi, de moins d'un million de tonnes dans les années 50, l'aquaculture a produit environ 59,4 millions de tonnes en 2004, pour une valeur estimée à 51 milliards d'euros. La part de l'Afrique subsaharienne dans cette production reste insignifiante : 0,16% du tonnage et 0,36% en termes de valeur. Pourtant l'aquaculture a été introduite dans le continent africain au cours des années 1940-1950 par les colonisateurs occidentaux en raison des immenses potentialités aquacoles naturelles de l'Afrique. La valorisation à des fins aquacoles de ces potentialités biophysiques aurait sûrement dû être un puissant facteur de développement économique durable, de sécurité alimentaire et de lutte contre la pauvreté et la misère en Afrique.

Malheureusement, malgré de multiples efforts entrepris dans le passé par les gouvernements et les bailleurs de fonds internationaux pour stimuler sa croissance d'une part, et d'autre part de nombreuses tentatives par l'entremise du carnaval de projets pour son expansion, le secteur aquacole africain est resté à la traîne et n'a pas pu atteindre les résultats escomptés. Paradoxalement, la plupart des pays africains ont recours aux importations de poissons pour satisfaire la demande locale en produits halieutiques. Ainsi, Brummett *et al*. (2008) estiment que les états africains importent environ 4,2 millions de tonnes de poissons par année pour une valeur estimée à 2,2 milliards d'euros. Dès lors, la nécessité de développer ce secteur s'impose avec acuité afin d'inverser cette tendance.

Plusieurs facteurs sont tenus responsables de la mauvaise performance de l'aquaculture africaine. Les plus cités sont l'absence et/ou l'insuffisance de juvéniles de qualité, le manque d'aliments piscicoles, la mauvaise conduite des élevages piscicoles, l'inadéquation des méthodes de vulgarisation et l'accès difficile aux capitaux d'investissements (Moehl *et al*. 2005). Ces paramètres, au niveau de chacun des états, semblent plutôt être des corollaires d'une part des politiques et approches inadaptées, et d'autre part de l'absence de stratégie et de plan nationaux de développement durable du secteur aquacole. Par conséquent, l'aquaculture en Afrique demeure essentiellement une activité artisanale, sans possibilité d'offrir des opportunités professionnelles. La production est relativement faible et destinée à la consommation familiale et/ou à la commercialisation à petite

échelle. Ce système de production utilise peu d'intrants et la gestion est individuelle ou familiale. Toutefois, l'émergence d'une aquaculture commerciale à petite ou grande échelle, avec pour objectif de maximiser les profits, est d'actualité dans plusieurs pays africains. Certains auteurs mettent ainsi en exergue l'importance de travailler désormais avec des petites et moyennes entreprises aquacoles et de soutenir les initiatives privées (Brummett *et al*. 2008) avec une politique de microcrédits à taux d'intérêt nul par exemple. Des pays comme le Nigéria, le Ghana et le Zimbabwe avec une contribution très appréciable dans le tonnage peuvent être cités en exemple. Dès lors, le défi corollaire des états africains serait donc d'induire le passage de cette aquaculture rurale à motivation sécurité alimentaire vers une aquaculture dont le moteur principal serait le profit. Le Cameroun a signifié, dans son dernier plan directeur du développement de son secteur aquacole, son orientation marquée vers cette voie (FAO, 1993).

Située au fond du golfe de Guinée, la République du Cameroun couvre une superficie de 475.650 km² entre les 2ème et 13ème degrés de latitude Nord. Le pays dispose d'une façade maritime sur l'Océan Atlantique de 360 km de long entrecoupée de nombreux estuaires et criques. Son plateau continental, relativement étroit, s'étend sur près de 14.000 km², tandis que la superficie de sa zone économique exclusive (ZEE) est estimée à quelques 15.400 km². Il possède un réseau hydrographique riche et varié comprenant fleuves, rivières, lacs, retenues et zones d'inondation. Le Cameroun comporte une variété de paysages, de zones climatiques et géomorphologiques qui peuvent être regroupées en cinq entités régionales ou zones agro-écologiques (Figure 1.1).

Au début de la décennie 80, le Cameroun était classé parmi les pays africains à revenu intermédiaire. De 1986 à 1996, le pays a été confronté à la récession économique avec des répercussions socio-économiques néfastes notamment sur les revenus des ménages et les emplois. La conséquence principale a été une extension de la pauvreté, faisant ainsi passer la nation du rang de 134ème sur 174 en 2002 à celui de 147ème sur 177 parmi les pays les plus pauvres en 2005, suivant le classement de l'indice de développement humain des Nations Unies.

Le poisson constitue la principale source de protéines pour une partie considérable de la population camerounaise. Il représente 40% de l'apport protéique d'origine animale dans sa ration alimentaire et couvre 9,5% de ses besoins totaux. Pour la couche de la population la moins nantie, il est l'unique source de protéines d'origine animale. La consommation moyenne annuelle de poisson par habitant est de 16,2 kg au Cameroun (MINEPIA 2008), largement supérieure à celle de la moyenne africaine évaluée à 7,6 kg (FAO 2006). Cette demande des ménages camerounais varie de 28 kg à

proximité des zones de pêche (côte maritime, plans d'eaux continentaux) à 8 kg par année dans les localités éloignées des points de captures. Celle-ci semblerait plus élevée dans les grands centres urbains, à savoir 25 et 33 kg/habitant/an respectivement à Yaoundé et à Douala.

Les besoins annuels en poissons des populations camerounaises ont été estimés à 185.000, 215.000, 243.000, 255.000, 298.000 et à 320.000 tonnes respectivement pour les années 1990, 1995, 2000, 2002, 2005 et 2007 (MINEPIA 2008). Ces chiffres illustrent clairement l'augmentation incessante des besoins des ménages camerounais en produits halieutiques. En revanche, la production annuelle de la pêche et de l'aquaculture au Cameroun n'a pas suivi la même tendance et est actuellement estimée aux alentours de 180.000 tonnes de poissons dont 93.000 de la pêche artisanale maritime, 75.000 de la pêche continentale, 7.000 de la pêche industrielle et 5.000 de la pisciculture (MINEPIA 2008).

De cette analyse, il ressort inéluctablement que pour satisfaire sa demande locale, le Cameroun doit recourir annuellement à l'importation d'importantes quantités de poissons congelés ; en 2007, ces importations ont été évaluées à 140.000 tonnes pour une valeur estimée à 40 milliards de FCFA, soit 62 millions d'euros.

Face à cette croissance récurrente du déficit de l'offre locale en poissons, le gouvernement du Cameroun a élaboré en 1992 un plan directeur des pêches et de l'aquaculture avec l'assistance technique de la FAO. Ce plan visant le développement rationnel et durable du secteur de la pêche et de l'aquaculture a été axé sur la réalisation de quatre objectifs stratégiques, à savoir la mise en place d'un système de gestion en vue de réduire la pression sur l'exploitation des espèces démersales et des crevettes, la concentration des efforts sur les pêcheries sous exploitées, la réduction des pertes après captures et le développement de l'aquaculture.

Dans le souci d'opérationnaliser l'axe du secteur aquacole de ce schéma-directeur, les autorités camerounaises ont élaboré en 2003 un cadre stratégique pour un développement durable de l'aquaculture avec, une fois de plus, l'assistance technique de la FAO. Il en est ressorti que le secteur public devrait davantage jouer le rôle de facilitateur, au détriment de celui d'investisseur direct, dorénavant dévoué au secteur privé. Cette stratégie ne pouvant être mise en œuvre sans plan de développement, cette action est actuellement poursuivie par le projet TCP/CMR/3103 (D), financé sur 18 mois par la FAO et exécuté depuis septembre 2008 par le gouvernement camerounais avec la collaboration technique de la FAO. Les résultats majeurs escomptés à terme sont la disponibilité d'un plan de développement durable de l'aquaculture au Cameroun et la carte digitale aquacole retraçant les potentialités piscicoles du pays par province.

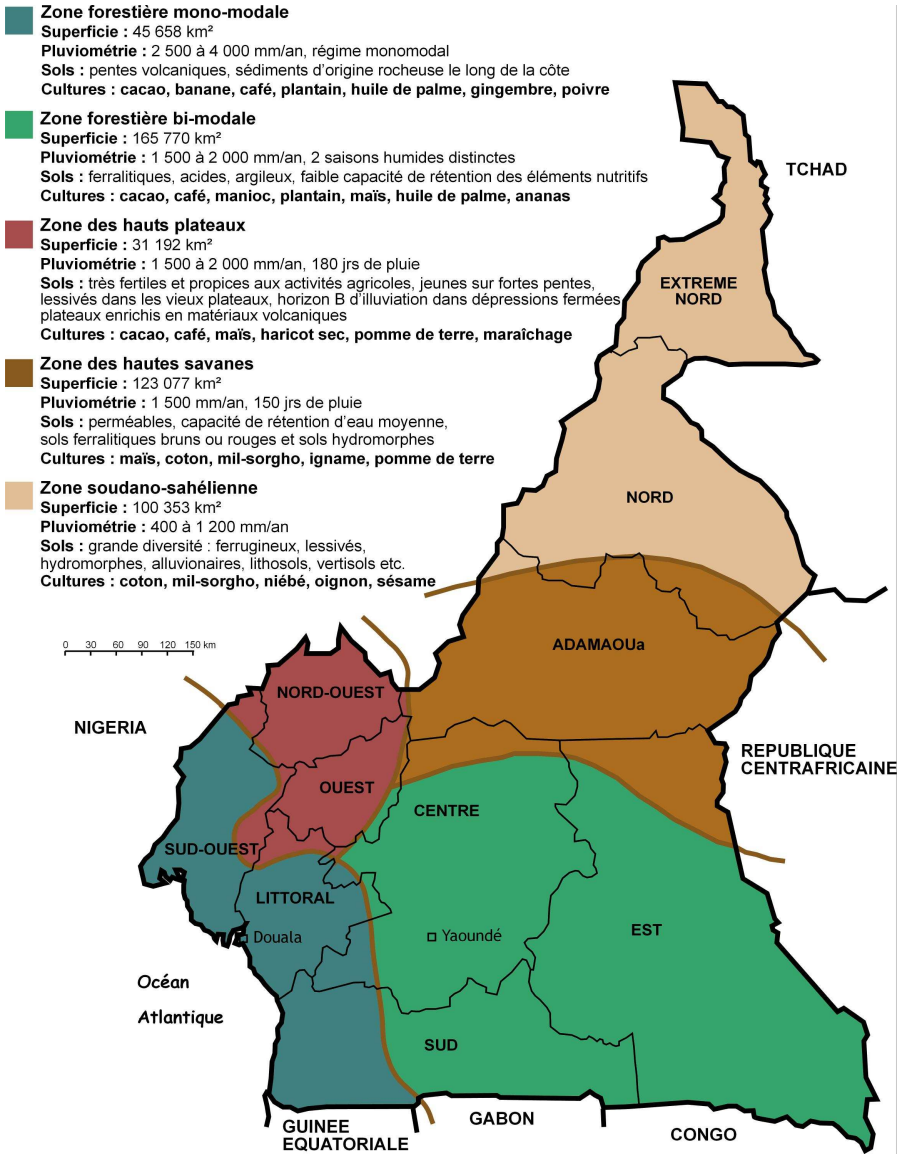

Fig. 1.1. Les différentes zones agro-écologiques du Cameroun (Source : PNSA 2007)

Toutefois, les priorités de la stratégie sectorielle aquacole du gouvernement camerounais sont axées sur l'amélioration du cadre institutionnel, la modernisation de l'appareil de production par l'intensification et la spécialisation de la filière piscicole actuelle, l'amélioration du cadre incitatif,

la gestion durable des ressources naturelles, la promotion de l'émergence des spéculations aquacoles autres que la pisciculture (surtout la crevetticulture et la conchyliculture) et la domestication de nouvelles espèces de poissons qui présentent des potentialités aquacoles. C'est dans ce dernier contexte que les pouvoirs publics, les chercheurs, les vulgarisateurs et les producteurs locaux fondent actuellement une partie de leurs espoirs sur la domestication de *Heterotis niloticus*.

L'aquaculture extensive en étang de barrage a été introduite au Cameroun en 1948. L'engouement suscité s'est illustré par la construction de près de 10.000 étangs ruraux par les paysans et la mise en place respective de 32 stations aquacoles publiques et d'un service national de vulgarisation. De multiples interventions bilatérales ou multilatérales se sont succédé de 1954 à nos jours avec peu de résultats pertinents et pérennes. De plus, toutes les politiques proposées convergeaient vers la promotion d'une pisciculture rurale de subsistance, artisanale et peu productive. Le constat d'échec est dès lors incontournable. En revanche, le projet « Development of Integrated Aquaculture-Agriculture Systems for small-scale farmers in the forest margins of Cameroon » a récemment permis d'accroître la productivité des étangs de 500-1500 kg ha^{-1} à un peu plus de 2525 kg ha^{-1} (Brummett *et al*. 2005) en testant une approche nouvelle : chercheurs et vulgarisateurs ont ainsi travaillé en partenariat avec les pisciculteurs pour la résolution in-situ des problèmes spécifiques en introduisant le moins possible des itinéraires techniques et/ou des intrants extérieurs à l'exploitation agricole.

Au Cameroun, 95% des pisciculteurs actifs possèdent un à deux étangs de 350 m² de superficie moyenne et la tendance générale observée en Afrique subsaharienne sur les systèmes aquacoles actuels reste valable dans ce pays. Les exploitations piscicoles sont de type familial, extensif et semi-intensif avec parfois une intégration aux animaux domestiques (poulets, porcs, lapins). La production aquacole nationale reste minime alors que le pays regorge d'énormes potentialités biophysiques dont 4 millions d'hectares de plans d'eaux intérieurs, 2700 km² de mangrove et 15400 km² de plateau continental.

Les principales espèces de poissons utilisées en pisciculture camerounaise appartiennent aux familles des Cichlidae et des Clariidae. La plus utilisée est incontestablement le cichlidae *Oreochromis niloticus*, localement connu sous le nom de Tilapia. L'origine de la souche présente au Cameroun demeure inconnue (Nguenga & Pouomogne 2006). Les Clariidae d'élevage du Cameroun sont représentés par deux espèces *Clarias gariepinus* et *Heterobranchus longifilis*. Ces deux poissons-chats africains présentent des potentialités de croissance et de survie extraordinaires (Micha 1973 ; Viveen *et al*. 1985 ; Legendre 1991 ; Hecht *et al*. 1996 ; Imorou Toko 2007 ;

Nyinawamwiza 2007). Ils sont très adaptés à la pisciculture camerounaise, et capables de supporter de fortes densités d'élevage (Avit & Luquet 1995 ; Hecht *et al*. 1996). De ce fait, ils devraient jouer un rôle prépondérant dans l'expansion future de l'aquaculture au Cameroun. La carpe *Cyprinus carpio*, le poisson-vipère *Channa obscura*, *Hemichromis fasciatus* et *Heterotis niloticus* sont utilisés en polyculture dans une proportion relativement moindre.

Heterotis niloticus est un poisson omnivore, originaire des grands fleuves et lacs d'Afrique centrale et de l'Ouest (D'Aubenton 1955; Daget 1957; Moreau 1982; Levêque *et al* 1990, Li & Wilson 1996; Mbega 2004; Adite *et al*. 2005). Il a été introduit avec succès au Sud du Cameroun (Depierre & Vivien 1977), en Côte d'Ivoire (Moreau 1974; Lazard 1980) et en République Démocratique du Congo. Ce poisson de la famille des Arapaimidae, est exploité par la pêche continentale en Afrique subsaharienne. La taille des individus capturés varie entre 2 et 7 kg, avec une sérieuse tendance à la baisse. Les captures annuelles d'Heterotis ont été estimées au Cameroun à 1520 tonnes pour une valeur de 5.221.375 USD (Kemgang 2007), et au Benin à 742 tonnes pour une valeur de 1.485.000 USD (Gbaguidi & Pfeiffer 1996). Cette espèce présente des qualités intéressantes (taux de croissance élevé, chaîne alimentaire courte, double respiration, bonne résistance aux manipulations et aux transports, délicieuse qualité de la chair fraîche ou fumée, prix de vente relativement élevé), qui en font un excellent candidat pour l'aquaculture tropicale. Il s'adapte bien en polyculture avec le tilapia du Nil, le poisson-chat africain et la carpe commune dans les systèmes piscicoles extensifs de plusieurs pays d'Afrique noire francophone (Pouomogne *et al*. sous presse).

Les recherches entreprises sur cette espèce ont démarré dans les années 50, puis ont régressé vers la fin des années 60, avant de connaitre récemment un regain d'intérêt. Les investigations ont porté sur la biologie (D'Aubenton 1955; Daget 1957; Omorinkoba *et al*. 1991; Okoye & Abubakar 1996; Fagbenro 2001; Achionye-Nzeh & Omoniyi 2002; Adite *et al*. 2006), l'écologie (Moreau 1974; Moreau & Moreau 1982; Adite *et al*. 2005) et sur les possibilités d'élevage en étang (Tillon 1957; Lemasson 1957; Tillon 1959; Bard 1960 ; Olaniyan & Zwilling 1963; Reizer 1964 ; Reizer 1966). Les résultats préliminaires obtenus sur les Heterotis semblent ainsi des plus encourageants. Il convient de mentionner : (i) la reproduction naturelle observée en étang de faible ou grande superficie (Tillon 1957 ; Reizer 1964) ; (ii) le mode d'alimentation proche de celui du Tilapia du Nil : il est ainsi confirmé que cette espèce n'est pas carnassière et peut même être nourrie artificiellement (Reizer 1966) ; (iii) des taux de croissance spécifique de 2,77 et 3,48 sont rapportées respectivement pour des juvéniles d'Heterotis de 30-50 g et de 3-6 g (Tillon 1959 ; Bard 1960) ; (iv) la facilité de

transporter les alevins par avion et par voie terrestre (Reizer 1964). Néanmoins, les mortalités massives des alevins souvent observées peu après la naissance empêchent toujours la production contrôlée des juvéniles. Ces résultats prometteurs sont confortés par des performances de croissance remarquables : avec une alimentation artificielle, *Heterotis niloticus* peut atteindre en polyculture avec le tilapia, 3 à 4 kg en 12 mois d'élevage (Bard, 1973).

Dans l'état actuel des connaissances, les données sur l'élevage larvaire et les besoins nutritionnels des Heterotis sont quasi-inexistantes. Or, tout processus de domestication d'une espèce de poisson transite nécessairement par la maîtrise de la production massive des juvéniles de qualité et par la formulation d'aliments spécifiques à cette espèce. Dès lors, il devient indispensable d'une part d'entreprendre d'avantage de recherches sur le comportement de reproduction des Heterotis en captivité et sur les stratégies d'élevage de leurs larves afin de remédier à la forte mortalité larvaire observée ; et d'autre part, de mener des études visant à déterminer les besoins nutritionnels et alimentaires spécifiques des Heterotis à différents stades ontogénétiques. C'est dans cette perspective que la présente étude a été initiée.

1.2 Objectifs et méthodologie générale de la thèse

1.2.1 Objectif global et objectifs spécifiques

La présente recherche doctorale s'inscrit dans une approche prospective de domestication de l'Arapaimidae *Heterotis niloticus* en Afrique subsaharienne. Il s'agit spécifiquement d'une part d'établir les variables nutritionnelles de base relatives au pré-grossissement des juvéniles de cette espèce, et d'autre part d'examiner la possibilité de substituer la farine de poissons par des matières premières végétales dans son alimentation.

Il est toutefois important de souligner que cette étude prévoyait également d'aborder certains aspects primordiaux à différents niveaux de l'ontogenèse larvaire de *Heterotis niloticus*, surtout l'impact de l'alimentation sur la mise en place des structures digestives en vue de contribuer à la détermination des stratégies alimentaires des larves de ce poisson basées sur des critères ontogénétiques et physiologiques. Cet objectif n'a pas pu être atteint en raison des fortes mortalités larvaires observées durant les multiples tentatives menées au début de notre travail.

1.2.2 Méthodologie générale

1.2.2.1 Matériel animal

Les adultes, immatures, juvéniles et larves de *Heterotis niloticus* ont été collectés avec la collaboration des pêcheurs locaux en milieu naturel (Photos 1 et 2) dans le fleuve Nyong à Akonolinga (3°47'N and 12°15'E), située à une centaine de km de Yaoundé (Cameroun). Après les phases de localisation des nids actifs et le suivi quotidien des sorties-promenades des alevins dans les zones de marais du fleuve, les juvéniles expérimentaux (poids moyen < 1 g) ont toujours été collectés à l'aide de l'épervier ou d'une grande épuisette. Les spécimens capturés ont de même été acheminés à la station de Melen à Yaoundé par voie terrestre dans une caisse étanche de 150 litres fabriquée avec du contreplaqué doublé de polystyrène et pourvue d'un aérateur à batterie, à une densité d'environ 200 juvéniles par 10 litres d'eau. Avant le transfert, les juvéniles ont toujours été conservés à jeûn pendant 48 heures. Aucune mortalité n'a été enregistrée durant les différents transports. Dès leur arrivée, les poissons ont de même été stockés durant deux semaines dans des étangs fertilisés (3 jours auparavant) de la station aquacole de Melen à Yaoundé, sans une alimentation artificielle.

1.2.2.2 Dispositif expérimental

Les tentatives d'élevage larvaire ont été effectuées dans des aquariums de l'écloserie de la station de recherche spécialisée IRAD à vocation internationale de Koupa Matapit (Foumban, Ouest-Cameroun). Les expériences du présent travail ont quant à elles été réalisées à la station aquacole MINEPIA de Melen (Yaoundé, Cameroun).

Après la période en étang fertilisé, 50 juvéniles ont toujours été stockés de manière aléatoire par hapa et nourris 2 fois par jour avec un aliment composé contenant 30% de protéines durant deux (sections 3 et 5) et trois semaines (section 6). Au terme de cette phase d'acclimatation, les poissons ont de même été répartis aléatoirement au nombre moyen de 27 dans les hapas expérimentaux, après la pesée individuelle de 50 individus échantillonnés au hasard. Chacun des traitements a été testé en triplicat. Les morts ont été quotidiennement décomptés, enlevés et pesés. A la fin des expériences, tous les juvéniles ont toujours été individuellement pesés.

Les hapas d'élevage, en toile moustiquaire, de forme rectangulaire et de volume utile (500 L en moyenne), ont toujours été installés dans des étangs en terre (300-600 m², 1,2 m de profondeur) de la station de Melen (Photos 3 et 4). Les étangs expérimentaux étaient des étangs de dérivation avec des tuyaux d'amenée d'eau en PVC de 100 mm de diamètre et des moines pour la vidange complète par gravité. Ces étangs ont été alimentés par de l'eau

captée également par gravité d'un ruisseau voisin. Le circuit d'élevage était un circuit ouvert avec un léger débit d'écoulement d'eau en permanence ; les faces externes et internes des hapas ont été brossées bimensuellement pour lutter contre un éventuel développement algal. Au cours des expériences, les poissons ont été soumis à la photopériode naturelle. Les mesures de la température, du pH et de la conductivité de l'eau d'élevage ont été prises quotidiennement avec un multimètre C 830 de marque Consort NV. Ces paramètres ont varié sans relation significative avec les traitements dans les intervalles suivants : température entre 24 et 33°C avec une moyenne de 27,6°C ; pH entre 6,5 et 7, tandis que la conductivité moyenne a été de 32,13 (± 3,1) $\mu s\ cm^{-1}$.

1.2.2.3 Aliments expérimentaux et nourrissage

Lors de la formulation des aliments expérimentaux, nous avons utilisé des ingrédients importés et des matières premières locales. La farine de poisson, l'huile de poisson, le CMC et les premix vitaminique et minéral ont toujours été obtenus respectivement chez Coppens International BV (Helmond, Pays-Bas), Sigma-Aldrich (Bornem, Belgique), INVE Aquaculture (Belgique) et INRA (Belgique). Le reste des ingrédients (tourteaux de soja et de coton, farine de maïs, son de blé, huile de palme) ont été achetés au niveau du marché local (Cameroun). Les sous-produits végétaux ont toujours été soumis à tour de rôle à un traitement thermique, 1 à 3 heures de cuisson suivant la source de combustible (bois ou gaz de cuisine). Ces cuissons ont de même été suivies successivement de séchages au soleil et de passages au moulin pour l'obtention de fines farines, avant toute incorporation dans les régimes expérimentaux.

Suivant les régimes, les ingrédients protéiques et glucidiques ont toujours été pesés et mélangés jusqu'à l'obtention d'une poudre homogène, à laquelle étaient ajoutées les proportions respectives de premix vitaminique et minéral, de CMC et d'huiles. De l'eau était ensuite ajoutée au mélange de manière à obtenir une pâte destinée à être transformée en spaghettis (diamètre, 2-3 mm) à l'aide d'un robot de cuisine (Kenwood KM 800, Havant, UK) (Photo 5). Les spaghettis obtenus ont toujours été séchés au soleil (28-35°C) durant 3 jours avant d'être concassés manuellement. Avant leur distribution, les aliments ont de même été stockés à -20°C. Après la pesée quotidienne, les granulés étaient distribués manuellement jusqu'à la satiété apparente, deux fois par jour (9h30 et 14h30), en deux passages à chacune des fois.

1.2.2.4 Echantillonnage et analyses chimiques

Des échantillons de poissons ont toujours été prélevés aléatoirement au début et à la fin de chaque essai pour réaliser des analyses dans le but de

déterminer respectivement les compositions corporelles initiale et finale des poissons expérimentaux. Des analyses similaires ont également été réalisées sur les ingrédients et les régimes expérimentaux.

