Isotopic evidence for biogenic molecular hydrogen production in the Atlantic Ocean

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Abstract

Oceans are a net source of molecular hydrogen (H₂) to the atmosphere. The production of marine H₂ is assumed to be mainly biological by N₂ fixation, but photochemical pathways are also discussed. We present measurements of mole fraction and isotopic composition of dissolved and atmospheric H₂ from the southern and northern Atlantic between 2008 and 2010. In total almost 400 samples were taken during five cruises along a transect between Punta Arenas (Chile) and Bremerhaven (Germany), as well as at the coast of Mauritania. The isotopic source signatures of dissolved H₂ extracted from surface water are highly deuterium-depleted and correlate negatively with temperature, showing δD values of (-629±54) ‰ for water temperatures at (27±3) °C and (-249±88) ‰ below (19±1) °C. The results for warmer water masses are consistent with biological production of H₂. This is the
first time that marine H$_2$ excess has been directly attributed to biological production by
isotope measurements. However, the isotope values obtained in the colder water masses
indicate that beside possible biological production a significant different source should be
considered.

The atmospheric measurements show distinct differences between both hemispheres as well
as between seasons. Results from the global chemistry transport model TM5 reproduce the
measured H$_2$ mole fractions and isotopic composition well. The climatological global oceanic
emissions from the GEMS database are in line with our data and previously published flux
calculations. The good agreement between measurements and model results demonstrates that
both the magnitude and the isotopic signature of the main components of the marine H$_2$ cycle
are in general adequately represented in current atmospheric models despite a proposed
source different from biological production or a substantial underestimation of nitrogen
fixation by several authors.

1 Introduction

Molecular hydrogen (H$_2$) is the second most abundant reduced compound in the atmosphere
after methane (CH$_4$). H$_2$ is not a radiatively active gas itself, but – via its role in atmospheric
chemistry – it indirectly influences the lifetime of the greenhouse gas CH$_4$ and several air
pollutants (Prather, 2003; Schultz et al., 2003; Tromp et al., 2003; Warwick et al., 2004;
Jacobson, 2008; Feck et al., 2008; Ehhalt and Rohrer, 2009, Popa et al. 2015). The main H$_2$
sources are photo-oxidation of CH$_4$ and non-methane volatile organic compounds (NMVOC)
in the atmosphere and combustion processes at the surface, whereas soil deposition and
oxidation by hydroxyl radicals (HO') are the main sinks. Oceans are a minor but significant
source to the global H$_2$ budget with a mean estimated contribution of 7 %. However,
estimates of the oceanic contribution range from 1 % to 15 % in different studies, indicating
high uncertainties (Novelli et al., 1999; Hauglustaine and Ehhalt, 2002; Ehhalt and Rohrer,
2009, and references herein, Pieterse et al. 2013).

Oceanic H$_2$ production is assumed to be mainly biological, as a by-product of nitrogen (N$_2$)
fixation (e.g. Conrad, 1988; Conrad and Seiler, 1988; Moore et al. 2009, 2014). H$_2$ is
produced during N$_2$ fixation in equimolar proportions, but also reused as an energy source.
The H$_2$ net production rate during N$_2$ fixation depends on environmental conditions and also
on microbial species (Bothe et al., 1980; 2010; Tamagnini et al., 2007; et al. 2010a). Besides
Nitrogen fixation, abiotic photochemical production from chromophoric dissolved organic matter (CDOM) and small organic compounds such as acetaldehyde or syringic acid has also been found to be a source of hydrogen in the oceans (Punshon and Moore, 2008a, and references therein).

Unfortunately, measurements that constrain the temporal and spatial patterns of oceanic H₂ emissions to the atmosphere are sparse. Vertical profiles display highest concentrations in the surface layer (up to 3 nmol L⁻¹) and a sharp decrease with depth towards undersaturation, where the reasons for the undersaturation are not fully understood yet (e.g. Herr et al., 1981; Scranton et al., 1982; Conrad & Seiler, 1988). Tropical and subtropical surface waters are supersaturated up to 10 times or even more with respect to atmospheric H₂ equilibrium concentrations, and therefore a source of H₂ to the atmosphere. This is in contrast to temperate and polar surface waters, which are generally undersaturated in H₂ (Scranton et al., 1982; Herr et al., 1984; Herr, 1984; Conrad and Seiler, 1988; Seiler and Schmidt, 1974; Herr et al., 1981; Lilley et al., 1982, Punshon et al., 2007, Moore et al. 2014).

Additional information to constrain the global H₂ budget and to gain insight into production pathways comes from the analysis of the H₂ isotopic composition (quantitatively expressed as isotope delta value, δD, see section 2.2). Different sources produce H₂ with characteristic δD values. Moreover, the kinetic isotope fractionation in the two main removal processes, soil deposition and reaction with HO²⁻, is different. The combined action of sources and sinks leads to tropospheric H₂ with a δD of +130 ‰ relative to Vienna Standard Mean Ocean water (VSMOW), (Gerst and Quay, 2001; Rhee et al., 2006; Rice et al., 2010; Batenburg et al., 2011). In sharp contrast, surface emissions of H₂ from fossil fuel combustion and biomass burning have δD values of approximately -200 ‰ and -300 ‰, respectively (Gerst and Quay, 2001; Rahn et al., 2002; Röckmann et al., 2010a; Vollmer et al., 2010). As originally proposed by Gerst and Quay (2001), isotopic budget calculations require the photochemical sources of H₂ to be enriched in deuterium, with δD values between +100 ‰ and +200 ‰ (Rahn et al., 2003; Röckmann et al., 2003; Feilberg et al., 2007; Nilsson et al., 2007; Pieterse et al., 2009; Nilsson et al., 2010; Röckmann et al., 2010b). Biologically produced H₂ has the most exceptional isotopic composition with δD of approximately -700 ‰ (Walter et al., 2012), reflecting strong preference of biogenic sources for the lighter isotope ¹H.

The aim of the study was I) to determine the δD of dissolved H₂ and gain more information about possible sources, and II) to get a high-resolution picture of the distribution of
atmospheric $\text{H}_2$ along meridional Atlantic transects during different seasons and compare it with global model results. Samples were taken on four cruises along meridional Atlantic transects in the southern and northern hemisphere and on one cruise at the coast of Mauritania. A total of almost 400 atmospheric and 22 ocean surface water samples were taken, covering two seasons between 2008 and 2010.

2 Methods

2.1 Cruise tracks

During four cruises of RV Polarstern and one of RV L’Atalante between February 2008 and May 2010, air and seawater samples were collected (see Fig. 1, Table 1). The cruises of RV Polarstern were part of the OCEANET project (Autonomous measuring platforms for the regulation of substances and energy exchange between ocean and atmosphere, Hanschmann et al. 2012). They covered both hemispheres, between Punta Arenas (Chile, 53° S / 71° W) and Bremerhaven (Germany, 53° N / 8° E). South–north transects were carried out in boreal spring (April / May) and north–south transects in boreal autumn (October / November). The transects followed similar tracks as the Atlantic Meridional Transect (AMT) programme (http://amt-uk.org/) and crossed a wide range of ecosystems and oceanic regimes, from sub–polar to tropical and from euphotic shelf seas and upwelling systems to oligotrophic mid–ocean gyres (Robinson et al. 2009, Longhurst 1998).

The RV L’Atalante followed a cruise track from Dakar (Senegal) to Mindelo (Cape Verde), covering a sampling area along the coast of Mauritania and a transect to the Cape Verde Islands. This area is characterized by strongly differing hydrographical and biological properties with an intensive seasonal upwelling. Area and cruise track are described in more detail in Walter et al. (2013) and Kock et al. (2008).

2.2 Atmospheric air sampling

Discrete atmospheric air samples were taken on–board RV Polarstern at the bridge deck, using 1 L borosilicate glass flasks coated with black shrink-hose (NORMAG), with 2 Kel–F (PCTFE) O–ring sealed valves. The flasks were pre–conditioned by flushing with $\text{N}_2$ at 50 °C for at least 12 hours; the $\text{N}_2$ remained in the flask at ambient pressure until the sampling.

During sampling the flasks were flushed for 4 minutes with ambient air at a flow rate of 12 L min $^{-1}$ using Teflon tubes and a membrane pump (KNF VERDER PM22874–86 N86ANDC).
The sample air was dried with Drierite® (CaSO₄). The flasks were finally pressurized to approximately 1.7 bar, which allows duplicate measurements of the H₂ isotopic composition of an air sample.

