The Relationship Between Ammonia Production and Oxygen Concentration in Water and the Biomass of Eels and Level of Protein in the Diet of Anguilla anguilla L.

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ABSTRACT

This study examined the influence of weight and protein level in their diet, on water quality (ammonia and oxygen concentrations, nitrate level, and pH) in the containers in which the eels were kept. A linear correlation was found between the time the eels were in the water, and ammonia production by them grouped by weight. The equation is \( A = 0.0782 W - 0.0456 \) where \( A \) = ammonia concentration (in ppm) per hour, and \( W \) = the weight (g) of eels. One kg of eels produced 24.99 mg ammonia per hour. The correlation between ammonia concentration, pH and nitrate did not change during the period of measurement in the present study.

Ammonia concentration increased with an increase in the protein level in the diet. The equation is \( A = 0.01 D - 0.05 \) where \( A \) = ammonia concentration in water and \( D \) = % protein in the diet.

The oxygen concentration in the water was inversely correlated with the weight of the eels, and the allometric equation for the relationship between body weight of fish and oxygen concentration (0 ppm) is \( 0 = -0.1925 W + 7.7760 \). The allometric equation for the relationship between oxygen concentration (0, ppm) and percent protein in the diet \( (D) \) is \( 0 = 0.016 D + 6.8790 \).

INTRODUCTION

The major constraints in intensive aquaculture are the depletion of dissolved oxygen (DO) and the accumulation of ammonia and other toxic products of metabolism (Websters & Pratt, 1977; Boyd & Tucker, 1979; Armstrong & Boyd, 1982). Both DO and ammonia concentrations are...
dependent on density, fish metabolism and water exchange in the fish culture system (Soderberg et al., 1983; Degani et al., 1985).

Eels (Anguilla anguilla) are stocked at various densities: 1-2 kg m$^{-2}$ (Degani & Levanon, 1983), 4-30 kg m$^{-2}$ (Degani et al., 1985), and as high as 60 kg m$^{-2}$ or 150-250 kg m$^{-2}$ (Liewes, 1978). However, the effect of density on ammonia production or DO has not been sufficiently investigated (Degani et al., 1985).

The water quality of eel cultures has been studied mostly in ponds with Japanese eels (A. japonica) (Usui, 1979). Normal daily variation of oxygen ranged between 1 and 10 ml liter$^{-1}$, pH between 7 and 8 and nitrate between 0 and 100 mg liter$^{-1}$.

The reduction in fish growth caused by exposure to a constant high concentration of ammonia is widely documented and was summarized by Soderberg et al. (1983). The ammonia production in American elvers (A. rostrata) has been described by Gallagher et al. (1984).

The purpose of this work was to study the relationship among density, protein in diet, and ammonia production, and to develop equations which will be of help in calculating the ammonia production at different densities of eel culture with different percentages of protein in the diet.

**MATERIALS AND METHODS**

**Animals**

European glass eels (Anguilla anguilla), netted during their winter migration in coastal waters off France during February 1985, were held for three months in indoor containers as described by Degani & Levanon (1983). At the end of the initial period, the elvers were divided into three groups: slow-growing elvers (0-4–1 g), normally-growing elvers (1-3 g) and fast-growing elvers ( > 3 g). Only the normal and fast-growing elvers were used in this study.

**Experimental conditions**

The experiment was conducted in indoor containers measuring 2 x 0.4 m x 0.4 m as described previously by Degani & Levanon, 1984. The water volume was 0.32 m$^3$ (Fig. 1). Each container was covered with a net to prevent the eels from escaping. Water flow was controlled by a flow meter, and compressed air was supplied throughout the experimental period. Dissolved oxygen was measured by a digital oxygen analyzer (model 58, Yellowstone Scientific Inst., Yellow Springs, Ohio,
USA) provided with a DO probe (no. 5739). The temperature was maintained at $23 \pm 1^\circ$C by passing hot water ($80^\circ$C) in a closed system through a pipe in every container. The flow through the pipe was controlled by a solenoid-operated thermostat. Ammonia and nitrate were
determined according to Weatherburn (1967). The pH was monitored by means of a research pH meter, model 84 (Radiometer, Copenhagen).

In the first experiment eels (5 g mean weight) were stocked, in indoor containers with a water volume of 0.32 m$^3$, at different densities: 1 kg m$^{-2}$, 2 kg m$^{-2}$, 4 kg m$^{-2}$, 7 kg m$^{-2}$, 14 kg m$^{-2}$ and 19 kg m$^{-2}$ (2.5 kg m$^{-3}$, 5 kg m$^{-3}$, 10.1 kg m$^{-3}$, 17.8 kg m$^{-3}$, 36.3 kg m$^{-3}$ and 49.9 kg m$^{-3}$ respectively). Every 2 h a water sample was taken to determine the ammonia concentration. Air was supplied to each container throughout the experiment by a compressor.

