

Prokaryotic Horizontal Gene Transfer in Freshwater Lakes: Implications of Dynamic Biogeochemical Zonation

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ABSTRACT

The highly adaptive nature of prokaryotic communities in the face of changing environmental conditions reflects in part their ability to share advantageous genetic information through horizontal gene transfer (HGT). Natural freshwater lacustrine (lake) systems are a vital and finite resource, and the influence of HGT on their quality (e.g. enabling the spread of antibiotic resistance and xenobiotic catabolism genes) is likely significant. Laboratory and *in situ* studies indicate that the dynamic physical, chemical, and biological conditions that structure freshwater systems can influence HGT within freshwater prokaryotic communities. Thus, understanding how biogeochemical parameters impact HGT in freshwater lakes is an emerging knowledge gap with potential implications for ecosystem and human health on a global scale. In this review, we provide a general synopsis of what is known about HGT in freshwater prokaryotic communities, followed by an integrated summary of current knowledge identifying how biogeochemical factors may influence prokaryotic HGT in freshwater lacustrine systems.

Keywords: Gene Transfer; Freshwater; Water Quality; Prokaryotic; Biogeochemistry

1. Introduction

Intercellular horizontal gene transfer (HGT), the movement of genetic information between cells by mechanisms operating independently of cell division (*i.e.* vertical gene transfer), has contributed to the macroevolution of prokaryotes and their attainment of tremendous structural and functional diversity [1,2]. Further, the highly adaptive nature of prokaryotic communities in the face of changing environmental conditions reflects in part their ability to share advantageous genetic information through HGT. The horizontal spread of antibiotic resistance genes among pathogenic bacteria [3], catabolic genes among bacteria inhabiting soil contaminated with organic pollutants [4,5], and foreign genes carried by genetically engineered organisms into natural prokaryotic communities [6,7] highlights the breadth of the environmental impact of prokaryotic HGT.

Prokaryote-mediated biogeochemical processes are fundamental to aquatic ecosystem functioning [8], such that functional community changes resulting from HGT may have substantial repercussions. The dissemination of introduced genetic information in aquatic prokaryotic communities via HGT can potentially impact water quality by such diverse processes as altering their health hazard potential (e.g. acquisition of introduced pathogenicity-enhancing genes) or ability to carry out particular

metabolic pathways (e.g. degradation of recalcitrant organic pollutants). Natural freshwater lacustrine (lake) systems are a vital and finite resource, collectively accounting for 94% of the world's fresh surface water by volume [9]. As water use increases and water quality decreases, it is increasingly clear that our freshwater resources have gone beyond a critical status on a global scale, where many countries are without adequate safe and secure water supplies. Reflecting the links between water quality and HGT, the growing spread of clinically derived antibiotic resistance genes, an emerging class of environmental contaminants [10,11], in freshwater prokaryotic communities by HGT has been the focus of recent attention [12-14]. Laboratory and *in situ* studies indicate that the dynamic physical, chemical, and biological conditions that structure freshwater systems can influence the nature, extent and rates of HGT within freshwater prokaryotic communities. However, mechanistic understanding of how biogeochemical parameters impact HGT in freshwater lakes is an emerging knowledge gap for both ecosystem and human health on a global scale. This review provides an integrated summary of current knowledge identifying how biogeochemical factors influence the horizontal flow of genetic information in freshwater lacustrine prokaryotic communities.

2. Prokaryotic Horizontal Gene Transfer in Freshwater Systems

Intercellular HGT consists of the acquisition, stable assimilation, and expression of foreign DNA via specific cellular mechanisms. Foreign DNA derived from a donor cell is introduced into a recipient cell predominantly via 1) direct intercellular exchange (conjugation); 2) virus-mediated transport (transduction); or 3) uptake from the extracellular environment (transformation). As the emphasis of this review is on the regulation of HGT by variable biogeochemical parameters within freshwater lacustrine systems, the reader is referred to specific reviews covering conjugation [15], transformation [16], and transduction [17] for more detailed discussion of the mechanisms themselves.

Single-cell DNA sequencing of bacteria inhabiting freshwater lakes has revealed evidence for inter-phyla HGT [18] and the three principal mechanisms of intercellular HGT have been observed *in situ* in freshwater environments (e.g. [19-21]); however little is known about the magnitude and relative contributions of each mechanism, the indigenous participant prokaryotes, or the overall impact of freshwater HGT on water quality and prokaryotic microevolution. In the Baltic Sea, genomic sequence analysis of *Shewanella baltica* isolates from various depths within the water column indicated that genes enabling adaptation to particular environmental conditions (e.g. anaerobic metabolism genes) were horizontally exchanged to a greater extent at more similar depths (*i.e.* biogeochemical conditions) as well as over seasonal time frames [22]. This finding suggests that HGT rates may vary with water column depth-dependent biogeochemical zonation within freshwater systems, and that the horizontal transfer of ecologically relevant genes occurs over timescales that are sufficiently rapid to have appreciable system impacts.

Most studies of aquatic HGT have been carried out in microcosms, which while capable of providing approximate *in situ* transfer rates [23] and permitting some control of individual environmental parameters, cannot entirely replicate the complexity of *in situ* conditions [24]. These studies have predominantly utilized culture-dependent approaches, although more recent investigations have utilized molecular microscopy approaches to directly assay HGT within freshwater prokaryotic communities, albeit under laboratory conditions. Shintani *et al.* [25] detected the conjugative transfer of a plasmid tagged with a fluorescent protein gene in river water samples. Kenzaka *et al.* [26] directly detected transduction in a freshwater river prokaryotic community mediated by three *Escherichia coli*-infecting phages, including one previously isolated from a freshwater river, using a DNA amplification-based *in situ* microscopy technique. A similar approach was used to detect the uptake and ex-

pression of an introduced plasmid tagged with a fluorescent protein gene by indigenous prokaryotic communities in two Japanese rivers up to three weeks following plasmid introduction [27,28]. A small number of field studies of freshwater HGT have been performed to date, and will be briefly described in the following section.

2.1. Conjugation

Conjugative plasmids have been isolated from bacterial communities in freshwater lakes [29] and rivers [30-33], indicating that conjugation is common in freshwater systems. O'Morchoe *et al.* [20] detected conjugative plasmid transfer *in situ* between strains of the freshwater bacterium *Pseudomonas aeruginosa* introduced into a semi-permeable chamber suspended in the upper pelagic waters of a freshwater lake. Conjugative plasmid transfer has also been demonstrated *in situ* in biofilms on the submerged surfaces of river stones (epilithon) between introduced strains of *P. aeruginosa* [30-32] and from an indigenous freshwater epilithic prokaryotic community to an introduced recipient *Pseudomonas* strain [32,34].

