Towards the construction of a carbon fluxes inventory of tropical waters: a unifying method pipeline

Hacia el inventario de flujos de carbono en aguas tropicales: unificar métodos

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Abstract
The relevance of inland waters in the global carbon cycle has been stressed recently, particularly because of a reassessment of their capacity for carbon exportation to the atmosphere and to the sediments. Global surveys have also highlighted the acute lack of information on tropical systems, which are exposed to crescent problems in the Global Change panorama, such as contamination and eutrophication, as well as important impacts related to water management strategies and water supply (e.g., water level fluctuations). Oxygen dynamics, a method left behind in the past, has been revised and is now being increasingly implemented to estimate primary production and ecosystem respiration due to the urgency to understand carbon fluxes in aquatic systems. Therefore, the details (advantages and disadvantages) of modern implementation of oxygen dynamics are revised and discussed here, particularly oriented to facilitate and promote their application in tropical aquatic systems, where it seems an adequate strategy. We suggest a unifying method pipeline in order to obtain comparable results among systems, towards the construction of a carbon flux inventory at larger (spatial and temporal) scales. This effort would contribute to understand the role and responses of tropical aquatic systems and regions (particularly as carbon sources or sinks) facing Global Change.

Keywords: Metabolism, oxygen, biogeochemistry, heterotrophic, production, respiration, Global Change, upscaling

Resumen
Recientemente se ha revalorado la relevancia de los sistemas acuáticos epicontinentales en el ciclo global del carbono, ya que éstos tienen una gran capacidad de ser tanto sumideros de carbono hacia sus sedimentos como fuentes de carbono atmosférico. Sin embargo, la información
existente sobre los sistemas tropicales es aún heterogénea y escasa, lo cual es particularmente crítico, dado que estos sistemas enfrentan problemas crecientes en el panorama del cambio global, incluyendo la contaminación y en particular la eutrofización, así como impactos de las estrategias de manejo (e.g., cambios en el nivel de agua). El estudio de la dinámica del oxígeno, criticado en el pasado, ha sido retomado y hoy es nuevamente utilizado ampliamente para estimar la producción primaria y la respiración ecosistémica ante la urgencia por estimar los flujos de carbono asociados a los sistemas acuáticos. Se revisan aquí, por ello, los aspectos metodológico-conceptuales asociados a la implementación moderna del método de evolución del oxígeno y se discuten las ventajas y los problemas asociados a esta metodología, buscando facilitar y extender su aplicación a los sistemas tropicales, donde su implementación es muy conveniente. Se propone también una secuencia metodológica unificadora para hacer posible la comparación entre estudios, y el escalamieneto de los flujos de carbono, tanto espacial como temporalmente. Los inventarios de carbono en diferentes escalas (espaciales y temporales) son necesarios para entender la participación y las respuestas de los sistemas acuáticos y regiones tropicales (en particular su papel como fuentes o sumideros de carbono) frente al cambio global.

Palabras clave: metabolismo, oxígeno, biogeoquímica, heterotrofía, producción, respiración, cambio global, escalamiento.

