Effect of operating conditions on water quality control in intensive shrimp mariculture ponds in developing countries by sterile seaweed

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不稔性海藻による開発途上国型集約エビ養殖池の水質制御に対する操作条件の影響

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モデル海水中においてアオサ(横浜産)のアンモニア窒素(AN)摂取速度ならびに成長速度を実測した。アオサのAN摂取ならびに成長を確認した。AN、炭素、リンの濃度、光合成光量子束密度の増加、温度の上昇とともに、摂取、成長いずれの速度も増加した。AN濃度が特に高い範囲においてアオサの白化が観察された。これらの実験結果に基づいた計算において、エビ養殖池のAN濃度を低く抑えることができるとともに副産物として大量のアオサを得ることができた。

1 Introduction

Since the 1990s, the shrimp industry, which has given high profit and foreign exchange to developing countries, has suffered many viral disease outbreaks [1]. To avoid entering viruses from external water, some shrimp farmers introduced none or quite small water exchange culture system. However, this system caused the serious water deterioration and the water quality control became more important. Especially, ammonia-nitrogen (AN) is toxic to shrimp [2][3]. So, to control AN concentration is important for shrimp mariculture. However, because of low shrimp value, shrimp mariculture in developing countries can't pay away a lot of money for water quality control. In previous study [4], water quality control of shrimp ponds using sterile seaweed was proposed. However this system is affected on environmental conditions. So to examine effect of operating conditions is important. In this study, we examined effect of operating conditions on water quality control in intensive shrimp mariculture ponds in developing countries by sterile seaweed. First, we measured AN uptake rate by and growth rate of sterile seaweed and examined effect of operating conditions for growth rate. Second, we calculated water quality control and growth of seaweed in shrimp pond.

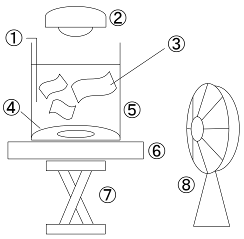
2 AN uptake by and growth of sterile seaweed

2.1 Materials

Sterile *Ulva* sp. collected from Umi no koen (Marine park) in Yokohama, Japan was used as sterile seaweed. Commercial sea salt was used to prepare artificial seawater (30 ‰). NH_4Cl, $Na_3PO_4{\cdot}12H_2O$, $NaHCO_3$ were used as sources of total ammonia-nitrogen (TAN), dissolved inorganic phosphorus (DIP), and carbon (DIC) in the seawater.

2.2 Experimental

Fig. 2.2-1 shows experimental apparatus. We measured TAN uptake rate by and growth rate of seaweed, and examined effect of total TAN concentration in culture medium, photosynthetic photon flux density (PPFD), and water temperature for growth rate. TAN concentration in liquid solution was determined by the indophenol blue method and growth rate of seaweed was calculated from mass increment of biomass.



① Temperature indicator
② Metal halide lamp
③ Sterile *Ulva* sp.
④ Nylon mesh
⑤ Plastic beaker
⑥ Magnetic stirrer
⑦ Jack
⑧ Fan

Fig. 2.2-1 Experimental apparatus

2.3 Results and discussion

2.3.1 TAN uptake rate of sterile seaweed

The initial specific uptake rate of TAN by seaweed, $\pi_{TAN,0}$, was obtained by

$$\pi_{\mathrm{TAN,0}} = -\frac{1}{\rho_{\mathrm{U}}}\frac{\mathrm{d}C_{\mathrm{TAN}}}{\mathrm{d}t}\bigg|_{t=0} \quad (1)$$

Fig. 2.3.1-1 shows the effect of initial TAN concentration on the initial specific uptake rate. It was reconfirmed that sterile *Ulva* sp. could take in TAN. The uptake rate increased, as TAN concentration increased. The relation between TAN concentration and uptake rate was fitted by Michaelis-Menten equation,

$$\pi_{\mathrm{TAN,0}} = \frac{\pi_{\mathrm{max}} C_{\mathrm{TAN}}}{K_{\mathrm{M}} + C_{\mathrm{TAN}}} \quad (2)$$

with the maximum specific uptake rate of TAN, π_{max}, and the half saturation constant of TAN concentration for uptake, K_M, as shown by the solid line in Fig. 2.3.1-1. The relation was well represented by this equation with $\pi_{max} = 11.6 \times 10^{-3}$ kg-N kgDM^{-1} h^{-1} and $K_M = 2.3 \times 10^{-3}$ kg-N m^{-3}.

