

Review Article

Organismal and cellular interactions in vertebrate–alga symbioses

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Photosymbioses, intimate interactions between photosynthetic algal symbionts and heterotrophic hosts, are well known in invertebrate and protist systems. Vertebrate animals are an exception where photosynthetic microorganisms are not often considered part of the normal vertebrate microbiome, with a few exceptions in amphibian eggs. Here, we review the breadth of vertebrate diversity and explore where algae have taken hold in vertebrate fur, on vertebrate surfaces, in vertebrate tissues, and within vertebrate cells. We find that algae have myriad partnerships with vertebrate animals, from fishes to mammals, and that those symbioses range from apparent mutualisms to commensalisms to parasitisms. The exception in vertebrates, compared with other groups of eukaryotes, is that intracellular mutualisms and commensalisms with algae or other microbes are notably rare. We currently have no clear cell-in-cell (endosymbiotic) examples of a trophic mutualism in any vertebrate, while there is a broad diversity of such interactions in invertebrate animals and protists. This functional divergence in vertebrate symbioses may be related to vertebrate physiology or a byproduct of our adaptive immune system. Overall, we see that diverse algae are part of the vertebrate microbiome, broadly, with numerous symbiotic interactions occurring across all vertebrate and many algal clades. These interactions are being studied for their ecological, organismal, and cellular implications. This synthesis of vertebrate–algal associations may prove useful for the development of novel therapeutics: pairing algae with medical devices, tissue cultures, and artificial ecto- and endosymbioses.

Introduction

External and internal surfaces on our bodies, such as our skin and gastrointestinal tract, are colonized by diverse microbes from bacteria and archaea [1] to microbial eukaryotes [2] forming what is commonly referred to as our microbiome. This is true of all vertebrates [3], and most animals [4] with few exceptions [5]. Beyond surfaces, internal to tissues and cells, the microbiomes of invertebrate and vertebrate animals can diverge sharply. Invertebrate animals can host beneficial microbes, including bacteria and eukaryotic algae, within their tissues and cells [2,6], while such interactions are largely absent from vertebrates where most inter-/intracellular microbes are transient pathogens [7].

There is one known exception to this dichotomy: the tissues and cells of a salamander (*Ambystoma maculatum*) can house a green alga (*Oophila amblystomatis*) during early development [8,9]. Aspects of this novel interaction, such as presumed fermentation by the alga [10] resemble intracellular pathogens of vertebrates, aligning the alga with detrimental invaders. However, classic studies characterized *O. amblystomatis* as a mutualist during its co-habitation of the salamander egg capsules [11]. In its role outside of the embryo, the alga oxygenates the egg and removes nitrogenous waste to the benefit of the developing embryo [12–14]. Upon hatching, large numbers of free-living algae from egg capsules are released into the environment. The fates of the algae that enter salamander tissues and cells

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are unknown and consequently, the adaptive value of tissue and cellular invasion to the alga is unclear. Additionally, any roles that the intratissue and intracellular algae play during salamander development are not resolved.

Endozoic photosymbioses, where a host harbors algal symbionts in its cells or tissues and benefits from algal photosynthetic products (reviewed by Venn et al. [15]), are especially common in Poriferans (sponges) and Cnidarians (sea anemones and corals) among metazoans [6], and broadly span the eukaryotic tree of life [6]. The absence of endozoic photosymbioses in vertebrates is therefore conspicuous and may be related to the unique vertebrate adaptive immune system [16–18]. In this review, we consider vertebrate–alga interactions across the entire vertebrate tree of life to explore molecular, cellular, and organismal roles algae play in their interactions with vertebrate hosts.

We categorize the associations reviewed here through a gradient of internal to external relationships: intracellular < extracellular < endozoic (endosymbiotic) < epizoic (ectosymbiotic). We follow the latest consensus taxonomy of subphylum Vertebrata (Figure 1) [19] with an emphasis on existing members within the node Euteleostomi (OTT: 114654, ‘bony vertebrates’). We found algal associations within four main groups: Mammalia (OTT: 244265), Sauropsida (OTT: 639642), Amphibia (OTT: 544595), and Actinopterygii (OTT: 773483).

