



## Ecology of feeding Nile tilapia under *Azolla* cover in earthen ponds: an assessment using structural equation modelling

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### Abstract

The mechanisms by which Nile tilapia (*Oreochromis niloticus*) grow under *Azolla* cover (AC) in semi-intensive system was evaluated for 90 days using structural equation modelling and pathway analysis. The following *Azolla* cover (AC) extensions were applied: 0%, 15%, 30%, 45%, 60%, 75% and 90% of the surface. Fingerlings (initial mean weight: 15.8 ± 0.2 g) were additionally fed with the same diet formulated with local ingredients and containing 20% of *Azolla* meal. To quantify the relative contribution of AC and artificial feed in growth of fish, two models were defined, in which: (1): *Azolla* in ponds was considered as direct food for fish and a macrophyte on water surface; (2): *Azolla* acts as macrophyte only. Pathway analysis was done using standardized fortnight data and a coefficient was calculated for each causal relationship. Survival rate did not show any significant difference and values were higher than 84%. From the two competing models, indirect effects of AC on fish growth via phytoplankton biomass was similar and was the most important in the first month of experiment, the average ratio calculated being -0.68. As fish size increases, phytoplankton becomes limited and feeding on artificial food progressively grows in importance and influences growth. The coefficient of this direct effect on fish growth can reach 1.36, whilst the indirect effects from AC was -0.58 at the same period. The ratio of the relative contribution of the direct effect of feed versus the indirect effect of AC on Nile tilapia growth was therefore 1.36/-0.58. Summary, in ponds covered with *Azolla*, fingerlings of Nile tilapia fed agricultural by-products feed primarily on phytoplankton at the beginning, and artificial food gains prominence with time, beneficially in tropical aquaculture where there is a need to reduce the feed cost in that enterprise.

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## Introduction

In Asia and in African rural areas, there is ongoing practice of covering fish pond with the aquatic fern *Azolla*. This atmospheric nitrogen-fixing fern naturally rich in nitrogen (Micha, 2000) is recently valorised in fish farming as a potential ingredient to replace fishmeal in omnivorous and phytophagous fish feed (Leonard, 1997; Shiomi and Kitoh, 2001; Kanangire, 2001; Micha and Leonard, 2001-2; Fiogbé *et al.*, 2004; Abou *et al.*, 2007a,b). When covering a fraction of pond surface, this fern induces the development of two sub-ecosystems, differing in their ecological characteristics: under *Azolla*, and in *Azolla*-free waters. Many research studies under laboratory conditions have largely underlined the effects of AC on physicochemical characteristics of the water underneath (Kröck, 1987; Villegas and Valentin, 1989; Kröck *et al.*, 1988; Lejeune *et al.*, 1999; Vlek *et al.*, 1995; Gilmont, 1997; de Macale *et al.*, 2002). But published research on the effects of AC on dynamic of pond organisms, especially on fish growth and production, is lacking. Very recently, Abdel-Tawwab (2006) has studied its effects on Nile tilapia and common carp polycultured in fertilized ponds, and did not find any significant difference between fish reared at control (*Azolla*-free ponds) and 25 % AC. However, one could consider that growth in this system is influenced by the AC acting as macrophyte and/or feed and by the artificial feed provided. Indeed, this fern could serve directly as food for fish hence influencing directly the growth, and its mat on the water surface could have indirect effect on growth through their negative impact on the phytoplankton productivity. Also, artificial food provided could be another factor that induces variation in growth. It is therefore imperative to quantify the contribution of each factor on growth of fish. For this purpose, an evaluation that considered the whole system, in which many ecological processes may exist and would be linked with many biological mechanisms, is needed to better understand the mechanisms that drive fish growth under AC in ponds.

The aims of this study are to investigate the effects of AC, and its interactions with feed, on the variation of growth in Nile tilapia supplementary fed with the fern *Azolla*. The practical objectives are to analyse direct or indirect effects on growth in such fish farming systems.