Photo 1 : Heterotis adulte

Photo 2 : Pêcherie dans le Nyong

Photo 3 : Etang d'acclimatation

Photo 4 : Hapas en installation

Photo 5 : Fabrication de granulés

Les protéines ont été déterminées en utilisant la méthode de Kjeldahl (N x 6,25) après une digestion acide. Les lipides totaux ont été estimés par la méthode de Folch *et al*. (1957), les cendres par incinération de l'échantillon dans un four à moufle (550°C, 12h) et l'humidité après séchage de l'échantillon à 105°C durant 24h. Ces analyses ont été réalisées au laboratoire de l'Unité de Recherche en Biologie des Organismes (URBO) des Facultés Universitaires Notre-Dame de la Paix de Namur (FUNDP) en Belgique.

Les dosages des acides aminés ont été effectués à l'Unité de Chimie Biologique Industrielle de la Faculté Universitaire des Sciences Agronomiques de Gembloux en Belgique. Les acides aminés totaux ont été déterminés suivant la méthode de la Commission Européenne (Commission Directive 98/64/EC du 3 Septembre 1998). Ainsi, les acides aminés ont été déterminés dans un analyseur automatique (Biochrom 20 plus) après une hydrolyse acide de 100 mg d'échantillon dans 10 ml 6 N HCL (+ 0,1% phénol) à 110°C pendant 22h. Les acides aminés soufrés ont été dosés après une oxydation performique avant l'hydrolyse acide. Le tryptophane a été obtenu après une hydrolyse alcaline selon la méthode décrite par Fontaine *et al*. (1998).

1.2.2.5 Evaluation de la croissance et de l'utilisation des aliments

Des résultats obtenus, plusieurs paramètres ont été calculés pour évaluer respectivement la croissance et l'efficacité alimentaire. Ces paramètres sont le Taux de croissance spécifique (TCS), le gain de poids (GP) exprimé en pourcentage, l'efficacité alimentaire (CEA), le Coefficient d'Efficacité Protéique (CEP) et la rétention protéique (RP). Les formules de calcul de ces facteurs sont :

$$TCS\ (\%\ j^{-1}) = 100\ (\ln P_f - \ln P_i)/\Delta T$$

$$GP\ (\%) = 100\ [(P_f - P_i)/P_i]$$

$$CEA = (\text{Gain de poids des poissons incluant la biomasse des morts})/Q$$

$$CEP = (\text{Gain de poids des poissons incluant la biomasse des morts})/P$$

$$RP = 100\ [((P_f \times PC_f) - (P_i \times PC_i))/(Q \times PA)]$$

avec P_f = Poids final (g) ; P_i = Poids initial (g) ; ΔT = Durée de l'expérience (jour) ; Q = Quantité d'aliments distribuée (g) ; P = Quantité de protéines alimentaires distribuée (g) ; PC_f = teneur corporelle protéique finale ; PC_i = teneur corporelle protéique initiale ; PA = teneur protéique de l'aliment.

1.2.2.6 Analyses statistiques

Les données obtenues et les paramètres calculés sont soumis à une analyse de la variance (ANOVA) à un ou deux critères dans le but de comparer les différents traitements après vérification de l'homogénéité des variances avec le test de Hartley (Dagnelie 1975). En cas de différence significative au seuil de 5%, on a eu recours au test de la plus petite différence significative. Toutes les analyses statistiques ont été réalisées à l'aide du logiciel Statview (Version 5.0.1.0., SAS Institute Inc.) et les graphiques avec Microsoft Office Excel 2007.

1.3 Organisation de la thèse

Cette thèse est organisée en sept sections.

Section 1 : intitulée « Introduction générale », elle présente le contexte, la justification, les objectifs et la méthodologie globale de la recherche.

Section 2 : dénommée « Biologie et Aquaculture de *Heterotis niloticus* », cette section synthétise l'état des connaissances actuelles sur la biologie, l'écologie et les acquis aquacoles de cette espèce d'une part, et elle relève d'autre part les obstacles majeurs à franchir tout en proposant les thématiques de recherche futures en vue de son utilisation durable et rentable en aquaculture tropicale.

Sections 3 et 4 : elles sont intitulées « Nutrition protéique de *Heterotis niloticus* en phase de pré-grossissement ». La section 3 analyse la réponse des juvéniles d'Heterotis nourris avec des régimes isoénergétiques ayant des teneurs croissantes en protéines et estime leurs besoins spécifiques en protéines. Quant à la section 4, elle s'intéresse au profil corporel en acides aminés des œufs, larves, juvéniles, immatures et adultes d'Heterotis, et estime également les besoins en acides aminés indispensables de cette espèce à différents stades ontogénétiques.

Section 5 : dénommée «Rapport protéine/énergie chez les juvéniles de *Heterotis niloticus* », elle examine les effets de différents rapports protéine/énergie sur les performances de croissance et la composition corporelle des juvéniles de cette espèce.

Section 6 : intitulée « Valorisation des sous-produits végétaux dans l'alimentation des juvéniles de *Heterotis niloticus* », cette section évalue les réponses des juvéniles de ce poisson nourris avec des régimes ayant des taux

graduels de substitution de la farine de poissons par des tourteaux oléagineux disponibles localement.

Section 7 : cette section ultime « Discussion générale, conclusion et perspectives » est une synthèse de l'ensemble du travail : elle visualise les résultats obtenus en fonction des objectifs de la thèse afin de mettre en exergue les acquis majeurs de cette recherche, puis d'en tirer des conclusions incluant des perspectives de recherche-développement sur *Heterotis niloticus*.

1.4 Références

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La section suivante a été rédigée avec l'intention de fournir au lecteur une synthèse de l'état des connaissances disponibles sur la biologie, l'écologie et les possibilités d'élevage de *Heterotis niloticus* dans le but de mieux appréhender l'espèce étudiée et la problématique globale de ce travail. Ainsi, elle regroupe d'abord les données disponibles sur la reproduction et la croissance de cette espèce en milieu naturel et en captivité. Ensuite, elle relève les obstacles majeurs à surmonter en vue de son utilisation durable en aquaculture tropicale. Enfin, elle propose des axes de recherche-développement sur ce poisson tout en suggérant quelques solutions pratiques facilement mises en œuvre.

2

BIOLOGIE ET AQUACULTURE DE *Heterotis niloticus*

Biology and prospect for aquaculture of African bonytongue, *Heterotis niloticus* (Cuvier, 1829): A review

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2.1 Abstract

This paper first reviews the available data on the biology of *Heterotis niloticus*, and then the prospect for sustainable aquaculture of this species. Previous researches principally investigated its reproduction and growth in natural environment. Thus, Heterotis has an unpaired gonad located on left side; the spermatogenesis and oogenesis of this species are similar to those of most teleosts. Heterotis has an elaborated reproductive behaviour (coupling, nest construction and parental guarding). The species is classified within the opportunistic omnivorous fish category and consume a variety of food resources, ranging from aquatic invertebrates to small seeds, including small benthic organisms, fishes, shrimps, plant remains and terrestrial insects. In captivity, remarkable growth performances have been reported, individual mean body mass reaching up to 3 to 4 kg in 12 months. However, its use for profitable fish-farming in Africa relies on the knowledge of ecological, behavioural and nutritional factors which condition its reproduction, the resolution of massive mortality during early ontogeny, the estimate of its nutritional needs at various ontogenetic stages and the identification of an efficient breeding. The prospect for Heterotis contribution to the rise of African aquaculture depends on the solutions which will be found to the mentioned crucial problems.

KEY WORDS: *Heterotis niloticus*, biology, aquaculture, nutrition, growth

2.2 Introduction

Fish provides 22 percent of the protein intake in sub-Saharan Africa (FAO, 2006). This share, however, can exceed 50 percent in countries where other sources of animal protein are scarce or expensive. Fish consumption per capita in sub-Saharan Africa is the lowest in all regions and is the only part of the world where it is declining. The main reason is the upper limit of capture of fisheries production and the growing population. Since fishing products cannot meet the demand for fish, aquaculture will have to play a crucial role.

Aquaculture production in the sub African continent accounted for 0.2 percent of the total world production, estimated at 59.4 millions tonnes (FAO, 2006). This region, despite its enormous natural potentials, continues to play a minor role in the world aquaculture production. To maintain the current consumption level for fish in Africa, the aquaculture sector would have to increase by 267 percent by 2020 (FAO, 2006). Consequently, it is necessary to promote the expansion of this sector.

Presently, African fish farming is based mostly on the breeding of Nile tilapia. Unfortunately results obtained on fish ponds are very low. In order to overcome the challenge of African aquaculture, it is necessary, on one hand, to intensify and integrate the actual techniques of Nile tilapia breeding in Africa, and, on the other hand, to develop aquaculture in Africa, including the domestication of species with high aquaculture potentialities. One of the species with such a potential is the African bonytongue *Heterotis niloticus*, a species belonging to the Arapaimidae family (Ferraris, 2003).

The African bonytongue is exploited by fisheries in Cameroon River Basins (essentially in Nyong River) and others (Benin, Central African Republic, Chad, Congo, Democratic Republic of Congo, Ivory Coast, Madagascar, Nigeria and Senegal). The Heterotis marketing size actually varies from 2 to 7 kg body mass and annual captures in Cameroon have been estimated at 1520 tonnes valued at U.S. $5,221,375 (Kemgang, 2007). The African bonytongue presents undeniable characteristics which justify the present interest, such as remarkably high growth rate, air-breathing characteristic, omnivorous diet and good market potential. In fertilised pond, this fish exceeds 500 g within four months meanwhile at the same age, Nile tilapia difficultly attains 150 g. Individual mean body mass of 3 up to 4 kg within a twelve-month cycle is very encouraging. However, its use for intensive and sustainable aquaculture should be envisaged with precaution as numerous problems persist. The present article summarises the available data on the biology of *H. niloticus*, and presents its potential in relation to missing data for its sustainable and profitable aquaculture in Africa.

2.3 Systematics

2.3.1 Taxonomy and morphology

According to Blache (1964), the valid scientific name is *Heterotis niloticus* (Cuvier, 1829). Previously, Heterotis formed part of the Osteoglossidae family. This family included four genera distributed around the world (*Arapaima* and *Osteoglossum* in the Amazonian basin, *Heterotis* in tropical Africa and *Scleropages* in Australia, Asia and India). Currently, Osteoglossidae was divided into two different families: Osteoglossidae (*Osteoglossum* and *Scleropages*) and Arapaimidae (*Arapaima* and *Heterotis*) (Ferraris, 2003).The genus *Heterotis* contains only one species: *Heterotis niloticus*.

Heterotis has a compressed body covered with large and strong scales (Paugy, 1990). These scales are corrugated with a more or less vermiform sculpture and oval in shape with the thicker portion exposed. African bonytongue is characterized by dorsal and anal fins, which are spineless, elongated and posteriorly positioned. The caudal fin is small and rounded while the pelvic are abdominally positioned. The lateral line extends in a straight line from above the operculum to the middle of the caudal peduncle. Heterotis possesses premaxillary and maxillary teeth but no pharyngeal. Villiform teeth are observed on the tongue.

The dorsal fin possesses 33 to 37 soft rays and start a little behind from anal fin (34 to 38 soft rays) (Moreau, 1982; Mbega, 2004). The number of gill rakers increases with the fish length; 33 (young) to 98 on the ceratobranchial and 21 (young) to 76 on the epibranchial. Young specimens possess external gills. In addition, the number of vertebrae oscillates between 66 and 69. The maximum body mass and size observed vary according to geographical regions (Table 2.1).

2.3.2 Anatomical specificity

There exist spiral tubular structures rooting from the 5th branchial arc and opening at the pharyngeal roof. These snail-like organs similar to those of *Chanos chanos* have multiple functions. The suprabranchial organ plays a role in gaseous exchange, alimentary or sensory aspects. D'Aubenton (1955) estimates that these structures could have a mechanical part in nutrition and respiration thanks to the presence of mucus cells and sensory buds.

African bonytongue has a tripartite air bladder which communicates with the pharynx by a sphincter. The anterior part of the bladder, near the sphincter, is not very vascularized. The second part, in the contrary, is much more vascularized, whereas the third coupled part with the kidneys forms a

vascularized spongy mass by the pulmonary artery and the posterior cardinal veins. Heterotis uses this bladder to breathe on the surface of water, thus allowing the oxygenated blood to return to the general circulation. This anatomical specificity permits its life in muddy water relatively poor in oxygen. Embryos and larvae of African bonytongue have external gills of endodermal origin (Budgett, 1901; Moreau, 1982). These gills increase respiratory surface area to volume ratio.

Table 2.1: Maximum body mass and size (SL, standard length) in natural environment

Geographical area	Maximum		Reference
	Mass (kg)	Size (SL, cm)	
Lake Tchad	10.15	98	Blache (1964)
Nile River	6.50	80	Bishai (1970)
Ubangui	4.00 – 5.00	-	Micha (1973)
Nyong (Cameroon)	5.06	91	Depierre and Vivien (1977)
Nigeria	-	100	Olaosebikan and Raji (1998)

2.3.3 Geographical distribution

Heterotis niloticus is present in large rivers and lakes of the Nilo-Sudanian area, Central and West Africa (e.g., Nile, Niger, Gambia, Oueme, Benoue, Senegal or Lake Chad) (D'Aubenton, 1955; Daget, 1957; Levêque et al, 1990, Li and Wilson, 1996; Mbega, 2004; Adite et al., 2005) and has been successfully introduced in many rivers and aquaculture stations in Africa (Table 2.2).

Several countries reported adverse ecological impact after introduction. In Gabon for example, it was introduced in 1959 for fish farming due to its high growth rate and good meat quality (smoked or salted). Following the accidental introduction into the river Ogowe in 1980 (Mbega, 2004) this species more and more predominates in artisanal fishing. The consequence has been a drop in the capture of favourite species such as tilapia sp. The Gabonese authorities attribute this drop to feed competition between

introduced Heterotis and local fish species, uncontrolled occupied area and difficulties related to fish net.

Table 2.2: Introduction in various river basins

Introduction area	From	Year / Period	References
Nyong and Sanaga (South Cameroon)	North Cameroon	1958	Depierre and Vivien (1977)
Ubangui River(CAR)[1]	South Cameroon	1960	Micha (1973)
Ogowe (Gabon)	South Cameroon	1980	Mbega (2004)
Madagascar	Cameroon	1964	Moreau (1982)
Lake Kossou and Ayame (IC)[2]	Benue Cameroon	1959	Moreau (1982)
Congo (DRC)[3]	Congo	1966	Moreau (1982)

[1]CAR=Central African Republic; [2]IC=Ivory Coast; [3]DRC=Democratic Republic of Congo

2.3.4 Diet

Arapaima, Scleropages and Osteoglossum fishes consume a variety of invertebrates, terrestrial vertebrates and particularly fish. Numerous authors characterized these species as carnivores, and more specifically, piscivores (Goulding, 1980; Rainboth, 1996; Allen et al, 2002). On the other hand, African bonytongue of all sizes consume a variety of food resources, ranging from aquatic invertebrates to small seeds, including macrophytes, plant remains, aquatic insects and fishes (Lauzanne, 1976; Micha, 1976; Moreau, 1982; Mbega, 2004; Adite et al., 2005).

The principal food items recorded in Lake Hlan and Sô River in Benin were detritus, aquatic insect, microcrustacea, molluscs and substrate particles (Adite et al., 2005). Microcrustacea consumed were mostly Ostracoda, Cladocera (mainly Daphnidae), Copedoda, Amphipoda and Eubranchipoda. Aquatic insects were mostly immature stages of Diptera (Chironomidae, Ceratopogonidae, Syrphidae, Tipulidae and Empididae), Ephemeroptera, Hemiptera, Odonata, Heteroptera and Plecoptera. Chironomidae larvae are the most appreciated (Lauzanne, 1976; Adite et al., 2005).

Recurrent gastropod molluscs in the stomach contents of African bonytongue are Limnidae, Planorbiidae, Physidae and Hydrobiidae (Moreau, 1982; Adite et al., 2005). Minor diets recorded by several authors were shrimps, fishes, terrestrial insects (Coleoptera, Hymenoptera), invertebrate eggs, plant tissues, nematode worms, chitin fragments, algae (diatoms, cyanobacteria) and Rotifera (Lauzanne, 1976; Micha, 1976; Moreau, 1982; Mbega, 2004; Adite et al., 2005).

The diet varies with the ontogenetic stage. Larvae and young consume exclusively phytoplankton and zooplankton (Daget, 1957; Lauzanne, 1976; Micha, 1976; Durand and Levêque, 1981; Moreau, 1982), while adults consume small benthic organisms, water-column invertebrates, aquatic insects, fishes, shrimps, and seeds (Fagade and Olaniyan, 1973; Lowe-McConnell, 1975; Lauzanne, 1976; Hickley and Bayley, 1987). African bonytongue has been characterized as microphagous (Daget, 1957; Lowe-McConnell, 1975, 1987; Depierre and Vivien, 1977; Moreau, 1982), invertebrate strict feeder, or omnivore (Lauzanne, 1976; Micha, 1976; Durand and Levêque, 1981; Moreau, 1982; Mbega, 2004; Adite et al., 2005). According to the diversity in the composition of its diet as well as in its seasonal variation, Heterotis can be considered as an opportunistic omnivore.

2.4 Reproduction

2.4.1 Sexuality and gonad

The morphology does not permit external sex determination. Therefore, autopsy or stripping (to expulse genital sperm or ovules) is still frequently used. The gonads of Heterotis are similar to those of the other bonytongues (*Mormyrus kannume* and *Scleropages formosus*) and this species has an unpaired gonad located on its left side (Moreau, 1982).

The single testis is subdivided in lobules similar to those observed in other species. The lobules with irregular form are separated by conjunctive tissue. The conjunctive tissue approximately occupies 40% of the gonad volume. The periphery of these lobules is covered by a membrane which is not always distinctive of near-by conjunctive tissue. Lobules harbor sexual cells disposed in a disorderly manner. These types of lobules are especially located at the periphery of the testis and are observed throughout the year. In the reproductive season, the central lobules are richer in spermatozoa than the peripheral. The testis length reaches 7 cm, weighs 3-4 g (gonado-somatic ratio < 1%) and the gonado-somatic ratio of males is lower than 1%, even during the spawning season.

The maximum diameter of a mature oocyte is 2.5-2.8 mm (Daget, 1957; Olaniyan and Zwilling, 1963; Reizer, 1964; Rakotomanampison, 1966). The maximum weight of the ovary varies between 70 and 90 g, accounting for about 5% of total fish.

2.4.2 Gametogenesis

The spermatogenesis and oogenesis of Heterotis are similar to those of most teleosts (Moreau, 1982; Adite et al., 2006). The primary are large cells measuring 18 to 28 µm in their greater dimension, and 15 to 21 µm in their smaller dimension. Their round or elliptic nucleus (13 µ diameter) contains a nucleolus. Primary can be very abundant in a testis at the beginning and they represent up to 27% of gonad volume. The secondary resulting from the division of primary, are irregularly observed because this stage is short. Primary spermatocytes (23 µm) are distributed in all lobules. No study clearly identified and described the secondary spermatocytes of Heterotis, probably due to the massive degeneration of sexual cells observed during this stage. The spermatozoa are present rarely more than 8% of the testis volume and the head of the spermatozoon is close to 3µm in diameter.

Primary oocytes in meiotic prophase contain low cytoplasm. At the end of meiosis the nucleus contains several nucleoli. The nucleoplasmic ratio varies with the oogenesis stages, 0.30 for oocytes of 250 µm of diameter, it passes to 0.25 for oocytes of 500 µm and reaches 0.20 in oocytes of 1000 µm. The follicular appears around the oocytes (300 µm) and differentiates around oocytes of 500-µm size to which oocytes start to fill up with yolk. In oocytes of 1000 µm, cytoplasm is filled of yolk and the nucleus rich in nucleoli is still at the centre of oocyte. The size structure of oocytes in a mature ovary is multimodal (Moreau, 1974).

2.4.3 Maturity

In natural environment, the observed size at first maturity varies according to ecological areas. In Ubangui, it was observed when Heterotis weighed about 600 g, corresponding to a total length of 400 mm (Micha, 1973). In Ivory Coast, 35% of specimens (800-1000 g) are mature in the artificial lakes of Kan and Ayame, whereas all individuals of more than 1 kg are mature (Moreau, 1974). It is generally believed that this species reach maturity in the course of the second year (Daget, 1957) and the age at first maturity seems later (24-30 months) in high altitude (e.g., Madagascar). Reizer (1964) observed the reproduction of 24 to 36-months old fish in captivity and suggesting that the sexual maturity would occur at a more advanced age in pond breeding conditions.

2.4.4 Fecundity

Absolute fecundity, expressed by the number of mature oocytes in the ovary, increases with size ($F = 6\ 10^{-7}\ (TL)^{3.65}$; $r^2 = 0.47$ where F = fecundity and TL = Total length in mm) and mass ($F = 5.74W-748$; $r^2 = 0.43$ where W = body mass, in g) (Adite et al., 2006). This author reported that the absolute fecundity varies from 2697 to 27,508 for females of 50.0-73.5 cm SL, 1.33-4.65 kg body mass. Other studies reported from 3572 to 15,246 for females of 56.0-82.0 cm SL in Ubangui (Micha, 1973) and from 4200 to 12,000 for females of 1.5-4.7 kg in Ivory Coast (Moreau, 1982).

Relative fecundity, expressed as number kg^{-1} body mass, varies from 2028 to 5916 (Moreau, 1982; Adite et al., 2006). However, the real fecundity, expressed as number of effectively-laid eggs, averaged about 3000 eggs per female (1.8 kg, mean body mass).

2.4.5 Coupling and spawning

Many authors have described the elaborated reproductive behaviour of Heterotis (Daget, 1957; Tillon, 1957; Mvogo, 1962; Reizer, 1964; Rakotomanampison, 1966). During the annual reproductive season, adults search for favourable egg-laying areas. These nesting places cannot be too deep, but rich in grass. The mature fish frequent the grassy banks of the rivers or flooded zones during this period. The acquisition of a favourable nesting place and the meeting of a sexual partner induce the reproductive behaviour.

Gonad maturation and reproductive behaviour seem to be two distinct phenomena, which would not necessarily depend on the same external stimuli. Indeed, the complete maturation of oocytes seems to be conditioned by floodal favourable spawning areas and nests construction by males; while three factors seem to influence the reproductive behaviour (onset of the rainy season, lengthening of photoperiod and lowering of pH) (Moreau, 1974).

Before selecting a partner, male creates a circular nest (100-150 cm in diameter) in swamps. The basin-like nest is surrounded by vegetation and provided with an opening allowing the passage of breeders. The average depth in the middle of the nest varies according to the authors, 20-34 cm according to Svensson (1933) in Moreau (1982), 35-55 cm according to Daget (1957. This stage is relayed by an alternative nest parental guarding during 24 to 48 hours. After that, batches of eggs are laid and fertilized during a spawning clasp. The ripe ovarian eggs are yellow to orange and demersal fertilized eggs adhere together in one mass when deposited. When spawning is completed, both male and female guard the nest. Each spawning

episode can give approximately from 3000 (Moreau, 1982) to 6125 larvae (Adite et al., 2006).

2.4.6 Reproductive season

Numerous authors recognize a correlation between Heterotis reproduction and the rainy season, involving water rise and flood grassy zones (Reizer, 1964; Rakotomanampison, 1966; De Kimpe, 1967; Vincke, 1971; Micha, 1973; Moreau, 1974). This species has one reproductive season per year that varies according to area; July-September in the Niger, September-December in the Nyong, August-October in the Chad basin, September-October in the Senegal. However, the reproduction period does not obligatorily correspond to the approach of rain; and spawning may continue in the dry season even at water temperature below (De Kimpe, 1967; Moreau, 1974), or over 30°C (Rakotomanampison, 1966). In addition, the spawning can be obtained naturally in fish pond containing vegetation (Reizer, 1964; Rakotomanampison, 1966) or by simulating a rising-water in the pond (De Kimpe, 1967; Vincke, 1971).

2.5 Growth

Few studies have described the growth from birth to adult in natural environment or in pond. However, the larval development has been precisely described from hatching to 8 days post-hatch (Daget, 1957).

2.5.1 Hatching

At hatching the chorion tears, releasing the caudal area, the cephalic area and the yolk-sac, respectively. The duration between egg laying and hatching is not exactly reported. Some authors observed that hatching would occur between 24 and 48 hours at 30°C (Budgett, 1901; Daget, 1957) or in less than 24 hours in ponds at unspecified temperatures (Lemasson, 1957).

2.5.2 Larval growth

Larval growth has been described in ten subdivided stages (Figure 2.1) and synthesizes in Table 2.3.

Table 2.3: The embryonic and larval growth of *Heterotis niloticus* from hatching to 8 days post-hatch (Daget, 1957)

Stage	Time post-hatching	Larval length (mm, TL)	Main characteristics
1	Just after hatching	7.5	• Embryonic curved-body • Visible and strongly pigmented eye • Developed abdominal and caudal areas compared to the cephalic area • Presence of yolk-sac • Small pectoral fins and myomeres are distinguished • Unpaired embryonic fin begins dorsally • Anal tract is quite visible ventrally at the posterior limit of yolk-sac • Caudal limit of the body is clearly raised • Mouth and the five branchial arches are open • Some-well-developed, short or less-developed and starting-up filaments exist in the first, second and third branchial arc, respectively
2	18 hours	9	• All larval parts are greatly increased in volume except yolk-sac, body

			myomeres and anal tract • Curved-body structure is less marked • Caudal limit increasingly raised • The branchial filaments are more numerous and longer • The filaments of the first three branchial arcs are well formed and those of the fourth arc start to appear in tubercular form
3	42 hours	10	• Much voluminous cephalic and longer caudal areas • The branchial filaments exist on the four arches and those of the first arc reach the anterior side of the eye
4	2 days	10	• The early stage of caudal fin appears • The opercula fold is not formed yet • Branchial filament lengths exceed the head
5	3 days	10.5	• The larva resembles a young fish • The body no longer presents traces of embryonic curve • The caudal fin separates • The branchial filaments regress in length • Yolk-sac remains voluminous

			• The lips are well formed but the larva does not feed yet
6	4 days	11.5	• The yolk-sac decreases considerably • The head presents its final aspect including the mouth • The opercula fold is not completely developed • The larvae appear black in colour while fingerlings and adults acquire scale-grey colour (black in exceptional case as in Nyong River)
7	5 days	12	• The nascent anal fin organized • Much more decreased yolk volume • Little developed opercula fold
8	6 days	12.5	• Total disappearance of the yolk • No ventral and dorsal fins
9	7 days	13	• The appearance of the early stages of ventral and dorsal fins
10	8 days	14	• The ventral fins are well formed but still very small • Rudimentary rays are visible with the dorsal and pectoral fins.

