Effect of Stocking Density on the Energy Budget of Juvenile Soft-Shelled Turtles (*Pelodiscus sinensis*)

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Abstract.- The present work investigates the effect of stocking density on the energy budget of juvenile soft-shelled turtles (*Pelodiscus sinensis*). Turtles (body weight: 16.22±0.28 g) were stocked at densities of SD1 (8 animals/m^2, 0.14 kg/m^2), SD2 (48 animals/m^2, 0.81 kg/m^2) and SD3 (96 animals/m^2, 1.62 kg/m^2) in aquaria in triplicate for each treatment. The experiment lasted for 35 days. Survival rate, coefficient of size variation, productivity, and apparent digestibility coefficient were not significantly different at the three stocking densities. While there were no significant differences between treatments SD2 and SD3, turtles in group SD1 showed a lower excretion rate and significantly higher food intake and growth rate. Turtles in group SD1 also showed higher crude lipid content and lower crude ash content. No significant differences were found among the treatments in body moisture and crude protein.

Keywords.- Survival, growth, food consumption, stress, body composition.

Introduction

Stocking density is one of the most important biotic factors in aquaculture because it directly influences survival, growth, behavior, health, feeding, and production. High densities may interfere with intra-population interactions and eventually affect biomass gain. The relationship between stocking density and growth for fish has been shown to be positive (Papst et al., 1992), negative (Hengsawat et al., 1997; Irwin et al., 1999) or density-independent (Fairchild and Howell, 2001; Rowland et al., 2004; Rowland et al., 2006), depending on different experimental density ranges. In the fish farming industry, it is very important for the farmer to know the optimum stocking density of the animals being reared to maximize production and profitability.

The soft-shelled turtle (*Pelodiscus sinensis*) is a commonly cultured aquatic reptile species in China with a yield of more than 140,000 tons in 2004 (Shen et al., 2006; Zhang, 2005). Despite the fact that the aquaculture of this species is widespread, scientific studies concerning the effects of stocking density on biological characters are limited (Mayeaux et al., 1996). The objective of the present study is to evaluate the effect of stocking density on the energy budget of juvenile *P. sinensis*.

Materials and Methods

Turtles and rearing conditions. - Juvenile *Pelodiscus sinensis* (body weight: 16.22±0.28 g) were obtained from a commercial turtle farm in Beijing. Turtles were reared in rectangular aquaria (80 length [L] × 35 width [W] × 30 cm height [H]), with 11 individuals per aquarium, at a water depth of 15 cm. Water temperature was maintained at 29.5±0.5ºC by a thermo-controlled heater. Aquaria were supplied with dechlorinated water. The dissolved oxygen level was over 5 mg/L and the pH was 7.95. Natural photoperiod was followed. Turtles were fed to satiation once daily at 1500 h. Commercial turtle food was used with 0.5% Cr_2O_3 added for the apparent digestibility coefficient assay. Proximate dry matter composition of the diet was as follows: moisture 3.97%; crude protein 40.27%; crude lipid 7.04%; and crude ash 15.73%. Energy content was 16.06 kJ/g. Turtles were allowed to acclimate to the laboratory conditions for three weeks before the experiments began.

Experimental process. - Healthy turtles were randomly stocked at initial densities of SD1 (8 animals/m^2, 0.14 kg/m^2), SD2 (48 animals/m^2, 0.81 kg/m^2) and SD3 (96 animals/m^2, 1.62 kg/m^2) in aquaria (40 L ×30 W ×30 cm H) in triplicate for each treatment. There were no significant differences in initial average body weight or coefficient of size variation within each aquarium among the treatments. The experiment lasted for 35 days. The final densities were 0.34 kg/m^2, 1.20 kg/m^2 and 2.26 kg/m^2, respectively. To maintain a constant numbers of animals, an alternative turtle with approximately the same body weight was added when an initial turtle died. All the water in the tanks was replaced by an equal amount of fresh water daily after surplus food was removed. The aquaria were inspected once daily for mortalities and dead turtles were removed immediately after detection.

