

Research Article

Reaction Network Modeling of Complex Ecological Interactions: Endosymbiosis and Multilevel Regulation

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Endosymbiosis is a type of symbiosis where one species of microscopic scale inhabits the cell of another species of a larger scale, such that the exchange of metabolic byproducts produces mutual benefit. These benefits can occur at different biological levels. For example, endosymbiosis promotes efficiency of the cell metabolism, cell replication, and the generation of a macroscopic layer that protects the organism from its predators. Therefore, modeling endosymbiosis requires a complex-systems and multilevel approach. We propose a model of endosymbiosis based on reaction networks, where species of the reaction network represent either ecological species, resources, or conditions for the ecological interactions to happen, and the endosymbiotic interaction mechanisms are represented by different sequences of reactions (processes) in the reaction network. As an example, we develop a toy model of the coral endosymbiotic interaction. The model considers two reaction networks, representing biochemical traffic and cellular proliferation levels, respectively. In addition, the model incorporates top-down and bottom-up regulation mechanisms that stabilizes the endosymbiotic interaction.

1. Introduction

Endosymbiosis is a particular kind of symbiosis which occurs when an organism (endosymbiont) lives within another (host) in a mutualistic relationship. This relationship is given by the coupling of the host's and endosymbiont's metabolisms through exchange of useful metabolites [1, 2]. The endosymbiotic concept was proposed by Lynn Margulis, who developed *Serial Endosymbiosis Theory* (SET) to explain the origin of plastids and mitochondria as organelles in the eukaryotic cell, promoting the emergence of eukaryotic cell complexity [3]. Endosymbiosis is recognized as a widespread mechanism in nature and is taking relevance as an evolutionary mechanism in different lineages [4]. Symbiotic interactions allow alternative ways of evolution, for example, through natural selection, in evolving complexity for a population of individuals. Endosymbiosis implies the coupling of two or more species in an intimate relationship.

Such relation leads to an increase of phenotypic complexity at the genomic, physiological, and morphological levels. Therefore, endosymbiosis enables ecological expansion into different and new niches. As Darwin said in his book *The Origin of Species*: “*Natural Selection cannot possibly produce any modification in a species exclusively for the good of another species; although throughout nature one species incessantly takes advantage of, and profits by, the structures of other*” [5].

One of the examples of endosymbiosis corresponds to the relationship between corals (Cnidaria, Anthozoa) and a photosynthetic algae of the genus *Symbiodinium* or *Zooxanthellae* [6], in which the latter lives within the gastrodermis coral cells. This relationship is typical when the environmental food is scarce [7, 8], and they (the *Symbiodinium* and the coral) can feed back through their metabolisms, by the nutrient acquisition via the cycling of organic compounds which supplies extra energy to both

endosymbiont and host. The latter provides an ecological advantage and thus promotes survival because the metabolic coupling between coral cells and *Symbiodinium* promotes cellular proliferation implying coral's growth. Interestingly, such growth process is regulated by the release of an inhibition factors by the endosymbiont. Namely, when certain byproducts of the energy generation process that might harm the cells surpass a threshold, inhibition factors are released, stopping the growth process [9, 10]. Thus, endosymbiosis is a multilevel and self-regulated process.

The establishment of endosymbiosis gives rise to new structural and morphological properties and thus to new evolutionary competences. However, the experimental study of endosymbiotic systems is extremely complicated, and it cannot be properly achieved by analyzing the interacting species separately [1]. For this reason, a modeling framework for the establishment of the endosymbiosis relationship is of crucial importance from an ecological perspective [11].

Such modeling framework of endosymbiosis should incorporate both the multiple levels at which the interaction takes place as well as bottom-up and top-down regulation mechanisms. Interestingly, these features are relevant not only for endosymbiosis but also for embryonic development [12], ecological trophic networks [13], and also during evolutionary processes [14, 15]. For this reason we aim at proposing a process-based and multilevel approach to the representation of endosymbiosis.

Reaction networks, and equivalent languages such as Petri Nets [16], are the main representational languages in systems biology [17, 18]. They allow for a description of the dynamical properties of complex biochemical reaction system of multilevel nature, using methods that exploit both the structure and stoichiometry of the network [19, 20]. Reaction networks can also describe processes whose entities are not only biochemical [21]. Indeed, reaction networks have been applied to model the exchange of economic goods [22], the influence of political decisions [23], the evolution of cooperation [24], and other game-theoretical settings [25]. Therefore, reaction networks can not only be applied outside biochemical settings, but also have the potential to become an intuitive language to describe and study problems of multidisciplinary nature from a systemic perspective [26, 27]. The latter can have novel and powerful applications particularly in the modeling of ecological interactions [28].

In the present study, we provide a simple mathematical model of the interaction between anthozoan corals and symbiont algae of the genus *Symbiodinium* by representing the mechanisms of their endosymbiotic interaction using reaction networks. In particular, we propose two reaction networks modeling endosymbiosis at a biochemical traffic (lower level) and cellular proliferation (upper level). We point to how endosymbiosis occurs at each level and focus on representing the multilevel regulatory mechanisms that are known to happen in this two-layer interaction process.

The paper is organized as follows. In Section 2, we review in detail the endosymbiotic mechanisms known up to date. In Section 3, we introduce the basics of ecological modeling using reaction networks. In Section 4, we propose a toy model of the endosymbiotic as well as of the multilevel

regulatory mechanisms of the *Symbiodinium*. We complement the latter with a discussion of how we could scale up to more realistic models of the interaction, and we end with a conclusion.

2. Endosymbiotic Mechanisms and Their Levels of Interaction

Corals are ancestral animals formed by only four epithelial layers: oral ectoderm, oral endoderm, aboral endoderm, and calcidodermis (Figure 1). In conditions of food limitations, corals (Cnidaria) can engulf photosynthetic dinoflagellates (algae) of the genus *Symbiodinium* from the surrounding water. *Symbiodinium* cells engulfed by the coral are hosted gastrodermis layer of the coral that surrounds the gastric cavity (oral endoderm), where they can still perform photosynthesis. Namely, the *Symbiodinium* acquires nutrients and metabolic residual compounds from the host and produces organic compounds. These organic compounds that are product of the photosynthesis not only foster the growth and respiration of endosymbionts, but also are used to produce nearly up to 95% of the host's energy demand [29].

