Integrative and Comparative Biology

Integrative and Comparative Biology, volume 54, number 2, pp. 323–335
doi:10.1093/icb/icu045

Society for Integrative and Comparative Biology

SYMPOSIUM

An Integrated Modeling Approach to Assessing Linkages between Environment, Organism, and Phenotypic Plasticity

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From the symposium "A New Organismal Systems Biology: How Animals Walk the Tight Rope between Stability and Change" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

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Synopsis Many of the most interesting questions in organismal biology, especially those involving the functional and adaptive significance of organismal characteristics, intrinsically transcend levels of biological organization. These organismal functions typically involve multiple interacting biological mechanisms. We suggest that subdisciplinary advances have led both to the opportunity and to the necessity to reintegrate knowledge into a new understanding of the whole organism. We present a conceptual framework for a modeling approach that addresses the functioning of organisms in an integrative way, incorporating elements from environments, populations, individuals, and intra-organismal dynamics such as physiology and behavior. To give substance to our conceptual framework, we provide a preliminary focal case study using phenotypic plasticity in the tooth morphology of snails in the genus *Lacuna*. We use this case study to illustrate ways in which questions about the evolution and ecology of organismal function intrinsically span all organizational levels. In this case, and in many others, quantitative approaches that integrate across mechanisms and scales can suggest new hypotheses about organismal function, and provide new tools to test those hypotheses. Integrative quantitative models also provide roadmaps for the large-scale collaborations among diverse disciplinary specialists that are needed to gain deeper insights into organismal function.

Introduction

Biology is an enormously broad field, dealing with all aspects of living systems and the diversity of life. Over the past several decades, we have seen increasing specialization, especially within subdisciplinary boundaries, in biology. This is evident in the explosion of new journals and an increase in the number of publications within each of the subfields of biology, which some argue result in even more specialization because the need to keep up with the information-explosion prohibits being generalized (Pautasso 2012). Although specializations address narrower subsets of the whole span of biological organization, this concentrated focus has led to major breakthroughs and deep insights within these subdisciplinary specialties.

By comparison, organismal biology—our understanding of the biological functioning of the whole organism in the context of its external environment, the dynamics of its internal state, and its morphological and behavioral components—has not kept pace. The functional, or adaptive, significance of most organismal characteristics, and the functional consequences of variations in those characteristics, cannot presently be quantified. Many, if not most, recent insights into environmental processes, population dynamics, physiology, behavior, and all the other elements comprising organismal biology have not yet been incorporated into our analysis of whole-organism function (Padilla et al. 2013, 2014). This is in large part due to the complexity of mechanisms operating at each of the various levels of biological organization drawn on by organismal biology, and the feedbacks and influence among levels of organization. Integration among levels into a unified

Advanced Access publication May 26, 2014
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organismal perspective increases the complexity of the problems exponentially, beyond the point where the traditional approaches of organismal biology are effective (Padilla and Tsukimura 2014). A key question for organismal biologists as a community is whether or not this is a problem—will the current style and substance of organismal biology be sufficient to advance us in the foreseeable future significantly beyond our present knowledge? Can organisms be understood as a sum of their parts, and will deep understanding of the parts through specialized or subdisciplinary approaches lead to understanding of the functioning and responses of whole organisms? If not, what additional or alternative approaches will yield maximum benefits?

Here, we advance two key arguments. First, we suggest that many of the most interesting questions in organismal biology, especially those involving the functional significance of organismal characteristics, intrinsically transcend levels of biological organization. These organismal functions typically involve multiple interacting biological mechanisms, often spanning wide ranges of temporal and spatial scales, and transcending traditional disciplinary boundaries within which data are collected and models are developed. Second, we suggest that current approaches to organismal biology are limited because of our inability to adequately integrate new disciplinary knowledge to investigations of many, if not most, organismal functions. Our arguments imply that disciplinary advances have led both to the opportunity and to the necessity to reintegrate disciplinary knowledge into a new understanding of the whole organism. However, this cannot happen without new and more effective tools that can couple essential elements and information from diverse levels of biological organization without becoming hopelessly complex. First and foremost among these tools, we believe, are systems-type approaches based on quantitative modeling tools that make explicit the interacting roles of components, and that clarify the data and expertise required of subdisciplinary collaborators contributing to systematic, collaborative, and comprehensive investigations of organismal function.

In this article, we present a conceptual framework for one modeling approach that addresses organismal function in an integrative way, incorporating elements from environments, populations, individuals, and intra-organismal dynamics such as physiology and behavior. To give substance to our conceptual framework, we provide a sample case study of phenotypic plasticity in the tooth morphology of snails in the genus *Lacuna* (Padilla 1998, 2004). We use this case study to illustrate the ways in which questions about the evolution and ecology of organismal function intrinsically span all these organizational levels. Including and directly linking diverse organizational levels within a quantitative, integrated modeling framework led us to testable hypotheses about connections between biological mechanisms that we would not have found had we confined ourselves to studies within traditional subdisciplinary boundaries.

A modeling framework for organismal biology in context

In our conception of an integrated, quantitative approach to organismal biology, we envision the relevant biological dynamics as being loosely segregated into a hierarchy of organizational levels. For example:

- Environment—external to the organism, but with direct feedbacks to other levels of organization, and in some cases, may be affected or modified by organisms (e.g., ecosystem engineering; Jones et al. 1994).
- Population—groups of the same species occurring and interacting in the same place, including mating groups, as well as potential competition or group defense or feeding. Again, properties at the level of the population are affected by, and affect, other levels of organization.
- Organism—the individual, and its characteristics such as location, habitat history, and cumulative reproductive success, which are affected by internal and external biotic and abiotic conditions, across ontogenetic stages.
- Intra-organism—processes and conditions of internal systems including genetic makeup, physiological and developmental processes, behavior and neurological processes, and reproductive state, all of which are affected by, and affect, other levels in the hierarchy.

These levels are illustrated schematically by linked boxes in Fig. 1. Most biological processes and mechanisms can be placed more or less clearly within one of these boxes. Some mechanisms could plausibly fall within either of two adjacent boxes. For some questions, subdividing boxes into smaller functional units (e.g., tissues or organs and gene networks) may be important. Therefore, there is no uniquely correct implementation of our framework to a given biological question. Nonetheless, we believe a framework of this type accomplishes three key purposes First, the framework makes explicit the relationships among many different subdisciplines of natural science, which are all relevant to questions in organismal biology, but which typically are investigated in isolation by distinct scientific communities (e.g.