Table 1 gives an overview of the sampling scheme for discrete H₂ samples. In total 360 samples were collected, regularly distributed over the transects at 4 to 6 hour intervals. In 2009 the resolution of sampling was enhanced to one sample per two hours and focused on five sub-sections of the transect, in an attempt to resolve dial variability.

Samples were always taken at the downwind side of the ship to exclude a possible contamination by ship diesel exhaust. One atmospheric sample was taken directly inside the ship's funnel of RV Polarstern to determine the mole fraction and δD of ship diesel exhaust as a possible contamination source. This first measurements for ship diesel exhaust gave an H₂ mole fraction of (930.6±3.2) nmol mol⁻¹ and a δD of (–228.6±5.0) ‰. In the following, we will use the abbreviation "ppb" = 10⁻⁹ in place of the SI unit "nmol mol⁻¹".

2.3 Headspace sampling from surface waters

In addition to the atmospheric air samples, 16 headspace samples from surface water were taken during the RV Polarstern cruise ANT-XXVI/4 in April / May 2010 and 6 samples during the RV L'Atalante cruise in February 2008. The experimental setup (Fig. 2) was a prototype, and deployed for the first time to extract headspace air from surface water for isotopic composition measurements of molecular H₂. It consists of a glass vessel (10 L) and an evacuation / headspace sampling unit.

The glass vessel was evacuated for at least 24 h before sampling, using a Pfeiffer vacuum DUO 2.5A pump, with a capacity of 40 L min⁻¹ (STP: 20°C and 1 bar). Water samples were taken from 5 m depth (RV Polarstern cruises) or 10 m depth (RV L'Atalante cruise) using a 24-Niskin-bottle rosette with a volume of 12 L each. Sampling started immediately after return of the bottle rosette on-board and from a bottle dedicated to the H₂ measurements. The evacuated glass vessel was connected to the Niskin bottle by Teflon tubing, which was first rinsed with approximately 1 L surface water. Then, 8.4 L water streamed into the evacuated flask (Fig. 2), using a drip to enhance the dispersion of the sample water. After connection of the headspace-sampling unit, the lines were first evacuated and then flushed with a makeup gas several times. During the RV L'Atalante cruise a synthetic air mixture with an H₂ mixing ratio below threshold was used as makeup gas. The makeup gas used during the RV
Polarstern cruises was a synthetic air mixture with an H$_2$ mole fraction of (543.9±0.3) ppb and a δD of (93.1±0.2) ‰. The mole fraction of the makeup gas was determined by the Max Planck Institute for Biogeochemistry and is given on the MPI2009 scale (Jordan and Steinberg, 2011). The glass vessel was pressurized to approximately 1.7 bar absolute with the makeup gas and the total headspace (added makeup gas plus extracted gas from the water sample) was then flushed to a pre-evacuated sample flask. The flasks were of the same type as for the atmospheric sampling: 1 L borosilicate glass flasks (NORMAG), coated with black shrink-hose to minimize photochemical reactions inside and sealed with 2 Kel-F (PCTFE) O-ring sealed valves. All flasks were previously conditioned by flushing with N$_2$ at 50 °C for at least 12 hours and evacuated for at least 12 hours directly before use. The whole sampling procedure took around 15 minutes: (4.0±0.5) min flushing surface water to the evacuated glass vessel, (8.0±1.0) min to connect the glass vessel to the sampling unit and evacuate the lines, and (3.0±0.5) min to add and pressurize the glass vessel with the makeup gas and take the headspace sample. The surface water temperature was on average (0.9±0.6) °C higher than the air temperature. Given that most of the apparatus was at air temperature and that the headspace will adjust to ambient temperature relatively quickly during equilibration the air temperature was used for calculations. Since the temperature dependence of H$_2$ solubility is less than 0.3 % per K for seawater between 16 °C and 30 °C (as encountered here) and view of the large H$_2$ saturations (see below), the error associated with this assumption is negligible.

Flasks were stored in the dark until measurement. At the same location of headspace sampling also atmospheric samples were taken (Table 4).

2.4 Measurements

2.4.1 Atmospheric H$_2$ and δD (H$_2$) in discrete samples

The mole fraction and isotopic composition of molecular H$_2$ was determined using the experimental setup developed by Rhee et al. (2004) and described in detail in Walter et al. (2012, 2013) and Batenburg et al. (2011). The D/^{1}H molar ratio in a sample, $R_{\text{sample}}$($D$/H), is quantified as the relative deviation from the D/^{1}H molar ratio in a standard, $R_{\text{standard}}$($D$/H), as isotope delta δD value, and reported in per mill (‰):

$$\delta D = \frac{R_{\text{sample}}(D/H)}{R_{\text{standard}}(D/H)} - 1$$

(1)
The isotopic standard is Vienna Standard Mean Ocean Water (VSMOW). H₂ mole fractions are reported as mole fractions in nmol mol⁻¹, abbreviated ppb (10⁻⁹, parts per billion) and linked to the MPI2009 calibration scale for atmospheric hydrogen (Jordan and Steinberg, 2011). As working standards, atmospheric air from laboratory reference air cylinders and synthetic air mixtures were used (Walter et al. 2012, 2013, Batenburg et al. 2011); the H₂ mole fractions of the air in these cylinders were determined by the Max Planck Institute for Biogeochemistry, Jena, Germany. The atmospheric reference air and the synthetic isotope reference air were measured daily (atmospheric reference air at least twice) and results were used for correction of the sample measurements. The uncertainties reported here reflect random (i.e. repeatability) errors only and do not include possible systematic errors (Batenburg et al., 2011; Walter et al., 2012, 2013). Samples were measured in random order and analysed within 3 months (ANT-XXIV/4, ANT-XXV/5, ANT-XXVI/1) up to two years (ANT-XXVI/4) after sampling. Storage tests indicate that glass flasks equipped with Kel-F valves are stable for H₂ (Jordan and Steinberg, 2011). The mean measurement repeatability between the two measurements on the same flask was between ±3.2 ppb (ANT-XXV/5, n = 14) and ±6.4 ppb (ANT-XXVI/4, n = 108) for the mole fraction and ±3.4 ‰ (ANT-XXVI/4, n = 108) and ±5.0 ‰ (ANT-XXV/5, n = 14) for the isotopic composition.

H₂ and CO mole fractions were also measured by using a Peak Performer 1 RGA with synthetic air as a carrier gas, either continuously on-board (ANT-XXVI/4, see section 2.4.2) or from discrete flasks in the laboratory (ANT-XXV/5 and ANT-XXVI/1). The discrete RGA measurements were performed from the same glass flasks after measurement of the isotope system (see above). Due to a remaining slight overpressure in the flasks, an active pumping of the air into the RGA was not necessary and the flasks were simply connected to the RGA inlet by Teflon tubing. The remaining pressure was mostly sufficient to perform 8 to 10 measurements. A slight memory effect was observed and thus only the last 5 measurements were taken into account when stable. Samples with only three or less valid measurements were not used for evaluation. The standards were the same as those used for the isotope system. For both cruises (ANT-XXV/5 and ANT-XXVI/1), the mean measurement repeatability was better than ±0.8 % (H₂) and ±2 % (CO). A comparison between the H₂ mole fractions measured with the Peak Performer 1 RGA and the isotopic experimental setup reveals on average slightly lower RGA values of (7.5±23.8) ppb (see Fig. 3).
2.4.2 Atmospheric $\text{H}_2$ measured continuously

For the on-board continuous measurements of $\text{H}_2$ mole fractions a Peak Performer 1 RGA was used. The atmospheric air was drawn from the bridge deck to the laboratory in ¼ inch Decabon tubing. The CO mole fraction was also measured in the same measurement and will be reported here for information, but without further discussion.

In alternating order, 10 air samples and 10 aliquots of reference air were measured, using synthetic air as carrier gas. Due to small memory effects, only the last 5 measurements of each were taken into account when the values were stable. The mole fractions of $\text{H}_2$ and CO were calculated by using the mean of the enclosing standard measurements, with an estimated maximal error of ±5 %. For more details see Popa et al. 2014. The mean measurement repeatability for the air samples was ±1.7 % for $\text{H}_2$ and ±3.6 % for CO in ambient air, respectively ±0.8 % ($\text{H}_2$) and ±0.9 % (CO) for the reference air. Comparing the $\text{H}_2$ mole fractions measured continuously on the RGA with discrete samples measured on the isotope system and collected close in time, we found a mean offset of (–18.8±16.4) ppb for the RGA results.