The latter phenomenon occurs in the oral endoderm (yellow layer) within a membrane called symbiosome (white layer), produced by both the host and the *Symbiodinium* (green layer) in Figure 1.

The endosymbiotic interaction occurs in various phases: (1) initial contact; (2) engulfment; (3) sorting; (4) proliferation; (5) dynamic stability; and (6) dysfunction (Figure 2). The initial contact phase is characterized by the compatibility recognition between the host and the symbiont (Figure 2(a)). Namely, each coral lineage is compatible with one or more *Symbiodinium* lineages [31]. When compatible, the coral engulfs the algae (Figure 2(b)). After successful engulfment, the symbiosome membrane is developed promoting the stabilization and persistence of the symbiont within the cells of the coral [32]. Next, the persistence of the symbiont depends on the balance between host cell growth and proliferation of the symbiont population. The latter balance can be achieved by a series of sorting and proliferation steps, reaching the dynamic stability phase (Figure 2(c)). This last stage is characterized by the metabolic exchange and nutrient trafficking, where the products generated by dinoflagellate support coral metabolism, growth, reproduction, and survival and also promote the conservation and recycling of essential nutrients. In this phase, the typical calcium deposition of coral reef takes place. This phenomenon is strongly promoted by the endosymbiosis [33]. This calcium matrix deposition protects the coral against predators and decreases mechanical erosion of the marine environment, further promoting its persistence and growth [34] (Figure 2). The last phase of the endosymbiotic interaction is dysfunction, related to the bleaching of the calcium layer, which is explained by the *Symbiodinium* expulsion by exocytosis and coral mortality, and by host cell detachment or host cell apoptosis, generally due by the production of reactive oxygen species or cellular signaling [35]. Coral reef bleaching is increasingly

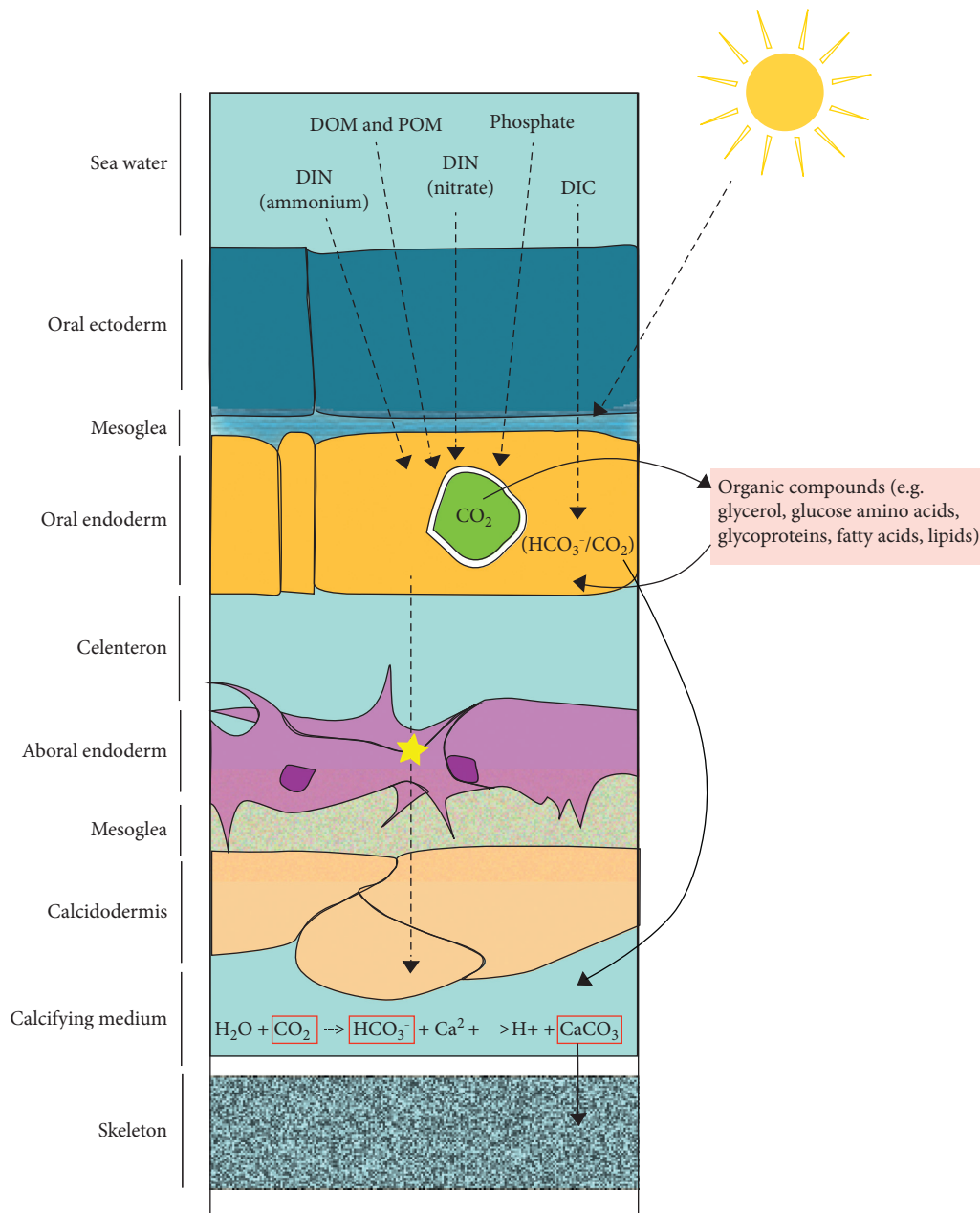


FIGURE 1: Metabolic interactions and calcification processes. DIC: dissolved inorganic carbon uptake; DOM: dissolved organic matter; POM: particulate organic matter; DIN: dissolved inorganic nitrogen.

threatened by recent changes in salinity, higher solar radiation, increased sedimentation or pollutants, elevated sea surface temperatures, and global warming [36].