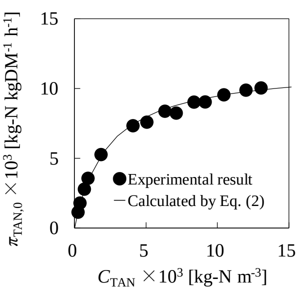


Fig. 2.3.1-1 Effect of TAN concentration on initial specific uptake rate

Table 2.3.1-1 Experimental conditions

$\rho_{U,0}$ [kgDM m^{-3}]	0.65
T [°C]	27 ± 1
I [μmol m^{-2} s^{-1}]	600
$C_{TAN} \times 10^3$ [kg-N m^{-3}]	0 - 13.1
$C_{DIP} \times 10^4$ [kg-P m^{-3}]	0
C_{DIC} [mM]	0

2.3.2 Effect of TAN concentration in culture medium on specific growth rate of sterile seaweed

The specific growth rate of seaweed, μ, was defined by,

$$\frac{\mathrm{d}\rho_{\mathrm{U}}}{\mathrm{d}t} = \mu\rho_{\mathrm{U}} \quad (3)$$

where ρ_{U} is density of seaweed in culture medium. Integration of this equation leads to,

$$\ln\frac{\rho_{\mathrm{U},t}}{\rho_{\mathrm{U},0}} = \mu t \quad (4)$$

where $\rho_{\mathrm{U},0}$ and $\rho_{\mathrm{U},t}$ are density of seaweed in culture medium at initial and t. **Fig. 2.3.2-1** shows time course of natural log of ratio of seaweed density to initial seaweed density in culture medium. The growth of the seaweed could be quantitatively detected in the range of this work. By eq. (4), the specific growth rate could be obtained from slope of Fig. 2.3.2-1.

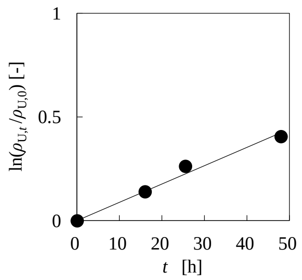


Fig. 2.3.2-1 Time course of natural log of ratio of seaweed density to initial seaweed density in culture medium

Table 2.3.2-1 Experimental conditions

$\rho_{\mathrm{U},0}$ [kgDM m^{-3}]	0.13
T [°C]	27 ± 1
I [μmol m^{-2} s^{-1}]	600
$C_{\mathrm{TAN}} \times 10^3$ [kg-N m^{-3}]	1.0
$C_{\mathrm{DIP}} \times 10^4$ [kg-P m^{-3}]	3.0
C_{DIC} [mM]	2.0

The specific growth rate is plotted against TAN concentration, C_{TAN}, in **Fig. 2.3.2-2**. The growth rate increased with increasing TAN concentration. In this study, effect of TAN concentration, PPFD, I and water temperature, T for specific growth could be described by,

$$\mu = \mu_{\max}\frac{C_{\mathrm{TAN}}}{K_{\mathrm{S}} + C_{\mathrm{TAN}}}\frac{I}{I_{\mathrm{S}} + I}\exp\left[-\frac{E}{R(T+273)}\right] \quad (5)$$

where $\mu_{\max}$ is the maximum specific growth rate. K_{S} and I_{S} are the half saturate constant of TAN concentration and PPFD for growth. E is the apparent activation energy for growth of seaweed, R is gas constant.

