

Review

Occurrence and Ecological Significance of Microalgae Species Associated with Egg Clutches of Aquatic Animals

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Abstract

The association of microalgae with aquatic animal egg masses plays an important role in the reproductive ecology of several species. The first descriptions of this phenomenon were reported in 1888 for the egg masses of the spotted salamander *Ambystoma maculatum*. The microalgae most commonly associated with animal egg masses are green algae belonging to the genus *Oophila*. For many years, *Oophila* sp. was considered the only known intracellular endosymbiont in vertebrates; however, subsequent research has expanded the list of microorganisms capable of forming such associations. Here, we reviewed 69 English-language scientific publications on the occurrence of microalgae in the egg clutches of aquatic animals. This clearly indicates that current knowledge of these organisms remains incomplete and requires further investigation. This review presents a general characterization of the occurrence of microalgae in aquatic animal egg clutches, summarizes the locations where this phenomenon has been documented, describes both the animal hosts and the microalgae involved, examines the benefits resulting from the presence of photosynthetic microorganisms within egg masses, and highlights future research directions, including potential practical applications of these associations.

Keywords: microalgae; *Oophila*; aquatic animal egg masses; egg capsules; green algae; symbiosis

1. Introduction

Cyanobacteria and microalgae are predominantly autotrophic organisms with a simple cellular structure [1,2] that play a crucial role in aquatic ecosystems. They are capable of colonizing a wide variety of surfaces and organic structures and can engage in diverse, often complex, interactions with other organisms. An interesting example of such interactions is the occurrence of microalgae within the egg clutches of aquatic animals. It is worth noting that, in salamander egg masses, relatively pure populations of microalgae can be studied both directly within the egg mass and after isolation under controlled laboratory conditions [3].

Gelatinous egg masses constitute a highly specialized environment. The gelatinous matrix surrounding developing embryos functions as a protective structure, safeguarding embryos not only against desiccation caused by sudden changes in environmental conditions or exposure to air but also against mechanical damage and invasion by potentially harmful microorganisms from both aquatic and terrestrial environments [4].



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The colonization of egg masses by microalgae plays an important role in the reproductive ecology of certain aquatic animals, as numerous studies have demonstrated that the presence of these photosynthetic microorganisms provides various benefits to developing embryos. The first reports of this phenomenon appeared in the nineteenth century when egg masses of *Ambystoma maculatum* were observed to exhibit a green coloration that is not typical of this species. Since then, increasing scientific attention has been devoted to these associations, particularly following the discovery of similar interactions in other aquatic organisms, including invertebrates.

Several detailed review papers on algal–animal symbioses have been published over the past century (i.e., [5–10]). However, most of these reviews have focused either on the unique association between the spotted salamander *Ambystoma maculatum* and the green alga *Oophila amblystomatis* or on algal–animal symbioses in a broader evolutionary or ecological context. To date, no comprehensive review has specifically compiled the available data on the occurrence of microalgae and heterotrophic organisms associated with the egg envelopes of aquatic animals across different taxonomic groups. In the present review, we bring together the available evidence on the diversity, distribution, and reported functions of these organisms, providing an integrative overview of this still understudied field.

2. General Characteristics of the Occurrence of Microorganisms in Animal Egg Masses

The first descriptions of the coexistence of microalgae and developing embryos within animal egg masses were provided by Henry Orr in 1888 [11], who reported the presence of spherical, unicellular green algae within the egg masses of the salamander *Ambystoma* sp. [12]. The microalgae most commonly associated with the egg masses of amphibious and fully aquatic animals are green algae belonging to the genus *Oophila* [13]. For many years, these algae were considered the only known intracellular endosymbionts in vertebrates [12]; however, advances in research have demonstrated that a broader range of microorganisms can form symbiotic associations with animal hosts. Furthermore, the relationship between microalgae and aquatic animal embryos demonstrates the evolutionary phenomenon of host switching, indicating that not all algae that form symbiotic associations are restricted to a single host species [14].

A review of the available literature revealed that information regarding the occurrence of microalgae associated with the egg masses of aquatic animals has been reported in 69 scientific publications (Figure 1, Table S1). The publications cited in this study were identified using Google Scholar and then reviewed manually. The literature search was conducted using combinations of keywords including *Oophila*, *Oophila amblystomatis*, “algae in animal eggs”, “algae in egg masses”, “egg-associated algae”, as well as searches based on the names of key authors in the field (e.g., Bishop). Additional relevant publications were identified by examining the reference lists of the retrieved articles. Only studies directly addressing the occurrence, ecology, physiology, or interactions of microalgae associated with aquatic animal egg masses were included in this review. The search included both historical and recent publications to provide a comprehensive overview of more than a century of research on this topic.

The World Register of Marine Species, AlgaeBase, and the Global Biodiversity Information Facility were used to determine the taxonomic classification of the identified species of algae and aquatic animals. The earliest publication dates from 1888, whereas the most recent study was posted in January 2026.

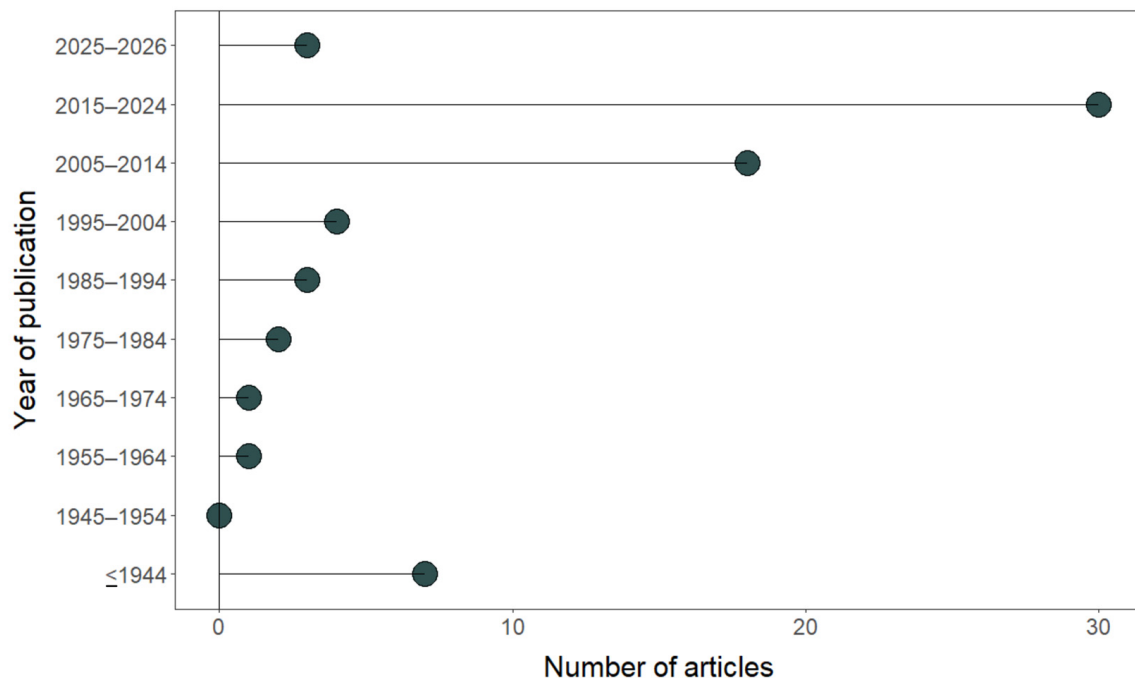


Figure 1. Distribution of scientific publications by year concerning the occurrence of microalgae associated with the egg clutches of aquatic animals.

Over the 130 years following the first discovery made by Henry Orr in 1888 [11], relatively few scientific publications were produced describing the occurrence of photoautotrophic organisms within the egg masses of aquatic animals. This may reinforce the assumption that the topic was known only to a narrow group of researchers in the United States and Canada. Interest in this phenomenon emerged only sporadically, resulting in a limited number of individual scientific studies. It was not until the 1980s that the occurrence of microalgae within aquatic animal egg masses gained broader scientific attention. However, until 2004, the cumulative number of publications did not exceed fourteen.

