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Phytoplankton pigment retrieval using remote sensing: methods, validation and evidence readiness

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Abstract

Optical observations of phytoplankton pigments can extend aquatic monitoring beyond bulk biomass, although their value depends on how spectral information, reference measurements and validation are connected. This review synthesizes pigment targets, observation systems and retrieval approaches in 107 studies published from 2020 to August 2025 and evaluates their evidence through six cumulative readiness levels. Chlorophyll-a and phycocyanin dominated the literature, while accessory pigments provided more detailed, often shared associations with community composition. Hyperspectral measurements supported spectral decomposition and multipigment estimation, whereas multispectral satellites provided repeated spatial coverage. Across these architectures, performance depended on optical conditions, measurement correspondence and the domain represented by validation. Structured independent validation was the first unmet requirement in 54.4% of direct retrieval configurations. No configuration met the operational criteria of the main rubric, although joint relaxation of transfer and implementation requirements changed this outcome. Cross-lake experiments and satellite applications showed that favourable predictive association could coexist with substantial concentration error, analytical variability or sensitivity to atmospheric correction. The findings support development that combines improved pigment discrimination with traceable analytical references and evaluation across relevant environments. Matching field sampling to the spatial and temporal support of

image products, while maintaining quality control and uncertainty characterization, is necessary to translate retrieval advances into reliable environmental monitoring.

Keywords: Chemotaxonomy; Bio-optics; Radiometry; Cyanobacteria; Eutrophication

Introduction

Phytoplankton regulate primary production, oxygen dynamics and nutrient cycling in aquatic systems, while their rapid responses to light, temperature and nutrient availability make them useful indicators of environmental change. Chlorophyll-a has consequently become a central variable in the assessment of trophic conditions and a widely used proxy for phytoplankton biomass, although its concentration alone cannot describe the composition or physiological state of an assemblage. Spatially resolved pigment observations extend this information by revealing bloom extent, patchiness and gradients that are difficult to represent through discrete sampling, providing a basis for directing field surveys and interpreting changes in water quality (Carlson 1977; Topp et al. 2020; McKibben et al. 2024).

The ecological information contained in a pigment assemblage becomes particularly relevant when environmental pressures alter community structure without producing a proportional change in total biomass. Nutrient enrichment, changes in water-column stability and climatic variability can modify the relative abundance of phytoplankton groups, with consequences for food webs, oxygen demand and the occurrence of harmful blooms (Boegehold et al. 2023; Pérez-González et al. 2023; Molner et al. 2024). Accessory chlorophylls, carotenoids and phycobiliproteins help distinguish some of these responses because their distribution differs among organisms; phycocyanin, for example, provides information about many cyanobacterial assemblages that is not available from chlorophyll-a alone. Their interpretation nevertheless depends on marker specificity and on the biological processes that determine pigment abundance within cells (Pyo et al. 2022; Lomas et al. 2024).

The relationship between pigmentation and the remotely observed signal is mediated by absorption, scattering and the optical contribution of the surrounding water. Pigment packaging alters absorption per unit concentration, while photoacclimation changes cellular pigment content and ratios in response to the light environment; the same concentration can therefore produce different optical expressions across communities or environmental conditions (Huan et al. 2021; Lain et al. 2023). Coloured dissolved organic matter (CDOM) and non-algal particles (NAP) add further spectral overlap, particularly in the blue-green region where several accessory pigments absorb. These interactions

explain why a relationship that is useful within one water body may require additional information when applied to a different optical regime, and why the recovery of a pigment concentration is a distinct problem from attributing that concentration to a taxonomic group (Castagna et al. 2021; Kramer et al. 2022).

Observation systems offer different ways of resolving these relationships. Multispectral satellites support repeated, spatially extensive measurements, but their band configuration and pixel size constrain the information available in small or heterogeneous waters. Hyperspectral instruments provide contiguous narrow bands that can separate overlapping features and support decomposition of the optical signal, while proximal radiometry and laboratory measurements allow specific mechanisms to be examined under more controlled conditions (Begliomini et al. 2023; Grunert et al. 2025). Combining these sources is scientifically valuable because laboratory pigment measurements establish analytical references, field observations characterize natural-water spectra and imagery places the resulting information in a spatial context. The benefit depends on correspondence among the measured target, the optical sampling volume and the spatial and temporal support of the image product. These relationships connect pigment retrieval to spatial measurement: a concentration observed in a bottle, a radiometer footprint and an image pixel need not represent the same water mass or acquisition interval.

Retrieval methods have developed along similarly complementary lines, ranging from empirical band relationships and physical inversion to machine learning and spectral unmixing. These approaches can represent different aspects of the bio-optical relationship, but a high correlation or coefficient of determination does not indicate how much concentration error remains, whether the analytical reference is comparable among datasets, or whether performance persists when an entire lake or region is withheld (Werther et al. 2022; Salmi et al. 2024; Naik et al. 2025). The distinction is especially important for environmental applications, where an algorithm must operate under changing optical conditions and produce information at an error level appropriate to the intended decision. Progress in spectral discrimination therefore needs to be considered together with the evidence supporting validation, transfer and repeated implementation.

Previous reviews establish an important basis for this assessment. Fang et al. (2025) synthesize phycocyanin optics, retrieval models, sensors and global sampling coverage, while Wu et al. (2025) examine the accuracy of remotely observed freshwater bloom indicators. Goodrich et al. (2026) explicitly assess validation evidence in 122 Sentinel-2 chlorophyll-a studies using NASA data maturity concepts, linking limited spatial coverage and reporting to early maturity. Evidence maturity is therefore already an established

concern in aquatic remote sensing. The contribution pursued here is a pigment-oriented specification that separates analytical-reference validity from retrieval error and test-domain independence, retains ecological interpretation as a parallel assessment, and links these properties through cumulative requirements across laboratory, proximal and image-based observations. Table 1 places this contribution alongside the related reviews.

Table 1 *Related reviews and the focus of this evidence synthesis*

Review	Scope	Assessment focus
Fang et al. (2025)	Phycocyanin in inland waters	Optics, retrieval models, sensors and global sampling coverage
Wu et al. (2025)	Freshwater cyanobacterial blooms	Monitoring accuracy across indicators, sensors and methods
Goodrich et al. (2026)	Sentinel-2 chlorophyll-a; 122 studies	Validation evidence assessed using NASA data maturity concepts
This review	Multiple pigments and observation chains	Separate analytical, retrieval and transfer evidence; parallel ecological interpretation; cumulative criteria and sensitivity analysis

This review examines how pigment targets, observation systems, retrieval methods and validation designs shape the information available for aquatic environmental assessment. Its objectives are to characterize the methodological and environmental coverage of the selected pigment literature, evaluate the optical and ecological meaning of the main products, and identify the evidence supporting their use beyond the calibration domain. An evidence-readiness assessment complements the methodological synthesis by distinguishing spectral feasibility, quantitative retrieval, independent evaluation, cross-system validation and operational demonstration. This perspective connects advances in pigment sensing with the conditions under which their resulting spatial products can support reliable monitoring.

Methodology

Literature search and study selection

The review covered English-language research articles and data papers published from 2020 to 14 August 2025. Searches were conducted in Scopus, Web of Science and ScienceDirect on 14 August 2025. The search combined four concept groups

representing optical observations, phytoplankton organisms, pigment compounds and aquatic environments. For Scopus and Web of Science, the conceptual expression was (spectroscopy OR spectral* OR absorbance OR absorption OR reflectance OR "remote sensing") AND (phytoplankton OR cyanobacteria) AND (pigment* OR carotenoid* OR phycobiliprotein*) AND water. ScienceDirect used the interface-adapted expression (spectroscopy OR absorbance OR absorption OR reflectance OR "remote sensing") AND (phytoplankton OR cyanobacteria) AND pigment AND water. Document-type and publication-period filters were applied, with title, abstract and keyword restrictions where supported.

Chlorophyll-a was not included as a standalone search term because the review emphasized diagnostic pigments, phycobiliproteins and taxonomically informative applications beyond conventional biomass estimation. Chlorophyll-a studies retrieved by the broader terms were retained when they met the eligibility criteria. Eligible publications addressed remote or proximal optical sensing of aquatic pigments, or analytical, ecological and optical work directly relevant to its development and validation. Laboratory studies were included when they supported pigment detection, reference measurements or interpretation of optical signals; investigations limited to chemical or biological properties without an applicable optical-sensing component were excluded.

Records were normalized and deduplicated using Mendeley Reference Manager, followed by title/abstract screening and full-text assessment. Study selection was performed by one researcher and checked by a second, with the selection procedure documented according to PRISMA 2020 principles (Page et al. 2021). The resulting evidence base comprised 107 publications. Later reviews and service documentation were used to contextualize this evidence, including literature available in October 2026; they were not added to the publication-period counts or readiness denominators. Bibliographic information, study settings and methodological characteristics for the 107 included publications are provided in Online Resource 1, and the selection accounting is presented in Supplementary Fig. S1.

