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Synthesis of Lysoglycerophosphocholines from Crude Soybean Lecithins as Sustainable and Non-toxic Antifouling Agents against the Golden Mussel *Limnoperna fortunei*

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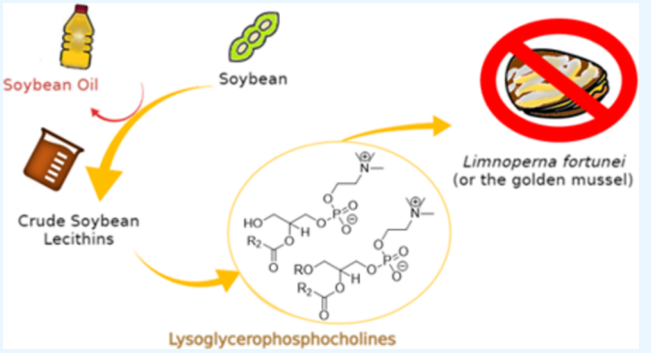
ABSTRACT: This research aimed to produce, on a multigram scale, a new class of non-toxic, halogen- and metal-free antifouling agents from the abundant lecithin byproducts of industrial soybean oil extraction. Three glycerophospholipid analogues were prepared by a facile methanolysis of crude soybean lecithins and a subsequent solvent-free O-alkylation: lysoglycerophosphocholines (LGPCs) and its ether derivatives *O*-alkyl lysoglycerophosphocholines (ALPCs). As efficient antiproliferative agents, LGPCs and ALPCs are an eco-friendly alternative to current commercial antifoulants which possess significant toxicity to aquatic life. *In situ* immersion tests of coated stainless-steel nets with previously incorporated automotive paint products, LGPCs and ALPCs (1-*O*-octadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine, ALPC18, and 1-*O*-hexadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine, ALPC16), in an aquaculture reservoir in SP-Brazil revealed significant growth inhibition against macrofouling species, especially the epibiotic golden mussel (*Limnoperna fortunei*), when compared with the control. These results promise a more sustainable and ecologically innocuous approach to combating the biofouling phenomenon and the deeply concerning dissemination of the golden mussel which has provoked an economic crisis in the energy and aquaculture sectors.



1. INTRODUCTION

Biofouling is defined as the deposition of micro- and macro-organisms on natural and artificial surfaces that are either completely or partially submerged. This spontaneous phenomenon starts with an accumulation of macromolecules (*e.g.*, proteins, polysaccharides, and lipids) onto this structure when the latter gets in touch with an aqueous medium, thus generating a nutrient-enriched substrate. Rapidly, a vast diversity of microorganisms (*e.g.*, unicellular bacteria and diatoms) colonize this surface.[1−3] Within weeks, this colonization then evolves into an even more complex biologic community by the attachment of macrofouling agents (*e.g.*, fungi, tunicates, barnacles, and mollusks) to the surface.

Although biofouling is a natural phenomenon, its repercussions can become a major burden to a myriad of economic sectors: from marine transportation, health, and energy production to food processing and aquaculture.

For instance, aquaculture, which is defined as the farming of aquatic organisms, suffers tremendously from the impacts of biofouling development. Since aquaculture's infrastructure invariably consists of submerged components (*e.g.*, nets, cages, floats, and ropes), all of them serve as surfaces for biofouling. Even shells in grown shellfishes can become a substrate for biofoulant settlement.[4]

Biofouling-induced damages to the aquaculture industry have raised deep concern due to the financial and social relevance exerted by this sector. This segment plays an important role as a major food supplier to a global population that never ceases to expand. In 2018, the sector was accountable for 82 million tons (USD 250 billion) of 179 million tons (USD 401 billion) of global fishery production.[5] In terms of human consumption, that same year, aquaculture accounted for 46% of the total fishery supply worldwide.

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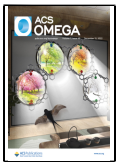

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Meanwhile, the direct cost of biofouling managing strategies was estimated to be 5−10% of aquaculture production.[6] Globally, this equates from USD 1.5 to 3 billion per year.[4]

Non-indigenous foulant species, which can be introduced to foreign environments through vessel biofouling and shipping, ballast water discharge, and shellfish translocation, might use aquaculture as a reservoir for their quick proliferation.[4] The spread of these invasive organisms is responsible for disrupting coastal ecosystems and homogenizing those habitats,[7] besides taking a toll on aquaculture productiveness.

Among the species with the most significant negative impact on biodiversity is the golden mussel or *Limnoperna fortunei*, a bivalve mollusk that originated from China and South-East Asia's rivers that can attach itself to any sort of substrate, even on autochthon mussels' shells. Since their spreading within South America (through the La Plata basin in the 1990s), economic losses have been alarming.[7] This small mussel has already infested Itaipu, the largest hydroelectric reservoir in the world, threatening local biodiversity and the energy sector efficiency.[8] *L. fortunei* rapid dissemination has then become a serious inconvenience to the aquaculture industry through substantial maintenance and production costs' increases.[9]

1.1. Biofouling Control Strategies. Since control of foulant infestation in natural open areas is virtually impossible, their attachment prevention has become the main strategy against the mussel's dissemination.[8] Although physical control has shown some efficiency against invasive organisms through mechanical removal, chemical mitigators are, in general, more cost-effective and have higher success rates.[10] Chlorine is one of the most common chemicals used to avoid foulant dissemination due to its low cost, easy acquirement, and local authority licensing (*e.g.*, in Brazil, CONAMA, and in the US, USEPA).[8] However, as a non-specific poison, chlorine usage might be more detrimental to non-targeted organisms than to foulants themselves.[10] For bivalves' control, such as the golden mussel, chlorine can also show a low efficiency. These species can detect concentrations less than 10 $mg{\cdot}L^{-1}$ of chlorine, closing their shells and isolating themselves from the environment for up to 2 weeks, a strategy that grants them tolerance and immunity to this substance.[8]

Nowadays, the most employed marine aquaculture fouling's mitigation technique also includes chemical antifoulants as its main components. Metal derivatives such as cuprous oxide and zinc oxide are incorporated into paints and coatings as biocide agents, therefore preventing the settlement and growth of undesired species to painted surfaces.[11,12] Due to the corrosive character and low durability of these compounds, especially the copper-based ones, a group of additives have been included to enhance antifoulant formulations' efficacy: the "booster biocides".[13]

Even though "booster biocides" are marketed as environmentally friendly, there are still concerns regarding their ecological threat.[13] Typically, pesticides and herbicides used in agriculture and some "boosters" have shown non-targeted toxicity to the aquatic life.[14] For instance, Zineb can be harmful to some fishes' development, while Sea-nine 211 endangered crustaceans' development.[15]

Since commercially available antifouling paints and coatings are toxic to the aquatic environment, the need for "greener" antifouling alternatives has recently increased. "Eco-friendly" antifouling alternatives comprise multiple strategies such as silicone-based and recoverable magnetic ferroferric oxide nanoparticle (Fe_3O_4-NP) coatings. The first's application to aquaculture textiles (*e.g.*, ropes and nets) remains a challenge,[16] whereas the latter has shown good results in controlled aquatic environments.[17] Therefore, a low-cost, efficient procedure, to yield non-toxic antifoulants, is still under development.