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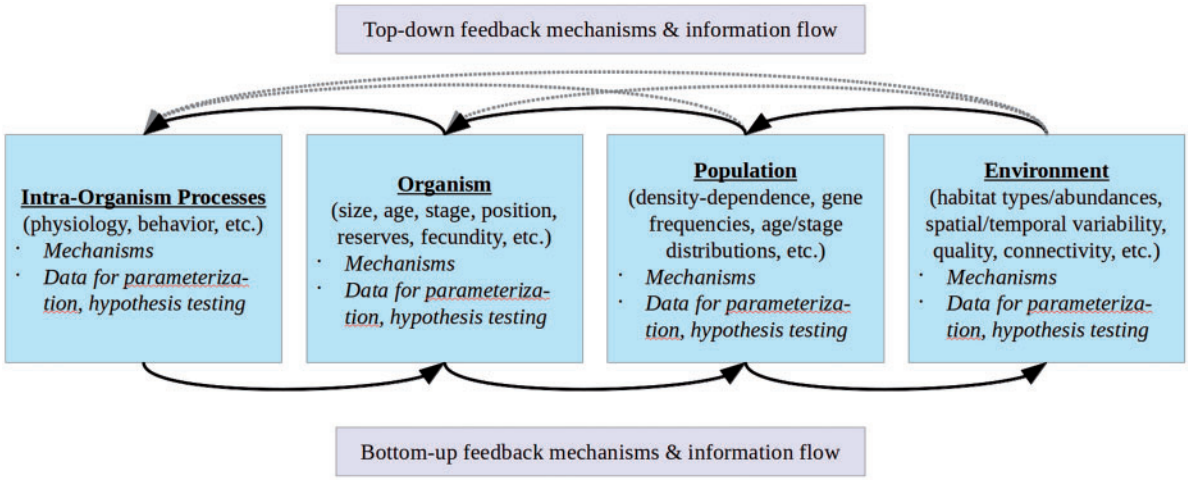

Fig. 1 Schematic of an integrative modeling framework. Boxes represent conceptual levels of biological organization. Traditional biological disciplines typically focus on topics within one box, some examples of which are provided. The graphic is intended to convey the idea of a quantitative approach to organismal biology that encompasses multiple levels, with explicit quantitative representations of interactions and feedbacks among boxes. Key attributes of this framework include that each mechanism or process be explicitly associated with a mathematical formulation governing dynamic simulations, and data for estimation of parameters and testing of hypotheses (Table 1). We expect that most direct interactions are mediated as bottom-up or top-down feedbacks or information streams between adjacent boxes, illustrated here as solid arrows. For example, we expect that physiological processes directly affect individual organismal responses, with effects ultimately propagating to population and environmental levels indirectly through secondary feedbacks. However, there may be exceptions, as for example when populations or environments have direct impacts on physiological responses or behaviors that are not mediated at the organismal level (dashed arrows).

developmental biologists, physiologists, endocrinologists, morphologists, population ecologists, and behaviorists). Diverse relevant mechanisms, from regulation of transcriptional factors to long-term climatic trends, have identifiable places in this conceptual framework. Second, a quantitative approach to such a complex scenario usually requires numerical modeling. The framework guides the programmer or analyst in designing code structure that, because it reflects the underlying mechanisms, is efficient and easy to construct. Third, because no single investigator or laboratory generally possesses the broad base of knowledge and skills represented in our framework, collaboration among a team of experts in key disciplines is necessary to accomplish an integrative analysis. The framework clarifies the roles and relationships of a collaborative team, and highlights potential problems such as differences in interpretations of jargon across disciplines.

Case study: phenotypic plasticity in *Lacuna*

Phenotypically plastic morphology of teeth in the gastropod genus *Lacuna* is an example of a biological phenomenon that cannot be understood at any single organizational level, discipline, or spatio-temporal scale. Although much remains to be learned about this plasticity, considerable information is known about this system, enabling us to investigate this plasticity in our modeling framework (Martel and Chia 1991a, 1991b, 1991c; Martel and Diefenbach 1993; Padilla et al. 1996; Padilla 1998, 2001, 2004; D. K. Padilla, unpublished data). Marine snails in the genus *Lacuna* are in the family Littorinidae, along with the better-studied and ecologically important genus *Littorina*. Like most littorines, *Lacuna* are small-bodied grazers (generally 1–9 mm), and are often extremely abundant in habitats where they occur. Unlike many littorines, they live exclusively on macroalgae or seagrasses, and are not found grazing on rocky substrates. When found on macroalgae, they graze directly on the macroalga, excavating deep gouges in the surface of the thallus as they graze. When found on seagrasses, which are angiosperms, they do not feed directly on the seagrass, but rather graze the epiphytes, primarily diatoms, from the surface of the seagrass. Two sympatric species of *Lacuna*, *Lacuna variegata* and *Lacuna vincta*, are found in the eastern Pacific on shores in Washington state, USA, and British Columbia, Canada (Kozloff 1996). These two species are regularly found on all mid-shore, low-shore, and subtidal macroalgae, including kelp, as well as on surfgrass (*Phyllospadix scouleri* and *Phyllospadix torreyi*) attached to rocky substrates and eelgrass,

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Table 1. Examples of key mechanisms, processes, or phenomena affecting the costs and benefits of phenotypic plasticity in the tooth morphology of Lacuna (left column), characteristic types of observations or data (middle column), and some details of implementation in our integrated model (right column)

Mechanism, process, or phenomenon	Observation or data	Implementation of the model
Intra-Organism		
Physiological time lags in phenotypic plasticity of tooth morphology	Microscopic examination of radulae of snails grown under manipulated food regimes (number of rows of teeth (time) and tooth morphology)	Model of tooth configuration of radula for each individual, updated daily as a function of current type of food
Feeding rates for matched and mismatched tooth morphologies	Laboratory observations of feeding rates as a function of size, age, and type/amount of food	Feeding rated on alternative types of food, expressed in common units, as a function of match/mismatch of teeth
Dispersal behavior by voluntary mucus-thread "jumping"	Flume observations of "jumping" behavior under conditions of manipulated food	Jumping probability per day as a function of type of habitat and time since change of habitat type
Organism		
Position, ambient conditions, and history of habitat type occupied	Field surveys and analysis of the morphologies of field-collected individuals	Agent-based model of individual organisms
Reproductive success	Laboratory observations of fecundity as a function of size, age, and type/amount of food; field densities of egg masses	Production of egg masses regulated by size, food type, and feeding rate due to tooth shape
Population		
Density-dependent regulation of abundance and condition of juveniles, adults, and egg masses of *Lacuna*	Time series censuses of *Lacuna* populations within kelp and eelgrass habitats across seasonal and long-term variations	Crowding into unfavorable or exposed parts of habitat, leading to increased rates of dislodgement and dispersal (involuntary "jumping")
Selective costs and benefits of organism-level traits	Time series of relative abundances of genetic, morphological, and behavioral variants	Lottery-type recruitment of metamorphosing larvae into randomly chosen habitats
Environment		
Habitat type, size, and spatio-temporal variability	Landscape-level surveys; time series of availability of food across types of habitat	Model of size and type of habitat; model of seasonal variability and long-term climatic trends
Connectivity of habitats to dispersing *Lacuna*	Pelagic sampling of dispersing juveniles and adults	Dispersal matrix specifying probability of dispersal between habitats
Disturbance rates (rates of involuntary "jumping" due to dislodgement)	Capture–recapture studies of marked individuals across habitat types and ambient conditions	Site-specific probabilities per day of involuntary jumping between habitats due to disturbance

Zostera marina, which inhabits soft muddy or sandy substrates, and typically forms large meadows.

