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Evidence of nitrification associated with globally distributed pelagic jellyfish

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Abstract

Often considered detrimental to the environment and human activities, jellyfish blooms are increasing in several coastal regions worldwide. Yet, the overall effect of these outbreaks on ecosystem productivity and structure are not fully understood. Here we provide evidence for a so far unanticipated role of jellyfish in marine nitrogen cycling. Pelagic jellyfish release nitrogen as a metabolic waste product in form of ammonium. Yet, we observed high rates of nitrification ($NH_4^+ \rightarrow NO_3^-$, 5.7–40.8 nM gWW^{-1} [wet weight] h^{-1}) associated with the scyphomedusae *Aurelia aurita*, *Chrysaora hysoscella*, and *Chrysaora pacifica* and low rates of incomplete nitrification ($NH_4^+ \rightarrow NO_2^-$, 1.0–2.8 nM gWW^{-1} h^{-1}) associated with *Chrysaora fulgida*, *C. hysoscella*, and *C. pacifica*. These observations indicate that microbes living in association with these jellyfish thrive by oxidizing the readily available ammonia to nitrite and nitrate. The four studied species have a large geographic distribution and exhibit frequent population outbreaks. We show that, during such outbreaks, jellyfish-associated release of nitrogen can provide more than 100% of the nitrogen required for primary production. These findings reveal a so far overlooked pathway when assessing pelagic nitrification rates that might be of particular relevance in nitrogen depleted surface waters and at high jellyfish population densities.

Jellyfish blooms are increasing in frequency and magnitude in several coastal regions around the world (e.g., Sea of Japan, Black sea, Benguela current, Antarctic; Brotz et al. 2015). The presence of jellyfish blooms in coastal waters can cause severe damage to economic activities such as fisheries (e.g., 2.1%–25% decrease in annual Korean fishery production every year; Kim et al. 2012), tourism (e.g., costing the Israeli coastal tourism industry an estimated annual monetary loss of €1.8–6.2 million every year; Ghermandi et al. 2015) and power generation (e.g., the closure costs of Torness nuclear plant in Scotland due to jellyfish bloom from 28th June to 1st July 2011: approximately £1 million d^{-1}; Kopytko 2015). Simultaneously, jellyfish outbreaks create ecological disturbances by altering the marine food chain structure (Pitt and Purcell 2009). Their voracious predation on zooplankton makes them competitors to planktivorous fish (Condon et al. 2011). The grazing pressure that jellyfish put on zooplankton grazers releases primary producers from predatory control, causing a trophic cascade that can results in phytoplankton blooms (Schnedler-Meyer et al. 2018). By preying on ichthyoplankton (eggs and larvae of fish), jellyfish even exert a top-down control on their competitors and predators (Gordoa et al. 2013). Overall, the complex interaction of jellyfish with the food web can have large impacts on ecosystem structure, function and resilience (Baum and Worm 2009).

The role of jellyfish as top-down predators has been widely studied (e.g.: Stone and Steinberg 2018), yet their bottom-up influence as a nutrient source on marine ecosystems is less clear. Ammonium excreted by jellyfish has been estimated to support up to 8%, 10%, and 11% of the phytoplankton

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Additional Supporting Information may be found in the online version of this article.

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nitrogen requirement in the Lake Illawarra (Australia), the Inland Sea of Japan and the Kiel Bight, respectively (Schneider 1989; Pitt et al. 2005; Shimauchi and Uye 2007). Ammonium and phosphate released by jellyfish more than doubled the phytoplankton biomass in a mesocosm experiment conducted in a saline lake (West et al. 2009). In addition, the release of organic matter in the form of mucus provides an extremely labile source of organic carbon for bacterioplankton (Condon et al. 2011). While there is clear evidence that jellyfish can alter both biogeochemical cycles and food web structure, their role in pelagic nitrogen cycling remains understudied.

Ammonia is an intensely contested compound in most of the world's sunlit oceans, where nitrogen availability limits primary productivity ($\sim$ 75% of the surface ocean; Moore et al. 2013; Bristow et al. 2017). Additionally, ammonia provides the substrate for ubiquitous chemolithoautotrophic nitrifying bacteria and archaea that generate energy by the stepwise oxidation of ammonia to nitrite and nitrate. The first step is mediated by ammonia oxidizing bacteria (Kowalchuk and Stephen 2001) and archaea (Hallam et al. 2006). Ammonia oxidizing archaea can reach high abundances especially in the dark ocean (> 30% of the microbial community; Karner et al. 2001) and appear to be the main drivers of marine ammonia oxidation (Wuchter et al. 2006). As for the second step, all known nitrite oxidizers belong to the bacterial domain (Spieck and Bock 2015) and are characterized by their often remarkable metabolic versatility (Füssel et al. 2017). Both ammonia oxidizers and nitrite oxidizers (collectively called nitrifiers) are ubiquitous in pelagic environments, where they contribute substantially to carbon fixation in absence of light (dark carbon fixation), influencing ocean carbon fluxes (Pachiadaki et al. 2017). Nitrifiers have also been shown to live in association with benthic invertebrates such as sponges (e.g., Subina et al. 2018), corals (e.g., Hoffmann et al. 2009), zoanthids (Sun et al. 2014), bivalves (Welsh and Castadelli 2004), ascidians (Martínez-García et al. 2008), and insect larvae (Stief et al. 2009). As part of invertebrate microbiomes, nitrifiers can provide a source of nutrition for their host when phagocytosed (Martínez-García et al. 2008), preventing the loss of nitrogen into the environment by recycling the excess of ammonium trapped in the mucus (Rädecker et al. 2015). Understanding the role of these associations is important for accurate mapping of marine nitrogen biogeochemistry and may help to improve our ability to predict future change (Pajares and Ramos 2019).

Jellyfish are densely populated with microorganisms (Weiland-Bräuer et al. 2015; Lee et al. 2018; Kramar et al. 2019), which play a beneficial role in the fitness of the host and contribute to the ecological features of the jellyfish (Stabili et al. 2018; Tinta et al. 2019). The epithelial mucus layer of a jellyfish is an attractive niche for microbes, providing them with both a habitat and a high-quality energy source (Kramar et al. 2019). By attracting profitable bacteria and preventing colonization by potentially harmful microorganisms (via interferences with bacterial quorum sensing), the host maintains a healthy microbiome providing immune system functions (Weiland-Bräuer et al. 2019). In addition, jellyfish microbiomes are production hotspots of chemical compounds (e.g., exopolysaccharides, vitamins, enzymes, toxins, antibiotics; Tinta et al. 2019) and harbor microbes closely related to known drivers of major elemental cycles (e.g., nitrogen cyclers, chemolithoautotrophs, methylotrophs, methane oxidizers, and polycyclic aromatic hydrocarbon degraders; Lee et al. 2018). In terms of the nitrogen cycle, two species of nitrifiers (the ammonium oxidizing bacterium *Nitrosospira multiformis* and the nitrite oxidizing bacterium *Nitrospira moscoviensis*) have been found in association with the jellyfish *Chrysaora*. *plocamia* (Lee et al. 2018) and *Aurelia*. *aurita* (Weiland-Bräuer et al. 2015). This discovery leads to the suggestion that these two nitrifiers are ubiquitous members of the microbiome of these two genera (Lee et al. 2018) and indicates that jellyfish could contribute to marine nitrogen cycling beyond the excretion of ammonia via their microbiome.