Among the most promising candidates, the natural-based lysoglycerophosphocholines (LGPCs) and their ether-analogues *O*-alkyl-lysoglycerophosphocholines (ALPCs) are there.

1.2. Glycerophospholipids as Natural Biocides. LGPCs and ALPCs are glycerophospholipids (GPLs), a class of lipids whose structures comprise a glycerol backbone in which a phosphodiester and a choline head group are attached to the *sn*-3 position. Unlike other GPLs, LGPC lack an acyl group in either the *sn*-1 or *sn*-2 positions, while ALPC possesses an ether group linked to the glycerol chain's *sn*-1 and/or *sn*-2 sites (Figure 1). Usually, the term "LGPC" makes

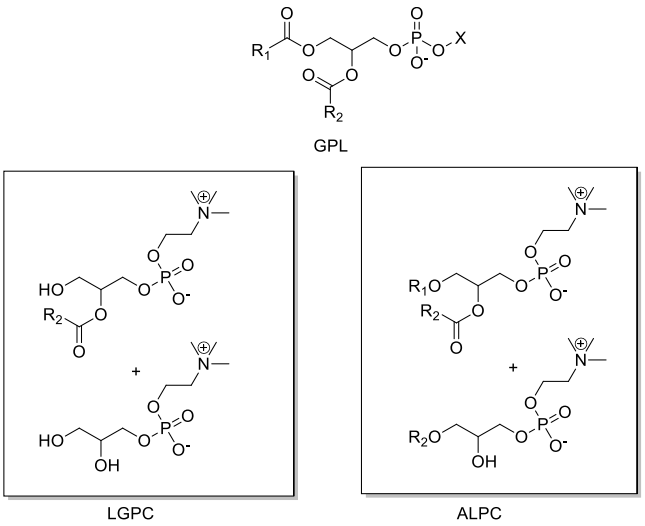

Figure 1. GPL and its derivatives (LGPC and ALPC); X = choline, ethanolamine, serine, or inositol and R1 and R2 = alkyl/alkenyl chains.

reference to either *sn*-1 or *sn*-2 monoacylated glycerophosphocholines; in this work, however, LGPC is used for a mixture of (non/*sn*-2)-acylated of compounds, as shown in Figure 1.

GPL, once immersed in an aqueous environment, can self-assemble into monolayers and nanotubules which might be applied to control the release of antifouling agents. With feasible and a good scale-up price, these structures might serve as an interesting vehicle for sustained delivery of substances in paints and other antiadhesive coatings.[18,19] As a base for materials engineering, self-assembled GPLs (especially choline derivatives), due to their amphiphilic and zwitterionic character, have shown great non-foulant ability. This capacity is possibly related to the fact that GPL's polar moieties strongly hold water molecules, therefore forming a hydration layer that non-specifically repels protein and cell adsorption onto surfaces.[20] The water barrier generated by ionic solvation in addition to hydrogen bonds between GPL's neutral and polar groups and those water molecules could then be responsible for interfering with the latter interfacial forces which promote antiadhesiveness.[20]

Conversely, GPL's adsorption to potentially incrusted structures is generally promoted by hydrophobic interactions.[3] Nonetheless, through a delicate balance between these non-charged and electrostatic forces, chiral self-assembled nano-

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tubules can perform as good adsorbents of cationic, anionic, and hydrophobic species (*e.g.*, gold NPs) of highly specific surface area.[21] Since the tubules are extremely versatile and easy to functionalize, they might be applicable as drug delivery carriers, packing materials for separation, and templates for metal nanomaterials as well.[21]

Thus, besides being promising antifoulants, GPL nanotubes may also serve as important deliverers of other substances that might act in synergy with their biocidal purpose.[22]

Moreover, LGPCs and ALPCs are important immunomodulators found in human plasma that have shown a variety of biological functions such as antimicrobial, antiparasitic, and antitumor.[23−26] Although not completely elucidated, LGPC's and ALPC's cytotoxicity mechanism is often associated with their amphiphilic and zwitterionic characters, that is, their chemical structure which consists of a combination of nonpolar and polar groups: a long hydrocarbon chain and a (positive and negatively) charged phosphocholine radical, respectively. These structural properties grant LGPCs and ALPCs "detergent" abilities as they insert themselves and destabilize organisms' plasma membranes' lipid bilayer conformation until cell lysis.[27] Also, ALPC's ether bonds make them resistant to phospholipases, therefore promoting their accumulation in cells' membranes and eventual cellular death.[28]

Therefore, GPL's antifouling capabilities might be a combination of biocidal roles toward non-desired epibiotic species performed both directly (*e.g.*, through membranal destabilization, intracellular accumulation, and/or triggering of an apoptotic signalization[28]) and indirectly (*e.g.*, when promoting a physical repulsion which culminates on a non-adhesive environment that prevents the mentioned organisms' adsorption[18]).

Given GPL's biocidal prospective, our research group developed a simple and feasible preparation method to generate LGPCs and ALPCs from soybean lecithins: an affordable and abundant starting product.[29,30] Synthetic LGPCs thus showed significant algaecide activity against the growth of foulants *Tetraselmis striata*, *Dunaliella tertiolecta*, and *Skeletonema costatum*.[29] Meanwhile, ALPC 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (ALPC16), obtained through an alkylation treatment of LGPCs, was able to reduce marine bacterial proliferation (*e.g.*, *Shewanella putrefaciens*, *Vibrio estuarians*, *Pseudoalteromonas elyakovii*, and *Pseudomonas fluorescens*)[29,30] and was proved to be as effective as an antifoulant as the commercial "booster biocide" Econea.[31] Additionally, LGPCs showed biocide activity against marine microfoulants similar to that observed for copper sulfate. When compared to commercial biocides such as menadione and Irgarol 1051, however, LGPCs was proved to be less toxic than those products. Also, because of its low potential of persistence and bioaccumulation in the environment,[30,32] LGPC presents itself as an "eco-friendlier" biocide, hence a safer alternative to the biofouling problem.