Recived: 23/01/2017
Accepted: 23/06/2018

Introduction

The study of oxygen dynamics to evaluate primary production (PP) and community metabolism (defined as the balance between production and respiration) became a landmark in the understanding of fluxes of matter and energy of ecosystems (see Odum, 1956). The development of other
techniques such as $^{14}$C incorporation also favored and enriched the study of PP, however the interpretation of these results is complex due to re-fixation of respired $^{14}$C or to its excretion (Marra, 2002), besides variable underestimation of the rates, which are in between gross and net photosynthesis (Bender, Orchard, Dickson, Barber, & Lindley, 1999) amongst other type of complexities. Even so, the extended use of the $^{14}$C method limited the metabolic balances studies in spite of their potential for assessing (through oxygen dynamics) autotrophy or heterotrophy at the ecosystem level. This also implied a decrease in the assessment of community respiration during decades (Smith & Kemp, 1995; Behrenfeld & Falkowski, 1997). In contrast, the relevance of inland aquatic systems in the global carbon cycling has been recently highlighted (Duarte & Prairie, 2005; Cole et al., 2007) because these systems exhibit larger rates of carbon exportation to the sediments, compared to ocean systems (Tranvik et al., 2009). It has now become evident that this information is key to face the challenges of environmental change (e.g. changes in precipitation patterns, in eutrophication thresholds, and in the role of ecosystems as carbon sinks or atmospheric sources) from planetary to regional scales (Guimarais-Bermejo, Merino-Ibarra, Valdespino-Castillo, Castillo-Sandoval, Ramírez-Zierold, 2018). Additionally, whether inland aquatic systems have a net autotrophic or heterotrophic functioning (Cole et al., 2007; Tranvik et al., 2009) remains an open question. Moreover, it has been pointed out that the scarcity of carbon fluxes data for tropical systems (St. Louis et al., 2000; Tranvik et al., 2009) is one of the main limitations to answer this and other presently open questions.

Comparisons amongst tropical and temperate aquatic systems are also needed to test general hypotheses of Ecological theory and Limnology. For example, it has been hypothesized that nutrient cycling should be more intense in tropical systems than in temperate ones (Boulton et al., 2008); if this is a generalized behavior, and how it affects metabolic balance of ecosystems, are questions that –if they are not addressed through integrative ecosystem approaches- will require an intense experimental effort to assess all the processes involved.

**Assessment of the metabolism of aquatic ecosystems**

*Tecnología y ciencias del agua*, 10(1), 68-84, DOI: 10.24850/j-tyca-2019-01-09
Because of this, an increasing number of scientists aim to foster the assessment of the metabolism of aquatic ecosystems (Staehr et al., 2010) – including lakes (Solomon et al., 2013), estuaries and coral reefs – through oxygen dynamics because it is a simple, useful and trustworthy method that allows the evaluation of respiration and, therefore, to separately assess both gross and net productions (McKinnon, Logan, Castine & Duggan, 2013). This renewed interest is also due to the need of finding a unifying approach to study tropical aquatic systems in the panorama of environmental change (Staehr et al., 2012; Solomon et al., 2013; Cloern, Foster & Klekner, 2014), because the multiplicity of methods and strategies used so far is an important source of variation of results, hindering their interpretation and data integration (Cloern et al., 2014).

The scarcity (St. Louis et al., 2000; Tranvik et al., 2009) and heterogeneity of the information available on tropical aquatic ecosystems are exhibited in Table 1, derived from a comprehensive review of literature on this subject. The way in which results are reported reveals the heterogeneity of the way studies have been carried out, which hinders both comparison among studies and the scaling of the information. The low number of studies that report respiration and metabolic balance (P:R in Table 1) is particularly noticeable. Most of them only report primary production data. Moreover, many of the studies report metabolic rates only in volumetric units (lower section of Table 1), which means that both vertical variability and the integration on this axis were neglected. Similarly, rates are reported on hourly basis and do not inform if the necessary integration over a full day and night period was done or not. As discussed in detail further ahead, both of these aspects are crucial to obtain sound comparisons among systems and correct regional scaling of the information.