2.2. Transduction

Viruses are ubiquitous in freshwater environments, typically exceeding bacteria in concentration by an order of magnitude [35]. Phages (for the purposes of this review, viruses that infect prokaryotes) constitute the majority of freshwater viruses, exhibit high diversity, and consistently infect a substantial portion of freshwater prokaryotic cells, indicating that transduction is an important form of HGT in freshwater systems [36]; however to date this remains largely unconstrained. Most known species of bacteria can be infected by phages, some of which can infect more than one species or even genus [37]. Many prokaryotic cells in freshwater systems are lysogenic, meaning that phage DNA capable of being reactivated to produce viral particles has been integrated into their chromosomes as inducible prophage [19]. Induction of prophage in lysogenic cells can potentially stimulate both transduction and transformation by increasing the availability of phage particles and extracellular DNA, respectively, which are released following cell lysis. Phage particles may be rapidly inactivated in aquatic environments [36], so the frequency and induction of lysogeny in prokaryotic cells are important determinants of transduction potential in aquatic environments [19].

Transduction of chromosomal and plasmid DNA has been demonstrated *in situ* between strains of *Pseudomonas aeruginosa* intentionally infected with species-specific phages and introduced into a semi-permeable chamber suspended in the upper pelagic waters of a freshwater lake [19,38-40]. Transduction between introduced lysogenic bacterial strains following natural induction of

lysogeny has also been demonstrated *in situ* in river epilithic biofilms [41].

2.3. Transformation

Transformation in aquatic environments is contingent upon the availability of genetically competent (able to uptake extracellular DNA) prokaryotes and extracellular DNA sharing homology with the intracellular DNA of recipient competent cells. The reader is referred to Nielsen *et al.* [42] for a detailed review of the release and fate of extracellular DNA in aquatic environments. Dissolved (<0.2 μm) extracellular DNA including fragments of sufficient size to encode prokaryotic genes has been measured at concentrations on the order of 10-1 to 101 $\mu\text{g/l}$ in bulk water from freshwater lakes, reservoirs, ponds, and rivers [43-45], with transformable free DNA molecules (as opposed to viral or colloid-associated DNA) constituting a major form of dissolved DNA in aquatic systems [44,46]. Extracellular DNA is also a component of the extracellular polymeric substances that surround prokaryotic cells in biofilms [47]. Sources of prokaryotic extracellular DNA in aquatic systems include excretion by living cells and release from dead cells following grazing or lysis (e.g. phage-induced) [48,49]. Aquatic extracellular DNA is degraded by extracellular and cell-associated nucleases, genotoxic chemicals, as well as solar ultraviolet radiation [50]. In particular, the activity of extracellular nucleases can strongly influence the persistence of extracellular DNA in freshwater environments [51].

In situ transformation in freshwater systems remains poorly characterized. The transformation of an introduced recipient strain of *Acinetobacter calcoaceticus* by co-introduced extracellular DNA from cell lysates or intact donor *A. calcoaceticus* cells has been demonstrated *in situ* in river epilithic biofilms [21].

While these studies indicate the occurrence of prokaryotic HGT within freshwater systems, their relevance is limited by two important factors. Firstly, they were restricted to the lighted and well-oxygenated temperate environments of either biofilms on the submerged surfaces of river stones or the upper offshore open waters of lakes, poorly representing the range of habitats and extensive biogeochemical variation found within freshwater systems. Secondly, they employed laboratory-grown strains of freshwater-inhabiting species of *Acinetobacter* or *Pseudomonas* bacteria (both belonging to the *Pseudomonadales* order of *Gammaproteobacteria*) as donors (cells, DNA, or phage particles from lysogens) and/or recipients. In stark contrast, environmental prokaryotic communities are well known to be highly diverse and remain largely uncharacterized [52,53] and *Gammaproteobacteria* are typically not major constituents of freshwater lake bacterial communities [54]. Of key signifi-

cance, prokaryotes utilize a wide variety of metabolic pathways to obtain energy and organic carbon for growth. Autotrophic prokaryotes (e.g. photoautotrophs, sulfur-oxidizing lithoautotrophs) are widespread in freshwater environments, and heterotrophs capable of using terminal electron acceptors other than oxygen (e.g. iron- and sulphur-reducing bacteria) can dominate prokaryotic communities in oxygen-poor and anoxic regions (e.g. bottom sediments and bottom waters of productive lakes). These metabolic guilds are capable of substantially altering their local environments (*i.e.* generating biogeochemical zonation) such that they and their neighbours encounter distinct biogeochemical conditions [55-58] that may differentially influence HGT within freshwater systems. Further, these prokaryotes are often challenging to grow under laboratory conditions owing to their oxygen, nutrient and/or consortial requirements and so are likely underrepresented among HGT studies to date. The potential influence of the particular metabolism a prokaryote is carrying out on HGT remains essentially undefined. Thus, the characterization of HGT based principally on only two species of bacteria of the same order in a highly limited range of habitats and biogeochemical conditions means that the full context of freshwater HGT has yet to be determined.

3. Biogeochemical Factors Influencing HGT in Freshwater Lakes

The biogeochemical conditions found in freshwater lacustrine systems can potentially influence all mechanisms of HGT (**Figure 1(a)**). Many of the factors known to influence HGT typically exhibit spatial and temporal variation among and within freshwater lakes. While the impacts of most of these factors have been individually assessed with respect to HGT, most studies have assessed impacts in isolation, impeding understanding of the role of environmental interactions or spatial/temporal variability of these factors within freshwater lakes.

Field studies of freshwater HGT to date have been highly limited in their scope of system characterization, and with the exception of a small number of studies examining temperature [21,30-32] or suspended particulate matter concentrations [40], lack data linking biogeochemical parameters to observed HGT rates in the particular freshwater compartment investigated. Most studies of biogeochemical factors influencing HGT have been conducted *in vitro*, utilizing a limited number of easily culturable chemoorganoheterotrophic bacteria grown aerobically in nutrient rich media under highly constrained laboratory conditions. Such an approach is poorly representative of the myriad permutations of biogeochemical conditions to which freshwater prokaryotic communities are typically exposed, as well as their sub-

stantial phylogenetic and metabolic diversity. This has limited the generation of new insight into environmentally linked HGT and reduced the relevance of any findings for environmental systems. Here we provide a summary of what has been shown for these factors in terms of their influence on HGT to date, providing context for what is known about their variability within freshwater lacustrine systems where possible.