Data extraction and analytical units

For each publication, we extracted its year, aquatic environment, geographic setting, pigment or related optical target, observation platform, sensor, spectral architecture, retrieval approach, reference measurement and principal findings. Primary environment was assigned once per study, whereas pigments, instruments, models and countries were recorded as non-exclusive categories. Instruments were distinguished according to their role in observation or validation, so that laboratory chromatography was treated as an analytical reference rather than a remote sensor. Retrieval approaches were

organized into empirical/statistical, physical/semi-analytical and machine-learning families, with decomposition, unmixing and hybrid processing identified as treatments that could overlap those families.

Bernardo T. A. Souza extracted the study information and classified the evidence units. Caio C. S. Mello independently classified the same evidence units against the source publications using the same assessment framework. The two sets of classifications were subsequently compared, and disagreements, including those concerning configuration boundaries and transferability, were resolved through discussion and consensus.

Because one paper can evaluate several targets or validation configurations, readiness was assessed at the level of publication × target × materially distinct retrieval/validation configuration. Separate units were assigned where the target, analytical reference, observation chain or validation design differed in a way that could affect the evidence assessment; minor algorithm variants evaluated under the same structure were retained together. Units were allocated to three tracks: direct pigment retrieval, analytical/optical support, and ecological/community inference. Only direct retrieval units entered the cumulative readiness classification, while the other tracks informed the interpretation of reference uncertainty, optical mechanisms and biological meaning.

Evidence-readiness assessment

The framework evaluates seven domains: spectral observability (SO), retrieval evidence (RE), analytical validity (AV), environmental robustness (ER), transferability (TR), ecological interpretability (EI) and operational implementation (OI). SO assesses whether the observations contain information relevant to the target; RE addresses performance characterization; and AV concerns the suitability, independence and reliability of the reference measurement. ER evaluates how retrieval behaves across optical or environmental variability, TR examines separation between calibration and evaluation domains, and OI considers repeatability, quality control and monitoring use. EI addresses the additional evidence required when pigment products are interpreted as taxonomic or community information. SO, RE, AV, ER and EI use ordinal scores from 0 to 3, while TR and OI range from 0 to 4; the complete definitions and scoring rules are provided in Online Resource 2.

Six cumulative readiness levels are assigned through the requirements summarized in Fig. 1. R1 requires a plausible or demonstrated target-relevant optical basis (SO ≥ 1), and R2 additionally requires quantitative retrieval development (RE ≥ 1). R3 requires evaluation on held-out or distinct observations with appropriate target-error characterization (RE ≥ 2), a fit-for-purpose reference (AV ≥ 1), and reference independence coded as Yes or Partial. R4 introduces structured temporal, spatial,

campaign or equivalent independence (TR ≥ 2). R5 additionally requires cross-system testing (TR ≥ 3), explicit evaluation of environmental heterogeneity (ER ≥ 2), and an observation chain extending beyond laboratory absorbance to at least natural-water reflectance. R6 requires the R5 conditions together with a reproducible, quality-controlled and uncertainty-aware monitoring workflow (OI ≥ 3; RE ≥ 3; AV ≥ 2).

The cumulative structure prevents performance in one domain from compensating for missing evidence in another. Reference independence is therefore assessed separately from test-set independence, and spectral reconstruction error is distinguished from error in the target concentration. Ecological interpretability is evaluated alongside the retrieval pathway because a pigment concentration can be valid without supporting a taxonomic claim, whereas a community product requires evidence for its biological endpoint. No universal accuracy threshold is imposed: readiness describes the completeness of the evidence chain, while fitness for use depends on the target, concentration range and monitoring purpose.

The rubric was refined using a purposive pilot of 15 studies covering different pigments, settings, observation systems and validation designs. Each unit retained its study identifier, source location and scoring rationale. Cases requiring interpretation of the observation chain or validation design were adjudicated before the final stage was assigned. Each configuration received the highest cumulative stage for which the required evidence was judged to be met. When published information did not establish a requirement, it was coded as not demonstrated; this designation does not establish that the corresponding procedure was absent from the original research.

Synthesis and performance interpretation

Readiness distributions were calculated by evidence unit and by publication. At publication level, each study contributed once at the maximum stage reached by its direct retrieval configurations; target-level profiles retained the individual units. The first unmet requirement was defined as the earliest cumulative condition preventing progression, identifying the next evidence needed without counting later deficiencies repeatedly. Relationships with targets and spectral architectures were examined descriptively, including an influence check for multipigment publications contributing many units to a single category.

To distinguish evidence frequency from the order of the cumulative gates, we also calculated the proportion of all eligible direct retrieval units satisfying each component requirement separately. These marginal frequencies permit overlapping unmet requirements and therefore do not sum to the number of units. Sensitivity analyses retained the main rubric and changed specified assumptions: the R5 transfer threshold

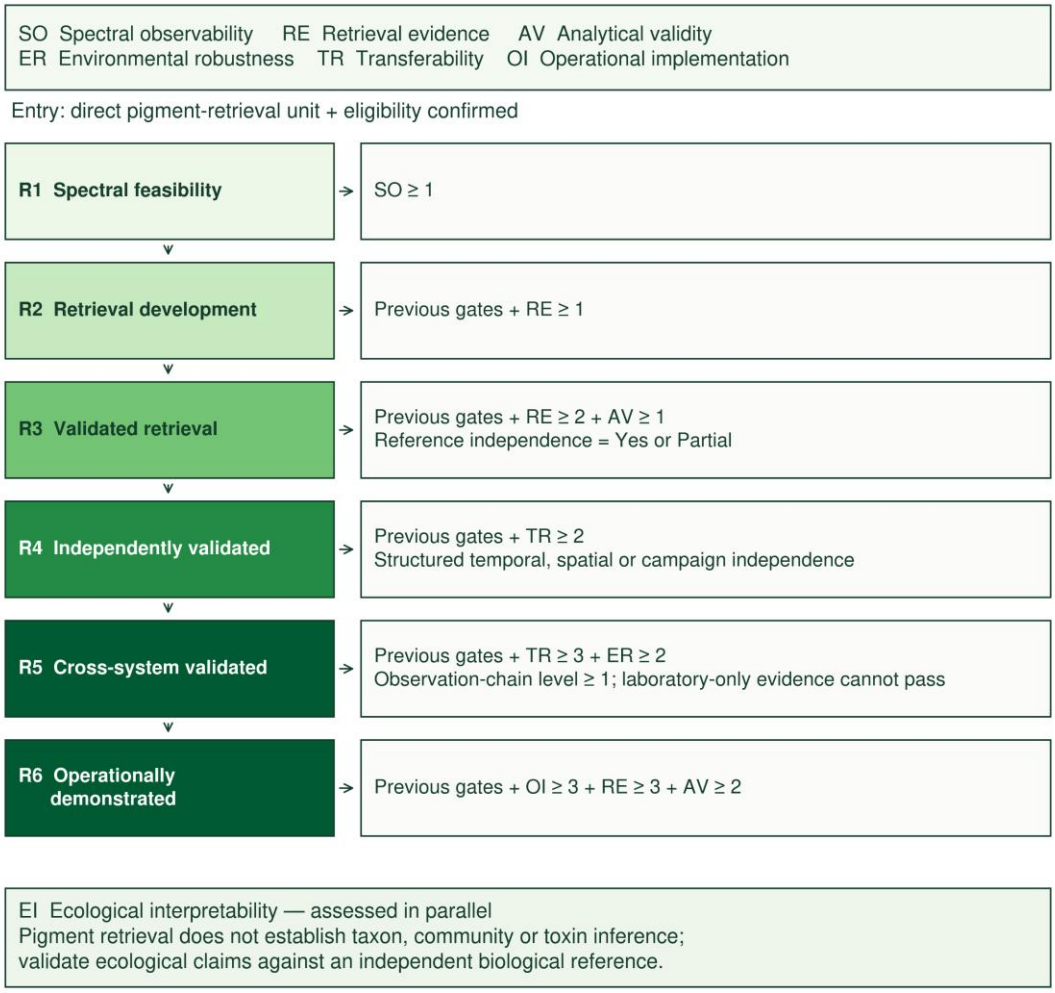
from TR ≥ 3 to TR ≥ 2; the R6 implementation threshold from OI ≥ 3 to OI ≥ 2; both thresholds jointly; and reference independence restricted to Yes rather than Yes or Partial. All other predicates remained fixed. The relaxed thresholds test whether structured within-domain evaluation and repeated application would change the distribution; they do not carry the cross-system and operational meanings assigned by the main rubric. Results were summarized by unit and by maximum stage per publication.

Performance was interpreted in relation to the actual endpoint, concentration range, analytical reference, measurement context and validation partition. Pearson correlation, explained variance, absolute error, percentage differences and multiplicative metrics were retained in their original forms, and simulated sensor configurations were distinguished from imagery-derived products. Differences in targets, error definitions and study designs precluded pooled meta-analysis. Numerical comparisons therefore describe specific experiments or evidence profiles rather than a common ranking of algorithms, pigments or sensors.