## Material and methods

### *Experimental design and feeding*

The experiment was carried out for 90 days at the rural area (6°29'15.12"N 2°37'6.42"E, and at 13 m above mean sea level) in Porto-Novo suburb, Benin (West Africa). The fern *Azolla* (*A. filiculoides* Lam.) was mass cultivated in two ponds of 150 m<sup>2</sup> each to initially fill the experimental ponds. The experimental ponds are eighteen stagnant earthen ponds of 30 m<sup>2</sup> each (10 m x 3 m x 1 m) and they were randomly assigned to six treatments T1, T2, T3, T4, T5 and T6, to represent six levels of pond surface covered by *Azolla*, namely 0% (control), 15%, 30%, 45%, 60% and 75%, respectively. The suitable surface areas were covered with dense mats of *Azolla* one week prior to the experiment. The fern was set in one corner (the same for all experimental ponds) of the ponds to mimic the method generally followed in rural areas, and the mats were constrained to these locations by *Bambusa* sticks planted into the banks.

The fish used in the study were male *O. niloticus* (initial mean weight = 15.8 ± 0.2 g) stocked in ponds at 2 fish m<sup>-2</sup>. All experimental fish were fed with a same practical *Azolla*-diet (crude protein: 29.2%; Gross energy: 16.9 kJ g<sup>-1</sup>) containing 20% of the meal of the fern. Fish were fed according to Melard (1986). Daily rations were divided into two parts, each hand-distributed at 8:00 h and 16:00 h, respectively. They were adjusted every two weeks according to fish biomass in each pond.

### *Path analysis of changes in fortnight means weight*

In this study, experimental fish were fed based on their mean weight, and the amount of feed supplied was adjusted every fortnight after weight control. Because of the influence of AC in ponds, fish growth

varied widely, influencing the amount of feed supplied in each experimental pond every two weeks. Hence, the following questions should be addressed: “which parameter, AC or the amount of feed most influences the growth of fish”? or “what is the relative contribution of each factor”? To analyze their contribution, we take into account not only the final data from the experiment but also the results in term of weight gain and variation in the amount of feed supplied during each two week period. Then, models were built for pathway analysis. The models were based on the *a priori* causal relationships existing in a pond food web, and the specification introduced here by the AC. Two models were constructed (Figs 1, (a) and (b)). We assume that there were two exogenous variables in the system: AC and food quantity. In the first model (Fig. 1, (a)), *Azolla* fern and the artificial diet could serve as food for fish, hence directly influencing the growth of fish, whereas in the second model (Fig. 1, (b)) *Azolla* was considered solely as a macrophyte (not as food) that could drive many ecological processes in the ponds. Indeed, in both models, AC could have indirect effects on fish growth via their impact on the dynamics of phytoplankton. We assumed that the level of AC in ponds would determine the abundance of phytoplankton, which could then influence the density of zooplankton (grazing process). As Nile tilapia are planktonophagous, phytoplankton and zooplankton are potential prey items. Finally, any variation in the amount of feed supplied could directly impact the growth of fish. Which of the two variables (AC or feed amount) is then the most important in growth of Nile tilapia? To answer that question, we used Structural Equation Modelling (SEM) to investigate the causal relationships using standardized variables. SEM is a multivariate statistical methodology that encompasses variables and path analysis, and is recommended in the cases where the knowledge of the natural history of a system is sufficient to construct *a priori* path diagrams (Palomares *et al.*, 1998). To perform the analysis, the two hypothetical diagrams shown in fig. 1 (Figs 1, (a) and (b)) were considered. For each connecting path, a coefficient was calculated. These

coefficients are equivalent to the standardized partial regression coefficients of multiple regression, and according to Mitchell (1992) represent the effect of one variable on another dependent variable, with all other variables are statistically held constant.

The pathway analysis was performed with the R software version 2.5.1 using the SEM library (Fox 2002).