2.4.3 Dissolved $\text{H}_2$ extracted from surface water

The discrete samples of extracted dissolved $\text{H}_2$ were measured as described for the discrete atmospheric samples in section 2.4.1. Details about assumptions and calculations to determine dissolved $\text{H}_2$ concentrations and isotope delta values and quantity symbols are given in detail in the Appendix.

Defining the extraction efficiency $\eta$ as

$$\eta = \frac{c_h V_h}{c_w V_w}$$

where $V_h$ and $V_w$ are the volume of the headspace and the water fraction, and $c_h$ the concentration of $\text{H}_2$ in the headspace. The initial concentration of $\text{H}_2$ in seawater, $c_{w0}$, can be calculated from

$$c_{w0} = \frac{c_h V_h}{\eta V_w}$$
The concentration in the headspace, \(c_h\), was not measured directly, but can be derived from the measured \(H_2\) mole fraction in the sampling flask. The sampling procedure following gas extraction under vacuum can be broken into three steps (see Methods section):

1. Expansion of the headspace into the gas transfer system
2. Addition of makeup gas
3. Expansion of the headspace / makeup gas mixture into a sample flask

As shown in the Appendix, the original concentration of \(H_2\) in seawater (in nmol L\(^{-1}\)) can be calculated using the following equation:

\[
c_{w0} = \frac{y_f \left[ \left( \frac{V_c}{V_h} \right) P_{H_2} - P_h(\text{H}_2O) \right]}{\eta \left( \frac{V_c}{V_h} \right) P_{H_2} - P_h(\text{air})} \tag{4}
\]

where \(y_f\) is the dry mole fraction of the air in the flask and \(y_m\) the mole fraction of the makeup gas = (543.9±0.3) ppb.

The extraction efficiency, \(\eta\) can be calculated from the following mass balance:

\[
V_c c_{w0} = V_c c_h + \alpha V_c c_h \tag{5}
\]

Assuming that headspace gas phase and water phase are in equilibrium, the ratio of the \(H_2\) concentration in water and in the headspace is given by the Ostwald coefficient (where the concentrations refer to in situ temperature):

\[
\alpha = \frac{c_h}{c_w} \tag{6}
\]

This gives for the extraction efficiency as defined in equation (2):

\[
\eta = \frac{1}{1 + \alpha \frac{V_c}{V_h}} \tag{7}
\]

In the present case, \(\alpha = \alpha(\text{H}_2)\) was equal to 0.0163±0.0001, which gives \(\eta = 92.12 (±0.013)\%\) for \(V_c/V_h = 8.4/1.6 = 5.25\).

Two alternative scenarios were considered to derive the \(\delta D\) of the dissolved \(H_2\), with scenario 1 assuming equilibrium isotopic fractionation between headspace and water, and scenario 2 assuming kinetic isotopic fractionation during extraction from Niskin bottle to glass vessel.

**Scenario 1:**

\[
\delta_{w0} = \delta_h + \epsilon (1 - \eta)(1 + \delta_h) \tag{8}
\]

**Scenario 2:**

\[
\delta_{w0} = \delta_h + \epsilon (1 - \eta)(1 + \delta_h) \tag{9}
\]
The equilibrium isotope fractionation between dissolved phase and gas phase is $\varepsilon = (37 \pm 1) \text{‰}$ at 20 °C [Knox et al., 1992].

Scenario 2: $\delta_{w0} = \frac{(1+\delta_h)\eta}{1-(1-\eta)^\varepsilon} - 1 \approx \delta_h + \varepsilon \left(1 - \frac{\ln(1-\eta)}{\eta}\right)$ (9)

The kinetic isotope fractionation during gas evasion is $\varepsilon_k = (-18 \pm 2) \text{‰}$ at 20 °C [Knox et al., 1992]. The approximation is not used and only shown to illustrate the small difference between $\delta_{w0}$ and $\delta_h$ when $\eta \approx 1$.

The temperature dependences of $\varepsilon$ and $\varepsilon_k$ are unknown and were neglected here.

The air saturation equilibrium concentration, $c_{sat}(H_2)$, was determined using the parameterization of Wiesenburg and Guinasso (1979). The $H_2$ saturation anomaly, $\Delta(H_2)$, was calculated as the difference between the measured $H_2$ concentration, $c(H_2)$, and $c_{sat}(H_2)$:

$$\Delta(H_2) = c(H_2) - c_{sat}(H_2)$$ (10)

Meteorological and oceanographic parameters (radiation, air and water temperatures, salinity, relative humidity) were measured using standard instrumentation and recorded and provided by the data system of the ships. More information about devices and sensor documentation can be found on the website of the Alfred Wegener Institute http://dship.awi.de/. Backward trajectories were calculated using the backward “Hybrid Single Particle Lagrangian Integrated Trajectory” (HYSPLIT, Schlitzer 2012) model of the National Oceanic and Atmospheric Administration (NOAA, http://ready.arl.noaa.gov/HYSPLIT.php).

### 2.5 Modeling

#### 2.5.1 TM5 model

We performed simulations of $H_2$ mole fractions and isotopic composition with the global chemistry transport model TM5 (Krol et al., 2005), and compared them with our measurement data (Fig. 5). The simulation setup was similar to the one of Pieterse et al. (2013) and only a short description is given here. The model version used employs the full hydrogen isotopic scheme from Pieterse et al. (2009) and uses ERA-Interim meteorological data. The chemistry scheme is based on CBM-4 (Houweling et al., 1998), which has been extended to include the hydrogen isotopic scheme (that is, for all chemical species that include hydrogen atoms, $H$ and $HD$ are treated separately and have different reaction rates). The $H_2$ sources and isotopic signatures are given as input; these and also the $H_2$ soil deposition velocities are identical to Pieterse et al. (2013).
The model has a relatively coarse spatial resolution of 6° longitude by 4° latitude, and a time step of 45 minutes. Daily average mole fraction fields are used for comparison to observations. The model results were interpolated to the time and location of the observations.

2.5.2 Global oceanic emissions

The climatological global oceanic emissions were calculated using the protocol of Pieterse et al. (2013), based on the GEMS database and an assumed mean oceanic H\(_2\) source of 5 Tg a\(^{-1}\) as given from global budget calculations (see Ehnhalt and Rohrer, 2009, and references therein, Pieterse et al. 2013). The spatial and temporal variability of oceanic H\(_2\) emissions caused by N\(_2\) fixation are adopted from the spatial and temporal distribution of oceanic CO (Erickson and Taylor, 1992).

3 Results and discussion

3.1 Atmospheric H\(_2\) transects

Our data set includes data of two hemispheres and two seasons between 2008 and 2010 (see Table 2, Fig. 4). The mean mole fraction of H\(_2\) ranged between (532.0±10.7) ppb and (548.5±6.8) ppb. In spring, the mean values were almost equal between the hemispheres with approximately 1 to 2 ppb difference, but they differed significantly in autumn. In this season, the mean values in the northern hemisphere (NH) were approximately 16 ppb or 3 % lower compared to the southern hemisphere (SH), with a distinct transition between the hemispheres at around 8° N. In contrast, δD differed significantly between the hemispheres in both seasons. In the southern hemisphere, absolute δD values were always between 9 and 27 ‰ higher than in the northern hemisphere, and generally remained within a narrow range between (140.5±21.1) ‰ and (145.4±5.3) ‰. In contrast to the mole fraction, isotope delta differences between the hemispheres were less pronounced in autumn than in spring. These two seasonal patterns, in the following defined as “summer signal” and “winter signal”, are mainly caused by biological processes and tropospheric photochemistry and driven by variations in the NH. They are in line with previously published data and model results (Rhee et al. 2006, Price et al. 2007, Rice et al. 2010, Pieterse et al. 2011, 2013, Batenburg et al. 2011, Yver et al. 2011, Yashiro et al. 2011).
The “summer signal”, observed in October, is characterized by lower H$_2$ mole fractions in the northern hemisphere and a less pronounced difference in $\delta$D between the hemispheres. Deposition by biological activity of microorganisms in the soils is the main sink of H$_2$ (Yonemura et al. 2000, Pieterse et al. 2013) and the sink strength in the northern and southern hemisphere depends on the distribution of landmasses and on season. With approximately 70% of landmasses in the NH and higher microbial activity in the summer, the mole fraction during this season is lower in the NH than in the SH. Due to the general preference of organisms for molecules with lighter isotopic composition, the $\delta$D values increase during summer in the NH and the interhemispheric gradient becomes less pronounced.