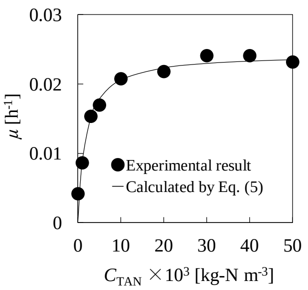


Fig. 2.3.2-2 Effect of TAN concentration on specific growth rate

Table 2.3.2-2 Experimental conditions

$\rho_{\mathrm{U},0}$ [kgDM m^{-3}]	0.13 - 0.18
T [°C]	27 ± 1
I [μmol m^{-2} s^{-1}]	600
$C_{\mathrm{TAN}} \times 10^3$ [kg-N m^{-3}]	0 - 50.0
$C_{\mathrm{DIP}} \times 10^4$ [kg-P m^{-3}]	3.0
C_{DIC} [mM]	2.0

2.3.3 Effect of PPFD on specific growth rate of sterile seaweed

The effect of PPFD on specific growth rate of seaweed is given in **Fig. 2.3.3-1**. When PPFD was 0 μmol m^{-2} s^{-1}, the seaweed did not grow. The specific growth rate increased with PPFD.

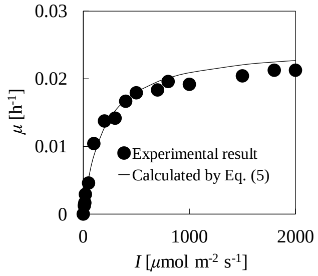


Fig. 2.3.3-1 Effect of PPFD on specific growth rate

Table 2.3.3-1 Experimental conditions

$\rho_{\mathrm{U},0}$ [kgDM m^{-3}]	0.13 - 0.18
T [°C]	27 ± 1
I [μmol m^{-2} s^{-1}]	0 - 2000
$C_{\mathrm{TAN}} \times 10^3$ [kg-N m^{-3}]	4.0
$C_{\mathrm{DIP}} \times 10^4$ [kg-P m^{-3}]	3.0
C_{DIC} [mM]	2.0

2.3.4 Effect of water temperature of culture medium on specific growth rate of sterile seaweed

Fig. 2.3.4-1 shows the effect of water temperature on specific growth rate. The growth rate had the maximum at around 30 °C over temperature. In higher temperature range, the chlorosis of seaweed was observed, which would result in the low growth rate. In this study, lower than 30 °C, effect of water temperature to the specific growth rate was fitted Arrhenius equation.

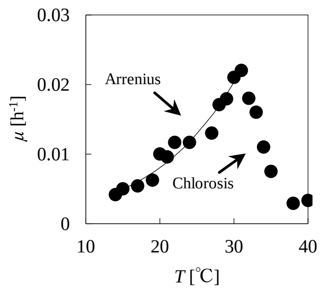


Fig. 2.3.4-1 Effect of water temperature on specific growth rate

Table 2.3.4-1 Experimental conditions

$\rho_{\mathrm{U},0}$ [kgDM m^{-3}]	0.13 - 0.18
T [°C]	14 - 40
I [μmol m^{-2} s^{-1}]	600
$C_{\mathrm{TAN}} \times 10^3$ [kg-N m^{-3}]	4.0
$C_{\mathrm{DIP}} \times 10^4$ [kg-P m^{-3}]	3.0
C_{DIC} [mM]	2.0

In this study, the maximum specific growth rate, $\mu_{\max}$, was 3 × 10^{10} h^{-1}. Half saturation constant of TAN concentration for growth, K_{S}, was 1.8 × 10^{-3} kg-N m^{-3}. Half saturate constant of PPFD for growth, I_{S} was 189 μmol m^{-2} s^{-1}. The apparent activation energy for growth of seaweed, E = 6.86 × 10^4 J mol^{-1}.