Interestingly, in recent years a marked increase in research activity has been observed (a total of 33 published studies after 2014), which may indicate the growing importance of this phenomenon in the context of environmental change and the ecology of organisms that form symbiotic associations with microalgae. Furthermore, studies concerning the potential benefits and risks associated with the presence of microalgae in aquatic animal egg masses may provide important insights into the functioning of aquatic ecosystems.

To date, two mechanisms of colonization of vertebrate egg masses by microalgae are known. The first involves intergenerational transmission of microalgae via the oviduct, which was confirmed by the detection of 18S rDNA in two of three oviduct samples previously collected from adult females of *A. maculatum* [15]. The second mechanism applies only to eggs deposited in aquatic environments and involves the penetration of free-living, waterborne microalgae from breeding habitats into egg masses. As observed by Goff and Stein [16], microalgae (presumably flagellated zoospores) accumulate on the outer surface of the egg capsule, after which they lose their flagella and attach to the inner membrane of the capsule, thereby entering the egg mass. Free-living *Oophila amblystomatis* were identified by Bishop and Miller [3] based on 18S rDNA from salamander breeding habitats, supporting the hypothesis that *O. amblystomatis* may colonize egg masses by entering from the surrounding environment.

Microalgae proliferating in the fluid within the egg during early stages of embryonic development may penetrate host tissues (in this case, *A. maculatum*) and be present

within the digestive tract. At later developmental stages, microalgal cells are most likely assimilated by the animal host [15].

It is worth noting that the presence of microalgae in aquatic animal egg masses may depend on environmental factors such as low light availability and the presence of chemical compounds in the water, which may limit algal population development. Additionally, the high thickness and low permeability of the host egg mass gelatinous matrix may also prevent colonization by microalgae. Initially, microalgae occur only in the outer egg envelope and are not always macroscopically visible. At later stages of embryonic development, they are present in all three egg envelope layers [12], whereas the green coloration of the egg mass is usually caused by algae abundantly present in the middle layer. In some cases, microalgae may even obscure the embryo, preventing its observation [17]. It should also be emphasized that not all egg masses are green; white and transparent egg masses are also observed [18]. This may indicate either a lower concentration of microalgae within the egg masses or the presence of algae that do not produce intense green pigmentation.

3. Study Sites and Methods

Regarding sampling locations and analyses, most studies have been conducted in North America, primarily in Canada and USA. A small number of publications originate from Japan, Germany, Poland, England, Finland, Sweden, Norway, Portugal, and Israel (Figure 2). A summary of the sampling locations reported in studies on microalgae associated with egg clutches of aquatic animals is provided in Table S2. It is highly probable that this phenomenon may be observed in many other regions of the world. It can be hypothesized that the distribution range of microalgae associated with aquatic animal egg masses is considerably broader than currently known; however, the lack of observations from other parts of the world prevents verification of this hypothesis.

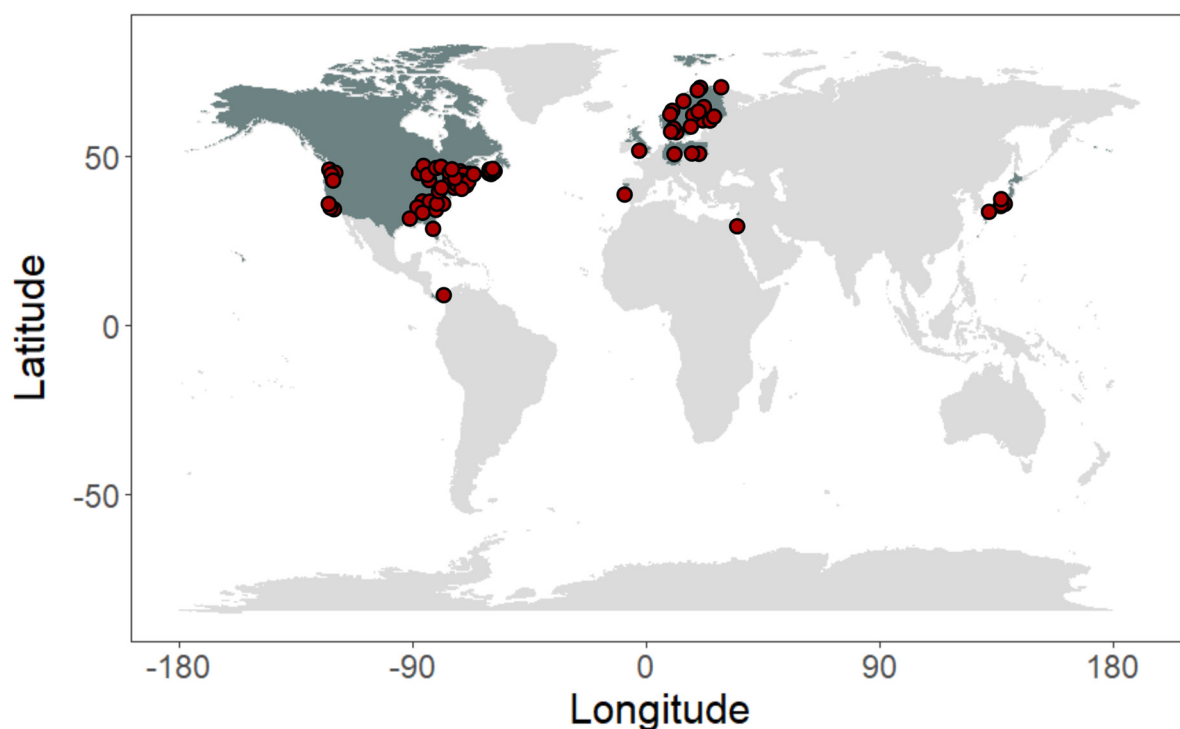


Figure 2. Documented geographic locations of the occurrence of microalgae associated with the egg masses of aquatic animals. The countries are marked in green, while the red dots indicate the exact locations of the places where the measurements were taken.

To investigate the occurrence of microalgae in aquatic animal egg masses, a variety of research methods are required, ranging from field observations to advanced experiments involving specialized laboratory equipment and genetic analyses. The methods described to date focus on characterizing this phenomenon from two perspectives: that of the microalgae (its physiology and taxonomy) and that of the embryos (their activity and survival). Sample collection is the first stage of research, and depending on the study system, the choice of sampling method can have a significant impact on the obtained results. A summary of the methods employed to investigate the presence of microalgae in animal egg masses, as reported in the available literature, is provided in Table 1.

When studying the effects of microalgae such as *O. amblystomatis* on aquatic animal embryos, researchers most often compare egg masses in which microalgae are visibly present with egg masses in which no such presence is detected [12,19]. In many cases, comparisons were made solely based on macroscopic observations, although more detailed analyses have also been conducted (e.g., [3,20]). According to available sources, the presence of green algae in embryonic and larval tissues can be detected using chlorophyll autofluorescence and amplification of microalgal 18S rDNA [15].

Attempts have been made to isolate *O. amblystomatis* by extracting it using a syringe or by dissecting the egg envelope, followed by culturing under various environmental conditions [14]. *Oophila* is notoriously difficult to isolate and maintain in laboratory cultures without its host. Recent study documented the successful isolation of *Oophila* from the eggs of the Japanese black salamander (*Hynobius nigrescens*) [21]. The cells were isolated using Holtfreter's solution and were observed to grow in AF6 medium. A detailed description of the isolation procedure is provided in the study by Genot et al. [21]. In addition, it has been observed that egg masses cultured in tap water at a constant temperature of 10 °C under a 14:10 light–dark cycle exhibit fluctuations in oxygen concentration, ranging from hyperoxic to anoxic conditions during light and dark cycles [22].