‘Algae’ are a paraphyletic assemblage of unicellular photosynthetic life [20]. The term describes prokaryotic cyanobacterial and multiple eukaryotic protist lineages. The latter have been historically defined by their plastids [21] being of primary (archaeplastida [22] and *Paulinella* [23]), secondary (chlorarachniophytes [24]; and stramenopiles, haptophytes and dinoflagellates [25]) or tertiary and above (e.g. some dinoflagellates [26]) symbiotic origins. Algal lineages differ in their propensity to form novel symbiotic associations. In this systematic look at alga–vertebrate interactions, we find that algae do form important associations with many vertebrate clades, and can be an integral part of the vertebrate microbiome.

Where do we find vertebrate–alga symbioses?

Mammalia

Mammalian-algal symbioses range from mutualisms and commensalisms to parasitisms. They vary in abundance from the isolated phenomena in zoos to persistent associations in wild populations.

An intriguing mammal-alga symbiosis occurs between two- and three- fingered sloths (*Bradypus spp.* and *Choloepus spp.*) and members of the green alga genus *Trichophilus spp.* [27]. The alga is ectopic to the sloth,

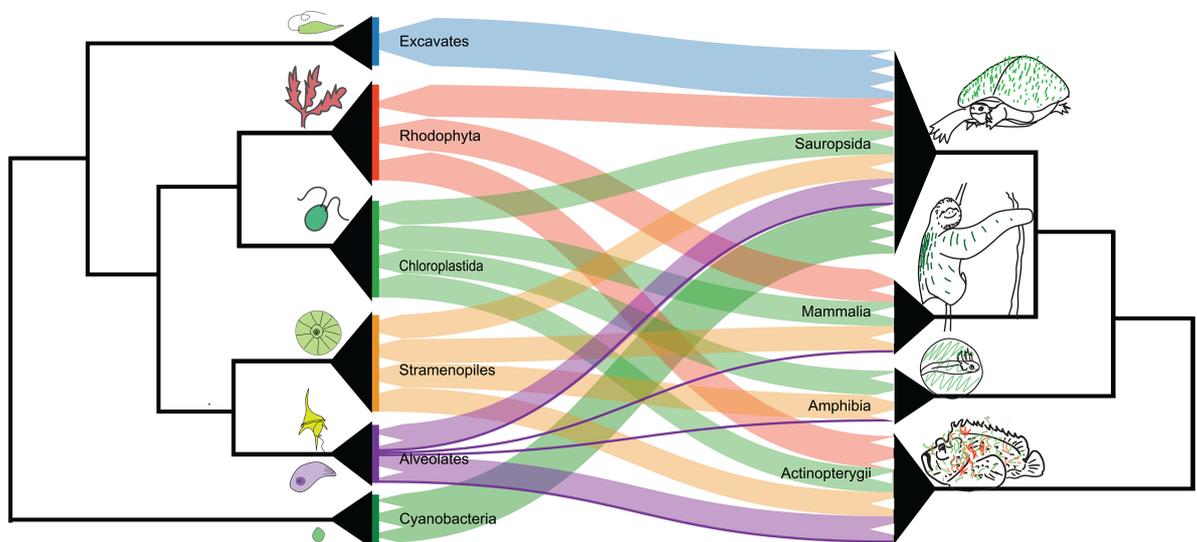


Figure 1. Sankey diagram made with <https://sankeymatic.com/> and Affinity Designer showing that diverse algae enter symbiotic interactions with representatives from all four major vertebrate groups.