3 Calculation of water quality control and seaweed growth in shrimp pond

3.1 Nitrogen balance and growth of seaweed in shrimp pond

Nitrogen as the form of the shrimp feed is supplied into pond. Much of feed is eaten by shrimp and a portion of feed is uneaten. Shrimp excretes AN through respiration and other nitrogen metabolite (feces, shed shell etc.). AN also generate from sediment decomposition. On the other hand, in this study, AN in water column was removed by seaweed uptake only. Growth of seaweed is affected by AN concentration and environmental conditions (PPFD, water temperature).

3.1.1 Basic equation

Material balance;

The material balance of TAN in a unit volume of shrimp pond is given by,

$$\frac{dC_{TAN}}{dt} = r_{total,TAN} - r_{total,remove} \quad (6)$$

where $r_{total,TAN}$ is total generation rate of TAN, $r_{total,remove}$ is total removal rate of TAN.

3.1.2 TAN removal

The removal rate of TAN in a unit volume of shrimp pond is given by,

$$r_{total,remove} = \rho_U \pi_{TAN} \quad (7)$$

where ρ_U is density of seaweed in shrimp pond, π_{TAN} is the specific uptake rate of TAN by seaweed.

Growth yield of seaweed against TAN;

The specific uptake rate of TAN by seaweed, π_{TAN}, is described by,

$$\pi_{TAN} = \frac{\pi_{max} C_{TAN}}{K_M + C_{TAN}} \quad (8)$$

the maximum specific uptake rate of TAN, π_{max}, in eq. (8) is described by,

$$\pi_{max} \propto \mu_{max} \frac{I}{I_S + I} \exp\left[-\frac{E}{R(T + 273)}\right] \quad (9)$$

given $K_S = K_M$, π_{max} could be represented by,

$$\pi_{max} = \frac{\mu_{max}}{Y_G} \quad (10)$$

where growth yield of seaweed, Y_G, was 2.1 kgDM kg^{-1}-N.

The specific growth rate of seaweed;

The specific growth rate of seaweed is given by,

$$\mu = \mu_{max} \frac{C_{TAN}}{K_S + C_{TAN}} \frac{I}{I_S + I} \exp\left[-\frac{E}{R(T + 273)}\right] \quad (11)$$

the value of specific growth rate is changed with TAN concentration and environmental conditions in shrimp pond.

Environmental conditions;

Time course of PPFD in shrimp pond;

Time course of PPFD in shrimp pond is given by,

$$I = I_{max} \sin^2\left[\frac{\pi(t - 6)}{12}\right] \quad (6\ \mathrm{h} \le t < 18\ \mathrm{h}) \quad (12)$$

$$I = 0 \quad (0\ \mathrm{h} \le t < 6\ \mathrm{h},\ 18\ \mathrm{h} \le t < 24\ \mathrm{h}) \quad (13)$$

with PPFD at the time of maximum solar height (midday), I_{max} is 1800 μmol m^{-2} s^{-1} in fine days, is 100 μmol m^{-2} s^{-1} in cloudy or rainy days.

Time course of water temperature in shrimp pond;

Time course of water temperature in shrimp pond is given by,

$$T = T_{min} + (T_{max} - T_{min}) \sin\left(\pi \frac{t - LSH + \frac{DL}{2}}{DL + 2P}\right)$$
$$(6\ \mathrm{h} \le t < 18\ \mathrm{h}) \quad (14)$$

$$T = \frac{T_{min} - T_S \times \exp\left(-\frac{\eta}{\tau}\right) + (T_S - T_{min}) \times \exp\left(-\frac{t - t_s}{\tau}\right)}{1 - \exp\left(-\frac{\eta}{\tau}\right)}$$
$$(0\ \mathrm{h} \le t < 6\ \mathrm{h},\ 18\ \mathrm{h} \le t < 24\ \mathrm{h}) \quad (15)$$

with maximum daily water temperature, T_{max} = 30 °C, minimum daily water temperature, T_{min} = 25 °C, the time of maximum solar height (midday), LSH = 12 h, day length, DL is 12 h, the delay in T_{max} with respect to LSH, P = 2 h, sunset temperature, T_S = 28.5 °C, sunset time, t_S = 18 h, time coefficient, τ = 4 h^{-1} and night length η = 24 h - DL. **Fig. 3.1.2-1** and **Fig. 3.1.2-2** show time course of PPFD and water temperature in shrimp pond.