Hutchison and Hammen [23] investigated oxygen production by microalgae isolated from aquatic animal egg masses and/or embryos of *A. maculatum* using a Warburg respirometer. In several cultures, oxygen concentration was measured; however, due to the unnaturally high temperature (25 °C) used in the experiments, their conclusions were later questioned by subsequent studies employing more precise microelectrode measurements directly within egg masses [22,24].

There have also been studies in which embryos were subjected to X-ray analysis. Prior to fixation on X-ray film, embryos were homogenized and incubated for one hour at 23 °C in pond water. The water contained $\text{NaH}^{14}\text{CO}_3$ which was used as a photosynthetic radioactive tracer [23].

As mentioned above, contemporary research focuses on determining the taxonomic composition of microalgal species present in aquatic animal egg masses using genetic methods. Advanced molecular techniques for taxon identification, combined with appropriately designed sampling systems, have enabled testing of the role of spatial and temporal factors in shaping microbial presence within egg masses.

Genetic analyses have shown that in most cases, a single group of green algae, designated as *Oophila* 'Clade B' dominates within egg masses. However, more recent phylogenomic studies have revealed that this 'Clade B' is itself composed of multiple distinct phylogenetic lineages (subclades I, III, IV, J1, etc.) that show varying degrees of specificity towards different amphibian hosts [21,25]. Unfortunately, there are limitations to environmental metabarcoding when identifying symbiotic algae in the eggs of aquatic animals. Amphibian and mollusk egg clutches are encased in a hydrophilic gelatinous matrix that rapidly sorbs environmental DNA (eDNA) from the surrounding water. Due to its ultra-sensitivity, DNA metabarcoding often detects hundreds of transient microalgal

species that do not actively participate in the symbiosis, generating significant background ‘noise’ and false-positive results. In genetic study, researchers sample egg fluid or clean egg capsules for genetic studies of these types of symbionts [21].

To assess the diversity and relative abundance of microalgae and other unicellular eukaryotes, as well as their survival within egg masses, researchers perform sequencing of 18S rDNA obtained from cells within egg capsules or from water in *A. maculatum* breeding habitats. Recent population-level analyses use polymorphic genetic markers such as SNPs (Single Nucleotide Polymorphisms), AFLP (Amplified Fragment Length Polymorphism), and microsatellites to determine whether microalgae present in egg masses co-evolve with their hosts [15].

Using both PacBio and Illumina 18S rRNA gene sequencing, Wallace et al. [26] investigated whether algal symbionts are restricted to the *Oophila* clade and evaluated geographic variation in *Oophila* diversity. They also reanalyzed previously published 18S rRNA gene (V4 region) data using modern denoising approaches.

Furthermore, Genot and Burns [27] have applied transformation techniques to the symbiotic green algae associated with this system, a significant methodological breakthrough. The authors established a transformation system for the symbiotic alga *O. amblystomatis*, demonstrating stable transgene expression through heritable antibiotic resistance and green fluorescent protein expression. This methodological advance provides an essential tool for reverse genetic studies of the *A. maculatum* and *O. amblystomatis* symbiosis, allowing functional validation of genes implicated in the establishment and maintenance of this unique vertebrate–microalga association.

Table 1. Overview of methods used to detect microalgae species associated with egg clutches of aquatic animals based on the available literature.

Methods Used	Reference
Historical field observations; larval morphological comparison	[28]
Light microscopy; field observations	[12]
Experimental manipulation (light vs. dark incubation); developmental observations	[29]
Experimental manipulation; oxygen measurements	[23]
Laboratory culturing techniques and field research	[30]
Light microscopy; physiological and biochemical analyses	[16]
Light microscopy; fluorescence microscopy; long-term culture observations; monitoring of sexual cycle	[31]
Ultrastructural/histological analysis; electron microscopy; laboratory strain comparisons	[32]
Laboratory manipulation experiments; multi-population sampling; embryonic staging (Harrison stages); field collection and controlled rearing	[33]
Field observations; nest surveys; reproductive success analysis	[34]
Field observations; experimental analysis of egg masses	[4]
Laboratory culture; histological analysis; transmission electron microscopy (TEM)	[35]
Laboratory oxygen manipulation experiments; controlled fluctuating oxygen treatments; measurements of embryonic development, hatching time, developmental stage, and egg capsule oxygen conductance (G _{O2})	[36]

Table 1. Cont.

Methods Used	Reference
Laboratory light manipulation experiments; measurements of embryonic rotation, muscular contractions, hatching success, developmental stage, and hatching timing	[37]
Laboratory exposure experiments using environmentally relevant atrazine concentrations; assessment of algal persistence, embryonic survival, hatching success, and developmental stage	[38]
Laboratory experiments manipulating light intensity and oviposition substrate; measurements of embryonic development rate, hatchling size, and microphyte fouling; field observations	[39]
Fluorescence imaging (chlorophyll autofluorescence); algal 18S rDNA amplification; FISH; ultrastructural analysis; histology	[15]
Field measurements of oxygen gradients in natural breeding ponds; laboratory pH manipulation experiments; analyses of oxygen, ammonia, lactate, embryo mass, and developmental microenvironment	[40]
Field surveys; breeding site monitoring; mtDNA sequencing	[17]
Light microscopy, transmission electron microscopy (TEM), histology, ribotype (molecular) analysis	[41]
Field collection, algal population quantification, radiolabeled carbon (^{14}C) tracer experiments, developmental and growth measurements	[42]
Laboratory culture of isolated algae, atrazine toxicity assays (96 h), chlorophyll fluorescence (PSII yield), optical density and growth rate measurements, recovery assays	[43]
Light microscopy, cell counts, immunocytochemistry, chlorophyll <i>a</i> quantification, pulse-amplitude modulated (PAM) fluorometry	[3]
Field collection, radiolabeled carbon (^{14}C) tracer experiments, developmental stage analysis	[44]
Field sampling of amphibian egg masses, algal isolation and culture, DNA extraction, 18S rDNA sequencing, phylogenetic analyses	[14]
Isolation of algae from salamander egg masses, algal culturing, optimization of growth media and culture conditions, 18S rDNA sequencing, laboratory toxicity assay using Zn^{2+}	[45]
Egg capsule manipulation (capsulated vs. de-capsulated embryos), oxygen and ammonia measurements under light and dark conditions, microscopy of algal localization during embryogenesis	[20]
In vivo exposure of egg masses to graded atrazine concentrations (up to 338 mg/L), measurement of PSII yield, hatching success, developmental timing; laboratory algal culture and 96 h toxicity assays	[46]
Water sampling and filtration, PCR amplification, 18S rDNA sequencing, phylogenetic assignment within <i>Oophila</i> clade	[47]
Strain-based culture experiments under light/dark conditions, growth assays in different media (BBM $\pm$ glucose/galactose), physiological comparison of heterotrophic capability; taxonomic review and epitype designation using historical and molecular data	[48]
Dual RNA-seq (host + symbiont transcriptomics), comparison of intracellular vs. extracellular algal states, gene expression profiling of salamander cells, algal culturing and metabolic inference	[49]

Table 1. Cont.