In the view of LGPC's and ALPC's potential as biocides without the toxicity of the metal-based and organohalogen compounds (*i.e.*, booster biocides) currently on the market, this report intended to accomplish the generation of a cost-effective and sustainable synthetic method for LGPC and ALPC production at a multigram scale for their future incorporation to antifouling paints as a new class of biocide additives.

Following significantly high reaction yields of previously synthesized LGPCs and ALPCs (1-*O*-octadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine, ALPC18, and 1-*O*-hexadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine, ALPC16), biocidal abilities against biofilm's formation and the golden mussel's (*L. fortunei*) dangerous infestation were assessed through immersion tests with LGPC- and ALPC-coated nets in an aquaculture reservoir in São-Paulo, Brazil, a region where there had been prior observations of extremely concerning drawbacks from the mollusk's quick spread.

2. MATERIALS AND METHODS

2.1. General Information. Except for crude soybean, all reagents and solvents were obtained commercially and used without further purification.

Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates (60F-254) using an acid solution of cerium sulfate, and heat, as a visualizing agent. The structures of compounds were determined by nuclear magnetic resonance (NMR), where ^{1}H and ^{13}C spectra were recorded on 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or 300 (^{1}H, 300 MHz; ^{13}C, 100 MHz) or 200 (^{1}H, 200 MHz; ^{13}C, 100 MHz) using tetramethylsilane as an internal standard. For LGPCs, the deuterated solvent was D_2O (deuterium oxide), while for ALPCs, the deuterated solvent was $CDCl_3$ (chloroform-*d*). Additional structural confirmation was performed through infrared spectral analysis (PerkinElmer 1600 FTIR spectrometer). IR spectra were recorded using KBr pellets for solid compounds or as liquid films in the case of oily samples.[30,31,33] Further structural confirmation was performed through coupled liquid chromatography/mass spectrometry (LC/MS) following the procedure described by Batista *et al.*[29,31]

2.2. Preparation of LGPCs: Methanolysis Reaction. Soybean lecithins were donated by a food company as a major industrial grain oil generation byproduct. Before all experiments, to obtain the crude lecithins, soybean oil was treated with water, under conditions wherein the gums (GPL and lecithin's enriched fraction) were precipitated from the solution, separated by centrifugation, and finally dried.[34]

LGPC preparation followed an adaptation of our previously published synthetic procedure.[31] To a 2000 mL glass reactor with five joints of 24 × 40*N* coupled with a mechanical stirrer EUROSTAR POWER-B IKA, methanol (1500 mL), non-purified soybean lecithin (1 mol), and the alkaline agent (either NaOH, Na^0 or NaOMe) (2.4 mol) were added. The reaction mixture was stirred at room temperature for 72 h. Afterward, product extraction was initiated by the resulting suspension filtration *in vacuo* (with the aid of a Buchner funnel) followed by the liquid phase concentration (also *in vacuo*). To further remove non-desired side products (*e.g.*, methyl esters), the resulting yellowish solid was solubilized in hexane and put to rest for 2 h. LGPCs were finally yielded after this mixture of filtration and concentration *in vacuo*, of which the major product generated was 1-hydroxy-2-*O*-acyl-*sn*-glycero-3-phosphocholine (97%).

2.3. Preparation of *O*-Alkyl-Lysophosphocholines (ALPCs). *2.3.1. Under Solvent-free Conditions.* To previously synthesized LGPCs (1 mol), a phase transfer catalyst (tetrabutylammonium hydrogen sulfate, TBAHS; tetrabutylammonium bromide, TBAB; tetrabutylammonium iodide, TBAI; cetyltrimethylammonium bromide, CTAB; methyltriphenylphosphonium bromide, MTPB; phenyltrimethylammonium tribromide, PTMTB; or tetrabutylphosphonium bro-

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Table 1. Limnological Parameters of Aquaculture Reservoir's Water

limnological parameters	March/20	April/20	May/20	June/20	November/20
temperature (°C)	29.06	23.53	23.19	21.69	23.70
pH	6.69	6.34	6.98	5.85	7.32
conductivity (μS cm^{-1})	59.00	54.00	60.00	59.00	57.00
turbidity (NTU)	2.83	2.00	1.00	0	2.90
DO (mg·L^{-1})[a]	11.03	7.79	8.31	8.14	5.85
DO saturation (%)[a]	144.50	_	99.40	94.9	70.60
TDS (mg·L^{-1})[b]	0.039	0.035	0.039	0.038	0.037
water Transparency (m)	2.50	_	5.00	4.10	3.2
chlorophyll-*a*(ug L^{-1})	6.17	6.85	6.93	7.14	8.52

[a]DO = dissolved oxygen. [b]TDS = total dissolved solids.

Table 2. Golden Mussel Individuals' Density and Size on each Coated-Net Sample after the 9 Month Immersion Period (on November/20)

			size (mm)		
net-sample composition (% = 0.1 g·mL^{-1})	individuals/225 cm^2	mean density (individuals/m^2)[a]	minimum	maximum	median
ALPC16 10%	3	133	6.12	8.54	7.21
base paint	14	622	2.73	17.87	5.91
non-coated	12	361	1.43	3.96	2.95
LGPC 10% + ALPC16 5%	19	844	2.55	11.22	8.02
LGPC 2% + linseed oil 5%	74	3289	2.33	22.84	7.56
LGPC 10% + ALPC18 5%	3	133	4.04	9.93	7.62
LGPC 5%	20	889	1.48	11.07	5.90
LGPC 10%	4	178	4.98	11.48	8.51
ALPC18 10%	10	444	2.1	10.54	8.15
PVC	183	8133	1.92	25.10	10.12

[a]Triplicates of each net sample composition were assessed regarding *L. fortunei* individual's population density and size through counting and measuring, respectively; ALPC18 = 1-*O*-octadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine and ALPC16 = 1-*O*-hexadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine; non-coated = stainless-steel net without any coating; and PVC = polyvinyl chloride.