Line drawings highlight representative taxa that participate in vertebrate–alga symbioses in each clade. In Alveolates, we distinguish photosynthetic algal associations (pale violet) from apicomplexan parasites (dark violet).

growing on its fur as part of a complex epi-microbiota. This has recently been described as a ‘model mobile ecosystem,’ which includes arthropods, fungi, bacteria and other algae [28]. *Trichophilus* spp. cluster in distinct clades that are linked to specific sloth species [29,30]. These sloth symbionts have not yet been found abundantly in the environment, and are potentially transmitted vertically from mother to offspring via fur–fur contacts [29]. Sloth hair morphology supports algal growth, suggesting co-evolution; however, this may be an expected interaction on the part of the algae [28]. *Trichophilus* spp. may act as camouflage [31] and provide nutritive or defensive benefits to the sloth [27,28], although these benefits are largely speculative. Additional study is needed to better understand this fascinating interaction.

In an apparent commensalism, growth of the red alga *Acrochaetium secundatum* was observed on the pelage of the sea otter *Enhydra lutris* [32]. The alga does not appear to affect host survival or thermal regulation and the alga may benefit from access to light and nitrogen from its association with the otter due to common attachment points on the ventral tail and perineal region [32]. The observed fur attachment may be early stages of a novel commensal interaction, or it may anticipate other alga–sea mammal interactions that have gone unnoticed [32]. Sparse reports also find green algae in the pelage of pinnipeds, notably a green alga, *Pringsheimiella scutata*, tints the pelage of the Hawaiian Monk Seal, *Monachus schauinslandi*, in certain regions such as around the eyes and mouth [33].

In another apparent commensalism, green algal growth was reported on polar bear fur in several zoos [34]. The putatively commensal algae reside in the hollow ducts in bear hair [35]. Algae on mammal pelage and fur gain a surface to grow on, potential access to nutrients, and avenues for dispersal, but, outside of the potential benefits to sloths noted above, they serve no apparent role with respect to their host animals.

Yellow films/patches on the skin surface of marine cetaceans (whales and dolphins) have been noted in the scientific literature for over 100 years [36]. Whalers also noted the coloration, using the term ‘sulfur bottom’ for blue whales with diatom pigmented skin [36]. The cause of the coloring was identified as diatoms forming films and patches on the cetacean’s skin associated with extended periods of feeding in cold Antarctic waters [37–39]. Researchers hypothesize that in the cold-water feeding grounds, cetaceans are unable to shed their skin due to the need to reduce blood flow to the skin for thermal protection [40]. The diatoms, particularly the species *Bennettella ceticola*, and associated epibionts accumulate on this old skin causing the yellow coloring [40]. Research suggests that the spectacular long-range whale migrations to warm, tropical waters occur in part because the whales need the warmer temperatures to molt, shed and rejuvenate their skin, relieving the accumulation of old skin, diatoms, and associated microbes [40].

Another group of marine mammals, manatees, order Sirenia, also have macro- and micro-algal epibionts on their skin including heterotrophic, apochloric diatoms that have abandoned photosynthesis and may receive organic nutrients from shed manatee skin [41,42], and red algae from at least two families, Rhodomelaceae and Delesseriaceae [43,44]. Remarkably, the red alga *Melanothamnus maniticola* sp. nov. seems to have developed a unique anchoring thallus to live attached to the host epidermis. The algae may be obligate symbionts of the manatees, relying on the animal hosts for attachment, nutrition, and dispersal, but are presumably commensal with respect to the manatee with no known roles in manatee physiology [41,43,44].

Algal parasitisms are also known in mammals. The alga-infectious disease protothecosis is caused by a group of ubiquitous achlorophyllous species from the green algae genus *Prototheca* [45,46]. *Prototheca* infect a variety of mammals aligned with human activities (e.g. [47–50] see Supplementary Table S1 for details), including humans [51]. Most cases of animal infections involve farm animals drinking from heavily contaminated stagnant water sources while human cases are usually associated with infection of open wounds and co-morbidities that involve a weakened immune system, like cancer [47,50–52]. However, a recent discovery of *Prototheca* cells in the stool of apparently asymptomatic humans invokes the possibility that *Prototheca* could act as a commensal microbe in addition to its established role as a parasite [53]. *Prototheca* infections are typically subcutaneous and associated with granulomas, areas of inflammation caused by macrophage invasion and isolation of the pathogen away from the surrounding tissue [46,54].