Use of AI-assisted technologies

During the preparation of this work, the authors used generative AI and AI-assisted technologies (ChatGPT and Gemini) in order to improve the English language and semantic clarity, assist in summarizing the reviewed literature, and aid in constructing the code to generate the graphs. The authors confirm that AI was strictly not used to write the core manuscript, interpret data, or draw scientific conclusions. After using these tools, the authors thoroughly reviewed and edited the content as needed and take full responsibility for the final content of the publication.

***Fig. 1** Evidence-readiness framework for phytoplankton pigment retrieval*



Results and discussion

Research landscape and environmental coverage

The 107 publications were distributed across 2020 (15 studies), 2021 (14), 2022 (26), 2023 (23), 2024 (12) and 2025 (17, through the publication cutoff). Lakes were the most frequent primary setting, with 25 studies, followed closely by oceans (24) and coastal/estuarine waters (19); reservoirs accounted for 11 studies, mixed aquatic systems for ten, other inland waters for seven, and rivers and laboratory/synthetic settings for five each, with one setting insufficiently specified (Fig. 2a). Clearly inland categories represented 48 studies (44.9%), rising to 58 (54.2%) when mixed settings containing inland components were included. The distribution places inland-water applications within a wider bio-optical literature that also provides oceanic pigment datasets, controlled optical experiments and analytical support.

This environmental breadth is important because the conditions that favour pigment discrimination differ among systems. Reservoir and lake applications often concern cyanobacterial blooms, drinking-water surveillance and fine-scale heterogeneity, while marine studies supply broader pigment assemblages and applications to phytoplankton functional types or community structure (Xi et al. 2020; Kramer et al. 2022; Lomas et al. 2024). The same pigment can therefore be evaluated for different purposes: a chlorophyll-a product may support trophic assessment in a reservoir, act as a normalization variable for marine accessory pigments, or provide a reference against which fluorescence or absorption is examined. These roles explain why descriptive pigment occurrence and direct retrieval evidence have different frequencies.

Geographic coverage was concentrated in a limited set of countries. Among studies with identifiable national locations, the United States appeared in 14, China in 11, South Korea in seven, Canada in six and Brazil in five; Belgium and Italy each appeared four times. Multi-country studies contributed to more than one location, while regional and global datasets extended beyond national categories. The geographic imbalance matters for transfer because water colour, suspended particles, dissolved material and community composition vary across environments; a geographically broad training dataset can improve coverage, but its contribution to generalization depends on whether evaluation actually withholds entire systems or regions. Environmental representation and validation independence thus need to be considered together when interpreting the applications represented by the corpus.

Pigment targets and ecological interpretation

Chlorophyll-a and phycocyanin were the most frequent descriptive targets, appearing in 63 and 37 publications, respectively, and together accounting for 100 of 156 pigment or related-parameter tags (64.1%). Fucoxanthin occurred in eight publications, chlorophyll-b in seven, chlorophyll-c and zeaxanthin in six each, 19′-hexanoyloxyfucoxanthin in five, and alloxanthin and peridinin in four each (Fig. 2b). Their unequal coverage reflects both the broad use of biomass indicators and the more specialized analytical and spectral requirements of accessory-pigment applications. Table 2 summarizes the principal associations relevant to interpreting these targets, including the distinction between a pigment occurring in a group and a retrieved concentration providing reliable evidence of that group's abundance.

Fig. 2 *Aquatic environments (a) and selected pigment targets (b) in the 107-study evidence base*

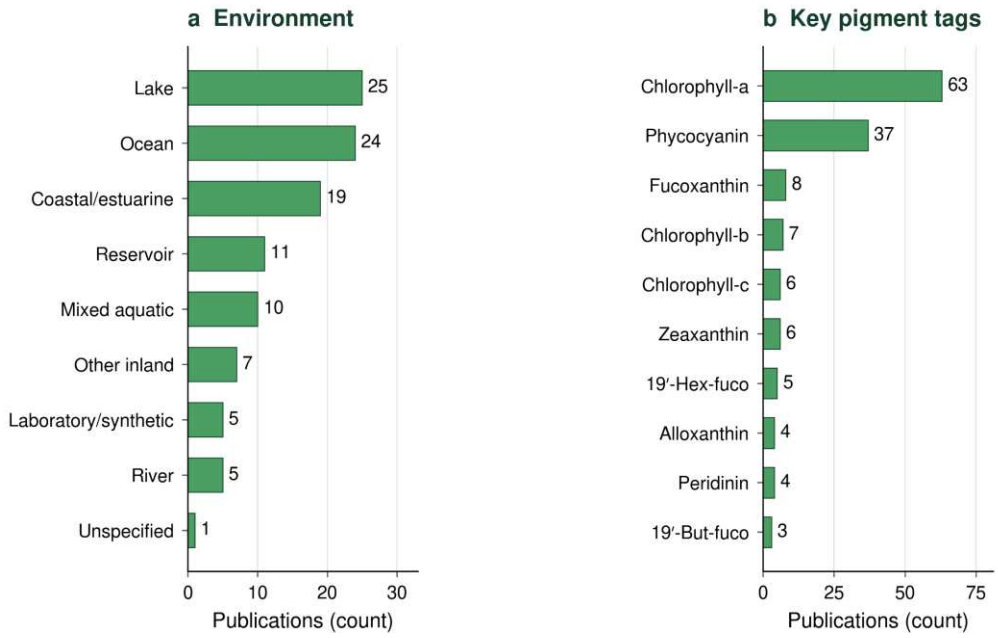


Table 2 *Principal pigment associations and limits on ecological interpretation*

Pigment or pool	Association and interpretation	References
Chlorophyll-a	Broad phytoplankton indicator; not group-specific or a fixed conversion to carbon biomass. Define whether the measured pool includes divinyl forms or derivatives.	Kramer et al. (2022); Lomas et al. (2024)
Fucoxanthin	Diatoms and other fucoxanthin-bearing groups, including several haptophytes/chrysophytes. Interpretation depends on assemblage context.	Meler et al. (2020); Castagna et al. (2021)
Chlorophyll-c forms	Several lineages, including diatoms, haptophytes, dinoflagellates and cryptophytes. c1/c2/c3 are not a uniquely diatom-specific pool.	Castagna et al. (2021); McKibben et al. (2024)
Monovinyl / divinyl chlorophyll-b	Monovinyl forms are associated with chlorophyll-b-bearing green lineages and others; divinyl forms have Prochlorococcus-associated interpretation.	Kramer et al. (2022); McKibben et al. (2024)
Divinyl chlorophyll-a	Prochlorococcus-associated marine cyanobacteria rather than a general marker for every cyanobacterial lineage.	Kramer et al. (2022)
19′-But-fuco	Pelagophytes and selected haptophyte/chrysophyte groups; a shared pigment requiring joint interpretation.	Meler et al. (2020); Kramer et al. (2022)
19′-Hex-fuco	Many haptophytes; occurrence and abundance vary among members and conditions.	Kramer et al. (2022); Castagna et al. (2021)

Pigment or pool	Association and interpretation	References
Alloxanthin	Cryptophyte-associated information; group abundance requires evaluation against a community reference.	Meler et al. (2020); McKibben et al. (2024)
Peridinin	Peridinin-containing dinoflagellates. Absence of the pigment does not exclude all dinoflagellates.	Meler et al. (2020); McKibben et al. (2024)
Phycocyanin	Many cyanobacteria, particularly phycocyanin-rich assemblages. Does not independently quantify species identity or toxin concentration.	Pyo et al. (2022); Legleiter et al. (2022)
Zeaxanthin	Cyanobacteria and several green lineages; use in conjunction with pigment patterns and ecological context.	Meler et al. (2020); Pyo et al. (2022)
Lutein / neoxanthin / violaxanthin; myxoxanthophyll	The first three occur in several green lineages; myxoxanthophyll is cyanobacteria-associated. Physiological function and taxonomic occurrence are not interchangeable.	Kramer et al. (2022); Pyo et al. (2022)

Associations are indicative and non-exclusive. They do not independently establish optical separability or a validated group-abundance product. 19′-But-fuco and 19′-Hex-fuco denote 19′-butanoyloxyfucoxanthin and 19′-hexanoyloxyfucoxanthin.

Chlorophyll-a provides a common quantitative reference across environments because it captures a major component of phytoplankton absorption and is routinely measured by several established methods. Its interpretation remains sensitive to which analytical pool is reported, including monovinyl and divinyl forms or degradation products, and to physiological variation in chlorophyll relative to cellular biomass. Phycocyanin adds information about many cyanobacterial assemblages through its absorption in the orange-red region near 620 nm, but its optical contribution can be small relative to chlorophyll-a or other constituents in mixed communities (Pyo et al. 2022; Yuan et al. 2025). A model may consequently achieve useful pigment prediction by exploiting local covariance even when the target-specific spectral contribution is weak, creating a reason to evaluate observability separately from predictive association.