In the second experiment eels (4 g mean weight) were stocked at different densities: 1.6 kg m$^{-2}$, 6 kg m$^{-2}$, 12 kg m$^{-2}$ (4.0 kg m$^{-3}$, 15.2 kg m$^{-3}$ and 30.9 kg m$^{-3}$).

Water flowed through the containers (0.32 m$^3$) at a rate of 120 liters h$^{-1}$, and was replaced nine times a day. All the eels were fed dough equal to 2% of their body weight twice a day (at 7:00 and 15:00 h) throughout the experiment. A water sample was taken every day to determine the DO, ammonia and pH. This was continued for three weeks.

In the third experiment, eels (mean weight 5 g) were stocked at three different densities (0.75 kg m$^{-2}$, 6 kg m$^{-2}$ and 8.8 kg m$^{-2}$), with three replicates per density. A water sample was taken every 2 h for 24 h for water analysis.

In the fourth experiment eels (1-2 g mean weight) were stocked in outdoor containers (1 m$^3$ volume) under conditions described by Degani

**TABLE 1**
Composition of the Diet Used in the Experiment

<table>
<thead>
<tr>
<th>Groups (% weight)</th>
<th>25%</th>
<th>35%</th>
<th>45%</th>
<th>55%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn meal</td>
<td>57</td>
<td>42</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Chicken meal</td>
<td>25</td>
<td>46</td>
<td>70</td>
<td>47</td>
</tr>
<tr>
<td>Fish meal</td>
<td></td>
<td></td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Soy bean meal</td>
<td>12</td>
<td>8</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Chicken oil</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Calcium</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Chemical analysis (%) dry matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic matter</td>
<td>92.1</td>
<td>90.0</td>
<td>92.2</td>
<td>93.0</td>
</tr>
<tr>
<td>Crude protein (gN x 6.25 g kg$^{-1}$)</td>
<td>25.0</td>
<td>35.1</td>
<td>44.8</td>
<td>54.9</td>
</tr>
<tr>
<td>Lipid</td>
<td>5.9</td>
<td>8.9</td>
<td>10.0</td>
<td>13.1</td>
</tr>
</tbody>
</table>
et al. (1985). Fifty fish (stocking density of 0.06 kg m\(^{-2}\)) were put into each container. Four containers of 500 liter capacity were used for each experimental diet. They encompassed four iso-caloric diets with the four protein levels: 25%, 35%, 45%, and 55%. The composition of the diet is presented in Table 1. The food pellets were given at 4% of the body weight of the fish per day, and divided into two meals per day (at 7:00 and 17:00 h). Water samples were taken every day during the three month growth period at 10:00 h.

![Graph](image)

**Fig. 2.** The correlation between ammonia concentration in the water and the length of time the eels were in the containers, at different densities.

![Graph](image)

**Fig. 3.** The relationship between ammonia concentration in the water and pH in containers stocked at 19 kg m\(^{-2}\) (experiment 1).
RESULTS

The correlation between total ammonia (NH₃ + NH₄) concentration and the length of time the eels were in the containers at different densities is presented in Fig. 2. A linear correlation was found between the time and the total ammonia concentration of each weight of fish (Fig. 1). Table 2 shows the total ammonia concentration per hour for each density. The equation is \( A = 0.0782 \ W - 0.0456 \) (where \( A \) = ammonia concentration).

### TABLE 2

The Correlation Between Ammonia Production and Length of Time (h) the Eels were Stocked at Different Densities. The Time Limits are Represented in Fig. 2.

<table>
<thead>
<tr>
<th>Density (kg m⁻²)</th>
<th>Equation*</th>
<th>r</th>
<th>P (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( A = 0.0427T - 0.145 )</td>
<td>0.99 (8)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>2</td>
<td>( A = 0.1213T - 0.1862 )</td>
<td>0.99 (9)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>4</td>
<td>( A = 0.2853T - 0.0048 )</td>
<td>0.90 (6)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>7</td>
<td>( A = 0.4405T - 0.1780 )</td>
<td>0.97 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>14</td>
<td>( A = 1.0725T - 0.600 )</td>
<td>0.99 (4)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>1-14</td>
<td>( B = 0.0782W - 0.0456 )</td>
<td>0.99 (5)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

* \( A \) = ammonia concentration change in water, \( T \) = time (h), \( P \) = AT⁻¹, \( W \) = weight (g).

### TABLE 3

The Relationship Between Ammonia and Nitrates Concentration (ppm) in Containers of Eels Stocked at Different Densities (Experiment 3). The Water was Replaced in Every Container Nine Times a Day, pH = 7.4. The Flow (Continual) was 1.5 liters h⁻¹