Table 3. ALPC Synthesis: Reactions' Yields under Solvent-Free Conditions

reaction's product/catalysts[a]	CTAB (%)	MTPB (%)	PTMTB (%)	TBAB (%)	TBAHS[b] (%)	TBAI (%)	TBPB (%)
ALPC12	78	38	66	13	79	72	50
ALPC14	36	40	53	18	54	27	22
ALPC16	84	47	73	86	87	75	59
ALPC18	70	62	71	83	84	32	56

[a]Solvent-free reactions performed at 100 °C. [b]Phase-transfer agent as depicted in Batista *et al.*[31]

Table 4. ALPC Synthesis: Reactions' Yields (with a Solvent)

reaction's product/catalysts[a]	CTAB (%)	MTPB (%)	PTMTB (%)	TBAB (%)	TBAHS[b] (%)	TBAI (%)	TBPB (%)
ALPC12	77	15	45	85	31	53	55
ALPC14	40	10	22	82	18	54	30
ALPC16	80	12	24	60	36	15	22
ALPC18	36	54	41	80	73	38	37

[a]Solvent = dichloroethane. [b]Phase-transfer agent as depicted in Batista *et al.*[31]

mide, TBPB) (0.003 mol), NaOH (1.5 mol), and the respective alkyl bromide (1-bromododecane, 1-bromotetradecane, 1-bromohexadecane, or 1-bromooctadecane) were carefully added to a glass reactor with five joints of 24 × 40*N*. The solid mixture was heated at 100 °C under constant agitation with the aid of a mechanical stirrer Eurostar Powerb IKAWERKE. After 72 h and reaction completion (follow-up through TLC analysis), the final mixture was washed three times with brine solution. Product (ALPC) liquid−liquid extraction was performed with hexane. Excess water was then removed through Na_2SO_4 addition followed by the salt filtration. Finally, the exceeding solvent removal was later performed *in vacuo*. The major ALPC [1-*O*-dodecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine (ALPC12), 1-*O*-tetradecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine (ALPC14), 1-*O*-hexadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine (ALPC16), and 1-*O*-octadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine (ALPC18)] product yields are summarized in Table 3.

2.3.2. With Solvent. ALPC syntheses were conducted according to our previous methodology.[31] Nonetheless, alternative phase-transfer catalysts (TBAB, CTAB, TBAI, MTPB, PTMTB, and TBPB) (0.05 mol) were also tested as replacements for TBAHS (0.05 mol). Product yields are summarized in Table 4.

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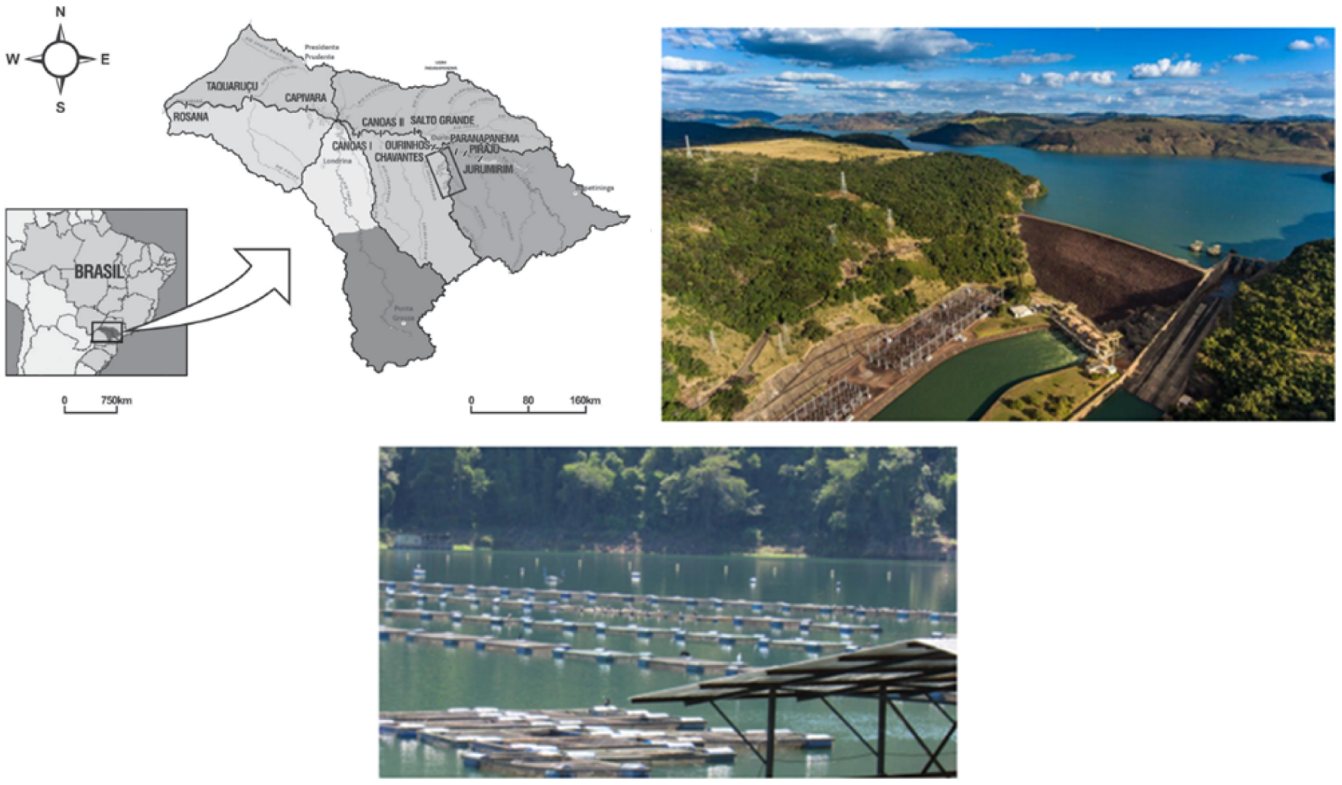
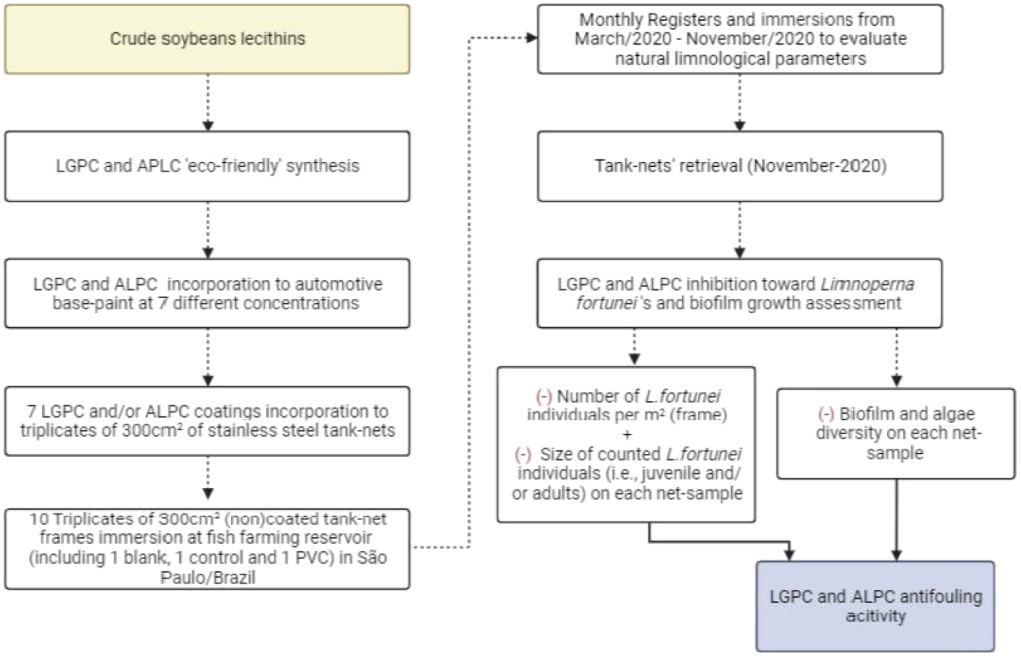