Accessory pigments can make that distinction more demanding because their absorption overlaps with other pigments and with CDOM. Fucoxanthin is informative about diatoms in many assemblages, yet it also occurs in other groups, including several haptophytes and chrysophytes, so a fucoxanthin map cannot be translated into a unique diatom map without additional ecological constraints (Meler et al. 2020; Castagna et al. 2021). Chlorophyll-c forms likewise occur across several lineages, and combining c1, c2 and c3 can erase differences relevant to community interpretation. By contrast, separating monovinyl from divinyl chlorophyll-b preserves the distinction between green-lineage-

associated information and the Prochlorococcus-associated divinyl pigments represented in marine datasets (Kramer et al. 2022). Analytical definition is therefore part of ecological interpretation, rather than a detail that can be added after retrieval.

The limitations of marker specificity are illustrated by Castagna et al. (2021), who examined chlorophyll-c3-related signals in the Belgian Coastal Zone and showed that an apparently useful bloom indicator could also respond to co-occurring organisms with similar pigmentation. In Chesapeake Bay, McKibben et al. (2024) found support for cryptophyte and/or cyanophyte differentiation, whereas diatom and dinoflagellate discrimination remained inconclusive. Their comparison with CHEMTAX-derived community fractions evaluated correspondence between optical and pigment-based reconstructions, providing ecological evidence while retaining assumptions associated with the pigment reference itself. These examples show how informative community products can emerge from shared markers, but also why the appropriate validation endpoint is a group fraction or composition measure rather than the concentration of one diagnostic pigment alone.

Photoacclimation and pigment packaging add a further source of variability even when marker identity is well established. Cells adjust pigment abundance and ratios to the available light, and intracellular self-shading changes the absorption expressed per unit concentration; relationships calibrated under one community or light regime may therefore respond differently elsewhere (Huan et al. 2021; Lain et al. 2023). The ecological value of accessory pigments is greatest when several markers, optical information and environmental context are interpreted jointly, as opposed to assigning each pigment an invariant biomass conversion. This supports the use of a parallel ecological-interpretability domain: the retrieval of a concentration and the inference drawn from it can be assessed at their own evidential levels without dismissing either contribution.

Observation systems and the spatial measurement chain

The literature contained 172 non-exclusive sensor, platform and validation-instrument tags. Sentinel-3 OLCI was the most frequently recorded spaceborne instrument (24 studies), followed by MODIS (15) and Sentinel-2 MSI (13), while laboratory UV-visible spectrophotometry and HPLC/UHPLC each appeared in 13 studies. Proximal instruments, including ASD/FieldSpec and TriOS RAMSES, were recorded alongside UAV, airborne and other satellite observations, reflecting frequent combinations of measurement scales. These combinations serve different functions: field radiometry characterizes the optical signal, chemical analysis anchors pigment concentration and

imagery identifies spatial structure. Their integration is most informative when the validation target is traced through the entire sequence.

Spatial and spectral resolution impose different constraints on that sequence. Sensors with finer pixels can better represent narrow reservoirs and separate water from adjacent land, but a limited band configuration may not resolve the features required for accessory-pigment recovery. Ocean-colour sensors provide more targeted spectral sampling and repeated regional coverage, although coarser pixels can average bloom patches, shoreline contamination and different optical water types within a single observation (Beal et al. 2024; Fu et al. 2023). The resulting trade-off is not simply between more bands and smaller pixels: retrieval also depends on the fraction of water represented by each pixel, the relationship between the sampled water volume and the optical footprint, and whether the target varies over the acquisition interval.

Hyperspectral instruments address the spectral part of this problem by supporting derivatives, feature analysis and decomposition of overlapping absorption, particularly for multipigment targets. Spaceborne PRISMA and other imaging spectrometers extend these measurements to spatial products, while airborne and UAV systems can examine heterogeneity at scales closer to field sampling (Pyo et al. 2022; Begliomini et al. 2023; Fabbretto et al. 2024). Yet narrow sampling alone does not determine how much target information is recoverable: spectral response, band width, signal-to-noise ratio and processing all affect the effective information content. Matthews et al. (2023), using synthetic data, found that approximately nine optimally positioned bands could approach full-spectrum performance for their tested cyanobacterial products, indicating that band configuration can be as important as band number within a defined optical problem.

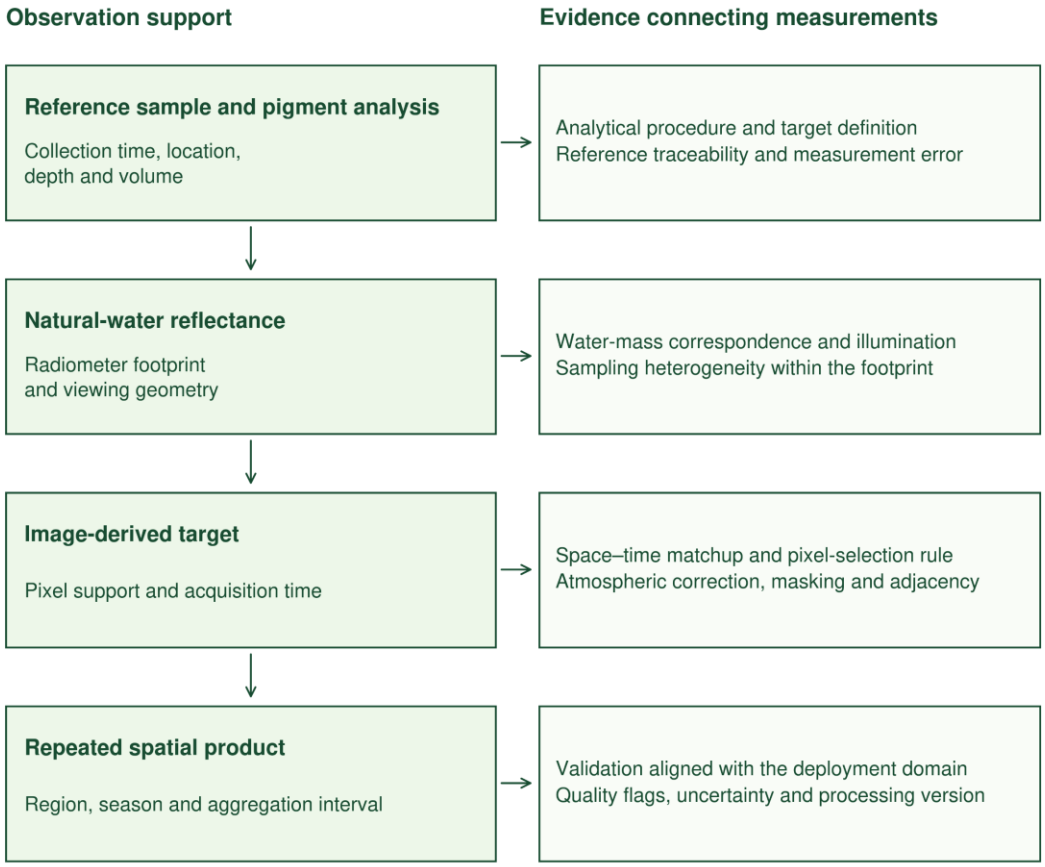
The distinction between spectral feasibility and a validated spatial product becomes clear in the PRISMA application of Begliomini et al. (2023). Their Billings Reservoir dataset contained 115 phycocyanin samples, while satellite evaluation used 24 radiometric matchups and 13 independent pigment-map matchups. The standard Level-2C reflectance product achieved errors below 20% over 500-700 nm under the study's log-based metric, and the PRISMA/random-forest phycocyanin product achieved 45% error on the independent orbital comparison. These results demonstrate a complete mapping application while also identifying separate sources of radiometric and pigment error; the error in reflectance cannot be read as an equivalent error in concentration. The study's multiplicative mean absolute error is calculated in logarithmic space and reported as percentage deviation, so these values express relative rather than arithmetic concentration error.

Laboratory and proximal observations remain essential to interpreting such satellite results rather than merely preceding them in technological development. Concentrated-

sample absorbance can isolate spectral mixtures and test endmember models, while natural-water reflectance introduces scattering, vertical structure and interference from dissolved or suspended material. Laboratory transfer between lakes can therefore be rigorous within its measurement domain without establishing performance for a satellite image, and a satellite map can provide spatial coverage without resolving the source of each pigment relationship. Maintaining this distinction makes the separate contributions of analytical support, spectral experiments and remote retrieval visible within the same evidence base.

The correspondence between field and image measurements consequently belongs within the validation design rather than being treated only as a processing detail. A discrete sample represents a collection location, time and depth interval; proximal radiometry integrates a viewing footprint and illumination geometry; an image product integrates the sensor response over a spatial support and acquisition time. Advection, vertical stratification, shoreline adjacency and subpixel patches can separate these observations even when their coordinates appear close. A defensible matchup therefore reports the collection depth, time difference, pixel-selection rule and spatial variability around the sampling location, together with masking and atmospheric-correction choices. Where the intended output is a seasonal or regional map, the test partition must additionally preserve those temporal or geographic boundaries. These correspondences connect analytical reference quality to validation of the final spatial product (Goodrich et al. 2026; Zolfaghari et al. 2023), as summarized in Fig. 3.