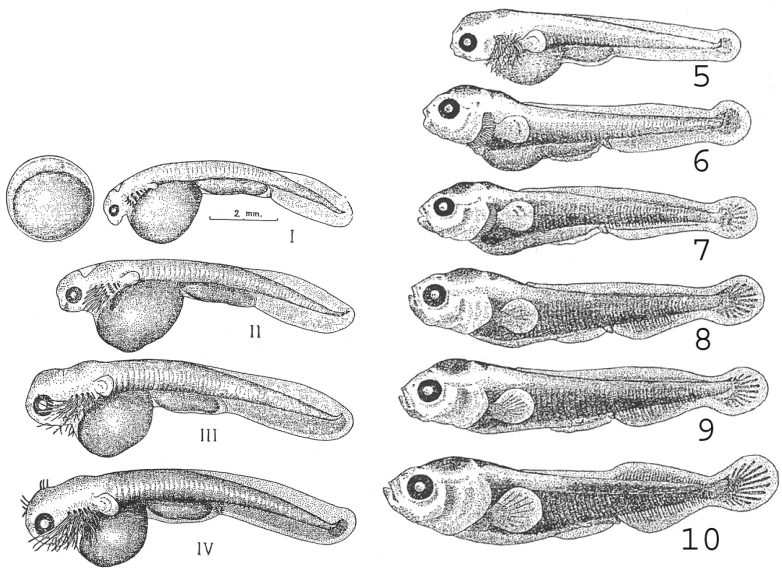

Fig. 2.1. Diagrammatic representation of yolk-sac larva and post yolk-sac larva (adapted from original drawing in Daget, 1957).

At the later stages, larvae acquire essential adult organs. However, the scales are not formed yet and there are remains of protopterygia in front of the dorsal and anal fins. Except for an increase in the total length, no other important phenotypical characteristics are observed until the 18th day post-hatching.

In the course of the first three weeks of life, the growth in length is variable: a rapid increase in length during the first 24 hours, then a relatively slow growth until the yolk reserves are depleted, and lastly, an acceleration of the growth rate when larvae start the exogenous feeding (Figure 2.2).

2.5.3 Fingerling growth

The growth of fingerlings has not been fully studied in natural environment. Observations indicated that juveniles also live in swarm and high mortality rates are recorded until 2 months of age. Tillon (1959) recorded a SGR value of 3.5 for fish of 3 to 6 g fed in ponds with cotton seed at 100 $kg.ha^{-1}.day^{-1}$. SGR values of 0.47 (Okoye and Abubakar, 1996) and 1.27 (Omorinkoba et al., 1991) have been reported for fingerlings of 5 g reared in fertilized ponds and in polyculture with Nile tilapia and African catfish irregularly fed with pellets (25% dietary protein).

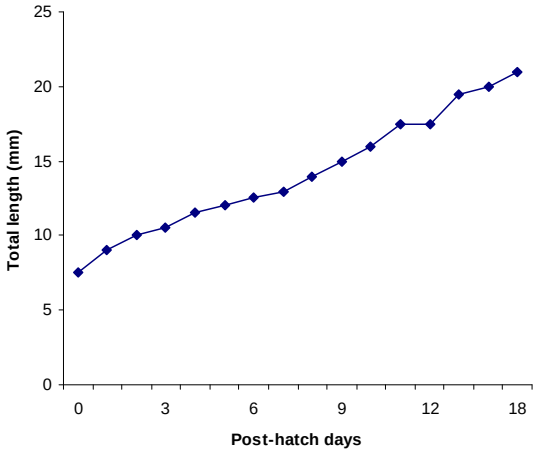

Fig. 2.2. Growth of young *Heterotis niloticus* during the first three weeks (adapted from Daget, 1957).

2.5.4 Adult growth

Data on adult growth in natural environment or captivity are rare. The only criteria used were the scales (Daget, 1957; FAO/UNDP, 1970) and size frequency (Depierre and Vivien, 1977). The results obtained from various natural environments are shown in Figure 3.3.

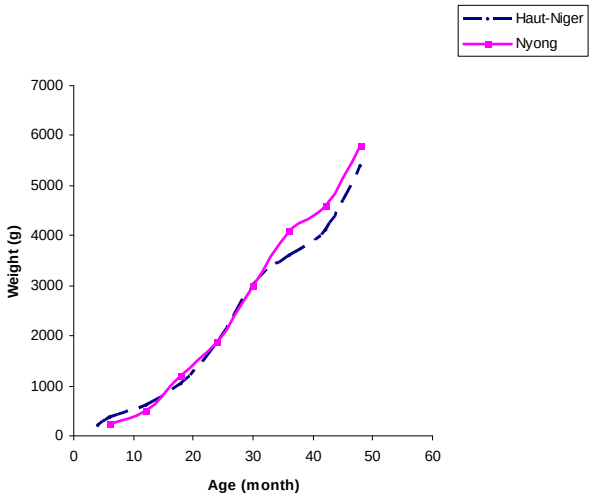

Fig. 3.3. Growth of *Heterotis niloticus* in two different ecological environments (redrawn from Daget, 1957; Depierre and Vivien, 1977).

Several authors studied the relationship between the length and the body mass, expressed as $W = aL^b$ (W = body mass, in g; L = total length, in mm). For specimens (300-800 mm SL, 0.5-5 kg body mass) this relationship varies according to ecological areas (Table 2.4).

These relationships between the length and the body mass have been established in rivers (Ubangui and Nyong) and lakes (Ivory Coast and Madagascar) (Table 2.4). Hydrology of rivers is strongly influenced by seasonal rainfall and during the flood season, water from rivers covers extensive floodplains, which probably provide abundant food resources (microcrustacea, molluscs, aquatic insect, shrimps, and fishes). In lakes, floating plants occupy large areas and also provide habitat for many aquatic organisms, including small fishes. But, seeds from submerged and emergent terrestrial vegetation are only available for Heterotis during the wet and flood seasons. Consequently, its diet varies with the ecological area and the season. This probably explains the differences observed in a and b values, respectively (Table 2.4).

Table 2.4: Relationship between the body mass (W, in g) and the length (L, in mm) expressed as $W = aL^b$

Ecological area	a	b	References
Ubangui River	1.19×10^{-5}	2.95	Micha (1973)
Nyong River	1.30×10^{-4}	2.58	Depierre and Vivien (1977)
Madagascar	4.33×10^{-2}	2.61	Moreau (1982)
Ayame (IC)	1.23×10^{-2}	1.78	Moreau (1982)
Bouake (IC)	4.26×10^{-2}	2.59	Moreau (1982)
Niger	-	2.56	FAO/UNDP (1970)

[2]IC=Ivory Coast

2.6 Population

2.6.1 Sex ratio

Results obtained by various authors on the sex-ratio converge towards equal proportions between the two sexes. In Niger, within a population (44 to 78 cm SL), Daget (1957) observed a balanced sex-ratio. This tendency is confirmed by the results of other studies (Reizer, 1964; Micha, 1973;

Moreau, 1982) while Adite et al. (2006) reported proportions of 53.7% and 46.3% for males and females, respectively (7.4 to 76.5 cm SL; 5 to 5840 g).

2.6.2 Recruitment

Recruitment occurs towards 6 months of age, when juveniles first migrate out of laying places. The recruited fingerlings depend on female fecundity and survival rate of young. The available data on larval and fingerling mortality in natural environment are scarce. However, observations reported a high mortality at these two stages. This aspect was better studied in captivity.

In fish-farming, authors reported low survival rates of larvae and fingerlings (mean weight < 2g). During the first week post-hatch, massive mortalities up to 80% were recorded (De Kimpe, 1967). Observations have shown that total disappearance of larvae can occur within a few hours (Olaniyan and Zwilling, 1963; Reizer, 1964; Rakotomanampison, 1966; Vincke, 1971). According to Reizer (1964), this massive mortality occurred especially between the 5th and the 7th days post-hatching.

A series of hypothesis were suggested to explain this massive mortality: change of diet during weaning (Daget (1957), insufficiency of food resources and high stocking density (Rakotomanampison, 1966; Micha, 1973), predation of larvae by other fish, batrachians or aquatic insects (Reizer, 1964), impact of physicochemical environmental modifications and parasitosis (Reizer, 1964). Taken together, it would be probable that several reasons interact simultaneously in this problem, which remains still unresolved.

2.6.3 Social life and migration

Reproduction occurs during the rise of water level and the key-stages of natural reproduction consist of couple constitution, nest construction, nest and offspring parental guard (Daget, 1957; Mvogo, 1962; Reizer, 1964; Rakotomanampison, 1966, Moreau, 1982). After hatching, larvae remain grouped at the bottom of the nest. At the 3rd post-hatch day, larvae execute a continuous up and down movement for aerial respiration. Similarly, they leave the nest daily under alternative parental guarding. During these outings, the number of larvae falls considerably. The characteristic gregarious remains until larvae reach the fingerling stage (body mass < 3-5 g) where the swarm of juveniles disperses and young fish leave spawning zones during the first river migration. No complete study on the dynamics of populations is available. However, some preliminary results indicated annual feeding migrations of fingerlings and adults, from spawning areas to rivers or lakes; and reproductive migrations of mature towards

laying places (Daget, 1957; Depierre and Vivien, 1977; Moreau, 1982; Adite et al., 2005).

2.7 Elements of aquaculture

The first studies dealing with its culture started in 1950. This preliminary work concerned the behaviour and reproduction in small ponds, the growth and the nutrition, with encouraging results (Tillon, 1957; Reizer, 1964). Unfortunately, this interest was blurred with African states independence and then studies are scarce. Recently, this species interests again fish farmers and researchers.

2.7.1 Reproduction in captivity

As mentioned before, reproduction occurs naturally in pond (Lemasson, 1957; Tillon, 1957; Mvogo, 1962; Olaniyan and Zwilling, 1963; Reizer, 1964; Rakotomanampison, 1966) but the results are uncertain (Moreau, 1982). The pond (from 200 to 10,000 m^2) should contain herbaceous vegetation or at least vegetable remains, and low water depths (15 to 30 cm) in certain places. In captivity, it displays similar reproductive behaviours as in natural environment (couple constitution, nest construction and parental guard). However, sexual maturity occurs tardily (Tillon, 1957; Reizer, 1964).

No studies clearly demonstrated that females spawn several times per year in pond. The annual reproductive season is rather limited to a few months (Tillon, 1959, Reizer, 1964). Moreover, few data concerning the effects of environmental factors on its reproductive physiology are available (Moreau, 1974). Thus, ecophysiological studies have to be considered. Such studies would increase the knowledge of the influence of ecological factors (temperature, photoperiod, oxygen, pH, turbidity, quality of water) on gonad maturation, gamete quality, fecundity, reproductive season, spawning, embryogenesis, sex ratio and reproductive behaviour. Likely, nutritional impact on reproduction should be examined and finally research on the artificial propagation possibilities be initiated.

2.7.2 Larval rearing

No complete study exists on larval rearing. Many authors agree on massive mortality of larvae during the first days post-hatching. As mentioned before, several hypotheses were emitted to explain this observation. In spite of preliminary research initiated, this problem remains still currently

unresolved. Consequently, it is imperative, considering its importance in fish farming, to explore all eventualities to overcome this obstacle.

The feeding plays a fundamental role in larval survival and development of several fish species. One crucial stage is the passage of the larvae from endogenous to exogenous feeding. This passage depends on the yolk utilization efficiency, the quantity of endogenous nutrient reserves and the developmental competence. Another critical stage is to habituate the larvae to accept and use efficiently compound diets, a stage usually named weaning. In order to evaluate the impact of diet during larval mortality of Heterotis, several investigations are still required to evaluate the effects of different diets (live and dry diets) on survival and growth, and then to determine its optimal weaning age.

For larval feeding strategies, it is necessary to study larval development based on the evolution of yolk reserves, digestive tract or lipid metabolism during the very early life stages. These studies should indicate the onset of functional digestive structures. Investigations need also to evaluate the impact of physicochemical environmental modifications on larval mortality.

2.7.3 Fingerling and adult rearing

Studies on fingerlings and adult growth in captivity are scarce. Preliminary research examined the effect of diet containing cotton seed (fed to 100 $kg.ha^{-1}.day^{-1}$) on growth in pond. The results showed an exceptional monthly increase, 372 g in fish from 530 to 3880 g (Tillon, 1959), but this author observed that the best growth period occurred during the first 15 months.

Other studies reported a remarkable growth of 3 to 4 kg in a twelve-month cycle (initial body mass < 5 g; 10 fish per 100 m² pond) (Tillon, 1957; Bard, 1973). Some preliminary observations indicated high survival rate (from 69 to 96%, Tillon (1959); from 83% to 100% (Reizer, 1966) of juveniles (mean weight > 2 g) and adult. Okoye and Abubakar (1996) reported a daily growth rate of 2.8, 1.6 and 0.1 g respectively for Heterotis, African catfish and Nile tilapia during a 20 months period.

The growth performances of *Heterotis niloticus*, *Oreochromis niloticus* and *Cyprinus carpio* reared polyculturally under a semi-intensive system (only organic fertilization) were reported. The mean daily growth rates were 3.0, 1.11 and 0.38 g, respectively (Omorinkoba et al., 1991; Akande and Omorinkoba, 1994). These studies suggested also that these three fish species exhibited positive interactions within the pond environment.

No studies reported cannibalism, deformity and disease in captivity. The overall prevalence of gastrointestinal helminth infections were 38.9% and 50% for wild male and female, respectively (Akinsanya et al., 2007). These authors specified the infection with a trematode *Brevimulticaecum heterotis* in the liver, *Tenuisentis niloticus* and *Sandonella sandoni* in the intestine. Heterotis are also frequently infected with crustacean parasites of the genus *Lernaogiraffa* in the gills (Micha, 1973), *Lernaea* (Moreau, 1982) and by nematodes worms (Bard, 1973), without any remarkable consequence on growth. Large cysts (3 mm in diameter) of genus *Myxobolus sp.* have often been observed on its gills, usually leading to some respiratory complications in the case of severe infection (Paperna, 1982).

It appears clearly that the studies undertaken were first concerned with growth and survival rate, without approaching nutrient requirements, dietary impact on body composition and other factors. Therefore, basic information on its nutrient requirements is needed in order to develop a well-balanced and a cost-effective feed. In consequence, researches have to be carried out to estimate nutriment requirements at different ontogenetic stages and to evaluate the effects of dietary protein and lipid levels on growth, feed utilization and body composition in order to estimate the optimal P/E ratio of fingerlings and adults. Moreover, considering its omnivorous feeding habit, the valorization of local plant by-products must be envisaged.

Heterotis being bred in extensive and semi-intensive pond farming system with the potential natural food availability, tremendous research work is still needed to understand the mechanisms prevailing in fish culture ponds in terms of fish interactions within polyculture, stocking density and organic fertilization in order to produce efficient but cost effective fish feed at maximizing benefits.

Up to now, rearing densities were usually low, but values varied considerably between authors, from 3 to 500 fish per 100 m² pond, according to Moreau (1982) and Bard (1973), respectively. No studies clearly demonstrated the effect of stocking density, but negative correlation has been reported (Vincke, 1971; Micha, 1973). Therefore, more studies are still required to investigate the possibility of its breeding at higher stocking densities, in order to confirm its use for semi-intensive or intensive pond farming.

The cultivation of Heterotis dates back to about 1918 (Olaniyan and Zwilling, 1963), a very recent thing when compared with the common carp *Cyprinus carpio*, which was cultivated in China back from 473 before Christ. African fish farmers use Heterotis in polyculture with Nile tilapia, African catfish and sometimes common carp, in earthen ponds of generally higher than 200 m² in surface area (Department of Fisheries and

Aquaculture, 2007). Taking into account the problems and prospects already emitted for this species, let us suggest some practical solutions, relatively easy to implement now.

With respect to reproduction, the major difficulties are the sex determination and production of fingerlings. Therefore, it is practical to introduce a good number (around 10) of mature fish (2.2-3 kg body mass) into one or more spawning ponds (about 200 m²) preferably during water flooding season. This method has been successfully used (Tillon, 1957; Bard, 1960; Olaniyan and Zwilling, 1963; Reizer, 1964). 15-30 days after spawning, fingerlings can be collected with a landing net (35-40 cm diameter) or 45 days later (Tillon, 1957; Reizer, 1964). It would be also possible to stock several mature specimens in extensive pond (5 000 m² minimum) and collect juvenile regularly with cast net. Fingerlings captured must be stocked in fertilized pond in mono or polyculture with Nile tilapia. Therefore, the installation of private stations is required in order to provide fingerlings (5 g minimum) for fish farmers. These stations should also specialize in the massive production of others fingerlings (Tilapia and African catfish) to maximize its profits. Aside this controlled natural reproduction in ponds, some efforts should be put into a better control of gonad maturation and artificial spawning based on hormonal or environmental induction, as it has been done with other species like carps, Asian and African catfish.

Aquaculture of Heterotis can be envisaged in small or large ponds (more than 500 m²) and natural or artificial lakes. Moreover, its probable escapement from ponds to natural environment seems to pose an ecological disaster considering its auxiliary branchial air breathing organs and omnivorous diet. In introduction areas, resulted populations were established and their relatively low proportions in the annual captures of fisheries vary according to region: 16% in Lake Ayame, Ivory Coast (Lazard, 1980), 19% in South Cameroon (Kemgang, 2007) and 10% in Lake Tchad (Moreau, 1982). This indicates a minor environmental impact, not comparable to what has been observed with the introduction of some invasive species like Nile perch (*Lates niloticus*) in Lake Victoria, Common carp in Australia, and Nile tilapia in Sri Lanka.

2.8 Conclusion

In order to increase the African freshwater fish aquaculture production, the culture of new species of high potential is needed. Several fish species have been investigated, and *Heterotis niloticus* is considered among the most promising species for fish farming. The African bonytongue presents a remarkably high growth rate (3 to 4 kg in a 12-month cycle), an omnivorous

diet and a relatively higher market price. However, some obstacles restrict its production, in particular the difficulty in sex determination, massive mortality of larvae during the first day post-hatching and lack of basic information on nutritional requirements. This paper illustrates the real expansion possibilities of its aquaculture in relation to available biological data, and presents the specific studies required. Finally, its intensive and sustainable culture remains among the best hope for inland aquaculture in Africa.

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3 et 4

NUTRITION PROTEIQUE DE Heterotis niloticus EN PHASE DE PRE-GROSSISSEMENT

Influence of dietary protein levels on growth performance and body composition of African bonytongue fingerlings, *Heterotis niloticus* (Cuvier, 1829)

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3.1 Abstract

Two experiments were conducted in order to examine the influence of dietary protein levels on growth and carcass proximate composition of Heterotis fingerlings. Four isoenergetic practical diets were formulated to contain dietary protein levels from 250 to 400 g kg^{-1} diet. Replicate groups of young Heterotis (initial live weight 3.96 g and 26.40 g in experiments 1 and 2, respectively) were handfed twice daily to apparent satiation for a period of 42 and 28 days, respectively. Statistical analysis revealed that growth rate were significantly affected by dietary protein level ($P<0.01$). The highest weight gain was observed in fingerlings fed with 300 and 350 g protein kg^{-1} diet for fish size ranging between 3-15 g and 26-62 g, respectively. There was no significant difference between groups fed with 300, 350 and 400 g protein kg^{-1} diet for *Heterotis* fingerlings (3-15 g) in the one hand; in the other hand significant differences were found between fish (26-62 g) fed with 350 g protein kg^{-1} diet and those receiving 300 and 400 g protein kg^{-1} diet, with no significant difference between each other. The specific growth rate varied from 2.4 to 3.1 $\%.day^{-1}$. The whole-body protein, lipid, moisture and ash contents were not significantly affected by dietary protein levels ($P>0.05$). The relationships between percentage weight gain and dietary protein levels suggested very similar dietary protein requirement (about 310 g crude protein per kg diet) for Heterotis ranging between 3 and 62 g. The maximum growth occurred at about 345 g protein per kg diet.

KEY WORDS: *Heterotis niloticus*, dietary protein level, feed utilisation, growth, protein requirement, whole-body composition

3.2 Introduction

In west and central Africa, fishing remains the main source for indigenous fish supply to the population. Obviously, these fishing grounds have attained maximum sustainable levels and for the last 15 years, there is even a decline trend. To meet up with the increasing demand for animal protein from the growing human population, without increased fish importation, there is an imperative need to develop aquaculture in the African continent. Apart from Nigeria, aquaculture sector contributes to less than 5% of local fish demand in most African countries. Despite its aquaculture potential, Sub Saharan Africa lags far behind. It is therefore indispensable to stimulate the development of this sector through diversification including the culture of indigenous species such as African bonytongue (*Heterotis niloticus*). This species has a relatively high commercial value and strong aquaculture potential, but very few data are available on its farming.

Heterotis niloticus originated from tropical Africa (Greenwood 1973; Li & Wilson 1996). It is found in big rivers (Senegal, Gambia, Niger and Nile) and in some tropical African lakes like Lake Chad (D'Aubenton 1955; Levêque *et al*. 1990). Moreover, it was successfully introduced in the south of Cameroon (Depierre & Vivien 1977), in Ivory Coast (Moreau 1974; Lazard 1980), in the Democratic Republic of Congo, in Gabon (Mbega 2004) and in Madagascar.

Heterotis niloticus is a warm water bony fish well appreciated for human consumption in Sub Saharan Africa. It possesses an important potential market, with a commercial value twice higher than that of tilapia. The rapid growth (3 to 4 kg in a twelve-month cycle), late sexual maturity, short food chain and natural reproduction of this species in small and large ponds make it a good candidate for aquaculture production. Initial research on this species began in the 1950s. Unfortunately, the number of studies diminished considerably just after independence in 1960, before the recent regain of interest. These studies were conducted to better understand the biology (D'Aubenton 1955; Daget 1957; Omorinkoba *et al*. 1991; Okoye & Abubakar 1996; Fagbenro 2001; Achionye-Nzeh & Omoniyi 2002; Adite *et al*. 2006), the ecology (Moreau 1974; Moreau & Moreau 1982; Adite *et al*. 2005) and the culture (Tillon 1957; Lemasson 1957; Tillon 1959; Olanyan & Zwilling 1963; Reizer 1964) of this species. Heterotis was classified in the omnivorous fish category (Micha 1976; Mbega 2004; Adite *et al*. 2005).

Protein is a main component in fish feed. Increasing protein level in feed can lead to improved fish production, but excessive dietary protein level is not economical for fish culture. Valid information on the protein requirement of fish is essential for any new aquaculture attempt. It is therefore not

surprising that protein requirement studies are usually among the first fish nutrition experiments to be conducted when a new fish species is being considered for aquaculture. Dietary protein requirements has been investigated in a number of fish species (NRC 1993), but no research has been conducted so far on *Heterotis niloticus*.

The present study was designed to investigate the effects of dietary protein levels on growth and carcass proximate composition of Heterotis fingerlings, and to estimate their specific dietary protein requirement. As a consequence, an appropriate supply of dietary protein would allow supporting high growth rate and reducing nitrogen wastes.

3.3 Materials and methods

3.3.1 Rearing conditions

The trial was conducted in hapas that were constructed with nets of fine texture. These hapas with rectangular dimensions (1m x 0.5m x 1.1m; vol. = 400 L) were placed in a rectangular fish pond (300 m², 1.2 m deep) located at the Government Aquaculture Station in Melen (Yaounde Cameroon). The pond was free from aquatic vegetation, completely independent, well exposed to sunlight and had a well-designed system of inlet and outlet to maintain the water level in the hapas at 0.8m for the duration of the experiment. During the feeding trial, fingerlings were exposed to natural photoperiod (6.15 a.m. to 6.45 p.m. daylight followed by night). Water temperature ranged from 25 to 31°C while pH ranged from 6.5 to 7.0. The experimental durations were 42 days (from December 2006 to January 2007) and 28 days (from September to October 2006) for experiments 1 and 2, respectively.

3.3.2 Experimental fish

Heterotis fingerlings weighing < 1g were caught in river Nyong near the town of Akonolinga (Cameroon), and transported to the Melen aquaculture station where they were stored for several weeks in a fertilized fish pond. After this phase, 50 fish were randomly distributed into each hapa and fed diet containing 300 g protein kg^{-1} diet for two weeks (during a pre-experimental period). After this conditioning period, each diet was tested on 25 fish per hapa, in triplicate for experiment 1 and duplicate for experiment 2. Before the fish allotment**,** 50 fish were randomly sampled and individually weighed (initial mean weight: 3.96 ± 0.13 g for experiment 1 and 26.40 ± 0.64 g for experiment 2). At the end of the experiment, all fish were individually weighed and total length measured.

3.3.3 Diet formulation, preparation and feeding

Diet formulations are shown in Table 3.1. Four isocaloric experimental diets for each trial were formulated to contain graded levels of protein (250, 300, 350 and 400 g kg^{-1} diet) (Tables 3.2 and 3.3 for experiment 1 and 2, respectively). These protein contents were chosen based on the results of the protein requirements of other omnivorous fish species such as tilapia (Shiau 2002) and channel catfish (Robinson *et al.* 2000). Fish meal was obtained from Coppens International BV (Helmond Netherlands). Apart from fish oil, other ingredients were obtained from local markets. Fish and soybean meals were used as the main protein source. Maize meal, wheat bran and fish oil levels were adjusted accordingly to make the diets iso-energetic. Gross energy levels of the diets were calculated based on 23.7, 39.5 and 17.2 kJ g^{-1} for protein, lipid and nitrogen-free extract, respectively (Guillaume *et al*. 1999).