Chlorellosis is another emerging disease, caused by infection with algae in the genus *Chlorella* — green algae closely related to *Prototheca* [55] — in a variety of domestic animals (Supplementary Table S1), especially sheep and cattle [52]. At least two separate cases were reported in humans [56,57]. Both *Prototheca* and *Chlorella* infection cause cutaneous lesions or systemic disease, depending on the infected tissue. *Prototheca* are most frequently associated with bovine mastitis [58] while *Chlorella* often target organs such as liver, lung, and lymph nodes [59]. Due to the presence of chlorophyll, infected organs often have a green discoloration [52].

Sauropsida

Reptiles and birds also experience a wide range of algal symbioses. These include many instances of ornamentation as well as emerging wildlife diseases.

Epizoic algae living on reptiles are best known, perhaps, from the carapace of turtles, some of which are deemed ‘moss-backs’ due to the wispy green algae anchored to the turtle shells. One genus of green algae is exclusively adapted to and associated with turtle shells, *Basycladia*, and is responsible for the mossy-appearance of some turtle carapaces [60,61]. In addition to the macro-alga *Basycladia*, morphological and DNA surveys have revealed a large diversity of microalgae associated with turtles, including 77 epizoic algal species (from *Bacillariophyta*, *Chlorophyta*, *Cyanophyta*, *Euglenophyta*, *Dinophyta* and *Xanthophyta* taxa) on the European pond turtle (*Emys orbicularis*) carapace and plastron [62]. Several other species of turtle were also found to have epizoic algae including: *Caretta caretta*, *Chelonia mydas*, and *Chelydra serpentina* [63–65]. It has to be noted that *Komvophoron sp.* (cyanobacteria) is able to form colonies on the base of the Blanding’s turtle legs, *Emys blandingii* [64]. For the algal symbiont, it is hypothesized that these associations largely help species dispersion in the environment (epizoochory) while the host may gain camouflage that would influence predators and prey senses [62–65].

Epibiont algae from the genera *Trichodesmium* and *Epistylis* (diatoms) have been identified on the undersides of juveniles of the American crocodile (*Crocodylus acutus*) [66] with unknown significance. In a fascinating case of both algae and a land plant living on the head of a lizard, epizoic algae (*Chlorophyceae* and *Cyanophyceae*, together with the liverwort plant *Taxilejeunea obtusangula*) were found on the head of the lizard *Corythophanes cristatus* in a Mexican rainforest. *C. cristatus* is noted to be slow moving and to remain still for hours as well as having a concave region on its head where the algae and plant were found [67]. These anatomical and behavioral adaptations may enable the association with algae and plants in this case as the green patch may offer camouflage for the lizard in its habitat of moss- and algae-covered tree trunks [67].

Sea birds are thought to be involved in marine microorganism dispersion [68]. In line with this hypothesis, several studies mention bird-associated algal species [69–72]. Forty-eight algal species (cyanobacteria, green algae and chrysophytes) were found on sea gull’s feet, feathers, and beak but also in the esophagus and colon [69]. Diatoms were identified on diving seabirds [70,71] as well on mallard ducks in a recent study [72]. While some algae were reported to be epizoic, it is not yet clear whether any species can live inside host tissues or cells. These attachments are a likely mechanism of ectozoochory (dispersion via fur or skin of animal) instead of lasting symbioses. The algal cells do not stay attached to their hosts for a long period of time and so far, birds have not been shown to benefit from the algae.