Fig. 3 *Spatial and temporal correspondence along the pigment-observation chain*



Retrieval approaches and the meaning of reported performance

Empirical and statistical relationships were the most frequent model tags (30 studies), followed by neural-network/deep-learning labels (19) and semi-analytical/physically based approaches (18). Random forests occurred in eight studies, convolutional networks and gradient boosting in five each, support-vector methods in four, and spectral decomposition/unmixing in five. The methodological relationships summarized in Table 3 explain why these labels overlap: a convolutional network belongs to a broader learned-model family, whereas spectral decomposition can be embedded in either a physical or a learned retrieval. The main distinction is how each approach represents the optical mixture and how its parameters are constrained by the available observations.

Table 3 *Retrieval families and complementary spectral treatments*

Family or treatment	Implementation	Interpretive and validation requirement
Empirical/statistical	Ratios, indices, regressions and splines	Compact calibration; the optical relationship must remain stable across the domain of application.

Family or treatment	Implementation	Interpretive and validation requirement
Physical / semi-analytical	Inversion of absorption, scattering and reflectance	Optical interpretation depends on parameterization and separability of mixed constituents.
Machine / deep learning	Forests, support-vector methods, boosting, neural and convolutional models, mixture density networks	Nonlinear relationships and conditional error can be represented; environmental covariance requires independent evaluation.
Decomposition / unmixing	Gaussian features, spectral endmembers and learned mixture models	A cross-cutting treatment; spectral reconstruction and concentration error are distinct endpoints.
Hybrid configurations	Optical-water-type routing and physical constraints combined with learned retrieval	Both routing and target estimation require testing on the deployment domain.

Categories overlap: a convolutional model is part of a wider neural-model family, while decomposition can be embedded in physical or learned retrieval. They are not additive study totals.

Empirical band relationships remain useful where the target has a consistent spectral expression and calibration spans the conditions of interest, offering compact models that can be applied efficiently to repeated imagery. Their apparent simplicity does not eliminate dependence on environmental covariance, however, especially when changes in suspended particles or dissolved absorption modify the relationship between a selected band ratio and pigment concentration. Physical and semi-analytical inversion addresses this problem by linking the observed signal to absorption and scattering components, but its performance depends on how well those components and their variability are parameterized. For accessory pigments, ambiguity can arise even in a physically interpretable model when different constituents produce similar spectral features or when the analytical target is more detailed than the recoverable optical component (Holtrop and Van der Woerd 2021; O'Shea et al. 2023; Grunert et al. 2025). Machine-learning methods can exploit nonlinear relationships across full spectra and heterogeneous datasets, including interactions that are difficult to represent with a small number of empirical terms. This flexibility is useful for complex waters, yet it also permits accurate predictions based on associations that may not persist outside the training domain; uncertainty and external evaluation consequently become central to interpreting model performance rather than optional additions to a high R^2. Hybrid methods attempt to improve this relationship by routing observations through optical-water-type classes or combining physical information with learned estimation (Lomeo et al. 2025; Xia et al.

2025; Zhu et al. 2025). Their scientific advantage is a more explicit treatment of heterogeneity, provided that both the classification and the subsequent retrieval are evaluated on the environments to which the workflow will be applied.

Naik et al. (2025) demonstrate how endmember-guided learning can improve the interpretation of overlapping pigment spectra. Using absorbance measurements from 20 Scottish lakes, their guided autoencoder recovered relationships with measured chlorophyll-a, fucoxanthin and phycocyanin that were not equally resolved by the blind architecture. For Loch Leven, which was excluded from training on the other 19 lakes, the reported mean Pearson correlations were approximately 0.99, 0.96 and 0.85 for the three pigments, compared with 0.60, 0.38 and 0.66 across additional lake holdouts. The results identify substantial differences among external test domains while demonstrating the usefulness of endmember constraints; because performance was expressed through abundance association and model stability, they characterize successful spectral separation more directly than absolute concentration error.

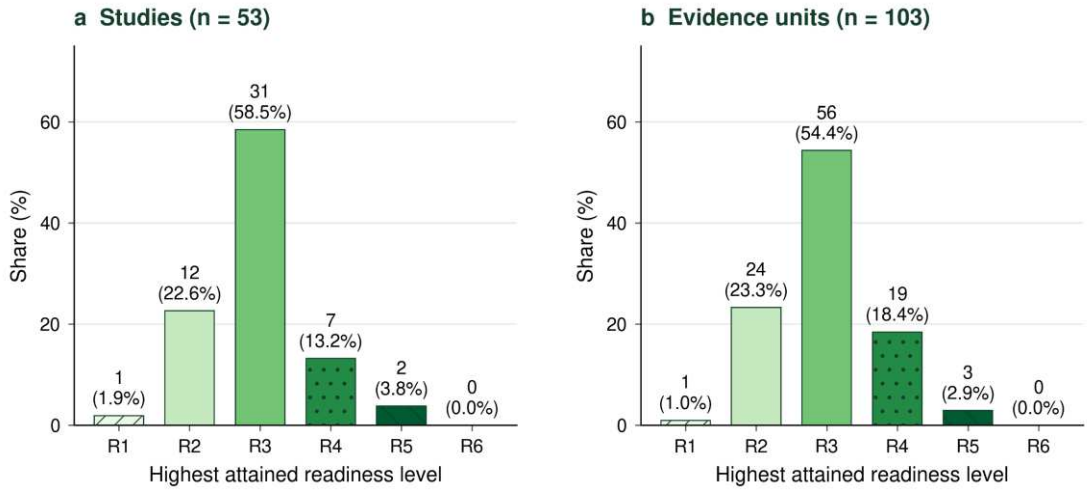
Salmi et al. (2024) provide a complementary example in which target error was explicitly quantified for concentrated-sample spectral imaging with one-dimensional convolutional networks. With Loch Leven withheld, median absolute percentage differences were 15% for chlorophyll-a, 36% for pooled carotenoids and 102% for phycocyanin, whereas leave-one-lake-out results for the other lakes were 26%, 27% and 75%. Both sets of results came from lake-excluded evaluation, so the differences describe the effect of the tested domain and target rather than a simple penalty for moving from internal to external validation. Their phycocyanin median symmetric accuracy was 107% in Loch Leven, a separate statistic from the 102% median absolute percentage difference. Together, these studies show why a retrieval claim should retain the target, metric and partition design: spectral correspondence, concentration accuracy and transfer are related properties, but they answer different questions.

Evidence readiness from retrieval development to independent validation

The evidence base comprised 157 target/configuration units: 103 direct pigment-retrieval units, 28 analytical/optical support units and 26 ecological/community-inference units. Fifty-three publications contributed direct retrieval evidence and formed the publication-level readiness distribution. At their maximum attained stage, one study was R1 (1.9%), 12 were R2 (22.6%), 31 were R3 (58.5%), seven were R4 (13.2%) and two were R5 (3.8%); none reached R6 (Fig. 4a). At evidence-unit level, the corresponding counts were one, 24, 56, 19, three and zero (Fig. 4b). The concentration of studies at R3 indicates

that quantitative evaluation against a suitable reference is well represented, while the smaller number reaching R4 and R5 identifies a more restricted evidence base for structured independence and transfer across systems.

Fig. 4 *Evidence-readiness distribution by publication (a; n = 53, maximum stage per study) and target/configuration unit (b; n = 103)*



This pattern has a substantive connection with the retrieval results discussed above. Validation against held-out observations can establish concentration prediction within a sampling domain while leaving its persistence under different communities, optical regimes or acquisition conditions unresolved. Of the primary publications, 44 of 53 (83.0%) did not progress beyond R3, even though the literature includes detailed spectral analysis, flexible models and spatial applications. That distribution does not diminish those advances; it locates the additional evidence needed when their conclusions extend from the conditions evaluated to another period, water body or instrument. The distinction is especially relevant when several targets are produced by one model, because the best-supported target cannot establish equivalent readiness for every output.

The two R5 studies illustrate what more extensive evidence contributes. Werther et al. (2022) compared 13 chlorophyll-a algorithms using 346 observations from 53 lakes and reservoirs with concentrations below 10 mg m^{-3}, examining optical controls on error and removing training-data overlap for the independent mixture-density-network evaluation. O'Shea et al. (2023) assessed multiple optical and biogeochemical outputs using regional/dataset holdouts and satellite-related comparisons, drawing on 5740 chlorophyll-a and 940 phycocyanin reference pairs. Their comparative generalization gains varied by endpoint, with phycocyanin an exception to matching or exceeding the comparison algorithms, despite being externally evaluated. These studies support stronger statements about where transfer works and where it remains difficult, rather than establishing uniformly low error across targets or environments.

Readiness therefore adds information that cannot be inferred from the highest predictive metric alone. Naik et al. (2025), for instance, provide a structured lake-transfer design and strong target associations, while the absence of an appropriate concentration-error measure limits progression through the earlier validated-retrieval requirement. Salmi et al. (2024) combine comparable laboratory transfer with target-error metrics, but their absorbance chain does not demonstrate recovery from natural-water reflectance and imagery. The framework makes these contributions distinguishable without treating either laboratory experiment as a deficient satellite application: each resolves part of the scientific problem, and each supports a different boundary for subsequent use.