3.1. Temperature

Conjugative plasmid transfer rates for laboratory cultures of Gram-negative bacteria have been observed to be temperature sensitive, potentially reflecting the limited expression of conjugative machinery within certain temperature ranges independent of optimal temperature ranges for growth [59,60]. The relationship between temperature, optimal growth, and HGT in natural freshwater prokaryotic communities is likely to be appreciably more complex. In stream epilithic biofilms, rates of conjugation and transformation between laboratory strains of bacteria have been positively correlated with temperature over seasonal timeframes, suggesting that HGT increases during summer months in temperate climates, particularly in surface waters warmed by solar radiation, and consistently occurs with greater frequency in warmer climates [21,30-32]. However, the observed influence of temperature on HGT in these studies may simply reflect the optimal temperature range for growth of the mesophilic bacterial strains that were used. Temperature was positively correlated with the abundance, production, and specific growth rate of marine heterotrophic bacteria throughout Chesapeake Bay during non-summer seasons [61], suggesting that it may similarly control the growth of prokaryotes in temperate freshwater lakes and their metabolic capacity for participating in HGT.

Transduction may be influenced by temperature, but the direction and magnitude of this relationship are currently poorly defined. Viral decay rates are generally reduced at lower temperatures in aquatic systems [36], suggesting that transduction rates are greater in colder water due to greater phage availability. However, viral abundance has been significantly positively correlated with temperature in a thermally stratified freshwater reservoir [62] and prophage induction was demonstrated *in vitro* to increase by several orders of magnitude with an increase in temperature from 22°C to 28°C in the presence of ultraviolet radiation [63], so the relationship between temperature and transduction in freshwater systems remains unclear.

Transformation rates may be greater in cooler waters as the reduced activity of nucleases at lower temperatures increases the availability of transformable extracellular DNA [64]. Using water collected from different regions within a thermally stratified freshwater lake during

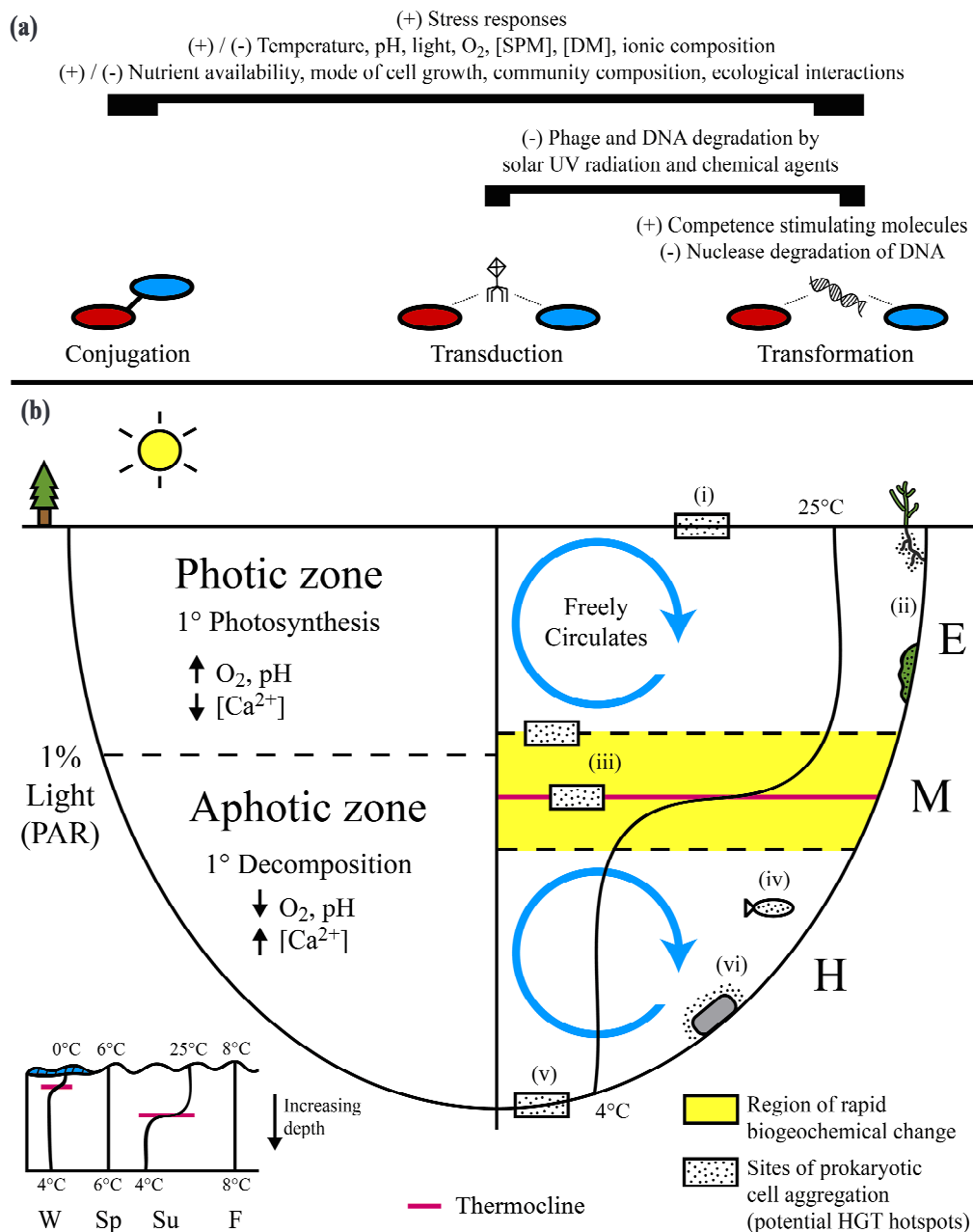
summer, Matsui *et al.* [65] found that degradation of introduced exogenous plasmid DNA with a concurrent decrease in transformation efficiency was observed over several days in epilimnetic water (18.0°C) but not in hypolimnetic water (7.8°C), both held at the same temperature as measured in the field. Taken together, these observations indicate that rates of transformation may be higher in summer hypolimnetic regions of temperate lakes. However, what is not known is whether these findings are directly translatable to rates of transformation within the naturally occurring prokaryotic communities within these regions.