Amphibia

Amphibians specialize in a variety of habitats, ranging from arboreal, to terrestrial and aquatic. They lay individual eggs, eggs in large masses, or even have eggs that develop internal to the mother [73]. Large egg masses, in particular, pose challenges to developing embryos with respect to oxygen availability within eggs [74]. The embryos of wood frogs (*Rana sylvatica*) [75], northern red-legged frogs (*Rana aurora*) [75], European agile and common frogs (*Rana dalmantina* and *R. temporaria*, respectively) [76], spotted and northwestern salamanders (*Ambystoma maculatum* and *A. gracile*, respectively) [8,77,87–90], potentially *Ambystoma jeffersonianum* [78], and the Japanese black salamander, *Hynobius nigrescens* [79], all form associations with a clade of green algae, *Oophila amblystomatis*, within their egg capsules. Of these, the most well studied association is that between *A. maculatum* and *O. amblystomatis*. The association was first described over 130 years ago [9], and has been established as a mutualism where the embryos benefit from increased partial pressure of oxygen within their egg capsules [80], and the algae may benefit from an increase in available nitrogen, released as waste from the embryos [13]. However, no studies to date investigate the mutualism outside Amblystomatid salamanders, including the potential benefits of *Oophila* in any of its ranid frog hosts.

Recent research on this association has focused on: (1) the algal symbiont, including its molecular taxonomy [75,76,81,82], origin [83,84], material benefits [85,86], and latent effects on embryo physiology [87]; and (2) the host embryo, including host specificity [88], morphology impacts [12,86], and spatio-temporal factors of the habitat [82,84]. The discovery of an intracellular component to this association [8] brought a new frontier in this symbiosis and raised new questions (e.g. host-symbiont immune and metabolic responses [10]). In addition, *Oophila* DNA was found in the adult female *A. maculatum* oviduct [8], however no cells have been found in reproductive structures. The potential for vertical transmission in *A. maculatum* is still unknown.

Recent work has found that amphibian eggs can also have opportunistic associations with diverse algal groups, including other green algae, diatoms and other ochrophyte algae [76,82]. However, evidence supports that members of one clade of symbiotic algae, *Oophila amblystomatis* ('clade B'), resides within spotted salamander (*A. maculatum*) egg capsules to the exclusion of other algae [82] despite the presence of other Chlamydomonas or Chlorococcum algae living in association with *A. maculatum* and other amphibian eggs [81,89].

In post-hatching stages, there are only a few observations of association between algae and amphibians. A fortuitous mutualism was proposed between the green alga *Chlorogonium* and tadpoles of dwarf American toad *Bufo americanus charlesmithi*, in which the algae alleviate the oxygen stress in warmer water and increase the critical thermal maximum of the tadpoles [90]. Although not investigated, algal congregation was also found on tadpoles of gray treefrogs (*Hyla versicolor*) and cricket frogs (*Acris crepitans*) [90].

Actinopterygii

Over 50% of all vertebrate species are ray-finned fish. They exhibit a tremendous range of aquatic habitat preferences, life history modes, and microbial associations. Undoubtedly, more symbioses are to be discovered than the known actinopterygian-algal associations summarized here.

Some members of the order Scorpaeniformes are slow moving, highly camouflaged, ambush predators, with algae attached to their skin [91–93]. The algae may act as lures for prey or as camouflage [92]. Researchers hypothesize that the fish recruit algae from their surroundings with secretions and specialized skin structures [91,92].

There are reports of associations between diatoms and eggs of several fish species off the Pacific Northwest coast of North America (cabezon-*Scorpaenichthys marmoratus*, red Irish lord-*Hemilepidotus hemilepidotus*, kelp greenling-*Hexagrammos decagrammus*, lingcod-*Ophiodon elongatus*, buffalo sculpin-*Enophrys bison*) [94,95]. At least one author hypothesized that the algae may have a role in aiding fish development similar to the oxygen benefits and waste removal in amphibian eggs [94]. However, no follow up studies have substantiated those claims and some have hypothesized that diatom-associated algae in *O. elongatus* may be harmful [96]. Atlantic herring (*Clupea harengus*) fish eggs are negatively affected by the filamentous brown alga *Pyraliella littoralis*, while other algae are neither harmful nor beneficial (e.g. the green alga *Ulva intestinalis*) [97].