Table 3.1: Ingredient and composition of the experimental diets (g kg^{-1})

	Diets (g protein kg^{-1} diet)			
Ingredients	**250**	**300**	**350**	**400**
Fish meal	130	180	235	285
Soybean meal	130	180	235	285
Whole maize meal	320	275	220	170
Wheat bran	320	275	220	170
Menhaden fish oil[1]	50	40	40	40
Vitamin premix[2]	20	20	20	20
Mineral premix[3]	20	20	20	20
Carboxymethylcellulose[4]	10	10	10	10

[1,4]Sigma-Aldrich products (Bornem, Belgium)
[2]Vitamin Mix Fish 0.5% INVE Aquaculture, Belgium (Composition per kg: vitamin A, 2 500 000 IU; vitamin D3, 500 000 IU; vitamin E, 30 000 mg; vitamin K3, 2 000 mg; vitamin B1, 2 000 mg; vitamin B2, 5 000 mg; panthotenic acid, 10 000 mg; Niacin, 5 000 mg; vitamin B6, 4 000 mg; folic acid, 2 000 mg; vitamin B12, 4 mg; vitamin C, 20 000 mg; biotin, 200 mg and inositol, 80 000 mg)
[3]Mineral Mix MLNP 763, INRA Belgium (Composition per kg: dibasic calcium phosphate, 500 g; calcium carbonate, 215 g; sodium chloride, 40 g; potassium chloride, 90 g; magnesium hydroxide, 124 g; iron sulphate, 20 g; zinc sulphate, 4 g; manganese sulphate, 3 g; cobalt sulphate, 0.02 g; potassium iodide, 0.04 g; sodium selenite, 0.03 g and sodium fluoride, 1 g)

Soybean meal was boiled in a pressure cooker for two hours followed by sun drying. The different experimental diets were made by adding appropriate volumes of water to ingredients. The resulting paste was transformed into spaghetti (2mm for experiment 1 and 3mm for experiment 2) with the aid of food blender (Kenwood KM 800, Havant, UK). After sun drying at a temperature of 28-35°C for about three days, the spaghetti were manually broken and converted into pellets. The pellets were stored at -20°C until use.

Table 3.2: Chemical composition of the experimental diets (experiment 1; g kg^{-1} dry diet)

	Diets (g protein kg^{-1} diet)			
Constituents[5] (‰)	**250**	**300**	**350**	**400**
Moisture	89	84	83	83
Crude protein	253	295	347	393
Total lipid	62	54	58	62
Ash	59	70	76	93
NFE[6]	538	497	436	370
Gross energy ($kJ.g^{-1}$)	18	18	18	18

[5]Values are the mean of three replicate analyses.
[6]Nitrogen-free extract (NFE) calculated as: 1000 – (‰ moisture + ‰ protein + ‰ lipid + ‰ ash).

Fish were fed by hand twice a day (09h30 – 10h00 and 14h30 – 15h00) to apparent satiation. Pellets were distributed slowly, allowing all fish to eat. The daily feed supply was recorded.

3.3.4 Sample collection and methods for chemical analysis

Initially, ten fish were sampled for initial whole-body proximate composition. At the end of the experiment, 7 fish from each treatment were randomly selected for final analysis of body composition. All samples were stored at – 20°C prior to analysis.

Proximate composition of feed ingredients, experimental diets and fish were analysed following Association of Official Analytical Chemists methods (AOAC 1999). Crude protein (total nitrogen x 6.25) was measured using the Kjeldahl method after acid digestion. Total lipid content was estimated using

the Soxhlet apparatus method according to Folch, Lees & Sloane-Stanley (1957). Moisture was determined by drying the sample at 105°C for 24h to a constant weight. Ash was determined by incinerating the dried sample in a muffle furnace at 550°C for 12h. Due to problems of conservation, proximate composition of final fish (experiment 2) was not analysed.

Table 3.3: Chemical composition of the experimental diets (experiment 2; g kg^{-1} dry diet)

	Diets (g protein kg^{-1} diet)			
Constituents[7] (‰)	**250**	**300**	**350**	**400**
Moisture	100	105	101	103
Crude protein	259	301	357	396
Total lipid	61	53	59	60
Ash	64	71	80	89
NFE	517	471	404	352
Gross energy ($kJ.g^{-1}$)	17.4	17.3	17.7	17.8

[7]Values are the mean of three replicate analyses.
[8]Nitrogen-free extract (NFE) calculated as: 1000 – (‰ moisture + ‰ protein + ‰ lipid + ‰ ash).

3.3.5 Data processing and statistical analysis

From these data, final mean weight, specific growth rate (SGR; $\%.day^{-1}$) (100 x (ln final body weight – ln initial body weight))/duration of experiment (days)), weight gain (WG) ((final body weight – initial body weight) (g) x 100/initial body weight (g)), feed efficiency (FE) ((final body weight – initial body weight) (g)/total feed intake (g)), protein efficiency ratio (PER) ((final body weight – initial body weight) (g)/total protein intake (g)), protein deposition (PD) (100 x (final body weight x final body protein – initial body weight x initial body protein)/total feed intake x dietary protein) were determined.

Data were analysed for comparison among different dietary treatments using one-way analysis of variance (ANOVA) after verifying the homogeneity of variance using Hartley's test (Dagnelie 1975). Differences among means were tested by least significance difference (LSD). The significance level was 5%. Percentage data were transformed to arcsine values before analysis.

In order to determine the levels of dietary protein for maximum growth, the relationship between dietary protein and weight gain (WG) were fitted using a second-order polynomial equation, where WG was a function of dietary protein, using the formula $Y = a + b_1X + b_2X^2$ (Espinós *et al*. 2003). An estimation of the dietary protein requirement, based on percentage weight gain, was done by the broken line model (Robbins *et al*. 1979).

3.4 Results

3.4.1 Experiment 1

3.4.1.1 Growth, survival and feed utilization

Weight gain, specific growth rate, feed efficiency and survival rate of Heterotis fingerlings during the feeding trial are shown in Table 3.4. Weight gain and specific growth rate were significantly affected by dietary protein level ($P<0.01$). Heterotis fingerlings fed with 300 g protein kg^{-1} diet displayed the highest growth rate, but values did not differ significantly from those of fish fed with 350 and 400 g protein kg^{-1} diet ($P>0.05$). However, the weight gain and SGR of this group were significantly ($P<0.01$) higher than those of fingerlings fed with 250 g protein kg^{-1} diet, which displayed the poorest growth.

Survival in all treatments ranged from 59% to 76% and was affected by dietary protein level. The survival rate of fish fed with 300 and 350 g protein kg^{-1} diet did not differ from each other, but survival of fingerlings fed with 350 g protein per kg diet was significantly ($P<0.05$) lower than those of the fish fed with 250-400 g protein kg^{-1} diet. Feed intake decreased progressively with graded dietary protein level and was found to differ significantly ($P<0.001$) between groups fed with 250-300 g kg^{-1} and those receiving 350-400 g protein kg^{-1} diet. Feed efficiency increased progressively with graded dietary protein level and was found to differ significantly ($P<0.05$) between fingerlings fed with 250-350 g and 400 g protein kg^{-1}diet. On the contrary, protein efficiency ratio and protein deposition did not differ significantly between treatments ($P>0.05$) (Table 3.4).

3.4.1.2 Body composition

The whole body composition of Heterotis fingerlings is presented in Table 3.4. Percentage moisture, ash content, whole-body protein and lipid content were not significantly affected by dietary protein level ($P>0.05$).

Initial whole body composition of fish contained less moisture, protein and lipid than final body composition of fish, regardless of experimental diets.

3.4.1.3 Estimation of protein requirement

The influence of increased dietary protein levels on the growth response was examined by fitting dose-response data to a polynomial curve (Espinós *et al*. 2003). In that way, the polynomial equation obtained between weight gain and dietary protein level was:

$$Y = -1.2339X^2 + 83.991X - 1145.5 \ (r = 0.85)$$

The maximum weight gain was observed when the dietary protein level was 340 g kg^{-1} diet. Using the broken line model of Robbins *et al*. (1979), the dietary protein requirement for the Heterotis fingerlings (3-15 g) based on percentage weight gain was estimated to be 306 g protein kg^{-1} diet (Fig.3.1).

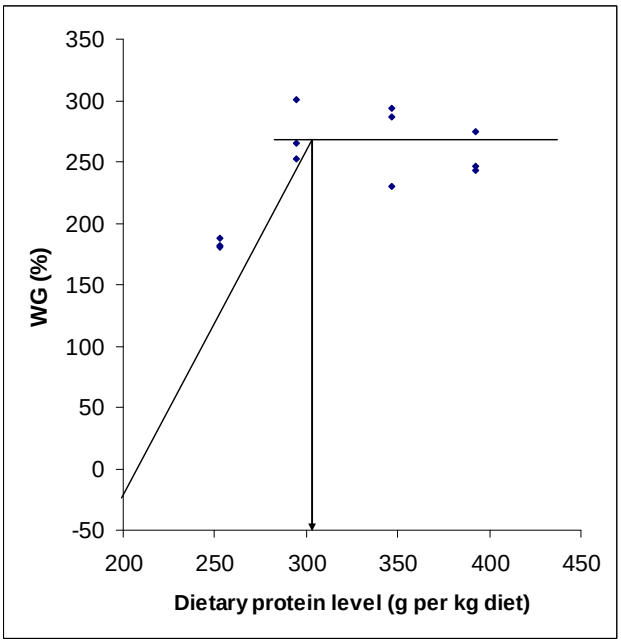

Fig. 3.1. Estimation of the dietary protein requirement of Heterotis fingerling according to the broken line model (initial average weight 3.96 g).

Table 3.4: Feed utilization, growth, survival performance and body composition of *Heterotis niloticus* fingerlings fed with diets containing graded levels of protein (initial average weight 3.96 g)

	Diets (g protein kg^{-1} diet)			
	250	**300**	**350**	**400**
Final weight (g)	11.2 ± 0.5^{a}	14.8 ± 1.1^{b}	14.6 ± 1.0^{b}	14.1 ± 0.9^{b}
Weight gain (%)	183 ± 2^{a}	272 ± 14^{b}	269 ± 20^{b}	254 ± 10^{b}
SGR (%.day^{-1})	2.5 ± 0.0^{a}	3.1 ± 0.1^{b}	3.1 ± 0.1^{b}	3.0 ± 0.1^{b}
Feed Intake[9]	50 ± 3^{a}	46 ± 1^{a}	34 ± 2^{b}	33 ± 2^{b}
FE	0.4 ± 0.0^{a}	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}	0.8 ± 0.0^{b}
PER	1.52 ± 0.16^{a}	1.86 ± 0.34^{a}	1.61 ± 0.18^{a}	2.12 ± 0.11^{a}
PD	22.4 ± 2.7^{a}	28.2 ± 4.8^{a}	24.0 ± 2.1^{a}	30.1 ± 1.6^{a}
Survival (%)	76 ± 2^{a}	68 ± 6^{ab}	59 ± 3^{b}	76 ± 2^{a}
Moisture[10]	80.5 ± 0.1	79.6 ± 0.3	80.3 ± 0.5	80.5 ± 0.5
Protein[10]	13.6 ± 0.2	14.0 ± 0.2	13.6 ± 0.2	13.5 ± 0.2
Total lipid[10]	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
Ash[10]	4.0 ± 0.1	4.1 ± 0.3	3.5 ± 0.1	3.8 ± 0.1

SGR = Specific growth rate; FE = Feed efficiency; PER = Protein efficiency ratio; PD = Protein deposition.
Values are mean ± SE of three replicates. Means in a row with different superscript letters are significantly different ($P<0.05$).
[9]Feed intake (g.week^{-1})
[10]Values are means ± SE of three replicates and expressed in (%). Initial whole body composition was 79.5% moisture, 12.2% protein, 0.87% lipid and 5.1% ash.

3.4.2 Experiment 2

3.4.2.1 Growth, survival and feed utilization

Weight gain and specific growth rate were significantly affected by dietary protein level ($P<0.05$) (Table 3.5). Fish fed with 250 g protein kg^{-1} diet displayed the lowest growth rate. The weight gain and specific growth rate of fingerlings fed with 300 and 400 g protein kg^{-1} diet did not differ from each other, but were significantly ($P<0.05$) lower than those of fish receiving 350 g protein kg^{-1} diet.

Similarly, survival was affected by dietary protein level. The survival rate generally showed a decreasing trend with increasing dietary protein level and was significantly higher ($P<0.05$) in fingerlings fed with 250-300 g protein kg^{-1} diet than in fish receiving 350-400 g protein kg^{-1} diet (Table 3.5). Feed efficiency increased progressively with graded dietary protein level and was found to differ significantly ($P<0.01$) between fingerlings fed 250-300 g protein kg^{-1} and 350-400 g protein kg^{-1} diet. There was no significant ($P>0.05$) difference between fish receiving 250 and 300 g protein kg^{-1} diet, but fingerlings fed with 350 g protein kg^{-1} diet had significantly ($P<0.01$) lower feed efficiency than those of fish receiving 400 g protein kg^{-1} diet. The highest protein efficiency ratio was obtained with diets containing 250 and 400 g protein kg^{-1} diet while the lowest PER was obtained with diet containing 350 g protein kg^{-1} diet.

3.4.2.2 Estimation of protein requirement

As in experiment 1, the effect of increased dietary protein levels on the growth response was examined by fitting dose-response data to a polynomial curve. The polynomial equation obtained between weight gain and dietary protein level was:

$$Y = -0.457X^2 + 31.875X - 423.24 \quad (r = 0.987)$$

The maximum weight gain was observed when the dietary protein level was 349 g kg^{-1} diet. Using the broken line model of Robbins *et al*. (1979), the dietary protein requirement for the Heterotis fingerlings (26-62 g) based on percentage weight gain was estimated to be 311 g protein kg^{-1} diet (Fig.3.2).

Table 3.5: Feed utilization, growth and survival performance of *Heterotis niloticus* fingerlings fed diets containing graded levels of protein (initial average weight 26.40 g)

	Diets (g protein kg^{-1} diet)			
	250	**300**	**350**	**400**
Final weight (g)	51.7 ± 1.8^{a}	58.6 ± 2.6^{b}	61.6 ± 2.2^{b}	58.9 ± 1.6^{b}
Weight gain (%)	96 ± 0^{a}	122 ± 4^{b}	133 ± 1^{c}	123 ± 0^{b}
SGR (%.day^{-1})	2.4 ± 0.1^{a}	2.9 ± 0.1^{b}	3.0 ± 0.0^{c}	2.9 ± 0.0^{b}
Feed intake[11]	230 ± 7	208 ± 18	152 ± 16	97 ± 1
FE	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	0.8 ± 0.0^{b}	1.0 ± 0.0^{c}
PER	2.53 ± 0.03^{a}	2.26 ± 0.03^{b}	2.18 ± 0.05^{c}	2.43 ± 0.02^{a}
Survival (%)	88 ± 2^{a}	84 ± 4^{ab}	76 ± 4^{bd}	72 ± 2^{cd}

SGR = Specific growth rate; FE = Feed efficiency; PER = Protein efficiency ratio.
Values are means ± SE of two replicates. Means in a row with different superscript letters are significantly different ($P<0.05$).
[11]Feed intake (g.$week^{-1}$)

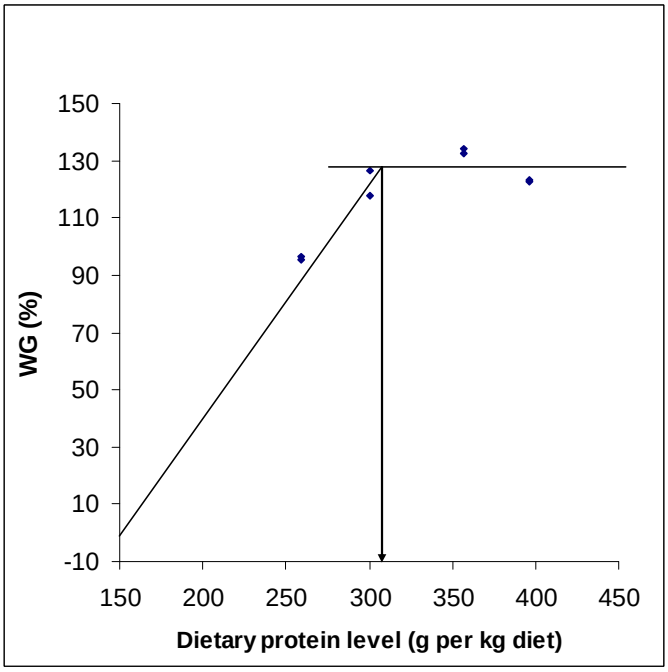

Fig. 3.2. Estimation of the dietary protein requirement of Heterotis fingerling by broken line model (initial average weight 26.40 g).

3.5 Discussion

In this study, an estimation of the dietary protein requirements of *Heterotis niloticus* fingerlings was done by formulating diets incorporating readily available and cheap local ingredients. In a majority of similar studies, researchers employed various purified and semi purified diets with high-quality protein sources such as casein, gelatin or synthetic amino acids that yielded more precise values. These protein sources being relatively expensive and not readily accessible for an average fish nutritionist in developing countries, our choice was guided by the amino acid composition of proteins (experimental ingredients) and their digestibilities in the one hand; in the other hand by similar estimations of dietary protein requirements of tropical bagrid catfish, *Mystus nemurus* fingerlings demonstrated by Khan *et al.* (1993) and by Ng *et al*. (2001). These authors reported requirements of 420 g kg^{-1} and 440 g kg^{-1} diet in this species when using practical and semi purified diets, respectively. Identical results were

obtained in other studies involving tilapia juveniles (Jauncey 1982; El-Sayed & Teshima 1992; Gunasekera *et al*. 1995; Al Hafedh 1999). Despite the slight difference, the similarity observed permits the use of practical diets in the determination of fish protein requirements. Moreover, in our study experimental diets were formulated by adjusting levels of fish and soybean meals with maize meal and wheat bran; the part of protein which comes from fish meal was highly digestible and its amino acid composition was close to requirements; the digestibility of other protein which comes from practical ingredients depends on digestibility of dietary amino acids which varies among feed ingredients. So, it is crucial to underline that the amount of nonprotein energy in the feed and the quality of the protein influences the growth response of fish fed with diet containing different levels of protein.

In this study the relationship between dietary protein levels and weight gain (WG) were fitted using a second-order polynomial equation (Espinós *et al*. 2003). The maximum weight gain was observed when the dietary protein level was 340 g kg^{-1} diet (initial average weight 3.96 g) and 349 g kg^{-1} diet (initial average weight 26.40 g). The dietary protein requirement of *Heterotis niloticus* fingerlings between 3 and 15 g was estimated to be 306 g kg^{-1} diet and 311 g kg^{-1} diet for fish between 26 and 62 g when fish meal and soybean were the major protein sources. These values are slightly lower than those obtained for other omnivorous fish species (*Oreochromis niloticus* and *Cyprinus carpio*) of similar body weight (350-420 g protein kg^{-1} diet), as recommended by Tacon (1987). These low dietary protein requirements of Heterotis fingerlings can be correlated to the natural feeding of this species. In fact, juveniles of this species naturally ingest zooplankton, phytoplankton, seeds, aquatic insect and other small benthic organism (Daget 1957; Lauzanne 1976; Micha 1976; Adite *et al*. 2005).

Protein requirements between fish species is complicated by difference in species, size and age of fish, diet formulation, stocking density, protein quality, hygiene and experimental conditions between studies (NRC 1993). However, the dietary protein requirement of young Heterotis (3-62 g), as estimated in the present study, were slightly lower or very close to the range determined for fingerlings of other omnivorous species, such as cyprinid fish *Spinibarbus hollandi* (327 g protein kg^{-1} diet; Yang *et al*. 2003), common carp *Cyprinus carpio* (310 g protein kg^{-1} diet; Takeuchi *et al*. 1979), jundia *Rhamdia quelen* (326 g protein kg^{-1} diet; Meyer & Fracalossi 2004), channel catfish *Ictalurus punctatus* (280-320 g protein kg^{-1} diet; Robinson *et al*. 2000) and Nile tilapia (280-300 g protein kg^{-1} diet; De Silva *et al*. 1989; 300-360 g protein kg^{-1} diet; Shiau 2002).

Dietary energy has a major impact on the utilization of dietary protein in fish, and energy affects quantitative requirements for protein (Wilson 2002). In the present study, isoenergetic experimental diets were formulated by adjusting levels of fish and soybean meals with maize meal, wheat bran and fish oil. The protein to energy (P/E) ratio also may influence the dietary protein requirements. Therefore, further study is needed to evaluate the response of Heterotis fingerlings to diets containing various protein and lipid levels, in order to establish the optimal dietary protein to energy ratio and to determine the maximum inclusion level of dietary lipid to spare protein for growth. Numerous investigations were conducted to elucidate the protein-sparing effect of many fish species (Hillestad & Johnsen 1994; Thoman *et al*. 1999; De Silva *et al*. 2002; Lee *et al*. 2002; Espinós *et al*. 2003; Meyer & Fracalossi 2004; Kim & Lee 2005).

From 250 g protein kg^{-1} to 300-350 g protein kg^{-1} diet, statistical analysis on juveniles of 3 to 15 g indicated a significant increase in weight gain and specific growth rate with increasing levels of dietary protein. Above this value, we observed a plateau in the growth rate. A similar trend was revealed in juveniles of 26 to 62 g, except that growth decreased above diet containing 350 g protein kg^{-1} diet. Investigations with other fish species have reported the same kind of growth profile, fish growth either attaining a maximum output (Shiau & Huang 1989; Al Hafedh 1999; Lee *et al*. 2001; Kim *et al*. 2001; Yang *et al*. 2002; Giri *et al*. 2003; Islam & Tanaka 2004; Meyer & Fracalossi 2004) or decreasing (Henken *et al*. 1986; Yang *et al*. 2003; Ng *et al*. 2001) above a plateau varying from species to species.

In juveniles with mean body weight varying from 9 g to 44 g, SGR values obtained in this study are 3.13. These growth rates are higher than those reported in other young omnivorous species such as Nile tilapia (0.49-0.75 according to Al Hafedh (1999) and 1.90-2.20 according to Abou *et al*. (2007)) and jundia (1.84-2.61) according to Meyer & Fracalossi (2004). However, these values are in the same order of magnitude as those reported for juveniles of African catfish *C gariepinus* (2.17-3.10) according to Nyina-wamwiza *et al*. (2007). The SGR values obtained in this study are very much higher than those reported on the same species by different authors. Actually, SGR values of between 1.36 and 2.77 were reported for juveniles of Heterotis (30 to 50 g) fed with groundnut cake and brewer's grain and 0.87 according to Bard (1960) under unfavourable feeding conditions; values of 0.47 (Okoye & Abubakar 1996) and 1.27 (Omorinkoba *et al.* 1991) have been reported for Heterotis fingerlings of 5 g reared in fertilized ponds and in polyculture with Nile tilapia and African catfish irregularly fed with pellets of 250 g protein kg^{-1} diet. The good result recorded in this study is explained by the use of fish meal and soybean meal as major protein sources.

Nevertheless, Tillon (1959) recorded an SGR value of 3.48 for Heterotis fingerlings (3 to 6 g) fed with cotton seed at 100 $kg.ha^{-1}.day^{-1}$.

Feed efficiency values obtained in this study are 0.96, which are higher than those reported for young tilapia of similar sizes (Al Hafedh 1999). However, these values are in the same order of magnitude as those reported for juveniles of jundia *R quelen* (0.47-0.87) according to Meyer & Fracalossi (2004). On the other hand, feed efficiency values obtained in this study are lower than those reported for fingerlings of African catfish *C gariepinus* of similar sizes (0.85-1.29) according to Nyina-wamwiza *et al*. (2007). From the two experiments, feed efficiency values obtained with Heterotis fingerlings (3-15 g) were slightly lower than those obtained for juveniles (26-62 g). These data suggest that feed efficiency increased with the size of fingerlings in *Heterotis niloticus*. Nonetheless, this hypothesis has to be further studied at other ontogenetic stages of this species.

From 250 g protein kg^{-1} to 300-350 g protein kg^{-1} diet, statistical analysis on juveniles of 26 to 62 g indicated a significant increase in weight gain and feed efficiency with increasing levels of dietary protein. Above this value the weight gain does not significantly decrease, while feed efficiency still increases significantly. A similar trend was observed in fingerlings of 3 to 15 g. This kind of growth and feed efficiency profile obtained in this study can be explained by the decreasing feed intake in fish receiving dietary protein (from 250 to 400 g kg^{-1} diet). Moreover, energy balanced experimental diets were obtained by adjusting levels of fish and soybean meals with maize meal and wheat bran. These data suggest that the amount of nonprotein energy in the feed probably affects intake in Heterotis fingerlings; and then dietary carbohydrate affects diet digestibility and growth in Heterotis fingerlings (3-62 g). In omnivorous fish such as common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and channel catfish (*Ictalurus punctatus*), dietary carbohydrate utilization is more important (Wilson 1994). Therefore, more research is needed to study carbohydrate utilization in Heterotis fingerlings, in order to estimate the maximum dietary starch content acceptable without significant reduction of growth.

The whole-body protein, lipid, moisture and ash contents were not significantly affected by dietary protein levels. Similar results were observed in the carcass composition of Nile tilapia (El-Saidy & Gaber 2005). Other studies in which fish meal was used as main protein source for investigating the dietary protein requirement of fish showed that body ash content was not influenced by the dietary protein level (Shiau & Huang 1989; Yang *et al*. 2002; Yang *et al*. 2003).

3.6 Conclusion

This study is the first controlled nutritional research in *Heterotis niloticus*, a promising species for Sub Saharan Africa aquaculture exploitation. The results of the present study indicate that the maximum growth of Heterotis fingerlings (3-62 g) was achieved at about 345 g protein kg^{-1} diet when fish and soybean meals were used as the major sources of protein. Using the broken line model, the dietary protein requirement for *Heterotis niloticus* fingerlings (3-62 g) was estimated to be 310 g kg^{-1} diet.