Cnidarian jellyfish excrete ammonium, a by-product of their metabolism, by diffusion through their body surface (Lőw et al. 2016). Though they are not known to directly produce nitrite or nitrate, low rates of nitrate release have been observed in association with pelagic jellyfish (< 2% of total inorganic nitrogen released; Pitt et al. 2009). For benthic jellyfish that live in symbiosis with zooxanthellae (photosynthetic dinoflagellates), experiments have shown nitrite/nitrate release rates equivalent to 21.5% of the total dissolved inorganic nitrogen release, indicating a substantial colonization by nitrifying microorganisms (Welsh et al. 2009). While the authors suggested the association with nitrifiers to be specific to zooxanthellate jellyfish, we hypothesize that nitrifying microorganisms that benefit from the excreted ammonium are commonly associated with jellyfish and play a significant role in the nitrogen cycling. To test this hypothesis, we chose four species of non-zooxanthellate scyphozoan jellyfish, *A*. *aurita*, *Chrysaora hysoscella*, *Chrysaora fulgida*, and *Chrysaora pacifica* from four contrasting environmental conditions (brackish lake, both North and South Atlantic Ocean coastal waters, and artificial seawater), representing a wide range of environmental conditions (Dawson et al. 2005; Morandini and Marques 2010). All of these species exhibit population outbreaks in coastal areas (Lucas 2001; Lynam et al. 2006; Makabe et al. 2015; Abato 2017) leading to high population biomass ultimately disturbing human activities. We measured the release rates of ammonium, nitrite, nitrate and phosphate in association with all four jellyfish species in order to assess the global prevalence of an association between nitrifiers and jellyfish as well as its potential role in the marine nitrogen cycle.

Materials

Adult medusae of *A*. *aurita* (n = 5), *C*. *hysoscella* (n = 5), and *C*. *fulgida* (n = 2) were sampled from Horsea lake (UK), the

Rame Peninsula (UK), and Walvis Bay (Namibia), respectively (Table 1). Medusae were collected carefully from near-surface waters using a 10-liter bucket and kept in approximately 5 liter of ambient water during transportation to the laboratory. The water temperature was kept as close to in situ conditions as possible (maximum fluctuations: ±2°C from in situ conditions; Table 1). Maximum transportation time was 4 h. All jellyfish survived transportation and were transferred to the lab in good condition, indicated by regular swimming pulse. Once in the lab, jellyfish were transferred to their respective experimental conditions (Table 1). *C. pacifica* specimen (n = 5) were collected from the London aquarium. The medusae were produced from polyps cultured in artificial conditions (artificial seawater with continuous UV-treatment and filtering system) and had not been in contact with natural seawater.

Sampling sites and species

Horsea Lake (Portsmouth, United Kingdom; 50°83′68.26″ N, 1°10′19.11″W) is a shallow (6–7 m), brackish (salinity: 19–23 g L^{-1}), semi-enclosed man-made body of water connected to Portsmouth Harbor via a controlled pipe and valve (Lucas 1996). The lake is oligotrophic with annual surface temperatures between 5°C and 23°C (Lucas 1996). It lacks a riverine input and is replenished with seawater 2–3 times a year during high water spring tides (Lucas et al. 1997). The moon jellyfish *A. aurita* is found in Atlantic boreal waters and in the Black Sea (Dawson 2003; Fig. 1). The species was previously associated with a cosmopolitan distribution and is now known to be formed by many regional "cryptic" species spread globally (Scorrano et al. 2016, Fig. 1). The medusae of *A. aurita* can reach bell diameters up to 40 cm (Arai 1996) and are often found in high densities in coastal and brackish waters such as estuaries and bays (Lucas 2001). They are present in Horsea Lake throughout the year (Lucas 1996).

The Rame Peninsula (Cornwall, United Kingdom) is located on the south-west coast of England. Medusae of the species *C. hysoscella* were collected in waters characteristic of the English Channel (50°19′54.5″N, 4°11′59.2″W). The mean monthly surface temperature ranges from 9.2°C to 16.5°C and the mean monthly surface salinity ranges from 35.1 to 35.3 g L^{-1} (based on 5-year running median time-series analyses of station E1; Smyth et al. 2010). Medusae of *C. hysoscella* are of medium sizes (15–25 cm in bell diameter) and are found in the North Sea, the English Channel and the Mediterranean Sea (Morandini and Marques 2010; Fig. 1), where they can form dense populations (Abato 2017). They appear in the English Channel during the summer months (Pikesley et al. 2014).

Walvis Bay is a large bay located on the coast of Namibia (22°57′22″S, 14°30′ 29″E). The water conditions of the bay are dictated by the Northern Benguela Upwelling System, which is a highly productive eastern boundary ecosystem. The seawater temperature in Walvis Bay varies between 10°C and 22°C and the salinity mainly ranges between 34.5 and 35.5 g L^{-1} (Pryor et al. 2009). *C. fulgida* is an exclusively marine species found along the west coast of Africa (Fig. 1) with medusae of medium size (10–20 cm in diameter). This species has previously been identified as *C. hysoscella* due to their morphological similarities (Morandini and Marques 2010). In Walvis Bay, *C. fulgida* medusae occur throughout the year and frequently reach significant population densities during the summer months (Skrypzeck 2019).

Medusae of the species *C. pacifica* are slightly smaller (typically 10–15 cm in diameter) than the two studied species of *Chrysaora* described above, and occur in the Northern Pacific Ocean in the vicinity of Japan (Fig. 1; Morandini and Marques 2010). Since the beginning of the century, the number of *C. pacifica* medusae in the Inland Sea of Japan has been growing, and the population now has recurring annual blooms (Makabe et al. 2015; Takasu et al. 2019).

Experimental structure

Prior to the experiment, all equipment was acid washed in 10% hydrochloric acid and rinsed three times with ultra-high purity water (MilliQ ≥ 18.2 10^6 Ω cm^{-1}, Millipore, UK). The incubators consisted of 5-liter high-density polyethylene

Table 1. Jellyfish collection and incubation details.

Species	*A. aurita*	*C. hysoscella*	*C. fulgida*	*C. pacifica*
Origin	Horsea Lake (UK)	Rames peninsula (UK)	Walvis Bay (Namibia)	London Aquarium
Date (DD-MM-YY)	23-10-2018	21-08-2018	21-07-2019	27-02-2019
Bell diameter (cm)	12–16	9–13	12–16	6–9
Wet weight (g)	73–190	100–278	180–279	35–59
Number of specimens	5	5	2	5
In situ conditions				
Temperature (°C)	14	18	14	16
Salinity (g L^{-1})	25	35	35	30
Experimental condition				
Temperature (°C)	15	20	14	16
Salinity (g L^{-1})	25	35	35	30

