Carbon and Calcium Carbonate Export Driven by Appendicularian Faecal Pellets in the Humboldt Current System off Chile

A. Eduardo Menschel & Humberto E. González

The role of appendicularian faecal pellet (FPa) size fractions on coccolithophore-derived particulate organic carbon (POC) and calcium carbonate (CaCO₃) export to the deep sea was assessed from sediment traps within a period of ten years (1995–2004) off Coquimbo (CQ, 30°S) and five years (2005–2009) off Concepción (CC, 36°S) in the Humboldt Current System (HCS) off Chile. The composition and size distribution of 1,135 FPa samples from sediment traps deployed at 2,300 and 1,000 m depths showed non-linear, inverse relationships between the FPa size-fractions and their volume-specific POC and CaCO₃ contents, which were up to ten times higher for small (<100 µm in diameter) than large (>100 µm) FPa. On average, 13 and 2% of the total POC and CaCO₃ fluxes, respectively, were contributed mainly by small FPa (90%), with maxima during the autumn and summer. Thus, a non-linear, exponential model of volume-specific POC and CaCO₃ contents of FPa substantially improved vertical flux rate estimates. In the HCS, annual carbon flux based on a non-linear FPa carbon load was double the estimate assuming a linear-volume to carbon load for FPa (345 and 172 kton C y⁻¹). We recommend a widespread consideration of this non-linear model in global carbon estimates.

The Humboldt Current System (HCS) off Chile is one of the world's most productive coastal upwelling systems, originating from the West Wind Drift at circa 40–45°S and including the Coquimbo (30°S) and Concepción (36°S) upwelling centres. The coastal and oceanic regions between 30°S and 36°S are dominated by Sub-Antarctic Waters (SAAW) down to 150 m depth and approximately 700 nm westward. This part of the HCS is highly dynamic with a net northward flow of the SAAW, which bifurcates into two tongues (oceanic and coastal currents) and border the southward Chile-Perú counter current. Below the SAAW down to 400 m depth lay the cold (<10 °C), nutrient rich, oxygen depleted (<1 mL L⁻¹) Equatorial Subsurface Water. Between 400 and 1,000 m depths predominate the Antarctic Intermediate Waters and the Deep Pacific Water. The region between 30–36°S is characterized as a transition zone with high kinetic energy (˃40 cm² s⁻²) in a wide band parallel to the coast that can extend up to 800 km westward.

The wind stress that favours upwelling along the coast off Chile predominates during spring-summer and contributes to fertilization (e.g. nitrate, phosphate) that promotes high primary production (>20 g C m⁻² d⁻¹) and phytoplankton biomass (>5 mg m⁻³) in the surface waters of the HCS. The importance of the continental margins in the productivity and carbon cycles is widely recognized; however, the little information on the function of the biological pump is restricted to specific upwelling centres within the vast area covered by the HCS.

The biological carbon pump is an important component of the global carbon cycle in which the factors influencing zooplankton faecal material export, particularly FPa, remain poorly understood. In the coastal areas of the HCS, the vertical flux of POC is driven mainly by diatoms and copepods; however, in oceanic areas, the carbon flux is driven mainly by appendicularian/euphausiid faecal pellets and the carbonate flux by foraminifers and FPa loaded with coccolithophore shells. Thus, in oceanic areas of the HCS off Chile, appendicularians and coccolithophores are important functional groups in the carbonate pump. Overall, the magnitude and
export production, playing significant roles in the export of the particulate organic carbon (POC) and calcium carbonate (CaCO₃) to the deep sea. However, they can process large amounts of food in a short time and produce numerous faecal pellets (FP) that contribute to the carbon biogeochemical cycle. This plethora of information demonstrates the importance of zooplankton FP in the export of particulate organic carbon from the photic zone to the deep sea.

The size-spectrum of FP sizes (diameter and length) and volumes were analysed from 5,102 FP isolated from 201 sediment trap samples from CQ and CC in the HCS, covering most of the study period. We found that 89% of the FP were between 10 and 100 μm in diameter, highlighting the pivotal role of small FP in fluxes of carbon and calcium carbonate in the oceanic region of the HCS off central Chile (Fig. 1).