The radula is the major organ used by gastropods for feeding. It consists of a long chitinous ribbon with repeated rows of teeth. Throughout the lifetime of a snail, new rows of teeth are produced at a relatively constant rate, while the oldest rows are shed (Fig. 2). The shape of teeth affects their efficiency when grazing (Padilla 1985), and tooth shape is determined when the formative cells, odontoblasts, first produce a chitinous template (Fig. 2). Tooth shape cannot be changed once that template is built. Other tissues associated with the radula then deposit materials, producing a mature tooth that is then capable of feeding. Only the anterior-most rows of teeth are used for feeding. Radular ribbons can be quite long, reflecting the time to initiate and then build teeth and how rapidly they wear during feeding and need to be replaced. Thus, there is a time lag between the initial formation of a tooth and its first use for feeding.

In these two species of *Lacuna*, new teeth are produced at a constant rate of three rows per day, independently of diet (Padilla et al. 1996). The length of the radular ribbon varies among individuals. For *L. vincta*, it typically ranges from 47 to 94 rows of teeth, with an average of 69.0 rows ($N = 125$; Padilla et al. 1996). The length of the radula is only loosely correlated with shell length overall, and is not significantly correlated with shell length

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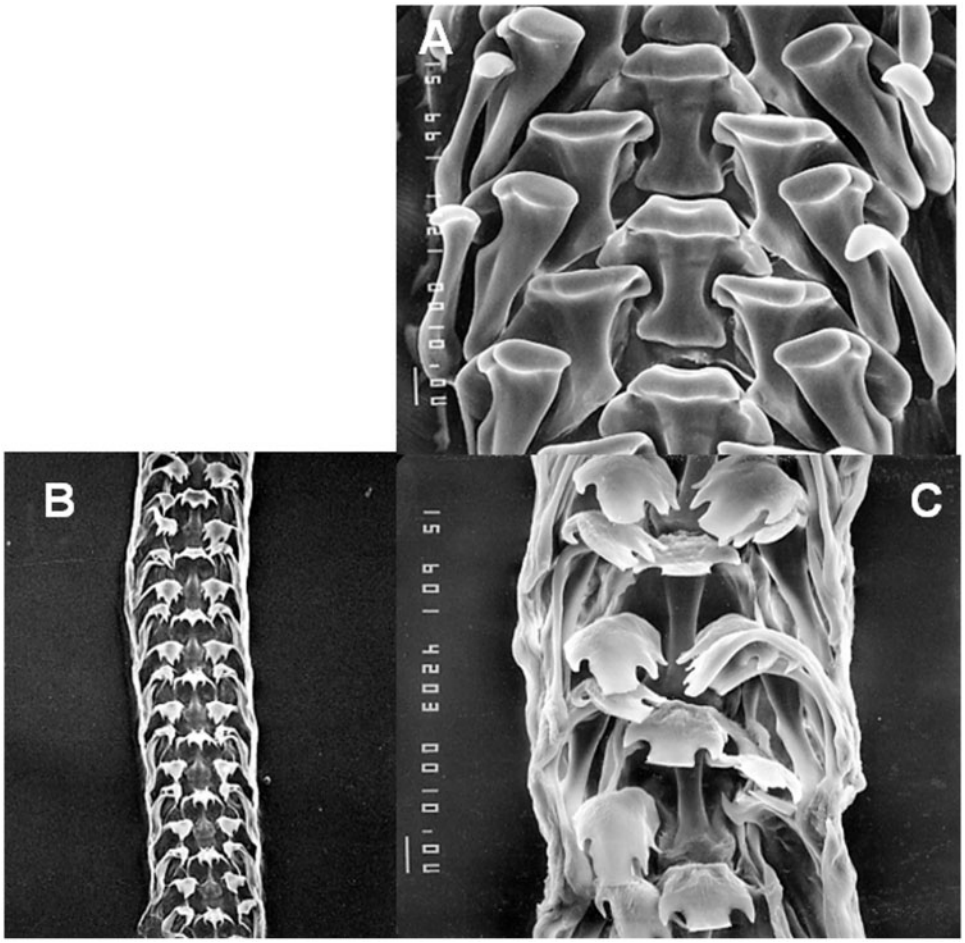

Fig. 2 Scanning electron micrographs of the radular teeth of *Lacuna variegata*. *Lacuna vincta* produces similarly shaped teeth in a similar way. (**A**) Anterior-most teeth are used for feeding, and display wear. Each row has six teeth across, and is most identifiable by the central, rachidian tooth. Worn rows of teeth are shed as new teeth are produced at the posterior end of the ribbon (**B** and **C**). The shape of the teeth is determined when they are first deposited (B-pointed, C-blunt), and cannot be modified once deposited. Scale bars are 10 μm.

for females. *Lacuna variegata* tends to have a longer radula, with an average of 77.8 rows of teeth, and a range of 53–99 rows (Padilla et al. 1996). In *L. variegata*, the length of the ribbon is only loosely correlated with shell length, and is not significantly correlated for females.

When feeding on macroalgae, both species of *Lacuna* produce pointed-cusped teeth (Fig. 3), which have been shown to be the most effective shape of tooth for excavating fleshy algae (Padilla 1989). When feeding on diatom-covered eelgrass, both species produce blunt-cusped teeth (Fig. 3). These are shaped similarly to teeth of other gastropods that are known specialists feeding on epiphytes of eelgrass (Lindberg 1981), and are the shape most effective for this type of food. Intermediate shapes of teeth are not found in nature. Experiments have shown that individual snails will produce the type of tooth appropriate for the corresponding diet, and are capable of changing tooth morphology throughout their lives. Thus, phenotypic plasticity of teeth is inducible and reversible throughout the life of the snail. Recent work suggests that in the absence of cues from benthic food, snails produce pointed teeth (Yee et al. 2014). Of 100 field-collected larvae from different habitats, all had pointed cusps (D. K. Padilla, unpublished data). This indicates that teeth with pointed cusps are probably a ''default'' or ancestral morphology, and that the production of blunt teeth requires cues from the available diet in eelgrass environments (Yee et al. 2014). In the field, although most snails in a given habitat have tooth morphologies that are most effective for the prevailing type of food, it is not uncommon to find animals with the other tooth morphology, many of which are producing new teeth that morphologically match the immediately available food (D. K. Padilla, unpublished data). *Lacuna* typically live for 6–12