Table 1. Survival, specific growth rate, food consumption, apparent digestibility coefficient, and excretion of juvenile soft-shelled turtles (*Pelodiscus sinensis*) held at different stocking densities (Mean ± S. E.)[1].

Treatment	SD1	SD2	SD3	*P*-Value
Initial average weight (g)	16.90±0.45	16.27±0.23	16.15±0.16	0.294
Final average weight[3] (g)	40.30±1.96[a]	24.09±0.38[b]	22.49±0.70[b]	0
Survival rate (%)	100±0.00	91.67±8.33	77.78±10.02	0.1
Coefficient of size variation (%)	—	30.82±5.24	26.84±2.72	0.504
Specific growth rate[2] (%/day)	2.48±0.22[a]	1.12±0.09[b]	0.94±0.11[b]	0.002
Productivity (g/day/m²)	5.57±0.57	7.08±4.96	7.60±7.98	0.963
Food consumption[3] (mg/day/g)	40.54±0.46[a]	25.76±0.43[b]	24.34±0.62[b]	0
Apparent digestibility coefficient (%)	74.29±2.12	75.60±0.10	78.14±1.44	0.319
Excretion (mg N/day/kg)	60.44±10.64[b]	104.36±5.75[a]	108.59±3.54[a]	0.012

[1]Values in each row with different superscript letters are significantly different ($p < 0.05$).

[2]Means are significantly different among treatments ($p < 0.01$).

[3]Means are extremely different among treatments ($p < 0.001$).

Turtles were weighed to an accuracy of 0.1 g before and after the experiment following three days starvation. Six turtles at the beginning of the experiment and all turtles remaining at the end of the experiment were sacrificed and dried at 65ºC to constant weight for analysis of body biochemical composition. Crude protein was determined by the Kjeldahl method, crude lipid was extracted by ether, and crude ash was determined after 12 h of burning at 550º in a muffle furnace. Energy contents were measured using a calorimeter (CA-4P, Shimadzu, Japan). All samples were analyzed in triplicate.

Measurements of various components of the energy budget. - A weighed excess of feed pellets was fed to the turtles once daily (at 1500 h) with a fraction of feed retained for determination of dry matter content. Uneaten food was collected an hour later and dried. Food intake was determined as the difference between the food supplied and the food left uneaten.

Fresh complete feces were collected once daily. Cr_2O_3 content in the diet and feces were determined by the method described in detail by Bolin et al. (1952). The apparent digestibility coefficient (ADC) and the energy lost via feces (F) were calculated by the following expressions:

$$ADC\ (\%) = 100 \times (1 - Cr_2O_3 \text{ content in diet}/Cr_2O_3 \text{ content in feces})$$

$$F = I \times (100 - ADC) \times E_f/100$$

where I and E_f are food consumption in dry weight and feces energy content, respectively.

The coefficient of variation in body weight (CV) within each aquarium, specific growth rate (SGR), and productivity were calculated by the following formulae:

$$CV\ (\%) = 100 \times \text{Standard deviation/Average body weight}$$

$$SGR\ (\%/day) = 100 \times (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

$$Productivity\ (g/day/m^2) = (W_{t2} - W_{t1})/S/(t_2 - t_1)$$

where W_2 and W_1 express final average weight at time t_2 and initial average weight at time t_1 in days, respectively. W_{t2}, W_{t1} and S express biomass at day t_2, biomass at day t_1, and aquarium surface area, respectively. Energy allocated to growth was calculated from weight gain (g) and energy content (kJ/g) of the whole body.

Energy lost via excretion was calculated from the ammonia and urea excreted using energy equivalents for ammonia (24.83 J/mg N) and urea (23.03 J/mg N) (Elliott, 1976). Ammonia and urea concentrations inside the water were measured by firstly catalyzing urea to ammonia using urease, and then assaying the total ammonia via standard Nessler's colorimetric technique. The turtles were kept in a given amount of renewed experimental water for 48 h and fasted during the measurement. Water samples were taken before and after this period.