**Table 1.** Gross primary production (GP), net primary production (NP) and community respiration (R) in tropical inland aquatic systems. On the first half of the table, studies that report area/photoperiod integrated rates (gC m⁻² day⁻¹). In the lower part, studies that only report volumetric and/or hourly rates, and the units used in each report.
| System                          | Trophic state* | Depth | GP     | NP     | R       | P:R   | Units           | Reference                                      |
|--------------------------------|----------------|-------|--------|--------|---------|-------|-----------------|------------------------------------------------|
| Alchichica, México             | Oligotrophic   | D     | 2.9    | 0.84   | 2.029   | 4.39  | gC m⁻³ day⁻¹    | Oseguera et al. 2015                           |
| Chapala, México                | Oligo-mesotrophic | S     | 0.27   |         |         |       |                 | Lind et al. 1992                               |
| Shahidullah, Bangladesh        | Mesotrophic    |       | 4.2    | 3.7    | 1.15    |       |                 | Khondker and Kabir 1995                        |
| Valle de Bravo, México         | Eutrophic      | D     | 2.1    | 0.49   | 3.9     | 0.54  |                 | Valdespino-Castillo et al. 2014                |
| Trophogenic                    |                |       | 2.1    | -1.1   | 6.1     | 0.34  |                 |                                                 |
| System                         |                |       |        |        |         |       |                 |                                                 |
| Quebrada Seca, Venezuela       | Hypertrophic   |       | 1.8 – 3.5 |     |         |       |                 | González et al. 2003                           |
| La Mariposa, Venezuela         |                |       | 0.9 – 2.6 |     |         |       |                 |                                                 |
| Pao Cachinche, Venezuela       |                |       | 2.5 – 6.8 |     |         |       |                 |                                                 |
| Lake Xolotlán, Nicaragua      |                |       | 1.0 - 6.8 | 0.5 - 3.9 | 0.3 - 5.2 |       |                 | Erikson et al. 1998, 1999                     |
| Ganges River, India            |                |       | 0.9 – 1.02 |     |         |       |                 | Natarajan 1989                                 |
| Chang Jiang, Yangtze, China   |                |       | 1.15 – 3.61 |     |         |       |                 | Liang et al. 1988                              |
| Lake Lanao, Filipinas          |                |       | 2.6    | 1.7    |         |       |                 | Lewis 1974                                     |

**Tropical ponds, India**

| System                          | GP     | NP     | R       | Units           | Reference                                      |
|--------------------------------|--------|--------|---------|-----------------|------------------------------------------------|
| Poza Othakadai                 | 1.5 – 15.8 |     |         |                 | Vijayaraghavan 1971                           |
| Teppakulam Pond                | 2.0 – 8.0 |     |         |                 |                                                 |
| Poza Yanamalai                 | 1.05 – 5.4 |     |         |                 |                                                 |
| Ololden, Kenya                 | 1.58 – 4.54 |     |         |                 | Allanson 1990                                  |
| Bosomtwe, Ghana                | 4.73   | 0.38   | 4.34    | 1.1            | Awortwi 2010                                   |

**System Trophic state**

- **Oligotrophic**: 2.9 - 0.84 - 2.029 - 4.39
- **Oligo-mesotrophic**: 0.27
- **Mesotrophic**: 4.2 - 3.7 - 1.15
- **Eutrophic**: 2.1 - 0.49 - 3.9 - 0.54
- **Hypertrophic**: 1.8 - 3.5 - 0.9 - 2.6 - 2.5 - 6.8 - 1.0 - 6.8 - 0.5 - 3.9 - 0.3 - 5.2
- **Eutrophic**: 2.1 - 0.49 - 3.9 - 0.54

**Reference**

- Oseguera et al. 2015
- Lind et al. 1992
- Khondker and Kabir 1995
- Valdespino-Castillo et al. 2014
- González et al. 2003
- Dávalos-Lind and Lind 2001
- Erikson et al. 1998, 1999
- Natarajan 1989
- Liang et al. 1988
- Lewis 1974
- Allanson 1990
- Awortwi 2010
- Vijayaraghavan 1971

**Units**

- **gC m⁻³ day⁻¹**
- **mgC l⁻¹ h⁻¹**
- **gC m⁻³ day⁻¹**
- **mgC m⁻³ h⁻¹**
- **mgC m⁻³ h⁻¹**
- **mgC m⁻³ h⁻¹**
- **mgC m⁻³ h⁻¹**

**Area integrated rates**

**Volumetric rates**

**Trophic state**

- **Oligotrophic**: D
- **Oligo-mesotrophic**: S
- **Mesotrophic**: D
- **Eutrophic**: D
- **Hypertrophic**: D
*Data are ordered by trophic state (when reported), by integrated rated (area and daylenght); and volumetric measurements are shown in the lower part. S=shallow; D=deep.