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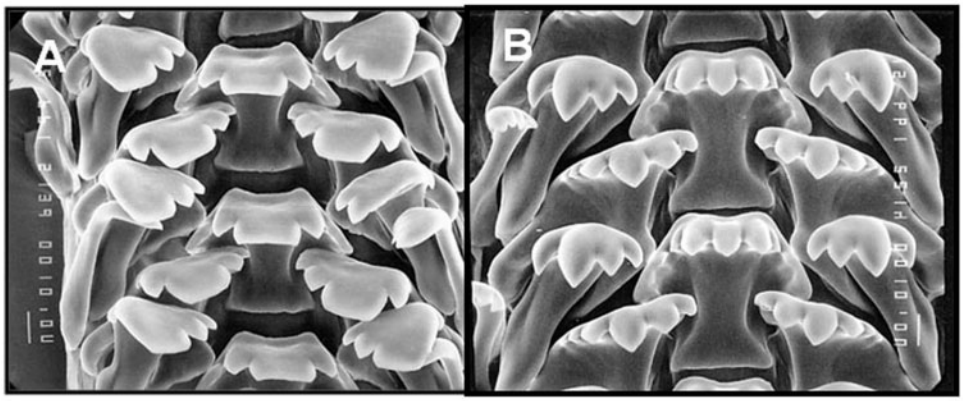

Fig. 3 Scanning electron micrographs of teeth of *Lacuna variegata* when feeding on diatoms that epiphitize eelgrass (**A**) or macroalgae (**B**). Scale bars are 10 μm.

months, have separate sexes, internal fertilization, and females lay gelatinous egg masses on algae or eelgrass (Martel and Chia 1991a). Females are reproductive year-round, and egg-mass production increases with females' body size (D. K. Padilla, unpublished data). Planktotrophic veliger larvae hatch from eggs after 5 (*L. variegata*) or 6 (*L. vincta*) days. Larvae are in the plankton probably for 8–24 weeks, depending on water temperatures and abundance of phytoplankton. Larvae are transported by currents, and settle into both habitat types at about 1 mm shell size. Juveniles and adults are capable of dispersal (jumping) through the production of mucus parachutes, which enable them to passively drift on water currents (Martel and Chia 1991b, 1991c; Martel and Diefenbach 1993). Snails will drift on these parachutes more frequently if food is reduced in abundance or degraded in quality, if certain predators are present, or when physically disturbed (Martel and Diefenbach 1993; D. K. Padilla, unpublished data). However, they cannot control where water currents will carry them.

Macroalgae and diatoms are of different quality as food for *Lacuna*. Snails feeding on diatoms appear to grow faster and reproduce sooner, but die younger, than those feeding on macroalgae. However, because they live longer, the lifetime fecundity of snails feeding on macroalgae is similar to that of snails feeding on diatoms (D. K. Padilla, unpublished data). Macroalgae are abundant year-round; although ephemeral algae are seasonal, there is an abundance of biannual and perennial algae on which snails can graze throughout the year. Diatoms, however, are highly seasonal, and most abundant only when day lengths are long in the spring, summer, and early fall.

Phenotypically plastic teeth raise a number of compelling questions about the functional consequences of diverse aspects of *Lacuna*'s organismal biology. These questions also illustrate both top-down and bottom-up links among physiology, behavior, and reproductive biology, and the characteristics of the local environment. For example, there is a minimum time lag imposed on this system between the time when the environment changes and when a new morphology can be used. Teeth with the new morphology cannot be instantaneously available to snails because the processes of building the tooth take time. This lag time results in costs of reduced feeding efficiency and all of the subsequent effects such as reproduction and growth being limited by lower availability of food and energy. However, in nature, we see animals that vary widely in the length of their radular ribbon, and hence in their lag time. To what extent is the lag time a hard constraint in the process of producing a functional row of teeth? In what ways is it shaped by evolutionary costs and benefits, and is there strong selection to reduce this lag time? Is the dispersal behavior observed in field and laboratory studies an outcome of evolutionarily adaptive behaviors, or does it primarily reflect purely local, short-term mechanisms such as jumping to escape immediate threat of predation or being knocked loose from a precarious foothold? How does dispersal behavior affect exposure of individual snails to sequential habitats, and how does an individual's foraging history affect its subsequent dispersal behaviors? The prevalence of macroalgal and eelgrass habitats, and their proximity and connectivity to animals that are passively dispersed by ambient currents, strongly affect the fraction of time spent in either habitat type and the typical duration of foraging between changes in

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habitat type. How does this environmental structure influence the relative costs and benefits of alternative tooth morphologies, and the benefits conferred by plastic, rather than constitutive, morphologies? Large grazing populations affect the availability of food, the potential impacts of predators and pathogens, and many other aspects of *Lacuna*'s life history. Through which mechanisms do crowding, competition, and other density-dependent effects directly or indirectly impact individuals, and how do those mechanisms affect costs and benefits of the phenotypic plasticity of teeth?

We believe that answering questions like these is central to exploiting the untapped potential in organismal biology provided by disciplinary advances. However, those answers cannot be obtained without an integrated quantitative modeling such as outlined above. Such a model needs to include the entire system—the individual organisms and their traits, and the populations and environments in which they are found—as a whole. In the next section, we provide a preliminary but illustrative example of how such a model might be implemented and the types of results that might then be obtained.

An integrated model of phenotypic tooth morphology in *Lacuna*

Previous general modeling of phenotypic plasticity found that lags between the change in habitat and the time when a new phenotype is produced or is usable were disadvantageous. Such lags reduce the adaptive value of an inducible phenotypic plasticity and the amount of reduction depends on the rate of environmental change (Padilla and Adolph 1996). That is, the longer the lag time between an environmental change and the phenotypic response to it, the greater the fraction of time an organism must cope with an inappropriate morphology. The relative adaptive value of a phenotypically plastic trait is greatly reduced if the time scale of environmental change is less than the lag time; then, the morphology may be inappropriate most, or even all, of the time.

Padilla's and Adolph's results are general and intuitively plausible, and seem applicable to many examples of phenotypic plasticity, especially morphological plasticities, in which the new phenotype cannot be produced instantaneously. In the context of inducible radular morphology in *Lacuna*, their model suggests that increased radular length, which corresponds to increased time lag between exposure to cues of a new habitat and the availability of teeth with a phenotype appropriate to that habitat, should be generally disadvantageous. Thus, the results of Padilla's and Adolph's (1996) model suggest there should always be positive selection for reducing the lag time by reducing the length of the radular ribbon.