3.2. Dissolved Oxygen

As depth-related variation in the concentration of dissolved oxygen can often mirror thermal stratification in freshwater lakes (**Figure 1(b)**), it is inherently difficult to tease out the separate effects of temperature and oxygen concentration on freshwater HGT. Further, no *in situ* studies examining the influence of dissolved oxygen concentration on HGT in freshwater systems have been published to date. As discussed previously, under low oxygen and anoxic conditions prokaryotes often utilize terminal electron acceptors other than oxygen, and so may distinctly change their local environments, in turn influencing HGT. In a laboratory setting, the efficiency of competence development and frequency of transformation were both reduced in the facultative anaerobe *Streptococcus pneumoniae* under anaerobic conditions [66]. Similarly, lower rates of conjugation have been observed under anaerobic conditions relative to aerobic conditions in laboratory studies utilizing the facultative anaerobe *Escherichia coli* [67,68]. Conjugation rates were observed to increase 10-fold following increased aeration of a laboratory culture of *E. coli* [69]. Notably, these organisms are not typically dominant or key players in freshwater prokaryotic communities observed within freshwater systems and thus the relevance of these findings to environmental prokaryotes, many of which are autotrophic and/or obligate anaerobes, is unknown. However, collectively, these findings indicate that conjugation and transformation may be inhibited in anoxic zones, both in bulk (e.g. hypolimnion of highly productive thermally stratified lakes) and microenvironments (e.g. within biofilms), among facultatively anaerobic members of inhabitant prokaryote communities. Laboratory research clearly indicates that HGT among bacteria capable of both aerobic and anaerobic growth is influenced by dissolved oxygen concentration; however *in situ* studies are required to characterize this regulation in freshwater lacustrine systems.

3.3. Chemical Agents

A suite of natural and anthropogenic chemicals can



Figures 1. Physical, chemical, and biological parameters capable of regulating horizontal gene transfer (HGT) can vary appreciably over space and time within freshwater lacustrine systems. (a) Biogeochemical parameters as a whole remain poorly characterized with respect to their *in situ* influence on HGT. Many parameters may alternatively stimulate (+) or inhibit (-) all three principal mechanisms of gene transfer (conjugation, transduction, and transformation) in freshwater systems based on their magnitude and interactions with other parameters. In general, activation of stress responses can stimulate HGT. Degradation of phage particles and extracellular DNA, the external vehicles for transduction and transformation, respectively, can inhibit these mechanisms. Competence stimulating molecules, produced by prokaryotes and algae, can promote transformation; (b) Biogeochemical parameters capable of influencing horizontal gene transfer can vary appreciably within freshwater lakes. In particular, the vertical physical separation of chemical and biological processes by depth-dependent changes in light availability and thermal density can lead to the formation of distinct local environmental conditions that likely have a specific cumulative influence on HGT. In general, HGT is enhanced in surficial aggregations of metabolically active cells (*i.e.* biofilms). Sites representing potential hotspots for HGT within freshwater lakes include 1) the air-water interface; 2) aquatic plants and algae; 3) vertical zones of rapid density and subsequent biogeochemical change within the metalimnion (e.g. thermocline); 4) aquatic animals; 5) bottom sediments and the sediment-water interface; and 6) submerged abiotic surfaces (e.g. rocks). SPM = suspended particulate matter, DM = dissolved matter; PAR = photosynthetically active radiation; W = winter, Sp = spring, Su = summer, F = fall; E = epilimnion, M = metalimnion, H = hypolimnion.

potentially influence HGT in freshwater lakes, although their *in situ* effects largely remain uncharacterized. Chemicals can damage prokaryotic cells, phage particles, and extracellular DNA, limiting their participation in HGT. Detergents, mutagens, and antibiotics have been observed to inhibit conjugation *in vitro*, either directly or indirectly via induction of plasmid degradation and/or selective toxicity targeting plasmid-containing cells [70, 71]. Conversely, *in vitro* exposure to chemicals has been demonstrated to stimulate HGT between bacterial species otherwise unlikely to exchange genetic information, likely by inactivating restriction enzymes that normally limit interspecific HGT [71-73].

Genotoxic chemicals including antibiotics and trace elements can promote all three principal mechanisms of HGT *in vitro* by activating bacterial stress responses (e.g. SOS response), leading to the induction of competence [74,75], prophage [76,77], and expression of conjugation machinery [78]. In particular, prophage induction via activation of the SOS response in lysogenic strains of both Gram-positive and Gram-negative bacteria has been demonstrated *in vitro* for a variety of chemicals at sublethal concentrations including antibiotics, antineoplastic drugs, halogenated organics, polycyclic aromatic hydrocarbons, and trace elements [79-83]. SOS response activation was observed in cultures of the aquatic bacterium *Vibrio cholerae* following exposure to subinhibitory concentrations of most major groups of clinically used antibiotics [84], indicating that antibiotic-mediated stimulation of transduction can occur in freshwater prokaryotic communities. Collectively, *in vitro* investigations of the influence of chemical agents on HGT via bacterial stress response activation suggest that freshwater lake compartments containing biologically accessible genotoxic chemicals at concentrations sufficient to induce stress responses are sites of higher HGT rates. Supporting this conclusion, a genotoxicity assay based on prophage induction in *Escherichia coli* produced positive results for bulk water samples collected at sites along a freshwater river most highly contaminated by trace elements [85].

Horizontally mobile genetic elements including integrons, transposons, and plasmids were found to be more abundant in freshwater river sediments exposed to waste water containing relatively high concentrations of antibiotics [86]. Similarly, exposure to trace elements has been associated with increased abundance of IncP-1 plasmids in freshwater river sediments [87] and class 1 integrons in freshwater river epiphytic biofilms, bulk surface water, and bottom sediments [88,89]. The general extent of freshwater system contamination with chemicals has been positively correlated to the incidence of potentially mobile plasmids in bacterial isolates [90]. As the abundance of mobile genetic elements can be used as an indicator of relative HGT potential in prokaryotic com-

munities [88], these findings support the notion that freshwater lake compartments with relatively high concentrations of antibiotics and trace elements may be hotspots for HGT.

Chemical agents capable of influencing HGT are principally introduced into freshwater lacustrine systems as a consequence of anthropogenic activities [91,92]. Generally speaking, the lowest concentrations of trace elements and antibiotics necessary to stimulate HGT *in vitro* are several orders of magnitude greater than concentrations typically found in freshwater environments [93,94], so it is unclear whether bacterial stress response activation by these chemicals is relevant to HGT regulation in most freshwater systems. However, chemicals may be sufficiently enriched in compartments within lakes that they can locally influence HGT. Trace elements and organic pollutants can accumulate within bottom sediments and at the sediment-water and air-water interfaces of aquatic systems [95-97], and aquatic biofilms can sorb a wide variety of inorganic and organic chemicals [47,98,99], suggesting that these compartments could be hotspots for HGT within freshwater lakes. Antibiotics are naturally produced by common freshwater microorganisms including *Actinobacteria* and filamentous fungi, and so may influence HGT in freshwater habitats in which these microorganisms are active and abundant. As discussed previously, prokaryotes are capable of a wide variety of metabolisms, and particular metabolisms may distinctly alter biogeochemical conditions, in turn influencing HGT, although this remains essentially undefined. For example, iron-reducing bacteria in anoxic lake bottom sediments can degrade recalcitrant organic pollutants and liberate trace elements through the dissolution of iron minerals [58], potentially resulting in their differential exposure to chemicals that can regulate HGT via activation of stress responses. Prokaryotes can also transform the redox state of trace elements such as arsenic, altering their mobility, bioavailability, and toxicity [100], and ultimately their influence on HGT.