Methods Used	Reference
Laboratory rearing of clear vs. white egg mass morphs under light (algae present) and dark (algae absent) conditions; field deployment across temperature and dissolved oxygen gradients; comparison of embryo development and hatching success	[50]
Male-removal field experiment combined with 16S rRNA gene amplicon sequencing comparing egg microbiomes with and without paternal attendance	[51]
Microscopy (morphological characterization); PCR-DGGE; 18S rRNA sequencing; phylogenetic analysis across populations	[18]
RNA-Seq transcriptome analysis comparing developmental stages before and after plastid acquisition; differential gene expression analyses	[52]
Field pairing of clear and white egg masses; measurement of algal density in egg capsules; larval morphometrics; swim performance trials; mesocosm predation/survival experiment; statistical comparison between morphs	[53]
Cross-species algal transplant experiments (swapping cultured symbionts between hosts); embryo co-culture assays; microscopy of tissue entry; comparison of cultured vs. endogenous algal strains; transcriptomic re-analysis of algal gene expression	[54]
Nuclear SSU rRNA phylogenetic analysis of algal isolates; mitochondrial phylogeography of host; herbicide toxicity assays (atrazine and 2,4-D); 96 h exposure and recovery growth experiments	[55]
Carbon fixation assays under light/dark conditions; separation of host vs. algal carbon fixation contributions; photosynthesis inhibition experiments; comparative metabolic tracing within egg capsules	[56]
18S rRNA phylogenetic analysis; large-scale photobioreactor cultivation; growth rate and areal productivity measurements; biochemical composition profiling (proteins, lipids, carbohydrates, pigments, fatty acids)	[57]
Field sampling of egg capsules and pond water over two breeding seasons; nested sampling design; environmental DNA extraction; 18S rRNA amplicon sequencing; microbial community analysis	[19]
Field sampling of 149 egg masses across four breeding seasons; quantitative PCR (qPCR) using newly developed <i>Oophila</i> -specific primers; phylogenetic analysis; linear mixed-effects modeling	[58]
Egg masses reared under three light regimes (0 h, 14 h, and 24 h light) to manipulate algal photosynthesis; measurements of embryonic growth, survival, metabolic rate under hypoxia, larval growth over two weeks, metabolic rate, and starvation tolerance	[59]
DNA metabarcoding (multiple genetic markers); 18S rDNA sequencing; phylogenetic analyses of 18S rDNA and 18 nuclear genes (transcriptomes); comparison of microbial communities from egg clutches, pond water, sediment, and submerged leaves	[60]
Bacterial cultivation; 16S rRNA (V4/V5) amplicon sequencing; comparison of bacterial communities from individual egg capsules and surrounding pond water across two ponds and sampling periods	[61]
Deep-sea specimen collection (ROV); sex identification of juveniles; histology; light and electron microscopy; fluorescence in situ hybridization (FISH) to localize bacterial symbionts during oogenesis	[62]

Table 1. Cont.

Methods Used	Reference
Field surveys of breeding ponds (2018–2020); egg clutch searches; DNA barcoding; PCR-based mitotyping to distinguish <i>R. dalmatina</i> from <i>R. temporaria</i> and <i>R. arvalis</i>	[63]
Algal transformation/transfection; foreign DNA expression; antibiotic selection; GFP reporter assays; RT-PCR for transgene expression; fluorescence microscopy	[27]
Sampling of intracapsular fluid from individual egg capsules across embryonic developmental stages; 16S rRNA gene amplicon sequencing; bacterial diversity and community composition analyses	[64]
Phylotranscriptomics; RNA-seq; analysis of 76 single-copy nuclear protein-coding genes; inclusion of draft genomes; multi-locus phylogenetic reconstruction and comparison with previous clade frameworks	[65]
DNA extraction from historical herbarium/phycological material; PCR with <i>Oophila</i> -specific 18S rRNA primers; cloning and sequencing; phylogenetic analysis including modern reference sequences	[25]
Citizen science data (435 egg clutch observations); phylogenetic analysis of environmental and clutch-associated algae; fluorescence microscopy to test for intracellular invasion; comparative sequence analysis	[66]
High-throughput amplicon sequencing (PacBio and Illumina); 18S rRNA gene metabarcoding; ASV-based denoising of 16.37 M reads; haplotype network analysis; re-analysis of previous datasets; comparative culture sequencing	[26]
Field sampling of egg masses; algal isolation and culturing; morphological characterization; phylogenetic analysis of cultured isolates; comparative clade assignment	[21]

4. Description of Associated Aquatic Animals and Microalgae

4.1. Characteristics and Description of Animal Species

The described phenomenon of microalgae occurring within aquatic animal egg clutches represents an extremely complex interaction between animal organisms belonging to different taxonomic groups and photoautotrophic organisms that form symbiotic associations with their hosts [67]. The overview of host species and associated microalgae, including their phylum-level classification, is presented in Table S3.

The most thoroughly documented cases concern amphibians, particularly salamanders of the genus *Ambystoma* (*Ambystoma maculatum* (i.e., [14,65,68]), *Ambystoma gracile* (i.e., [14]), *Ambystoma jeffersonianum* [12,69]), *Ambystoma punctatum* [12] and *Dicamptodon ensatus* [70] as well as an endemic species occurring only in Japan (*Hynobius nigrescens*) [18,21,65]. The literature also reports the presence of microalgae in the egg masses of frogs (*Rana dalmatina* [17,63,65], *Rana temporaria* [65,66], *Rana (Lithobates) sylvatica* [14,22,65] and *Rana (Lithobates) aurora* [14,65]), as well as in invertebrates such as polychaetes (*Axiiothella mucosa*, *Arenicola cristata* [4]), snails (*Haminoea succinea*, *Haminoea elegans*, *Haminoea antillarum* [4]), hydrozoa (*Hydra viridissima* [31,32] and acoe flatworms (*Waminoa litus* [41]). The benefits provided by microalgae in this relationship are likely species-specific; however, it can be assumed that, regardless of host species, oxygen production by photosynthetic organisms under conditions of limited gas exchange is of primary importance for embryo development. Characterizing the features of each taxon involved in this interaction may contribute to a better understanding not only of the phenomenon itself, but also of its distribution and

underlying mechanisms. This information may serve as a starting point for further research on the role of microalgae in the development, survival, and reproductive adaptations of selected aquatic animals.

4.2. Characteristics and Composition of Microalgae

Aquatic animal egg masses, regardless of whether they occur in marine or freshwater environments, may serve as habitats for microalgae. The observed green coloration of egg masses indicates their presence; however, not all authors who have described this phenomenon have attempted species-level identification. There are numerous publications in which authors report the presence of “green algae” (e.g., [12,29]), sometimes providing limited morphological descriptions, but their primary focus was the animal host rather than the algal symbionts. The lack of taxonomic resolution is partly due to the limited knowledge of microalgae that form symbiotic associations within egg masses. It should also be noted that there is no certainty that the observed microalgal taxa were correctly identified in all cases, further emphasizing how poorly understood this phenomenon remains. Figure 3 illustrates representative examples of microalgal species that have been identified in the egg capsules of various animal species.

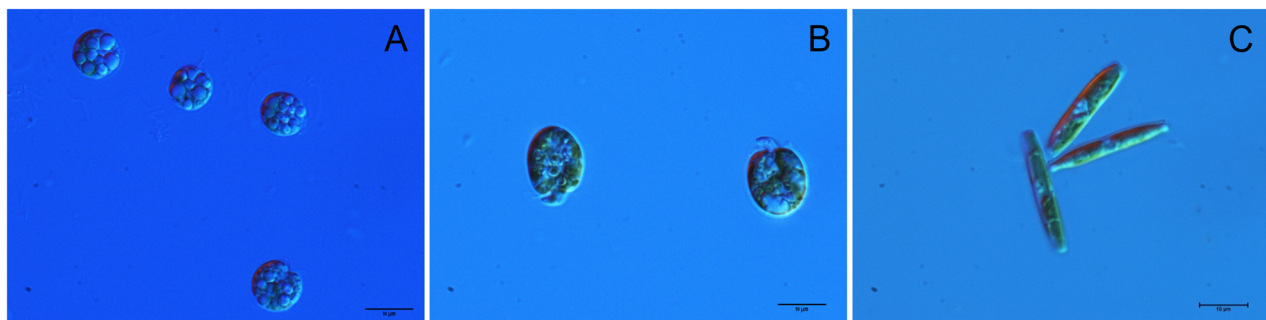


Figure 3. Photographs showing different microalgae that may be found in aquatic animal egg masses: *Chlamydomonas* sp. (A), *Amphidinium* sp. (B), *Nitzschia* sp. (C). Scale bar = 10 µm.

Only one publication from 2010 reports the presence of cyanobacteria *Prochloron* sp. in the eggs of *Lissoclinum punctatum* [71]. Unfortunately, the publication is not available, so we were unable to determine the exact research theses. In addition, Peyton et al. [4] have reported filamentous cyanobacteria colonizing the intra-gelatinous space of some polychaete egg masses; however, their taxonomic identity was also not determined.