The “winter signal” observed in April is defined by almost equal mole fractions and more pronounced differences in $\delta$D values between the hemispheres. In winter, molecular hydrogen is accumulating in the NH hemisphere, and the main source is fossil fuel combustion with a depleted isotopic composition of $-170$ ‰ to $-270$ ‰ (Gerst and Quay, 2001; Rahn et al., 2002). This leads to nearly equal mole fractions in both hemispheres and a more pronounced $\delta$D gradient, with isotopically lighter H$_2$ in the NH. The contribution of source and sink processes in the SH to the seasonal patterns is less pronounced than for the NH (Pieterse et al. 2011, 2013). As a result, the H$_2$ seasonal cycle in the SH is much weaker compared to the NH. The SH isotopic H$_2$ signature is caused by mainly emissions and chemical loss with an isotope delta of approximately $+190$ ‰, which explains the generally higher $\delta$D values. The Intertropical Convergence Zone (ITCZ) separates the two hemispheres and is clearly visible, not only in the H$_2$ distribution, but also in the CO distribution.

Simulations of H$_2$ mole fractions and isotopic composition using the global chemistry transport model TM5 (Krol et al., 2005) compared with our atmospheric data reveal that the model simulates the H$_2$ mole fractions quite well (Fig. 5), with a slight overestimate of up to 20 ppb (which means up to 4 %). The model results are less variable on small spatial scales, due to the low spatial resolution, and possibly to local influences that are not included in the model (e.g. ocean emissions in the model are less variable in time and space than they could be in reality). The largest differences between the modeled and measured H$_2$ occur between 30° S and the equator. This seems a systematic feature and could be due to a slight overestimation of sources or underestimation of sinks by the model. Despite these small differences, the model is consistent with measured H$_2$ mole fractions and simulates them well. Large-scale features are
clearly visible, like the sharp gradient around 10° N during cruise ANT-XXVI/1 (Fig. 5, top, third plot), or the decrease in δD towards northern mid-latitudes (most evident for the cruises ANT-XXIV/4 and ANT-XXVI/4, first and last plots in Fig. 5, top). A slight overestimate of the H₂ mole fractions was also noted by Pieterse et al. (2013). This might be explained by an overestimate of photochemical sources in the model, which would influence only the mole fractions but not the δD values.

The model simulates the isotopic composition of H₂ even better than the mole fractions. The most important features are the general decrease from south to north, and the sharp gradient around the equator. As most sources and sinks of H₂ have very different isotopic signatures, this good comparison indicates that the model represents well both the magnitude and the isotopic signature of the main components of the H₂ cycle. Similar to Pieterse et al. (2013) we also observe a slight underestimate of the δD at high southern latitudes, which is possibly due to underestimating the isotopic composition assumed for H₂ returning from the stratosphere in the latitude band 60° S to 90° S.

### 3.2 Spatial and temporal high–resolution transects during ANT-XXV/5

In April 2009 the sampling resolution was increased to approximately one sample per two hours for five selected sections of the transect during ANT-XXV/5 (Fig. 4, Table 3): three in the southern hemisphere, one crossing the equator and one in the northern hemisphere. These transects were chosen based on previously published data (Herr et al. 1984, Conrad and Seiler, 1988) and with the aim to get an indication of small–scale sources or diurnal cycles of atmospheric H₂ for further investigations.

All transects showed neither a diurnal cycle nor a correlation with radiation and a range of δD values within or only slightly outside a 2 σ range around the mean, except for the one between 23.5° S to 15.7° S (Fig. 6a). Here the highest H₂ mole fractions of (631.9±3.2) ppb, combined with the lowest δD values of (20.9±5.0) ‰, were found around 16° S. Due to the limited spatial resolution and therefore low number of data points a Keeling plot analysis (Fig. 6b) of the data between 15° S and 18° S was made with either 5, 7, or 9 data points to get a reasonable range for the source signature. It reveals a mean source signature of ~561.5 in a range of ~530 ‰ to ~683 ‰ (n = 7±2, \( R^2 = 0.85±0.01 \)). The correlation coefficient is a mean of the three analyses.
HYSPLIT trajectories for the samples collected on this transect during the 28 April 2010 and 1 May 2010 (21.8° S to 15.7° S) reveal the same origin of air masses coming from the direction of Antarctica. Oceanographic parameters such as water temperature and salinity are similar and do not correlate with H₂ mole fractions and δD values. These findings indicate a strong but local source, and the low δD value for the source obtained by the Keeling plot analysis points to biological production (Walter et al. 2012). Such local and temporal patchiness of high H₂ mole fractions in surface waters was reported previously in correlation to high N₂ fixation rates (Moore et al. 2009, 2014). Although reported for other oceanic regions the H₂ mole fractions and δD values here do neither show a diurnal cycle (Herr et al. 1984) nor they are correlated with radiation indicating photochemical production (Walter et al. 2013), and most of the values were observed during night. Wilson et al. (2013) recently showed that H₂ production and uptake rates clearly depends on microbial species, and also on their individual day–night rhythm, but the contribution of different diazotrophs to the marine H₂ cycle is unknown (e.g. Bothe et al., 2010; Schütz et al., 2004; Wilson et al., 2010a and 2010b; Punshon and Moore, 2008b; Scranton 1983, Moore et al., 2009).

Around 21.2° S one single sample with a low mole fraction of (393.9±3.2) ppb in combination with a high δD of (322.45±5) ‰ value was observed. As mentioned before HYSPLIT models reveal the same origin of air masses on this transect, thus this sample indicates probably a local sink. However, this interpretation depends on only one single measurement point and although neither instrumental parameters indicated an outlier nor meteorological or oceanographical parameters differed from other samples, we cannot exclude an artefact due to sampling, storage, or analyses. A simple Rayleigh fractionation model reveals a fractionation factor of $\alpha = 0.646±0.002$, which is close to the value of oxidation by HO $\cdot$ ($\alpha = 0.58±0.07$, Batenburg et al. 2011). An estimate of the δD value by using an HO oxidation fractionation factor would lead to an increase by 125 ‰ or 149 ‰, respectively. The observed increase of δD seems reasonable when assuming oxidation by HO $\cdot$, but with respect to the HO mole fraction and the slow reaction rate of H₂ + HO $\cdot$ it is questionable whether the H₂ decrease here can be explained by this.

### 3.3 Dissolved H₂

A new method has been presented to extract H₂ from surface waters for isotopic determination. Before discussing the measurement results, we will give an overview of the
possible main errors and their effects. To show the effect of the errors on the measurements, we will present error factors, thus how much the final data differ by shifting the respective parameter by 1% and also the absolute assumed error.

For the extraction method several error sources could be identified: the determination of pressure, especially in the sampling vessel before adding the make–up gas and during extraction, the temperature of air and water, respectively the difference between them when the sample is extracted from the headspace, and the volume of the set–up and the sample. The determination of pressure in the sampling vessel would be one issue of further improvement, because the error caused by pressure deviations for the total pressure after adding the make–up gas is about a factor of 0.7 for concentrations and 0.2 for the isotopic values. The error based on temperature of air, water and sample is negligible due to high–precision measurements and the short handling time between water sampling and headspace extraction. The error for the volume parameter for the set–up is negligible due to the high volume, the precise determination of the glass vessel volume by weighing, and the calculation of the tubing volume. The main error source is the water volume of the sample, which counts by a factor of 5.9 for the concentration, but with negligible effect on the isotopic values. Although the relative error factor is quite high the absolute value is assumed to be around 0.5% due to the sample size, which has also been weighed at the home lab. The H\textsubscript{2} measurement procedure is the same as for atmospheric samples and possible errors are described in the respective sessions or related literature. However, the error caused by the determination of the dry mole fraction itself seems to have the main input by a factor of 5.3 for concentration and 4.6 for the isotopic values of dissolved H\textsubscript{2}. Errors of the determination of the isotopic value are much less significant and count by a factor of 0.2.

Taking measurement and handling errors during the extraction as well as errors in the determination of the dry mole fraction into account, we assume a robust overall uncertainty of ±6.9% for the dissolved H\textsubscript{2} mole fractions and ±4.7% for the isotopic values by calculating the root of the sum of the squared uncertainties. As shown in Table 4 we also tested the effect of equilibrium isotopic fractionation and kinetic isotopic fractionation. The effect is less than 0.2%.