In the dynamic stability phase, coral and algae metabolisms couple controlling each other's growth, promoting functional efficiency [37, 38]. Typically, coral gastrodermis regulate their density to maintain up to two *Symbiodinium* per cell [39, 40]. Such regulation incorporates various mechanisms, such as the expulsion and degradation of *Symbiodinium*, signaling factors affecting the cellular cycle such as a host release factors (HRFs) [9, 10], and other processes and biochemical factors that act as inhibitory factors (IFs) released by the host, hindering the growth of

symbionts [41–43]. The release of IFs depends on the concentration of photosynthetic byproducts in the metabolism of the endosymbiont. Thus, this factor is relevant to achieve a steady growth and nutrient traffic state.

In steady state, *Symbiodinium* is benefited by acquisition of inorganic carbon, inorganic nitrogen, and inorganic phosphate by the coral, which gives the *Symbiodinium* greater availability of these nutrients for its own growth. In turn, as a product of the photosynthesis of *Symbiodinium*, diverse compounds are generated that nourish the coral, such as glycerol, amino acids, glucose, maltose, Krebs cycle molecules, pyruvate, zooxanthellamine, and zooxanthellatoxins [44]. In this way, the coral develops mechanisms and processes that

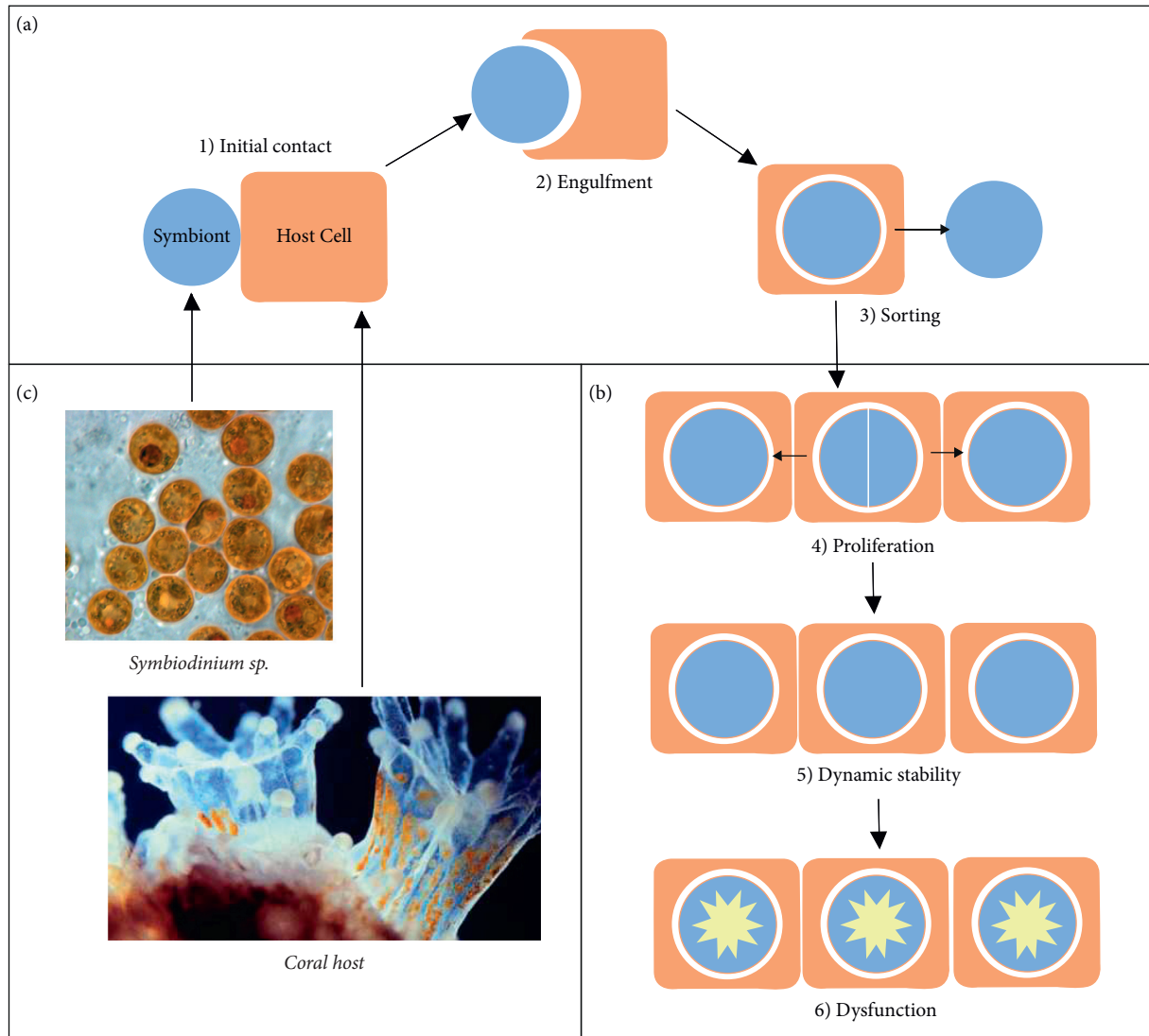


FIGURE 2: Phases of endosymbiosis establishment and persistence in cnidarian-*Symbiodinium* interaction (modified from [30]).

benefit endosymbiont and vice versa. These mechanisms can affect other processes occurring at other cellular layers of the host. For example, in the carbon metabolism, coral actively transports carbon from outside to gastrodermis cells and concentrates CO_2 near the endosymbiont from the enzyme carbonic anhydrase (CA) located around the symbiosome, fostering the algae photosynthetic process. Another example is in the nitrogen metabolism, which is necessary to the algae in the form of ammonium (NH_4^+) and nitrate (NO_3^-), to form amino acids, and which are also a resource to the coral because they are transported back to the host as a nutrient. Calcification also is promoted as a consequence of the metabolic coupling of both organisms, since dissolved inorganic carbon necessary for photosynthesis by *Symbiodinium* is also necessary to produce calcium skeleton, which is mainly composed of calcium carbonate (CaCO_3) crystallized in an organic matrix. Calcification is enhanced by light in presence of *Symbiodinium*, and this affects the balance of dissolved inorganic carbon, taking CO_2 for photosynthesis reactions, which directly promotes the precipitation of

CaCO_3 . In addition, organic products of photosynthesis are precursors of the synthesis of organic matrix, and energy and oxygen supplementation to the host increases its metabolism and promotes faster calcification [33,45]. Moreover, corals presenting *Symbiodinium* are capable of living in clear and shallow waters, where they are constantly threatened by high intensity solar radiation. Despite the fact that light levels are harmful, symbionts produce UV-absorbing sunscreen compounds, like mycosporine amino acids (MAAs), which act as light-harvesting pigments and free radical scavengers [46].