In what ways might an integrative modeling approach enhance and enlarge Padilla's and Adolph's analysis? Here, we develop a preliminary integrated model of *Lacuna*'s tooth plasticity as an illustrative case study. The translation of the conceptual hierarchy from Fig. 1 to the *Lacuna* case study is illustrated schematically in Fig. 4. We use this model to ask whether the time lag between initiation of the tooth, when its morphology is determined by response to environmental conditions, and the tooth's availability for feeding is selectively beneficial, costly, or near-neutral under a variety of different environmental conditions. Our presentation here is intended as an illustration of the approach, and, therefore, many details of model assumptions and parameters and a more complete description of the model and of the statistical results will be presented elsewhere (D. Grünbaum and D. K. Padilla, in preparation).

The model in its present form is implemented as an individual-based model, in which post-recruitment organisms (juvenile and adult stages) are considered to be individual agents. The model tracks only females; we assume that there are always sufficient males to ensure fertilization but do not account for males explicitly. Each individual is a member of a population, which we interpret here as corresponding to a species (either *L. vincta* or *L. variegata*), but may in general be a subset of a larger population within a species. Variations among individuals within populations are implemented as a set of organism "types"—these types encode parameters determining physiology, behavior, reproductive biology, feeding, and other aspects of *Lacuna*'s life history. Here, we interpret types as corresponding to genotypes, but other interpretations are possible. Types may be present at the beginning of the simulation, or be dynamically created during simulations (e.g., as the outcome of mutation and selection). In addition to types, individuals possess a number of state variables, such as radular length and tooth morphology, feeding rate and metabolic reserves for reproduction, size, current, and previous types of habitat, rate of egg production, and cumulative fecundity.

Pre-recruitment stages (eggs and planktonic larvae) are accounted for as "super-individuals" representing egg masses and larval cohorts. Due to *Lacuna*'s high fecundity, the number of eggs and larvae at a given time may be orders of magnitude

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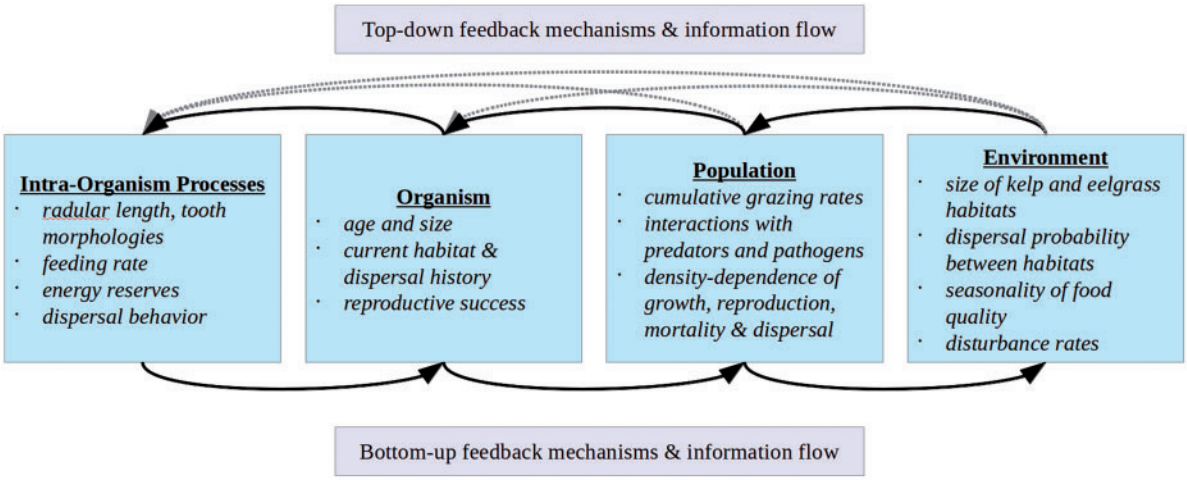

Fig. 4 Selected elements of an integrative modeling framework of phenotypic plasticity of tooth morphology in snails in the genus *Lacuna*. This graphic presents the framework of Fig. 1 and reflects elements of phenotypically plastic tooth morphology in *Lacuna*. The subset of mechanisms and interactions presented are focused on the question: are time lags between formation of phenotypically plastic teeth and their availability for use in selective feeding costly, beneficial, or neutral in terms of individuals' reproductive success (a proxy for fitness)? These time lags are associated with the length of radulae; longer radulae produce longer lag times between initiation of the formation of a tooth and its subsequent use. In addition to time lags, matching or mismatching of the morphology of the tooth to the type of habitat occupied is affected by other intra-organismal processes (e.g., dispersal behavior and feeding rate); organism-level processes (e.g., the organism's age and dispersal history); population-level processes (e.g., density-dependent effects on growth, mortality, and reproduction and the impacts of grazing on the habitat); and environment-level processes (e.g., size and seasonality of habitats). These interactions suggest that assumptions of the model and its parameters at each of these levels may strongly influence the estimated costs and benefits of time lags in the phenotypic plasticity of *Lacuna*'s teeth.

larger than the number of juveniles and adults. Accounting for pre-recruitment stages as super-individuals forestalls modeling extremely large numbers of pre-hatching and post-hatching individuals, most of which will die before settlement.

We model *Lacuna*'s phenotypic plasticity by assuming that each organism has a radula of a predetermined length, and replaces teeth at a fixed rate determined from laboratory experiments (Padilla et al. 2001). We also use laboratory data to parameterize feeding rates on kelp or eelgrass with matched or mismatched shapes of teeth. Type of tooth is determined by the kind of habitat type in which the animal occurs when the tooth forms, and feeding rate is determined by the type of habitat and the type of anterior-most teeth. Dispersal by jumping can occur by voluntary decisions to enter the water column; the probabilities per day of voluntary dispersal decrease with length of time in a given habitat type, as determined by laboratory observations. In addition, individuals may be dislodged or otherwise disturbed, causing them to enter the water column involuntarily. Once in the water column, survival and transport to new habitats are equal for voluntary and involuntary dispersal.

The environment is defined as a set of habitats, currently of either kelp or eelgrass. A transition matrix determines the probabilities of dispersal between habitats and the probability of mortality during dispersal. Each type of habitat has characteristics determined from observations when possible (e.g., abundance, quality, and seasonal variability of food and maximum population density) or assigned tentative values subject to future sensitivity analysis and exploration of parameter space when data are unavailable (dispersal rate and destination, etc.). Within each population, individuals are distributed across habitats in various proportions that arise from dispersal behavior, recruitment, and demography.