Therefore, recommendations for the extraction method are to additionally measure parameters such as the initial pressure in the glass vessel and to ensure a precise determination of the
sample volume. Besides this we recommend high-precision IRMS measurements and to consider multiple sampling for better statistics on the data.

3.3.1 H₂ concentration

In total 16 headspace samples were taken during the RV Polarstern cruise in April / May 2010 along the transect 32.53° W / 18.79° S to 13.00° W / 36.54° N and 6 samples during the RV L’Atalante cruise in February 2008 between 23.00 – 17.93° W to 16.9 – 19.2° N to analyse the H₂ mole fraction and the isotopic composition (see Table 4).

Although our setup was a prototype with possibilities for improvement, the mole fractions are in line with previously published data. The H₂ excess, Δ(H₂), exceeds 5 nmol L⁻¹, the saturation differ from close to equilibrium to 15-fold supersaturation. Highest supersaturation was found in the southern hemisphere between 16° S and 11° S and in the northern hemisphere around the Cape Verde islands and the coast of Mauritania (Fig. 7a, Table 4).

Herr et al. (1984) reported patchy enhanced H₂ concentrations in the surface water with up to 5-fold supersaturation in the subtropical south Atlantic (18 – 31° W and 29 – 42° W). This is comparable to what Conrad and Seiler (1988) found in the Southern Atlantic, on a similar cruise track as the RV Polarstern. Around the equator they measured H₂ surface water concentrations up to 12-fold supersaturation. In the Southern Pacific, Moore et al. (2009) combined H₂ surface water measurements with N₂ fixation measurements. They reported a strong correlation between these parameters, a patchy distribution and a steep maximum of H₂ concentrations up to 12.6 nmol L⁻¹ around 14° S.

The recently published data by Moore et al. (2014) show similar patterns across the Atlantic as we found, with highest values around the southern and northern subtropics. However, our saturations are lower than the ones given by them, especially in the northern hemisphere. Such differences might be caused by experimental issues such as overestimated extraction efficiency or can be due to real temporal variability as the sampling seasons differed. The extraction efficiency has been estimated as 92.12 (±0.013)% (see Appendix) and was incorporated into the calculation of the original seawater concentration. With respect to the assumption of biological production as main production pathway it is more likely that due to the different sampling seasons less H₂ was produced in April than in October / November because of less microbial activity especially on the northern hemisphere in boreal winter.
3.3.2 Isotopic composition of H$_2$

Additional information about H$_2$ sources comes from the analysis of the H$_2$ isotopic composition. In the literature only one experimental value of dissolved marine δD exists, δD = −628 ‰ (Price et al. 2007, Rice et al. 2010), but the origin of this value is unclear and it is based on unpublished data. Nevertheless, this value has been used as representative for oceanic emission in several global budget calculations (e.g. Price et al. 2007, Pieterse et al. 2011). Other authors (e.g. Rahn et al. 2003, Rhee et al. 2006) used a theoretical value of −700 ‰, as expected for thermodynamic isotope equilibrium between H$_2$ and H$_2$O based on the calculations of Bottinga (1969). The results presented here are the first well-documented experimental results for isotope analysis of dissolved H$_2$ in seawater.

From the measurement of the isotopic composition of H$_2$ in the headspace we calculate the isotopic composition of H$_2$ that was originally dissolved in the sea water as described in section 2.4.3 and in the Appendix, using two different assumptions for fractionation between dissolved H$_2$ and H$_2$ in the gas phase. The results shown in Table 4 reveal δD values for the dissolved H$_2$ that vary within a wide range of −112 ‰ to −719 ‰ for both fractionation scenarios. Interestingly, δD shows two distinct groups of samples that can be separated by the water temperature (Fig. 7b). In water masses with a temperature above 21 °C the δD values are (−629±54) ‰ (n = 14), in water masses with a temperature of 20 °C or below δD values are (−249±88) ‰ (n = 8). There is no correlation of δD with salinity (Fig. 7c), but the high temperature (and low δD) waters show also a generally higher saturation than the low temperature (high δD) waters (Fig. 7d).

The very depleted isotope signature of the H$_2$ in the warmer water masses is consistent with the values expected for biological production. The slight enrichment compared to the value of ≈−700‰ that is expected for biologically produced H$_2$ in equilibrium with ocean water (Bottinga, 1969, Walter et al., 2012) may be caused by a partial consumption within the water, which would enrich the remaining fraction. The relatively smooth distribution of the isotopic composition of H$_2$ in the atmosphere strongly indicates that the contribution from atmospheric variability cannot be a main contributor of the isotope variations observed in dissolved H$_2$, even within the group of the depleted samples.

To our knowledge this is the first time that oceanic production of H$_2$ has been directly attributed to biological processes by using isotope techniques. For the samples collected from warm surface waters, our results verify the general assumption of a biological production.
process as a main source of oceanic H\textsubscript{2} to the atmosphere rather than photochemical or other sources (Herr et al. 1981, Conrad, 1988; Punshon and Moore, 2008, Moore et al. 2009). The dominance of biological formation at higher temperatures is qualitatively consistent with the general understanding of the temperature dependence of N\textsubscript{2} fixation rates for N\textsubscript{2} fixers such as e.g. Trichodesmium spec., which exhibit highest N\textsubscript{2} fixation rates within a temperature range between 24 °C to 30 °C (Breitbarth et al. 2007, Stal 2009). In fact, the saturations also show a correlation with temperature, but less clear than for \(\delta D\) (Fig. 7d), presumably due to simultaneous uptake and consumption processes in a complex microbial community.

However, this clear attribution is only valid in water masses with higher temperatures and the unexpectedly high \(\delta D\) values in cooler waters indicate the influence of other processes. The isotopic enrichment that is expected for removal of H\textsubscript{2} (Chen et al., 2015, Rahn et al., 2003, Constant et al., manuscript in preparation) is highly unlikely to cause a shift of almost 400 ‰ in \(\delta D\) from an assumed pure biological source, because in this case the removed fraction would have to be unrealistically large, as also recently argued for soil emitted H\textsubscript{2} (Chen et al., 2015). We suggest that a source of H\textsubscript{2} must exist in these surface waters, which produces H\textsubscript{2} that is out of isotope equilibrium with the water. This can be either one single source with an isotopic signature of approximately -250 ‰, or an even more isotopically enriched source that mixes with the depleted biological source.

Punshon and Moore (2008a, and references therein), reported abiotic photochemical H\textsubscript{2} production from CDOM and small organic compounds such as acetaldehyde or syringic acid. Walter et al. (2013) indicated, that biologically active regions such as the Banc d’Arguin at the coast of Mauritania could act as a pool of precursors such as VOCs for atmospheric H\textsubscript{2} with high \(\delta D\) values. It is thus possible that abiotic photochemical production in the surface water might be an alternative source of H\textsubscript{2} excess, which is not isotopically equilibrated with water, especially in regions with high radiation and biological activity, and less N\textsubscript{2} fixation.

Given the fact that the two groups of warm and cold waters are relatively well separated and there is not a continuous mixing curve between two end members, the explanation of a single different source seems more straightforward. Isotope analyses are a powerful tool to distinguish this source from biological production. Additional measurements are needed to determine the isotopic signature of such a source and investigate to which extend photochemical production contributes to the oceanic H\textsubscript{2} budget in colder water masses, and also update the current models. However, with an isotopic signature of approximately -250
‰, or an even more isotopically enriched, such a source would not significantly impact the current models.

Based on their $H_2$ measurements, Moore et al. (2014) suggested a substantial underestimation of oceanic $N_2$ fixation, especially due to high $H_2$ supersaturations measured in the southern hemisphere. By using direct measurements of $N_2$ fixation rates a systematic underestimation by approximately 60 % was also proposed by Großkopf et al. (2012) who suggested a global marine $N_2$ fixation rate of $(177 \pm 8)$ Tg N a$^{-1}$. In order to identify a possible significant mismatch between $N_2$ fixation rates and total marine $H_2$ production, we calculated the climatological global oceanic emissions from the GEMS database using the protocol of Pieterse et al. (2013), and an assumed mean oceanic $H_2$ source of 5 Tg a$^{-1}$ as given from global budget calculations. The estimated emission rates and distributions in the Atlantic Ocean (Fig. 8) are in line with the calculations of Moore et al. (2014), who reported $H_2$ sea-to-air fluxes mostly in the range of $(10 \pm 5)$ mmol m$^{-2}$ a$^{-1}$ and an almost equal distribution between the hemispheres.