In summary, there are both metabolic coupling and regulation between *Symbiodinium* and host cells. The latter promotes their growth, reproduction, and survival at different organizational levels. We organize the levels of interaction of the endosymbiotic interaction as follows (see Table 1):

(1) Biochemical traffic level: related to the biochemical relationship and nutrient traffic inside the cells of the coral, and between the coral and *Symbiodinium* cells.

TABLE 1: Representing ecological interactions using reaction networks.

Interaction	Positive	Neutral	Negative	Reaction
Amensalism	—	x	y	$x + y \longrightarrow x$
Antagonism	x	—	y	$x + y \longrightarrow 2x$
Mutualism	x, y	—	—	$x + y \longrightarrow 2x + 2y$
Commensalism	y	x	—	$x + y \longrightarrow x + 2y$
Competition	x, y	—	x, y	$x + y \longrightarrow x$ and $x + y \longrightarrow y$

(2) Cellular proliferation level: related to the cellular reproduction to achieve the dynamic stability state between the cells biomass of both partners.

(3) Organismic level: related to the growth of the coral and the protection from biological and mechanical erosion due to the calcidodermis production generated in lower levels.

Therefore, when the endosymbiotic interaction is established, the above explained processes at these three levels intertwine benefiting the persistence of endosymbiosis.

3. Reaction Network Modeling of Ecological Interactions

A reaction network is defined by a pair (M, R) , where $M = \{a, b, c, \dots\}$ is a set of molecular species, and $R \subseteq \mathcal{P}_m(M) \times \mathcal{P}_m(M)$ is a set of reactions, where $\mathcal{P}_m(M)$ denotes the set of multisets of M . For example, consider

$$\begin{aligned}
 M &= \{a, b, c\}, \\
 R &= \{r_1, r_2, r_3, r_4, r_5, r_6\}, \text{ with,} \\
 r_1 &= a \longrightarrow 2a, \\
 r_2 &= a + c \longrightarrow c, \\
 r_3 &= b + c \longrightarrow b + 2c, \\
 r_4 &= a \longrightarrow \emptyset, \\
 r_5 &= c \longrightarrow \emptyset, \\
 r_6 &= b + a \longrightarrow \emptyset.
 \end{aligned} \tag{1}$$

Note that reaction $r_1 = a \longrightarrow 2a$ represents a self-reproduction process of species a , reaction $r_2 = a + c \longrightarrow c$ represents the destruction of species a out of the interaction of species a and c , and $r_3 = b + c \longrightarrow b + 2c$ represents the reproduction of species c catalyzed by b . Similarly, the destruction and disappearance of a and c are represented by r_4 and r_5 , respectively, and the mutual destruction of a and b is represented by r_6 .

The dynamics of a reaction network can be modeled using difference, stochastic, or differential equations [47], and a vast amount of literature is devoted to complement these approaches with structural analysis of the network [18–20].

Recently, reaction networks have been proposed to represent ecological interactions and ecosystems [28, 48]. In particular, contrary to traditional network approaches, which represent different ecological interactions by different types of links, reaction networks characterize types of ecological interactions by the way in which combinations of

inputs produce combinations of outputs. This opens up an exponentially wider range of interacting processes. Typical ecological interactions such as depredation, cooperation, and parasitism are easily expressed by means of reaction networks. For example, reaction $r_3 = a + c \longrightarrow c$ of the reaction network equation (1) corresponds to an amensalistic interaction, since a is destroyed in the presence of c without altering c . Likewise, $r_4 = b + c \longrightarrow b + 2c$ is a commensalistic relation, because c benefits from its interaction with b without altering b . In Table 1, we represent ecological interactions using minimal reaction networks.

Interestingly, when one is interested in representing an ecological interaction in more detail, the model of the interaction is made by a collection of reactions representing the interaction mechanism. Since the interaction mechanisms of different interaction might share some species at both resource and product levels, an ecological system is modeled by a large reaction network composed by the coupling of multiple subnetworks, each of these subnetworks representing one of the ecological interactions of the ecosystem [28].

In addition to the structural transformational relation specified by a reaction, there is usually a parameter which indicates its likelihood to happen. This value, known as kinetic parameter or kinetic rate, is indicated above the arrow which specifies the reaction. For example, in the case of competition, the reactions $r_1 = x + y \xrightarrow{k_x} x$, $r_2 = x + y \xrightarrow{k_y} y$ constrained to $k_x < k_y$, would indicate that y has a competitive advantage over x , as reaction r_2 is more likely to happen than r_1 .

Moreover, the empty set symbol \emptyset represents the environment, i.e., what is not part of the system under study. For this reason, reactions $\emptyset \xrightarrow{k} a$ and $a \xrightarrow{k} \emptyset$ indicate the *inflow* (born, entrance, spontaneous formation) of a in the system while indicates the *outflow* (death, exit, decay) of a from the system.

4. Multilevel Reaction Network Model of Endosymbiotic Interactions

In this section, we develop a reaction network model of the biochemical traffic and cellular proliferation levels of the endosymbiotic interaction depicted in Table 2. Next, we explain how to represent the regulation mechanisms occurring between these two levels. This model extends previous work [49].

4.1. Biochemical Traffic Level. At this level, endosymbiosis is characterized by the possibility to metabolize elements that coral cells alone cannot metabolize, and by the increase of

TABLE 2: Levels of interaction and processes that are enhanced by endosymbiosis at each level.

