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# The effect of food conditioning on feeding and growth responses to variable rations in fast and slow growing spat of the Manila clam (*Ruditapes philippinarum*)

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ABSTRACT

Two groups of clam spat (*Ruditapes philippinarum*) belonging to different growth categories of fast (F) and slow (S) growers were each conditioned to diets of low and high phytoplankton concentration (0.3 and 1.0 mg POM $l^{-1}$, respectively), and their components of energy balance measured across a range of food concentrations, in order to ascertain the combined contribution of endogenous and exogenous (food ambient) factors to growth performance. Analysis of the physiological traits indicates that both higher food acquisition and higher metabolic efficiencies were responsible for faster growth rates recorded with F spats in the tested range of food concentration (0.3 to 1.1 mg POM $l^{-1}$). In both types of clams acute response to increased food availability involved ingestion regulation by adjusting clearance rates was observed, thus helping to maintain nearly constant absorption efficiency values (around 70%) across food levels. Optimal ration (that providing maximum ingestion and scope for growth values) was found to rise from mid- (0.65 mg POM $l^{-1}$) to high food concentration (1.1 mg POM $l^{-1}$) following the change in conditioning from low to high food levels. Increased capacity to process food efficiently is accounted for by greater digestive investments in clams conditioned to high ration that results in increased metabolic costs of maintenance (recorded as rates of oxygen consumption after 4 days starvation), and associated to decline in growth performance during transitory exposure to poor food conditions.


## 1. Introduction

Effects of availability and composition of suspended food on feeding physiology and growth of marine bivalves has been the subject of extensive research for many years (see reviews by Bayne, 1998; Gosling, 2003; Jørgensen, 1990, 1996). Most studies on this subject were based on field and laboratory experiments involving short-term exposures to variable diets, and so much less attention has been paid to physiological responses over longer time-scales. Brood-stock conditioning of bivalves for aquaculture purposes serves as an example in which long-term effects of varying food ration has been broadly examined (Berntsson et al., 1997; Delaporte et al., 2006; Hendricks et al., 2003; Utting and Millican, 1997), but selection of traits for analysis (either fecundity or quality of eggs and larvae) tends to overlook basic physiological processes underlying the energetics of growth and reproduction.

Bayne (1993) has reviewed the characteristics of physiological compensation by filter feeding bivalves of changes in suspended food over different time-scales; while short-term responses (minutes to hours) are mainly based on adjustments of the ciliary machinery involved in clearance and selection of particles over the gills and palps (i.e., the pre-ingestive level). Adaptive responses over longer temporal scales (days to months) would rely on the ability to modulate processes within the digestive system, resulting in changes at functional (digestive enzyme investments) and even structural levels (morphology of digestive tubules). Experiments designed specifically to test the effects of previous feeding history on physiological behavior of bivalves are scarce. However, results are rather conclusive regarding the effects of acclimation to different diets on time-dependent increments in rates of absorption (Bayne et al., 1993; Ibarrola et al., 1998a, 2000a,b; Navarro et al., 2009). The suit of digestive adjustments accounting for these effects involved different mechanisms acting co-ordinately: from changes in the production of digestive enzymes (Hawkins and Bayne, 1992; Ibarrola et al., 1999, 2000c; Navarro et al., 2009) and associated modification of morphometric parameters of digestive diverticula (Ibarrola et al., 2000c), to time-dependent increments in the gut volume that is occupied by the ingesta (gut fullness). Such adjustments facilitate increases in the absorption efficiency with little change in gut passage time of food (Bayne et al., 1987; Ibarrola et al., 2000a; Navarro et al., 2009).

This phenotypic flexibility in feeding and digestive behavior contrasts with the persistence of physiological differences among groups of individuals conforming differentiated growth categories of possible genetic origin (Bayne, 1999a,b; Bayne et al., 1999a,b; Pace et al., 2006; Pernet et al., 2008; Toro and Vergara, 1998). According to

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Bayne (2004), characteristics of this contrast would be "considerable behavioral flexibility in response to changes in the food environment, linked with a strong genetic component to growth through a complex synergy of physiological traits" (p. 425). Thus, an outstanding approach concerning the adaption capabilities of bivalves to the food environment would be to assess the extent to which physiological behavior underlying growth performance is genetically determined and to question how much of this behavior can, on its side, be environmentally modulated in order to achieve a more effective exploitation of available food resources within the limits set by the genetic constitution of individuals.

In a precedent contribution (Tamayo et al., 2011) we describe an experimental approach that suits the requirements for the combined assessment of endogenous and exogenous (food conditioning) factors affecting the physiological components of growth in Manila clams. Two groups of clam spat – fast (F) and slow (S) growth – were grown in the laboratory and fed on two different food rations, henceforth high ration (H) and low ration (L). Combination of both factors resulted in differentiated growth trends for specimens belonging to each of the 4 combinations of F and S clams maintained with H and L food rations, respectively. In the course of these experiments, F clams maintained under L rations were caught up in size by S clams fed on H rations. Nevertheless, subsequent feeding of these F with H rations resulted in restoration of original size differences between growth groups that became statistically significant after ~25 days, thus indicating the persistence of an endogenous factor in growth differentiation. Physiological differences between F and S clams accounting for this endogenous component were found to involve parameters regulating both energy acquisition and metabolic costs of growth (Tamayo et al., 2011).