Alikas et al. (2023) illustrate the distinction between method comparison and structured independent validation. Their satellite chlorophyll-a configurations were compared quantitatively with other measurements, while lake-specific coefficients and algorithm selection were not linked to a documented evaluation set withheld from fitting and selection (Sections 2.2.3 and 2.4; Tables 2 and 4). This supports R3 for the represented configuration; coverage of different lakes or years alone does not establish the R4 separation requirement.

Yala et al. (2020) evaluated the fucoxanthin/total-chlorophyll-a ratio using random partitions of the DPIG calibration database and a separate UPSEN campaign, supporting structured campaign independence (TR = 2, R4). The external comparison comprises five paired VIIRS-based retrieval and HPLC ratio values from 21 February 2013; the authors describe this confirmation as qualitative, and no error statistics are reported for that external domain. Although the change from SeaWiFS to VIIRS extends the sensor context, the published global calibration map does not establish a region-withholding design or exclude regional overlap sufficiently to assign cross-system transfer (TR = 3). The comparison therefore supports an independent campaign test while leaving broader geographic transfer unresolved.

Configuration-level assessment also distinguishes evidence within the same publication. Castagna et al. (2021) calibrated an absorption-based chlorophyll-c3 fraction algorithm with cultures and evaluated it against field pigment measurements, supporting R4. Their reflectance-based configuration used a random division of field observations and remained at R3. Keeping those configurations separate preserves the distinction between a structured change in the evaluation domain and an internal holdout, while the publication-level result records the highest supported stage.

The sensitivity results distinguish a stable lower-stage pattern from threshold-dependent upper categories (Table 4). Across the threshold and reference-independence scenarios, 44 of 53 publications (83.0%) remained at R3 or below. Relaxing only the R5 transfer threshold increased stage-5 units from three to 18, whereas relaxing only the

implementation threshold left the main distribution unchanged. Joint relaxation placed ten units from the multipigment study of Sun et al. (2022) at scenario stage 6, corresponding to one publication; a further eight units remained at scenario stage 5. Restricting reference independence to Yes did not change the final stages. The changed outcome under joint relaxation means that the absence of R6 cannot be treated as a threshold-invariant property of the literature. The main criteria remain useful because they distinguish cross-system evaluation from structured within-system testing, and a reproducible quality-controlled workflow from repeated application, but those distinctions must accompany the reported stage frequencies.

Table 4 *Exploratory sensitivity of upper-stage classifications*

Scenario	Stage 4 units	Stage 5 units	Stage 6 units	Stage 5 / 6 studies
Main rubric	19	3	0	2 / 0
Transfer threshold TR ≥ 2	4	18	0	7 / 0
Implementation threshold OI ≥ 2	19	3	0	2 / 0
Both threshold relaxations	4	8	10	6 / 1
Reference independence Yes only	19	3	0	2 / 0

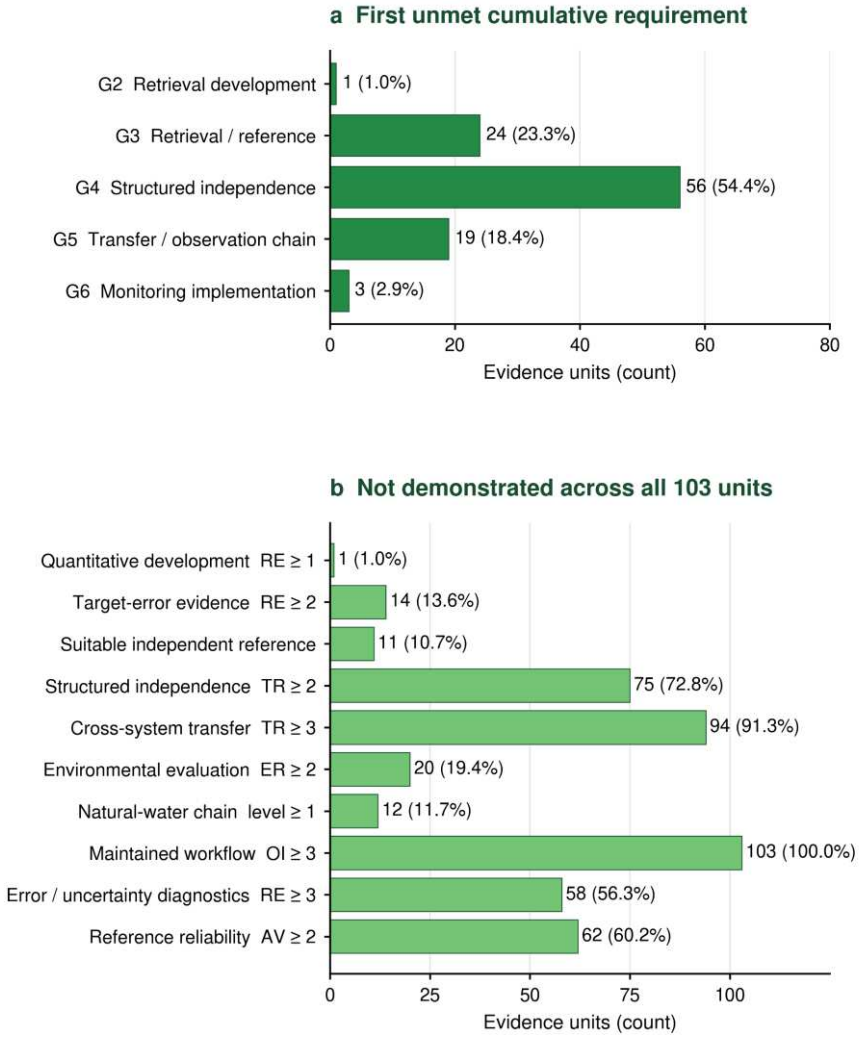
Unit denominator, 103; study denominator, 53, at maximum stage per publication. R1–R3 counts are unchanged across the threshold and reference-independence scenarios. Relaxed stage numbers do not inherit the main rubric meanings of cross-system validation or operational demonstration. The ten stage-6 units under joint relaxation all belong to Sun et al. (2022).

Validation requirements and sources of uncertainty

Structured independent validation was the first unmet requirement in 56 of 103 direct retrieval units (54.4%), followed by validated retrieval/reference in 24 (23.3%), cross-system transfer/observation-chain requirements in 19 (18.4%), operational implementation in three (2.9%) and quantitative development in one (1.0%) (Fig. 5a). This sequential distribution identifies the earliest unmet condition, rather than the prevalence of every missing requirement. When each component was assessed across all 103 units, structured independence (TR ≥ 2) was not demonstrated in 75 (72.8%), cross-system transfer (TR ≥ 3) in 94 (91.3%), comprehensive retrieval evidence (RE ≥ 3) in 58 (56.3%), and the stronger analytical-reference criterion (AV ≥ 2) in 62 (60.2%). No unit demonstrated OI ≥ 3, even though implementation appeared first for only three units in the cumulative sequence (Fig. 5b). These overlapping frequencies show that requirements can be missing together and prevent the first-unmet-gate result from being interpreted as evidence that later requirements are generally satisfied. A random holdout

from the same sampling pool may characterize interpolation adequately, but applications across seasons, campaigns or water bodies require partitions that preserve those sources of variation in the test set. The relevant validation design consequently follows the intended application rather than the nominal proportion of data reserved for testing.

Fig. 5 *Sequential and marginal unmet requirements among 103 direct retrieval units*



The independent-validation boundary is connected to the mechanisms that shape the optical mixture. If the relationship among chlorophyll-a, phycocyanin, particles and dissolved material is similar in training and test observations, a model can perform well without encountering the combinations most likely to cause failure elsewhere. In low-biomass lakes and reservoirs, Werther et al. (2022) reported median absolute percentage differences above 37% in their algorithm assessment and separately identified sensitivity to CDOM and NAP, especially for learned estimates. Their findings indicate that representative environmental coverage needs to be evaluated through the target error rather than inferred from sample number or the geographic extent of a pooled

dataset. They also give a physical rationale for optical stratification: a retrieval must remain identifiable when the dominant source of absorption changes.

Analytical validity introduces another requirement upstream of the remote model. In their comparison of HPLC measurements from oligotrophic Western Mediterranean waters, Canuti et al. (2025) reported differences of 85.5% for chlorophylls c and 56.6% for peridinin, while total chlorophyll-a differed by approximately 6.1% in the summary comparison. These are differences between the evaluated laboratory methods and samples, but their contrast among pigments shows that a well-behaved bulk-chlorophyll reference does not guarantee equivalent reliability for an accessory-pigment panel. If calibration targets vary because of extraction, detection or quantification procedures, part of the apparent retrieval error may originate in the reference, and differences among algorithms can be difficult to interpret without information on analytical comparability.

Phycocyanin further illustrates the value of analytical studies within a remote-sensing evidence base. Canuti (2025) developed an UHPLC method for C-phycocyanin in filtered samples, reporting approximately 10% analytical accuracy, 9.8% reproducibility and a detection limit of 0.025 mg L^{-1} under the tested protocol. This work does not constitute a remotely retrieved pigment product, but it addresses a reference problem that can constrain subsequent calibration and validation, particularly where the sample format differs from the liquid measurements used by conventional optical assays. The supporting track thus captures a practical route to stronger evidence: improving the reference can be necessary before a more sophisticated retrieval model can be evaluated reliably.