Westberry and Siegel (2006) estimated the global nitrogen fixation rate by *Trichodesmium* blooms by using satellite ocean color data at 42 Tg N a$^{-1}$ and an additional 20 Tg N a$^{-1}$ under non–bloom conditions, suggesting that *Trichodesmium* is likely the dominant organism in the global ocean new nitrogen budget. The good agreement between our measurements of $H_2$ concentrations and $\delta D$ and the model results from the TM5 model indicate that the oceanic emissions of $H_2$ to the atmosphere are actually well represented in current atmospheric models (Pieterse et al. 2013 and references herein). The proposed underestimate of oceanic $N_2$ fixation and a possible additional $H_2$ release during this process seems already be incorporated in the current atmospheric budgets of $H_2$. Thus, supposing that both an assumed total oceanic $H_2$ source of 5 Tg a$^{-1}$ to the atmosphere and a total global nitrogen fixation rate of approximately $177$ Tg N a$^{-1}$ are correct, our calculations clearly support the suggestion of Großkopf et al. (2012) that $N_2$ fixers other than *Trichodesmium* have been severely underestimated in the global picture and that the oceanic release ratio of $H_2$ to fixed $N_2$ clearly needs more attention. Besides *Trichodesmium*, several other $N_2$-fixing organisms are known for their potential to produce hydrogen (Wilson et al., 2010a; Falcón et al., 2002, 2004; Zehr et al., 2001; Kars et al., 2009; Barz et al., 2010), and even non-$N_2$-fixing organisms might play a role (Lilley et al. 1982).
4 Conclusions

Identifying sources is important to consider budgets and gain insight in production and consumption processes. Although H₂ has been assumed reasonably to be produced mainly biologically in the oceans, direct evidence was lacking. Our results verify a biological production as a main source of H₂ in oceanic surface water, especially in warmer water masses. As seen from the transects, local sources are difficult to spot due to their patchiness, this should be taken into account when planning the sampling strategy.

The unexpectedly high δD values in colder temperate water masses indicate the significant influence of processes other than biological production, and additional information e.g. by isotopic composition is needed to distinguish and verify possible sources and supersaturations of dissolved oceanic H₂. Especially the investigation of the isotopic composition of possible production pathways such as abiotic photochemical H₂ production needs further attention and should be an upcoming issue.

The pattern of mole fractions and isotopic composition of H₂ along a north–south Atlantic transect clearly depends on season and hemisphere and are consistent with previous published data and models. A possible significant underestimation of N₂ fixation as assumed by several authors could – providing a net H₂ release rate – go along with higher H₂ emissions. However, a comparison with the TM5 model and the calculation of the climatological global oceanic emissions based on GEMS database reveal that the oceanic contribution to the global H₂ budget is reasonable and in general reproduced well and therefor a proposed underestimation in the oceanic N₂ fixation seems already be corrected (from atmospheric considerations) in the current atmospheric budgets of H₂. This also indicates, with respect to the proposed source different than biological production in colder temperate water masses, that such a source would probably not significantly impact the current models.

Besides the isotopic composition of photochemically produced H₂ the composition of N₂ fixer communities and the release ratio of H₂ to N₂ fixed needs more investigation to understand the general processes and distributions of oceanic H₂ in more detail.

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InGOS (GA number 284274), and the BMBF (Bundesministerium für Bildung und Forschung) project SOPRAN, grant FKZ 03F0462, grant 03F0611 and grant 03F0662.
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tropospheric molecular hydrogen budget using atmospheric observations and variational

Zehr, J.P., Waterbury, J.B., Turner, P.J., Montoya, J.P., Omorogie, E., Steward, G.F., Hansen,
Table 1: Overview of sample distribution during the cruises: type A are discrete atmospheric samples, type H are headspace samples extracted from the surface water. The sample numbers in brackets give the number of measured samples in the northern (NH) and southern (SH) hemisphere.

<table>
<thead>
<tr>
<th>Cruise</th>
<th>Date</th>
<th>Position (start – end)</th>
<th>Nr. of Samples (NH / SH)</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANT-XXIV/4</td>
<td>18.04. – 20.05.2008</td>
<td>59.15 °W / 46.13 °S – 06.21 °W / 47.96 °N</td>
<td>95 (44 NH / 51 SH)</td>
<td>A</td>
</tr>
<tr>
<td>ANT-XXV/5</td>
<td>11.04. – 24.05.2009</td>
<td>50.99 °W / 40.82 °S – 23.05 °W / 16.55 °N</td>
<td>91 (30 NH / 61 SH)</td>
<td>A</td>
</tr>
<tr>
<td>ANT XXVI/1</td>
<td>16.10. – 25.11.2009</td>
<td>12.05 °W / 37.96 °N – 47.28 °W / 37.43 °S</td>
<td>60 (29 NH / 31 SH)</td>
<td>A</td>
</tr>
<tr>
<td>ANT XXVI/4</td>
<td>07.04. – 17.05.2010</td>
<td>58.14 °W / 43.75 ° S – 04.46 °E / 53.15 °N</td>
<td>114 (56 NH / 58 SH)</td>
<td>A</td>
</tr>
<tr>
<td>L’Atalante ATA-3</td>
<td>03.02. – 20.02.2008</td>
<td>17.83 °N / 16.56 °W – 17.60 °N / 24.24 °W</td>
<td>6 (6N H / 0 SH)</td>
<td>H</td>
</tr>
<tr>
<td>Cruise</td>
<td>IRMS – $\text{H}_2$ mole fraction [ppb]</td>
<td>Southern Hemisphere</td>
<td>IRMS – $\text{H}_2$ mole fraction [ppb]</td>
<td>Northern Hemisphere</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------</td>
<td>------------------</td>
<td>----------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>ANT- XXI/4</td>
<td>543.4 ± 7.3</td>
<td>145.4 ± 5.3</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>April 2008</td>
<td>528.8 – 568.5</td>
<td>135.4 – 155.7</td>
<td>(2 values excluded)</td>
<td>(2 values excluded)</td>
</tr>
<tr>
<td>ANT- XXV/5</td>
<td>553.9 ± 38.7</td>
<td>140.8 ± 21.1</td>
<td>520.4 ± 24.0</td>
<td>59.9 ± 17.7</td>
</tr>
<tr>
<td>April 2009</td>
<td>350.2 – 631.9</td>
<td>20.9 – 166.1</td>
<td>432.5 – 548.1</td>
<td>41.6 – 119.6</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>60</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>ANT</td>
<td>548.5 ± 6.8</td>
<td>143.2 ± 4.2</td>
<td>546.4 ± 7.4</td>
<td>59.9 ± 10.5</td>
</tr>
<tr>
<td>XXVII/1</td>
<td>535.9 – 563.4</td>
<td>135.5 – 149.3</td>
<td>531.4 – 563.0</td>
<td>47.7 – 85.8</td>
</tr>
<tr>
<td>October 2009</td>
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<td>30</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>ANT</td>
<td>541.6 ± 16.3</td>
<td>143.7 ± 11.5</td>
<td>523.1 ± 20.1</td>
<td>47.2 ± 8.8</td>
</tr>
<tr>
<td>XXVII/4</td>
<td>496.0 – 579.6</td>
<td>89.3 – 161.8</td>
<td>481.5 – 606.8</td>
<td>36.2 – 121.8</td>
</tr>
<tr>
<td>April 2010</td>
<td>58</td>
<td>58</td>
<td>617</td>
<td>617</td>
</tr>
</tbody>
</table>

Cruise: Antartic cruise.
IRMS: Isotopic ratio mass spectrometry.
RGA: Radiocarbon gas analyze.