Each habitat has a quantifiable number of resident juveniles and adults, which, in relation to its capacity, determines grazing and density-dependent effects. Based on observed demography, we believe that density-dependent mechanisms operate to constrain maximum densities of *Lacuna* on kelp and eelgrass substrates. However, we have no empirical data other than population censuses to constrain these mechanisms. We opted to implement density dependence with what to us seemed minimal assumptions: we assumed that kelp and eelgrass substrates are variable both in the quality of local food and in the risk of dislodgement, and that crowding forces individuals into unfavorable positions with greater frequency than prevails when populations

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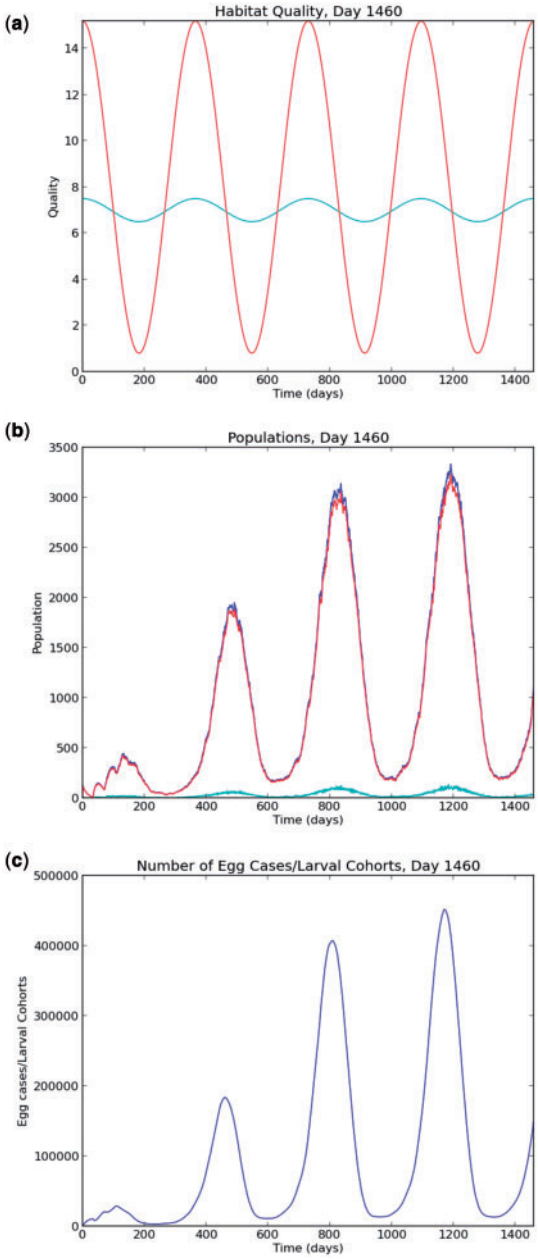

Fig. 5 Simulated time series of habitat and population changes in the integrated model of phenotypic plasticity in the radula of *Lacuna*. (**a**) Seasonal variation in quality of the simulated habitat. Vertical axis represents quality of the habitat (cyan-kelp habitat; red-eelgrass habitat) expressed in terms of the availability of food in units proportional to the maximum number of egg cases laid per day by an adult *Lacuna* female with habitat-appropriate tooth morphology. Horizontal axis represents time in units of days, across a 4-year period. Variations reflect seasonal changes, which are more pronounced for diatoms grazed by *Lacuna* in the eelgrass habitat than for macroalgae such as kelp. (**b**) Time series of simulated populations of adults and of juveniles of *Lacuna* in an environment dominated by eelgrass. Vertical axis represents number of individuals (cyan-kelp habitat; red-eelgrass habitat; blue-total population). Horizontal axis represents time in units of days, across a 4-year period. Initial populations were deliberately set far from equilibrium and much lower than capacity for both environments. The time series reflect increase in the population to the point where density-dependent mechanisms become significant in regulating the populations. In this simulation, the ratio of kelp-to-eelgrass habitat is 1:20. (**c**) Time series of simulated numbers of egg cases and of larval cohort populations in an environment dominated by eelgrass. Vertical axis represents number of individuals (cyan-kelp habitat; red-eelgrass habitat; blue-total population). Horizontal axis represents time in units of days, across a 4-year period. Upon hatching, egg cases are tracked as larval cohorts until metamorphosis; the populations plotted here represent the total number of egg cases and larval cohorts (not individual eggs and larvae). Upon recruitment, larval cohorts convert to the corresponding number of surviving juvenile individuals. In this simulation, the ratio of kelp-to-eelgrass habitat is 1:20.

are sparse. In essence, we assumed that the involuntary jumping rate (and the mortality associated with dispersal) increases for populations in crowded conditions.

Estimating the consequences of time lags

To investigate the consequences of time lags in the shape of *Lacuna*'s teeth, we initialized our model simulations with equal numbers of four types of individuals. These types differed in radular length (corresponding to delays of 3, 11, 21, and 29 days) but were identical in all other respects. We used the relative abundance of each type after 4 simulated years as an assay of selective costs and benefits of time lags between the changes in cues from the habitat and availability of phenotypically appropriate teeth. More specifically, we expected the phenotypes that result in a selective advantage to become more abundant in the simulated population; those with less adaptive variations to become less abundant; and neutral variations to undergo relatively small random changes reflecting stochasticity in recruitment, dispersal, and mortality.

Consistent with observations, we assumed that kelp substrates have relatively weak seasonal variations in availability of food, but that eelgrass habitats are strongly seasonal (Fig. 5). We explored the effects of environment-level variations by simulating environments in which either kelp or eelgrass dominated, or both were of equal abundance.

Typical population trajectories in simulations of eelgrass-dominated habitats (1:20 kelp-to-eelgrass abundance) are shown in Fig. 5. The total populations in these simulations were highly seasonal, reflecting the high proportion of individuals in eelgrass and the strong seasonal variation in fecundity of

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those individuals. In these simulations, the *Lacuna* population in kelp also shows seasonal variations, even though the quality of food in that habitat is relatively constant. Seasonality in the kelp habitat reflects the relatively high dispersal rates between habitats in our simulations. Figure 5 shows the corresponding numbers of egg cases and larval cohorts for each population. In these simulations, we assumed that *Lacuna* can lay up to three masses of eggs per day (each containing 800 eggs, half of which are female). We assumed eggs hatch after 5 days, and larvae achieve competence at 28 days. Hence, we expect the number of egg cases and larval cohorts to be roughly two orders of magnitude larger than the adult population, an expectation that is borne out by the simulations.

The relative frequencies of individuals with different radular length after 4 simulated years for the eelgrass-dominated environment are shown in Fig. 6. The simulation suggests a strongly disproportionate representation of individuals with short radulae compared with long radulae. This suggests that a short time lag between cues from a new habitat and the availability of morphologically-appropriate teeth is selectively advantageous over long time lags. Consistent with expectations from Padilla and Adolph (1996), these simulations suggest that over moderately longer time scales a hypothetical variant with a short radula would likely become fixed in the *Lacuna* population.