The scarcity and heterogeneity of studies on tropical aquatic ecosystems stands out against the urgent need to address the open questions and to assess if the capacity of these ecosystems of exporting carbon to the sediments or to the atmosphere is changing as temperature rises or in response to other consequences of global change. In that regard, one of the few long-term studies in Mexican tropical inland waters reports a metabolic balance tending towards net heterotrophy (P:R <1), in which respiration is higher than in many temperate inland systems; (Guimarais-Bermejo et al. 2018), therefore questioning the high carbon exportation to the sediments of inland waters derived in global surveys (Duarte & Prairie 2005; Cole et al., 2007).

**Studies on aquatic metabolism through the evolution of oxygen**

Considering all of this, we here endorse the proposal to multiply the studies on aquatic metabolism through the evolution of oxygen (Staehr et al., 2010; Solomon et al., 2013; Cloern et al., 2014) in order to contribute effectively to the understanding of carbon fluxes. In particular, we consider that, among the various options to evaluate oxygen dynamics (Staehr et al., 2012), light and dark bottle incubations method offers some relevant advantages to study tropical aquatic inland systems, such as: 1) it is a high precision method that requires only basic infrastructure, 2) its application is particularly adequate for systems with advanced trophic state (Zhang, Berberian & Wanninkhof, 2002), 3) it is the most appropriate method to use for systems influenced by wind, in which atmospheric oxygen diffusion affects measurements performed in open waters (Nöel et al., 2010) (Fig. 1).
To foster the extended, effective and comparable use of this approach in tropical aquatic ecosystems, in figure 1 we have put together the conceptual and technical aspects that we consider relevant and useful for their application in diverse systems. These aspects, crucial to obtain good quality and comparable information, are discussed next.

The first step for the successful application of a metabolic balance study is that it is designed accordingly to the characteristic limnology of the system to be studied. In particular, its oxygen distribution must be considered to design the incubation plan. Other important definitions and precautions are related to the critical issues of the light and dark incubations method, which -if not addressed- may lead to some of the disadvantages of using it: a) it is a laborious technique that requires a previous training of the operators, so preparation training is a key component to its successful application. b) It is known that proliferation of microbes on the bottle walls (bottle effect) may lead to respiration overestimations (Lee & Fuhrman 1991). This effect can be minimized by using low rugosity (glass) bottles and also by avoiding the use of small bottles; we recommend a minimum volume of 60 ml, c) In oligotrophic systems, long incubation times (~full day) are needed to register significant differences in the oxygen concentration (Fig. 1).
**Information of the system**

- With/without wind influence?
- Shallow or deep?
- Mixing regime. Monomictic, polymictic?
- Depth of the mixing layer. Anoxic hypolimnion?

### Oligotrophic system
- Light and dark bottles\(^1\)
- (long incubations)\(^2\)
- Experimental and analytic replicates
- Sensors *in situ*

### Eutrophic system
- Light and dark bottles\(^1\)
- (shorter incubations)\(^3\)
- Experimental and analytic replicates
- Sensors *in situ* (difficult implementation)\(^*\)

#### Depth
- Euphotic zone, various depths *in-situ* incubations (well-suited)\(^5\)
- Reproduction of light environment in Lab incubations (well-suited)\(^6\)

#### Spatial heterogeneity
- ¿PP of macrophytes/benthos?, dominant primary producers?
- ¿Phytoplankton/bacterioplankton?, consider separate incubations?
- Characterization of phyto or bacterioplankton?