Another type of interaction between fish and algae is that of the longfin damselfish (*Stegastes diencaeus*), which is involved in a domesticator-domesticate relationship with a shrimp and alga [98]. In this specialized mutualism, the damselfish protects planktonic mysid shrimps (*Mysidium integrum*) against fish predators. In return, the shrimps fertilize an algal 'farm' composed of turf-algae and brown algae (*Dictyota* and *Panina* genus), providing a better-quality food for the damselfish.

In addition to the above interactions, there are also reports of pathogenic fish-alga relationships. One case report of protothecosis on carp was published in 2017 [99]. Damaged tissues may expose bones and tissues to potentially pathogenic algal colonization [100].

Discussion

Diverse algae occasionally have specific, lasting interactions with vertebrate animals (Figure 1, Supplementary Table S1). Those interactions range from epizotic farming through an intermediate as in the damselfish (vertebrate) → mysid shrimp → brown alga mutualism [98], to epizotic colonization as in sloths with the green alga that grows on their fur [27], to endozoic parasitism in multiple vertebrate clades by the parasitic *Prototheca* [46] and the green alga *Chlorella* [55], to the endozoic, endosymbiotic association between the green alga *Oophila amblystomatis* and embryos of the spotted salamander, *Ambystoma maculatum* [8,87]. Algae, therefore, like other microbes, can be integrated into vertebrate microbiomes as parasites, commensals, or mutualists. Where we see divergence with other clades of animal and life broadly, is in endosymbioses where algae, or occasionally just chloroplasts, are domesticated by invertebrate animals who then receive the benefit of direct photosynthesis in their tissues. What is the reason for this divergence?

Shared traits of invertebrate photosymbioses and their occurrence in vertebrates

One way to approach this question is to consider how shared organismal and ecological characteristics of established photosymbioses apply to vertebrates. Researchers studying the history and origins of photosymbiosis in corals have detailed common traits of heterotrophic hosts that enable, or stem from successful photosymbioses.

Those traits can be examined in the context of vertebrates to consider whether some vertebrate species may present characteristics that would, in principle, make photosymbiosis a viable and beneficial trophic strategy. Those characteristics span host anatomy, behavior, and habitat [101].

Anatomical features of photosymbiotic host animals include thin, transparent tissues, low energy budgets, simple anatomy, and facultative asexual reproduction [101,102]. Some researchers hypothesize that these anatomical features are vital pre-adaptations of photosymbiotic interactions [101,103]. Vertebrates at least partially pre-adapted to photosymbiosis by anatomy are rare, but examples include the glass frog (Centrolenidae [104]), icefish (Channichthyidae) larvae [105,106], and leptocephalus larvae of eels and tarpon [107] that all have translucent to transparent skin. No vertebrates are known to reproduce asexually by budding or whole animal fission like some corals [101], limiting modes of generational symbiont transfer to horizontal acquisition or development of specialized modes of transfer as in ascidians [108].

Habitat features include life in tropical regions where light is available year-round and does not exhibit large fluctuations in irradiance. Indeed, corals in polar regions, even reef-building species, are azoothermophilic, fully dependent on feeding rather than photosynthesis for nutrition and energy [109]. Presumably algal symbionts exert a high cost when sufficient light is not available to meet their energy demands. Many vertebrates, including the transparent glass frogs and eel larvae, live in suitable regions with respect to light availability to make use of photosymbioses, but some such as icefish do not.

Behavioral features include exposure to sunlight for long periods of the day and a low metabolic demand consistent with the amount of energy available from symbionts. Vertebrates are ecto- or endotherms where, on average, ectotherms require less energy [110]. Vertebrate ectotherms (e.g. fish and amphibians) have resting energy needs lower than that of many aquatic invertebrates [110]. The comparison suggests that some vertebrates may have low enough basic energy demands to benefit from energy captured and transferred by photosymbionts if other conditions of successful photosymbioses are met. However, the closest evolutionary relatives to vertebrates that engage in nutritive photosymbioses are ascidian tunicates (Urochordates). The Urochordates are the extant outgroup to vertebrates. They include sessile members of the Didemnidae which enters photosymbioses with the cyanobacteria *Prochloron* sp. [108].