Figure 2. Map of Brazil with emphasis on Paranapanema river in the southern region, featuring its 11 reservoirs' complex (top left) and a picture of the Chavantes hydroelectric powerplant located in the highlighted zone (top right); the Chavantes aquaculture reservoir featuring its net cages (bottom)—adapted in part with permission from D.M.M.R.A. (2012). Caracteristicas limnológicas em áreas sob influência de piscicultura em tanques-rede no reservatório da UHE Chavantes, rio Paranapanema, SE/S, Brasil. PhD. Thesis. Universidade Estadual Paulista, Centro de Aquicultura. Available at: ⟨http://hdl.handle.net/11449/100158⟩. Copyright 2012. Dr Daercy Maria Monteiro de Rezende.

Scheme 1. Experimental Steps from LGPC and ALPC Synthesis Until Antifouling Evaluation[a]



[a](-) indicates a reduction or a growth inhibition.

2.4. Antifouling Activity Assay. *2.4.1. Study Area.* The Paranapanema river is a natural divider between the States of São Paulo and Paraná. Chavantes is part of a complex of 11 reservoirs built on the Paranapanema river for hydroelectric energy production purposes, under the concession of China Three Gorges Corporation—CTG do Brazil (Figure 2).

The dam of the hydroelectric plant is located at the coordinates 23° 07′00″ S and 49° 44′00″ W. The reservoir is

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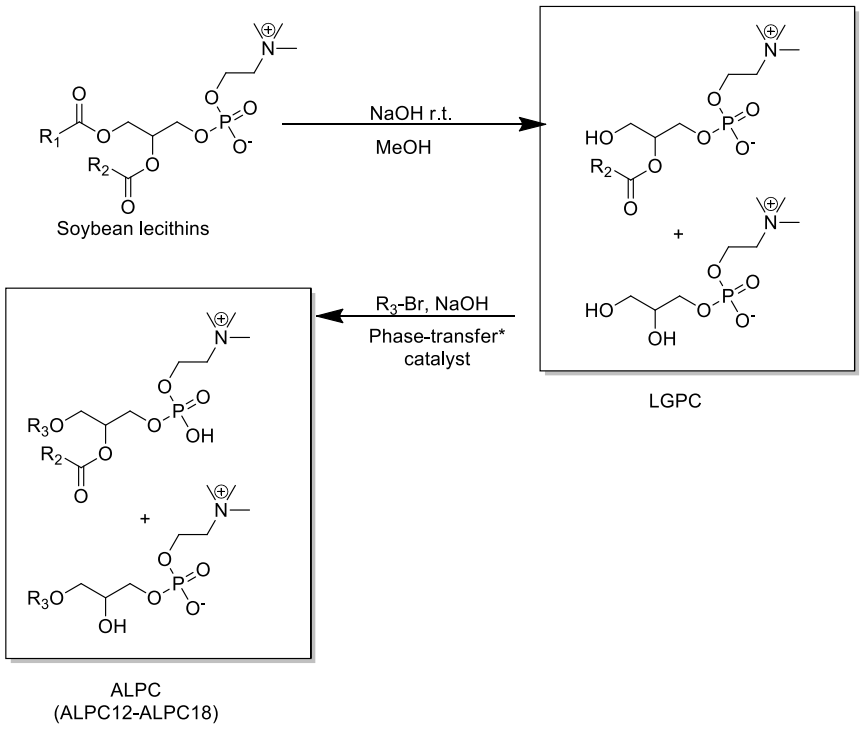
Scheme 2. Synthesis of LGPCs and ALPCs (ALPC12-ALPC18) from Soybean Lecithins[a]



[a]For LGPCs: R1 and R2 = blend of alkyl and alkenyl chains—(16:0/22:2), (18:3/18:2), (18:2/18:2), or (18:2/16:0); r.t. = room temperature. For ALPC: ALPC12—$R_3 = C_{12}H_{25}$; ALPC14—$R_3 = C_{14}H_{29}$; ALPC16—$R_3 = C_{16}H_{33}$; ALPC18—$R_3 = C_{18}H_{37}$; phase-transfer catalyst: CTAB, MTPB, PTMTB, TBAHS, TBAB, TBAI, or TBPB; * solvent-free step: 100 °C; with solvent step: dichloroethane, r.t.

the accumulation type because its main function is to store water during the dry period for the hydroelectric production of downstream power plants. This feature implies a great seasonal variation of the water level and total flow rate (spillway and turbine discharge), influenced by the plants' operating system.[35] Chavantes' total area is of 400 km^2 and features great depths and residence time (around one year). The immersion assays were carried out in a fish farm, which was implemented in 2006, in the municipality of Ipaussu, SP, comprising an area of 5.0 ha of water mirror of tilapia (*Oreochromis niloticus*) farming.