**Results**

**FP size-spectrum.** The size-spectrum of FP sizes (diameter and length) and volumes were analysed from 5,102 FP isolated from 201 sediment trap samples from CQ and CC in the HCS, covering most of the study period. We found that 89% of the FP were between 10 and 100 μm in diameter, highlighting the pivotal role of small FP in fluxes of carbon and calcium carbonate in the oceanic region of the HCS off central Chile (Fig. 2).

The remaining FP (11%) were between 101 and 390 μm.

**Carbon and calcium carbonate contents in FP.** The inverse relationship between the FP size and their volume-specific POC content (mg C mm⁻³) was best represented by a power function (f(x) = 0.0087x⁻⁰.⁵⁵⁶, R² = 0.85). This was obtained from measurements of 1,135 FP isolated from sediment trap samples collected at stations CQ and CC during most of the study period. The POC in FP was highly variable (0.02–0.77 mg C mm⁻³), with the highest volume-specific values (~0.2–0.77 mg C mm⁻³) in small FP (Fig. 3a).

---

**Table 1.** Station positions of the sediment traps off Coquimbo (CQ) and Concepción (CC) sites, trap depths, bottom depths at the trap locations, numbers of samples retrieved without problem, deployment periods and sampling intervals. Sample numbers less than 20 indicate periods when the sediment trap malfunctioned. The total samples obtained from sediment traps (CQ and CC) were 201. In the column "Samples", the numbers < 20 indicate malfunction of the sediment trap.
and the lowest (0.02–0.2 mg C mm\(^{-3}\)) in large FPa (Fig. 3). We also expressed the average concentration of carbon per FPa (mg C FPa\(^{-1}\)) for each size group (small, medium, and large) as functions of their average biovolumes. Different slopes in each of the linear equations among the three size groups denote a highly non-linear and inverse relationships between FPa carbon content and their biovolumes in FPa (Fig. 3b–d).

Coccolith abundance, CaCO\(_3\) content, and coccolithophore composition within FPa. The number of coccolithophore plates in the FPa matrix were quantified by species. The FPa ranged between 0.08–0.35 mm in diameter and 0.0006–0.080 mm\(^3\) in biovolume. The coccolith abundance and coccolithophore-derived calcium carbonate (CCa) concentration showed non-linear inverse exponential functions when related with its biovolume-specific (per mm\(^3\)) value at both sites. At CQ, the mean coccolith and CCa contents were 5.1 to 24.3\(\times\)10\(^6\) coccoliths mm\(^{-3}\) and 0.1 to 0.3 mg CaCO\(_3\) mm\(^{-3}\), respectively. The maximum biovolume-specific abundance of coccoliths and CCa were observed in the smallest FPa (2.1\(\times\)10\(^7\) coccoliths mm\(^{-3}\); 0.24 mg CaCO\(_3\) mm\(^{-3}\)) and the minimum in the largest (1.3\(\times\)10\(^7\) coccolith mm\(^{-3}\); 0.18 mg CaCO\(_3\) mm\(^{-3}\)) (Fig. 4a,b). In contrast, at the CC site, these parameters ranged from 7.8\(\times\)10\(^6\) coccoliths mm\(^{-3}\) and 0.091 mg CaCO\(_3\) mm\(^{-3}\) to 2.9\(\times\)10\(^6\) mm\(^{-3}\) and 0.027 mg CaCO\(_3\) mm\(^{-3}\), respectively. The smallest FPa showed higher coccolith abundance and CCa concentration (6.2\(\times\)10\(^6\) coccolith mm\(^{-3}\); 0.064 mg CaCO\(_3\) mm\(^{-3}\)); in contrast, the largest FPa had the least (5.5\(\times\)10\(^6\) coccoliths mm\(^{-3}\); 0.056 mg CaCO\(_3\) mm\(^{-3}\)) (Fig. 4a,b).