In studies concerning microalgae associated with aquatic animal egg masses, the most frequently reported organism is the photoautotrophic green alga *O. amblystomatis*, also referred to in the literature as *Chlorococcum amblystomatis* (Table S3). Furthermore, Goff and Stein [16] observed *Chlamydomonas* sp. in the egg masses of *Ambystoma gracile*. There are also reports about *Chlorella* sp. in *Hydra viridissima* oocytes [32]. All taxa belong to the division Chlorophyta.

Recent molecular analyses have clarified the taxonomic identity of the algal symbiont associated with salamander egg masses. By sequencing DNA extracted from Lambert’s original type material collected in 1905, Bishop and Garbary [25] confirmed that *O. amblystomatis* represents a distinct monophyletic lineage associated with *A. maculatum* egg masses. These findings verified the identity of the symbiotic alga originally described by Lambert [13] and provided molecular evidence supporting its classification within the *Oophila* clade, while rejecting its proposed transfer to the genus *Chlorococcum*.

There are also a few reports documenting the presence of diatoms (division Heterokontophyta). The following taxa were recorded: *Vaucheria litorea* [52], *Nitzschia* sp. [60] and at

least three unspecified taxa reported by Peyton et al. [4]. One example is the colonization of egg masses by diatoms observed in the Indian River Lagoon, FL, USA [4].

Interestingly, in addition to the above-mentioned groups of organisms, dinoflagellata (*Symbiodinium* sp. and *Amphidinium* sp.) have also been detected in developing oocytes of acoel worms (*Waminoa litus*) [35,41].

It should be noted here that although some diatoms are known to positively influence animal embryonic development [4], diatom communities colonizing the surface of fish eggs may exert detrimental effects. For example, Nilsson et al. [72] reported that benthic diatoms, dominated by *Fragilaria*, *Melosira*, *Nitzschia*, and *Tabellaria*, significantly reduced the hatching success of pike (*Esox lucius* L.) eggs in the Baltic Sea. Their findings suggest that diatom communities may adversely affect embryonic development and survival in fish. Therefore, further research is needed to elucidate the effects of benthic diatoms on egg development, as these microorganisms may influence the reproductive success and early development of many ecologically and economically important species.

Interestingly, studies have shown that microalgae, regardless of species, may penetrate from the external environment into developing embryos, particularly in the vicinity of the blastopore [73]. In the case of diatoms, colonization of egg masses occurs if light availability at the oviposition site is not limited, the embryonic development period is sufficiently long (approximately 3 days), and/or the egg mass has a mucilaginous consistency [4]. However, there is no basis for assuming that every host organism interacts with photoautotrophic organisms in the same manner or under the same environmental conditions [15].

For *O. amblystomatis*, it is known that its cells can be transmitted across generations, as demonstrated by the detection of microalgae within the reproductive system of adult *A. maculatum* [15]. The occurrence of *O. amblystomatis* in environmental samples indicates that this species does not require a specific host and is capable of existing independently outside egg masses [15]. Detailed studies (e.g., [57]) and microscopic observations (e.g., [16]) have demonstrated that *O. amblystomatis* exhibits high protein content and produces polyunsaturated fatty acids, which may explain its importance for embryo development [57].

Interesting findings were reported by Pinder and Friet [22], who described algae in the egg masses of the wood frog *Rana (Lithobates) sylvatica*. They observed that photosynthetic cells were responsible for the development of hyperoxic conditions within egg masses. In the egg capsules of *A. maculatum* and *A. gracile*, a characteristic feature of algal growth is the transition of motile cells into non-motile forms that attach to the egg membrane [16], although not all cells undergo this transformation [3]. Kim et al. [14] presented light microscopic images of the three major cell types of *O. amblystomatis*: free-swimming biflagellate cells (corresponding to zoospores or gametes), cells enclosed within a mother cell wall (likely representing asexually dividing zoospores), and larger non-motile zygotes. What is more, detailed analyses of photosynthetic pigment content [3] showed that chlorophyll *a* content decreased in cells attached to the egg membrane, while it increased in suspended cells; however, these values also depended on the developmental stage of the embryo.

Based on observations and comparisons with the life cycle of *Chlamydomonas* sp. [74], it was determined that suspended, motile microalgal cells with a double cell wall are zoospores or gametes, large and suspended cells are zygotes, which are relatively easy to identify [3], and cells with a quadruple wall that are immobile and attached to the egg membrane are cells undergoing conjugation. After examining numerous suspended cells at different stages of embryonic development, sporangia were never observed [75], whereas they were frequently observed in isolates cultured outside the egg envelope [3,14].

It has also been shown that numerous microalgal cells are associated with the inner surface of the egg mass and likely contribute to zoospore production via meiosis, thereby increasing the number of suspended cells during embryonic development [15]. This

suggests that only part of the life cycle of *O. amblystomatis* occurs within aquatic animal egg masses. The full life cycle of *O. amblystomatis* has not yet been fully documented; however, existing observations suggest that most cells present within egg masses are zygotes [3].

It is also important to note that microalgae are not the only organisms that can be found inside the egg mass. Wallace et al. [26] found cercozoan protists and rhizophydialean fungi inside egg masses of the spotted salamander *A. maculatum*. What is more, Bishop et al. [61] and Burgess and Bishop [64] demonstrated that heterotrophic bacteria can also be present in the egg mass. Bishop et al. [61] showed the interaction between *Oophila* and beneficial bacteria in the egg mass microbiome. Moreover, bacterial diversity was found to decrease over the course of embryonic development, while bacterial community structure differed with developmental stage, pond, and year [64]. A hypothesis should be formulated in which *Oophila* interacts with heterotrophic bacteria, framing the egg mass as a complex holobiont. The study highlighted the need for further research to clarify the ecological and functional roles of heterotrophs within this symbiotic system.

5. Benefits Resulting from the Symbiosis Between Microalgae and Aquatic Animals

The penetration of photosynthetic microorganisms into host cells and tissues represents a unique association between animal organisms and microalgae, in which both partners derive benefits from the relationship. Therefore, in the scientific literature, this interaction is often described as a symbiosis. A detailed overview of the major changes occurring in both salamander and algal cells, together with their potential interrelationships, was provided by Burns et al. [49]. What is more, depending on the host species, the benefits associated with the presence of microalgae may vary (Figure 4). A comprehensive overview of the reported benefits associated with microalgae in aquatic animal egg clutches is presented in Table S4. A comprehensive list of species, along with their taxonomic affiliations used for the construction of Figure 4 and the associated literature, is presented in Table S3.

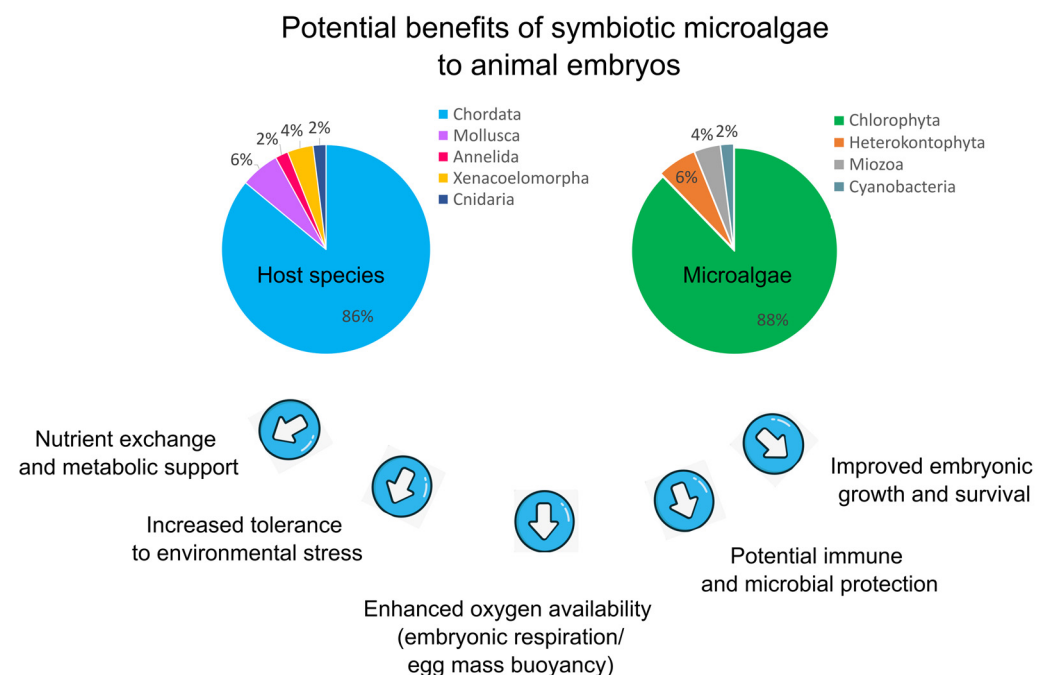


Figure 4. Percentage distribution of aquatic animal hosts and associated microalgae reported in the literature, together with the major benefits of their symbiotic interactions.