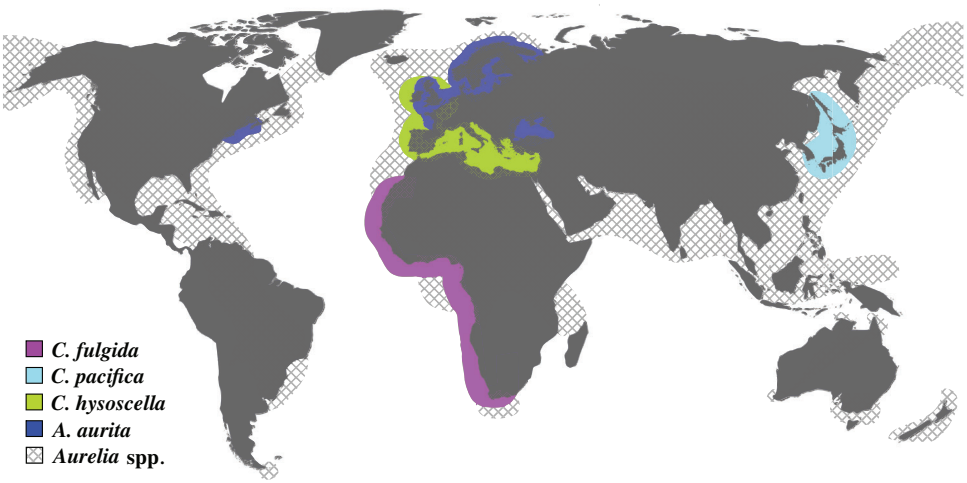

Fig 1. Geographic distribution of the four jellyfish species investigated in this study (*A. aurita*, *C. hysoscella*, *C .fulgida*, and *C. pacifica*; based on Dawson et al. 2005 and Morandini and Marques 2010) and of the cryptic genus *Aurelia* (based on Dawson and Martin 2001).

buckets filled with artificial seawater (ASW; ultra-high purity water + Tropic Marin synthetic sea salt; detailed preparation available in Data S1). A maximum number of five healthy and undamaged adult medusae were selected for each experiment. The health of a jellyfish was evaluated based on the swimming rhythm with active swimming behavior indicating good health. Two hours before the experiment, selected jellyfish were individually transferred to an incubator filled with 4 liter of ASW. The purpose of this first "acclimation/egestion" phase was to allow the medusae to egest any food they might have held in their gastric pouches. The experiment consisted of an initial Release phase, followed by an incubation phase with four incubation treatments: Jellyfish (ASW + jellyfish), Jellyfish-Control (ASW only), Mucus (ASW + mucus + ammonium), and Mucus-Control (ASW + ammonium; Fig. 2). First, the jellyfish were gently transferred by hand to the Release incubators (2 liter of ASW) using sterile vinyl gloves, while trying to minimize the amount of water transferred with it. The Release phase allowed mucus and its associated microbes to be released into the water. After 1 h, the jellyfish along with half of the volume of the water in the Release incubator (1 liter) were transferred to the Jellyfish incubators (3 liter of ASW; final volume = 4 liter). The other half of the water was transferred to the Mucus incubators (3 liter of ASW; final volume = 4 liter). The controls (Jellyfish-Control and Mucus-Control) consisted of incubators containing only ASW (3 liter of ASW).

As ammonia is continuously excreted by jellyfish, the nitrification rates associated with jellyfish in ASW (continuously increasing ammonium concentrations) would not be directly comparable to those associated with mucus in ASW (ammonium concentration of < 0.1 μM). To allow direct comparison of nitrification rates in the Mucus and Jellyfish treatments, we simulated jellyfish ammonium excretion in both the Mucus and the Mucus-Control treatments by adding ammonium (NH_4Cl, Fisher Scientific, UK) to the incubators after each sample collection. The amount of ammonium added was estimated based on literature (Pitt et al. 2005) and previous trial experiments. The expected increase in ammonium concentrations ranged from 0.5 to 2.5 μM h^{-1} (Data S1, Table II) depending on species, size of the jellyfish and temperature.

Rate measurements

Water samples for nutrient analysis were collected every hour. Before collecting each sample, the water was stirred gently to homogenize it. Two sets of 15-mL samples (one for nitrate, nitrite and phosphate, and one for ammonium) were collected using a 20 mL polypropylene syringe. The sample was filtered through a 0.22-μm polyethersulfone sterile syringe filter (33-mm diameter, Millipore, UK) with the first 5 mL discarded to wash the filter. The remaining 10 mL were collected in centrifuge tubes (polypropylene conical centrifuge tubes, 15 mL volume, Fisher Scientific, UK). For each treatment, a dedicated syringe was used to avoid cross-contamination. In between sample collection, the incubators were covered with a lid to avoid contamination. Based on initial experiments and findings of a previous study measuring ammonia release in *C. mosaicus* (Pitt et al. 2005), we decided an incubation volume and duration of 4 liter and 6 h as ideal to measure a significant rate of nutrients release without causing excessive stress to the jellyfish. The jellyfish were then removed from the incubators, and the jellyfish bell diameter and the wet weight (WW) was measured using a ruler and a balance (FireKingdom SF-400, ±1 g). Water sample collection continued for 2 h after removal of the jellyfish, resulting in a total experiment duration of 8 h.

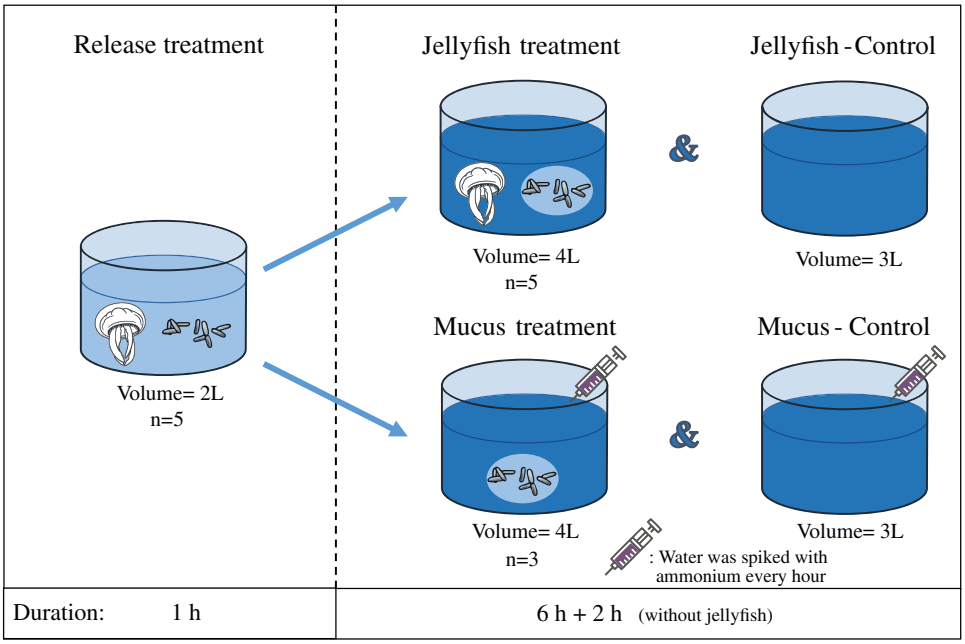

Fig 2. Experimental setup. Jellyfish were incubated for 1 h in the release treatment. Subsequently, the jellyfish along with half of the volume from the release phase were transferred to the jellyfish treatment; the other half was transferred to the mucus treatment. Controls for both experiments consisted of incubators containing only artificial seawater (ASW). The mucus and control-mucus incubators were spiked with ammonium every hour (Data S1, Table II).