Level of interaction	Processes driven by endosymbiosis
Biochemical traffic	Biochemical interrelationship, nutrient traffic, energy flow, and calcidodermis biochemical production.
Cellular proliferation	Cellular reproduction and death of <i>Symbiodinium</i> and host cells.
Organismic	Coral growth and protection against predators and other physical threats

calcium production, which leads to a macroscopic calcium layer that protects the macroscopic coral from predators and erosion. The following reaction network represents in a simplified way the endosymbiotic interaction at the biochemical traffic level:

Species set:

R_H : host cell resource

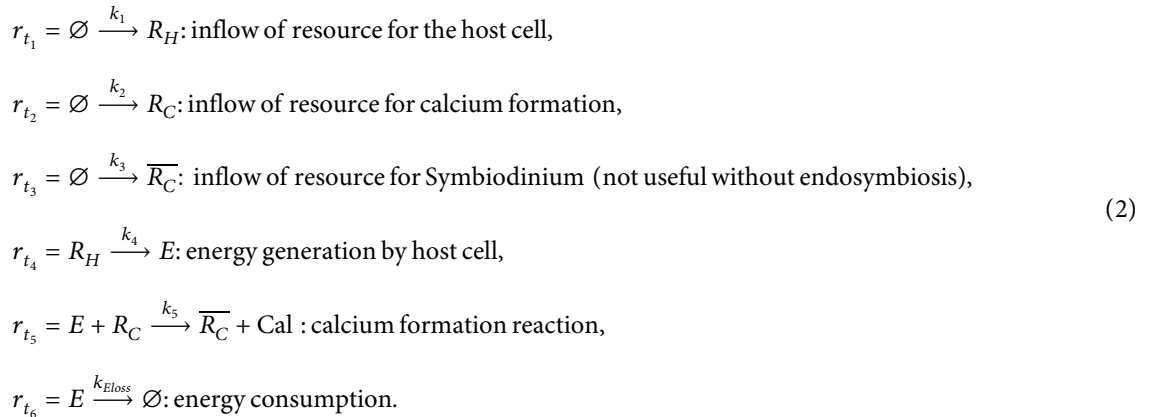
E : energy

R_C : resource to form calcium layer

\overline{R}_C : resource that is product of the calcium formation reaction

Cal_C: calcium

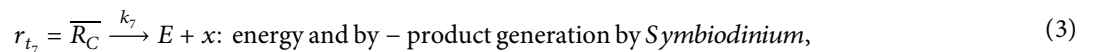
The reactions of a cell without *Symbiodinium* in it are



Note that in the model given by equation (2) there is an energy generation pathway given by the reaction sequence (r_{t_1}, r_{t_4}) , which in turns activate the calcium formation pathway (r_{t_2}, r_{t_5}) . For simplicity, the energy consumption pathway is represented by r_{t_6} . Note that if k_1 is too small, meaning that there is not enough R_H available in the

environment, the energy generation process driven by r_{t_4} might not compensate for the energy consumption represented by r_{t_6} .

When the *Symbiodinium* is present in the cell, we add the following reaction to the model:



meaning that *Symbiodinium* is capable of generating energy for the cell from the resource \overline{R}_C and creating x , which correspond to photosynthetic byproducts of algae metabolism, that can be sensed by the host. This last reaction activates a new energy generation pathway (r_{t_3}, r_{t_7}) by using

up the byproduct \overline{R}_C of r_{t_5} , and hence metabolizing \overline{R}_C to produce more Cal. The latter modifies the chemical equilibrium between R_C and \overline{R}_C by taking up more \overline{R}_C for its photosynthetic metabolism, which directly favors the precipitation of Cal by Le Chatelier's principle [33].

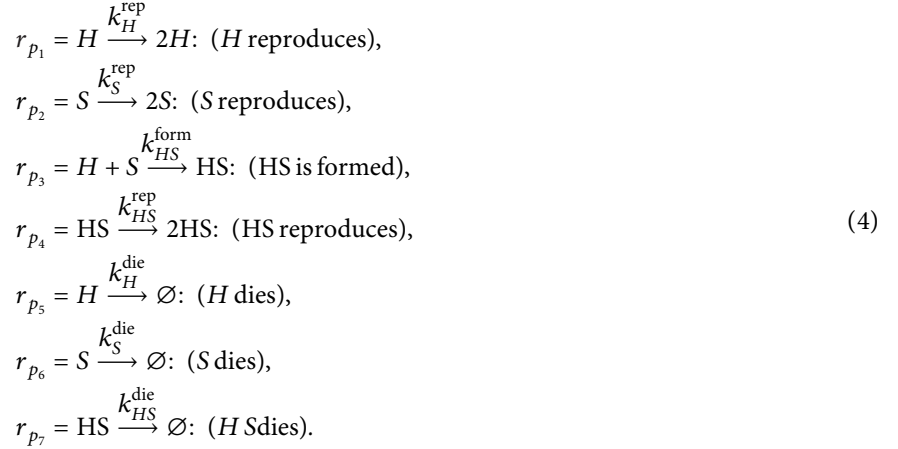
4.2. *Cellular Proliferation Level.* At the cellular proliferation level, we identify three types of cells:

H : host

S : *Symbiodinium*

HS: symbiont (host and *Symbiodinium* interacting in endosymbiosis)

Similar to the previous case, we will keep the model very simple, so we introduce reactions that account for the most basic interactions between cells, cell proliferation, and endosymbiosis. Other aspects such as the multiple manners in which the cells can die, or the environmental conditions that foster their reproduction, are not included.



Under appropriate internal cell proliferation conditions $k_{HS}^{\text{rep}} > k_S^{\text{rep}}$, and $k_{HS}^{\text{rep}} > k_H^{\text{rep}}$. This is because the *Symbiodinium* is supposed to have more energy due to its more efficient metabolic activity. Similarly, $k_{HS}^{\text{die}} < k_S^{\text{die}}$, $k_{HS}^{\text{die}} < k_H^{\text{die}}$ because HS has a larger calcium layer, which protects it best from predators, and because HS has a more diverse metabolism. On the other hand, under cell proliferation conditions that become harmful to the coral we expect $k_{HS}^{\text{rep}} < k_S^{\text{rep}}$, and $k_{HS}^{\text{rep}} < k_H^{\text{rep}}$ because the organism releases inhibitory factors that reduce its tendency to reproduce, and $k_{HS}^{\text{die}} > k_S^{\text{die}}$, and $k_{HS}^{\text{die}} > k_H^{\text{die}}$ due to *Symbiodinium* cell expulsion or degradation of HS.