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Nous venons d'examiner l'influence de différents aliments ayant des teneurs graduelles en protéines sur la croissance et la composition corporelle des juvéniles de *Heterotis niloticus* à 2 tailles différentes (3-15 g et 26-62 g). Ceci nous a permis d'estimer avec le modèle de la ligne brisée, sur base de résultats chiffrés, les besoins quantitatifs en protéines de cette espèce pour une croissance optimale et maximale dans la tranche de poids étudiée. Cependant en nutrition des poissons, les besoins quantitatifs en protéines sont certes importants pour formuler les aliments piscicoles, mais ils ne nous renseignent pas sur la qualité des protéines. La meilleure indication à ce propos est fournie par les acides aminés, surtout les acides aminés indispensables. Ainsi, la connaissance des besoins spécifiques d'une espèce en ces acides aminés est également importante pour sa croissance et sa bonne santé ; raison pour laquelle, la détermination des besoins en acides aminés indispensables succède logiquement à celle des besoins quantitatifs en protéines. La démarche poursuivie dans la section suivante, est donc d'analyser le profil corporel en acides aminés de *Heterotis niloticus* à différents stades ontogénétiques, et d'estimer ses besoins spécifiques en acides aminés indispensables.

Egg and whole body amino acid profile of African bonytongue (*Heterotis niloticus*) with an estimation of their dietary indispensable amino acids requirements

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4.1 Abstract

African bonytongue, *Heterotis niloticus* is a river fish from the Central and West Africa basin. The species presents a great potential for fish farming and has been increasingly raised in Central and South Cameroon. The total amino acid and proximate composition of the whole body of egg, larva, juvenile, immature and adult Heterotis were determined. Ash, moisture, whole-body protein and lipid contents were significantly affected by size ($P<0.05$). On the other hand, the amino acid composition of the whole body tissue, when expressed as a percentage of dietary protein, was not significantly different among ontogenetic stages (ranging from 0.2 g to 400 g mean body mass). The amino acid composition of the eggs was quite different to the one of whole body tissue with lower levels of methionine, proline, and glycine, and higher levels of arginine, histidine, isoleucine, leucine, threonine, valine, serine, and alanine. The A/E ratios of adult Heterotis muscle tissue are similar to those obtained for other fish species, except for histidine and tryptophan. Based on whole body or muscle tissue indispensable amino acids (IAA) to A/E ratios, the IAA requirement profiles for Heterotis (from larva to adult) were estimated and are similar to those of other omnivorous fish species, except for tryptophan and histidine.

KEY WORDS: *Heterotis niloticus*, amino acid composition, indispensable amino acid requirements, A/E ratios, whole body composition

4.2 Introduction

Proteins are indispensable nutrients for the structure and function of all living organisms, including fishes. Inadequate intake of protein results in a reduction or cessation of growth and a loss of weight due to withdrawal of protein from less vital tissues to maintain the function of more vital ones (Wilson 2002). However, if too much dietary protein is supplied, only part of it will be used to synthesize new tissues and the remainder will be converted into energy (Shiau 2002).

Fish do not have a true protein requirement but have a requirement for a well-balanced mixture of indispensable and non-indispensable amino acids. Actually, the requirement for indispensable amino acids (IAA) is important for the proper growth, development and health of fish. Although crude protein values are important for formulating fish diet, they may not allow the determination of protein quality. Thus, the balance of the indispensable amino acids is also fundamental since fish have quantitative requirements for each IAA.

Numerous investigators have determined the quantitative amino acid requirements for several fish species (Klein and Halver 1970; Nose 1979; Wilson et al. 1980; Santiago and Lovell 1988; Akiyama and Arai 1993; Borlongan and Coloso 1993). Most of the amino acid requirement values have been estimated based on the dose-response studies, which are costly and time consuming, especially when determining the requirement for all essential amino acids (Akiyama et al. 1997). Other authors used amino acid composition of the whole-body fish tissue or fish eggs to estimate the requirement data for the corresponding fish species (Moon and Gatlin 1991; Forster and Ogata 1998; Kaushik 1998; Meyer and Fracalossi 2005; Gurure et al. 2007). This method is less costly and constitutes a fast alternative when compared to amino acid dose-response experiments (Akiyama et al. 1997) and has been suggested for fish species whose IAA requirements have not yet been determined.

Heterotis niloticus (Cuvier, 1829) is a potential candidate for Sub Saharan Africa aquaculture since 1950 (Lemasson 1957; Tillon 1957, 1959; Mvogo 1962; Olaniyan and Zwilling 1963; Reizer 1964; Rakotomanampison 1966), but the studies aiming to estimate its nutritional requirements at different ontogenetic stages started recently (Monentcham et al. 2008). Heterotis is an omnivorous freshwater species usually found in big rivers and lakes of the Nilo-Sudanian area, Central and West Africa (D'Aubenton 1955; Daget 1957; Moreau 1982; Levêque et al. 1990, Li and Wilson 1996; Mbega 2004; Adite et al. 2005). This species has attracted a great interest of scientist and fish farmers because of its rapid growth, late sexual maturity, short food

chain, high market price and good meat quality (smoked or salted). There are few studies on the nutrient requirements of this species (Monentcham et al. 2008). So far the quantitative requirements for any of all ten indispensable amino acids are still unknown for Heterotis.

The present study was designed first to analyse the egg and the whole body amino acid composition at different Heterotis ontogenetic stages, and then to estimate the theoretical IAA requirements for larva, juvenile, immature and adult Heterotis.

4.3 Materials and methods

4.3.1 Sample collection and preparation

Samples of eggs and various sizes of Heterotis were collected in Cameroon basin river (Nyong river, 3°47'N and 12°15'E) between June and December 2007. The fish collected were classified into four categories (Table 4.1), randomly sampled and killed. The intestines were removed and the gut contents were flushed out for analysis prior to homogenization of the total carcass with a food mixer. Eggs, pooled tissue and fish samples were stored at – 20°C until analysis, then freeze-dried and rehomogenized to a fine powder prior to amino acid analysis.

Laying-eggs (9), larvae (6000), fingerlings (34), juvenile (20), immature (12) and adult (10) were randomly selected for amino acid and body composition analysis, respectively.

4.3.2 Analytical procedures

Proximate composition of eggs and fish were analysed following the Association of Official Analytical Chemists methods (AOAC 1999). Crude protein (total nitrogen x 6.25) was determined using the Kjeldahl method, after acid digestion. Total lipid content was estimated using the Soxhlet apparatus method according to Folch et al. (1957). Moisture was determined by drying the sample at 105°C for 24h to a constant weight. Ash was determined by incinerating the dried sample in a muffle furnace at 550°C for 12h.

Table 4.1: Categories of Heterotis used to determine whole body or muscle tissue amino acid composition.

Fish stage	Weight class (g)	Number of fish or laying-egg collected	Previous diet
Egg	-	13	-
Post yolk-sac larva	0.1 – 0.3	7500	Natural[1]
Fingerling	2 - 4	48	Natural[1]
Juvenile	50 - 60	33	Natural[1]
Immature	350 - 450	21	Natural[2]
Adult[3]	4000 - 5000	14	Natural[2]

[1]Exclusively zooplankton and phytoplankton (Daget, 1957; Lauzanne, 1976; Micha, 1976; Durand and Levêque, 1981; Moreau, 1982)
[2]Aquatics insects, fishes, shrimps, seeds and small benthic organisms (Fagade et Olaniyan, 1973; Lowe-McConnell, 1975; Lauzanne, 1976; Hickley and Bayley, 1987; Adite et al., 2005)
[3]Except for adult (AA analysis based on muscle tissue), AA analysis based on whole body

Total amino acid profiles of eggs, tissue and whole body were determined according to the adapted method of the European Commission (Commission Directive 98/64/EC of 3 September 1998). The sample (100 mg) was hydrolysed in 10 ml 6 N HCl (+ 0.1 % phenol) at 110°C for 22 h. Amino acids were analysed in automatic amino acid analyser (Biochrom 20 plus). Sulfur amino acids were established after performic acid oxidation before acid hydrolysis. Tryptophan was performed after alkaline hydrolysis, using high-performance liquid chromatography (HPLC) techniques (X Terra RP18, 4.6 x 150, 3.5µm, Column Waters, Made in Ireland) following the procedures described by Fontaine et al. (1998).

4.3.3 Data processing and statistical analysis

In order to compare the amino acid composition of Heterotis muscle tissue to those from other fish species, and to estimate their IAA requirements at different ontogenetic stages, the concentration of each specific indispensable

amino acid was expressed relative to the total indispensable amino acid content of the sample following the formula after Arai (1981):

A/E ratio = [(indispensable amino acid content x (total indispensable amino acid content including cysteine and tyrosine)$^{-1}$) x 1000].

Since no data on the lysine requirement are available for Heterotis, the mean value of the lysine requirements for Nile tilapia 5.1 g/16 g N (Santiago and Lovell 1988) and Channel catfish 5.1 g/16 g N (NRC 1993) was used to calculate requirements for all other IAA in Heterotis using the A/E ratio of whole body and muscle tissue determined in this study as:

IAA requirement = (requirement for Lysine x specific A/E) (A/E for lysine)$^{-1}$

For adult Heterotis, another estimated IAA requirements values were obtained using a formula similar to that described by Kaushik (1998) and Meyer and Fracalossi (2005):

IAA need = [(individual IAA content in Heterotis muscle tissue) x (average Sum IAA requirement among Common carp, Nile tilapia and Channel catfish)] / (Sum IAA Heterotis muscle tissue).

Amino acid analyses were performed in duplicate on each sample (two samples per fish stage). Data for individual amino acids are expressed as mean ± SD. Data were analysed to one-way analysis of variance ANOVA and when significant, differences between means were tested using least significance difference LSD (Dagnelie 1975).

4.4 Results and Discussion

Crude protein, total lipid, ash and moisture of Heterotis eggs, larva, fingerling and adult, respectively, are shown in Table 4.2. Ash, moisture, whole-body protein and lipid contents were significantly varied with the developmental stage ($P<0.05$).

The whole body or egg amino acid composition determined for all groups of Heterotis are shown in Table 4.3. There were no significant differences in the amino acid composition of the whole body tissue from the different ontogenetic stages of Heterotis. These data suggest that the whole body amino acid composition of African bonytongue is not affected by body size, at least within the size ranging from larva (0.2 g, mean body mass) to immature fish (400 g, mean body mass) analysed in this study. Similar results were obtained with other fish species as Siberian sturgeon *Acipenser baeri* (Kaushik 1991), European seabass *Dicentrarchus labrax*,

gilthead seabream *Sparus aurata* and turbot *Psetta maxima* (Kaushik 1998). The data obtained in this study also are in agreement with data from Channel catfish *Ictalurus punctatus* (30-863 g), for which Wilson and Poe (1985) reported that the amino acid composition of the whole body tissue should not change with increasing size of the fish.

Table 4.2: Proximate composition of egg and whole body for four different stages of *Heterotis niloticus*

Ontogenetic stage	**Composition**[4] (% dry matter)			
	Moisture	**Protein**	**Total lipid**	**Ash**
Egg	59.8 ± 0.8^{a}	22.2 ± 0.7^{d}	8.2 ± 1.2^{d}	1.7 ± 0.1^{a}
Larva (0.2 g, b.m.)	86.6 ± 0.8^{e}	9.3 ± 0.4^{a}	1.0 ± 0.2^{a}	1.8 ± 0.1^{a}
Juveniles (3 g, b.m.)	84.3 ± 0.7^{d}	10.7 ± 0.4^{a}	0.7 ± 0.1^{a}	2.6 ± 0.1^{a}
Juveniles (55 g, b.m.)	76.4 ± 0.4^{c}	13.6 ± 0.5^{b}	2.4 ± 0.3^{b}	4.1 ± 0.1^{b}
Immature (400 g, b.m.)	72.9 ± 1.1^{b}	16.3 ± 0.6^{c}	4.6 ± 0.2^{c}	4.6 ± 0.1^{b}

[4] Values are mean ± SD of two replicates. Means in a row with different superscript letters are significantly different ($P<0.05$).
[5] b.m. = body mass

The amino acid composition of the eggs was quite different to the whole body tissue with lower levels of methionine, proline, and glycine, and higher levels of arginine, histidine, isoleucine, leucine, threonine, valine, serine, and alanine. Similar trends were observed in channel catfish, *Ictalurus punctatus* (Wilson and Poe 1985). Other authors have also reported differences between the amino acid composition of eggs and whole body tissue in other fishes (Arai 1981; Ketola 1982; Ogata et al. 1983).

The indispensable amino acid composition, A/E of adult Heterotis muscle tissue and of other fish species are shown in Table 4.4. The A/E ratios of Heterotis muscle tissue are similar to those obtained for other omnivorous and carnivorous fish species, except for histidine and tryptophan (Table 4.4). Investigations with jundia *Rhamdia quelen* have reported the same similarities between the amino acid profile of this species and other fish

species, except in this case for tryptophan and methionine+cysteine (Meyer and Fracalossi 2005). Tryptophan and histidine values obtained in our study are lower and higher, respectively, than those reported for other fish species. Histidine and particularly tryptophan are largely required for synthesis of other compounds besides muscle protein (NRC 1993). Several deficiency signs resulting from tryptophan deficiency observed in salmonids were scoliosis and lordosis (Halver and Shanks 1960; Kloppel and Post 1975; Poston and Rumsey 1983; Walton et al. 1984; Akiyama et al. 1985), renal calcinosis (Kloppel and Post 1975), caudal fin erosion, cataracts and short gill opercula (Poston and Rumsey 1983), and increased liver and kidney levels of calcium, magnesium, sodium and potassium (Walton et al. 1984). Therefore, further study is needed to confirm the estimated A/E ratios of tryptophan and histidine obtained in our study.

Table 4.3: Amino acid composition of eggs or whole-body from different developmental stages of *Heterotis niloticus*

	Composition (% protein)									
	Egg		**Larva** (0.2 g, b.m.)		**Juvenile** (3 g, b.m.)		**Fingerling** (55 g, b.m.)		**Immature** (400 g, b.m.)	
Amino acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Indispensable										
Arginine	7.99	0.01	6.62	0.23	6.82	0.35	7.32	0.36	7.45	0.04
Histidine	5.07	0.03	3.46	0.25	3.56	0.18	3.76	0.24	4.23	0.39
Isoleucine	6.97	0.26	4.91	0.14	4.82	0.64	5.29	0.71	5.23	0.57
Leucine	9.35	0.06	7.77	0.04	7.48	0.11	8.03	1.09	7.97	0.41
Lysine	9.39	0.41	8.57	0.07	8.39	0.95	9.09	1.26	9.14	0.24
Methionine	3.02	0.44	2.83	0.54	3.50	0.23	3.72	0.52	3.53	0.35
Phenylalanine	3.68	0.52	3.42	0.05	3.23	0.60	3.28	0.19	3.34	0.22
Threonine	6.06	0.18	5.27	0.33	5.05	0.28	5.46	0.72	5.35	0.15
Tryptophan	1.05	0.12	0.89	0.06	0.89	0.11	0.75	0.15	0.92	0.10
Valine	7.87	0.46	6.06	0.08	5.74	0.44	6.28	0.82	5.90	0.49
Non-indispensable										
Serine	7.38	0.01	5.24	0.30	5.04	0.28	5.41	0.73	5.02	0.14
Glutamic acid	13.19	0.22	12.93	0.24	12.65	0.59	13.86	0.62	13.47	0.11
Aspartic acid	9.41	0.07	9.06	0.20	8.79	0.48	9.50	0.37	9.39	0.26
Proline	4.22	0.55	4.46	0.46	5.03	0.69	5.83	0.12	5.08	0.23
Glycine	4.35	0.28	5.97	0.36	6.55	0.70	7.47	0.46	6.54	0.27
Alanine	7.91	0.48	5.83	0.41	5.97	0.43	6.70	0.54	6.27	0.58
Cysteine	1.09	0.06	0.83	0.08	0.80	0.13	0.70	0.09	0.85	0.14
Tyrosine	4.66	0.31	4.86	0.42	4.86	0.39	5.02	0.01	4.91	0.51

Table 4.4: A/E ratios of Heterotis adult muscle tissue and of other omnivorous and carnivorous fish species

Indispensable amino acid	Heterotis		Omnivorous: Channel catfish[2, 3]	Omnivorous: Nile tilapia[4, 5]	Omnivorous: Jundia[3, 6]	Carnivorous: Seabass[5, 7]	Carnivorous: Seabream[5, 7]	Carnivorous: Rainbow trout[5, 8]
	Comp.[1,3]	A/E	A/E ratios					
Lysine	9.51	154.7	168.0	160.2	180.2	153.6	149.6	176.3
Arginine	7.42	120.7	132.0	142.0	115.5	146.6	162.4	125.5
Histidine	4.85	78.9	43.0	44.0	40.8	49.8	49.9	57.4
Isoleucine	5.54	90.1	85.0	93.6	78.8	84.0	78.9	83.8
Leucine	8.4	136.6	146.0	160.0	156.1	138.8	134.3	146.6
Met + Cys	4.7	76.4	75.0	72.1	96.4	72.5	73.1	52.8
Phe + Tyr	8.75	142.3	147.0	129.2	148.3	83.4	86.4	149.3
Threonine	5.54	90.1	87.0	89.3	93.2	86.3	84.8	94.0
Tryptophan	0.63	10.2	15.0	18.3	8.4	19.4	18.5	17.7
Valine	6.15	100.0	102.0	91.3	82.4	91.2	88.5	96.7

[1]Amino acid composition of Heterotis adult (% protein); [2]Wilson and Poe, 1985; [3]Muscle tissue composition
[4]Portz (2001) in Meyer and Fracalossi, 2005; [5]Whole body composition; [6]Meyer and Fracalossi, 2005
[7]Kaushik, 1998; [8]Wilson and Cowey, 1985.

Considering the importance of Heterotis as a promising species for African aquaculture, it is necessary to determine their amino acid requirements. So, an estimation of the indispensable amino acid requirements of *Heterotis niloticus* in this study was done by using the correlation reported between the indispensable amino acid requirements of certain fish and the indispensable amino acid pattern of the whole body composition of that fish (Arai 1981; Ogata et al. 1983; Cowey and Tacon 1983; Wilson and Poe 1985; Mambrini and Kaushik 1995). Among the techniques used to determine amino acid requirement of fish species, this approach seems to be the fastest and the most cost effective.

As shown in Tables 4.3 and 4.4, lysine is the most abundant indispensable amino acid in the whole body and the muscle tissue. On the basis of data obtained in this study, A/E ratios and theoretical requirement values of indispensable amino acid were calculated for Heterotis larva, juvenile, immature and adult (Table 4.5). The indispensable amino acid requirements obtained in our study show considerable similarity at different Heterotis ontogenetic stages (from larva to adult). These results suggest that the amino acid needs, when expressed as a percentage of dietary protein, may not be affected by body size in *Heterotis niloticus*.

Another estimation of indispensable amino acid requirements for Heterotis adult was done using a formula similar to that described by Meyer and Fracalossi (2005). This methodology used the amino acid requirements for other freshwater omnivorous fish species in the calculations. The data obtained are presented in Table 4.6. These values are very similar to those reported in Table 4.5. The tryptophan values obtained in this study were lower than those reported for other omnivorous fish species. On the other hand, histidine values were higher. These data indicate that indispensable amino acids requirements of Heterotis are similar to those of other omnivorous fish species, except for tryptophan and histidine. Numerous investigations were conducted to determine the lysine requirement value by growth studies in various fish species, such as Japanese flounder *Paralichthys olivaceus* (Forster and Ogata 1998), Large yellow croaker *Pseudosciaaena crocea R.* (Zhang et al. 2008) and Cobia *Rachycentron canadum* (Zhou et al. 2007). Therefore, further research is needed to determine the lysine requirement of Heterotis through dose-response experiments.

Table 4.5: An assessment of indispensable amino acid requirements for *Heterotis niloticus*

Indispensable amino acid	Larva (0.2 g)		Fingerlings (2-60 g)		Immature (400 g)		Adult (4-5 kg)	
	A/E	Requirements	A/E	Requirements	A/E	Requirements	A/E	Requirements
Lysine	154.4	5.0	153.5	5.0	155.4	5.0	154.7	5.0
Arginine	119.3	3.9	124.2	4.0	126.7	4.1	120.7	3.9
Histidine	62.4	2.0	64.3	2.1	71.9	2.3	78.9	2.5
Isoleucine	88.5	2.9	88.8	2.9	88.9	2.9	90.1	2.9
Leucine	140.0	4.5	136.2	4.4	135.5	4.4	136.6	4.4
Met + Cys	66.0	2.1	76.6	2.5	74.5	2.4	76.4	2.5
Phe + Tyr	149.2	4.8	144.0	4.7	140.3	4.5	142.3	4.6
Threonine	95.0	3.1	92.3	3.0	91.0	2.9	90.1	2.9
Tryptophan	16.0	0.5	14.4	0.5	15.6	0.5	10.2	0.3
Valine	109.2	3.5	105.6	3.4	100.3	3.2	100.0	3.2

Table 4.6: Amino acid requirements for Common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and Channel catfish (*Ictalurus punctatus*), and another estimation of indispensable amino acid requirements for *Heterotis niloticus*

Indispensable amino acid	Requirements (% of dietary protein)			Heterotis muscle tissue composition	Heterotis estimated requirement
	Common carp[1]	Nile tilapia[2]	Channel catfish[3]		
Lysine	6.0	5.1	5.1	9.51	5.1
Arginine	4.4	4.2	4.3	7.42	4.0
Histidine	1.5	1.7	1.5	4.85	2.6
Isoleucine	2.6	3.1	2.6	5.54	3.0
Leucine	4.8	3.4	3.5	8.40	4.5
Met + Cys	2.7	3.2	2.3	4.70	2.5
Phe + Tyr	5.7	5.5	5.3	8.75	4.7
Threonine	3.8	3.8	2.0	5.54	3.0
Tryptophan	0.8	1.0	0.5	0.63	0.3
Valine	3.4	2.8	3.0	6.15	3.3
Sum IAA	35.7	33.8	30.1	61.49	

[1]Ogino (1980)
[2]Data resulting from growth experiments (Santiago and Lovell, 1988)
[3]NRC (1993)

4.5 Conclusion

This study is the first prediction of dietary requirements for indispensable amino acids of *Heterotis niloticus*, a promising species for Sub Saharan Africa fish farming. The results of the present study indicate a lower and higher requirement for tryptophan and histidine, respectively, compared with other omnivorous fish species. Until data from dose-response experiments are available for Heterotis, the estimated values proposed in this study can be

used when formulating experimental and practical diets for *Heterotis niloticus*.

4.6 References

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Les apports émergents des sections précédentes sont les données nutritionnelles de base nécessaires à la formulation des aliments pratiques et expérimentaux couvrant les besoins spécifiques des juvéniles de *Heterotis niloticus* en protéines et en acides aminés indispensables. Les deux dernières investigations de cette étude ont été menées en se basant sur ces résultats. Compte tenu de l'importance du rapport protéine/énergie dans l'optimisation de la rétention azotée et la réduction des coûts de l'aliment chez les poissons, nous avons cherché à évaluer la capacité des juvéniles de cette espèce à épargner les protéines alimentaires par une utilisation judicieuse d'une source d'énergie non-protéique en l'occurrence les lipides (Section 5). La farine de poissons, fréquemment utilisée dans les expériences des sections 3 et 5, est un ingrédient onéreux, difficilement accessible dans les pays du Sud et en voie de raréfaction. C'est conscient de cela que la section 6 étudie la possibilité de la remplacer par des sous-produits oléagineux (disponibles localement et moins chères) dans le régime des juvéniles de ce poisson. Dans cette optique, nous avons substitué partiellement la farine de poissons par un mélange de tourteaux de soja et de coton.

5

RAPPORT PROTEINE/ENERGIE CHEZ LES JUVENILES DE Heterotis niloticus

Growth, feed utilization and body composition of African bonytongue, *Heterotis niloticus* fingerlings fed diets containing various protein and lipid levels

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5.1 Abstract

In order to evaluate the effects of dietary protein and lipid levels on growth, feed utilization and body composition of Heterotis fingerlings, a factorial experiment with three replicates was conducted. Six experimental diets containing three crude protein levels (28%, 32% and 36%) and two crude lipid levels (6% and 13%) were tested. Heterotis fingerlings weighing 2.34 g initial live weight were fed the experimental diets to apparent satiation, twice a day. After 8 weeks, statistical analysis revealed that weight gain, specific growth rate, feed efficiency and protein retention were significantly affected by dietary protein and dietary lipid levels, respectively ($P<0.01$). The highest weight gain, specific growth rate and feed efficiency were observed for juvenile fed the diet containing 36% protein and 6% lipid, but no significance difference was found between groups fed with the following diets: P28L13 (28% protein and 13% lipid), P32L6, P32L13, and P36L13. A significant interaction between dietary protein and lipid was observed for weight gain, specific growth rate, feed efficiency and protein retention. The whole-body protein, lipid, moisture and ash content were not significantly affected by dietary lipid levels, but body protein and lipid content were significantly affected by dietary protein. The dietary protein-sparing effect was clearly demonstrated when the dietary energy of lipid increases from 17 to 19.6 kJ g^{-1} at 28% crude protein on Heterotis.

KEY WORDS: *Heterotis niloticus*, protein, lipid, P/E ratio, growth, feed utilization

5.2 Introduction

African bonytongue, *Heterotis niloticus* (Cuvier, 1829), is an omnivorous freshwater fish well appreciated for human consumption in Central and West Africa. It is found in some lakes like Lake Chad and in large rivers (Senegal, Gambia, Niger and Nile) (D'Aubenton 1955; Levêque, Paugy & Teugels 1990). The rapid growth, late sexual maturity, short food chain and good meat quality of this species make it an excellent candidate for inland aquaculture in Africa. Recently, Heterotis has been successfully cultured in earthen ponds under a semi-intensive polyculture with *Oreochromis niloticus* (Linnaeus), *Clarias gariepinus* (Burchell) and *Cyprinus carpio* (Linnaeus), although no specific technique has been developed to raise this species. Individual mean body weight of 3 to 4 kg within a twelve-month cycle is very encouraging. Commercial feedstuffs, formulated to satisfy the nutritional requirements of other warmwater species such as Nile tilapia and African catfish have been used for Heterotis raised in ponds. Aside the control of reproduction and larval rearing, the limited knowledge on the nutritional requirements of Heterotis has become an obstacle for further development of its aquaculture production.