3.4. Solar Ultraviolet Radiation

As with chemicals, solar ultraviolet radiation can potentially stimulate or inhibit all three principal mechanisms of HGT by activating bacterial stress responses [101] and damaging participating bacterial cells [102], phage particles [102-104], and DNA [105], respectively. *In situ* effects of solar ultraviolet radiation on HGT in freshwater lacustrine systems are not well characterized. Ultraviolet wavelengths are rapidly attenuated within water bodies and thus their impacts will largely be restricted to the upper 1 - 2 meters of a lake. Prophage induction in lysogenic prokaryotic cells following exposure to solar radiation has been observed in water samples collected from freshwater lakes [106,107]. Exposure of microcosms

prepared *in situ* from seawater to solar radiation increased prokaryotic mortality and production of viral particles, suggesting an increase in transduction rates following prophage induction [37]. Consistent with the surficial limitation of ultraviolet radiation impacts, Tapper and Hicks [106] determined that the surface microlayer at the air-water interface of a freshwater lake was enriched in virus particles relative to the underlying epilimnion water, indicating the occurrence of appreciable prophage induction by the high intensity of solar ultraviolet radiation incident on the water surface. Ozone-driven increases in solar ultraviolet-B radiation over the last several decades with concurrent negative impacts on aquatic ecosystems [108] indicate that solar ultraviolet radiation will continue to increase in importance as a regulator of HGT in lacustrine systems.

3.5. Suspended Particulate Matter and Dissolved Matter

Suspended particulate matter and dissolved matter (typically operationally defined as materials suspended in the water column with diameters greater and less than 0.45 μm , respectively) can influence rates of HGT in freshwater lakes through a number of processes. Both contribute to the attenuation of solar radiation and thereby limit the vertical region in which it can directly influence HGT. In particular, dissolved organic matter, a major form of dissolved matter in most freshwater systems, can strongly reduce ultraviolet penetration [109]. Photo-oxidation mediated by solar ultraviolet-B radiation in humic (organic carbon rich) lakes was observed to be restricted to the top couple of centimeters [110], suggesting that the vertical region in which solar ultraviolet radiation can appreciably influence HGT is further restricted in systems with higher dissolved organic matter concentrations. Concentrations of dissolved organic matter are generally higher in highly biologically productive systems, reflecting their *in situ* production. Dissolved organic matter is also higher in lake systems impacted by anthropogenic organic-rich wastewater inputs (e.g. sewage, agricultural runoff) or those receiving significant wetland inputs. The influence of dissolved organic matter on HGT may be increasingly pronounced in Boreal Forest lake systems impacted by climate change, as melting permafrost is driving significant exports of organic matter via surrounding organic-rich wetlands to downstream lakes.

Conversely, the presence of suspended particulate matter can potentially stimulate all three principal mechanisms of HGT by providing surfaces for the adsorption of prokaryotic cells, phage particles and extracellular DNA, which stabilizes and protects these HGT participants from damage by extracellular enzymes, toxic chemicals, and solar ultraviolet radiation while also increasing the probability of cell-cell, DNA-cell, and phage-cell

encounters and resultant HGT [40,51,111-114]. Furthermore, particulate-associated prokaryotes are typically more metabolically active than planktonic (free-living) prokaryotes [115,116], suggesting that the former can participate to a greater extent in HGT. Ripp and Miller [40] measured a 100-fold increase in the number of transductants when sterilized freshwater microcosms were prepared using water collected near a lake bottom and enriched in suspended particulate matter compared to surface water with little particulate matter. The addition of clay mineral particles has also been shown to enhance *in vitro* transduction rates [40]. The affinity of a phage particle for adsorption to a surface is dependent on the type of phage and surface involved [117]. Kernegger *et al.* [118] found that the abundances of adsorbed bacteria and viruses, as well as the ratio of adsorbed virus to adsorbed bacteria, were significantly higher for suspended organic particles compared to suspended mineral (inorganic) particles, suggesting that organic particles may be sites of higher HGT rates. Extracellular DNA adsorbs to clay minerals, sand particles, and natural organic matter [119] and in this form is available for transformation [111, 112,120,121]. The high affinity of DNA for organic and mineral phases present in bulk in aquatic environments and the protection that adsorption confers against degradation by nucleases and toxic chemicals [119] indicate that adsorbed DNA represents an important reservoir of transformable DNA in freshwater systems. Transformation frequencies of adsorbed DNA have been observed to be alternatively higher [111], lower [112], or similar [122] relative to dissolved DNA under laboratory conditions, and the type of DNA sorbent can influence transformation efficiency [122,123], so the net effect of DNA adsorption on transformation in aquatic systems is likely complex. Variability in the amount of different mineral and organic phases available for adsorption of cells, phages, and DNA within freshwater lacustrine systems may result in system and system compartment specific control of transformation rates (e.g. nutrient poor subglacial lakes with high suspended clay mineral concentrations compared to nutrient rich lakes with high concentrations of organic matter). Finer (smaller grain size) suspended matter possesses relatively greater surface area to volume ratios and typically stay in suspension longer, and so may enhance contact between HGT participants to a greater extent than more coarse particles due to a greater collective capacity for HGT participant adsorption. Plasmid transfer rates were observed to be greater in soils with high clay content (*i.e.* finer textured soils) [124], suggesting that HGT rates may be similarly enhanced in freshwater lakes with finer suspended matter (e.g. lakes receiving extremely fine glacial powder from runoff). Concurrent sorption of HGT participants and chemical agents capable of influencing gene transfer may

further modulate the effect of suspended particulate matter on freshwater HGT. In freshwater lakes, suspended floc, which are aggregates of cells, organic matter and minerals, may be highly enriched in trace elements relative to bottom sediments and the bulk water phase [125, 126], potentially resulting in localized stress-driven HGT changes. At the lower end of the particle size scale, nanomaterials in water are capable of influencing HGT. Nanoalumina has been demonstrated to increase *in vitro* conjugative plasmid transfer across bacterial genera via increased stress and alteration of gene expression [127].