### Results and discussion

The effects of AC and feeds on variations in mean weight, at each two weeks intervals for the both models constructed were summarized in Tables 1 and 2. The standardized coefficients for each pathway are shown. The significant reliability ( $P < 0.05$ ) between variables is indicated by bold values. During the first fortnight, when an identical amount of feed was supplied to all ponds, a significant negative effect of AC on phytoplankton biomass was recorded (Tables 1 and 2). The latter variable positively influences zooplankton abundance. In addition, results from the second model (Table 2) show that weight gain in fish greatly depends on phytoplankton biomass (coeff. = 0.74). From day 15 to day 30 in the two models, the same negative effects of AC on phytoplankton biomass and positive effect of the latter on zooplankton was also observed. However, the analysis failed to show any positive effect of Chl *a* on fish growth as previously recorded in the second model (Table 2). During the following fortnight, i.e from day 30 to day 45 (Tables 1 and 2), the indirect significant effects of AC were permanent; the originality being an additional significant positive effect of artificial feed on growth. The importance of this effect was quite similar in the results of both models (path coefficients: 0.56 and 0.62). Its importance had increased between day 45 and day 60 (Tables 1 and 2). A significantly influence of phytoplankton biomass (path coeff. = 0.67) on fish growth is also observed using the first model during the same period (Table 1). During the last month of experiment, both models revealed the permanent indirect effects of AC, and the direct effects of feed on growth, with however an unexpected negative effect of Chl *a* on growth, as shown in Table 2 at the last

two weeks. Considering all periods for the first model (Table 1), no significant direct effect of AC on growth of fish was signalled. During the first month in both models, the indirect effects of AC are predominant.

Fish weight was influenced by the effects of AC and feed during the last two months, with a variation in their relative contribution, as shown by the variation in their coefficient.

**Table 1.** Path coefficient estimates from the structural equation modelling analysis of the pathways in *a priori* model in which *Azolla* cover acted as food and weed macrophyte.

Relationship	$d_0 - d_{15}$	$d_{15} - d_{30}$	$d_{30} - d_{45}$	$d_{45} - d_{60}$	$d_{60} - d_{75}$	$d_{75} - d_{90}$
<i>Azolla</i> cover - Chl <i>a</i>	<b>-0.92</b>	<b>-0.93</b>	<b>-0.92</b>	<b>-0.87</b>	<b>-0.85</b>	<b>-0.85</b>
<i>Azolla</i> cover - fish	-0.65	-0.47	-0.49	0.94	-0.07	0.40
Chl <i>a</i> - zoopl.	<b>0.86</b>	<b>0.86</b>	<b>1.03</b>	<b>1.07</b>	<b>1.02</b>	<b>1.10</b>
Chl <i>a</i> - fish	0.28	0.016	-0.011	<b>0.67</b>	0.06	-0.28
Zoopl. - fish	-0.29	0.27	-0.25	-0.11	0.02	0.46
Feed - fish	-0.12	0.21	<b>0.56</b>	<b>1.36</b>	<b>0.84</b>	<b>1.04</b>
$\chi^2$ (chi square)	2.83	0.24	0.39	8.61	8.20	6.06
d.f (degree of liberty)	2	2	2	2	2	2
$P(>\chi^2)$	<b>0.243</b>	<b>0.889</b>	<b>0.823</b>	0.013	0.017	0.048

Significant pathways and  $P(\chi^2) > 0.05$  are in bold.  $d_x - d_y$  means day x to day y; chl *a* means chlorophyll *a* and zoopl. means zooplankton.

**Table 2.** Path coefficient estimates from the structural equation modelling analysis of the pathways in *a priori* model in which *Azolla* cover served only as macrophyte.