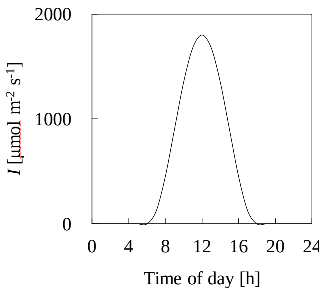


Fig. 3.1.2-1 Time course of PPFD

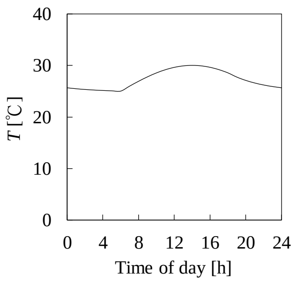


Fig. 3.1.2-2 Time course water temperature

Water balance in shrimp pond;

Water balance in shrimp pond is given by,

$$\Delta h = f_{Rain} + f_{Run} + f_{In} - f_{Evap} - f_{Out} - f_{Seep} - f_{Over} \quad (16)$$

where f_{Rain}, f_{Run}, f_{In}, f_{Evap}, f_{Out}, f_{Seep}, f_{Over} are water flow rate to and from shrimp pond, each is rainfall, runoff, inflow, evaporation, outflow, seepage, and overflow. In this study, f_{Run} = 1.25 × 10^{-5} m h^{-1}, f_{Evap} = 1.29 × 10^{-4} m h^{-1}, f_{Seep} = 2.17 × 10^{-4} m h^{-1}, f_{In} = f_{Out} = f_{Over} = 0 m h^{-1}, and on rainy days, f_{Rain} = 5.20 × 10^{-4} m h^{-1} and f_{Evap} = 0 m h^{-1}.

3.1.3 TAN generation

Total generation rate of TAN is given by,

$$r_{total,TAN} = r_{Shr,TAN} + r_{Sed,TAN} \quad (17)$$

where $r_{Shr,TAN}$ is rate of TAN excreted by shrimp respiration, $r_{Sed,TAN}$ is rate of TAN generated by sediment decomposition. $r_{Shr,TAN}$ is given by,

$$r_{Shr,TAN} = q_{TAN} r_{mtb,N} \quad (18)$$

where q_{TAN} is TAN fraction from shrimp respiration to total nitrogen metabolite. $r_{mtb,N}$ is excretion rate of nitrogen metabolite from shrimp per unit volume of shrimp pond and given by,

$$r_{mtb,N} = a_{mtb,N} \rho_S M_S^{\gamma} \quad (19)$$

where $a_{mtb,N}$ is nitrogen metabolite from shrimp per unit shrimp mass. ρ_S is culture density of shrimp. M_S is average shrimp mass and estimated from growth model. γ is allometric scaling of shrimp metabolism. On the other hand, $r_{Sed,TAN}$ is given by,

$$r_{Sed,TAN} = r_r M_{Sed,N} \quad (20)$$

where r_r is rate constant of decomposition. $M_{Sed,N}$ is amount of nitrogen contained in sediment per unit volume of shrimp pond. $M_{\mathrm{Sed,N}}$ include the sediment derived from uneaten feed and nitrogen metabolite from shrimp.

3.2 Main calculation conditions

In this study, *Penaeus monodon* was selected as model shrimp and cultivated 90 days. $C_{\mathrm{TAN},0}$ and $M_{\mathrm{Sed,N},0}$ were estimated 0 kg-N m^{-3}. $\rho_{\mathrm{U},0}$ was 1.0 kgDM m^{-3} and culture density of shrimp, $\rho_{\mathrm{S},0}$ was 40 m^{-3}. Pond area, *A* and depth, *h* were 4000 m^{2} and 1.0 m.