Sample analysis

The duplicate sample for ammonium was analyzed using the o-phthalaldehyde fluorometric method (Taylor et al. 2007). The ammonium measurements were performed the same day using a Turner design Trilogy fluorometer (model 7200, US) with a UV module (7200-047). The duplicate sample for nitrite, nitrate and phosphate was immediately frozen for later analysis. Frozen samples were thawed at room temperature and phosphate, nitrate and nitrite concentrations were measured using standard gas segmented continuous flow spectrophotometric techniques (QuAAtro, Seal Analytical). The baseline of the auto-analyzer was determined using the same ASW as used in the experiment (except for *C. fulgida* samples, for which we used ultra-high purity water as baseline; detailed descriptions of the calibrations and detection limits in Data S1).

Our hourly sampling regime, which provides a relatively low temporal resolution, was determined by the time it takes to collect the sample and the sample volume removed relative to the incubation volume. To determine the release rates at a higher temporal resolution, for one of the specimen of *A. aurita* incubations, nitrite and nitrate were measured at high-resolution (every 20 min) using a microfluidic lab-on-chip analyzer (Beaton et al. 2012). This novel application of lab-on-chip microfluidic analyzers allowed high-resolution measurements with small sample volumes and avoiding the need for sample storage. The nitrate and nitrite concentrations measured using the "manual" and lab-on-chip method agreed well (Data S1, Fig. V), as shown by a linear regression between the two methods (auto-analyzer = 1.04 ± 0.06; lab-on-chip = 0.15 ± 0.04; $R^2 = 0.98$, $p < 0.001$, $n = 8$; Data S1, Fig. VI). For both techniques, gas segmented continuous flow spectrophotometric and lab-on-chip, the combined (random + systematic) analytical uncertainty associated with nitrate + nitrite and phosphate measurements was < 5% (details in Data S1; Birchill et al. 2019).

Statistical analysis

Contamination, wall effects and production/absorption by microorganisms were accounted for by subtracting the changes in concentrations observed in the ASW controls from the treatments. In order to account for the loss of liquid due to the collection of nutrient samples, the total number of moles of nutrient released at each time point was calculated using the equation:

$$n_{[t]} = n_{[t-1]} + V_{[t-1]} \times (c_{[t]} - c_{[t-1]})$$

where n is the number of moles released at a certain time point (t) since the beginning of the experiment, V is the volume of the incubator, and c the molar concentration of nutrients (Giering et al. 2012).

The rates of nutrient (ammonium, phosphate, nitrite, and nitrate) release per incubator (or per jellyfish for the jellyfish treatment) were calculated using linear regression for each replicate. The rates were then normalized by the wet weight of the jellyfish and their differences were investigated by an analysis of covariance (ANCOVA; results are presented in Data S1).

The rates of nutrient release per species were calculated by averaging the rates of the replicates for each species. Finally, the differences in weight-specific rates of nutrient release caused by the differences in experimental temperatures were standardized using Q_{10} temperature coefficient factors from the literature. For ammonium and phosphate release, a Q_{10} of 3.1 was used for *A. aurita* (Møller and Riisgård 2007), and the general Q_{10} of 2.66 was used for the other jellyfish species (Ikeda 2014). For nitrite and nitrate release rates, a Q_{10} of 2.2 was used for all species (Zheng et al. 2017), corresponding to the temperature coefficient factor of nitrifying microorganisms. Rates were adjusted to the median temperature of the experimental conditions (16°C) and N : P ratios were calculated as the sum of ammonium, nitrite and nitrite over phosphate. The temperature-corrected nutrient production rates were plotted against the wet weight of the jellyfish, and a linear regression was fitted to investigate the allometric relationships between body weight and nutrient release rates. Finally, estimates of inorganic nitrogen release by jellyfish blooms were calculated using the allometric equations together with jellyfish densities from two case studies. The uncertainty range of these estimates were determined from the error on the allometric exponents and the temperature. All statistical analyses were carried out using R Statistical Software (R Core Team 2019).

Results and discussion

Nutrient excretion and nitrification

To determine rates of nitrification catalyzed by members of the jellyfish microbiome, we performed incubation experiments with four species of non-zooxanthellate scyphozoan jellyfish, *A. aurita*, *C. hysoscella*, *C. fulgida*, and *C. pacifica*. We measured rates of ammonium and phosphate excretion along with partial ($NH_4^+ \rightarrow NO_2^-$) and complete ($NH_4^+ \rightarrow NO_3^-$) nitrification associated with these jellyfish species. Ammonium and phosphate concentrations increased continuously in all incubations with jellyfish, whereas nitrite and nitrate concentrations increased only in the presence of three of the four species (see next paragraph; Fig. 3). For all nutrients, concentrations stabilized or decreased once the jellyfish were removed (Fig. 3, Table 2; see Data S1 for absolute concentrations, Fig. I). In the presence of mucus alone, rates of nitrification were negligible for all investigated jellyfish species ($< 2.0 \times 10^{-3}$ nmol L^{-1} h^{-1}; Data S1, Table III), strongly suggesting that the observed rates of nutrient release were directly related to jellyfish metabolism and the associated microbiome. Mass-specific release rates of ammonium ranged from 22.8 to 86.2 nmol NH_4^+ gWW^{-1} h^{-1} at experimental temperatures (27.9–86.1 nmol NH_4^+ gWW^{-1} h^{-1} when normalized to 16°C), which falls within the range of previous observations (2–111 nmol NH_4^+ gWW^{-1} h^{-1}: Pitt et al. 2013). The observed intraspecies variability of ammonium excretion was relatively low, with *C. hysoscella* showing the highest variation (14%) in release rates across specimens. In contrast, excretion rates between different jellyfish species varied widely (up to 3.7-fold). Mass-specific release rates of phosphate ranged from 3.2 to 11.9 nmol PO_4^- gWW^{-1} h^{-1} at experimental temperatures (3.7–11.9 nmol PO_4^- gWW^{-1} h^{-1} when normalized to 16°C). Excretion rates of phosphate were linearly correlated with ammonium excretion rates (all species included, not taking into account ammonium conversion; $p < 0.001$, $R^2 = 0.60$; $n = 17$; Data S1, Fig. VII). Ammonium: phosphate excretion ratios ranged from 2.7 to 15.2 with an average of 7.4, in accordance with previous reports (8.2 for *A. aurita*, Shimauchi and Uye 2007; 8.7 for *C. mosaicus*, Pitt et al. 2005; 7.5 for *P. noctiluca*, Malej 1991).