Concentrations of suspended particulate matter may vary substantially over time and space within freshwater lakes. Climatic variability (e.g. storms), anthropogenic point and non-point sources (e.g. combined sewer outfalls, agricultural runoff), phytoplankton blooms, as well as seasonal events including the turnover of stratified systems and springtime melting of snow and ice in temperate climates, may temporarily and locally or generally stimulate HGT by increasing concentrations of suspended particulate matter in freshwater systems via the resuspension of settled particles or increased particulate matter loads in inflowing waters. Concentrations of suspended particulate matter, and rates of HGT, may be relatively high in the metalimnion of thermally stratified lakes due to the accumulation of particulates from the epilimnion at the thermocline where maximal thermally related density differences inhibit mixing of water layers. Microbial communities are commonly concentrated in this region, taking advantage of the local build up of organic carbon and nutrients as well as the unique biogeochemical and energetic conditions that are often present. Similarly, suspended particulate matter is typically enriched at the air-water [128] and sediment-water interfaces of freshwater systems, and may also be generally elevated in the hypolimnion of stratified freshwater lakes due to internal seiches or large water circulation movements driving the resuspension of settled particles [129].

3.6. pH

No *in situ* studies examining the influence of pH on HGT in freshwater systems have been published to date. Rates of *in vitro* transformation and conjugation involving bacteria from freshwater river water or epilithic biofilms as donor cells were not significantly affected by pH in the range (6 - 8) found in most freshwater lakes [130-133]. *In situ* bacterial production was similar across acidified and limed humic lakes (pH range 5 - 7), suggesting that pH does not appreciably influence HGT via a general effect on prokaryotic metabolic activity [134]. However, freshwater pH may influence HGT by controlling the net surface charge present on prokaryotic cells, phage particles, and extracellular DNA, as well as the mineral and organic surfaces they adsorb to, altering surface-fac-

ilitated interactions between mediators of HGT and recipient cells and protecting HGT participants from degradation. Romanowski *et al.* [51] observed that the adsorption of plasmid DNA to sand and groundwater aquifer material in microcosms was inversely dependent on the pH of the solution (pH range 5 - 9), reflecting the increasing negative charge on mineral particle surfaces typically observed with increasing pH, which repulses the negatively charged DNA molecules [51]. This finding suggests that less DNA may be available for transformation in freshwater lacustrine systems and system compartments with higher pH due to the lack of protection from degradation conferred by adsorption to mineral surfaces. The bioavailability of trace elements, which dictates their potential for influencing HGT rates in freshwater prokaryotic communities, is also pH-dependent. The pH of a freshwater lake reflects both its chemical and biological characteristics, and is variable over depth dependent spatial scales as well as diel, seasonal and annual timeframes. This variability may be reflected in changing freshwater HGT rates. For example, diel variation in HGT may occur in lacustrine zones of high photosynthetic activity, as fluctuations in pH may be substantial (*i.e.* 1 - 2 pH unit shifts) over this timescale, with pH increasing occurring during lighted hours and decreasing at night when respiration dominates [135].

3.7. Ionic Composition

As with pH, the total dissolved ion concentration (salinity) of a freshwater environment can affect the adsorption of prokaryotic cells, phage particles, and extracellular DNA to mineral and organic surfaces, and in turn, the potential for HGT [36,51,136,137]. Freshwater systems, in contrast to marine systems, are highly dilute with the highest concentrations of the most abundant typical ion, Ca^{2+} , in the mM range (in contrast to water itself at 25 M). The adsorption of DNA to sand and groundwater aquifer material in microcosms was enhanced in the presence of divalent cations, with concentrations for half-maximum DNA adsorption of 0.5 mM for Mg^{2+} and 0.4 mM for Ca^{2+} [51,112,120]. *In vitro* transformation of the soil bacterium *Azotobacter vinelandii* by DNA adsorbed to natural organic matter significantly increased in the presence of 1 mM Ca^{2+} [122]. Similarly, *in vitro* transformation of *Bacillus subtilis* was optimal with media containing 20 mM Mg^{2+} [138]. Together, these findings suggest that transformation rates in freshwater prokaryotic communities may be influenced by the concentrations of divalent cations in their local environment. Metal cations, particularly Ca^{2+} and Mg^{2+} , are involved in competence development, and Ca^{2+} is necessary for transformation to occur in bacteria [139-141]. Trombe *et al.* [142] determined that 1 mM Ca^{2+} was sufficient to induce competence *in vitro* using *Streptococcus pneu-*

moniae, while Page and von Tigerstrom [139] found that 0.5 to 1 mM Ca^{2+} resulted in optimal competence induction *in vitro* using *Azotobacter vinelandii*. Transformation of *Escherichia coli* with plasmid DNA was demonstrated *in vitro* using fresh river water containing concentrations of Ca^{2+} as low as 1.4 mM, with rates of transformation generally being directly related to Ca^{2+} concentration [140]. The authors noted that rivers sampled in a calcareous area had Ca^{2+} concentrations of 2 - 2.5 mM, while those from a granitic area (minerals with low [Ca^{2+}]) had concentrations of 0.3 - 1.5 mM, suggesting that transformation rates may be higher in lacustrine systems with drainage basins rich in calcium carbonate rock [140]. Concentrations of Ca^{2+} can also vary appreciably with depth in stratified hardwater (*i.e.* Ca^{2+} rich) lakes, with photosynthetically driven calcium carbonate (CaCO_3) precipitation (associated with increasing pH) decreasing concentrations in the euphotic regions of the epilimnion and metalimnion during lighted hours, and decomposition (commonly decreases pH through CO_2 generation which increases CaCO_3 solubility) increasing concentrations in the hypolimnion. In highly productive hardwater lakes, Ca^{2+} concentrations can substantially decrease during periods of intense biological activity, which can strip out the Ca^{2+} in CaCO_3 precipitates, potentially inhibiting transformation.