Vertebrate adaptive immunity

The adaptive immune system and its facilitation of immunological memory are jawed-vertebrate synapomorphies [111]. In 2007 Margaret McFall-Ngai proposed the idea that rather than acting only as a novel defense against pathogens, adaptive immunity may have ‘evolved in part to recognize and manage complex communities of beneficial microbes living on or in vertebrates’ [17]. She points out that long-lived invertebrates, with only innate immunity, do not seem less able to resist pathogens than vertebrate counterparts postulating that resistance to pathogens does not explain the full scope of adaptive immune function. Indeed, recent work and meta-analyses support the idea that organisms with more complex adaptive immune systems host a more diverse internal (e.g. gut) microbiome [18]. A tradeoff, however, may be that immune memory precludes the ability to host *endosymbionts* by blocking frequent colonization of internal tissues and cells due to circulating antibodies after an initial encounter. In fact, the *A. maculatum-Oophila* endosymbiosis is initiated *prior* to activation of adaptive immunity by the salamander, which occurs at a later developmental stage [112]. In this way, embryonic salamanders may function more like invertebrates regarding immunity during their close associations with the algae in their eggs.

Embryonic interactions

Surprisingly little is known about the diversity of embryo associated microbiomes across vertebrates. The healthy embryonic microenvironments of fish, reptiles, birds and mammals have all been described as sterile in the literature, with isolated exceptions from PCR-based metabarcoding (reviewed in [113]). We don’t know yet whether the entry of *Oophila* into spotted salamander embryos is an isolated phenomenon across amphibian-*Oophila* associations, or whether less conspicuous symbionts are capable of vertebrate embryo endosymbioses. Even the fate of these algal cells remains a mystery, with only limited evidence of algal DNA in adult oviducts [8]. Therefore, the implications of establishing an embryonic endosymbiosis remain to be resolved.

Algal diversity in vertebrate–alga symbioses

Vertebrate–algal symbiotic interactions span major algal groups (Figure 1). Algae that are adapted to living on surfaces like many raphid diatoms [114,115], and red, brown, green, and cyanobacterial micro- and macro-

algae [116,117], find occasional niches on vertebrate substrates like fur, skin, and hard surfaces (e.g. turtle shells). The interactions may begin as colonization of a novel niche. For instance, the presumably introduced red algal species *Acrochaetium secundatum*'s apparently recent colonization of the fur of the Southern sea otter, *Enhydra lutris nereis* [32], followed by evolution towards increased fitness and specialization with respect to the new habitat, e.g. *Trichophilus* green algae on sloth-fur and *Oophila* algae of amphibian eggs. Endozoic algae of vertebrates are more restricted, and only come from green algal groups like the parasitic *Prototheca* and *Chlorella* examples and the endosymbiotic *Oophila*. Why some green algae are able to form endozoic interactions with vertebrates while members of other algal clades do not seem to do so is unknown and an excellent area for future research [46].

Algal parasites of vertebrates

It is worth noting certain protist parasites of vertebrates are presumably derived from photosynthetic algae, but have converted to obligate heterotrophic, parasitic lifestyles. These include the green alga genus *Prototheca* as discussed previously and the related *Helicosporidium*, which is a colorless parasitic green alga that infects invertebrates [117], as well as the apicomplexan parasites that cause the diseases malaria and toxoplasmosis [118,119], and possibly the oomycete parasites that cause 'water-mold' infections in fish (although the presence of an ancestral plastid in Oomycetes is controversial) [120]. Apicomplexans in particular are very successful parasites, infecting *all* vertebrate groups and many invertebrates [121–123] and doing so via intracellular invasion [124].