It is generally assumed that in this relationship, microalgae obtain a suitable habitat with favorable conditions for growth, as well as essential nitrogen compounds [15] and carbon dioxide [12,54] released by the developing host embryo. It has also been demonstrated that an increase in embryonic respiratory activity correlates with an increase in microalgal population size, indicating a close interdependence between the two organisms [20]. Following removal of the embryo from the egg masses, algal growth is inhibited, which further confirms that it is the embryo, rather than the egg mass itself, that supports microalgal proliferation [14].

The benefits for embryos are also substantial and are primarily associated with increased oxygen concentration within egg masses [4,19,56,59]. This is particularly important for embryos located in deeper layers of the egg mass, where oxygen diffusion is limited [17]. Recent research has expanded our understanding of the role of the *A. maculatum* and *O. amblystomatis* symbiosis under hypoxic conditions and its consequences beyond embryonic development [59]. Embryos developing in the absence of the symbiotic alga exhibited reduced growth and survival, confirming the importance of algal photosynthesis in mitigating hypoxia during development. Although larvae originating from algae-free egg masses displayed compensatory growth after hatching, chronic exposure to continuous light, which promoted persistent algal photosynthesis, resulted in negative latent effects, including reduced body mass, lower metabolic rates under normoxic conditions, and decreased starvation tolerance [59].

Elevated oxygen concentrations resulting from algal photosynthesis [12,23,24,76] also increase the buoyancy of egg masses, allowing them to float toward the water surface and avoid hypoxic zones. In the case of non-buoyant egg masses of *Haminoea succinea* and *Arenicola cristata*, increased buoyancy may reduce the risk of predation by benthic predators such as *Nassarius vibex* [4]. Enhanced buoyancy may also facilitate dispersal of these species within their habitats [4]. Moreover, algal photosynthesis is believed to significantly increase the partial pressure of oxygen within egg masses, transforming hypoxic or anoxic conditions into hyperoxia.

Elevated oxygen levels are also associated with the reduction of UV-B radiation effects, which have been shown to cause deformities in *A. gracile* and increase embryonic mortality [54]. Microalgae also remove nitrogenous waste in the form of ammonia from the fluid surrounding developing embryos and contribute to regulating its accumulation [54]. The presence of microalgae reduces ammonia concentration by converting it into storage compounds, which may be utilized by the embryo [22].

The combined effects of ammonia reduction and carbon availability resulting from photosynthesis may explain the accelerated developmental rates of embryos. It has been demonstrated that the presence of algae within egg masses correlates with increased embryo size [33], resulting from the supply of growth-stimulating substances by microalgae [16], earlier hatching [12,37], and reduced mortality [3,4,22,33,37,56]. Algae are also responsible for carbon transport, which constitutes an additional energy source for the embryo [44]. A positive effect of microalgae on the reduction of toxic metabolites produced by embryos has also been reported [19], as well as inhibitory effects on bacterial growth, which protects embryos from pathogenic microorganisms [44]. An increase in embryonic motility associated with algal presence has also been observed [37].

It is worth noting that algal cells within egg masses are not randomly distributed but tend to concentrate in specific regions. During embryonic development, some algal cells migrate, while others are ultimately digested, thereby contributing to the nutritional intake of the embryo [20].

It has been observed that *A. maculatum* egg masses containing algae did not hatch under conditions of limited light availability, whereas egg masses lacking microalgae were

able to hatch successfully under such conditions [12]. It has also been shown that the presence of diatoms in the egg masses of the polychaete *Axiiothella mucosa* has a positive effect on the condition of newly hatched juveniles, which utilize these microalgae as a food source [4].

In summary, the benefits for embryos proposed by researchers include: (I) increased oxygen availability within egg masses; (II) removal of ammonia excreted by the embryo; (III) prevention of acidification through CO₂ uptake; (IV) production of antimicrobial compounds; and (V) mitigation of oxygen limitation effects within egg masses. The proposed benefits for algae include: (I) access to nitrogen and CO₂, and (II) provision of habitat and protection from predators.

6. Summary and Future Research Directions

Research on the occurrence and ecological role of microalgae in the egg envelopes of aquatic animals is still in its early stages, despite the first reports dating back more than 100 years.

To provide a comprehensive overview of the current state of knowledge, the key findings reported in the available literature are summarized in Table 2. This compilation highlights the major advances in understanding the diversity, ecology, transmission, and functional significance of microalgae associated with aquatic animal egg clutches, while also emphasizing the remaining knowledge gaps that require further investigation.

Table 2. Overview of the key findings from studies on microalgae associated with egg clutches of aquatic animals based on the available literature.

Key Finding	Reference
Embryos of salamanders and frogs were collected for developmental observations; the exact identity of the <i>Amblystoma</i> species could not be confirmed	[11]
The authors propose that the larvae reported by Storer (1925) were likely misidentified and, based on morphological and developmental evidence, more plausibly belong to <i>Ambystoma gracile</i> rather than <i>Dicamptodon ensatus</i> .	[28,70]
The authors demonstrated that unicellular green algae colonize all three egg envelopes after oviposition and proposed a symbiotic association with developing <i>A. maculatum</i> embryos	[12]
The presence of the alga significantly reduced embryo mortality, accelerated embryonic development, increased larval size at hatching, and supported a mutualistic relationship between the alga and salamander embryos	[29]
The study demonstrated that photosynthetic oxygen produced by <i>Oophila amblystomatis</i> contributes to embryonic development, supporting a mutualistic exchange between the alga and salamander embryos	[23]
The authors proposed the life cycle of <i>Oophila amblystomatis</i> based on laboratory cultures and field observations, describing its developmental stages associated with salamander egg masses	[30]
The study demonstrated that symbiotic algae assimilate ammonia excreted by developing embryos, reducing nitrogen accumulation in the perivitelline fluid and promoting embryonic development	[16]

Table 2. Cont.