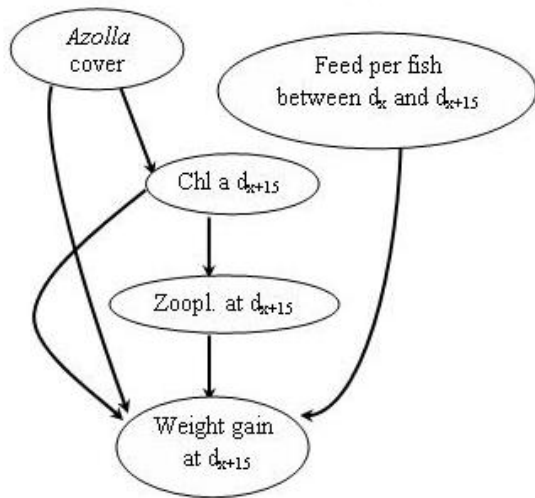
Relationship	$d_0 - d_{15}$	$d_{15} - d_{30}$	$d_{30} - d_{45}$	$d_{45} - d_{60}$	$d_{60} - d_{75}$	$d_{75} - d_{90}$
<i>Azolla</i> cover - Chl <i>a</i>	<b>-0.92</b>	<b>-0.93</b>	<b>-0.92</b>	<b>-0.87</b>	<b>-0.86</b>	<b>-0.85</b>
Chl <i>a</i> - zoopl.	<b>0.86</b>	<b>0.86</b>	<b>1.03</b>	<b>1.07</b>	<b>1.02</b>	<b>1.10</b>
Chl <i>a</i> - fish	<b>0.74</b>	0.44	0.17	0.31	0.09	<b>-0.42</b>
Zoopl. - fish	-0.14	0.24	0.007	-0.43	0.04	0.30
Feed - fish	-0.04	0.29	<b>0.62</b>	<b>1.02</b>	<b>0.87</b>	<b>0.91</b>
$\chi^2$ (chi square)	4.42	1.97	0.941	11.69	8.25	7.33
d.f (degree of liberty)	3	3	3	3	3	3
$P(>\chi^2)$	<b>0.220</b>	<b>0.580</b>	<b>0.816</b>	0.009	0.041	0.062

Significant pathways and  $P(\chi^2) > 0.05$  are in bold.  $d_x - d_y$  means day x to day . chl *a* means chlorophyll *a* and zoopl. means zooplankton.

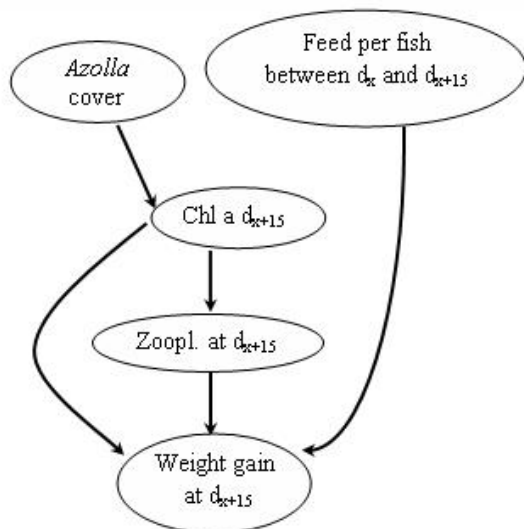
Survival rates were higher, and values were similar in all experimental ponds, suggesting that the fish had adapted to the AC system in ponds, even up to the level of 75%. In a water surface covered with macrophytes, there is different biological mechanisms, especially out-competition with phytoplankton, which are associated with the presence of macrophytes on the water surface (Abdel-Rahman *et al.*, 2002; Meerhoff *et al.*, 2003). In the case of this experiment, AC competes

phytoplankton for light when shading 15, 30, 45, 60 and 75% of the pond surface, and also for nutrients absorption. These could reduce phytoplankton abundance and therefore Chl *a* concentration, as seen in ponds with AC higher than 15% on the water surface.

(a) *Azolla* cover served as direct food for fish and as macrophyte



(b) *Azolla* cover acted as macrophyte only



**Fig. 1.** The hypothesized causal relationships linking exogenous variables (*Azolla* cover and food quantity), natural foods (phytoplankton and zooplankton) and fish growth (fortnightly weight gain). Arrows indicate the direction of causality assumed in each model.  $d_x$  means day X; chl *a* means chlorophyll *a* and zoopl. means zooplankton.