Atmospheric correction and acquisition geometry add uncertainty when the observation chain extends to imagery. In the common 26-sample pigment comparison of Zolfaghari et al. (2023), OLCI-based chlorophyll-a estimates had median symmetric accuracy below 37% for iCOR and ACOLITE inputs, whereas phycocyanin exceeded 100% with all three tested processors. Because the same correction can affect targets differently, acceptable radiometric behaviour in selected bands cannot establish an acceptable pigment product without an endpoint-specific evaluation. O'Shea et al. (2023) similarly reported median symmetric accuracy of 37.4-62.8% between products generated from co-located satellite and in situ reflectance; this measures the effect of changing the reflectance input, not an absolute concentration error against samples. Such comparisons are useful because they reveal how much of the retrieval changes along the measurement chain before any ecological interpretation is attempted.

The 19 units first constrained at the R5 requirement combine two different situations: 16 did not meet the cross-system transfer requirement, including one laboratory absorption configuration, while the remaining three met that transfer condition but remained

laboratory-only measurements. Distinguishing them identifies different next steps, since one requires more independent environmental testing and the other requires evaluating the relationship through natural-water reflectance or imagery. The smaller number first constrained at implementation should be interpreted within the cumulative sequence: later missing requirements are not counted again when an earlier requirement is absent. Table 5 summarizes the additional evidence needed at each transition in terms that can be incorporated into a study design, from an error-characterized reference comparison to a repeatable monitoring workflow.

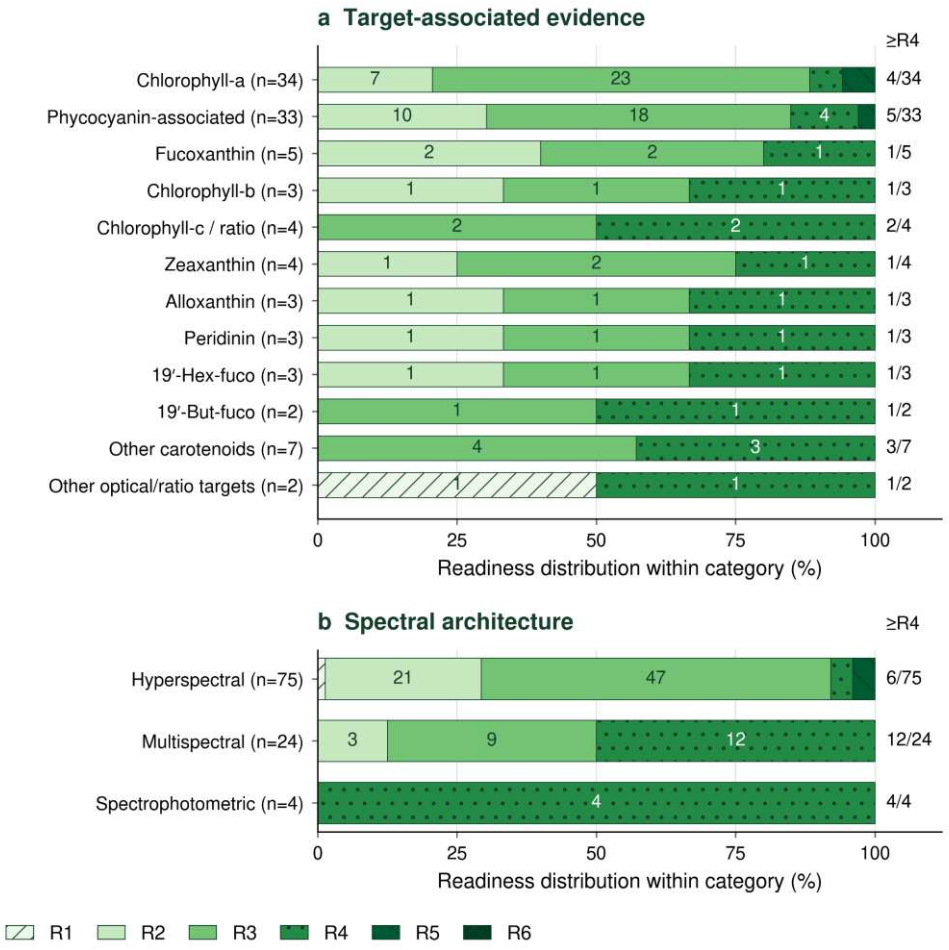
Table 5 *Evidence required for progression toward monitoring applications*

Transition	Additional evidence	Reporting needed
R2 → R3	Evaluate held-out/distinct observations against a suitable independent or partially independent reference.	Target-error metric, reference procedure, sample size, concentration range and measurement correspondence.
R3 → R4	Withhold complete time, spatial, campaign or equivalent units relevant to the application.	Partition unit, separation rule and target performance by the held-out domain.
R4 → R5	Test independent systems/regions through a natural-water observation chain and environmental heterogeneity.	Optical regimes, external errors, atmospheric/sensor context and applicability boundaries.
R5 → R6	Demonstrate repeatable monitoring with maintained reference quality, quality control and uncertainty.	Processing versions, quality flags, repeatability, validation maintenance and monitoring use.

Target coverage, spectral architecture and monitoring applications

The readiness profiles also clarify how the apparent breadth of the literature relates to particular products. Chlorophyll-a contributed 34 direct concentration units, of which four reached R4 or above (11.8%), while phycocyanin contributed 31 concentration units, with five at R4 or above (16.1%). Two additional configurations concerned a phycocyanin spectral proxy and a combined phycocyanin/cyanobacteria endpoint, bringing the broader associated group in Fig. 6a to 33. Fucoxanthin had five direct units, one at R4, and most other accessory-pigment groups contained only a few configurations. These small groups identify where evidence is sparse, but their percentages are highly sensitive to the design of individual multipigment studies; the more informative comparison is what was retrieved, against which reference and under what separation from calibration.

Fig. 6 *Readiness profiles by target-associated group (a) and spectral architecture (b). Values inside bars are unit counts; right-hand labels show R4 or above divided by the category total*



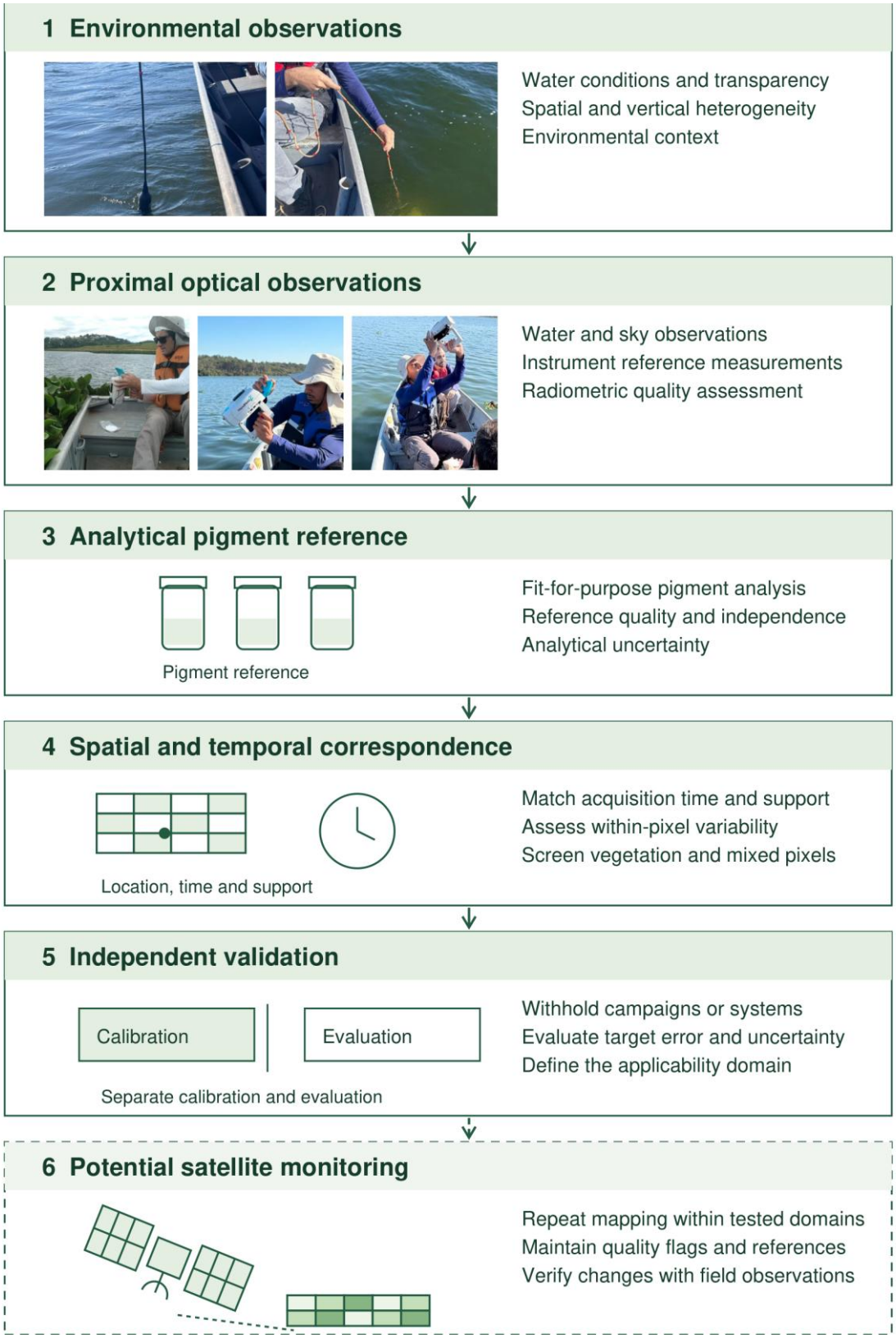
Among spectral architectures, six of 75 hyperspectral units (8.0%) and 12 of 24 multispectral units (50.0%) reached R4 or above. Ten of the 12 higher-stage multispectral units came from the multipigment satellite application of Sun et al. (2022), and removing that publication as an influence check leaves two of 14 units (14.3%). The comparison therefore reflects the configuration of available studies, including dependent targets and different purposes, rather than a direct experiment on spectral resolution. Hyperspectral measurements have an important role in accessory-pigment separation and optical-process research, whereas established multispectral archives have supported repeated spatial applications; both can contribute to monitoring, but the evidence needed to connect their strengths differs along the observation chain.