Key Finding	Reference
The study showed that algal and bacterial symbionts are released during sexual reproduction and transmitted extracellularly, allowing re-establishment of the symbiosis across generations; both symbionts were tracked throughout the sexual cycle of <i>H. viridis</i> under laboratory conditions	[31]
The study demonstrated that photosynthetic oxygen production by symbiotic algae exceeds the combined respiratory demand of embryos and algae, leading to rapid oxygen accumulation and even supersaturation within eggs under light conditions, despite hypoxic surrounding water	[24]
The study demonstrated a three-step process by which <i>Chlorella</i> symbionts migrate from endodermal digestive cells through extracellular spaces and are ultimately incorporated into developing oocytes via phagocytosis, establishing vertical transmission	[32]
The study showed that <i>Ambystoma maculatum</i> egg masses lack convective oxygen transport and rely on diffusion and algal photosynthesis, with <i>O. amblystomatis</i> producing sufficient oxygen to generate hyperoxic conditions in light and partially offset nocturnal hypoxia	[22]
The study showed that algal density within the egg perivitelline membrane influences embryonic development and survival in a population-dependent manner, indicating context-dependent effects of algal symbionts	[33]
The study showed that dense filamentous algal growth altered the breeding habitat of three-spined sticklebacks, increasing the density of breeding males and promoting a more even distribution of eggs among nests by influencing mating dynamics and sexual selection	[34]
Embryos grazed on diatoms within the egg masses, improving the fitness of newly emerged juveniles. Symbiotic algae increased egg mass buoyancy, potentially enhancing larval dispersal and reducing predation by benthic predators	[4]
The study provided the first definitive evidence of maternal transmission of dinoflagellate algal symbionts in a triploblastic organism by demonstrating the presence of symbionts within developing oocytes and embryos	[35]
The study demonstrated that naturally occurring diurnal oxygen fluctuations generated by <i>O. amblystomatis</i> influence embryonic development, with lower minimum oxygen levels delaying development and causing premature hatching	[36]
The study demonstrated that algal photosynthesis influences both embryonic development and behavior. Embryos associated with actively photosynthesizing algae hatched earlier and more synchronously, exhibited reduced ciliary rotation under oxygen-rich conditions, and decreased muscular activity under hypoxic conditions, indicating behavioral adaptations that optimize oxygen use during development	[37]
The study demonstrated that atrazine eliminated <i>O. amblystomatis</i> from salamander egg masses and significantly reduced embryonic survival and hatching success while delaying development	[38]

Table 2. Cont.

Key Finding	Reference
The study demonstrated that oviposition on the photosynthetic seagrass <i>Zostera marina</i> accelerated embryonic development under moderate and high light conditions, likely through increased oxygen production. Association with <i>Z. marina</i> limited microphyte growth in egg ribbons of <i>H. vesicula</i>	[39]
The study demonstrated that <i>O. amblystomatis</i> can invade embryonic salamander tissues and cells during development, with evidence of algal persistence from early embryonic stages and possible oviductal transmission between generations, indicating a more intimate, partly intracellular symbiosis than previously recognized	[15]
The study demonstrated that acidic water (pH 4.5) compromises the benefits of the <i>Ambystoma–Oophila</i> symbiosis by reducing oxygen availability, increasing ammonia and lactate accumulation, and inhibiting embryonic growth	[40]
The study documented the northeastern distribution of <i>Rana dalmatina</i> in Poland, identified new breeding localities, and proposed possible postglacial colonization routes based on mitochondrial DNA analyses	[17]
Demonstrated that accessory-follicle cells mediate the vertical transmission of two dinoflagellate symbionts into developing oocytes. The study also showed that <i>W. litus</i> maintains a specific <i>Symbiodinium</i> lineage independently of its coral host, indicating an active and highly regulated mechanism of symbiont inheritance	[41]
Demonstrated that higher intracapsular densities of <i>O. amblystomatis</i> accelerate salamander embryonic growth and development and provided the first direct evidence that photosynthetically fixed carbon is transferred from an algal symbiont to a vertebrate host	[42]
Demonstrated that isolated <i>Oophila</i> sp. is relatively tolerant to atrazine compared with standard algal test species and is unlikely to be inhibited at environmentally relevant atrazine concentrations	[43]
Demonstrated that <i>O. amblystomatis</i> undergoes a distinct life-history transition within salamander egg capsules, transforming from motile zoospores into non-motile capsule-associated cells, likely zygotes	[3]
Demonstrated that photosynthetically fixed carbon is transferred from <i>O. amblystomatis</i> to salamander embryos only during the second half of embryonic development	[44]
Demonstrated that amphibian egg-associated algae comprise a distinct <i>Oophila</i> clade with at least four phylogenetic subclades, showing partial host specificity and evidence that both host switching and co-speciation have shaped the evolution of amphibian–algal symbioses	[14]
Developed a reliable method for isolating and culturing <i>Oophila</i> sp. under near-standard laboratory conditions, providing a platform for ecotoxicological studies and demonstrating genetic variation among amphibian-associated algal symbionts	[45]
Demonstrated that algal photosynthesis supplies oxygen while algae consume embryo-derived ammonia, suggesting ammonia may limit algal growth	[20]

Table 2. Cont.

Key Finding	Reference
Atrazine reduced algal photosynthetic efficiency in a dose-dependent manner but did not significantly affect algal growth or salamander embryonic development. Both embryo development and algal populations showed high resilience and rapid recovery, indicating that the symbiosis is robust to environmentally relevant atrazine exposure	[46]
Demonstrated that pond water in amphibian breeding habitats contains <i>Oophila</i> lineages that match those found in egg masses, including host-associated subclades, supporting the hypothesis that environmental reservoirs serve as the source of symbiotic algae and suggesting possible host specialization	[47]
Demonstrated that genetically distinct <i>Oophila</i> strains vary in metabolic flexibility, including potential heterotrophic growth, suggesting strain-specific functional contributions to the symbiosis. Also clarified the taxonomic status of <i>Oophila</i> and proposed an epitype to stabilize future molecular and morphological identification	[48]
Showed that intracellular algae undergo strong metabolic reprogramming and stress responses, shifting toward fermentative metabolism, while potentially utilizing host-derived glutamine as a nitrogen source. Host salamander cells display moderated immune modulation rather than full immune activation, indicating a controlled interaction rather than pathogenic response	[49]
Clear egg mass morphs show higher embryo survival than white morphs under oxygen-stressed conditions because algae enhance oxygen availability; without algae (or under reduced light penetration), survival drops sharply, confirming algae-mediated protection against hypoxia in dense jelly masses	[50]
Egg clutches host a diverse and stable bacterial community; however, paternal care does not significantly shape or maintain egg bacterial assemblages, suggesting eggs acquire microbes through other environmental or maternal routes rather than direct male transmission	[51]
Egg capsules contain a consistent, likely unialgal symbiont forming a distinct subclade within the <i>Oophila</i> clade; this represents the first confirmed <i>Oophila</i> –amphibian association outside North America and suggests either vertical transmission or environmental acquisition depending on presence/absence in some egg masses	[18]
Acquisition of <i>V. litorea</i> plastids induces major changes in host gene expression, including upregulation of innate immune (MAMP-PPR) pathways, oxidative stress responses, DNA repair, and metabolic processes	[52]
No significant differences in algal density between clear and white egg masses, and no differences in larval performance, morphology, or survival; egg mass color morph effects are not mediated by algal density or embryo performance differences	[53]
Host identity strongly constrains algal tissue invasion: <i>A. maculatum</i> permits intracellular entry of both native and some foreign algae, while <i>A. gracile</i> resists invasion entirely. Intracapsular vs. cultured algae differ in gene expression, suggesting physiological shifts linked to life cycle state and host environment	[54]

Table 2. Cont.