As shown above, AC and feed could influence growth of fish. Results from the both models for the first month of the experiment revealed that the indirect effects of AC, namely its negative impact on phytoplankton productivity and the positive effects of the latter on zooplankton abundance was predominant. Competition between AC and phytoplankton for light, and the grazing of

phytoplankton by zooplankton are the main explanation for these findings. *Azolla* did not serve as direct food for fish, and feed amount did not determine significantly the growth of fish. The positive significant path coefficient found between Chl *a* and fish weight during the first two weeks revealed a great consumption of phytoplankton by fish. Because of the smaller size of Nile tilapia during that phase of the experiment, the abundance of phytoplankton and probably the large size and the lignification of *Azolla* fronds, it is not obvious that fish feed directly on the fern. Generally, fingerlings *Oreochromis niloticus* feed mainly on phytoplankton, and their filtration rate is known to increase with increasing cell concentration (Moriarty and Moriarty, 1973; Tudorancea *et al.*, 1988; Turker *et al.*, 2003). After a 30-days experiment, fish size is still smaller (average mean weight: 31-47 g) and the difference in the amount of feed per fish quite insignificant (21-23 g). This could lead to a continuously high fish pressure on phytoplankton, and an insignificant effect of the artificial feed provided. Although phytoplankton positively influences zooplankton throughout the experiment, the latter did not significantly contribute to fish growth, curiously. A possible reason could be an adaptation to fish predation. It is known that macrophytes in water surface provide a refuge for zooplankton against fish predation (Burks *et al.*, 2002). By acting as habitat, *Azolla* could reduce zooplankton abundance in *Azolla*-free area thus controlling zooplankton grazing and enhancing fish grazing on phytoplankton. From day 45 to day 60, the importance of the relationships AC-Chl *a* and Chl *a*-zooplankton was permanent and increased, this indirect effect of AC being equal for both models to  $-0.92 \times 1.03$  (Tables 1 and 3 at 30-45 days) and  $-0.87 \times 1.07$  (Tables 1 and 2 at 45-60 days). Also, a significant proportion of the weight gain was influenced by phytoplankton (Table 1 at 45-60 days). As the fish size increased, natural foods become limitant. A possible explanation could be an appearing of a regime shift, favouring feeding on artificial food distributed, that could then contribute to a larger part of fish growth. This importance of

feed on growth was maintained over the last phase of experiment. Closely, the results from the two hypothetical models were quite similar in explaining the way by which fish grow in AC systems, as well as the contribution of each exogenous variable to fish growth. Then, we attempted to calculate the ratio of the relative contribution of all effects of all exogenous variables to the changes in growth of Nile tilapia. For this purpose, we use the results that seem realistic and in which the indirect effect of AC through the influence on Chl *a* was significant until fish growth level. During the first period (Table 2 at 0-15 days), only the indirect effect of AC was significant, reaching  $-0.92 \times 0.74 = -0.68$ . In Table 2 from 45 to 60 days of experiment, the direct influence of artificial food on fish growth was 1.36, while the indirect effects of AC through Chl *a* was  $-0.87 \times 0.67 = -0.58$ . Overall, these results indicate that the negative effect of AC on phytoplankton, and thus on fish, is greater at the beginning of the experiment, and decreases progressively with the decrease in phytoplankton biomass and the contribution of feed.

AC in ponds generates a complex ecosystem that makes it possible to better understand and quantify the mechanisms by which plankton and feed interact in the growth of Nile tilapia in ponds, and particularly in ponds covered with *Azolla*. Fingerlings Nile tilapia feed primarily on phytoplankton at the beginning, and artificial feed gain importance with time. These results hold a great emphasis in rural tropical aquaculture where this kind of aquaculture is practiced and where there is a need to better managed fish feeding as the money to invest is already lacking.

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