TABLE 2: Hemispheric means of atmospheric $\text{H}_2$ and its isotopic composition along the four meridional Atlantic transects.
Table 3: Overview of means of atmospheric H\textsubscript{2} and its isotopic composition along the five high–resolution transects of AN\textsuperscript{T}-XXV/5, including the standard deviation and the range

<table>
<thead>
<tr>
<th>Transect (latitude)</th>
<th>Mole fraction [ppb]</th>
<th>δD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.8° S / 38.9° S</td>
<td>mean 515.5±37.7</td>
<td>141.4±6.2</td>
</tr>
<tr>
<td></td>
<td>range 448.4 – 566.9</td>
<td>129.3 – 151.0</td>
</tr>
<tr>
<td>33.0° S / 30.8° S</td>
<td>mean 521.4±33.3</td>
<td>152.9±3.9</td>
</tr>
<tr>
<td></td>
<td>range 350.2 – 551.9</td>
<td>142.8 – 166.1</td>
</tr>
<tr>
<td>23.5° S / 15.7° S</td>
<td>mean 536.9±38.4</td>
<td>144.1±41.4</td>
</tr>
<tr>
<td></td>
<td>range 392.9 – 631.9</td>
<td>20.91 – 322.45</td>
</tr>
<tr>
<td>2.0° S / 3.2° N</td>
<td>mean 537.3±36.2</td>
<td>119.5±12.6</td>
</tr>
<tr>
<td></td>
<td>range 466.9 – 592.2</td>
<td>89.1 – 135.5</td>
</tr>
<tr>
<td>9.9° N / 16.2° N</td>
<td>mean 537.0±12.2</td>
<td>122.5±3.0</td>
</tr>
<tr>
<td></td>
<td>range 511.0 – 560.3</td>
<td>118.4 – 131.0</td>
</tr>
</tbody>
</table>
Table 4: overview of headspace sample results from the ANT-XXVI/4 cruise (2010) and the L’Atalante ATA-3 (2008). $\delta_2$ is the measured supersaturation of the headspace in parts per billion (ppb = mole mole$^{-1}$). $\chi_2$ is the corresponding atmospheric mole fraction in ppb, $\delta_0$ and $\delta_0$ sat is the measured isotopic composition in permil [%]. The H$_2$ equilibrium concentration $c_2$(H$_2$) was determined by using the equations from Winstead and Guazzetti (1979), the initial dissolved H$_2$ concentration $c_{20}$ is calculated as given in Appendix 1, and the excess $\Delta$H$_2$ is the difference between them. $\delta_2$ sat and $\delta_2$ atm show the two scenarios to derive the initial isotope delta of dissolved H$_2$. $\delta_2$ atm is the concentration of H$_2$ in the surface water. The calculated extraction efficiency was $92\%$ (±0.01%). The calculations are given in the Appendix in more detail.

<table>
<thead>
<tr>
<th>Date / Time (UTC)</th>
<th>Sampling position</th>
<th>$\chi_2$ [ppb]</th>
<th>$\delta_0$ [‰]</th>
<th>$\delta_2$ [‰]</th>
<th>$\delta_2$(H$_2$) [ppb]</th>
<th>$c_2$(H$_2$) [mol L$^{-1}$]</th>
<th>$\delta_{20}$ [‰]</th>
<th>$\delta_{20}$(H$_2$) [ppb]</th>
<th>$\delta_{20}$(H$_2$) [mol L$^{-1}$]</th>
<th>$\delta_{20}$ atm [‰]</th>
<th>$\delta_{20}$ atm [ppb]</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.02.2008 16:05</td>
<td>-16.82° E</td>
<td>527.2</td>
<td>118.4</td>
<td>141.7</td>
<td>-224.09</td>
<td>0.35</td>
<td>1.57</td>
<td>1.22</td>
<td>-221.8</td>
<td>-221.0</td>
<td></td>
</tr>
<tr>
<td>16.02.2008 17:58</td>
<td>-16.81° E</td>
<td>538.5</td>
<td>115.3</td>
<td>550.4</td>
<td>-383.39</td>
<td>0.56</td>
<td>5.91</td>
<td>5.54</td>
<td>-381.6</td>
<td>-380.0</td>
<td></td>
</tr>
<tr>
<td>17.02.2008 17:03</td>
<td>-16.81° E</td>
<td>507.9</td>
<td>120.0</td>
<td>20.3</td>
<td>-100.51</td>
<td>0.37</td>
<td>0.50</td>
<td>0.12</td>
<td>-179.0</td>
<td>-179.0</td>
<td></td>
</tr>
<tr>
<td>18.02.2008 17:41</td>
<td>-16.69° E</td>
<td>458.4</td>
<td>120.0</td>
<td>31.0</td>
<td>-218.73</td>
<td>0.37</td>
<td>0.72</td>
<td>0.35</td>
<td>-217.3</td>
<td>-216.5</td>
<td></td>
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Figure captions

Figure 1: a) cruise tracks of the RV Polarstern, dots indicate positions of discrete atmospheric air sampling, b) positions of surface water headspace sampling during ANT-XXVI/4 (n = 16, green dots) and the RV L’Atalante ATA-3 cruise (n = 6, black dots)

Figure 2: Experimental setup for headspace sampling, a) sampling of the surface water into the glass vessel, connected to the Niskin bottle rosette, b) scheme of the experimental setup

Figure 3: Comparing the H2 mole fractions [ppb] measured with the isotopic experimental setup (x-axis) and the Peak Performer 1 RGA (y-axis) during ANT-XXVII/3 (red labeled) and ANT-XXV/3 (yellow labeled), y = 0.979x + 3.96, R² = 0.81, n = 147

Figure 4: δD (H2) [%] (first column), H2 mole fraction [ppb] (second column), and CO mole fraction [ppb] (third column), along the meridional cruise tracks of RV Polarstern, the mole fraction and δD of H2 are measured by IRMS, the CO mole fraction by RGA

Figure 5: Comparison of measurement results of H2 and CO mole fractions and δD with TM5 model results (given in red). Data are shown against latitude. The blue markers show results of flask samples, the green markers represent the continuous in-situ measurements (performed with the peak performer instrument on-board). CO has not been analysed in the flasks sampled during the last cruise. The model data were interpolated at the place and time of sampling or measurements.

Figure 6: a) H2 mole fraction [ppb] (black) and δD [%] (red) along the ANT-XXV/5 high-resolution transect 24° S -15° S; b) Keeling plot of the samples along the high-resolution transect north of 18° S. The three trend lines indicate the range of the Keeling plot analysis that was applied to determine the source signature.

Figure 7:

a) H2 saturation in the surface water (color coded) along the RV Polarstern cruise track of ANT-XXVI/4 and the RV L’Atalante cruise ATA-3, with maxima around the Cape Verde islands and 10-15° S. Note: each sample is represented by a single dot.

b) Comparing the δD (H2) at different water temperatures, the respective H2 saturation are color coded, sample dots marked with a diamond belong to the RV L’Atalante cruise, sample dots without to the ANT-XXVI/4 cruise; y = -35.2x + 360.9, R² = 0.66, n = 22

c) Distribution of δD (H2) (color coded) in correlation between water temperature and salinity

d) Correlation between water temperature and δD saturation, the δD (H2) color-coded, the exceptional high saturation has been excluded from the correlation calculation, y = 0.26x - 2.79, R² = 0.22, n = 21

Figure 8: Oceanic H2 emissions used in the TM5 model simulations (mmol m⁻² s⁻¹), based on the distribution provided by the project GEMS (Global and regional Earth-system (Atmosphere) Monitoring using Satellite and in-situ data) and scaled to a total oceanic source of 5 Tg a⁻¹ (Pieterse et al. (2013))
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b) Comparing the $\delta^D (\text{H}_2)$ at different water temperatures, the respective $H_2$ saturations are color coded, sample dots marked with a diamond belong to the RV L’Atalante cruise, sample dots without to the ANT-XXVI/4 cruise; $y = -35.2x + 360.9$, $R^2 = 0.66$, $n = 22$
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Interactive comment on “Isotopic evidence for biogenic molecular hydrogen production in the Atlantic Ocean” by S. Walter et al.

S. Walter et al.
s.walter@uu.nl

Received and published: 20 November 2015

Dear referee, thanks for your comments. Please find our reply in-line. Kind regards, S. Walter