Ammonia oxidation is usually considered the rate-limiting step in nitrification (Zhang et al. 2020): nitrite is immediately oxidized by free-living nitrite-oxidizing bacteria, preventing its accumulation at significant rates. We observed these expected dynamics in the presence of *A. aurita*, when nitrite concentrations did not increase whereas nitrate accumulated (5.7 ± 1.3 nmol NO_3^- gWW^{-1} h^{-1}; Table 2), indicating a tight coupling of both nitrification steps. However, this paradigm did not apply to nitrification in association with the other three jellyfish species that we investigated. In the presence of *C. hysoscella* and *C. pacifica*, accumulation rates were significant for both nitrite (*C. hysoscella*: 2.8 ± 1.9 nmol NO_2^- gWW^{-1} h^{-1}; *C. pacifica* 2.1 ± 0.4 nmol NO_2^- gWW^{-1} h^{-1}) and nitrate (*C. hysoscella*: 11.9 ± 6.0 NO_3^- nmol gWW^{-1} h^{-1}; *C. pacifica* 40.8 ± 3.1 nmol NO_3^- gWW^{-1} h^{-1}; Table 2). The decoupling was more pronounced in incubations with *C. hysoscella* (nitrite accumulation rate was 23% of the nitrate accumulation rate), whereas nitrite accumulation in association with *C. pacifica* was lower (5% of nitrate accumulation). During the incubations with *C. fulgida*, ammonia oxidation to nitrite was the only detectable nitrification process (1.0 ± 0.2 nmol NO_2^- gWW^{-1} h^{-1}; Table 2).

To our knowledge, two other studies investigated the nitrite + nitrate (NO_x, no distinction made) release by non-zooxanthellate scyphomedusae: Pitt et al. (2005) found that *C. mosaicus* released < 2% of the released nitrogen in form of NO_x, and Shimauchi and Uye (2007) did not observe significant release of NO_x associated with *A. aurita*. The latter study contrasts with our observation that 16% of the released nitrogen by *A. aurita* was in the form of nitrate. This discrepancy indicates a potential effect of past and present environmental conditions on the jellyfish-associated microbial community composition and, subsequently, on the balance of jellyfish-associated nitrification rates. In contrast to the hypothesis that nitrifiers are specific to zooxanthellate jellyfish (Welsh et al. 2009), our results suggest that both, zooxanthellate and non-zooxanthellate, jellyfish are potential hosts for nitrifiers and can thus be a source of nitrite and nitrate to the environment.

Together nitrite and nitrate release rates were 5–50% lower than ammonium excretion rates (Fig. 4) and

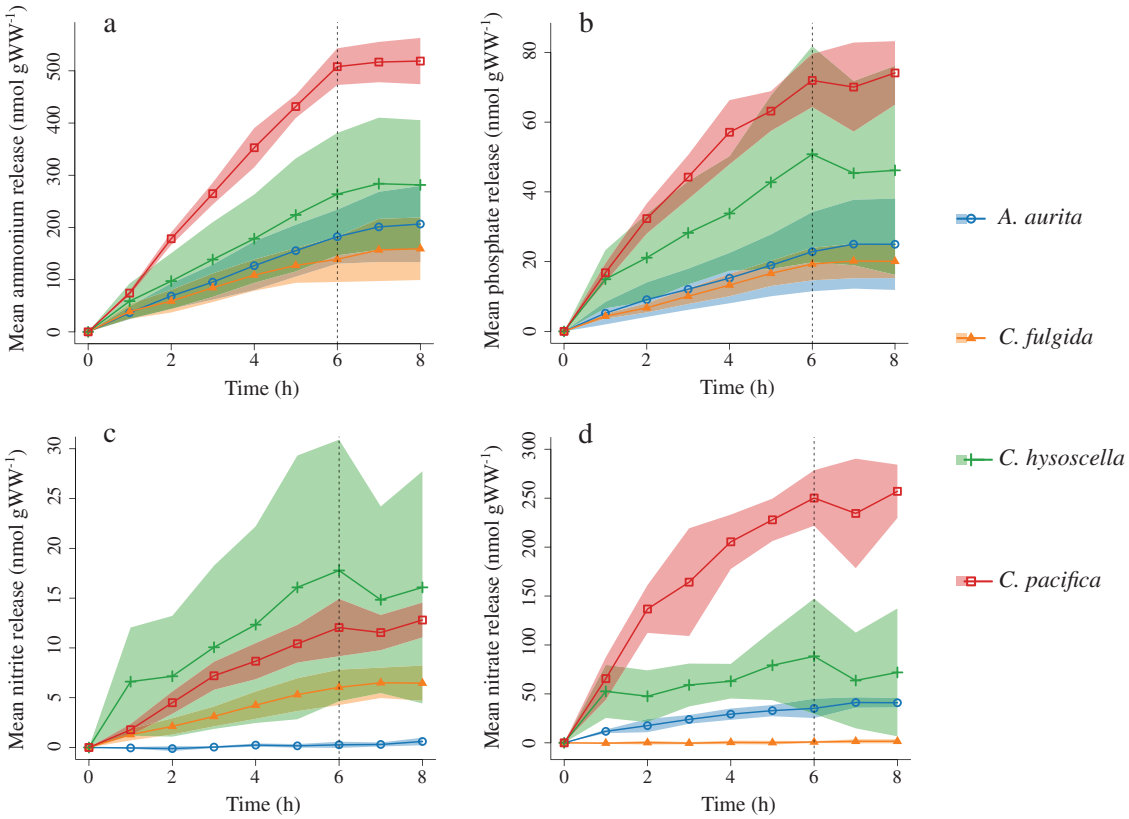

Fig 3. Mean cumulative release of (**a**) ammonium, (**b**) phosphate, (**c**) nitrite, and (**d**) nitrate by *A. aurita* (blue circle), *C. fulgida* (yellow triangle), *C. hysoscella* (green cross), and *C. pacifica* (red square), normalized to the wet weight (WW) of each specimen. Colored areas indicate uncertainty envelopes (standard deviation) of the mean cumulative release of nutrients. Vertical dotted line corresponds to the time when the jellyfish were removed from the incubators (6 h).

contributed 5–33% of the total inorganic nitrogen release. Under saturating substrate levels (ammonia and nitrite), nitrification reactions follow a zero-order kinetic (Chen et al. 2006), meaning that increases in substrate concentration do not increase the reaction rates. As ammonium excretion exceeded that of nitrite and nitrate substantially, we conclude that nitrification rates were not limited by ammonia availability in any of the experiments. Moreover, since the total ammonia concentrations of the incubators were well below toxicity levels for polyps and ephyrae (2 mg L^{-1}, Jian-Long et al. 2018), we are confident that the observed nitrification rates are reflective of in situ processes.

Between species, the rates of nitrification varied more than the excretion rates of ammonium and phosphate. For example, we observed a > six-fold difference between the nitrate release rates of *A. aurita* and *C. pacifica* (Table 2, Fig. 4). Both the inter- and intraspecies variabilities observed in ammonia and phosphate excretion as well as nitrification rates can partly be explained by allometric scaling of the mass-specific release rates to the wet weight of each individual (ammonium excretion: 80%, phosphate excretion: 73%, nitrification: 55%, Fig. 5; Data S1, Table VI). The allometric relationships for the ammonium, phosphate and nitrate-specific release (ASR, NSR, and PSR, respectively; nmol gWW^{-1} h^{-1}) were:

$$\mathrm{ASR} = 1.84\times10^{3}\pm1.6WW^{-0.82\pm0.10} \quad (p<0.001, R^2=0.80, n=17) \tag{1}$$

$$PSR = 369\pm1.9WW^{-0.90\pm0.13} \quad (p<0.001, R^2=0.73, n=17) \tag{2}$$

$$NSR = 2.84\times10^{3}\pm3.6WW^{-1.20\pm0.28} \quad (p<0.001, R^2=0.55, n=15) \tag{3}$$

The negative scaling exponents indicate that smaller specimens release more nutrients per gram of mass, and hence follow the expected allometric scaling. The high rates of nutrient excretion and nitrification associated with *C. pacifica* can therefore be partly explained by the small size of these specimens (35–59 gWW) compared to the other investigated species. Similarly, the high variability in *C. hysoscella* rates matches the wider range of wet weights per individual (100–278 gWW, Table 1). All scaling exponents (Eqs. 1–3; Data S1: Slope, Table VI) were lower than the −¼ allometric exponent commonly observed for other zooplankton mass-specific

Table 2. Release rates and regression statistics for the cumulative nutrient release by the four jellyfish species. Standard deviation of the slope = SD, number of observations = *n*. Rates at experimental temperatures and adjusted to 16°C are presented, as well as the N : P ratios at 16°C. The rate, SD, $\bar{R}^2$, and $\bar{p}$ are mean values from the replicates individual linear regressions. Levels of statistical significance are indicated by *, **, and *** ($p \leq 0.05$, 0.01, and 0.001, respectively).