Figure 6 also shows the relative frequency of different shapes of teeth across the entire simulated *Lacuna* population. In these simulations, blunt teeth appropriate for grazing diatoms of eelgrass are more common than pointed teeth appropriate for kelp, but only slightly so. This modest prevalence of blunt teeth, despite the overwhelming prevalence of eelgrass, reflects our assumption—based on laboratory observations—that individuals recruit with a full set of pointed teeth in their radulae. A population with many newly settled juveniles therefore has a disproportionately large representation of pointed teeth.

In Fig. 7, we present the corresponding frequencies of individuals with different radular lengths and shapes of teeth, in typical simulation in environments with equal amounts (1:1 kelp-to-eelgrass). The change of environments has several major consequences. One is the relatively small magnitude of changes in relative frequency of individuals with 3, 11, 21, and 29 days of lag, and the other is the direction of trend. Rather than an increase in the proportion of animals with a short lag time, individuals with longer time lags are more numerous at the end

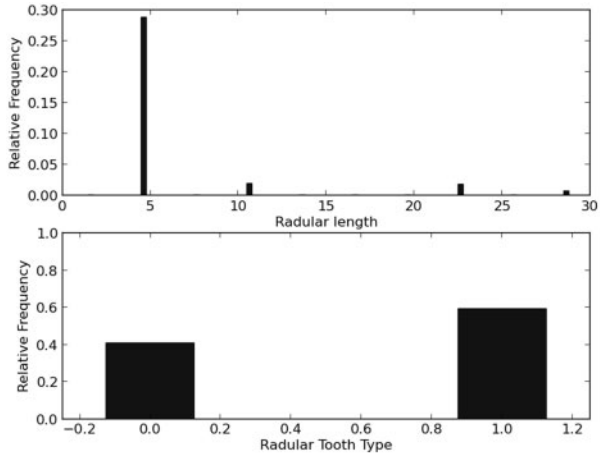

Fig. 6 Relative proportion of individuals with given radular lengths and tooth morphologies after 4 simulated years in an environment dominated by eelgrass. The top plot represents the relative proportion of the *Lacuna* population with radular lengths corresponding to time lags of 3, 11, 21, and 29 days. Initially, all radular lengths are equally represented. Hence, increases in relative abundance reflect a selective advantage within the framework of the model. This simulation, with a kelp-to-eelgrass habitat ratio of 1:20, suggests that a short radular length (i.e., a time delay of 3 days in deployment of phenotypically plastic teeth) confers a significant selective benefit over longer radular lengths. The bottom plot represents the total relative frequency of tooth shapes adapted to kelp and to eelgrass across the entire population after 4 simulated years. The shape of teeth appropriate for eelgrass represents ~60% of the total teeth, compared with the shape appropriate to kelp (~40%). Hence, in this eelgrass-dominated environment, eel-grass-type teeth are more abundant than kelp-type teeth, but the relative abundance of the two types of teeth is not as skewed as the habitat abundance might suggest. This is likely because the "default" shape of tooth is pointed, the tooth type associated with kelp; hence, all newly settled individuals have kelp-type teeth.

of this simulation, although that trend may be due to very low selective advantages resulting in stochastic differences among types. Contrary to Padilla's and Adolph's model, these results suggest absence of a significant selective disadvantage to longer radulae in kelp-dominated environments.

For environments in which kelp and eelgrass are relatively balanced in abundance, pointed teeth are overwhelmingly prevalent after 4 simulated years (Fig. 7). This, we believe, again reflects pointed teeth as the "default" morphology, which in this case is the appropriate morphology for most of the population.

How do we interpret the apparent influence of type of environment on the selective consequences of time lags in tooth plasticity? More specifically, why is there little, or no, apparent cost to longer

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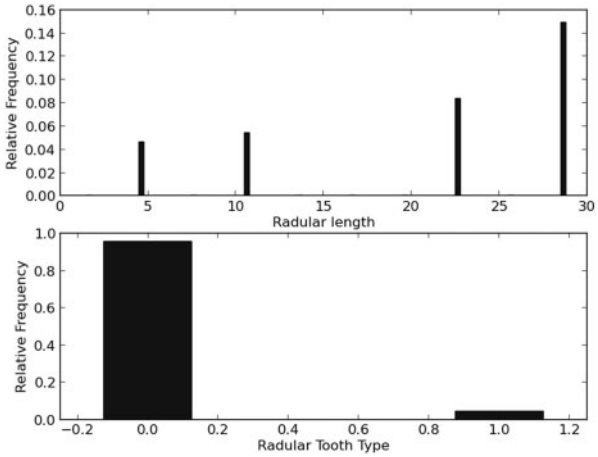

Fig. 7 Relative proportion of individuals with given radular lengths and shape of teeth after 4 simulated years in an environment in which kelp and eelgrass are balanced. The top plot represents the relative proportion of the *Lacuna* population with radular lengths corresponding to time lags of 3, 11, 21, and 29 days. Initially, all radular lengths are equally represented. Hence, increases in relative abundance reflect a selective advantage within the framework of the model. This simulation, with a kelp-to-eelgrass ratio of 1:1, provides little, or no, evidence that a short radular length (i.e., a time delay of 3 days in deployment of phenotypically plastic teeth) confers significant selective benefit over longer radular lengths. The bottom plot represents the total relative frequency of kelp-type and eelgrass-type teeth across the entire population after 4 simulated years. Despite the equal availability of eelgrass and kelp habitat in this simulation, the majority of teeth are of the pointed type appropriate for kelp. Again, this likely reflects the observation that newly settled individuals have kelp-type teeth.

lags in kelp-dominated environments, in contradiction to the apparently applicable predictions of Padilla's and Adolph's model?

Our interpretation is as follows: we distinguish between two situations in which time lags in phenotypic plasticity can lead to mismatches between habitat type and shape of teeth, which are costly in terms of empirically measured feeding rates. One occurs at recruitment of larvae, when individuals settle with a full complement of pointed teeth. Individuals settling onto eelgrass habitat always have morphologically-inappropriate teeth. This mismatch prevails until they disperse to kelp habitat, or until the time lag for eelgrass-induced teeth to become available has expired.

The other case of costly mismatches is the dispersal of jumping juveniles and adults that leads to a new type of habitat. In this case, the appropriateness of tooth morphology in the short term depends on the habitat to which the animal was exposed when it formed its currently-used teeth. For tooth morphology to correlate with habitat type—which is necessary for plasticity to confer a feeding advantage—individuals must typically remain within a given habitat over a time scale equal to, or exceeding, the time lag in phenotypic plasticity.