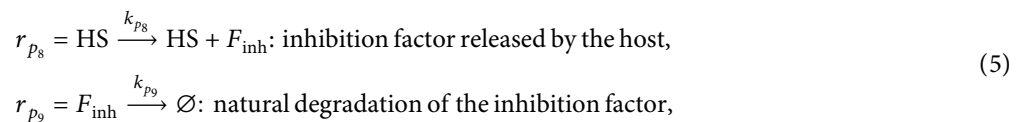
4.3. *Levels of Association and Regulation: An Example.* As seen in Section 2, it is possible to identify variables in one level that constrain or promote the activity at other levels. In this model, we consider only one top-down constraint, and one bottom-up promoter, by modifying the kinetic parameter k_{HS}^{rep} as an example of how to represent the multilevel control mechanisms.

For the bottom-up control mechanism, we have that the nutrient traffic level reactions r_{t_4} and r_{t_7} produce energy, and

that such energy is consumed by reactions r_{t_5} and r_{t_6} for metabolic survival. Therefore, when the production of energy is not enough to compensate its consumption, the cell HS will not reproduce, so r_{p_4} becomes inactive. Analogously, when the production of energy surpasses its consumption, r_{p_4} becomes active. Hence, we consider a threshold of total energy production in the lower level E^* , and when the energy in the nutrient traffic level surpasses this threshold, the kinetic parameter k_{HS}^{rep} activates.

For the top-down direction, we consider a signaling species that is known to regulate the proliferation of the *Symbiodinium* according to its sensitivity to some photosynthetic byproducts. The photosynthetic product is in this case represented by the species x at the nutrient traffic level (see r_{t_7} in equation (2)). Let the signaling species be F_{inh} , representing the factor that is released when the endosymbiont's byproducts x surpass a threshold x^* , and that is sensed by the host, deactivating the reaction r_{t_7} .

In order to provide a formal specification of the bottom-up and top-down regulatory mechanism of HS reproduction, we introduce the following reactions at the organismic level:



where $k_{p_8} = h(x - x^*)$, with $h(x - x^*)$ being the known Heaviside-step function, which is zero if $x - x^* \leq 0$ and a reference value (one for simplicity) if $x - x^* > 0$. Hence,

reaction r_{p_8} is inactive when the concentration of x is below the threshold x^* and once the concentration x surpasses the threshold x^* the inhibition factor is released,