The energy budget for juvenile animals can be described as:

$$C = F + U + R + G$$

where C is the energy in the food consumed, F is the energy lost in fecal production, U is the energy lost in nitrogenous excretory products, R is the energy spent in metabolism, and G is growth energy. In this study, C, F,

Table 2. Chemical composition of juvenile soft-shelled turtles (*Pelodiscus sinensis*) held at different stocking densities in 35-day experimental period (Mean ± S. E.)[1].

Treatment	Moisture (%)	Crude protein*(%)	Crude lipid* (%)	Crude ash*,[2] (%)
SD1	75.86±0.28	63.58±0.65	13.46±0.62[a]	19.16±0.15[b]
SD2	76.27±1.45	65.05±0.13	8.31±1.01[b]	22.39±0.54[a]
SD3	76.46±0.83	61.89±1.04	10.13±0.73[b]	23.11±0.22[a]

*Crude protein, crude lipid, and crude ash based on the contents in the dry matter.
[1]Values in each column with different superscript letters are significantly different $p < 0.05$).
[2]Means were extremely different among treatments ($p < 0.001$).

U, and G were determined directly, and R was calculated by the equation:

$$R = C - F - U - G.$$

Statistical analysis.- All data were analyzed with SPSS for Windows, Version11.0. A one-way ANOVA was used to test the differences among treatment means when assumptions of normality and homogeneity were met. When a significant treatment effect was found, the Least-Significant-Difference (LSD) test was applied to determine which specific pairs differed. The nonparametric Kruskal-Wallis test was applied when the required homogeneity of variance and normality were not satisfied. A regression analysis was carried out to estimate the relationship between stocking density and growth rate. The significant level was set at $p < 0.05$.

Results

All turtles in one aquarium of treatment SD2 died due to a malfunction of the thermo-controlled heater. This replicate was not taken into account for any statistical comparisons.

The effects of stocking density on survival, specific growth rate, food consumption, apparent digestibility coefficient, and excretion.- Survival rates were not significantly different among the three treatments (Table 1), but all showed a negative relationship with increased stocking density (r = -0.708, $p = 0.050$). Stocking density showed a clear influence on final body weight, specific growth rate, food consumption, and excretion, as identified by the statistical significance (Table 1). Turtles in group SD1 showed significantly higher food intake and growth rate than those held at the other two densities, which did not differ significantly from each other. Lower excretion rate was observed in group SD1 compared to groups SD2 and SD3. The apparent digestibility coefficients of juvenile turtles ranged from 74.29% to 78.14%. The relationship of SGR and stocking density (SD, animals/m^{2}) can be described as the lin-

ear model or the quadratic model:

SGR = -0.0171× SD + 2.4360
($p < 0.01$, $R^2 = 0.769$)

SGR = 0.0003 × SD[2] - 0.0531× SD + 2.8805
($p < 0.01$, $R^2 = 0.915$).

The effect of stocking density on body composition.- Body composition for each treatment group is shown in Table 2. There were significant differences in lipid and ash contents between treatments ($F_{2,5} = 11.520$, $p = 0.013$; $F_{2,5} = 64.577$, $p = 0.000$). Crude lipid contents of group SD1 were much higher than those of groups SD2 and SD3 while crude ash contents were lower. No significant differences were found among treatments in body moisture and crude protein ($F_{2,5} = 0.155$, $p = 0.860$; $F_{2,5} = 3.412$, $p = 0.116$).

The effect of stocking density on energy budget.- No marked differences were found among treatments in F/C and R/C (Table 3; $F_{2,5} = 0.058$, $p = 0.945$; $F_{2,5} = 2.561$, $p = 0.171$). Stocking density significantly influenced U/C and G/C ($F_{2,5} = 27.151$, $p = 0.002$; $F_{2,5} = 6.243$, $p = 0.044$). Energy budgets for the different treatments can be described as:

100C = 10.0F + 0.3U + 73.3R + 16.5G; SD1
100C = 10.3F + 0.7U + 78.3R + 10.8G; SD2
100C = 10.0F + 0.8U + 79.1R + 10.2G; SD3

Discussion

Stocking density has been considered to be chronically stressful to reared animals (Vijayan and Leatherland, 1988). Several studies have also demonstrated that increased stocking density has a negative effect on survival and growth (Penha-Lopes et al., 2006; Schram et al., 2006), except in some fish species that exhibit schooling behavior (Jørgensen et al., 1993; Papoutsoglou et al., 1998). This impaired growth by stocking density may be attributed to reduced food con-

Table 3. Energy budget of juvenile soft-shelled turtles (*Pelodiscus sinensis*) held at different stocking densities in 35-day experimental period (Mean ± S. E.)[1].