Species	Nutrient	Rate (nmol gWW^{-1} h^{-1})	SD	*n*	$\bar{R}^2$	$\bar{p}$	*n*	Rate at 16°C (nmol gWW^{-1} h^{-1})	SD	N : P
A. aurita	Ammonium	30.1	8.1	5	0.99	***	7	33.7	9.1	10.3
	Phosphate	3.6	1.5	5	0.98	***	7	3.9	1.7	
	Nitrite	0.1	0.0	5	0.31	0.22	7			
	Nitrate	5.7	1.3	5	0.89	**	7	6.2	1.4	
C. fulgida	Ammonium	22.9	4.5	2	0.97	***	7	27.9	5.5	7.89
	Phosphate	3.2	0.5	2	0.99	***	7	3.7	0.6	
	Nitrite	1.0	0.2	2	0.99	***	7	1.2	0.2	
	Nitrate	0.1	0.1	2	0.16	0.52	7			
C. hysoscella	Ammonium	42.9	17.0	5	0.99	***	7	29.0	11.5	6.95
	Phosphate	7.9	4.1	5	0.94	***	7	5.7	2.8	
	Nitrite	2.8	1.9	5	0.87	**	7	1.9	1.4	
	Nitrate	11.9	6.0	5	0.61	*	7	8.7	4.4	
C. pacifica	Ammonium	86.2	5.0	5	0.99	***	7	86.1	5.0	10.8
	Phosphate	11.9	1.2	5	0.96	***	7	11.9	1.2	
	Nitrite	2.1	0.4	5	0.98	***	7	2.1	0.4	
	Nitrate	40.8	3.1	5	0.91	***	7	40.8	3.1	

physiological processes (Arhonditsis et al. 2019). We suggest that this divergence relates to the jellyfish's high water contents and unique body plans (Pitt et al. 2013). The scaling exponent of the nitrate release allometric equation (– 1.20 ± 0.28, Eq. 3) being lower than the exponent for the ammonium release (– 0.82 ± 0.10, Eq. 1) indicates that, when wet weight increases, the nitrate-specific rate decreases faster than the ammonium-specific rate. This difference in scaling exponent is likely to be related to the changes in the jellyfish surface-to-volume ratio: the release of ammonium is likely more depending on the jellyfish's body volume as it is a metabolic waste product, whereas nitrate is likely more dependent on the jellyfish surface owing to the association with the microbiome living on the jellyfish. Our data show that release rates by jellyfish are highly variable between populations, yet, when normalized to wet weight, we observe strong allometric scaling. This observation highlights the potential for these pathways to be incorporated into models.

Evidence of active nitrifying microorganisms in jellyfish

Jellyfish host diverse microbial communities on their epithelium as their mucus provides an attractive niche for

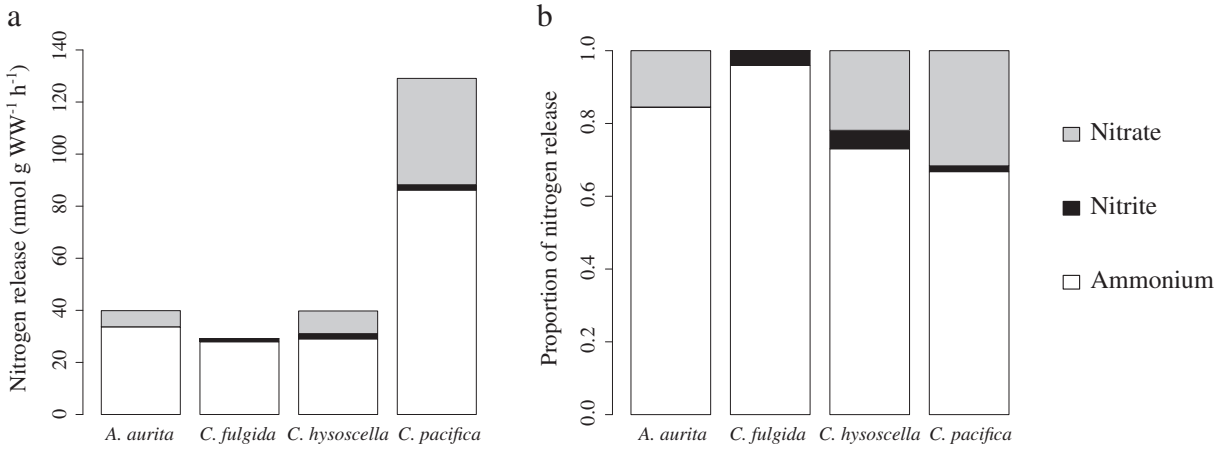

Fig 4. Inorganic nitrogen release rates of different jellyfish species (**a**) normalized by the wet weight of the specimens, and (**b**) as proportion of total inorganic nitrogen release. Wet weight = WW.

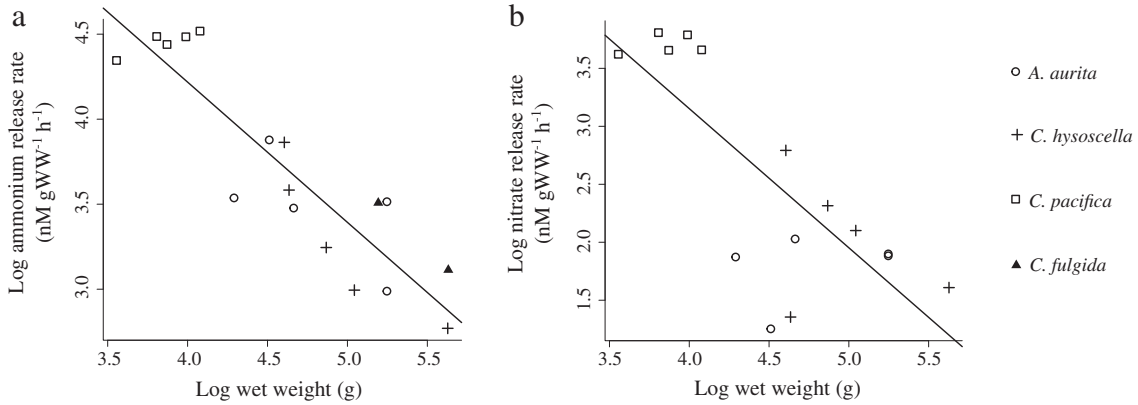

Fig 5. Effect of wet weight on the mass-specific release rates of ammonium (**a**; $p < 0.001$, $R^2 = 0.80$, $n = 17$) and nitrate (**b**; $p < 0.001$, $R^2 = 0.55$, $n = 15$) for the jellyfish *A. aurita (circle)*, *C. hysoscella (cross)*, *C. pacifica (square)*, and *C. fulgida* (triangle) at 16°C. The black line is the linear regression. No significant release of nitrate was observed for *C. fulgida*.