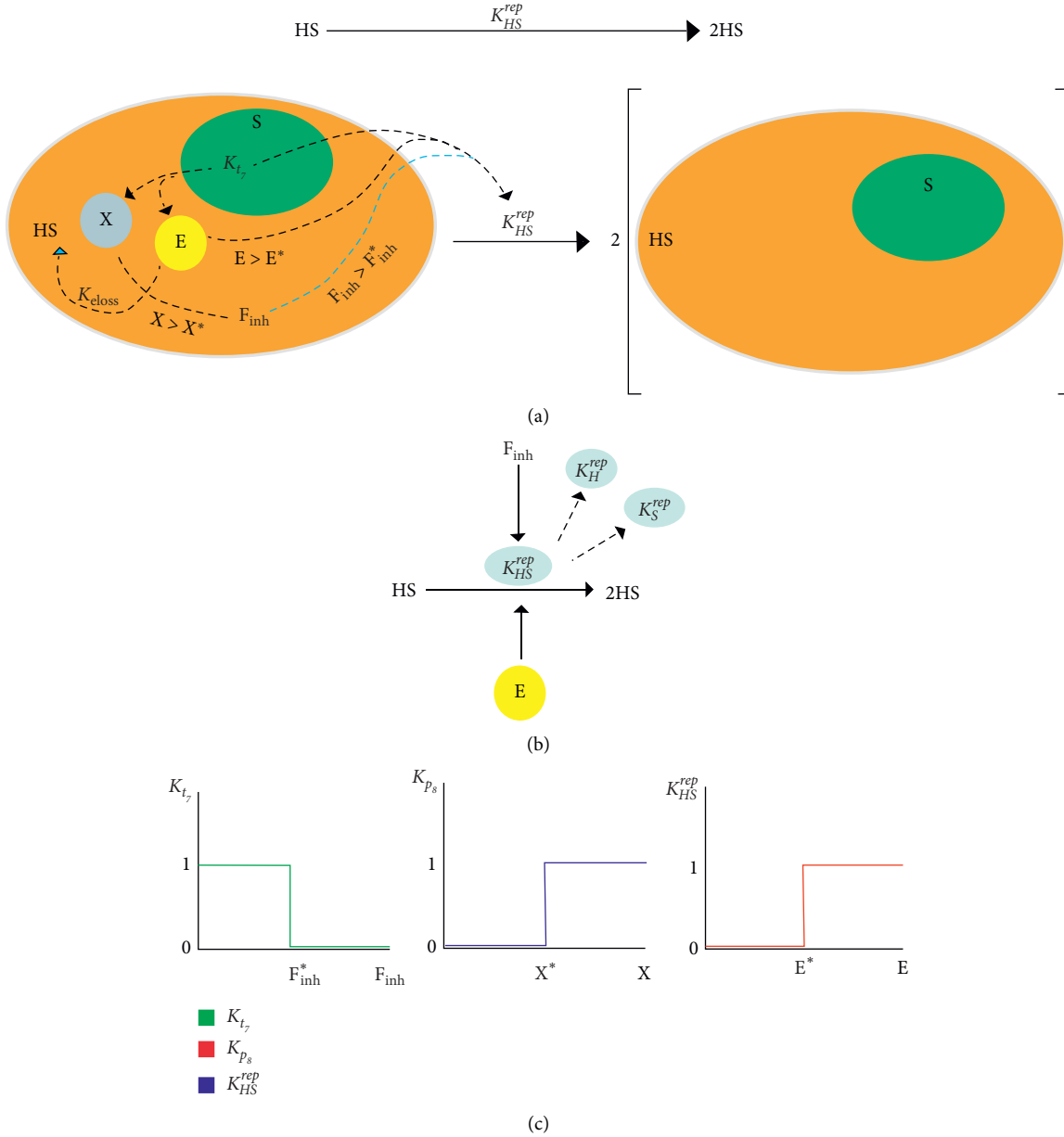


FIGURE 3: Schema of the regulation of species through two levels of interaction. (a) Representation of the interaction between the host (H in orange) and the *Symbiodinium* (S in green) in the reaction r_{p_4} , and how E , x , and F_{inh} affect the kinetic parameter k_{HS}^{rep} . Dotted lines represent ways of regulation of k_{HS}^{rep} , occurring when $E > E^*$, $x > x^*$, and $F_{inh} > F_{inh}^*$. Blue dotted line represents the release of inhibition factors by coral cells. (b) Regulation of k_{HS}^{rep} , by F_{inh} and E , showing the indirect regulation of k_S^{rep} and k_H^{rep} in dotted lines. (c) Dependency of k_{t_7} , k_{p_8} , and k_{HS}^{rep} on F_{inh} , x , and E , respectively.

while k_{p_9} is a constant rate controlling the degradation of F_{inh} .

Next, the kinetic parameters k_{t_7} becomes explicitly dependent on F_{inh} to control the extra production of energy and x :

$$k_{t_7} = h(F_{inh} - F_{inh}^*), \quad (6)$$

where F_{inh}^* is the F_{inh} tolerance threshold that once surpassed the production of extra energy is inhibited.

Finally, we modify k_{HS}^{rep} , making it dependent on E as follows:

$$k_{HS}^{rep} = h(E - E^*). \quad (7)$$

Therefore, when the nutrient traffic is intensified by the presence of the *Symbiodinium*, the extra energy produced regulates in a bottom-up manner the kinetic parameter k_{HS}^{rep} of the endosymbiotic interaction at the cellular proliferation level, activating its proliferation. Conversely, when proliferation of *Symbiodinium* surpasses the amount of the byproducts required for operating its metabolism, F_{inh} is released, inhibiting in a top-down manner the kinetic parameter k_{t_7} . We represent

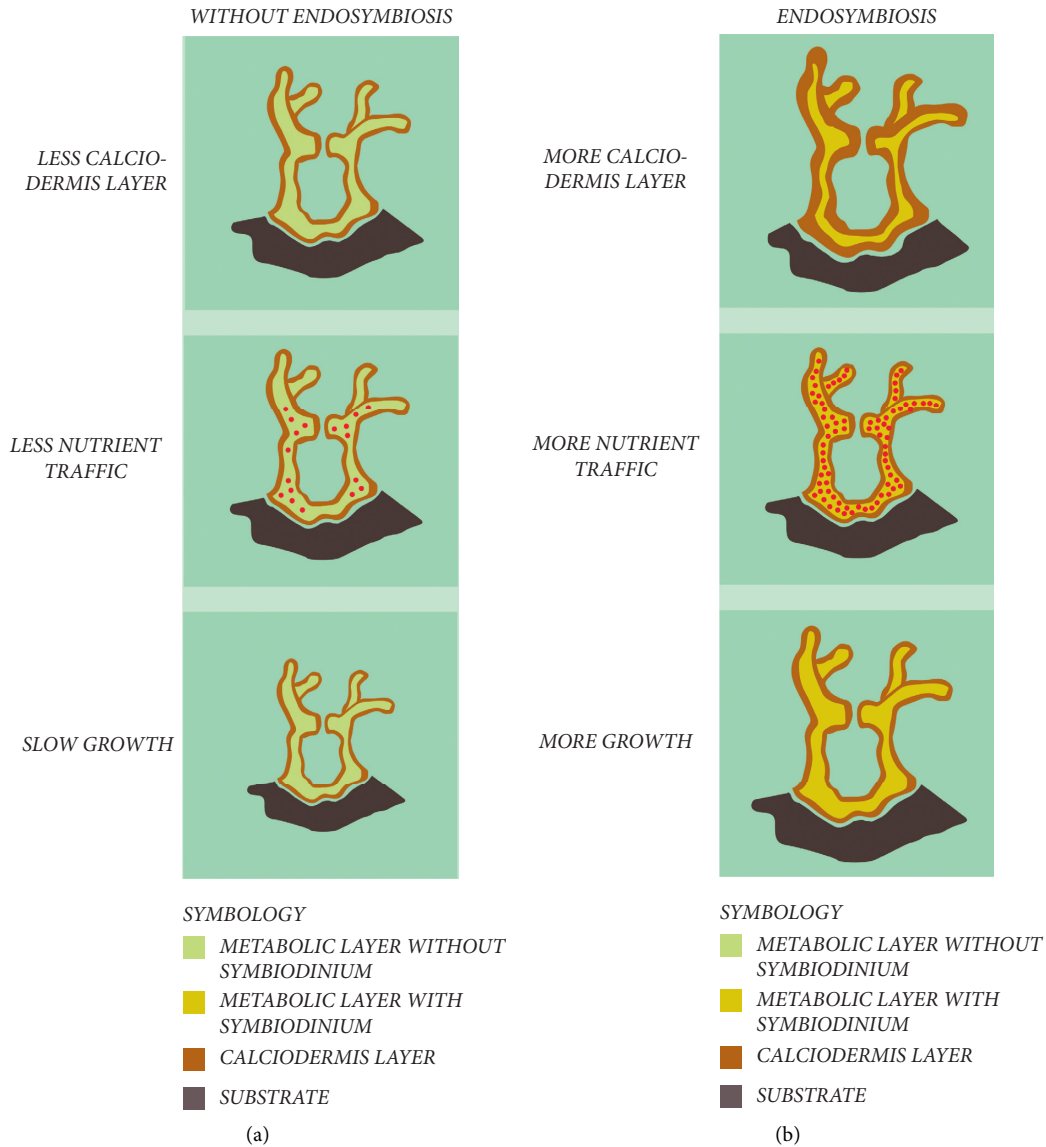


FIGURE 4: Organismal system processes, showing in a comparative scenario how different processes as calcidodermis production, nutrient traffic, and coral growth are enhanced by the endosymbiosis interaction (b) with respect to the coral without *Symbiodinium* (a).

these interrelationships in Figure 3, specifically the regulation for k_{HS}^{rep} .

