

carrying capacity via high biodeposit flux and suboptimal cage setup can negate growth despite adequate food, emphasizing the need to integrate hydrodynamic and deposition patterns into density decisions (Cutajar et al., 2022; Sadoul et al., 2022). Within each model, management measures such as feeding regime, cleaning frequency, and staged stocking adjust the effective carrying capacity over time. In intensive culture of *Holothuria scabra*, high-quality diets can partially offset density-related growth limitations by improving feed conversion and protein efficiency, but crowding still affects survival, health, and feeding, indicating that management can shift but not eliminate density thresholds. In ranching of *Holothuria atra*, repeated stocking events and monitoring of sediment pigments allowed managers to time low-density stockings to periods when microphytobenthos had recovered, thereby improving growth while maintaining acceptable survival at higher densities, demonstrating how adaptive stocking schedules and habitat monitoring can dynamically regulate carrying capacity in open systems (Hartati et al., 2020).

4 Growth Performance Indicators and Statistical Analysis

4.1 Construction of growth indicator systems (weight gain rate, specific growth rate, survival rate, etc.)

In density and feeding experiments on sea cucumbers and other aquaculture species, growth performance is typically quantified through a set of complementary indicators that capture both absolute and relative changes in body mass and population status. For juvenile *Holothuria tubulosa*, growth was monitored by wet weight and summarized using weight gain (WG), growth rate (GR), specific growth rate (SGR), relative weight gain (RWG), coefficient of variation (CV), and survival rate (SR) over the rearing period. Similar indicator suites have been applied for *Holothuria poli* in long-term integrated multi-trophic aquaculture, where relative weight gain, growth rate, SGR and survival rate were calculated from initial and final wet weights and numbers per cage. These indicators are derived from standardized formulas that facilitate comparison across treatments and studies. For *H. poli*, RWG is defined as $100 \times (W_2 - W_1) / W_1$, GR as $(W_2 - W_1) / t$, SGR as $100 \times (\ln W_2 - \ln W_1) / t$, and SR as $100 \times (n_2 / n_1)$, using initial and final mean wet weight (W_1 , W_2), culture duration (t), and animal numbers (n_1 , n_2) (Cutajar et al., 2022). In sea cucumber density trials, these metrics have been sensitive to stocking density, with SGR, RWG and SR often declining at higher densities, underscoring their suitability as core indicators for evaluating growth responses to crowding.

4.2 Measurement methods for feeding efficiency and energy utilization indicators

Feeding efficiency in sea cucumbers is commonly evaluated through food conversion efficiency (FCE), protein efficiency ratio (PER), apparent digestibility coefficients (ADCs) and related energy budget components. In a 60-day study on *Apostichopus japonicus*, diets with graded biofloc levels significantly affected SGR, FCE and PER, and allowed estimation of optimal dietary replacement levels using polynomial regression of SGR and FCE. Energy allocation was expressed using a full budget (e.g., $100C = 3.8G + 65.4F + 3.3U + 27.5R$), partitioning ingested energy (C) into growth, feces, excretion and respiration, which revealed how diet altered the balance between growth deposition and metabolic losses. Under combined feeding-frequency and density manipulation, energy budget indicators were further linked to crowding stress. In *A. japonicus*, feed intake, energy intake and feces production rate were significantly affected by both feeding frequency and stocking density, and high density reduced food conversion efficiency and protein efficiency ratio while decreasing apparent digestibility of crude protein and lipid. The same study quantified how crowding shifted energy allocation, with energy for growth decreasing and metabolic energy increasing as density rose, supported by endocrine indicators such as elevated cortisol and lactate.

4.3 Statistical analysis methods (ANOVA, multiple comparisons, and regression analysis)

Analyses of density effects on growth indicators in sea cucumbers and other cultured species rely heavily on ANOVA and multiple comparison procedures to test for treatment differences. For *Holothuria scabra*, two-way ANOVA was used to evaluate the effects of feeding regime and stocking density on total and monthly weight gain, with one-way ANOVA applied to daily weight gain and SGR; significance was set at $P < 0.05$ (Figure 1). Similar designs are widely used in density-growth studies of fish and invertebrates, typically followed by Tukey's or Duncan's multiple range tests to identify which stocking densities differ in growth, feed conversion or survival.