Major Comments: Page 16440-42, Section 2.4.3, diss. H2 measurements: Since the extraction method is new and presented for the first time, I would like to see a discussion about the overall measurement errors of both the diss H2 conc. and its isotope signature in seawater. Reply: The following discussion has been included in the manuscript: A new method has been presented to extract H2 from surface waters for isotopic determination. Before discussing the measurement results, we will give an overview of the possible main errors and their effects. To show the effect of the errors...
on the measurements, we will present error factors, thus how much the final data differ by shifting the respective parameter by 1 % and also the absolute assumed error. For the extraction method several error sources could be identified: the determination of pressure, especially in the sampling vessel before adding the make–up gas and during extraction, the temperature of air and water, respectively the difference between them when the sample is extracted from the headspace, and the volume of the set–up and the sample. The determination of pressure in the sampling vessel would be one issue of further improvement, because the error caused by pressure deviations for the total pressure after adding the make–up gas is about a factor of 0.7 for concentrations and 0.2 for the isotopic values. The error based on temperature of air, water and sample is negligible due to high–precision measurements and the short handling time between water sampling and headspace extraction. The error for the volume parameter for the set–up is negligible due to the high volume, the precise determination of the glass vessel volume by weighing, and the calculation of the tubing volume. The main error source is the water volume of the sample, which counts by a factor of 5.9 for the concentration, but with negligible effect on the isotopic values. Although the relative error factor is quite high the absolute value is assumed to be around 0.5% due to the sample size, which has also been weighed at the home lab. The H2 measurement procedure is the same as for atmospheric samples and possible errors are described in the respective sessions or related literature. However, the error caused by the determination of the dry mole fraction itself seems to have the main input by a factor of 5.3 for concentration and 4.6 for the isotopic values of dissolved H2. Errors of the determination of the isotopic value are much less significant and count by a factor of 0.2. Taking measurement and handling errors during the extraction as well as errors in the determination of the dry mole fraction into account, we assume a robust standard deviation of ±6.9 % for the dissolved H2 mole fractions and ±4.7 % for the isotopic values by calculating the root of the sum of the squared uncertainties. As shown in Table 4 we also tested the effect of equilibrium isotopic fractionation and kinetic isotopic fractionation. The effect is less than 0.2%. Therefore, recommendations for the extraction
method are to additionally measure parameters such as the initial pressure in the glass vessel and to ensure a precise determination of the sample volume. Besides this we recommend high-precision IRMS measurements and to consider multiple sampling for better statistics on the data. Absolute error assumptions:

- $\Delta$ T air–sea: 0.3% 
- Pressure sensor: 0.5% 
- Temperature air: 0.01°C 
- Temperature water: 0.01°C 
- V vessel: 0.5% 
- V tubing: 10% 
- IRMS measurements: 1%

p.16441, Eq.(6): H2 solubility in seawater is also depending on the salinity, thus, I am wondering why the ‘salt effect’ is not considered in Eq.6. Reply: The actual salinity has been taken into account in the concentration calculations.

p.16441, l. 16; Eq.(7): p.16449, l.6: The extraction efficiency is given as 92%. Is this a mean value? The extraction efficiency according to Eq.(7) is depending on the Ostwald solubility coefficient which, in turn, depends on the temperature. Was the temperature always the same? Please give details. Reply: the mean value is 92.12 (±0.013)%. Due to the very low variation we mentioned just one value without decimal places. We included this to the manuscript for completeness.

p.16441, l.16: Please cite a reference for the Ostwald coefficient. Reply: we included a reference for the Ostwald coefficient.

p.16448, l.13-15: ‘... possibilities for improvements ...’ are mentioned. However, what does that mean for the analytical error of the presented data? Or in other words are they just by chance in agreement with the literature data? Reply: The possible improvements include a better monitoring of additional data such as temperature and pressure in the vessel itself, this was not possible yet. As already mentioned in chapter 2.3, the temperature dependence of the H2 solubility is quite low with less than 0.3% per K, and also the used pressure sensors (Omega, PAAR21R) work within a low error range of 0.5% or even below. In combination with the high extraction efficiency and the standardized sampling procedure over the two cruises, we are convinced that the presented data sets are comparable and not just by chance in agreement with the
literature data. Please see the discussion about possible error sources above.

Table 4: I was surprised to see a positive (i.e. excess) H2 concentration differences resulting in undersaturations of 0.35 and 0.94. That does not seem to be logical at all: How can a concentration excess of H2 result in a H2 undersaturation? Please explain. Reply: We gave the supersaturation factor, but changed this to saturation in % as also suggested by referee #2 to avoid misunderstandings.

Discussion of oceanic sources of H2 (see Section 3.3.1; Table 4; Figure 7): It is obvious that the majority of H2 data pool associated with the low temperatures has been measured in the coastal upwelling system off Mauritania. Upwelled waters off Mauritania, however, are originating from water depths down to 150-200m (i.e. upper oxygen minimum zone, OMZ). Thus, it is not that surprising to see a different H2 signature because in OMZ waters there might different sources/sinks at work: E.g., the interplay between H2 production (i.e. by N2 fixation at depth) and associated H2 oxidizing bacteria (which live at oxic/anoxic interfaces in particles and sediments). Reply: We showed in Walter et al. 2012, that bacteria and algae species with quite different H2 pathways (C. saccharolyticus, A. brasiliensis, E. coli, C. acetobutylicum and C. reinhardtii) produce H2 with similar, highly depleted isotopic signatures of around –700 ‰. The tested species included a N2-fixer as well as fermentative bacteria and also a limnic green alga. As mentioned (Chen et al. 2015) an isotopic enrichment causing a shift of almost 400‰ just by removal of H2 is unlikely due to unrealistic large fractions needed to be removed. Although OMZ waters and borders are known as microbial very active areas and we therefore cannot exclude such high turn–over rates completely, we assume another source responsible for the differing isotopic signature. As suggested in Walter et al. 2013, photochemical degradation of VOCs could play a role. The up–welling areas are quite productive and could indirectly emit pre-cursors of H2. Unfortunately, up to now there are no data available about the isotopic signature of photochemically produced H2 in surface waters. The value given for atmospheric photochemically produced H2 is around +100 to +200‰ and would fit to this hypothesis.
Moerover, I am not really convinced by the argument against a H2 sink (see p.16451, lines 1-5) since the observed undersaturations point towards a net H2 sink (sink > source) they can, therefore, not result from a single source (see p.16451, l.17-20). Reply: We changed from reporting the supersaturation factor to the saturation to avoid misunderstandings.

Technical Comments: Mauretania should read Mauritania, please check spelling throughout the manuscript. Reply: spelling has been checked and corrected

Table 4: Xh and Da are mentioned in the table caption, but do not appear in the header of the table: I guess that Xm and Dm should read Xh and Da (or the other way round). Reply: this has been corrected

p.16439/40; Figures 4 and 5: Please remove the CO data when they are not essential for the discussion of the H2 data Reply: The CO data are indeed not essential for this publication, but CO data are used to model H2 emissions caused by N2 fixation. We therefore prefer to keep them in as general information, although we do not discuss them in detail.

Suppl. Material: There are citations in the text of the Supplement, but there is no list of references. Please add list with full references. Reply: we added the reference list.

Interactive comment on Biogeosciences Discuss., 12, 16431, 2015.
Interactive comment on “Isotopic evidence for biogenic molecular hydrogen production in the Atlantic Ocean” by S. Walter et al.

S. Walter et al.
s.walter@uu.nl

Received and published: 20 November 2015

Dear referee, thanks for your comments. Please find our reply in-line. Kind regards, S. Walter

p. 16441 l. 16-17: How exactly was the value of 92 % for the extraction efficiency determined? Is this a mean for all samples? What was the variability for the whole dataset? Reply: the mean value is 92.12 (±0.013)%. Due to the very low variation we mentioned just one value without decimal places. We included this to the manuscript for completeness.

p. 16448 l. 13-15: Please detail planned/recommended improvements and expected impact on measurements. Reply: The possible improvements include a better monitor-
ing of additional data such as temperature and pressure in the vessel itself, this was not possible yet. As already mentioned in chapter 2.3, the temperature dependence of the H2 solubility is quite low with less than 0.3% per K, and also the used pressure sensors (Omega, PAAR21R) work within a low error range of 0.5% or even below. In combination with the high extraction efficiency and the standardized sampling procedure over the two cruises, we are convinced that the presented data sets are reasonable. A more detailed discussion about uncertainties and their effect on the results has been added to the manuscript (see also reply to referee #1).

p. 16452 l. 21: Ist he global nitrogen fixation rate of 175 TgN a-1 a result from the GEMS database? Reply: This was a typo, it should have been the value given by Großkopf et al. (2012). The value has been corrected.

Table 2: Why were samples excluded? Were there any issues with the sample handling or contamination? Reply: 5 samples out of almost 200 were excluded as outliers (>2 σiÄô, however, we do have no explanation for the deviation. There was no indication for sampling or measurement errors.

Technical comments Consider reporting saturations in % instead of saturation factors/supersaturation throughout the manuscript to facilitate comparison with other publications. Reply: we changed the supersaturation factor to saturation.

Table 4: Caption differs from table headings for xh/xm, Da/dDa and Dh/dDm Reply: This has been corrected.

Supplement p. 2: Format citation for Green Carritt, 1967, add full reference to supplement. Reply: the full reference has been added and formatted

Supplement p. 4: Add full reference for Knox et al., 1992. Reply: the full reference has been added

Interactive comment on Biogeosciences Discuss., 12, 16431, 2015.