In the context of this review, these colorless parasites are of great interest because they suggest that algae that infect vertebrate animals might take a route very different from the mutualist photosymbioses seen in corals and other invertebrates. Evolutionary pressures in hosts with opaque tissues that move frequently, avoid the sun, and have large energy demands may trend towards plastid loss and obligate heterotrophy rather than shared photosynthesis [125]. Indeed, data showing suppression of photosynthesis in intracellular algae during the *O. amblystomatis*-*A. maculatum* symbiosis suggests that photosynthesis is not a beneficial strategy in the intracellular state [10]. However, there are multiple modes of interaction in the salamander-alga symbiosis. Photosynthesis is likely beneficial to free-swimming algae in egg fluid, which may relieve pressure to lose the plastid and adopt a parasitic lifestyle for *Oophila* lineages. But, given examples like apicomplexan parasites, one can envision a future evolution of parasitism within the *Oophila* lineage.

Artificial symbioses

Several studies have explored the idea of inducing artificial endosymbioses between vertebrates and algae [126,127]. In artificial ecto-symbioses where eukaryotic and cyanobacterial algae were introduced to novel vertebrate hosts (reviewed in [127], and [128]), exogenous algal cells elicit minimal inflammation or detectable immunological response [129,130], suggesting high levels of biocompatibility. In introduced endozoic interactions, algae, both cyanobacterial and eukaryotic, were injected into the yolk of developing zebrafish embryos, and co-cultured with mammalian cells [131,132]. In those studies, after tracking the algae for several days, the researchers found that algae persisted in vertebrate tissues for at least 5 days and that the introduced endobiotic algae were viable at the end of the experiment [132]. The artificial symbiosis studies suggest that the innate immune system, in non-native hosts, with no histories of co-evolution, largely ignores foreign algae when introduced at an early developmental stage. The results parallel what we see in the *A. maculatum*-*Oophila* endosymbiosis suggesting that early vertebrate embryos are accessible to endosymbionts in ways that their adult counterparts, with active adaptive immunity, may not be [10]. In an applied setting, the findings from natural and artificial vertebrate-alga endosymbioses are being used to inspire new biomedical devices to deliver oxygen to healing dermal wounds [133,134], ischemic heart tissue [135], hypoxic brain [136] and hypoxic tumors [130].

Summary

The varied interactions between vertebrates and algae mirror the types of interactions seen between vertebrates and other classes of microbes, often with characteristics favorable to algae receiving light (ectobionts), but occasionally with algae acting as osmotrophic parasites within opaque tissues (e.g. *Prototheca* and *Chlorella*, and Apicomplexans). There are no current examples of vertebrates relying on ecto- or endo-symbiotic algae for energy as happens in invertebrates like corals, however it is conceivable that adaptations present in some vertebrates could allow a parallel interaction to be favorable. In natural and artificial systems, we see that

endosymbiotic interactions between algae and vertebrates are possible, but so far, they have only been observed or induced in embryonic stages, where adaptive immunity is not active.

Perspectives

- The vertebrate microbiome can include algal partners. Vertebrate–algal interactions can be commensal where algae colonize vertebrate surfaces; pathogenic, where algae actively infect vertebrate tissues; and mutualistic, where algae and vertebrates benefit one another. The functional roles of algae and vertebrates in such interactions are poorly understood in most cases.
- The absence of intracellular interactions between adult vertebrates and mutualistic or commensal microorganisms points to a role for adaptive immunity in excluding this type of interaction. More research is needed to determine the fate of algae in the known intracellular alga–vertebrate symbiosis and the roles the immune system plays when algae and vertebrates encounter one another generally.
- With a deeper understanding of vertebrate–alga interactions, and how they compare to invertebrate–alga symbioses, researchers are better able to describe ecological roles of both host and symbiont, and are able to make use of unique algal properties to address issues of human health.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

H.Y. led the review effort and organized materials and outlined the manuscript. B.G., S.D., R.K., and J.B. contributed to discussions and writing of the manuscript.

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