2.4.2. LGPC and ALPC Antifouling Activity Preliminary Assays. The synthetic products LGPCs and ALPCs (ALPC16 and ALPC18) had their antifouling activity assessed regarding their inhibition toward fouling species —with special attention to the golden mussel (*L. fortunei*)—through an *in situ* assay where ALPCs and LGPCs were incorporated to an automotive paint (LAZZURIL—SHERWIN WILLIAMS)[a] at different concentrations, either uniquely added or in addition to the other and later applied to three segments of 300 cm^2 stainless-steel nets. To promote an increase in LGPC's solubility, linseed oil (a common coating diluent) was incorporated into one of the formulations.

The 10 coating systems were evaluated (as depicted in Table 2): eight corresponding each to the following compositions of LGPCs and/or ALPCs: [(a) ALPC16 10%; (b) ALPC18 10%; (c) ALPC16 5% + LGPC 10%; (d) ALPC18 5% + LGPC 10%; (e) LGPC 5%; (f) LGPC 10%; (g) LGPC 2% + linseed oil 5%; (h) LGPC 5%]. For control and blank purposes, respectively, one net sample was covered solely by the base automotive paint and another received no treatment whatsoever. Finally, one polyvinyl chloride (PVC)-coated net was also tested (Scheme 1).

The nets samples were submerged at the fish farm in Chavantes Reservoir. The campaigns started on March/2020 and ended in November/2020: the nine-month period sufficient for the organisms' development. Monthly immersions were performed during the mentioned period for visual analysis of the biofilm and macrofouling development (Figures S22–S25—Supporting Information).

At the end of the experiment, in November/2020, all nets were finally emerged. To assess the biofouling formation over each net sample, the adhered golden mussels were first removed at the central zone of each net. Later, the same surface was scrubbed to retrieve and collect the biofilm (*i.e.*, other periphytic and planktonic microbiota) material. All collected material was preserved in individual flasks filled with a 4% formaldehyde/alcohol solution.

The largest individuals of *L. fortunei* adhered to each net sample were counted and measured with the aid of digital calipers (accuracy 0.001 mm). Meanwhile, individuals of size larger than 250 μm length were obtained through a stereoscopic microscopy analysis.

L. fortunei individuals of size smaller than 2 mm were classified as juvenile, whereas those with more than 2 mm were considered adults.[36] Parameters evaluated for the mussel's development on each net were as follows: (a) number of juvenile and adults, (b) density of individuals (*i.e.*, number of individuals/m^2 of total mesh frame), and (c) size preliminary statistics (highest, lowest, and median values of measured individuals' sizes on each net sample population).

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All golden mussels were measured and counted at the Reference Laboratory Unit in Limnology, Fishering Institute, SAA (www.pesca.sp.gov.br/).

Additionally, the biofilm taxonomic identification was executed at the Research Center in Phycology, Institute of Botany, SMA (www.infraestruturameioambiente.sp.gov.br/institutodebotanica/) to evaluate the biodiversity of the remaining population post-treatment with the coatings' samples.

2.4.3. Limnological Parameters. From March/20 to November/20, the reservoir's water limnological parameters (Table 1) such as (a) temperature, (b) dissolved oxygen (DO) levels, (c) pH, (d) turbidity, (e) electric conductivity, (f) chlorophyll-*a*, (g) total dissolved solids, and (h) transparency were analyzed according to methodology depicted in the literature.[37−39]

3. RESULTS AND DISCUSSION

Lysoglycerophospholipids' (LPL) synthesis is usually a multistep and tedious process, thus making it hardly suitable for a large-scale preparation.[40,41] Although other strategies have been employed with glycerol derivates (*e.g.*, solketal and glycidol) as starting materials, natural lecithin is considered an ideal precursor for LPL's preparation due to its abundant and sustainable availability.[40]

To develop a facile process feasible at an industrial magnitude, this work started from crude soybean lecithins (Scheme 2), which are an extremely low-cost source of GPLs generated as a side product of soybean oil extraction. As observed in all vegetable sources, these crude soybean lecithins' most prominent lipids are phosphatidylcholines, followed by a minor presence of other GPL and, to an even lesser extent, some free fatty acids.[42,43] Due to their presence as major components, the crude lecithins are hereby referred to as phosphatidylcholines.

It is worth mentioning that even though the starting materials are obtained from a natural source and therefore have been reported to be enantiomeric pure,[36] the synthetic method herein presented does not ensure this degree of stereochemical purity. Nonetheless, this work's goal, for now, comprises the production of LGPCs and ALPCs in good yields and multigram scale despite its racemic composition.

First, these lecithins suffered a mild methanolysis reaction when in contact with an alkaline agent and methanol (Scheme 2). This reaction's final products are the methanol-soluble (non/mono) acyl-lysoglycerophosphocolines (LGPCs), whereas, as side products, the more hydrophobic methyl esters are also formed but easily removed through a simple filtration process. We[29,32] previously described a soybean lecithins methanolysis procedure where the precursor of the alkaline agent used was NaOMe generated *in situ* after Na^0 reaction with a methanolic medium. This process was herein reproduced with crude soybean lecithins (instead of previously purified products) as its starting material and showed good yields (90%) when generating LGPCs. However, due to the difficult manipulation of metallic sodium, this component's application can become a major obstacle later when scaling up this synthetic methodology to an industrial magnitude. The industrial size increase would be even riskier due to the release of flammable gas H_2 by the Na° drastic reaction.[29]

To reduce the risks associated with this *in situ* methoxide formation, commercial NaOMe was tested. This methanolysis reaction, despite the significant resemblance to metallic sodium employment, exhibited a decrease in product generation (*i.e.*, yields of 70%). Thus, the search for a cheaper and more manageable alkaline agent proceeded until NaOH was proven to produce LGPCs not only more efficiently (*i.e.*, yields of 98%) but through a more affordable, feasible, and easily adaptable multigram-scale strategy as well.