microorganisms (Tinta et al. 2012, 2019; Weiland-Bräuer et al. 2015; Kramar et al. 2019). Two species of nitrifiers, the ammonia-oxidizing bacterium *N. multiformis* and the nitrite-oxidizing bacterium *N. moscoviensis*, have been identified as members of the microbiome of jellyfish *C. plocamia* (Lee et al. 2018) and *A. aurita* (Weiland-Bräuer et al. 2015). However, neither of these nitrifiers were highly abundant (< 2% of total operational taxonomic unit; Lee et al. 2018). The high nitrification rates we observed strongly supports the presence of either highly active or highly abundant nitrifying microorganisms in the jellyfish microbiome. The low coupling between nitrification rates could be caused by poor diffusional connectivity between nitrifiers (Welsh et al. 2001), i.e., a fraction of the produced nitrite might diffuse directly to the water column rather than to a zone where it can be oxidized to nitrate. The differential production of nitrite and nitrate associated with the four jellyfish populations investigated strongly indicates variable community composition or distribution of the microbiome on the jellyfish depending on jellyfish species or environmental factors. While our findings are representative only of a subset of jellyfish populations, the diverse identity and origin of the investigated specimens strongly supports our hypothesis of a widespread association with nitrifying bacteria and archaea. The detailed nature of this association requires further investigations including molecular approaches to determine the identity and distribution of nitrifiers within the jellyfish microbiome.

Ecological implications

Jellyfish stimulate primary production through the excretion of ammonium and phosphate (Pitt et al. 2005). The average inorganic N : P ratio of the released nutrients (7.3–10.9, Table 2; Data S1, Fig. VIII) lies below the Redfield Ratio (N : P = 16; Redfield 1963) and substantially below the N : P ratios of their main diet, zooplankton (N : P > 20; Elser and Hassett 1994). Thus, the gelatinous biomass of these jellyfish appears to retain nitrogen efficiently, which is further supported by their low molar C : N ratio (4.4; Lucas Cathy et al. 2011) compare to other marine zooplankton organisms (4.8–6.2 for crustacean zooplankton; Pitt et al. 2013). By storing nitrogen over phosphorus, expanding jellyfish blooms may locally drive the ecosystem toward N-limitation (Sterner 1990). Whereas under starvation, while jellyfish consume up to 85% of their own nitrogen-rich tissues (Lilley et al. 2014; Pitt et al. 2014), the N : P ratio of the excreted nutrients would increase. Starvation, a major cause of jellyfish bloom decline (Pitt et al. 2014), could temporarily drive the ecosystem toward P-limitation. A large jellyfish bloom could thus act as a "nitrogen buffer," storing nitrogen over phosphorus when food is abundant and releasing nitrogen over phosphorus during its decay.

Our findings demonstrate that a substantial fraction of the excreted ammonium is shunted through partial or complete nitrification (ammonium: 80 ± 12%, nitrite: 3 ± 2%, nitrate: 17 ± 13%; Fig. 6), thereby fueling dark carbon fixation in the sunlit surface ocean. An association with jellyfish allows nitrifiers direct access to ammonium in the surface ocean, thereby bypassing competition with phytoplankton for this otherwise scarce resource (Zakem et al. 2018). During jellyfish blooms, the release of different forms of bioavailable inorganic (nitrite, nitrate and ammonium) has the potential to locally enhance surface primary production and even influence phytoplankton community composition (Fig. 6; Shilova et al. 2017). This effect on the community composition, in turn could impact the quantity and quality of organic matter that sinks to depth (Basu and Mackey 2018).

To explore the potential relevance of jellyfish blooms on surface nitrogen cycling, we extrapolated our nitrification rate measurements based on two jellyfish blooms, whose spatial extent was measured in high resolution (Lynam et al. 2006; Han and Uye 2009). The blooms were observed in (1) the shallow eutrophic and brackish Honjo lagoon, northwest of Lake Nakaumi, Japan (Han and Uye 2009) and in (2) the coastal area of Namibia representing the Northern Benguela

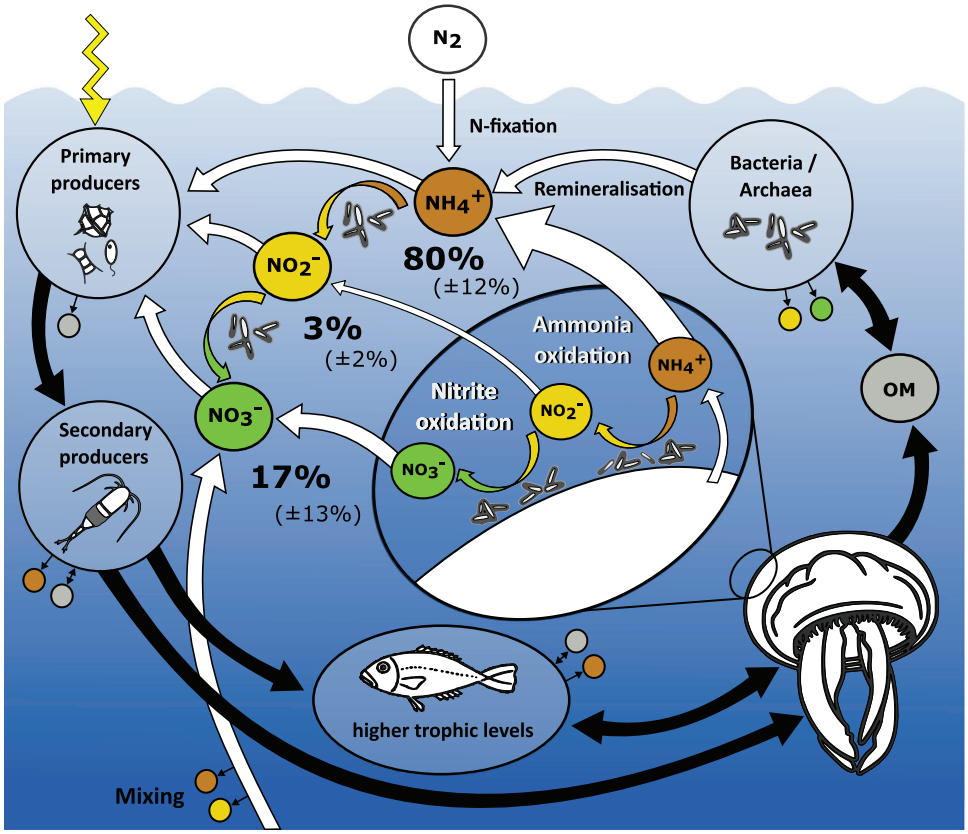

Fig 6. Conceptual diagram of the role and position of jellyfish in the surface marine nitrogen cycle. The flow of organic and inorganic matter is shown by black and white arrows, respectively. Colors indicate ammonium (NH_4^+, orange), nitrite (NO_2^-, yellow), nitrate (NO_3^-, green) and organic matter (OM, gray). Colored arrows represent ammonium-oxidation (orange-to-yellow) and nitrite-oxidation (yellow-to-green). Components linked to small colored circles release/assimilate nutrients of the same color. The average release of nitrogen forms are presented as percentage (± standard deviation) of total dissolved inorganic nitrogen released by jellyfish. The yellow zigzag arrow represent light. The large middle circle zooms in on the jellyfish epithelium.