Key Finding	Reference
<i>Oophila amblystomatis</i> is genetically paraphyletic and split into at least two major clades. The algae show relatively low sensitivity to atrazine and 2,4-D and recover fully after exposure, suggesting these herbicides are unlikely to directly disrupt the symbiont or indirectly affect host development via algal impairment	[55]
Salamander embryos themselves fix substantial inorganic carbon, especially in the dark, overwhelming detection of algal-to-host carbon transfer. Host and algal systems compete for inorganic carbon, making any direct photosynthate transfer from algae to embryo difficult to resolve under experimental conditions	[56]
Phylogenetically related to <i>Oophila</i> but proposed as <i>Chlorococcum amblystomatis</i> , supporting its potential as a high-value industrial microalgal strain rather than a strict amphibian symbiont	[57]
Egg capsules contained exclusively <i>Oophila</i> -clade green algae, supporting a highly specific symbiosis consistent with vertical transmission and/or competitive exclusion. Besides <i>Oophila</i> , only low-diversity chytrid fungi and cercozoan (allapsid) protists were consistently detected, indicating that salamander egg capsules are highly selective microbial habitats	[19]
The probability of detecting <i>Oophila</i> DNA and the amount of algal DNA within egg capsules increased with egg mass age, indicating progressive colonization during embryonic development. No significant differences among ponds, sites, or years were detected, and phylogenetic analysis confirmed that the Alabama algae belong to the established <i>Oophila</i> clades	[58]
Absence of algal photosynthesis (0 h light) reduced embryonic growth and survival, but larvae later showed compensatory growth. Continuous algal photosynthesis (24 h light) did not improve embryonic performance beyond normal light conditions and resulted in negative latent effects, including smaller larvae, reduced metabolic rates, and lower starvation tolerance. These findings suggest that the benefits of the <i>A. maculatum</i> – <i>Oophila</i> mutualism are optimized under intermediate, natural light conditions rather than maximal algal productivity	[59]
Frog egg clutches harbor distinct microbial communities compared with surrounding substrates. <i>Oophila</i> “Clade B” was found exclusively in egg clutches, supporting its specialization for amphibian eggs and suggesting it represents the “true” <i>Oophila</i> , whereas “Clade A” likely represents opportunistic <i>Chlorococcum</i> species	[60]
Egg capsules contained much lower bacterial diversity than pond water and harbored a consistent core microbiome dominated by members of Burkholderiales (especially <i>Herbaspirillum</i>) and Oligoflexales. These bacteria were repeatedly detected across ponds and sampling times, suggesting that the classic <i>Oophila</i> – <i>Ambystoma</i> association is likely multipartite, involving stable bacterial associates in addition to the algal symbiont	[61]
Symbiotic bacteria were localized on the outer surface of primary oocytes, but were absent from oogonia, indicating that maternal transmission occurs during oocyte development. The study provides new insight into the mechanism of vertical transmission of intracellular bacterial symbionts in deep-sea clams	[62]

Table 2. Cont.

Key Finding	Reference
Forty new breeding localities of <i>R. dalmatina</i> were discovered, extending the known northeastern range in Poland by approximately 100 km. The study identifies the Roztocze region as an important stronghold for this peripheral population and provides valuable information for its conservation	[63]
The authors developed a reliable method for transgene expression and presumed stable transformation of <i>Oophila amblystomatis</i> . Transformed algae exhibited heritable antibiotic resistance and GFP fluorescence, providing the first genetic manipulation toolkit for this symbiotic alga and enabling future functional studies of the unique <i>Ambystoma–Oophila</i> symbiosis	[27]
Bacterial diversity within egg capsules declined as embryos developed. Community composition was influenced by embryonic stage, pond, year, and their interactions, indicating that the <i>Ambystoma–Oophila</i> association is accompanied by a dynamic bacterial microbiome rather than being a simple bipartite symbiosis	[64]
<i>Oophila</i> is not monophyletic as previously assumed, and instead comprises multiple well-supported subclades associated with different amphibian hosts. Some amphibian-associated lineages are interspersed with free-living algae (<i>Chlamydomonas</i> , <i>Chlorococcum</i>), supporting a need for major taxonomic revision of Moewusinia and suggesting repeated or flexible origins of amphibian–alga associations across geography and host species	[65]
DNA from the original 1905 type-associated material clusters predominantly within the <i>Oophila</i> clade, confirming that <i>Oophila amblystomatis</i> is a valid, coherent lineage tied to historical type material. The results support retaining the species within <i>Oophila</i> and reject its transfer to <i>Chlorococcum</i> , resolving a key taxonomic debate	[25]
<i>Oophila</i> -like algae are present in <i>R. temporaria</i> egg clutches across Fennoscandia, representing multiple subclades. However, no evidence of intracellular algal invasion was detected in frog embryos	[66]
The symbiosis shows very high host–symbiont specificity, with no chlorophytes outside the <i>Oophila</i> clade detected. Genetic variation is dominated by two widespread intragenomic ASVs (A and B), whose relative abundance varies geographically (including with latitude). Evidence suggests these variants are intragenomic haplotypes capable of segregation, not distinct species-level lineages, refining understanding of global <i>Oophila</i> diversity	[26]
Egg masses consistently contain two distinct algal lineages, indicating multiple symbiotic <i>Oophila</i> -related taxa co-occurring in Japanese salamander eggs, including one lineage closely related to North American symbionts and another within a <i>Chlorococcum</i> -related group	[21]

With each new discovery, additional research directions and questions emerge that require further investigation. It is necessary to verify whether the association between microalgae and embryos within egg envelopes is beneficial at all developmental stages and how environmental conditions influence these processes [15]. Addressing these questions will require further detailed studies.

The fate of microalgal cells that fail to reach the interior of the embryo remains unknown, as does their role after larval hatching [3]. An important future research direction will be to track the fate of these cells, as well as their ecological function within ecosys-

tems. Kerney et al. [15] suggested that reduced frequency of embryonic rotation and increased muscular contractions may represent a response to elevated oxygen concentrations; however, to clarify the influence of microalgae on embryonic motility, experiments combining embryonic behavioral studies with analyses of algal metabolism and physiology are needed [37].

More detailed imaging studies of egg masses of *A. maculatum* and other aquatic species in which this phenomenon has been observed could provide a more precise understanding of how microalgae penetrate embryos, how they migrate through the egg envelope, and how potential vertical transmission occurs [15]. It also remains unclear why, as observed by Bishop and Miller [3], some algal cells remain attached to the egg membrane after larval hatching. Addressing this question will require studies conducted under natural, rather than laboratory, conditions.

Additionally, it is known that sampling location and timing may influence taxonomic composition; therefore, comparative studies across multiple geographic regions would be valuable to assess differences relative to those reported by Jurga et al. [19]. In addition, no studies have yet confirmed the presence of cyanobacteria, despite their likely occurrence within egg envelopes.

Future study should also focus on the practical applications of the symbiosis between algae and the eggs of aquatic animals. For example, Olivier and Moon [38] presented research focused on the use of *Oophila*–host system as a sensitive bioindicator for herbicide pollution. The eggs of the spotted salamander (*A. maculatum*) were exposed to atrazine exhibited a loss of symbiotic algae and lower hatching success in proportion to the herbicide concentration [38]. Another example of the potential practical applications arising from research on the *A. maculatum*–*O. amblystomatis* symbiosis is the development of algae-based biomedical technologies. Insights gained from this unique association may not only improve our understanding of the evolution of vertebrate photosymbioses but also contribute to the design of engineered symbioses for tissue engineering, regenerative medicine, and other biotechnological applications [10].

It should also be noted that, to date, the occurrence of microalgae in egg envelopes of aquatic animals has been reported only in a limited number of countries worldwide. It can be hypothesized that the geographic distribution of this phenomenon is considerably broader; however, if this is not the case, it would be important to determine the factors restricting its occurrence to specific regions. The table summarizing host species, identified microalgae, geographic location, type of reported benefit, methods used, key findings, and references is presented in Table S5, which additionally includes review articles and studies for which full-text versions were not accessible.

The occurrence of microalgae in the egg envelopes of aquatic animals is a highly intriguing yet poorly understood phenomenon. There is still uncertainty regarding the exact mechanisms governing the interaction between these organisms, as well as the full geographic extent of their co-occurrence. Some findings remain ambiguous or inconsistent with previously reported results. Future research should therefore provide a broader perspective and contribute to a better understanding of the ecological significance of this phenomenon in aquatic ecosystems, as well as its role in shaping the development of animal organisms through interactions with associated microalgae.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/phyecology6030093/s1>, Table S1: Current list of scientific articles on the occurrence of microalgae in animal egg capsules, along with the year of their publication. Table S2: Overview of sampling locations used in studies on microalgae associated with egg clutches of aquatic animals based on the available literature. Table S3: Overview of host species and associated microalgae, including their taxonomic affiliation at the phylum level, based on the available literature.

Table S4: Overview of the types of benefits reported for microalgae associated with egg clutches of aquatic animals based on the available literature. Table S5: Current list of scientific articles on the occurrence of microalgae in animal egg capsules, along with the year of their publication.

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