#### Physicochemical environment
- Temperature, oxycline depth, Secchi depth
- Light environment (intensity and photoperiod length)
- Deep chlorophyll maximum?; reported trophic state\(^8\)

**Light**
- Normalize by incubation light conditions\(^5\)
- or reproduction of temperature and light environment in Lab incubations\(^6\)
- Estimation of photorespiration and dark respiration (suggested)

**Rates integrations**
- Depth (spatial integration), report area rates (mg O\(_2\) m\(^{-2}\) h\(^{-1}\))
- Vertical integration up to PN = 0 (for production)
- Measurement and additional integration below this layer to estimate System´s total respiration

**Time integrations**
- Time period (day rates integration \(^7,8\)), report in mg O\(_2\) m\(^{-2}\) day\(^{-1}\)
- Integration per light hours (production)
- Integration per light + night hours (respiration)\(^*\)

**Conversion to carbon rates**
- Factor PQ, RQ \(^10,11\), report in g C m\(^{-2}\) day\(^{-1}\)

**Upscaling of production and respiration rates**
- Error propagation \(^12\) (associate error to each estimation)

**Time series**
- Annual rates (at least monthly sampling)\(^7\)

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\(^1\) Staehr et al. (2012); \(^2\) Lee y Fuhrman (1991); \(^3\) Zhang et al. (2002); \(^4\) Nöel et al. (2010); \(^5\) Valdespino-Castillo et al. (2014); \(^6\) Azebedo et al. (2006); \(^7\) Cloern et al. (2014); \(^8\) Behrenfeld y Falkowski (1997); \(^9\) Geider y Osborne (1989); \(^10\) Gazeau et al. (2005); \(^11\) Flores-Verdugo et al. (1988); \(^12\) Lehrter y Cebrián (2010).

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**Figure 1.** Proposed methods pipeline towards the construction of a carbon fluxes inventory in the tropics using oxygen dynamics as strategy.
The conceptual and technical aspects that we propose should be considered sequentially to obtain reliable and comparable data that can be up-scaled in space and time are presented.

Because metabolic rates are a function of temperature, and the photosynthetic rate is a function of the light availability, in situ incubations are most convenient. Nevertheless, since laboratory incubations also offer some advantages (Azevedo, Duarte & Bordalo, 2006), the natural habitat (light and temperature) must be emulated as much as possible when working in the laboratory (Fig. 1). In any case, multiple replicates should always be included (Fig. 1).

It is also very important to control the access of herbivores into the bottle, as well as to rigorously control the interaction of the water sampled with the atmosphere to avoid errors related to oxygen diffusion (Valdespino-Castillo et al., 2014). In this sense, it must be born in mind that other methods, such as the estimation of fugacity of CO₂, also require a similarly rigorous control of gas exchange. Furthermore, fugacity and carbon fluxes assessments using total alkalinity, dissolved inorganic carbon and pH require both high precision measurements as well as knowledge on the physicochemical particularities of the system studied, which drive these equilibria. Overall, the application of these methods involves a longer list of precautions, considerations and critical assumptions (Dickson & Goyet, 1994) than in the case of oxygen dynamics.

The analysis of oxygen dynamics data (either using bottles or sensors) requires the integration of instantaneous rates (per volume, per hour) to area and daily rates (integrated vertically and by the photoperiod or light hours) to allow system comparisons (Behrenfeld & Falkowski, 1997) (see Fig. 1). To do this, measurement of the community respiration during the night-time should also be accomplished, in order to avoid the use of indirect estimates, such as “dark respiration” (a fraction of photorespiration, see Geider & Osborne, 1989).

In any case, to obtain vertically representative integrated rates, the vertical distribution of in situ incubations should account for the intrinsic features of the system, because metabolism is a function of light penetration (affecting photosynthesis) as well as of the existing community respiration. Vertical integration should be extended down to the compensation point, where production and respiration rates become equal (and net production, PN= 0). This calculation provides the method with a conceptual and operative limit to fully assess the production zone.
(trophogenic layer), which is not vertically homogeneous; therefore multiple incubation levels should be included within it.