Upwelling System (Lynam et al. 2006). We applied our allometric equations for ammonium and nitrate release (Eqs. 1 and 3) to the average body characteristics of the jellyfish (Table 3), corrected for temperature (Table 3 and as described in methods), and multiplied by abundance.

In the Honjo District Lake, *Aurelia coerula* (a cryptic species to *A. aurita* and until recently named *A. aurita*) is highly abundant (up to 18 medusae m^{-3}) from June to November and are thought to ingest up to 47% of the daily mesozooplankton production (Han et al. 2009; Han and Uye 2009). During these months, average ammonium and nitrate levels are consistently low (≤ 0.01 mg L^{-1} for both ammonium and nitrate; Chugoku Regional Development Bureau 2018). We estimated that the large aggregation of *A. coerula* could have released up to 1.7 mmol N m^{-2} h^{-1} (uncertainty: 1.0–3.2 mmol N m^{-2} h^{-1}), of which 85% was in the form of

Table 3. Overview of case studies. Surface temperature at sampling time and body characteristics of jellyfish used to estimate inorganic nitrogen release.

Location	Species	Surface temperature (C°)	Mean WW (g)	Mean bell diameter (cm)	References
Honjo District	*A. coerula*	28–28.7	92.5[a]	13.1	Han and Uye 2009
Northern Benguela	*C. fulgida*	13[b]	1100[c]	27	Lynam et al. 2006

[a]Calculated from Han et al. (2009).
[b]Mean annual surface temperature in august from Junker et al. (2017).
[c]Calculated from Houghton et al. (2007).

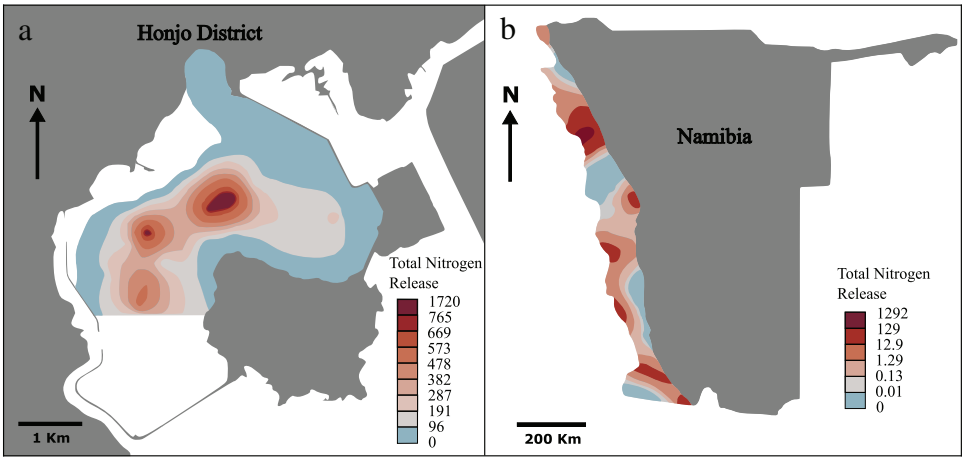

Fig 7. Heat map of estimated total inorganic nitrogen release associated with the densities of *A. coerula* in the Honjo District sea lake in Japan (**a**, linear scale: 0–1720 μmol N m^{-2} h^{-1}; map modified from Han and Uye 2009) and of *C. fulgida* along the coast of Namibia (**b**, exponential scale: 0–1292 μmol N m^{-2} h^{-1}; map modified from Lynam et al. 2006).

ammonium and 15% in the form of nitrate (Fig. 7a). On a daily basis, assuming Redfield ratio (C : N = 106 : 16; Redfield 1963), this nitrogen release would be able to support a primary production rate of 3.2 g C m^{-2} d^{-1} (uncertainty: 1.9–6.1 g C m^{-2} d^{-1}), equivalent to 463% (uncertainty: 275–884%) of the mean daily primary production of a typical estuarine-coastal ecosystems (global average: 252 g C m^{-2} y^{-1}; Cloern et al. 2014).

The Benguela Upwelling System is one of the four major coastal upwelling regions presenting the highest primary production of the world oceans (Carr 2001). Large jellyfish populations occur sporadically throughout the year with highest abundances observed in June–August (Flynn et al. 2012). The biomass of these blooms can at times exceed the biomass of fish by a factor of three (Lynam et al. 2006). We estimated that the *C. fulgida* blooms in August 2006 (Lynam et al. 2006) could have released up to 1.3 mmol N m^{-2} h^{-1} (uncertainty range: 0.7–2.7 mmol N m^{-2} h^{-1}; Fig. 7b), of which 95% was in the form of ammonium and 5% in the form of nitrite. Assuming the Redfield ratio (C : N = 106:16; Redfield 1963), this nitrogen release corresponded to a daily primary production of 2.5 g C m^{-2} d^{-1} (uncertainty: 1.3–5.2 g C m^{-2} d^{-1}), which is equivalent to 208% (uncertainty range: 108–433) of the average daily primary production of the Northern Benguela ecosystem (1.2 g C m^{-2} d^{-1}; Brown et al. 1991).

The densities observed in the Honjo District lake, although high, are not unusual for coastal habitats (e.g.: 36 ± 34 *A. aurita* m^{-3} in Limfjorden; Riisgård et al. 2010). Likewise, the jellyfish densities of the Northern Benguela Upwelling System are to our knowledge the highest currently on record, yet such high densities are predicted to become more common in some coastal areas of our changing ocean (Henschke 2019). For areas experiencing increases in jellyfish blooms, the two case studies provide a guide to understand how jellyfish and their associated microbiomes can impact the nitrogen cycle and supply nutrients for primary production.

Conclusion

Overall, our results suggest a widespread association between jellyfish and nitrifying microorganisms, which can oxidize up to one third of the ammonium excreted by jellyfish. While the identity of the nitrifiers and their distribution on the jellyfish remain unknown, it appears that their activity and abundance are constant in a given jellyfish population but likely vary between different environments. The allometric relationships obtained from our observations allow us to estimate the amount of nutrients released by a jellyfish population via extrapolation of the individual mass-specific release rates based on the abundance and size distribution of a population. This study highlights the importance and complex role of jellyfish blooms in coastal nitrogen cycling, where they can locally support high rates of surface ocean nitrification. Equally, the substantial release of ammonium likely supports phytoplankton growth and may locally impact phytoplankton community composition. Considering the widespread geographic distribution of bloom forming jellyfish species investigated in this study (Fig. 1) and the predicted future increase of jellyfish blooms, our findings point toward an increasing relevance of jellyfish on coastal nitrogen and carbon cycling.

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Conflict of interest

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