5. Discussion

A key concept in biological complexity is the emergence of properties that coregulate the persistence of processes occurring at different levels, and which are needed for organisms to live and reproduce. In this sense, the representation of biological phenomena as a hierarchy of intertwined dynamical processes is an important challenge to advance our understanding of biological complexity.

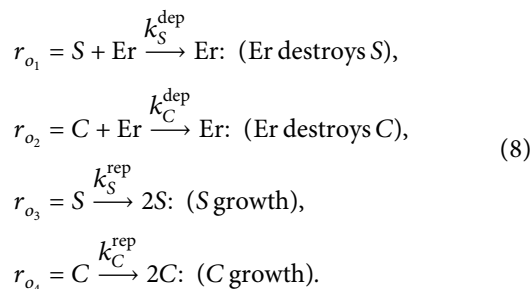
Coral endosymbiosis presents an interesting opportunity to study this issue. First, there is a concrete evidence of the microscopic interaction between the *Symbiodinium* and the coral host cell as well as evidence of the micro- and macroscopic consequences of such interaction. Secondly, levels

of interaction can be clearly delineated. Namely, in our model we considered a nutrient traffic and cellular proliferation levels, where the former is undoubtedly nested in the other. Thirdly, as explained in Section 4.3, even in our simple two-levels model, bottom-up and top-down regulatory mechanisms occur. Therefore, the coral-*Symbiodinium* endosymbiotic interaction exhibits structural features allowing a simple but explicative model of its mechanism.

Reaction network-like models of biological interactions have only recently been investigated [28, 48, 49], but seem to provide the necessary versatility to incorporate multilevel regulatory mechanisms in a sensible way. However, this approach is in its infancy and requires extensions.

We propose two ways in which our model can be extended. The first is to give a more detailed account of what occurs at each level, and the second is to incorporate more levels. For the former case, a more detailed account of the

reproduction of the symbionts within the endosymbiont could be developed. For example, we can introduce a new species HS_2 meaning that the host has two endosymbionts in it, and incorporate the reactions $HS \rightarrow HS_2$ and $HS_2 \rightarrow 2HS$ to represent the replication process of the endosymbiont. Analogously, it is possible to incorporate other necessary steps to provide a more realistic account of the phenomena such as regulation at the recognition level [50], symbiosome maturation [51], and regulation of symbiont biomass by symbiont expulsion and degradation [32, 52]. For the latter case, we can incorporate the macroscopic organismic level in our model, involving the coral's growth and survival. The simplest model of the endosymbiotic organismic activity lies at comparing the coral's ability to grow, with and without *Symbiodinium* (named S and C , respectively) under mechanical and biological erosion (Er), and its ability to reproduce. The coral's ability to grow is directly related to nutrient traffic and calcidodermis strengthening as depicted in Figure 4. For simplicity, we will provide an example focusing on the growth of C , in the presence of S , and erosion Er. Other aspects such as the reproduction of erosion factors (e.g., fish that predate the coral), or natural death of the S and C , are not incorporated.



The values k_S^{dep} , k_C^{dep} and k_S^{rep} , k_C^{rep} are the mechanical and biological erosion and growth rates of S and C , respectively.

Note that in this case the interactions that C and S have with the other species are equal. Therefore, the parameters k_S^{dep} , k_C^{dep} modulate their susceptibility to be destroyed by environmental phenomena or by predation, while k_S^{rep} , k_C^{rep} modulate their growth rate. For a more precise model of the general organismal situation, it is necessary to relate the organismal level to parameters of the lower levels such as the rate of production of the calcium layer (r_{t_5}), the free internal metabolic energy (related to the increase of the biochemical traffic), and the rate of proliferation of the cells.

On a more technical note, future models should apply reaction networks nested in compartments [53,54] instead of traditional reaction networks. Such models should be more realistic, but also grow in size and complexity. Thus, it will become necessary to apply pathway analysis methods such as flux-balance analysis [55] and chemical organization theory [19] to identify dynamical features that can be linked to the properties of the interactions, and to study how structural perturbations to the system such as the addition of new biochemical components, or new macroscopic species, affect the stability landscape of the system.

6. Conclusion

The complexity of biological processes is related to the integration of the different levels at which they occur and to the emergent properties that characterize the gaps between such levels. To address this, it is necessary to model not only the different levels at which interactions manifest, but also the interaction between these levels through multilevel regulation mechanisms. We developed a simple mechanistic model for the endosymbiotic interaction between corals and the algae (*Symbiodinium*) using reaction networks.

In our model, which complements a reaction network model of endosymbiosis between corals and dinoflagellates of the genus *Symbiodinium* [49], we incorporate biochemical species (R_H , R_C , \bar{R}_C , Cal, and x), thermodynamic species (E), cellular species (H , S , and HS), and a regulatory factor (F_{inh}). In addition, we incorporated a bilevel regulatory mechanism for the proliferation of the endosymbiotic cell HS which is fostered by the increase of its energy (occurring at the nutrient traffic level) but also regulated by the release of F_{inh} when the byproduct x of the energy production surpasses a threshold (occurring at the cellular proliferation level). This mechanism promotes the controlled organism's reproduction and is coherent with the knowledge of this interaction [11, 56].

The idea of developing multilevel interaction models for ecological interactions is relatively new in theoretical biology, as there are not many works that modulate kinetic parameters from the concentration of the system species at different levels [17]. The major innovation of this work is to consider that kinetic parameters at one level can be modulated by the presence of species (biotic or abiotic) at other levels, and thus providing a first approach to generate an integrative view of the multilevel nature of the endosymbiotic relationship.

As a final remark, we consider that this approach has the potential to describe the complexity of not only endosymbiosis, but also other complex multilevel biological interactions such as physiological approach in multiscale system biology [57, 58], cancer biology [59], and multiscale ecological approaches [60, 61].

Data Availability

Our study is theoretical. Therefore, no data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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