On average, seventeen genera/species of coccolithophore were found in the FPa from both sites; however, there were important differences between the CQ and CC sites. The abundance of coccoliths and CCa concentrations were ~3 and 1.6 times higher in CQ than in CC site. The main coccolith type observed was *Emiliania huxleyi*, which represented >70% of the abundance of all recorded species and was ~3 times more abundant in CQ than the CC site.
The CaCO$_3$ concentrations in FPa were dominated by coccoliths belonging to the species *Calcidiscus leptoporus*, which contributed ~45% of total carbonate biomass, while *E. huxleyi* and *Helicosphaera carteri* together contributed ~40%. These three species represented >80% of the carbonate biomass in FPa in both sites (Table 2).
POC, PIC/POC ratios and CaCO₃ fluxes mediated by FPA in CO and CC. Fluxes of POC and CaCO₃ in FPA at 2,300 and 1,000–2,300 m depths in the oceanic region off CO and CC varied greatly during time series stations measured (with gaps due to malfunctions of the traps) in time periods of ten and five years (1995–2004 and 2005–2009), respectively (Figs. 5 and 6). Overall in the CO site, the FPA-mediated fluxes averaged 18.1% of the total POC flux (average 4.7 mg m⁻² d⁻¹) (Fig. 5a,b) and 3.4% of the total FPA-mediated CaCO₃ flux (48.1 mg m⁻² d⁻¹) (Fig. 5c,d).

In the CO site, FPA-mediated fluxes averaged 19.8% (3.1 × 100)/51.6) and 4% (0.86 × 100)/21.36) of the total POC flux to the 1,000 m (average 15.6 mg C m⁻² d⁻¹) and 2,300 m (average 21.4 mg C m⁻² d⁻¹), respectively. While that the general average was 7.7% of the total POC flux (19.7 mg C m⁻² d⁻¹) (Fig. 5a,b and Table 3).

The total FPA-mediated CaCO₃ flux represented in average the 1.6% (0.81 × 100)/51.6) and 0.7% (0.32 × 100)/44.54) to the 1,000 m (average 51.6 mg CaCO₃ m⁻² d⁻¹) and 2,300 m (average 44.5 mg CaCO₃ m⁻² d⁻¹), respectively. However, considering both depth (1,000 and 2,300 m) the average was ~1% of the total FPA-mediated CaCO₃ flux (46.7 mg CaCO₃ m⁻² d⁻¹) (Fig. 5c,d).

The total PIC/POC ratios for the CC site (1.2) was higher than for CO site (0.28). On the other hand, the PIC/POC ratio was slightly higher at the CO site (0.33), from both depths, than for CC-site (0.22), respectively (Table 3).

In the CO site, maximum fluxes of FPA-mediated PIC ranged between 2.3 and 4.4 mg C m⁻² d⁻¹ and occurred during summer-autumn 1995 (Fig. 5b), while in the CC site, the maximum values (3.0–7.4 mg C m⁻² d⁻¹) occurred during the summer-autumn 2005–2006 and winter of 2006 (Fig. 6b). Similarly, fluxes of FPA-derived CaCO₃ for the CO site were highest in seasonal-summer of 1995, 1998, and 2002 (6.0–9.8 mg CaCO₃ m⁻² d⁻¹) (Fig. 5d), while high fluxes for the CC site (1.5–1.6 mg CaCO₃ m⁻² d⁻¹) occurred during the summer-autumn of 2005–2006 (Fig. 5d).

The average annual FPA-mediated PIC/POC fluxes in the CO site were highest during 1995 (1.78 mg C m⁻² d⁻¹) and 1999 (1.04 mg C m⁻² d⁻¹) and lowest (0.22 mg C m⁻² d⁻¹) during 2003 (Fig. 5d). For the CC site, the highest fluxes were recorded during 2005 (6.9 mg C m⁻² d⁻¹) and 2006 (2.4 mg C m⁻² d⁻¹) (Fig. 6b). Likewise, the average annual FPA-mediated CaCO₃ fluxes were highest during 1995 for the CO site (3.5 mg CaCO₃ m⁻² d⁻¹) and 2005 for the CC site (1.5 mg CaCO₃ m⁻² d⁻¹) and lowest during 2004 (0.32 mg m⁻² d⁻¹) and 2008 (0.1 mg m⁻² d⁻¹).

For the CO site, the FPA-mediated PIC/POC flux during 1995 (1.78 mg C m⁻² d⁻¹) was statistically different (Mann-Whitney test, n = 16, p < 0.01) from all other years combined; similarly, 1996–1998 (0.6–0.8 mg C m⁻² d⁻¹) was statistically different from 2002–2003 (0.3–0.2 mg C m⁻² d⁻¹) (Mann-Whitney test, n = 13, p < 0.05).
High seasonal variability in FPa-mediated fluxes of POC and CaCO₃ was observed, with maxima in summer/autumn (1.2 and 2.1 mg m⁻² d⁻¹) and minimum values in spring (0.4 and 0.6 mg m⁻² d⁻¹, respectively). Fluxes differed statistically (Mann-Whitney test, n = 31, p < 0.05) between summer and winter-spring and between autumn and winter-spring.