3.3 Results and discussion

In developing countries, recommended maximum TAN concentration in shrimp ponds is 1.0×10^{-3} kg-N m^{-3}. **Fig. 3.3-1** shows daily variation of TAN concentration in shrimp pond at 06:00 a.m. and 12:00 p.m. On each day, TAN concentration has large difference over the time of day. Since seaweed could take in TAN efficiency around 12:00 p.m., TAN concentration in shrimp pond was very low. On the other hand, from night to morning, seaweed could not take in TAN very well, so TAN concentration in the morning is high. However, using seaweed, TAN concentration was controlled under the recommended maximum concentration. **Fig. 3.3-2** shows time course of seaweed density in shrimp pond. Seaweed increased from 1.00 kgDM m^{-3} to 1.12 kgDM m^{-3} in 90 days. On this cultivation, we obtained 480 kgDM seaweed as by-product. However, since shrimp pond is large in area, seaweed density in shrimp pond did not increase excessively. So harvest of seaweed is not necessarily often times.

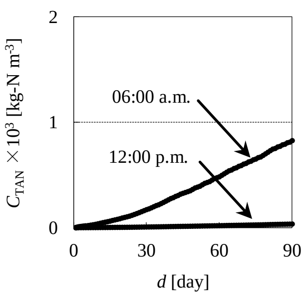


Fig. 3.3-1 Daily variation of TAN concentration in shrimp pond at 06:00 a.m. and 12:00 p.m.

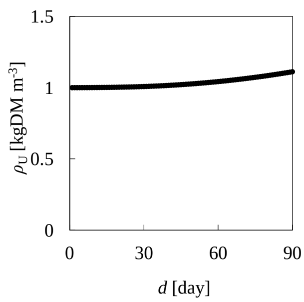


Fig. 3.3-2 Time course of seaweed density in shrimp pond

4 Conclusion

We could measure and quantitatively detect AN uptake and growth of sterile seaweed and examine the effect of operating conditions for specific growth rate and modeled mathematically. Next, the water quality control method by using sterile seaweed could remove AN in shrimp pond effectively and this method allows us to obtain a large amount of seaweed as by-product.