It is well known that formulation of balanced diets and their adequate feeding are important for successful aquaculture (Pillay 1990). Protein is one of the major dietary macronutrient affecting weight gain of fish and feed cost (Lovell 1989). Dietary energy has a major impact on the utilization of dietary protein in fish, and energy affects quantitative requirements for protein (Wilson 2002). Too little energy in the diet will result in the utilization of dietary protein for energy rather than for protein synthesis, which is economically wasteful. On the contrary, excess energy in the diets may result in a lower nutrient intake by the fish, due to lower feed consumption (Metailler, Aldrin, Messager, Mevel & Stephan 1981; Alsted & Jokumsen 1989); thus, the fish will not consume enough diet to meet their protein and other nutrient requirements, leading to reduced growth rate on the one hand, or in the other hand in fatty fish (Webster & Lim 2002). In addition, energy yielding nutrients, like lipids and carbohydrates, can theoretically reduce the oxidation of protein to energy and hence improve the utilization of dietary protein for growth. Therefore, providing the appropriate energy levels in feeds for fish is essential.

The dietary protein requirement for *H. niloticus* juveniles (3-62 g) was estimated to be 310 g kg^{-1} diet when diets containing protein levels from 250 to 400 g kg^{-1} with isoenergetic (17 $kJ.g^{-1}$ diet) were fed to fish twice a day to apparent satiation (Monentcham, Pouomogne & Kestemont 2008). Numerous investigations were conducted to evaluate the response of several fish species to diets containing various protein and lipid levels (Hillestad &

Johnsen 1994; Thoman, Davis & Connie 1999; De Silva, Gunasekera, Collins & Ingram 2002; Lee, Kim & Cho 2002; Espinós, Tomás, Pérez, Balasch & Jover 2003; Meyer & Fracalossi 2004; Kim & Lee 2005), but information on the effects of dietary protein and lipid on growth performances of *H. niloticus* fingerlings is lacking.

The present research was designed to assess the effects of dietary protein and lipid levels on growth, feed utilization and body composition of Heterotis fingerlings.

5.3 Materials and methods

5.3.1 Rearing conditions

The trial was conducted in hapas that were constructed with nets of fine texture. These hapas with rectangular dimensions (1.3m x 0.75m x 1.1m; vol. = 780 L) were placed in a rectangular fish pond (600 m², 1.2m deep) located at the Government Aquaculture Station in Melen (Yaounde, Cameroon). The pond was free from aquatic vegetation, completely independent, well exposed to sunlight and with a water inlet and outlet system designed to maintain the water level in the hapas at 0.8m for the duration of the experiment. During the feeding trial, fingerlings were exposed to natural photoperiod (6.15 a.m. to 6.45 p.m. daylight followed by night). Water temperature ranged from 26 to 33°C while pH ranged from 6.5 to 7.0. The experiment lasted 56 days, from October to December 2007.

5.3.2 Experimental fish

Heterotis fingerlings weighing <1 g were caught in Nyong River (3°47'N and 12°15'E) near the town of Akonolinga (Cameroon), and transported to the Melen aquaculture station where they were stored for several weeks in a fertilized fish pond. After this phase, 50 fish were randomly distributed into each hapa and fed diet containing 30% dietary protein during a pre-experimental period for two weeks. After this conditioning period, 50 fish were randomly sampled and individually weighed (initial mean weight: 2.34 ± 0.77 g) and 30 fish were redistributed into each hapa. At the end of the experiment, all fish were individually weighed and total length measured.

5.3.3 Diet formulation, preparation and feeding

Fish and soybean meals were used as the main protein source. Maize meal, wheat bran, Menhaden fish and palm oil were used as the primary

carbohydrate and lipid sources, respectively. Fish meal was obtained from Nutreco International BV (Boxmeer, The Netherlands). Except fish oil, other ingredients were obtained from local markets. Ingredient formulations are shown in Table 5.1. A 3 x 2 factorial experimental design with three replicates was adopted. Six experimental diets were formulated to contain three levels of protein (280, 320 and 360 g kg^{-1} diet) and two levels of lipid (60 and 130 g kg^{-1} diet) (Table 5.2). Gross energy levels of the diets were calculated based on 23.7, 39.5 and 17.2 kJ g^{-1} for protein, lipid and nitrogen-free extract, respectively (Guillaume, Kaushik, Bergot & Metailler 1999).

Soybean meal was boiled in a pressure cooker for two hours followed by sun drying. The different experimental diets were made by adding appropriate volumes of water to ingredients. The resulting paste was transformed into spaghetti (2mm) with the aid of food blender (Kenwood KM 800, Havant, UK). After sun drying at a temperature of 28-35°C for about three days, the spaghetti were manually broken and converted into pellets. The pellets were stored at -20°C until use.

Table 5.1: Ingredients and composition of the experimental diets (g kg^{-1})

	Protein levels (g kg^{-1})					
	280		320		360	
Lipid levels (g kg^{-1})	60	130	60	130	60	130
Ingredients						
Fish meal	185	185	226	226	266	266
Soybean meal	185	185	226	226	266	266
Whole maize meal	275	230	239	189	201	154
Wheat bran	275	230	239	189	201	154
Menhaden Fish oil[1]	15	50	10	50	8	45
Palm oil	15	50	10	50	8	45
Vitamin premix[2]	20	20	20	20	20	20
Mineral premix[3]	20	20	20	20	20	20
Carboxymethylcellulose[4]	10	10	10	10	10	10

[1,4]Sigma-Aldrich products (Bornem, Belgium)
[2]Vitamin Mix Fish 0.5% INVE Aquaculture, Belgium (Composition per kg: vitamin A, 2 500 000 IU; vitamin D3, 500 000 IU; vitamin E, 30 000 mg; vitamin K3, 2 000 mg; vitamin B1, 2 000 mg; vitamin B2, 5 000 mg; panthotenic acid, 10 000 mg; Niacin, 5 000 mg; vitamin B6, 4 000 mg; folic acid, 2 000 mg; vitamin B12, 4 mg; vitamin C, 20 000 mg; biotin, 200 mg and inositol, 80 000 mg)
[3]Mineral Mix MLNP 763, INRA Belgium (Composition per kg: dibasic calcium phosphate, 500 g; calcium carbonate, 215 g; sodium chloride, 40 g; potassium chloride, 90 g; magnesium hydroxide, 124 g; iron sulphate, 20 g; zinc sulphate, 4 g; manganese sulphate, 3 g; cobalt sulphate, 0.02 g; potassium iodide, 0.04 g; sodium selenite, 0.03 g and sodium fluoride, 1 g).

Table 5.2: Nutrient contents of the experimental diets

	Protein levels (g kg^{-1})					
	280		320		360	
Lipid levels (g kg^{-1})	60	130	60	130	60	130
Constituents[5]						
Crude protein (g kg^{-1})	284	281	322	321	363	359
Total lipid (g kg^{-1})	60	131	62	133	60	129
Ash (g kg^{-1})	77	75	78	80	83	85
Moisture (‰)	96	88	94	87	92	89
Nitrogen-free extract (g kg^{-1})[6]	483	425	444	379	402	338
Estimated Gross energy (kJ g^{-1})[7]	17.4	19.1	17.7	19.3	17.8	19.4
Protein: energy ratio (g protein MJ^{-1})	16.3	14.7	18.2	16.6	20.3	18.5

[5]Values are the mean of three replicate analyses
[6]Nitrogen-free extract (NFE) calculated as: 1000 – (moisture + protein + lipid + ash)
[7]Estimated Gross energy was calculated according to Guillaume, Kaushik, Bergot & Metailler (1999).

Fish were fed by hand twice a day (09h30 – 10h00 and 14h30 – 15h00) to apparent satiation. Pellets were distributed slowly, allowing all fish to eat. The daily feed supply was recorded.

5.3.4 Sample collection and chemical analysis

Initially, 10 fish were sampled for initial whole-body proximate composition. At the end of the experiment, 9 fish from each treatment were randomly selected for final analysis of body composition. All samples were stored at – 20°C prior to analysis.

Proximate composition of feed ingredients, experimental diets and fish were analysed following the Association of Official Analytical Chemists methods (AOAC 1999). Crude protein (total nitrogen x 6.25) was measured using the Kjeldahl method after acid digestion. Total lipid content was estimated using the Soxhlet apparatus method according to Folch, Lees & Sloane-Stanley (1957). Moisture was determined by drying the sample at 105°C for 24h to a constant weight. Ash was determined by incinerating the dried sample in a muffle furnace at 550°C for 12h.

5.3.5 Data processing and statistical analysis

From these data, final mean weight, specific growth rate (SGR; $\%.day^{-1}$) (100 x (ln final body weight – ln initial body weight))/duration of experiment (days)), weight gain (WG) ((final body weight – initial body weight) (g) x 100/initial body weight (g)), feed efficiency (FE) ((fish weight gain including weight of dead fish) (g)/total feed intake (g)), protein efficiency ratio (PER) ((fish weight gain including weight of dead fish) (g)/total protein intake (g)), protein retention (PR) (100 x (final body weight x final body protein – initial body weight x initial body protein)/total feed intake x dietary protein) were determined. Digestible protein intake and digestible energy intake were estimated by using the means values of apparent digestibility coefficient (ADC) for crude protein and gross energy of fish and soybean meals (the major protein sources in this study) by Heterotis (Fagbenro, 2001). $ADC_{crude\ protein}$ and $ADC_{gross\ energy}$ values are (92.8; 85.1) and (93.5; 77.4) for fish and soybean meals respectively (Fagbenro, 2001).

All percentage data were arcsine transformed before analysis. One-way and two-way analyses of variance (ANOVA) were applied to examine the significance of dietary treatment effects of the two main factors (dietary protein and lipid) and their possible interaction on growth parameters. Differences among means were tested by least significance difference LSD (Dagnelie, 1975). Differences were considered significant at $P<0.05$. The data are presented as mean ± S.D. of three replicate groups.

5.4 Results

Weight gain, specific growth rate, feed efficiency, protein efficiency ratio, protein retention and survival rate of Heterotis fingerlings during the feeding trial are shown in Table 5.3. Survival rates varied from 77% to 82%, independently to the dietary treatments ($P>0.05$). Weight gain, specific growth rate and protein retention were significantly ($P<0.01$) affected by dietary protein and dietary lipid levels. Except for survival rate, differences were significant for all protein x lipid interactions parameters ($P<0.05$).

The highest weight gain and specific growth rate were observed for juveniles fed the diet containing 36% protein and 6% lipid, but values did not differ significantly from those of fish fed the following diets: P28L13 (28% protein and 13% lipid), P32L6, P32L13, and P36L13. However, the weight gain and SGR of this group were significantly ($P<0.01$) higher than those of juveniles fed the diet containing 28% protein and 6% lipid, which recorded the poorest growth. Heterotis fingerlings fed the diet containing 28% protein and 13% lipid displayed the highest protein retention, whereas the lowest values were observed in fish receiving P36L13. Protein retention decreases significantly ($P<0.05$) with increasing levels of dietary protein at high lipid content.

Feed efficiency in all treatments, ranging from 0.90 to 1.16, was significantly affected by both dietary protein and lipid levels ($P<0.01$). The feed efficiency values of fish fed the following diets: P28L13, P32L6, P32L13, P36L6, P36L13 did not differ significantly from each other, but were significantly higher than those fed the diet P28L6. The dietary lipid levels significantly affected the protein efficiency ratio ($P<0.05$), with the highest values recorded in fish fed the diet containing 28% protein and 13% lipid.

Feed intake, digestible protein (DP) intake, digestible energy (DE) intake, protein and energy retention of juvenile Heterotis fed the experimental diets are shown in Table 5.4. The daily feed intake was not significantly ($P>0.05$) affected by both dietary protein and lipid levels. DP intake, protein retention and energy retention were significantly ($P<0.05$) affected by dietary protein level, but not by dietary lipid level. Protein retention efficiency and DE intake were not significantly ($P>0.05$) affected by dietary protein level, but only DE intake differ significantly ($P<0.01$) with dietary lipid level. Energy retention efficiency was significantly ($P<0.05$) affected by dietary protein and dietary lipid levels, respectively.

Table 5.3: Weight gain, feed efficiency and survival performance of *Heterotis niloticus* fingerlings fed with diets containing various protein and lipids levels (initial average weight 2.34 g)

Protein levels (g kg^{-1}diet)	Lipid levels (g kg^{-1}diet)	Final weight (g)	Weight gain (%)	SGR (%.day^{-1})	FE	PER	PR	Survival
280	60	18.1 ± 0.8^{a}	673 ± 11^{a}	3.64 ± 0.07^{a}	0.90 ± 0.02^{a}	3.21 ± 0.11^{a}	169 ± 5^{b}	78 ± 3
	130	21.8 ± 1.1^{b}	833 ± 13^{b}	3.98 ± 0.11^{b}	1.13 ± 0.04^{b}	4.02 ± 0.16^{c}	193 ± 6^{c}	77 ± 6
320	60	21.7 ± 0.6^{b}	832 ± 08^{b}	3.97 ± 0.05^{b}	1.14 ± 0.03^{b}	3.55 ± 0.14^{b}	177 ± 3^{b}	80 ± 3
	130	21.9 ± 0.5^{b}	836 ± 07^{b}	3.98 ± 0.09^{b}	1.15 ± 0.05^{b}	3.54 ± 0.26^{b}	176 ± 4^{b}	82 ± 2
360	60	22.0 ± 0.9^{b}	841 ± 12^{b}	4.00 ± 0.06^{b}	1.16 ± 0.07^{b}	3.61 ± 0.12^{b}	164 ± 5^{ab}	81 ± 5
	130	21.8 ± 0.7^{b}	834 ± 10^{b}	3.98 ± 0.03^{b}	1.15 ± 0.04^{b}	3.59 ± 0.09^{b}	161 ± 2^{a}	80 ± 4
Two-way analysis of variance[8]								
Protein		S	S	S	S	NS	S	NS
Lipid		S	S	S	S	S	S	NS
Protein x lipid		S	S	S	S	S	S	NS

Values (means ± SD of three replicates) in each column with different superscript letters are significantly different ($P<0.05$). [8] S = significant ($P<0.05$); NS = not significant ($P\geq0.05$)

Proximate composition of the whole fish fed diets with various levels of protein and lipid is presented in Table 5.5. The results of two-way analysis of variance showed that moisture content was not significantly ($P>0.05$) affected by dietary protein and lipid levels, respectively. Body protein and lipids content were significantly ($P<0.05$) affected by dietary protein level, but not by dietary lipid ($P>0.05$). The highest values of body lipid, measured in fingerlings fed the diet P36L13, were found to differ significantly ($P<0.05$) compared to fish groups fed the other experimental diets. Neither dietary protein nor lipid level had a significant effect on ash body content ($P>0.05$). There was significant interaction ($P<0.05$) between dietary protein and lipid and fish body composition, except for ash content.

Table 5.4: Daily feed intake, daily protein intake, protein and energy retention of the juvenile Heterotis fed the experimental diets containing various protein and lipid levels

Protein levels (g kg^{-1}diet)	Lipid levels (g kg^{-1}diet)	Feed intake (g/fish/day)	DP intake (g/fish/day)	Protein retention (g/fish/day)	PRE	DE intake (kJ/fish/day)	Energy retention (kJ/fish/day)	ERE
280	60	0.332	0.088^{a}	0.025^{a}	0.281^{a}	4.68^{ab}	1.71^{a}	0.343^{a}
	130	0.325	0.088^{a}	0.033^{b}	0.373^{cd}	5.03^{c}	1.82^{c}	0.377^{bc}
320	60	0.320	0.096^{b}	0.032^{b}	0.337^{bc}	4.59^{a}	1.71^{a}	0.373^{b}
	130	0.322	0.097^{b}	0.031^{b}	0.323^{b}	5.03^{c}	1.89^{c}	0.376^{b}
360	60	0.318	0.109^{c}	0.042^{c}	0.386^{d}	4.58^{a}	1.76^{b}	0.385^{c}
	130	0.317	0.105^{c}	0.030^{b}	0.287^{a}	4.99^{bc}	1.74^{ab}	0.346^{a}
Two-way analysis of variance[9]								
Protein		NS	S	S	NS	NS	S	S
Lipid		NS	NS	NS	NS	S	NS	S
Protein x lipid		NS	NS	S	S	NS	S	S

Values are means ± SD of three replicates. Means in a column with different superscript letters are significantly different ($P<0.05$)

[9] S = significant ($P<0.05$); NS = not significant ($P\geq0.05$); DP digestible protein, PRE protein retention efficiency = protein gain/total DP intake, DE digestible energy, ERE energy retention efficiency = energy gain/total DE intake.

Table 5.5: Proximate composition of *Heterotis niloticus* fingerlings fed diets containing various protein and lipids levels (initial average weight 2.34 g)

Protein levels (g kg^{-1}diet)	Lipid levels (g kg^{-1}diet)	Moisture (%)	Crude protein (%)	Total lipid (%)	Crude ash (%)	Energy (MJ kg^{-1})
280	60	77.9 ± 0.9^{a}	11.99 ± 0.52^{a}	1.32 ± 0.13^{a}	4.3 ± 0.8	4.14^{a}
	130	80.1 ± 0.2^{bc}	12.43 ± 0.11^{b}	1.21 ± 0.32^{a}	4.2 ± 0.1	3.78^{b}
320	60	78.8 ± 0.3^{ac}	12.41 ± 0.15^{b}	1.39 ± 0.25^{a}	3.8 ± 0.7	4.12^{a}
	130	78.2 ± 0.6^{a}	12.35 ± 0.07^{ab}	1.44 ± 0.16^{a}	3.9 ± 0.5	4.22^{a}
360	60	78.7 ± 0.4^{ac}	12.95 ± 0.18^{c}	1.32 ± 0.31^{a}	3.8 ± 0.6	4.14^{a}
	130	79.3 ± 1.2^{ac}	12.29 ± 0.24^{ab}	1.84 ± 0.21^{b}	3.8 ± 0.9	4.13^{a}
Two-way analysis of variance						
Protein		NS	S	S	NS	S
Lipid		NS	NS	NS	NS	NS
Protein x lipid		S	S	S	NS	S

Values are means ± SD of three replicates. Means in a column with different superscript letters are significantly different ($P<0.05$)

5.5 Discussion

Some authors reported high survival rate, 69-96% (Tillon 1959), 83%-100% (Reizer 1966) and 72-88% according to Monentcham *et al*. (2008) for Heterotis juveniles reared in earthen pond or cages. The survival rate values registered in this study, 77%-82%, are of the same order of magnitude. Specific growth rates reported in this research for juveniles Heterotis with mean body weight varying from 2 g to 22 g reached up to 4% day^{-1}. These growth rates are higher than those obtained on the same species for similar sizes such as 3.48% day^{-1} for fish of 3-6 g (Tillon 1959), 3.13% day^{-1} for fish of 9-44 g (Monentcham *et al*. 2008) and 2.77% day^{-1} for fish of larger sizes (ranging from 30 to 50 g, Bard 1960). However, these values are in the same order of magnitude as those reported for fingerlings of African catfish *Clarias gariepinus* (4.14 according to Imorou Toko, Fiogbé & Kestemont 2008).

At 28% dietary protein, statistical analysis indicated a significant increase in weight gain and specific growth rate with increasing levels of dietary lipid. Weight gain and specific growth rate did not increase significantly with 32-36% dietary protein at all levels of the dietary lipid tested (6% and 13%). A similar trend was observed in feed efficiency. The fact that weight gain and specific growth rate, within the protein level of 28%, were higher for juvenile fed the diet containing higher lipid level suggests that the diet provided more energy for the metabolization of protein to muscle tissue. In other words, at low protein level, protein may be utilized for growth rather than for energy in fingerlings submitted to a high dietary lipid level. A protein-sparing effect of dietary energy from lipids was thus clearly expressed in Heterotis juvenile fed 28% crude protein. Therefore, the optimum protein and lipid levels in the diet for growth of Heterotis fingerlings are 280 and 130 g kg^{-1} diet, respectively, with an estimated energy level of 19.1 kJ g^{-1} diet and protein to energy ratio of 14.7 g protein MJ^{-1}.

Monentcham *et al*. (2008) reported that the optimum dietary protein requirement for Heterotis juveniles from 3 to 62 g was 310 g kg^{-1} diet with an estimated energy level of 17 $kJ.g^{-1}$ diet. These two results suggest that the protein requirement of juvenile Heterotis (as a percentage of the diet can be reduced from 31% to 28 % by increasing the dietary energy (due to lipid) from 17 $kJ.g^{-1}$ diet to 19.6 kJ g^{-1} diet. The sparing effect of lipid on dietary protein has been reported for other fish species such as Common dentex *Dentex dentex* (Linnaeus) (Tibaldi, Beraldo, Volpelli & Pinosa 1996; Espinós *et al*. 2003), gilthead seabream *Sparus aurata* (Linnaeus) (Company, Calduch-Giner, Kaushik & Perez-Sanchez 1999; Vergara, Robainá, Izquierdo & de la Higuera 1996), ayu *Plecoglossus altivelis*

(Temminck & Schlegel) (Lee *et al*. 2002), red drum, *Sciaenops ocellatus* (Linnaeus) (Thoman *et al*. 1999), jundia *Rhamdia quelen* (Quoy & Gaimard) (Meyer & Fracalossi 2004), Malabar grouper *Epinephelus malabaricus* (Bloch & Schneider) (Shiau & Lan 1996), Murray cod *Maccullochela peelii peelii* (Mitchell) (De Silva *et al*. 2002), bagrid catfish *Pseudobagrus fulvidraco* (Richardson) (Kim & Lee 2005) and Atlantic salmon *Salmo salar* L. (Hillestad & Johnsen 1994).

In this study, maize meal and wheat bran were used as the primary carbohydrates sources and were the main nonprotein energy source in the experimental diets, except fish and palm oils. According to various authors, lipids are the first nonprotein energy source utilized by fish (Vergara *et al*. 1996; McGoogan & Gatlin 1999). However, many investigations reported that omnivorous species are able to utilize efficiently dietary carbohydrate as an energy source (Nile tilapia according to Ali & Al-Asgah (2001), Channel catfish *Ictalurus punctatus* (Rafinesque) according to NRC (1993) and Rohu *Labeo rohita* (Hamilton) according to Erfanullah (1995)). Therefore, more research is needed to study the effect of different carbohydrate/lipid ratios for Heterotis fingerlings, in order to determine which nonprotein energy source promotes the maximum protein-sparing effect for Heterotis.

Protein efficiency ratio obtained in this study range from 3.21 to 4.02 and tended to improve with the decrease of dietary protein level at high lipid levels. A similar trend was observed in juvenile ayu *P. altivelis* reared in seawater (Lee *et al*. 2002). Other studies reported a comparable result showing that protein efficiency ratio decreased with increase in dietary protein level (Danials & Robinson 1986; Company *et al*. 1999; Lee & Kim 1999; McGoogan & Gatlin 1999; Thoman *et al*. 1999). The highest protein efficiency ratio and protein retention were found in Heterotis fed the diet containing 28% protein and 13% lipid. Nevertheless, a protein sparing effect where supplementation of dietary lipid improves fish performance, protein efficiency ratio and protein retention (as shown by Weatherup, McCracken, Foy, Rice, Mckendry, Mairs & Hoey 1997; McGoogan & Gatlin 1999; Thoman *et al*. 1999; Vergara *et al*. 1996) was not observed in this study at all protein levels. It appears that *H. niloticus* requires relatively low dietary protein and high dietary lipid. As experimental diets did not contain lipid higher than 13% in the present study, more research are needed to revaluate the response of Heterotis fingerlings to diets containing various protein and lipid levels using diets containing higher levels of lipid than 13%, in order to determine the maximum inclusion level of dietary lipid to spare protein for growth.

Investigations with *I. punctatus* (Page & Andrews 1973), rainbow trout *Oncorhynchus mykiss* (Walbaum) *(*Lee & Putnam 1973), Japanese flounders

Paralichthys olivaceus (Temminck & Schlegel) (Lee, Cho & Kim 2000), *P. altivelis* (Lee *et al*. 2002) have clearly shown that feed intake is directly related to dietary energy level. Surprisingly in this study, statistical analysis indicated that daily feed intake remained relatively constant with increasing dietary lipid level for Heterotis fingerlings from 6 to 13%. This suggests again that *H. niloticus* probably requires dietary lipid higher than 13%. Therefore, further study is necessary to confirm this hypothesis.

The whole-body protein, lipid, moisture and ash content were not significantly affected by dietary lipid levels, but body protein and lipid content were significantly affected by dietary protein levels. Crude protein content of juvenile fed low lipid diet tended to be high at the same protein level, except for the juvenile groups fed the diet containing 28% protein. Crude protein content also tended to increase with increasing levels of dietary protein at low lipid diet. Similar results were observed in the carcass composition of ayu (Lee *et al*. 2002).

Body lipid content of fingerlings fed low lipid diet was not significantly different than those of fish fed high lipid diet at 28% dietary protein, but improved weight gain. These suggest that Heterotis fingerlings utilize the diet containing 28% dietary protein and high lipid more efficiently for growth, without body fat deposition.

5.6 Conclusion

This research is among the first in a series of studies on the controlled nutritional investigation in *Heterotis niloticus*, a promising species for inland aquaculture in Africa. The results of the present study indicate that the optimum protein and lipid levels in the diet for growth of Heterotis fingerlings seemed to be 280 and 130 g kg^{-1} diet, respectively, with an estimated energy level of 19.1 MJ kg^{-1} diet and protein to energy ratio of 14.7 g protein MJ^{-1}, when fish were fed to apparent satiation two times daily. The dietary protein-sparing effect was clearly demonstrated when the dietary energy of lipid increases from 17 to 19.6 kJ g^{-1} at 28% crude protein on Heterotis.