For the CC site, fluxes of FPa differed significantly (Mann-Whitney test, N = 18, p < 0.05) among all years, except when we compared the pairs 2006–2009 and 2007–2009 that were similar (Fig. 6b,d). No significant seasonal differences in FPa-mediated POC or CaCO₃ were observed (Mann-Whitney test, N = 35, p > 0.05) during El Niño, La Niña, or “normal” periods (see Figs 5 and 6) in either of the two study areas (Mann-Whitney test, N = 11, p > 0.05).

Discussion

The Biological Carbon Pump is an important component of the global carbon cycle in which the factors influencing zooplankton faecal material export efficiency and its controlling factors, particularly for PFa export, remain poorly understood. Overall, the magnitude and efficiency of the total POC flux in time and space depend on changes in the zooplankton and phytoplankton community compositions, as well as on changes in biological processes (i.e. zooplankton grazing rate, FP production rate, phytoplankton aggregation, microbial degradation) that affect the dynamics of particle flux from the euphotic zone in oceanic provinces and upwelling areas off central Chile. The bulk of the faecal material usually is removed quickly from the upper water column due to microbial degradation and zooplankton activity, although zooplankton faecal material still is a prominent fraction of total POC export in many areas of the world oceans.

The composition of the faecal material collected in sediment traps deployed at the CQ and CC sites was represented mainly by three functional groups: appendicularians, euphausiids, and undetermined (where high degradation, amorphous or broken pieces precluded classification).

Published estimates of the POC content of PFa and its contribution to carbon export from the HCS have assumed an average carbon:volume ratio of 0.048 mg C mm⁻³, but we demonstrated in this study that FPa size and both carbon and carbonate contents were related non-linearly (Figs 3a, 4b). For example, the mean carbon content per mm⁻³ in small FPa was five times higher than in large FPa (0.26 and 0.041 C mm⁻³, respectively). This result is important for quantitative estimates of the biological pump intensity, assuming that the FPa contents would be released in the same proportions as found in the sediment traps in this study, where more than 90% of the FPa were small. Our exponential conversion increased the contribution of the FPa-mediated carbon flux by ~50% (1.04 mg C m⁻² d⁻¹) over that obtained using a linear conversion factor between carbon content and FPa-size.
The calcareous components found in the matrix of FPa from the CQ and CC sites were mainly coccolith plates (mean $10.3 \times 10^6$ plates mm$^{-3}$, Fig. 4A), from which the most abundant coccoliths were *Calcidiscus leptoporus*, *Helicosphaera carteri* and *Emiliania huxleyi*, which together averaged 86% of the CCa or $2.4 \times 10^{-13}$ mg CaCO$_3$ µm$^{-3}$ in PFa isolated from sediment trap samples deployed at the CQ and CC sites (Table 2).

While existence of coccoliths and coccospheres embedded in the matrix of FPa have been reported, no prior quantitative information on their contribution to carbonate flux in the ocean has been previously made. The coccoliths and coccospheres within the matrices of FPa are important in the export of carbonate and increase the sinking velocity of FPa to the deep ocean. Previous work has demonstrated that 1-mm particles that are 90% carbonate by mass (and 10% POM) sink ~fivefold faster than particles that are 60% carbonate by mass (and 40% POM) and ~1.75 times faster than particles that are 90% silicate by mass (and 10% POM)\(^3\). An individual coccolith has a sinking velocity of 0.1 m d$^{-1}$ or slightly higher, depending on species types and calcification degree. For example, *E. huxleyi*, *G. oceanica*, and *C. leptoporus* coccolith settling velocities (0.3, 0.7, and 4.3 m d$^{-1}$, respectively) were higher than diatom sinking rates, which ranged from 0.07 m d$^{-1}$ (*Thalassiosira weissflogii*) to 0.2 m d$^{-1}$ (*Thalassiosira oceanica*). This information clearly suggests that FPa loaded with coccoliths and coccospheres can reach a higher sedimentation velocity than one loaded with diatom frustules, demonstrating the effectiveness of coccoliths and coccospheres in the transport of carbon and carbonate to the deep ocean.