<table>
<thead>
<tr>
<th>Stocking density</th>
<th>0.7 kg m⁻²</th>
<th>1.8 kg m⁻²</th>
<th>6 kg m⁻²</th>
<th>15.2 kg m⁻²</th>
<th>8.8 kg m⁻²</th>
<th>20.3 kg m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour of day</td>
<td>Ammonia</td>
<td>Nitrates</td>
<td>Ammonia</td>
<td>Nitrates</td>
<td>Ammonia</td>
<td>Nitrates</td>
</tr>
<tr>
<td>07.00</td>
<td>0.36</td>
<td>4.40</td>
<td>1.21</td>
<td>3.01</td>
<td>0.87</td>
<td>4.10</td>
</tr>
<tr>
<td>09.00</td>
<td>0.30</td>
<td>4.50</td>
<td>2.01</td>
<td>1.50</td>
<td>0.25</td>
<td>4.20</td>
</tr>
<tr>
<td>11.00</td>
<td>0.70</td>
<td>4.60</td>
<td>1.02</td>
<td>4.71</td>
<td>0.31</td>
<td>4.51</td>
</tr>
<tr>
<td>14.00</td>
<td>0.30</td>
<td>4.70</td>
<td>1.52</td>
<td>4.01</td>
<td>0.51</td>
<td>4.21</td>
</tr>
<tr>
<td>18.00</td>
<td>0.30</td>
<td>4.10</td>
<td>1.63</td>
<td>3.52</td>
<td>1.21</td>
<td>4.00</td>
</tr>
<tr>
<td>21.00</td>
<td>0.30</td>
<td>4.50</td>
<td>1.20</td>
<td>3.20</td>
<td>0.92</td>
<td>3.74</td>
</tr>
<tr>
<td>24.00</td>
<td>0.30</td>
<td>4.40</td>
<td>1.01</td>
<td>2.80</td>
<td>0.80</td>
<td>3.75</td>
</tr>
</tbody>
</table>
per hour, and $W=$ the total weight of the fish in grams). From these results it is possible to calculate the ammonia production per kg of eels per hour at each density: 1 kg of eels produce 24.99 mg h$^{-1}$ ammonia at 23°C.

The correlation between ammonia and nitrate in containers of eels stocked at different densities is presented in Table 3. The correlation between ammonia and pH in containers stocked at 19 kg m$^{-2}$ with a water flow at 50 liters h$^{-1}$ is presented in Fig. 3.

The ammonia concentration in water of eels fed different protein levels is shown in Fig. 4. The concentration increased with an increase in the protein level of the diet, and was in linear correlation with body weight: $A = 0.010 D - 0.05$, where $A =$ ammonia concentration (ppm) and $D = \%$ protein in the diet.

The oxygen concentration in water is in inverse correlation to the weight of eels (Fig. 5) and the allometric equation for the relationship between body weight $W$ (g), and oxygen concentration $O$ (ppm), is represented in the equation $O = -0.1925W + 7.7760$, $r = 0.99$.

**DISCUSSION**

The effect of density on ammonia or DO concentration is a very important parameter in eel culture. Previous studies (Degani et al., 1985)
in our laboratory have shown that in high density (30 kg m\(^{-3}\)) the weight gain of eels was more than 1% per day and the production in this condition is more than 100 kg per year. The only problem with high density is the water quality. For this reason it is very important to develop an equation that may help us to calculate the effect of densities on water quality.

In this study it was found that the correlation between total ammonia concentration and the length of time the eels were in the containers is linear.

An ammonia concentration of 1–2 ppm does not decrease the growth rate of European eels (*A. anguilla*) (Degani *et al.*, 1985) and Japanese eels (*A. japonica*) (Usui, 1979). In this study the pH was between 7 and 7.4. Soderberg *et al.* (1983) found that the growth of *Salmo gairdneri* was not significantly correlated with average ammonia concentration in a high density culture.

The total ammonia concentration in water is affected not only by the fish density but also by protein level in the diet (Gallagher *et al.*, 1984; Degani *et al.*, 1985). In this study it was found that the total ammonia concentration increased with an increase in the protein level of the diet.

The optimum level of protein in a purified diet has been studied with Japanese eels (*Anguilla japonica*) (Yamazaki, 1966; Arai *et al.*, 1971, 1972; Nose & Arai, 1972) and European eels (*A. anguilla*) (Degani *et al.*, 1985). Both Japanese and European eels seem to require about 45% protein in their diet for maximum growth. The natural diet of wild eels is composed mainly of protein and some fat (Lecomte-Finiger, 1983). The protein is used not only for growth but also as the main source of energy.
The percentage of protein in eels diet is very high compared with that of many other fish used in aquaculture and especially warm water fish (NRC-NAS, 1983).

The results of this study, together with those of previous studies in our laboratory show that reducing the percentage of protein in the diet by replacement with carbohydrate (Degani et al., 1986; Degani & Levanon, 1987) or with fat (Degani, 1986) improves the water quality by decreasing the ammonia concentration in the water.

In this study the allometric equation described the relationship between body weight of eels and oxygen concentration in water. The basal metabolic rates of various species of Anguilla have been the object of many investigations (Egusa, 1958; Tarr & Hill, 1979; Gallagher et al., 1984; Degani & Gallagher, 1985). The specific oxygen consumption rate increased with the increase in body weight.

In this study only juvenile eels were measured. It is possible that the allocation equation for the relationship between body weight and oxygen concentration in larger fish is different.

REFERENCES