For hyperspectral applications, the next advances may involve demonstrating that additional spectral information remains useful after realistic sensor response, atmospheric uncertainty and spatial mixing are introduced. For repeated multispectral products, the corresponding need may be to establish how a robust empirical or learned

relationship behaves across seasons and optical regimes that are poorly represented in the original calibration. These priorities follow from the same validation logic rather than a preference for a particular architecture: information that improves pigment identifiability should be retained, while evaluation should expose the environmental and instrumental variation encountered by the intended product. Coordinated campaigns pairing pigment analysis, field spectra and ecological observations provide a practical means of making that connection.

Monitoring protocols should connect field observations, optical measurements and analytical references through a validation design aligned with the intended application (Fig. 7). Environmental observations characterize the conditions influencing the spectrum, while pigment measurements provide a concentration reference whose quality and independence must be established. Spatial and temporal correspondence then links these measurements to the image product, and independent evaluation determines the domain over which the retrieval can support monitoring (Boegehold et al. 2023; Zolfaghari et al. 2023). Photographs from our field activities in Ibirité Reservoir, Brazil, illustrate the environmental and radiometric components of this workflow. Extension to repeated satellite mapping additionally requires maintained quality control, uncertainty characterization and field verification.

Fig. 7 Conceptual monitoring workflow, illustrated with field photographs from Ibirité Reservoir



Operational interpretation also depends on the decision variable. A pigment concentration map can identify a spatial anomaly and guide targeted sampling, while a

community product requires evidence for the biological composition it represents. Legleiter et al. (2022) showed how spectral mixture analysis could support genus-level bloom surveillance, offering information beyond a total biomass estimate, but the presence of a potentially toxic genus does not establish toxin concentration. Boegehold et al. (2023) likewise document the value of coordinated bloom monitoring without making pigment and cyanotoxin measurements interchangeable. Pigment-based early warning is therefore most useful when its relationship to subsequent field verification and management action is defined, rather than when a biochemical indicator is treated as a direct measure of every associated hazard.

Operational services provide a separate context for interpreting these classifications. Copernicus Land Monitoring Service distributes regularly updated lake-water-quality observations, including a global 300 m product based on Sentinel-3 OLCI, while NOAA provides routine automated processing and distribution of chlorophyll-a and cyanobacteria-index products (Copernicus Land Monitoring Service n.d.; NOAA n.d.). The latter index represents a biomass-related endpoint rather than phycocyanin concentration. These services demonstrate why the status of an operational product cannot be inferred solely from a selected research-paper corpus. They were used as contextual examples and were not assigned readiness scores through this assessment. Within the selected research configurations, none satisfied the full R6 requirements of the main rubric. This result concerns the joint documentation of cross-system validation, reference quality, comprehensive error characterization and a repeatable monitoring workflow, and is conditional on those requirements, as the sensitivity analysis demonstrates. Moving toward that combination requires sustained validation as processing systems, communities and environmental conditions change; processing a long image archive once or releasing algorithm code addresses only part of the task. The strongest contribution of the combined methodological and readiness evidence is to make those relationships explicit: development of new models, improved analytical methods and more discriminating observations remain valuable, while their environmental use depends on a validation chain proportionate to the product and its decisions.

Study limitations

The review was restricted to English-language publications retrieved from three databases and targeted complementary literature available through 14 August 2025. Because chlorophyll-a was not a standalone search term, the evidence base emphasizes diagnostic-pigment and taxonomically informative applications rather than providing an

exhaustive account of conventional chlorophyll-a retrieval. Environmental and geographic representation was uneven, with limited coverage of several tropical and pan-tropical regions, and the 2025 records cover only part of the year. These features constrain the extent to which the observed distributions describe the broader field or its subsequent development. The contextual comparison incorporates subsequent literature, but the quantitative corpus was not extended beyond August 2025 and cannot characterize the distribution of evidence emerging in 2026.

Studies differed in pigment pools, analytical procedures, concentration ranges, optical environments, observation support and validation metrics, preventing a common quantitative effect-size interpretation. The readiness assessment also involves judgement about reference independence, environmental heterogeneity and operational evidence. Comparison of the classifications and resolution of disagreements through discussion and consensus provided a common final assessment, but no inter-rater reliability estimate was calculated. Consensus therefore does not quantify the reproducibility of the rubric when applied by other researchers. Incomplete reporting in the original publications can still constrain the evidence established for a configuration, even after source checking. A requirement coded as not demonstrated therefore concerns the available report and should not automatically be interpreted as proof that the underlying procedure was not performed.

Readiness is consequently an assessment of documented evidence for a defined retrieval configuration, not a ranking of scientific quality or a certification of acceptable error. Early-stage experiments can provide strong mechanistic insight, while a rigorously tested external application can still perform poorly for a particular pigment. Small target groups, overlapping model labels and multiple units from one publication further limit architecture or method comparisons. The exploratory sensitivity scenarios show that upper-stage counts depend on the boundary between within-system and cross-system evaluation and between repeated application and a maintained quality-controlled workflow. The relaxed criteria change the meaning of the resulting stage numbers, so those counts cannot be presented as equivalent operational certifications. Operational services outside the selected research corpus were not comprehensively inventoried or scored.

Conclusions

The reviewed literature shows substantial advances in the use of optical measurements to estimate phytoplankton pigments and interpret their spatial distribution. Chlorophyll-a and phycocyanin remain the most frequent targets, while accessory pigments add

information about community structure through associations that depend on pigment identity, physiology and assemblage context. Empirical relationships, physical inversion, learned models and spectral decomposition offer complementary ways of extracting that information, with laboratory and proximal observations supporting the interpretation of airborne and satellite products.

The evidence-readiness assessment shows that quantitative retrieval is more widely demonstrated than structured independent validation and cross-system application. Of 53 studies contributing direct retrieval evidence, 44 (83.0%) did not progress beyond R3, and independent validation was the first unmet requirement in 56 of 103 configurations. These patterns identify a need to align evaluation with the environmental, spatial and temporal domain of use, while the influence of individual multipigment studies cautions against interpreting aggregate architecture percentages as sensor superiority. The absence of R6 under the main rubric is conditional on the requirements for cross-system testing and a reproducible quality-controlled workflow; joint relaxation of those requirements changes the upper-stage outcome for one multipigment publication. The operational conclusion therefore concerns the strength and completeness of documented evidence in the selected studies.

Further progress depends on developing retrieval methods and strengthening the measurements and validation that give their results meaning. Traceable pigment references, co-located optical and ecological observations, evaluation under changing optical regimes and maintained quality control can connect advances in spectral discrimination to repeated environmental assessment. By distinguishing concentration recovery, ecological interpretation and monitoring implementation, the framework provides a practical basis for designing those connections without requiring every study to address the same stage of application.

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Statements and Declarations

Author contributions: Bernardo T. A. Souza extracted the study information and classified the evidence units. Caio C. S. Mello verified the extracted information against the source publications and assessed the evidence units using the same framework. Bernardo T. A. Souza and Caio C. S. Mello compared the classifications and resolved disagreements, including those concerning configuration boundaries and transferability, through discussion and consensus.

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Ethics approval, consent to participate and consent for publication: Not applicable.

Data availability: The study-level extraction, evidence-unit classifications, domain definitions and analysis code are provided in Online Resources 1-3.

Supplementary information

Online Resource 1 contains the 107-study extraction and standardized descriptive categories. Online Resource 2 provides the 157 evidence units, domain definitions, readiness criteria and target-level classifications. Online Resource 3 contains the analysis and figure-generation code, marginal requirement frequencies, sensitivity results and Supplementary Fig. S1.

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