The magnitudes of costs from these two scenarios of mismatches depend differently on environmental and population characteristics. The post-settlement mismatch depends on the relative abundance of eelgrass—in eelgrass-dominated environments; this mismatch will likely result in significant costs, but in kelp-dominated environments the costs probably will be minor. The habitat-correlation mismatch, which is the focus of Padilla's and Adolph's model, depends on voluntary and involuntary dispersal rates. If dispersal rates are high, then typical intervals of time between changes in habitat type are very short—so short, in fact, that under some circumstances tooth morphology is uncorrelated with habitat type even for relatively short time lags in tooth plasticity. In our simulations, only time lags sufficiently short to maintain correlation between tooth morphology and habitat type confer feeding benefits, and consequently are selectively advantageous. All time lags long enough to lose correlation between tooth morphology and habitat type are functionally equivalent to each other. That is, plasticity with these time lags confers no feeding or selective benefits relative to a constitutive tooth morphology that is optimal for the prevailing habitat conditions.

Our assumptions about the density-dependent mechanisms that regulate densities of *Lacuna* over long periods of time are also important. As noted, we made a simple and plausible assumption that dispersal from a given habitat is an increasing function of crowding. A primary consequence of this assumption is that mortality during dispersal increases when populations are high, putting a limit on the maximum attainable population size. A secondary consequence, which we had not considered prior to our simulations, is that high dispersal rates when simulated populations are large could cause residence times in any given habitat to be shorter than even the shortest simulated time lags. This substantially reduces or eliminates potential benefits of variations in radular length for jumping individuals. Hence, in our integrated model, mechanisms of density-dependent regulation of populations within habitats interacted in (to us) unanticipated ways with dispersal behavior to strongly regulate the selective costs and benefits of time lags in phenotypic plasticity.

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Discussion, perspectives, and conclusions

In this article, we argue for a renewed focus among organismal biologists on integrative and quantitative "whole-organism" models that span current disciplinary boundaries. Such models make explicit the interactions among biological processes operating at different levels of biological organization through multiple mechanisms of interaction across diverse scales of time and space. We propose a conceptual framework for integrative modeling, which is not unique, but which we believe exemplifies an effective strategy for accomplishing this objective.

We developed a preliminary but illustrative case study of integrative modeling that focused on a complex organismal character, phenotypically plastic shapes of teeth in snails of the genus *Lacuna*. That the morphology of radular teeth is plastic and is induced by different feeding environments is well known from laboratory experiments. However, fundamental evolutionary, life-history and demographic questions concerning this plastic response remain unanswered: What is the adaptive value of extant radular forms, relative to hypothetical alternatives? In what ways, if any, are observed dispersal behaviors adaptive and how do dispersal patterns affect the selective benefits of phenotypically plastic teeth? How are the costs and benefits of alternative strategies affected by the variation of populations within habitats, and of habitats within environments? In our view, these questions and many others like them have not been answered about this, and most other, morphological plasticities. Indeed, we think they cannot be answered by traditional approaches to organismal biology, because those approaches are ill-suited to investigations spanning the requisite diversity of mechanisms, scales, and disciplinary knowledge.

We schematically illustrated our framework in general terms in Fig. 1 and in application to the phenotypic plasticity of *Lacuna* teeth in Fig. 4. Formulating our model enabled us to easily assess the logical consequences of our assumptions. Our simulations gave us new insights into indirect effects of assumed mechanisms, and provided testable predictions of quantitative characteristics with which to assess our assumptions. In our modeling, we adopted a very simple agent-based simulation approach that in our view maximized our abilities to represent key biological mechanisms and minimized unjustified assumptions. Further analysis could substantially improve this model. For example, population-level dynamics are frequently analyzed using ordinary or partial differential equations, leading to significant computational savings and important analytical results. Deriving differential equation models that accurately approximate agent-based models requires assumptions about underlying distributions of organismal characters, spatial distributions, encounter rates, and the like. The strength of a modeling approach initially based explicitly on individual organisms is that we can provide a rigorous justification for any particular equation that would be used for population level—or other—dynamics.

The case study we present is necessarily incomplete—indeed, one of our points is that collaborations among diverse specialists over a sustained period are required to properly design and implement an integrative model. Nonetheless, our preliminary results from this partially developed model were already sufficient to suggest previously unexplored linkages between environmental characteristics, population-regulating mechanisms, behavioral responses, and other biological elements that provide the context defining functional costs and benefits of the phenotypic plasticity of *Lacuna*'s teeth. The model also highlighted mechanisms and parameters that are poorly constrained. Because the effects of each mechanism we included in our model are reflected in many other characters at many different organizational levels, our model provided additional direct and indirect methods for estimating parameters and generating predictive tests of hypotheses. A future effort would clearly be strengthened significantly by this integrative modeling framework, even in its rudimentary state, and the results of that effort would strengthen the model and make it an even more effective tool going forward.

Central to an integrative approach that accomplishes these ends is the requirement that all modeled processes and parameters have been measured, or could be measured. This is a profound challenge for most biological systems, in which individual researchers tend to focus on one level of organization or process. For example, in our case study, field-sampling provided information on the absolute and relative densities of species in specific types of habitats at given times, and repeated sampling through time helped place bounds on the range of densities experienced. However, from such sampling it was not possible to determine dynamic processes such as dispersal rates, growth rates, reproduction rates, or mortality. Laboratory experiments helped us to determine the effects of type of food on growth and reproduction, and how quickly snails produced and replaced teeth. However, it was not possible by simply measuring them individually to link these

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effects to their consequences at the population and habitat levels, as is necessary to assess the functional consequences of the plasticity of *Lacunas*' teeth. We regard the integrative modeling framework as a continuously evolving expression of our most viable hypotheses about *Lacuna* organismal biology. As illustrated by our case study, this framework both quantifies the implied adaptive value of phenotypically plastic tooth morphology and provides us numerous means to test, falsify, and revise our current best understanding.

Acknowledgments

This article was inspired by discussion and results of a workshop: How organisms walk the tightrope between stability and change, February 28–March 3, 2013, The Banbury Center, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA. We would like to thank all of the participants of the workshop for discussion: Neda Bagheri, Alexa Bely, Zac Cheviron, Noah Cowan, Elizabeth Dahlhoff, Tom Daniel, Xinyan Deng, Manuel Diaz-Rios, Patsy Dickinson, Colleen Farmer, Kendra Greenlee, Melina Hale, Cheryl Hayashi, Laura Corley Lavine, Donal Manahan, James Marden, Kristi Montooth, Amy Moran, Fred Nijhout, David Plachetski, Matt Reidenbach, Scott Santos, Joel Smith, Eduardo Sontag, Billie J. Swalla, Lars Tomanek, Brian Tsukimura, and William G. Wright. We also thank William E. Zamer for advice and discussion.

Funding

We gratefully acknowledge support from National Oceanic and Atmospheric Administration Washington Sea Grant (NA10OAR-4170057 to D.G.), the National Science Foundation (NSF IOS 1243801 to D.K.P.), and the Velux Foundation and the Centre for Ocean Life, Danish Technical University (to D.G.), which made this research possible.

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