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6

VALORISATION DES SOUS-PRODUITS VEGETAUX DANS L'ALIMENTATION DES JUVENILES DE Heterotis niloticus

Partial substitution of fish meal with soybean and cottonseed oilcakes meals in diets for African bonytongue, *Heterotis niloticus* (Cuvier, 1829) fingerlings: effects on growth, feed efficiency and body composition

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6.1 Abstract

A feeding trial was conducted to examine the suitability of plant oil cakes as a partial substitute for the dietary protein supplied by fish meal for Heterotis fingerlings. Fish were fed with four isonitrogenous (350 g kg^{-1} crude protein) and isoenergetic (18.8 kJ g^{-1} GE) diets in which fish meal protein was gradually replaced by plant protein from a mixture of soybean and cottonseed oilcakes meals (0, 25, 50, 75% in diets 1, 2, 3 and 4, respectively). Triplicate groups of juvenile Heterotis (initial body weight of 5 g) were handfed twice daily to apparent satiation for 60 days. Growth performances of fingerlings fed diets containing 0, 25 and 50% plant protein were not significantly different ($P>0.05$). At 75% fish meal substitution, growth and feed utilization efficiency indicators were significantly reduced ($P<0.05$). The carcass composition were also significantly ($P<0.05$) affected by the replacement level of fish meal, except dry matter and ash. Our findings suggest that the dietary fish meal protein could efficiently be substituted by a mixture of soybean and cottonseed oilcakes meals up to 50%, without adverse effects on maximal growth in practical Heterotis fingerlings diets, when fish are fed to apparent satiation two times daily in hapas.

KEY WORDS: *Heterotis niloticus*, fish meal substitution, soybean oilcake meal, cottonseed oilcake meal, growth

6.2 Introduction

The African bonytongue, *Heterotis niloticus* (Cuvier, 1829), is present in large rivers and lakes of the Nilo-Sudanian area, Central and West Africa (Li & Wilson 1996; Mbega 2004; Adite, Winemiller & Fiogbé 2005). It is an excellent candidate for aquaculture production because of its fast growth rate, air-breathing characteristic, omnivorous diet, relatively high market price and good meat quality (smoked or salted) (Monentcham, Kouam, Pouomogne & Kestemont 2009a). A recent study conducted in hapas indicated that the dietary protein level for maximum growth of juvenile Heterotis is 34.5% when fish are fed with practical diets containing from 25 to 40% crude protein (Monentcham, Pouomogne & Kestemont 2008). Another research reported the quantitative indispensable amino acids requirements of the same species (Monentcham, Whatelet, Pouomogne & Kestemont 2009b).

Protein is the most expensive component in fish feed, and fish meal remains the major protein source because of its well-balanced essential amino acid profile, essential fatty acids, digestible energy, vitamins and minerals (Hertrampf & Piedad-Pascual 2000). The global aquaculture demand for fishmeal per year was estimated at 2.09 million tons (in 1999), and predicted to reach nearly 4.6 and 10.4 million tons of fishmeal by 2015 and 2030, respectively (New & Wijkström 2002). The actual annual world production of fish meal (6-7 million tons; FAO 2006) is expected to remain static or decrease slightly in the future. These observations imply that fish meal might constrain aquaculture expansion in the future. Moreover, fish meal is scarce and very expensive in developing countries. In this regard, the use of alternative less-expensive and locally available protein sources such as plant protein feedstuffs is considered an international research priority especially in African aquaculture.

Soybean and cottonseed oilcakes meals are widely incorporated as cheaper alternative protein sources in practical fish feeds because of their high protein content, favourable indispensable amino acid profiles and reasonable price. However these plant by-product meals may be deficient in one or more indispensable amino acids, especially methionine and lysine (Hertrampf & Piedad-Pascual 2000). Numerous studies have been conducted to partially (Shiau, Lin, Yu, Lin & Kwok 1990; Fagbenro & Davies 2001; Chou, Her, Su, Hwang, Wu & Chen 2004; Imorou Toko, Fiogbé & Kestemont 2007; Imorou Toko, Fiogbé & Kestemont 2008) or totally (Webster, Goodgame-Tiu & Tidwell 1995; El-Saidy & Gaber 2004) replace fish meal by soybean or cottonseed oilcakes meals in fish diets without reducing the growth and feed efficiency. The results have shown that soybean or cottonseed oilcakes meals could partially replace fish meal in fish

feeds, or entirely when supplemented with amino acid and fortified with minerals. Furthermore, it is better to replace fishmeal with simple or complex mixtures of vegetable ingredients (El-Saidy & Gaber 2003; Kaushik, Covès, Dutto & Blanc 2004; Borgeson, Racz, Wilkie, White & Drew 2006). It has also been found that the influence of replacing dietary fish meal on growth is species dependent (Hepher 1988). No studies have examined the effects of substituting fish meal with plant proteins in *Heterotis niloticus* feeds. Therefore, the present study was conducted to evaluate the possible effects of partial replacement of fish meal with a mixture of plant proteins (soybean and cottonseed oilcakes meals) on growth, feed efficiency and carcass proximate composition of Heterotis fingerlings.

6.3 Materials and methods

6.3.1 Experimental diets and feeding regime

The formulation and proximate composition of the experimental diets are given in Table 6.1. Four isonitrogenous (350 g kg^{-1} crude protein) and isoenergetic (18.8 kJ g^{-1} GE) diets were formulated to meet the maximum growth of Heterotis fingerlings (Monentcham *et al*. 2008). The mixture of plant ingredients (50% soybean oilcake meal (SBM) and 50% cottonseed oilcake meal (CSM)) was used as partial replacement of fish meal in experimental diets at levels of 25% (diet 2), 50% (diet 3) and 75% (diet 4). Fish meal was obtained from Nutreco International BV (Boxmeer, Netherlands). Except fish oil, other ingredients were procured from the local market (Yaounde, Cameroon). The diets were prepared, pelleted and stored as described by Monentcham *et al*. (2008).

The indispensable amino acid compositions of the experimental diets are shown in Table 6.2. This table shows that the indispensable amino acid content in all test diets, expressed in g/16 g N, were higher than the requirements of *Heterotis niloticus*.

Fish were hand fed twice daily (09h30 – 10h00 and 14h30 – 15h00) to apparent satiation. Pellets were distributed slowly, allowing all fish to eat. The daily feed supply was recorded.

Table 6.1: Ingredient and proximate composition of the experimental diets (g kg^{-1} dry diet)

	Diets			
	1	**2**	**3**	**4**
	(0%SBM+CSM)	(25%SBM+CSM)	(50%SBM+CSM)	(75%SBM+CSM)
Ingredient composition (‰)				
Fish meal	300	225	150	75
Soybean oilcake meal	0	70	160	250
Cottonseed oilcake meal	0	70	160	250
Wheat bran	300	270	220	145
Bean meal	210	175	120	90
Blood meal	80	80	80	80
Menhaden fish oil[1]	30	30	30	30
Palm oil	30	30	30	30
Vitamin premix[2]	20	20	20	20
Mineral premix[3]	20	20	20	20
Carboxymethylcellulose[4]	10	10	10	10
Proximate composition[5] (‰)				
Moisture	85	81	84	87
Crude protein	356	354	355	358
Crude lipid	99	93	87	81
Ash	81	79	78	75
NFE[6]	379	392	394	398
Gross energy ($kJ.g^{-1}$)[7]	18.9	18.8	18.7	18.6

[1,4]Sigma-Aldrich products (Bornem, Belgium).

[2]Vitamin Mix Fish 0.5% INVE Aquaculture, Belgium (Composition per kg: vitamin A, 2 500 000 IU; vitamin D3, 500 000 IU; vitamin E, 30 000 mg; vitamin K3, 2 000 mg; vitamin B1, 2 000 mg; vitamin B2, 5 000 mg; panthotenic acid, 10 000 mg; Niacin, 5 000 mg; vitamin B6, 4 000 mg; folic acid, 2 000 mg; vitamin B12, 4 mg; vitamin C, 20 000 mg; biotin, 200 mg and inositol, 80 000 mg).
[3]Mineral Mix MLNP 763, INRA Belgium (Composition per kg: dibasic calcium phosphate, 500 g; calcium carbonate, 215 g; sodium chloride, 40 g; potassium chloride, 90 g; magnesium hydroxide, 124 g; iron sulphate, 20 g; zinc sulphate, 4 g; manganese sulphate, 3 g; cobalt sulphate, 0.02 g; potassium iodide, 0.04 g; sodium selenite, 0.03 g and sodium fluoride, 1 g).
[5]Values are the mean of three replicate analyses.
[6]Nitrogen-free extract (NFE) calculated as: 1000 – (‰ moisture + ‰ protein + ‰ lipid + ‰ ash).
[7]Calculated using nutrient content: 23.7, 39.5 and 17.2 kJ g^{-1} for protein, lipid and nitrogen-free extract, respectively (Guillaume *et al*. 1999).
SBM = Soybean oilcake meal; CSM = Cottonseed oilcake meal.

Table 6.2: Indispensable amino acids contents of the experimental diets (expressed in g/16 g N)

	Diets				
	1	**2**	**3**	**4**	**Requirements[8]**
Indispensable amino acids					
Arginine	6.46	7.00	7.36	7.46	4.0
Histidine	3.92	3.95	4.03	4.00	2.1
Isoleucine	3.81	3.92	3.81	3.59	2.9
Leucine	7.79	7.81	7.87	7.44	4.4
Lysine	7.83	7.66	7.61	6.85	5.0
Methionine + Cystéine	4.17	3.99	3.72	3.40	2.5
Phenylalanine + Tyrosine	7.76	7.98	8.14	8.07	4.7
Threonine	4.67	4.60	4.58	4.27	3.0
Tryptophan	1.23	1.25	1.31	1.19	0.5
Valine	5.68	5.77	5.78	5.50	3.4

[8]From Monentcham, Whatelet, Pouomogne & Kestemont (2009b)

6.3.2 Experimental procedure

Heterotis fingerlings weighing <1 g were collected from River Nyong (3°47'N and 12°15'E) near the town of Akonolinga, 100 km away from Yaounde and transported to the Melen aquaculture station (Yaounde, Cameroon). The fish were acclimated to fish pond conditions for several weeks. After this phase, 50 fish were randomly distributed into each hapa and fed diet containing 35% crude protein during a pre-experimental period of three weeks. After this conditioning period, the acclimated lots were randomly stocked in experimental hapas at the rate of 30 fish per hapa with three replications per treatment. Before the fish allotment, 50 fish were randomly sampled and individually weighed. At the end of the experiment, all fish were individually weighed and total length measured.

The hapas with rectangular dimensions (1.2m x 0.5m x 1.1m; Vol. = 500 L) were placed in a rectangular fish pond (300 m², 1.2m deep) located at the Government Aquaculture Station in Melen (Yaounde Cameroon). The pond was free from aquatic vegetation, completely independent, well exposed to sunlight and had inlet and outlet system designed to maintain the water level in the hapas at 0.8m for the duration of the experiment. During the feeding trial, fingerlings were exposed to natural photoperiod (6.15 a.m. to 6.45 p.m. daylight followed by night). Water temperature ranged from 24 to 31°C while pH ranged from 6.5 to 7.0. The experimental durations were 60 days (from June to August 2008).

6.3.3 Sample collection and chemical analysis

Initially, ten fish were sampled for initial carcass proximate composition. At the end of experiment, eight fish from each treatment were randomly selected for final analysis of whole-body composition. Three fish from each treatment were also randomly selected and a portion of the dorsal muscle from each fish was sampled. All samples were stored at -20°C prior to analysis.

Proximate composition of feed ingredients, experimental diets and fish were analysed following the methodology described by Monentcham *et al*. (2008). Total amino acid profiles of experimental diets were determined according to the protocols described by Monentcham *et al*. (2009b).

Chemical analyses were conducted at the laboratory of the Research Unit in Organismal Biology (URBO, University of Namur, Belgium). Amino acid analyses were carried out in the Unit of Industrial Chemistry Biology (Gembloux Agricultural University, Belgium).

6.3.4 Data analysis and statistical methods

At the end of the experiment, variables such as final mean weight, specific growth rate, feed efficiency, protein efficiency ratio, protein retention and survival were determined as follows:

Specific growth rate (SGR; $\%day^{-1}$) = 100 (Ln final body weight – Ln initial body weight)/duration of experiment (days)

Feed efficiency (FE) = (fish weight gain including weight of dead fish) (g)/total feed intake (g)

Protein efficiency ratio (PER) = (fish weight gain including weight of dead fish) (g)/total protein intake (g)

Protein retention (PR) = 100 (((final body weight x final body protein) – (initial body weight x initial body protein))/(total feed intake x dietary protein))

Survival (%) = 100 (final number of fish per hapa/initial number of fish per hapa).

Mean results in fish growth, feeding efficiency, survival and carcass composition were subjected to one-way analysis of variance (ANOVA) after verifying the homogeneity of variance using Hartley's test. Individual differences between dietary treatments were tested by least significance difference (LSD). The level for statistical significance was set at 5%. When necessary, data were normalized by arc-sine transformation prior to analysis.

6.4 Results

6.4.1 Growth performance, nutrient utilization and survival

During the feeding trial, all fish were observed to feed actively. Growth performance, nutrient utilization and survival data are presented in Table 6.3. The specific growth rate of fingerlings fed diet 1 (0% SBM+CSM), diet 2 (25% SBM+CSM) and diet 3 (50% SBM+CSM) did not differ from each other, but were significantly ($P<0.05$) higher than those of fish receiving diet 4 (75% SBM+CSM).

Table 6.3: Growth response, feed utilization and cost-benefit analyses of *Heterotis niloticus* fingerlings fed with experimental diets (initial average weight 5.20 g)

	Diets			
	1	**2**	**3**	**4**
	(0% SBM+CSM)	(25% SBM+CSM)	(50% SBM+CSM)	(75% SBM+CSM)
Final weight (g)	35.1 ± 0.6^{b}	34.7 ± 0.5^{b}	33.6 ± 0.7^{b}	28.3 ± 0.6^{a}
Feed intake (g/fish/week)	2.45 ± 0.07	2.56 ± 0.21	2.44 ± 0.16	2.39 ± 0.17
SGR (%.day^{-1})	3.16 ± 0.05^{b}	3.14 ± 0.03^{b}	3.09 ± 0.04^{b}	2.80 ± 0.03^{a}
FE	1.13 ± 0.08^{c}	1.11 ± 0.04^{c}	1.05 ± 0.08^{b}	0.84 ± 0.07^{a}
PER	3.17 ± 0.03^{c}	3.13 ± 0.07^{c}	2.96 ± 0.06^{b}	2.35 ± 0.05^{a}
PR	42.7 ± 0.1^{d}	41.3 ± 0.3^{c}	38.9 ± 0.4^{b}	27.6 ± 0.2^{a}
Survival (%)	77 ± 2	80 ± 6	74 ± 4	70 ± 3

SGR = Specific growth rate; FE = Feed efficiency; PER = Protein efficiency ratio; PR = Protein retention.
Values are means ± SE of three replicates hapas allocated to each diet. Means in a row with different superscript letters are significantly different ($P<0.05$).

Table 6.4: Final whole body and dorsal muscle composition of *Heterotis niloticus* fingerlings fed experimental diets

	Diets			
	1	**2**	**3**	**4**
Parameters[9]	(0% SBM+CSM)	(25% SBM+CSM)	(50% SBM+CSM)	(75% SBM+CSM)
Whole fish				
Dry matter	20.8 ± 0.5	21.7 ± 0.2	20.9 ± 0.5	20.9 ± 0.3
Protein	14.1 ± 0.5^{c}	13.7 ± 0.4^{b}	13.9 ± 0.3^{c}	13.1 ± 0.1^{a}
Lipid	2.2 ± 0.1^{a}	2.1 ± 0.4^{a}	2.6 ± 0.3^{c}	2.5 ± 0.2^{b}
Ash	3.7 ± 0.2	4.0 ± 0.3	4.0 ± 0.1	3.8 ± 0.2
Dorsal muscle				
Dry matter	19.1 ± 0.6	20.1 ± 0.1	18.8 ± 0.5	18.9 ± 0.2
Protein	14.3 ± 0.2^{c}	14.0 ± 0.1^{ab}	14.2 ± 0.3^{bc}	13.8 ± 0.2^{a}
Lipid	1.06 ± 0.03	1.14 ± 0.02	1.05 ± 0.04	1.03 ± 0.03
Ash	0.90 ± 0.05	0.94 ± 0.04	0.98 ± 0.03	0.93 ± 0.02

[9]Values are means ± SE of three replicates and expressed in (%). Initial whole body composition was 20.5% dry matter, 11.2% protein, 1.02% lipid and 4.3% ash.

Feed efficiency, protein efficiency ratio and protein retention decreased significantly ($P<0.05$) with graded substitution level of fish meal, but no significant difference ($P>0.05$) was found between fingerlings fed diets 1 (0% SBM+CSM) and diet 2 (25% SBM+CSM) for feed efficiency and protein efficiency ratio, respectively. Survival did not differ significantly ($P>0.05$) between groups fed different levels of fish meal substitution.

6.4.2 Whole-body and dorsal muscle composition

Initial, final whole-body and dorsal muscle compositions of Heterotis fingerlings are shown in Table 6.4. Partial replacement of fish meal with a mixture of soybean and cottonseed oilcakes meals in diets for Heterotis did not significantly ($P>0.05$) affect final fish body composition in terms of dry matter and ash. On the contrary, the protein content of whole-body and dorsal muscle showed some significant variations ($P<0.05$). Heterotis fingerlings fed diet 1 (0% SBM+CSM) displayed the highest body and dorsal muscle protein content, while the lowest protein content was observed in fish fed diet 4 (75% SBM+CSM). The body lipid content of Heterotis were low and significantly different ($P<0.05$) among the treatments. Final whole body composition contained more protein and lipid than initial one.

6.5 Discussion

Several studies on fish meal replacements with alternative plant protein sources have been conducted in many fish species such as Nile tilapia *Oreochromis niloticus* (Linnaeus) (Fontainhas-Fernandes, Gomes, Reis-Henrigues & Coimbra 1999; El-Saidy & Gaber 2003; Borgeson *et al*. 2006), African catfish *Clarias gariepinus* (Burchell) (Fagbenro & Davies 2001; Nyina-wamwiza, Whatelet & Kestemont 2007; Imorou Toko *et al*. 2008), vundu catfish *Heterobranchus longifilis* (Valenciennes) (Imorou Toko *et al*. 2007), rainbow trout *Oncorhynchus mykiss* (Walbaum) (Gomes, Rema & Kaushik 1995; Drew, Ogunkoya, Janz & Van Kessel 2007), Atlantic salmon *Salmo salar* L. (Torstensen, Espe, Sanden, Stubhaug, WaagbØ, Hemre, Fontanillas, Nordgarden, HevrØy, Olsvik & Berntssen 2008), rohu *Labeo rohita* (Hamilton) (Afzal Khan, Jafri, Chadha & Usmani 2003), European seabass *Dicentrarchus labrax* (Linnaeus) (Kaushik *et al*. 2004), turbot *Psetta maxima* L. (Regost, Arzel & Kaushik 1999; Burel, Boujard, Kaushik, Boeuf, Van der Geyten, Mol, Kühn, Quinsac, Krouti & Ribailler 2000). A number of conventional plant oil meals have been evaluated including soybean, cottonseed, groundnut, sunflower, rapeseed and linseed meals. The results suggested that it is possible to partially or totally replace fish meal by plant protein sources without adverse effects on

growth, feed utilization and carcass composition. However, complete substitution of fish meal with individual plant proteins has generally induced fish growth depression (Mbahinzireki, Dabrowski, Lee, El-Saidy & Wisner 2001; Chou *et al*. 2004). In certain cases, the use of individual plant oil meals as fish meal replacement are better when diets are supplemented with methionine/lysine (Davies, Morris & Baker 1997) and/or fortified with minerals (El-Saidy & Gaber 2004). In order to decrease the negative effect of total substitution on fish growth, authors have used successfully simple or complex mixtures of plant ingredients to replace fish meal in aquaculture feeds (Kaushik *et al*. 2004; Borgeson *et al*. 2006). Indeed, substitution of fish meal by a mixture of plant proteins might reduce the exposure to individual antinutritional factors and therefore improve growth performance. Our study demonstrated that a mixture of soybean and cottonseed oilcakes meals can partially replace fish meal in practical diets up to 50% for Heterotis fingerlings without affecting maximal growth performances.

In this study, we used two conventional oilseeds meals (soybean and cottonseed oilcakes meals) to substitute fish meal in diet for Heterotis fingerlings. El-Saidy & Gaber (2003) found that a mixture of four plant protein sources (soybean, cottonseed, sunflower and linseed meals; at individual rate of 25% of dietary protein) could completely replace fish meal in diets for juvenile Nile tilapia, *O. niloticus*. Likewise, promising performances were recorded with Nile tilapia fed with fishmeal free diets in earthen pond environment (Pouomogne, 1994). Other results confirm that the growth performances are higher when complex mixtures of plant ingredients replace fish meal compared to simple mixtures in diets for Nile tilapia (Borgeson *et al*. 2006) and European seabass (Kaushik *et al*. 2004). Therefore, further research will be required to evaluate the growth response of Heterotis fingerlings and adults to diets containing graded substitutions of fish meal by a complex plant protein mixture containing more than two plants ingredients, in order to entirely replace fish meal protein in practical diets of Heterotis.

Specific growth rate values obtained in this study were slightly higher than those reported by Monentcham *et al*. (2008) on the same species of similar size. The present study also reveals that the lowest specific growth rate, feed efficiency, protein efficiency ratio and protein retention were observed in fish fed diet 4 (75% fishmeal replacement by oilcakes). These observations are similar to the findings of previous investigations which reported decreasing growth performances at high inclusion levels of one or two dietary plant proteins (Mbahinzireki *et al*. 2001; Chou *et al*. 2004). Researchers have attributed this reduction to amino acid deficiencies, especially regarding methionine/lysine content. In our study, the indispensable amino acid composition of diets indicated that all the diets

exceed the requirements of *H. niloticus* (Monentcham *et al*. 2009b) but the amino acid digestibility of soybean and cottonseed oilcakes meals for Heterotis are not determined yet. For Channel catfish *Ictalurus punctatus* (Rafinesque), the amino acid availability ranges between 71.2 and 90.6%, and between 78.7 and 96.7% for cottonseed and soybean oilcakes meals, respectively (Hertrampf & Piedad-Pascual 2000). Moreover, in our study plant ingredients were cooked in order to reduce the impact of some antinutritional factors, such as trypsin inhibitor. Therefore, poor utilization of a mixture of soybean and cottonseed oilcakes meals by Heterotis fingerlings, at 75% substitution level of fishmeal, may be partially attributed to the free gossypol content in cottonseed oilcake meal, and/or low availability of certain amino acids, especially lysine. However, the impact of other anti-nutritional factors present in soybean oilcake meal such as tannins, saponins and glycosides should not be excluded. Investigations with rainbow trout (Herman 1970) and Channel catfish (Dorsa, Robinette, Robinson & Poe 1982) have shown a growth depression in fish fed diets containing 290 and 900 mg free gossypol kg^{-1} diet, respectively. Imorou Toko *et al*. (2008) found that the dietary phytic acid resulting from the increase of soybean and cottonseed meals in the diet affected Ca, P and Zn content in carcass or fillet of African catfish. In Nile tilapia feeds, iron in the form of ferrous sulphate has been efficiently used to reduce the toxicity of free gossypol and to improve growth performances and feed utilization when cottonseed meal totally replaces fish meal (El-Saidy & Gaber 2004). Therefore, more research is needed in diets of Heterotis fingerlings, in order to estimate the possibility of total replacement of fish meal with soybean and cottonseed oilcakes meals supplemented with iron.

In fish diets, data on the digestible protein and digestible energy of conventional feedstuffs are essential for optimization of feed formulation. Investigations were conducted to determine the digestibility of macronutrients in commonly used feedstuffs by Heterotis (Fagbenro 2001), *C. gariepinus* (Fagbenro 1998; Nyina-wamwiza 2007) and *O. niloticus* (Hanley 1987; Hossain, Nahar, Kamala & Islam 1992). The apparent digestibility coefficients of protein and energy values of soybean and cottonseed oil cakes meals reported for Heterotis were similar to values determined for Nile tilapia, notably (93.5% and 77.4%) and (86.8% and 60.9%), respectively. Therefore, other investigations are needed to determine the digestibility coefficients for crude protein and gross energy in more conventional protein sources by Heterotis, in order to confirm this hypothesis. The whole-body protein and lipids, and dorsal muscle protein contents were significantly influenced by substitution levels of fish meal, but surprisingly no definitive trend with graded substitution of fish meal in diet for Heterotis fingerlings was detected.

6.6 Conclusion

This research is the first controlled nutritional investigation on *Heterotis niloticus* to evaluate the response of fish fed with plant ingredients as fish meal substitute. Our findings suggest that soybean and cottonseed oilcakes meals can be used to partially replace fish meal in Heterotis diets without reducing maximal growth. Further research is needed to examine the possibility of entirely replacing fish meal by a mixture of different plant oil meals in Heterotis diet.

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7

DISCUSSION, CONCLUSION ET PERSPECTIVES

7.1 Discussion générale

Cette recherche menée sur *Heterotis niloticus*, potentiel candidat à la pisciculture continentale en Afrique subsaharienne, avait pour objectif de réaliser en milieu contrôlé les estimations pionnières des besoins nutritionnels et alimentaires de base de ce poisson au stade juvénile (3-62 g). A cette fin, une série d'expériences ont été réalisées en hapas installés dans des étangs en dérivation entre mai 2005 et août 2008. Dans les lignes suivantes, nous essayerons de mettre en exergue les apports majeurs de l'étude tout en les recadrant avec la logique des objectifs spécifiques annoncés au début de notre travail. A terme, nous proposerons des axes d'investigations futures en relation avec nos résultats émergents.