In the present experiments feeding and growth responses to short-term changes in food availability were compared in these groups of clams in order to test the hypothesis that a) physiological variability accounts for by differential growth trajectories associated to each combination of growth category (F and S) and conditioning ration (H and L) and b) physiological traits involved in growth performance under variable food environments are modulated through a combination of endogenous and exogenous factors.

## 2. Material and methods

Juvenile clams (spat) of the species *Ruditapes philippinarum* belonging to a single cohort, and reared under uniform hatchery conditions, were selected for different rates of growth by choosing specimens at the extremes of their size distribution (percentiles $P_{15}$ and $P_{85}$). The resulting two groups of fast (F) and slow (S) growers were maintained for some 4 months in separate batches under identical feeding conditions (a ration of phytoplankton representing 5% of live weight per day; see Tamayo et al., 2011). These juvenile clams were supplied by the Marine Culture Farm of Tinamenor S.L. (Pesués, Cantabria, Spain) to be used in present experiments.

### 2.1. Experimental design

Physiological components of the energy balance and scope for growth (SFG) were compared for F and S clams under different feeding conditions that included different ration levels, both previous to taking physiological measurements (conditioning period) and during these measurements. This experimental design, aiming to assess the combined effect of prior and present dietary conditions on the physiological response of both types of clams, was performed in three independent experiments according to the scheme shown in Table 1. Experiment 1 served as a control for the physiological status of clams on arrival to laboratory, while Experiments 2 and 3 involved conditioning to low and high ration levels respectively, followed by physiological determinations in both conditioning groups under variable food conditions.

Recording of growth rates during permanence time of clams in the laboratory was undertaken by weekly determinations of individual live weight in four groups of 40 clams, each group corresponding to a combination of growth category (F or S) and level of ration (low or high concentrations of phytoplankton for Experiments 2 and 3 respectively; Fig. 1). Growth rates in each of these groups were found to be constant over the experimental period (Tamayo et al., 2011). Thus, the growth control ensured that clams in each conditioning group belonged to the same growth category, regardless of the moment in which physiological measurements were taken and the size attained at that moment.

Experiment 4: In order to analyze the influence of previous feeding history on levels of metabolism in F and S clams, oxygen consumption rates of individuals acclimated to low and high food rations (from Experiments 2 and 3, respectively) were recorded following 1, 2 and 4 days of starvation. Starvation was assumed to gradually suppress metabolic costs of activity (feeding and food processing), thus reverting metabolism to a standard level representative of maintenance costs.

### 2.2. Maintenance conditions and diet characteristics

Clams were maintained in the laboratory within feeding tanks inside large aerated aquaria (800 l) provided with re-circulating sea water regulated at 17 ± 1 °C and 33 psu salinity. Clams were fed the diets described below:

Food consisted of suspended particles of either monocultures of the alga *Isochrysis galbana* (T-ISO) (in maintenance diets) or mixtures of this alga and silt particles (in measurement diets). Use of silt in these diets aimed at fulfilling the requirements for an inorganic tracer in measurements of absorption efficiency with the Conover method.

**Table 1**
Experimental design: Conditioning parameters (ration and time), characteristics of experimental diets and mean sizes (soft-body dry weight) of fast (F) and slow (S) growing individuals used in Experiments 1, 2 and 3.

| Experiment | Conditioning ration (mg POM $l^{-1}$) | Conditioning time (days) | Measuring ration (mg POM $l^{-1}$) | O.C. (decimal) | Growth group | Soft body dry wt. (mg) | Diet notation |
|---|---|---|---|---|---|---|---|
| 1 | 1.00 | 2 | 1.16 ± 0.02 | 0.82 ± 0.01 | F | 28.92 ± 3.82 | H |
| | | | | | S | 7.82 ± 1.11 | |
| 2 | 0.33 | 30 | 0.32 ± 0.03 | 0.84 ± 0.08 | F | 25.01 ± 4.80 | LL |
| | | | | | S | 8.67 ± 1.64 | |
| | | 20 | 0.65 ± 0.10 | 0.83 ± 0.01 | F | 26.71 ± 4.38 | LM |
| | | | | | S | 8.88 ± 1.83 | |
| | | 40 | 1.02 ± 0.03 | 0.88 ± 0.02 | F | 27.41 ± 1.55 | LH |
| | | | | | S | 8.34 ± 0.44 | |
| 3 | 1.00 | 70 | 0.33 ± 0.01 | 0.86 ± 0.04 | F | 81.04 ± 13.87 | HL |
| | | | | | S | 30.10 ± 4.11 | |
| | | 60 | 1.08 ± 0.16 | 0.85 ± 0.04 | F | 78.44 ± 17.09 | HH |
| | | | | | S | 27.30 ± 2.08 | |

POM: particulate organic matter; O.C.: organic content.
Values are means (±SD). n = 10 for dietary parameters (Measuring ration and O.C.); n = 5 and 15 for sizes of F and S clams, respectively.