Nomenclature

A	Shrimp pond area [m^{2}]
$a_{\mathrm{mtb,N}}$	Nitrogen metabolite excretion rate [kg-N kg-shrimp^{-1} h^{-1}]
C_{TAN}	Total ammonia nitrogen (TAN) concentration [kg-N m^{-3}]
d	Cultivation time [day]
DL	Day length [h]
E	Apparent activation energy for growth of seaweed [J mol^{-1}]
f_{Evap}	Flow rate of evaporation water from shrimp pond [m h^{-1}]
f_{In}	Flow rate of inflow water to shrimp pond [m h^{-1}]
f_{Out}	Flow rate of outflow water from shrimp pond [m h^{-1}]
f_{Over}	Flow rate of overflow water from pond [m h^{-1}]
f_{Rain}	Flow rate of rainfall water to shrimp pond [m h^{-1}]
f_{Run}	Flow rate of runoff water to pond [m h^{-1}]
f_{Seep}	Flow rate of seepage water from pond [m h^{-1}]
h	Depth of shrimp pond [m]
I	Photosynthetic photon flux density (PPFD) [μmol m^{-2} s^{-1}]
$I_{\max}$	PPFD at the time of maximum solar height (midday) [μmol m^{-2} s^{-1}]
I_{S}	Half saturation constant of PPFD for growth [μmol m^{-2} s^{-1}]
K_{g}	Growth coefficient of shrimp [h^{-1}]
K_{m}	Existence coefficient of shrimp [h^{-1}]
K_{M}	Half saturation constant of TAN concentration for uptake [kg-N m^{-3}]
K_{S}	Half saturation constant of TAN concentration for growth [kg-N m^{-3}]
LSH	Time of maximum solar height [h]
M_{S}	Shrimp mass [kg-shrimp]
$M_{\mathrm{S},0}$	Shrimp mass at initial [kg-shrimp]
$M_{\mathrm{S,max}}$	Shrimp mass at maximum grown [kg-shrimp]
$M_{\mathrm{Sed,N}}$	Nitrogen in sediment per unit volume of pond water [kg-N m^{-3}]
P	Delay in $T_{\max}$ with respect to LSH [h]
q_{F}	Feed rate per shrimp mass [kg-feed kg-shrimp^{-1} h^{-1}]
q_{TAN}	TAN fraction from shrimp respiration to total nitrogen metabolite [-]
R	Gas constant [J mol^{-1} K^{-1}]
r_{F}	Feed rate per unit area of shrimp pond [kg-feed h^{-1}]
$r_{\mathrm{mtb,N}}$	Nitrogen metabolite excretion rate by shrimp [kg-N m^{-3} h^{-1}]
r_{r}	Rate constant of sediment decomposition [h^{-1}]
R_{S}	Fraction of shrimp assimilation in feed [-]
R_{Sed}	Fraction of leftover in feed [-]
$r_{\mathrm{Sed,TAN}}$	TAN generation rate from sediment [kg-N m^{-3} h^{-1}]
$r_{\mathrm{Shr,TAN}}$	Excretion rate of TAN by shrimp respiration [kg-N m^{-3} h^{-1}]
$r_{\mathrm{total,remove}}$	Total TAN removal rate [kg-N m^{-3} h^{-1}]
$r_{\mathrm{total,TAN}}$	Total TAN generation rate [kg-N m^{-3} h^{-1}]
t	Time [h]
T	Water temperature [°C]
$t_{\max}$	Cultivation time [h]
$T_{\max}$	Maximum daily water temperature [°C]
$T_{\min}$	Minimum daily water temperature [°C]
t_{S}	Sunset time [h]
T_{S}	Sunset temperature [°C]
Y_{G}	Growth yield of seaweed against TAN [kgDM kg^{-1}-N]
γ	Allometric scaling of metabolism [-]
η	Night length (24 h - DL) [h]
μ	Specific growth rate of seaweed [h^{-1}]
$\mu_{\max}$	Maximum specific growth rate of seaweed [h^{-1}]
$\pi_{\max}$	Maximum specific uptake rate of TAN [kg-N kgDM^{-1} h^{-1}]
π_{TAN}	Specific uptake rate of TAN [kg-N kgDM^{-1} h^{-1}]
$\pi_{\mathrm{TAN},0}$	Initial specific uptake rate of TAN [kg-N kgDM^{-1} h^{-1}]
ρ_{S}	Culture density of shrimp [m^{-3}]
ρ_{U}	Density of seaweed in culture medium [kgDM m^{-3}]
$\rho_{\mathrm{U},0}$	Density of seaweed in culture medium at initial [kgDM m^{-3}]
$\rho_{\mathrm{U},t}$	Density of seaweed in culture medium at t [kgDM m^{-3}]
τ	Time coefficient [h^{-1}]

Literature Cited

[1]Primavera, J.H.; "Tropical Shrimp Farming and Its Sustainability," *Tropical Mariculture*, S. S. De Silva ed., pp. 257-289, Academic Press, San Diego, U.S.A. (1998)

[2]Haywood, G.P.; "Ammonia Toxicity in Teleost Fishes: A Review," Can. Tech. Rep. *Fish Aquat. Sci*., 1177, 1-35 (1983)

[3]Chen, J. C. and C. C. Tu; "Influence of Ammonia on Growth of Penaeus monodon Fabricius Post–Larvae," *Aquaculture and Fisheries Management*, 22, 457-462 (1991)

[4]Sato, K., Eksangri, T., Egashira, R.; "Ammonia-Nitrogen Uptake by Seaweed for Water Quality Control in Intensive Mariculture Ponds," *J. Chemical Engineering of Japan*, 39, 247-255 (2006)

[5]Burford, M. A., Lorenzen, K., "Modeling nitrogen dynamics in intensive shrimp ponds: the role of sediment remineralization," *Aquaculture*, 229, 129-145(2004)