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Predicting phenotypic variation in growth and metabolism of marine invertebrate larvae

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ABSTRACT

Understanding the mechanisms that establish variation in growth and metabolism is fundamental in evolutionary and physiological ecology. Although a genetic basis is frequently invoked to explain variation in performance, it remains challenging to study such processes in marine animals due to the lack of genetically-enabled "model" organisms. The Pacific oyster *Crassostrea gigas* is a species for which pedigreed genetic lines have been established. In this study, a series of larval families was produced by crossbreeding pedigreed lines to yield large-volume larval cultures to provide sufficient biomass for biochemical and physiological analyses. Major phenotypic contrasts in larval growth rate were evident. A primary goal of this study was to investigate the physiological bases for this variation in growth and to identify biomarkers that are predictive of growth potential. To that end, measurements were undertaken to define the relationship between rates of growth, respiration, and ion transport by the sodium-potassium pump (in vivo Na^+,K^+-ATPase activity). The relationship of respiration and ion transport during larval growth showed that, on average, 17% of total energy demand was allocated to support ion transport. Further analyses of total Na^+,K^+-ATPase activity (in vitro enzyme assay) revealed that 41% of the total metabolic rate could be accounted for by this single process if all of the enzyme was physiologically active. Significant biological variation was evident, however, when size-specific comparisons were made across different larval families. These differences were up to (i) 2.2-fold in ion transport rates; (ii) 2.8-fold in the allocation of energy to support the metabolic demand of ion transport; (iii) 3.5-fold in total enzyme activity; (iv) 3.9-fold in the physiologically active fraction of total enzyme; and (v) 3.1-fold in gene expression. These differences among families highlight the need to distinguish genetic from environmental causes of biological variation. Notably, for inferences of physiological changes based upon molecular biological analyses, the measured rates of ion transport were not predicted from concurrent measurements of gene expression or enzyme activity. Size-corrected rates of ion transport were predictive of variation in growth rates among different larval families, supporting the application of physiological rates of ion transport as a predictor of growth differences. Evolutionary variation in physiological performance has important implications for understanding the ecology of larval forms. Developing physiological indices will be of value in predicting growth and metabolism and corresponding survival of larval forms of different genotypes in response to environmental change.

1. Introduction

Most marine animals have complex life cycles with larval phases of development. Understanding variability in growth and metabolism of dispersive larval stages is central to many marine ecological processes (Bergenius et al., 2002; Cushing, 1990; Hare and Cowen, 1997; Houde, 2008; Shima and Findlay, 2002; Widdows, 1991). Differences in size and maternal energy reserves, for instance, impact the duration of the larval phase and subsequent recruitment potential (Lannan et al., 1980; Marshall et al., 2010; Moran and Manahan, 2004). Such biological variation generally has both environmental and genetic components, the balance of which may determine evolutionary outcomes (Lynch and Walsh, 1998). Environmental manipulations (e.g., food and temperature) of growth and metabolism, using wild-type animals, have been a major theme in studies of the physiological ecology of larval forms (Kroeker et al., 2013; Marshall et al., 2010; Moran and Manahan, 2004; Widdows, 1991). However, such approaches limit the ability to separate the effects of exogenous environmental factors from endogenous, genetically-determined interactions.

It is widely accepted that developmental rates and larval growth are highly variable (Gallager and Mann, 1981; Loosanoff and Davis, 1963; Marshall and Keough, 2007; Strathmann, 1987), even among sibling larvae reared under similar environmental conditions (Pace et al., 2006). Endogenous variation in growth rate at many stages of the life cycle has a metabolic basis, since differences can be attributed to differential

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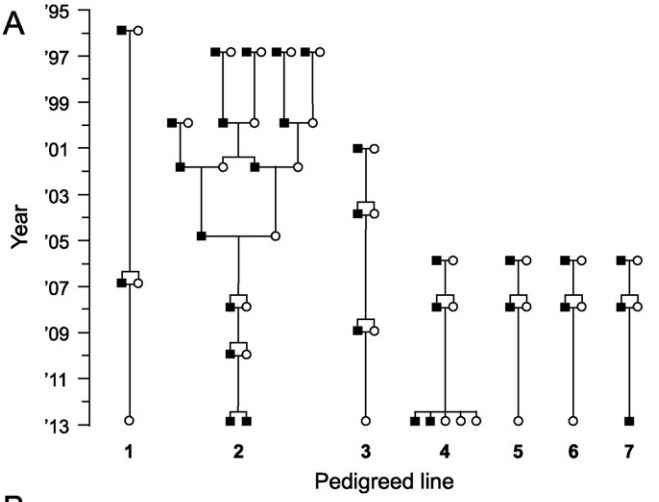
energy use by biological processes that support growth (Bayne and Hawkins, 1997; Hawkins and Day, 1996; Koehn and Shumway, 1982; Pace et al., 2006; Pan et al., 2015a). In our previous work with larval stages, specific physiological processes were identified that account for major components of metabolic energy demand during development and growth (Leong and Manahan, 1997; Marsh et al., 2001; Pace et al., 2006; Pace and Manahan, 2006; Pan et al., 2015b). One of these major energy-consuming processes is ion transport by the sodium pump (Na^+,K^+-ATPase). The regulation of ion gradients is required for many essential physiological processes of ecological relevance, such as osmotic balance (Evans et al., 2005) and nutrient uptake (Manahan, 1990). Notably, the energy required to support this single enzymatic process of Na^+,K^+-ATPase activity can account for up to 80% of the total energy requirements of a larva (Leong and Manahan, 1997, 1999).