When the metabolic balance is to be estimated at ecosystem level, it is important to also assess the aerobic respiration below the trophogenic layer and down to the bottom (Guimarais-Bermejo et al. 2018) when the oxygen availability allows its measurement. However, in stratified eutrophic systems, if the physical supply of oxygen (mainly by diffusion and vertical mixing) is not enough, the hypolimnion may remain anoxic. In these conditions, respiration becomes anoxygenic and can no longer be resolved using these methods. In this zone, microorganisms harness the redox chemistry, because some of them are metabolically able to obtain energy without oxygen (e.g., reducing nitrate).

For the full spatial integration, the potential role of benthos in the metabolic balance at ecosystem scale (including the interaction with the sediments) should also be assessed. Particularly in shallow water systems, oxygen dynamics studies will require methodologically more complex strategies, such as micro and mesocosm enclosures.

Another relevant aspect to consider when upscaling (from instantaneous to annual rates, and from profiles to ecosystem rates) is that the propagation of statistical errors has to be calculated (Lehrter & Cebrián, 2010; Valdespino-Castillo, Merino-Ibarra, Jiménez-Contreras, Castillo & Ramírez-Zierold, 2014).

Conclusions

Final considerations: In summary, here we have highlighted that community-to-ecosystem metabolic studies (the balance between photosynthesis and respiration) are a useful strategy to assess these fluxes in tropical aquatic ecosystems and to understand their role as carbon sinks or sources. To do this, comparable and reliable data are needed, and we offer a set of conceptual and technical considerations that will help achieve such data (see methods pipeline in Figure 1). So far, the information available in terms of metabolic balances and carbon exportation from tropical systems has been particularly scarce, and its
increase is urgently needed to upscale carbon balances (general and regional inventories) and to provide elements to face global change.

Finally, we want to close this proposal with two notes, one looking to past work and the other towards the future. Looking backwards, it must be acknowledged that the assessment of biomass using chlorophyll-a has been a useful proxy for the general trophic state of ecosystems. However it must also be outlined that it should not be considered directly as a proxy or measure of PP (gross or net) nor of community metabolism. This misinterpretation has often occurred in studies of primary production (Harris 1986) and may imply significant errors when interpreting results (Felip & Catalán, 2000).

It should be borne in mind that production and respiration rates are metabolic fluxes, therefore comprising a temporal component and a direction (i.e. they are vectors); in contrast, biomass is a static determination. Additionally, chlorophyll-a is itself a limited proxy for phytoplankton biomass: bio-volume:chlorophyll-a ratio is highly variable among phytoplankton species, depending on the structure and life histories of predominant species (Felip & Catalán, 2000), as well as within species (e.g., pigment content variation due to photo-acclimation; Longhurst & Harrison, 1989).

Looking toward the future of metabolism and production studies, it is interesting to visualize that we are near the dawn of understanding the relevance of carbon fixation through metabolisms different than oxygenic photosynthesis (anoxygenic photosynthesis and various chemosynthetic pathways). By then, it is desirable that metabolic studies using oxygen dynamics are a widely used approach in tropical aquatic ecosystems.

**Acknowledgements**

We acknowledge the students and collaborators of the Aquatic Biogeochemistry Laboratory PCML, ICML, UNAM (Emiliano Monroy, Gerardo Quintanilla, Monica Olson, Sofía Montalvo, Luz Ma. López, Mauricio Arteaga, Jorge Jiménez, Ma. Elena Valeriano, Gloria Vilaclara, Miroslav Macek, Irina Cruz, Guadalupe Pérez, Rebeca Sánchez, Claudia M. Saavedra, Lourdes M. Murueta, Flor Árcega, Alfredo Gallardo, Felipe Solís, Karina Castro, Andrea Guzmán, Mariel Barjau, Arantxa Sacristán and Federico Serna).
UNAM, CONACYT and SEMARNAT funded this work through projects PAPIIT-IN207702 and CONACYT-SEMARNAT C01-1125 awarded to M. Merino-Ibarra.

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