3.8. Prokaryotic Cell Density and Mode of Growth

Higher rates of HGT are typically found in more dense populations of prokaryotic cells, primarily due to the increased probability of contact between mediators of HGT and recipient cells [143]. Using a continuous culture model under conditions relevant to those found in freshwater environments, Replicon *et al.* [144] found that transduction rates were directly related to the recipient cell concentration. Prokaryotic cells are typically unevenly distributed throughout freshwater lacustrine systems. In the bulk water phase, prokaryotic cell concentrations can vary over space and time [145] but are typically relatively low, resulting in substantial spatial isolation that reduces the probability of HGT. Local regions of relatively high cell density include physical interfaces within the water column (e.g. boundaries of the metalimnion), the air-water and sediment-water interfaces, and biofilms both suspended within the water column (e.g. lake snow) and present at abiotic and biotic solid surfaces. Bacterial and viral abundance and production have been found to vary significantly among strata in stratified water columns, but consistently appear to be elevated in the metalimnion [146-148], indicating this region is a hotspot for HGT and that rates of gene transfer likely differ among epilimnetic, metalimnetic and hypolimnetic regions. A greater number of transconju-

gants was detected at the air-water interface relative to the bulk underlying water in a model freshwater system, suggesting that the surface microlayer represents a distinct microzone for HGT in freshwater lakes [29].

In aquatic environments, prokaryotes overwhelmingly tend to form and be found in biofilms at surfaces [149]. As these structures maintain a high density of metabolically active cells surrounded by a matrix containing potentially transformable extracellular DNA and capable of facilitating extracellular communication, providing protection from predation, and trapping nutrients and other chemicals, they are potential hotspots for HGT [150]. Underscoring the prevalence of HGT in biofilm settings, conjugation machinery [151], conjugative plasmids [152], and phage particles [153] have important roles in biofilm formation. Increased rates of HGT have been observed in biofilms relative to planktonic cells [154-157]. In a microcosm study designed to mimic a marine environment, conjugation frequencies were typically higher in biofilms compared to the bulk water phase, particularly at the biofilm surface in contact with the surrounding environment [158]. Biofilm stability and the stage of biofilm development may influence HGT. Regular disturbance of the spatial organization of prokaryotic cells in an *in vitro* biofilm strongly improved plasmid invasiveness, suggesting that the periodic breakup of biofilms (e.g. due to wind/solar driven turbulence) may promote HGT [157]. In cultured *Escherichia coli* and *Pseudomonas aeruginosa* mixed-biofilm communities, phage particles were observed by transmission electron microscopy to be closely associated with the biofilm matrix [159]. Following their release from lysogenic *Staphylococcus aureus* cells, phage particles remained detectable for longer periods in biofilm cultures than in planktonic cultures due to protection from degradation [160], suggesting that transduction may be more prevalent in freshwater biofilms relative to the surrounding bulk water phase.

Within biofilms, microscale gradients in oxygen, pH, and concentrations of nutrients, electron donors and acceptors, signalling molecules, trace elements, and antibiotics can produce microenvironments with distinct biogeochemistry [161-164] that may differentially influence local rates of HGT. Sites of biofilm formation and potential HGT hotspots in freshwater lakes include suspended particulate matter, submerged bulk abiotic surfaces (e.g. rocks) and biotic surfaces (e.g. epilithon of aquatic plants), and the internal structures of aquatic plants and animals (e.g. digestive tract of filter feeding bivalves) [23,165] (**Figure 1(b)**). As noted by Hermanson and Linberg [166] and Hill and Top [124], rates of HGT likely differ substantially among the numerous prokaryotic biofilms present in a lacustrine system, reflecting in part the highly variable environmental conditions that are present.

3.9. Nutrient Availability

The availability of nutrients for prokaryotic cell growth is potentially an important determinant of HGT rates in freshwater lakes. *In vitro* conjugation rates in pure cultures of heterotrophic bacteria have been positively correlated with the availability of rich nutrient sources containing phosphorus, nitrogen, and organic carbon [30,67], as well as with organic carbon alone [157,167]. Additionally, conjugation rates between introduced heterotrophic bacteria were found to be significantly higher in sterile nutrient-rich sewage water compared to sterile lake water [168]. Interestingly, the positive relationship between nutrient availability and conjugative plasmid transfer appears to be attenuated when nutrient concentrations rise above a particular threshold value *in vitro* [169,170], suggesting that a similar threshold may exist in natural environments. The latency period between phage infection and cell lysis, as well as the resulting number of released phage particles, are reduced in starved bacterial cells compared to actively metabolizing cells, indicating that transduction rates are reduced under conditions of nutrient limitation [171]. In addition to serving as a vector for transformation, extracellular DNA is a source of exogenous nucleotides, labile carbon, nitrogen, and phosphorus for microbial communities [119]. Reduced availability of these nutrients may increase the utilization of extracellular DNA by prokaryotes as a nutrient source may increase, potentially reducing the amount of transformable DNA available for HGT [172]. Collectively, these findings suggest that HGT rates in freshwater lacustrine systems are directly related to the availability of nutrients for prokaryotic growth and that HGT rates in heterotrophic prokaryotes are likely to be higher in nutrient/organic carbon rich systems. In freshwater lakes, bacterial production and growth rates can be constrained by the availability of nitrogen, phosphorus, and/or organic carbon [173], with phosphorus most commonly being the limiting nutrient [174]. It is important to note that most investigations of the effects of nutrient availability on HGT employed a single chemoorganoheterotrophic species of bacteria to assess the effects of organic carbon or general nutrient limitation, which precluded the investigation of other heterotrophic and autotrophic prokaryotes as well as any specific effects of phosphorus and nitrogen limitation on HGT in freshwater prokaryotic communities.

Nutrient limitation can also stimulate HGT, blurring the relationship between nutrient availability and HGT that has been established *in vitro* and exemplifying the likely complexity of HGT regulation within natural environments. As with DNA damage resulting from exposure to genotoxic chemicals or ultraviolet radiation, starvation can lead to the activation of bacterial stress responses that in turn can promote HGT. Amino acid and

thymine deprivation can induce prophage and thereby promote transduction [175]. The development of competence *in vitro* has been observed in cells in association with a reduction in metabolic activity, which can result from nutrient limitation [50,176]. Conjugation has been demonstrated in laboratory seawater microcosms even after starvation for up to 15 days [177], indicating that nutrient limitation does not preclude the potential for conjugation in a freshwater prokaryotic community. Hausner and Wuertz [154] found that conjugation rates were not appreciably diminished by nutrient limitation under laboratory conditions in bacterial biofilms, and suggested that although conjugation requires energy, starved cells that constitutively express their conjugation apparatus are still able to participate in HGT.