While the major metabolic processes that establish the cost of living in larval forms have been identified, the relationship between growth variability and such metabolic processes is not well understood. This latter issue is the focus of the current study. A starting premise for the study of standing genetic variation is to be able to produce genetically determined variation in phenotype for experimental analysis. The lack of genetically-enabled "model" marine organisms (cf. terrestrial organisms, such as the fruit fly *Drosophila melanogaster*, Mackay et al., 2012) has been a major constraint to understanding genotype-phenotype variation in marine larval biology (Applebaum et al., 2014; Munday et al., 2013). In the current study, pedigreed lines of the Pacific oyster *Crassostrea gigas* were used to produce larval families that were reared under similar, controlled environmental conditions. Larval families that showed contrasting growth and physiological phenotypes were used to investigate variation in a major energy-consuming process at the level of physiology (ion transport), biochemistry (enzyme activity), and molecular biology (gene expression), and to develop a potential cellular-level biomarker that is predictive of differential growth.



B

		Female (○)						
		1	4A	5	3	4B	4C	6
Male (■)	2A	x	x	x				
	4A		x					
	2B				x	x	x	x
	4B				x			x
	7				x	x	x	x

Fig. 1. (A) Pedigreed lines resulting from a 17-year breeding program were used for controlled crosses. Closed squares indicate individual males; open circles indicate females. (B) Controlled crosses using males and females from the seven pedigreed lines produced 14 viable larval families. Within a given line, individuals were selected depending upon the number of adults available from this long-term breeding program and the spawning condition of the broodstock (gamete abundance and viability). When more than one male or female from the same pedigreed line was used to start a family of larvae, such individuals were designated with a letter coding (e.g. male 2A, 2B; female 4A, 4B, 4C).

2. Materials and methods

2.1. Pedigreed lines and experimental crosses

Families with varying larval growth rates were produced by controlled crosses using pedigreed lines of the Pacific oyster *Crassostrea gigas*. The adult broodstock used in these crosses resulted from a 17-year breeding program carried out by Taylor Shellfish Farms, Quilcene, WA (J.P. Davis and D. Hedgecock, personal communication; Fig. 1A), in which genetic markers were used before each cross to confirm parentage (Hedgecock and Davis, 2007; Sun et al., 2015). Using males and females from seven different pedigreed lines (labeled 1–7 in Fig. 1A), a total of 14 larval families was produced (Fig. 1B). Within a given genetic line, males and females were selected depending upon the number of adults available from this long-term breeding program and, importantly, the spawning condition of the broodstock regarding gamete availability and viability. Herein, each larval family is named according to the parental lines used for crosses. For example, larval family 2A × 4A (male × female) was the result of crossing an individual male and female from lines "2" and "4", respectively. When more than one male or female from the same pedigreed line was used to conduct pairwise crosses and start different families of larvae, such individuals were designated with a letter coding (e.g. male 2A, 2B; female 4A, 4B, 4C).

2.2. Larval culturing

Eggs and sperm were removed directly from the gonads ("strip spawning") of gravid adults of known pedigree. Eggs were fertilized, checked for fertilization success, and placed at an initial concentration of 10 fertilized eggs ml^{-1} in 200-l culture vessels, each containing 0.2-μm (pore-size) filtered seawater at 25 °C. Larvae were reared in a dedicated culture facility at the University of Southern California Wrigley Marine Science Center (Santa Catalina Island, California). Continuously flowing ambient seawater was filtered and heated with (inert) titanium heat exchangers to the required rearing temperature of 25 °C. Throughout the many months required for these experiments, temperature data-loggers (HOBO U12, Onset Computer Corp., MA) were used to continuously monitor the seawater temperature in individual culture vessels. Over the experimental period tested for any given larval culture (range 10 to 20 days), the seawater temperature used to rear larvae was held constant at 25.2 °C ± 0.18 (± S.E.M.) (data based on 24-h daily averages of temperature recorded every 30 min). When veliger larvae reached feeding competency on day 2 post fertilization, they were fed the algae *Isochrysis galbana* at 30 cells μl^{-1}. This feeding ration was increased to 50 cells μl^{-1} as larvae grew (according to established protocols see Breese and Malouf, 1975; Helm et al., 2004). A complete replacement of the seawater in each of the 200-l culture vessels was performed every two days during all experiments.

2.3. Growth rate

The growth rates of 14 larval families were measured over a 10- to 20-day period (rearing duration and growth rate varied, depending on family). Larval sizes in each culture were documented by photomicroscopy. At least 50 randomly-selected larvae at each sampling interval were measured based on their shell length. Larval shell length was quantified as the distance from the anterior to posterior edge on calibrated photomicroscopic images using ImageJ (National Institutes of Health, MD). The precision of the size measurement was <2 μm (replicate measures of the same individuals). Growth rate was calculated for each larval family from the slope of the linear regression model describing the relationship between shell length and age. A