Treatment	C[3] (J/day/g)	F/C (%)	U/C[2] (%)	R/C (%)	G/C (%)
SD1	651.21±7.33[a]	10.04±0.83	0.31±0.06[b]	73.29±2.61	16.45±1.77[a]
SD2	413.83±6.89[b]	10.31±0.04	0.74±0.06[a]	78.32±0.63	10.75±0.63[b]
SD3	390.96±9.94[b]	9.96±0.65	0.79±0.03[a]	79.12±1.56	10.23±1.13[b]

C: food intake in energy; F: energy lost in fecal production; U: energy lost in nitrogenous excretion; R: energy lost in metabolism; G: energy allocated to growth.
[1]Values in each column with different superscript letters are significantly different ($p < 0.05$).
[2]Means are significantly different among treatments ($p < 0.01$).
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sumption, lowed food conversion rate or increased metabolic cost (Jørgensen et al., 1993; Li and Brocksen, 1977; Vijayan and Leatherland, 1988).

In the present study, an obvious trend of decreased survivorship of juvenile soft-shelled turtle with elevated stocking density was observed. This agrees with the results of Mayeaux et al. (1996), who reported that common snapping turtles (*Chelydra serpentine*) stocked at 58 animals/m^{2} exhibited greater mortality, lower weight gain, and higher food consumption compared to those stocked at 29 animals/m^{2}. Food consumption also reduced with increasing stocking density in the present study, however, conflicting with the above snapping turtle results. This discrepancy may be caused by differences in life habit or varying experimental conditions. Knights (1985) observed that more aggressive (and usually larger) eels of *Anguilla anguilla* ate more, while the feeding of smaller subordinates was inhibited, even when food was offered to excess. In the present experiment, despite food being divided between at several spots in each tank, a similar phenomenon was observed during the feeding process. It is likely that the appetite of the subordinate turtles was suppressed and their growth inhibited when the turtles were grouped at high density.

The lower feed intake in treatments SD2 and SD3 appear to explain the lower growth in the same treatments, because reduced food ingestion reduces the amount of energy available for growth (Table 1). Moreover, the proportions of food energy spent in growth (indicated by the gross energy efficiency, Table 3) in treatments SD2 and SD3 were obviously lower in relation to that in treatment SD1 (10.8% and 10.2%, compared to 16.5%). These results may suggest that the ingested energy was not efficiently converted to body reserves, especially at high stocking density.

Juvenile soft-shelled turtles tend to grab each other with their sharp claws, and grabbing activities often result in injuries of the toes and neck, and may even result in death. Agonistic interactions among individuals and elevated swimming activity also lead to increased metabolic expenditure. In the present experiment, turtles of groups SD2 and SD3 exhibited hyperactivity compared to group SD1, and had reduced lipid and higher ash contents. The difference in chemical composition in these turtles suggest that elevated stocking density may induce extra energy expenditure, subsequently allocating less energy to storage.

In conclusion, the pattern of energy allocation of the turtles in the present experiment was significantly influenced by different stocking densities. Turtles cultured at lower density had a relatively higher survival rate, distinctly higher growth rate and transfer more consumed energy to growth. The lower energy input and lower gross energy efficiency in treatments SD2 and SD3 may have contributed to their reduced growth rate. Furthermore, the excretion of nitrogenous wastes to the environment was relatively lower with reduced stocking density. Conversely, higher stocking density could result in higher productivity to some degree, since there were no significant differences in productivity among the treatments. We suggest that the turtle farmer pursue an optimal stocking density based on profitability, considering that lower stock densities are shown to be related to increased survivorship, growth rate and feed utilization, while also being associated with a reduction in nitrogenous wastes.

Acknowledgments

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