7.1.1 Acquis essentiels de la recherche et quelques suggestions

Durant les recherches bibliographiques relatives à cette étude, nous nous sommes heurtés maintes fois à la rareté des publications, des documents et même des rapports. Le peu d'articles disponibles étant en majorité rédigé en français, nous avons estimé opportun et nécessaire de synthétiser en langue anglaise (section 2) les connaissances disponibles sur Heterotis d'une part, et d'autre part de suggérer des thématiques futures de recherche-développement sur cette espèce en vue de son aquaculture durable et rentable. Les premières investigations initiées dès la fin des années 1950 ont porté sur le comportement reproducteur, la survie et les performances de croissance de cette espèce en étang. Les résultats préliminaires ainsi obtenus ont été des plus encourageants, mais aucune recherche relative aux besoins nutritionnels spécifiques de cette espèce n'a été alors initiée. Dans les entreprises aquacoles, le poste alimentation constitue près de 70% des charges (Pillay 1990). L'amélioration de la rentabilité de ces exploitations passe par la formulation d'aliments adéquats et spécifiques aux besoins de chacune des espèces de poissons. Des différents aspects envisageables pour aborder le problème de la détermination des besoins nutritionnels de *Heterotis niloticus*, nous avons sans hésitation opté pour les protéines pour diverses raisons, notamment leur coût élevé et surtout leur impact majeur sur la croissance. En effet, une augmentation de la teneur en protéine de l'aliment induit indubitablement un accroissement de la production jusqu'à un pallier où on dénote un plateau (Lee *et al*. 2001 ; Giri *et al*. 2003) ou une régression (Yang *et al*. 2003) de la croissance chez les espèces de poissons déjà étudiées. Ainsi, notre première démarche a consisté à étudier l'influence de différents aliments ayant des teneurs graduelles en protéines sur la croissance et la composition corporelle des juvéniles d'Heterotis à 2 tailles différentes, à savoir 3-15 g et 26-62 g. Cette approche semblait à notre sens, essentielle dans l'optique d'estimer les niveaux de protéines requis

respectivement pour une croissance optimale et maximale de ce poisson en phase de pré-grossissement.

Les résultats obtenus dans la troisième section ont indiqué une augmentation significative du gain de poids et du taux de croissance spécifique lorsque la teneur protéique de l'aliment augmente de 250 à 300-350 g protéines kg^{-1} d'aliment. Au-delà de cette valeur, la croissance plafonne et même diminue, respectivement chez les juvéniles de 3-15 g et 26-62 g. Des allures similaires de croissance ont été rapportées antérieurement chez le poisson-chat africain *Clarias gariepinus* (Henken *et al*. 1986), le tilapia hybride *Oreochromis niloticus x O. aureus* (Shiau & Huang 1989), le cyprinidé omnivore *Tor putitora* (Islam & Tanaka 2004), et le siluriforme *Rhamdia quelen* (Meyer & Fracalossi 2004). Les besoins en protéines des Heterotis ont été estimés dans cette recherche à 306 et 311 g protéines kg^{-1} d'aliment respectivement pour des poissons de 3-15 g et 26-62 g, lorsque les poissons sont nourris à satiété apparente en hapas. Ces estimations très similaires nous montrent que les besoins protéiques chez cette espèce ne diminuent pas significativement avec la taille du poisson dans la tranche de poids étudiée (de 3 à 62 g). Ainsi, nos résultats permettent de suggérer que les **besoins en protéines des Heterotis** (entre **3 et 62 g**) pour une **croissance optimale** sont identiques et de l'ordre de **310 g protéines kg^{-1} d'aliment**. De même, la teneur protéique recommandée pour atteindre la **croissance maximale des Heterotis** au même intervalle d'âge avoisine **345 g protéines kg^{-1} d'aliment**. Ces valeurs sont du même ordre de grandeur que celles de trois autres espèces de poissons omnivores notamment le Tilapia du Nil *Oreochromis niloticus*, 300 à 304 g protéines kg^{-1} d'aliment d'après Wang *et al*. (1985) et De Silva & Perera (1985) ; 310 g protéines kg^{-1} d'aliment chez la carpe commune *Cyprinus carpio* selon Takeuchi *et al*. (1979), et 280 à 320 g protéines kg^{-1} d'aliment chez le poisson-chat américain *Ictalurus punctatus* (Robinson *et al*. 2000). Les deux premières espèces citées sont usuellement élevées en polyculture dans les étangs ruraux en Afrique sub-saharienne (Pouomogne *et al*. sous presse) ; le même aliment composé équilibré peut de ce fait être utilisé dans ces étangs dans le processus d'intensification des élevages.

En nutrition des poissons, les besoins quantitatifs en protéines sont certes essentiels pour la formulation des aliments piscicoles, mais ils n'apportent aucune indication pertinente sur la qualité des protéines. Le principal baromètre utilisé à cette fin est le profil en acides aminés, surtout celui en acides aminés indispensables (AAI) des protéines. En effet, la synthèse corporelle des protéines nécessite la biodisponibilité de tous les acides aminés, indispensables et non-indispensables (Webster & Lim 2002). Dès lors, les besoins quantitatifs en protéines des poissons devraient toujours être associés avec ceux en AAI (les autres acides aminés pouvant être fournis par

voie métabolique) afin d'assurer une croissance maximale aux poissons. C'est ainsi que notre deuxième démarche a été focalisée sur l'estimation des besoins en AAI d'Heterotis à différents stades ontogénétiques.

Les résultats de la section 4 de notre étude révèlent de véritables similitudes des besoins en AAI des Heterotis à différents stades ontogénétiques, allant de la larve au poisson mature. Ceci nous enseigne que les **besoins spécifiques en AAI des Heterotis ne varient pas avec l'âge du poisson**. De même, ces **besoins sont proches de ceux d'autres poissons omnivores, à l'exception du tryptophane et de l'histidine qui sont respectivement plus faible et plus élevé**. Toutefois, ces estimations ayant été faites en utilisant une valeur des besoins de la lysine de 5g/16g N, basée sur la moyenne des besoins en lysine d'autres espèces omnivores, ces tendances observées demandent des confirmations ultérieures en déterminant le besoin en lysine avec la méthode dose-réponse. Avec ces principaux acquis des sections 3 et 4, les données nutritionnelles de base nécessaires à la formulation des aliments pratiques et expérimentaux couvrant les besoins spécifiques des juvéniles d'Heterotis en protéines et en AAI étaient dorénavant disponibles. Soucieux d'approfondir nos acquis et de réaliser davantage des économies substantielles en nutrition de ce poisson, notre préoccupation suivante a été focalisée sur la capacité de ce dernier à épargner les protéines par une utilisation efficiente des sources d'énergie non-protéiques. En effet, l'incorporation adéquate de lipides et/ou de glucides dans les aliments piscicoles réduit généralement l'utilisation des protéines alimentaires comme source d'énergie, au profit de la croissance. Par conséquent, la maîtrise du rapport protéine/énergie spécifique à chacune des espèces de poissons est indispensable afin de maximiser la rétention azotée et de réduire significativement les coûts de l'aliment.

L'évaluation de la réponse des juvéniles d'Heterotis recevant des régimes contenant trois niveaux de protéines (280, 320 et 360 g protéines kg^{-1} d'aliment) et deux niveaux de lipides (60 et 130 g lipides kg^{-1} d'aliment) nous a permis de mener cette étude au cours de notre troisième démarche (section 5). Nos résultats montrent que les **besoins en protéines des juvéniles d'Heterotis peuvent être réduits de 310 à 280 g protéines kg^{-1} d'aliment en augmentant le niveau énergétique de l'aliment de 17 à 19,6 MJ kg^{-1} d'aliment** (en incorporant davantage de lipides) **sans altérer les performances zootechniques**. Ce phénomène d'épargne des protéines par les lipides a été bien démontré chez plusieurs autres espèces de poissons telles que *Salmo salar* (Hillestad & Johnsen 1994), *Rhamdia quelen* (Meyer & Fracalossi 2004), *Sparus aurata* (Company *et al*. 1999; Vergara *et al*. 1996) et du *Clarias macrocephalus x C. gariepinus* (Jantrarotai *et al*. 1998). En dehors de la fourniture d'énergie, les lipides alimentaires assurent la couverture des besoins des poissons en acides gras

essentiels (AGE), acides gras (AG) non synthétisés par l'organisme et indispensables d'une part au maintien de l'intégrité des structures membranaires, et d'autre part au métabolisme cellulaire (Guillaume *et al*. 1999). En outre, les lipides servent de vecteur au cours de l'absorption des vitamines liposolubles et de précurseurs aux hormones stéroïdiennes et aux prostaglandines, améliorent la saveur de l'aliment, mais affectent la texture des granulés piscicoles et la composition corporelle en AG. Face à cette multitude de fonctions essentielles des lipides alimentaires dans la bonne croissance des poissons, il paraît dès lors indispensable d'envisager respectivement d'estimer les besoins en AGE des Heterotis à divers stades ontogénétiques, tout en examinant leurs capacités à bioconvertir les AG ; de déterminer le taux maximal d'incorporation des lipides dans leur alimentation et surtout d'évaluer leur capacité d'utilisation des huiles d'origine végétale en vue de leur substitution aux huiles de poissons. De telles études ont déjà été menées chez *O. niloticus* (Chou & Shiau 1996, 1999), *T. zillii* (Kanazawa *et al*. 1980). Les résultats obtenus, parfois contradictoires, semblent néanmoins retenir notre attention sur la nécessité des acides gras polyinsaturés à longue chaîne de la série n-3 et de la série n-6 pour une croissance maximale de ces poissons tropicaux.

La quatrième et dernière démarche de notre recherche a consisté à étudier la possibilité de remplacer la farine de poissons (FP), régulièrement utilisée dans les expériences précédentes par des sources protéiques végétales (disponibles localement et moins chères) dans le régime des juvéniles d'Heterotis (section 6). L'importance d'une telle étude tire ses origines dans le souci de contribuer à la formulation d'aliments pratiques à base d'ingrédients locaux, sans toutefois altérer ses performances zootechniques, en vue de son utilisation à long terme en aquaculture. De nombreux auteurs ont déjà attiré l'attention de la communauté internationale sur le danger qui plane sur une industrie aquacole basée sur les farines et les huiles de poissons, onéreuses et tendant à se raréfier (Tacon 1998 ; New & Wijkström 2002). Les points de vue sont ainsi unanimes sur la nécessité de substituer la FP par des sources protéiques alternatives, animales et surtout végétales, à condition qu'elles soient accessibles (Fagbenro and Davies 2001; Pouomogne 1994 ; Webster *et al*. 1995 ; Nyina-wamwiza 2007 ; Imorou Toko 2007). Pour atteindre donc l'objectif visé dans cette section, nous avons mené une expérience sur des juvéniles d'Heterotis nourris avec des régimes présentant des niveaux graduels de substitution de la FP par une combinaison de sous-produits locaux d'origine végétale. Les résultats obtenus suggèrent **qu'un mélange de tourteaux de soja et de coton peut remplacer partiellement (à hauteur de 50%) la farine de poisson dans l'alimentation des juvéniles d'Heterotis sans effet répressif sur leur croissance maximale**. Des résultats similaires ont été récemment obtenus chez *Clarias gariepinus* avec des tourteaux identiques (Imorou Toko *et al*.

2008). Des travaux antérieurs ont démontré un impact positif de l'ajout dans le régime de la lysine ou de minéraux comme le fer sur le niveau de substitution des FP respectivement par les tourteaux de soja et de coton chez *O. niloticus* (El-Saidy & Gaber 2004) et *O. mykiss* (Davies *et al*. 1997). Cette hypothèse mérite d'être examinée, de même que l'incorporation d'autres tourteaux tels que le tourteau d'arachide (46,2%) dans son alimentation en vue d'accroître sa capacité identifiée de substitution de la FP par ces sous-produits.

7.1.2 Analyses de la survie et des performances de croissance

Les taux de survie des juvéniles de Heterotis enregistrés durant nos quatre expériences ont varié entre 59 et 88%. Ces valeurs sont de même ordre de grandeur que celles obtenues chez les juvéniles de la même espèce élevée en étang, 69-96% (Tillon 1959), et relativement plus faibles que celles rapportées par Reizer (1966), 83-100%. Les différences observées au niveau des taux de survie de cette étude ont parfois été significatives sans tendance véritable avec les traitements respectifs et le poids. Toutefois, la survie des juvéniles (26-62 g) semble meilleure que celle des autres tranches de poids étudiées (2-36 g), suggérant ainsi que la mortalité s'atténue avec l'accroissement de l'âge, tout au moins dans l'intervalle étudiée. Cette hypothèse mérite des confirmations lors d'investigations ultérieures de plus longue durée.

Les taux de croissance spécifiques obtenus ont oscillé entre 2,5 et 4% j^{-1}. D'autres auteurs ont rapporté des résultats similaires chez *H. niloticus*, 3,48% j^{-1} pour des juvéniles de 3-6 g (Tillon 1959) et 2,77% j^{-1} pour des poissons de 30-50 g (Bard 1960). Néanmoins, les gains de poids quotidiens (GPQ) de ce travail, 0,29 à 1,29 g j^{-1}, sont 6 fois plus faibles comparativement aux données moyennes potentielles de l'espèce (Tillon 1957 ; Bard 1973). Cette réalité est attribuable d'une part à la relative courte durée des expériences, et d'autre part à la tranche de poids étudiée, correspondant à celle où la croissance exponentielle de cette espèce est moindre (se référer à la section 2).

7.1.3 Limites des contextes méthodologiques : réserves possibles quant à la validité des résultats

Les données obtenues dans cette étude doivent être considérées en tenant compte des limites de certains aspects de notre dispositif expérimental et du matériel animal. **La durée des expériences** a varié entre 28 et 60 jours (sections 3, 5 et 6) et la biomasse des poissons expérimentaux a toujours plus que triplé, excepté pour l'expérience dont la durée a été de 28 jours (section 3). Etant donné qu'il est recommandé que la biomasse soit au moins

triplée au cours d'une expérience de croissance d'une espèce de poisson donnée, nos résultats sont ainsi validés hormis à priori ceux relatifs aux besoins en protéines des Heterotis (26-62 g). Toutefois, la biomasse des juvéniles ayant au moins doublé au cours de cet essai, nous estimons que ces données devraient néanmoins être prises en considération car elles apportent des indications précieuses sur les besoins protéiques, inexistants pour ce poisson.

De même, la **taille expérimentale** des poissons entre 2 et 62 g, reste aussi une limite de ce travail au regard du poids maximal approchant la dizaine de kg atteint par ce poisson en milieu naturel (Vivien 1992 ; Stiassny et al. 2007). Compte tenu de son régime alimentaire omnivore, il serait probable que ses besoins en protéines soient plus faibles à des tailles supérieures à celle étudiée. Ceci nous suggère qu'en pratique, les résultats de croissance et d'efficacité alimentaire obtenus pourraient être améliorés par un apport supplémentaire d'aliment naturel, qu'elle valoriserait potentiellement bien. Ainsi, la suggestion actuelle de l'utiliser dans des étangs bien fertilisés en polyculture avec un supplément d'aliment artificiel s'avère opportune.

Toutes les expériences se sont déroulées dans des hapas en toile moustiquaire installés dans un étang. Ce système pourrait présenter certaines limites lorsqu'on tient compte de la **production primaire** potentielle de cet étang. Conscient de cela, diverses actions avaient été menées durant chacune des expériences pour atténuer cet apport supplémentaire de l'aliment naturel notamment le maintien permanent d'un faible renouvellement de l'eau de l'étang et le brossage bimensuel des faces externes et internes des hapas pour lutter contre un éventuel développement algal. En outre, le confinement des juvéniles dans des hapas limitait considérablement l'espace et donc la quantité d'aliment naturel disponible. Tous ces facteurs combinés concouraient à minimiser l'impact de l'aliment naturel sur la croissance de nos poissons expérimentaux et a pu ainsi nous permettre d'exclure une influence significative de ce facteur sur nos résultats. Cette réalité constitue à l'opposé une contrainte de leur applicabilité dans la majorité des étangs paysans, où la productivité naturelle de l'écosystème est loin d'être négligeable. Les investigations futures plus in situ pourraient tenir compte de cette composante.

L'utilisation de hapas installés dans des étangs, similaires aux étangs ruraux, constitue certainement un atout pour la valorisation des résultats obtenus dans des conditions du milieu écologique proches de celles des pisciculture paysannes. Toutefois, ce dispositif expérimental présente des limites supplémentaires qui doivent être émises. En l'occurrence, les conditions d'élevage n'ont pas permis la collecte des fèces pour des études de **digestibilité**, qui auraient à priori fournies plus d'informations sur

l'utilisation potentielle des nutriments apportés et sur les déchets d'origine alimentaire permettant ainsi de mieux appréhender l'impact de nos aliments sur la croissance des poissons et sur l'environnement. En outre, la vitesse de dissolution et la durée de flottabilité des granulés sont d'autres facteurs qui ont pu empêcher les juvéniles d'exprimer au maximum leur potentiel de croissance vis-à-vis des régimes. Un dispositif expérimental permettant une collecte des fèces et une meilleure observation visuelle du comportement alimentaire des juvéniles à l'exemple des aquariums pourrait être utilisé dans des recherches ultérieures pour surmonter ces limites. La totalité des réserves émises étaient à priori pressenties et notre choix a été orienté in situ par les contraintes de disponibilité des infrastructures.

7.2 Conclusion générale et perspectives

Arrivé au terme de ce travail de recherche, l'heure est venue de tirer des conclusions en fonction des objectifs fixés en début de thèse. La totalité des résultats obtenus constitue une avancée certaine dans la connaissance des besoins nutritionnels et alimentaires de l'Arapaimidae *Heterotis niloticus* en phase de pré-grossissement. S'agissant d'abord des besoins protéiques, il ressort que cette espèce omnivore nécessite 310 et 345 g protéines kg^{-1} d'aliment respectivement pour la croissance optimale et maximale de sujets pesant entre 3 et 62 g et que ses besoins spécifiques en acides aminés indispensables, excepté pour le tryptophane et l'histidine, sont similaires à ceux des autres espèces de poissons tropicaux omnivores. Ensuite, le phénomène d'épargne des protéines par les lipides alimentaires a été clairement démontré lorsque l'énergie a été augmentée de 17 à 19,6 MJ kg^{-1} d'aliment contenant 28% de protéines, permettant ainsi de suggérer que les besoins protéiques des juvéniles d'Heterotis peuvent être réduits de 310 à 280 g protéines kg^{-1} d'aliment dans les conditions expérimentales de cette étude. Enfin, les tourteaux de soja et de coton peuvent remplacer partiellement la farine de poisson dans l'alimentation des juvéniles de cette espèce.

L'objectif de départ de cette thèse était de contribuer significativement à la maîtrise des besoins nutritionnels et alimentaires des Heterotis. Dans l'état actuel de la recherche, nous avons effectivement pu obtenir des données alimentaires et nutritionnelles de base sur les juvéniles d'Heterotis nécessaires à l'optimisation de la formulation spécifique de leurs aliments. Alors, quelles seraient prioritairement les autres obstacles majeurs à surmonter pour avancer sur le chemin de la domestication de cette espèce piscicole ?

Sur le plan purement nutritionnel, des études similaires s'avèrent nécessaires sur les stades ontogénétiques des Heterotis non analysés dans la présente étude (larves, immatures et adultes, i.e. sur des spécimens de taille < 3 g ou > 62 g) afin de maîtriser les besoins nutritionnels spécifiques de ce poisson à toutes les phases de son cycle complet de production.

Sur le plan de la mise en place d'une filière de production massive de juvéniles d'Heterotis de qualité, les données disponibles convergent vers une reproduction naturelle (Mvogo 1962; Reizer 1964; Rakotomanampison 1966) mais irrégulière (Moreau 1982) en étang (200 à > 10.000 m²) des Heterotis et vers une forte et soudaine mortalité larvaire qui survient parfois en quelques heures au cours des jours post-éclosion (Olanyan & Zwilling 1963; Reizer 1964; Rakotomanampison 1966; Vincke 1971). Mes observations personnelles confirment cette tendance. Ces données mettent en exergue les difficultés majeures qui demeurent et entravent la réussite de la reproduction et de l'élevage larvaire de ce poisson en captivité, facteurs entre autres pourtant essentiels à la production massive d'alevins de qualité. Fort de cela, il est opportun d'estimer que la poursuite des recherches sur la voie de la domestication des Heterotis repose inéluctablement d'une part sur une meilleure compréhension du comportement reproducteur de ce poisson en captivité, et d'autre part sur une exploration approfondie des effets éventuels des différents facteurs susceptibles d'influencer son élevage larvaire afin d'éclaircir le mystère de la mortalité observée à ce stade.

S'agissant en premier lieu de la **reproduction**, Heterotis est caractérisé par un certain nombre de comportements reproducteurs naturels connus : formation de couples, construction de nids avec des débris végétaux et garde parentale, qui ont été confirmés en captivité (Tillon 1959 ; Reizer 1964). Toutefois, l'influence des caractéristiques physico-chimiques tels que la température, la photopériode, la turbidité, le pH, l'oxygène dissous sur le déroulement de ses cycles sexuels depuis la gamétogenèse jusqu'à la survie des premiers stades larvaires reste très mal connue. Des observations marginales de Moreau (1974) en milieu naturel révèlent l'influence probable de l'allongement de la photopériode, l'abaissement du pH et l'installation de la saison des pluies sur la maturation de ses gonades. Dans un futur très proche, ces grandes lacunes devraient être amoindries par les acquis des recherches entreprises au Sénégal depuis 2003 ([1]Daffé comm pers.). Des tentatives de reproduction artificielle devraient être initiées chez cette espèce en dépit de la relative grande taille et du manque de dimorphisme sexuel.

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S'agissant ensuite de **l'élevage larvaire**, les échecs des tentatives rencontrés dans ce travail confirment les tendances observées. Chez les autres espèces de poissons, la survie et le développement des larves sont assurés majoritairement par une alimentation adéquate. Excepté chez les salmonidés, elle est composée successivement de proies vivantes durant un temps relativement court, puis d'aliments artificiels. Le temps de passage de l'aliment vivant à l'aliment inerte varie considérablement selon les espèces. Chez Heterotis, plusieurs hypothèses explicatives de la forte mortalité larvaire ont été émises (se référer à la section 2). A la lumière de ce qui précède cependant, l'hypothèse la plus probable serait celle d'origine nutritionnelle et alimentaire. Dans cette perspective, quels pourraient être les facteurs limitants ? Les options suivantes, non exhaustives, pourraient être examinées : insuffisance des quantités d'aliments ingérées ; faible digestibilité des aliments ingérés, consistant essentiellement en zooplancton dans le milieu naturel ou dans l'étang piscicole ; cannibalisme ; besoins indispensables en proies vivantes (taille des larves d'environ 7,5 mm à l'éclosion) ; carences nutritionnelles ; déséquilibre nutritionnel des aliments ingérés.

Dans l'optique d'apporter des éléments de réponses à toutes ces questions, les futures recherches pourraient être orientées vers la connaissance du développement fonctionnel du tube digestif et des besoins nutritionnels des larves de *Heterotis niloticus*. A cette fin, diverses approches notamment zootechniques, biochimiques, histologiques et enzymatiques, utilisées antérieurement et avec succès chez d'autres espèces de poissons (Zambonino & Cahu 1994 ; Kestemont *et al*. 1996 ; Cuvier-Péres & Kestemont 2002) devraient alors être envisagées. L'étude des performances de croissance et de survie devrait se faire par une approche zootechnique usuelle, celle sur l'estimation des besoins nutritionnels spécifiques par une approche biochimique, celle sur la mise en place des structures digestives et l'impact du type d'aliment par une approche histologique et enfin, celle sur l'évaluation des capacités digestives par une approche enzymatique. Dans ce sens, des expériences visant à étudier l'âge et la période optimale de sevrage, la croissance et la maturation du tube digestif, ainsi que les mécanismes du métabolisme qui peuvent avoir un impact au niveau cellulaire sont nécessaires. Une telle approche pluridisciplinaire devrait nous permettre de détecter d'éventuelles perturbations de l'activité digestive des larves en reliant les changements ontogénétiques et morphologiques tels que l'ouverture de la bouche et la résorption des réserves vitellines, à des modifications aussi bien physiologiques, sécrétions enzymatiques notamment, qu'histologiques à l'instar du développement des glandes gastriques et de la fonctionnalité du foie et du pancréas. Les résultats obtenus à terme serviraient certainement à identifier une stratégie alimentaire des

larves des Heterotis basée sur des critères ontogénétiques et physiologiques, assurant ainsi leur bonne survie et leur excellente croissance.

A long terme, un accent devrait être mis dans son alimentation concernant la substitution des farines de poissons par des sous-produits d'origine végétale, disponibles et accessibles localement, sur la base des résultats pionniers de cette étude. Dans le même sens, le remplacement des huiles de poissons par des huiles végétales plus disponibles devrait faire partie de ces perspectives.

En somme, bien qu'ayant connu une progression substantielle avec les acquis de cette étude, le parcours des Heterotis sur la voie de la domestication semble encore jalonné d'embûches et de défis, synthèse d'un ensemble de données fondamentales et appliquées manquantes surtout au stade larvaire. Néanmoins, pour peu que les différentes approches soulignées ci-dessus soient mises en œuvre, il ne serait pas illusoire de réaffirmer notre conviction profonde et notre attachement réel à l'utilisation durable et rentable dans un avenir très proche de l'Arapaimidae *Heterotis niloticus* dans l'aquaculture au Cameroun et en Afrique au Sud du Sahara. La réalisation effective de cette perspective contribuerait efficacement à la diversification des espèces piscicoles élevées, à l'accroissement de la production aquacole de la région et par conséquent, à l'épanouissement des populations (renforcement de la sécurité alimentaire et augmentation des revenus), ainsi qu'à l'expansion de l'économie de cette région (création d'emplois).

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