For the second step and ALPC synthesis, alkylation of the previously produced LGPCs was performed through a Williamson-like reaction according to Batista *et al.*[31] This synthesis initiates with an alkoxide formation in an alkaline medium (NaOH as the alkali of choice) and is followed by its nucleophilic attack to an alkyl halide (either 1-bromododecane, 1-bromotetradecane, 1-bromohexadecane, or 1-octadecane), yielding a phosphocholine ether analogue as the final product (ALPC: **ALPC12**, **ALPC14**, **ALPC16**, and **ALPC18**).

To reduce waste and therefore adhere to a greener synthetic strategy, we investigated an alternative route toward ALPC formation. This methodology employed no halogenated solvent throughout the entire reaction sequence. Most importantly, the herein proposed alkylation reaction medium involved no solvent whatsoever; instead, its conduction was performed *via* reasonable heating (100 °C).

When compared to the literature precedents of lysolecithin alkaline O-alkylation,[31] which used dichloroethane as the solvent of choice and TBAHS as the phase-transfer catalyst, the solvent-free synthetic route herein described represents an environmentally friendlier and cheaper approach to ALPC production without significant loss of efficacy. Furthermore, this technique achieved completion with total consumption of the alkyl halide, requiring no additional agent (*e.g.*, ammonium hydroxide) to its quenching.

In fact, the reaction yields for ALPC16 and ALPC18 preparation were substantially higher under solvent-free conditions than those performed in an organochloride medium (86 and 83, and 36 and 34%, respectively). Conversely, ALPC12's and ALPC14's yields were significantly lower, with values that did not surpass 18% both under (non) and solvent-free conditions (Tables 3 and 4).

Because of the reduced yields for ALPC12 and ALPC14 formation in comparison to those observed for ALPC16 and ALPC18, further investigations on the effect of various phase-transference catalysts were conducted. TBAHS was then replaced with widely employed phosphonium or quaternary ammonium salts both under (non) and solvent-free conditions (maintaining Batista *et al.*[31] equivalents) to reach optimum conditions toward ALPC conversion.

Overall, both in (non) or solvent-free media, the ammonium salts (*i.e.*, CTAB, PTMTB, TBAB, and TBAI) showed greater efficiency than phosphonium salts (*i.e.*, TBPB and MTPB) (the former, with results that reached up to 85% of conversion, while the latter yielded up to 55%, as depicted in Tables 3 and 4). Among the ammonium catalysts, TBAB showed the highest values for ALPC12 and ALPC14 generation (*i.e.*, from 54 to 82%), whereas TBAI showed the lowest (*i.e.*, from 27 to 53%). TBAB's superiority over TBAI thus corroborates previous Williamson-like alkylations of which the maximum catalytic efficiency was obtained when "harder" counterion bromide was employed instead of "softer" iodide.[44,45]

Moreover, phosphonium ions' experimentally lower yields might be a result of their slight stability, especially under alkaline conditions.[46]

In regard to ALPC16 and ALPC18 formation, TBAB addition was also responsible for the greatest yields, especially

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under solvent-free conditions (87 and 84%, respectively). Analogously, for ALPC12 and ALPC14 production, the interaction between the majority of ammonium catalysts and the other reactants in dichloroethane had not resulted in significantly higher yields compared to those observed without the solvent.

Given the nature of this line of work, which consisted of repurposing crude soybean lecithins to produce LGPCs and ALPCs at a multigram scale, the herein depicted group of compounds (*i.e.*, a blend of 2-*O*-acyl glycerophosphocholines with either an OH- and/or *O*-alkyl moieties at *sn*-1, LGPC, and ALPC) was not purified. LGPC's and ALPC's components' structure formation and yields were thus confirmed through spectrometric analysis: IR, ^{13}C, ^{1}H NMR, and mass spectrometry.[29−31,33] These spectra are provided in Figures S2−S20—Supporting Information section.

3.1. Limnological Parameter Evaluation. The limnological parameters were monthly registered and compared with the literature's reporting requirements for these epibiotic species proliferation.[37,47−49] The results depicted in Table 1 demonstrated that, over the entire immersion assay duration, no variable seems to not follow the right criteria for the golden mussel's adequate development. For instance, throughout the whole experiment, the temperature levels (21.69−29.06 °C), which have been considered an essential factor for the mollusk proper growth, never surpassed the upper tolerance for adult individuals (35 °C) nor were found lower than 17 °C, the minimum required for *L. fortunei* reproduction.[48]

In terms of DO in the reservoir waters, no depletion of this vital component was registered (Table 1). For further analysis, a correlation between the water column's depth, temperature (Figure 3), and DO showed that while in March/2020, the

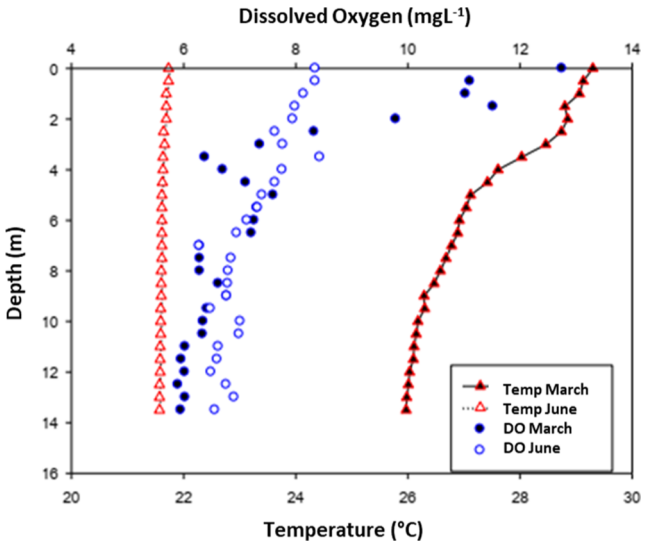

Figure 3. Vertical profiles of OD and temperature in the fish farming water column, in March and June 2020, in the Chavantes reservoir, Paranapanema river.

oxygen levels were stratified, on June/20, the oxygen profile became destratified. These results were already expected of this subtropical deep region of large time of residence. Additionally, the destratification is reasonably justified by the spontaneous "mixing" effect between the water column levels where the oxygen on the surface is transferred to deeper levels because of the reservoir's natural flow. Therefore, it is important to mention that this evaluation revealed that even the deepest levels of the water column were well oxygenated, allowing the macrofouling spread.

In conclusion, despite an expected reduction in larva development at naturally lower temperatures' periods, no environmental factor here measured is believed to have interfered negatively with the mussel's setting.