Viral infection rates and bacterial abundance and metabolic activity are generally higher in nutrient-rich freshwater systems [36,178], indicating greater rates of HGT. Within lacustrine systems, HGT rates may reflect spatial and temporal variations in nutrient availability. Epilimnetic waters of highly productive stratified lakes are typically depleted of nutrients during summer stratification as organic matter settles out of this compartment into the underlying metalimnion and hypolimnion [179]. The availability of phosphorus, nitrogen, and organic carbon is generally increased during turnover events, storm events, and the spring melting of snow and ice in temperate climates, as nutrient-rich material is introduced or redistributed within a system. Nutrient inputs may be particularly high in urban and agriculturally impacted areas, and point sources within lakes (e.g. wastewater treatment plant effluent) may create localized HGT hotspots. Concentrations of organic and inorganic nutrients tend to be greater in interfacial regions including the metalimnion, air-water interface [128,180], and sediment-water interface [181] compared to the bulk water column. Further, biofilms, commonly observed at such interfaces, can sorb and accumulate nutrients from the surrounding environment and the extracellular polymeric substances of which they are composed can serve as a nutrient source (e.g. extracellular DNA) [47].

3.10. Ecological Interactions

In freshwater lacustrine environments, interactions within prokaryotic communities and among prokaryotes and plants, algae, and protozoa can potentially influence rates of prokaryotic HGT. Bacterial quorum sensing (intercellular communication) systems can regulate conjugative transfer [182,183], prophage induction [184], and transformation [185]. Further, addition of *Escherichia coli* cell-free supernatant was observed to increase the frequency of transformation in *Bacillus subtilis* cells nearly ten-fold, suggesting that organic factors produced by one species in a freshwater prokaryotic community can sti-

multilate transformation in a distantly related species [138]. Investigations of HGT to date have largely utilized single bacterial species, such that the influence of interactions between different prokaryotic taxa on gene transfer remains poorly defined.

The rhizosphere of terrestrial plants has been identified as a hotspot for HGT, in part due to the availability of nutrient-rich plant root exudates [124,186]. Similarly, in lacustrine systems, aquatic plants and algae may enhance HGT in nearby prokaryotic communities. Conjugative plasmid transfer between bacteria in a microcosm was enhanced in the presence of the aquatic marsh plant *Echinochloa crusgalli* [187]. The presence of algae or algal exudates can enhance *in vitro* rates of conjugation [188] and transformation [189] in prokaryotes, suggesting that algal blooms may be hotspots for HGT. Under laboratory conditions, cocultivation of *Bacillus subtilis* with the ciliate protozoa *Tetrahymena thermophila* or metabolites from co-cultures decreased transformation rates, with the inhibitory effect of the metabolites being attenuated when the photosynthetic alga *Euglena gracilis* was present in culture [190]. In contrast, the presence of ciliates, which are ubiquitous in freshwater lakes, increased conjugation rates in cultures of *Escherichia coli* by facilitating contact between donor and recipient strains through co-accumulation in its vesicles [191]. Intercellular communication between bacteria is enhanced in biofilms [192] and freshwater biofilm communities typically include prokaryotes, algae, and protozoa, suggesting that they are important sites for ecological regulation of HGT in freshwater lacustrine systems.

More generally, low molecular weight organic acids (e.g. tartrate, citrate) derived from organic matter and associated with regions of high biological activity can compete with DNA for adsorption to cells, potentially inhibiting transformation [193]. Chitin, a biopolymer found in fungi, insects, molluscs, and crustaceans in aquatic environments, has been shown to induce competence in bacteria capable of colonizing and degrading it *in vitro* [194]. This suggests that transformation rates may be influenced by the distribution and abundance of chitin producing organisms within freshwater lakes.

3.11. Prokaryotic Community Composition

Generally speaking, HGT in the majority of freshwater prokaryotes remains uncharacterized and taxon-specific variables that may influence HGT remain to be determined. However, several lines of evidence suggest that the composition of freshwater prokaryotic communities influences the character of HGT taking place within them, although much more research is necessary to firmly establish such a relationship. First, metabolisms limited to particular taxa, as discussed previously, can differentially alter local biogeochemical conditions, in turn po-

tentially influencing HGT among impacted prokaryotes. Second, the formation of consortia dependent on the presence of particular community members may impact HGT rates through changes in donor and recipient cell numbers, metabolic activities, and proximity [124]. For example, chemoorganoheterotrophic bacteria tend to accumulate around cyanobacterial heterocysts, taking advantage of secreted organic carbon and nutrients [195]. Third, plasmids and phages can have specific host ranges [196] and transforming DNA can be tagged such that it will be preferentially taken up by specific taxa [197], such that the extent to which they mediate HGT within a prokaryotic community is dependent on the presence and relative abundance of particular taxa. High prokaryotic diversity can potentially inhibit transduction rates via cells not susceptible to infection by a particular phage interfering with initial virus-host contact [37]. Fourth, nuclease activity and DNA release may differ depending on prokaryotic community composition, influencing transformation rates via the availability of transformable DNA [176]. Finally, as discussed previously, communication within prokaryotic communities via extracellular signalling molecules with taxa-specific production and responses can differentially regulate HGT.

Prokaryotic community composition can differ substantially between freshwater lakes [198,199] and within systems can vary with depth and site location, as well as over seasonal and annual timescales [200-203]. Further, community composition can vary between epilimnetic and hypolimnetic regions within summer stratified lakes [204-206] and planktonic bacterial communities can differ from those associated with suspended particulate matter [207,208]. Finally, regular and episodic system perturbations (e.g. seasonal lake mixing events, addition of prokaryotes and nutrients via wastewater input) may appreciably alter community composition [206,209]. These spatial and temporal variations in lacustrine prokaryotic community composition may be associated with different HGT rates and participants.

4. Concluding Remarks

Although the three principal mechanisms of HGT have been demonstrated in freshwater environments, field studies linking HGT to specific biogeochemical parameters are relatively scarce and experimental studies assessing natural prokaryotic communities are equally rare. These linkages, although established in many cases using laboratory-based approaches with chemoorganoheterotrophic organisms, have yet to be confirmed in freshwater lacustrine systems. Consequently, how and to what extent individual biogeochemical factors influence HGT in freshwater lakes remains poorly understood. An appreciation for the substantial spatial and temporal variability in factors capable of influencing HGT within

freshwater lakes is necessary to fully characterize environmental gene transfer. In particular, interfacial regions, whether between thermally stratified zones of water, between bottom sediments and overlying water, or between the water surface and the atmosphere, are areas that should be targeted for further investigation of freshwater lacustrine HGT. Further, greater exploration of HGT within autotrophic guilds, a key component of environmental prokaryotic communities, is required to fully constrain these processes within freshwater systems. Culture-independent molecular techniques (e.g. [27,210,211]) provide a means of *in situ* assessment of HGT in freshwater prokaryotic communities. It is anticipated that the coupling of these techniques with biogeochemical characterization will further understanding of HGT occurrence, controls and impacts within freshwater lakes.

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