3.2. Using LGPCs and ALPCs as Antifouling Biocide Agents. As the formulation of assessed antifouling coating samples demanded a significant amount of LGPCs and ALPCs, the elected ALPCs for incorporation on the base paint of choice were those which had been produced in larger quantities (ALPC16 and ALPC18) in detriment of those more scarcely formed (ALPC12 and ALPC14).

After the 9 month period of immersion, as depicted in Table 2, the coated nets with the smallest population of juvenile and adult golden mussels were those covered with **ALPC16** 0.1 g·mL^{-1}; LGPC 0.1 g·mL^{-1} + **ALPC18** 0.05 g·mL^{-1}; and LGPC 0.1 g·mL^{-1} dissolved in methanol. On these net samples, no observed individual was smaller than 4 mm, which can be a sign that the mussels were developed elsewhere (*i.e.*, instead of over the coated-net). These results indicate that the aforementioned formulations exhibited a promising antiadhesive, therefore antifoulant, behavior toward the golden mussel.

The results also indicate that concentrations higher than 0.05 g·mL^{-1} might be necessary for antifouling formulations of biocide **ALPC16**. A promising formulation is herein presented at 0.1 g·mL^{-1} of **ALPC16** (without the co-additive LGPC), which showed an inhibition of the number of settled individuals 6.5 times higher than the formulation of **ALPC16** 0.05 g·mL^{-1} + LGPC 0.1 g·mL^{-1}.

Unlike **ALPC16**, **ALPC18** reached its highest antifouling capacity at concentrations lower than 0.1 g·mL^{-1} (*i.e.*, **ALPC18** at 0.05 g·mL^{-1}). When added to LGPC (0.1 g·mL^{-1}), the ether analogue **ALPC18** thus showed a synergic effect in the LGPC biocide activity. Combined with LGPC, ALPC18 was able to reduce even more the density of individuals settled on the coated-net promoted by the LGPC formulation alone.

On the other hand, on the net coated with LGPC 0.02 g·mL^{-1} on linseed oil as the diluent, the highest biofouling development was observed. This might have been provoked by a negative role of the linseed oil on the formulation's biocide capacity: in lieu of inhibiting the organisms' settlement, it could have acted as a substrate for their growth.

PVC fish farming coated net was the least active material regarding *L. fortunei*'s inhibition, showing the greatest individual density, even surpassing the control values. These results thus demonstrate that PVC as an antiadhesive fish farming net component, besides possessing a higher overall cost and lower resistance to collisions, is not suitable for the golden mussel's settlement control.

Moreover, the net that exhibited the most diverse algal community was coated with LGPCs at 0.1 g·mL^{-1}, which might be a sign of the little algaecide role promoted by the substance, most specifically, against green algae.

4. CONCLUSIONS

The golden mussel invasive spread in South America has quickly become a major concern to multiple economic sectors and to this day has not found an efficient mitigating strategy that does not endanger the aquatic environment more or at a similar level to this non-native mollusk. Therefore, the GPLs herein described, LGPCs and their 1-*O*-alkyl-derivatives

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ALPCs (1-*O*-hexadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine, **ALPC16**, and 1-*O*-octadecyl-2-*O*-acyl-*sn*-glycero-3-phosphocholine, **ALPC18**, Table 2), present themselves as a great environmentally friendly alternative—due to their natural occurrence, low toxicity potential, and bioaccumulation[30]—for the combat of *L. fortunei*. Furthermore, when compared to currently available commercial antifoulants, LGPC and ALPC16, as metal and halogen-free compounds, have already been proven less menacing than "booster biocides" such as Irgarol 1051 and Diuron.[30]

With special attention to the fish farming industry, this work's results elaborate on ALPC and LGPC activity against the golden mussel when applied to aquaculture nets and revealed that both LGPCs and ALPCs (**ALPC16** and **ALPC18**) are capable of inhibiting this organism's growth. This important discovery thus ratifies and deepens our previous investigations on LGPC and APLC16 antifouling abilities—which have shown so far a greater anti-epibiotic proliferation than commercial antifoulants Econea and copper-sulfate[31]—and still holds an immense promise as biocide-coating additives (https://lasape.iq.ufrj.br/eng/tinta_antincrustante.html).

Concerning LGPC and ALPC preparation herein depicted, the most suitable for an industrial-scale synthetic route for LGPC generation was the one with NaOH as its alkaline agent. Meanwhile, for ALPCs, the solvent-free procedure with TBAB as the phase-transfer catalyst proved to be the most efficient approach.

Overall, these methods serve the purpose of a low-cost, feasible, and sustainable synthetic pathway. Moreover, it availed the copious supply of an industrial crude side product (*i.e.*, the substantially cheap soybean lecithins) as its starting material and was able to produce at significant yields the desired products (LGPC and ALPC12-APLC18) through a facile process, with reduced waste generation—for example, without pollutant halogen solvent waste.

From a future perspective, ALPCs' "shorter" *O*-alkyl chains such as ALPC12 and ALPC14, antifouling activity must be evaluated to ensure their promising ability as non-toxic and sustainable non-foulant agents.

Finally, it is possible to conclude that the strategy of LGPC and ALPC use as antifoulants—from their production to their interaction with the aquatic environment—is an affordable and greener mitigation option for the golden mussel dissemination. LGPCs and ALPCs also possess great potential as biocide agents for the combat of other (micro- and macro-) foulants' proliferation.[25,41] ALPC and LGPC coatings may become an option for *L. fortunei* and other foulers' control at obstructed hydroelectric pipelines as well.

Given these compounds' remarkable antiproliferative capabilities, other (alkyl)glycerophospholipids may serve as a promising class of substances to be explored in the future for the development of more and better "eco-friendly" biocide alternatives.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05645.

LGPC and ALPC structures' identification, including ^{1}H and ^{13}C NMR, IR, and LC/MS spectra, and visual registers and photographs of immersion assay follow-up (PDF)

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Author Contributions

This work is a collaborative effort where all authors contributed to the conception, the writing, and the review of the paper.

Notes

The authors declare no competing financial interest.

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■ ADDITIONAL NOTE

[a]LAZZURIL-Sherwin Williams automotive paint was selected as a base for the synthesized biocides' incorporation, due to its low cost compared to commercial antifouling paints used to inhibit the marine biofouling process. Automotive paints as small boats' coatings are a popular measure employed by local fishermen in the Araruama lagoon, state of Rio de Janeiro, Brazil.

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