The absence of a linear relationship between FPa-size and POC and CaCO$_3$ content may be attributable to differences in the size spectrum of the food ingested, with small FPa being more uniform and compact than large FPa. To test this hypothesis, we isolated large and small FPa from a sediment trap deployed at 2,300-m depth during different seasons between 1995 and 2004 and analysed the particle content qualitatively. Large FPa contained many relatively large pieces of diatom frustules (20–63 µm diameter), mostly from the genus *Pseudo-nitzschia*, but also from the abundant *Thalassiosira* spp. and *Planktomisella sol*. In addition, the remains of radiolarians (groups Spumellaria, Nasselaria, and Phaeodaria), tintinnid loricae (genera *Codonellopsis*, *Dictiochyla*, *Protorhabdonella*, *Undella*, and *Dadayiela*), and silicoflagellates, such as *Dictiocha fibula*, were frequently observed. In contrast, microscopic analysis of small FPa demonstrated a preponderance of coccoliths, with occasional remains of diatoms and tintinnid loricae embedded in the FPa matrix. Large oikopleurid species have food-concentrating filters.
with a coarser mesh than small ones37, suggesting a direct relationship between pore dimensions of appendicularian filters and body size. Our study suggested that large appendicularians utilized a broad food-size spectrum (diatoms, coccolithophores, tintinnids, radiolarians, etc.), while the diet of small appendicularians was more restricted, which partially explained the small mineral skeletons in small FPas.

The FPas biovolume to POC (or CaCO3) content models that should be used to estimate the role of FPas in biomineral vertical fluxes follow size-dependent non-linear relationships, indicating that the FPa size fraction needs to be considered to improve the accuracy of these estimates. We strongly recommend a more taxa-specific analysis by microscopy, which is more time consuming but essential when dealing with size-dependent FPa-mediated POC and carbonate export processes. This view is supported by findings of changing composition of sinking particles across a region with unchanging carbon flux, suggesting that variability in the mechanisms of carbon flux38 or the role of phytoplankton biominerals as ballast39 may not be reflected by bulk measurements. In addition, coccolithophore calcium carbonate is usually considered to have a low carbon flux and high export efficiency41. Thus, CaC is generally not included in calculations of FPa carbon export, resulting in up to 4% underestimation of total carbon flux to deeper areas of the HCS.

High seasonal and inter-annual variability in POC flux was observed in the time series station off the CQ site (Fig. 5). The estimated average FPa-driven POC flux at the CQ site (0.86 mg C m⁻² d⁻¹) was slightly higher than that reported for the same area during 1993–1995 (~0.7 mg C m⁻² d⁻¹)44, while that recorded for the CQ site was substantially higher (1.5 mg C m⁻² d⁻¹) than at the CQ site (Figs 5b, 6b).

Overall, the use of the FPa-size-based carbon content model of this study increased the estimated contribution of FPAs to total POC export by a factor of 34% (CC site) and 46% (CQ site) over those obtained from a linear FPa-volume to carbon content model. The highest average FPa flux occurred during the productive summer period, coinciding with intensification of the winds favouring upwelling and Ekman transport45, which may enhance local productivity and carbon export. We did not find significant differences (p > 0.05) in the FPa-mediated fluxes of POC or CaCO3 among El Niño, La Niña, and “normal” periods (showed in Figs 5 and 6), suggesting that the diet of the appendicularians was not severely affected by these events.

Reports during the El Niño event in the HCS show that the seasonal upwelling ceased, the water column became warmer, and the thermocline and nutricline deepen significantly during the passage of coastal-trapped waves46,47. These events decrease phytoplankton productivity and alter the trophic chain, changing the biological composition and physical dynamics of the coastal and marine ecosystems18,41. Both phytoplankton and zooplankton shift toward small-sized species that have been claimed to prevail during El Niño17,18,40. Thus, it is highly probable that the composition and size spectrum of small food particles used by appendicularians, down to colloids (<0.2 μm)42, is little affected by El Niño events47,49, a situation that might be extrapolated to future global warming scenarios on the FPa-mediated carbon and CaCO3 export fluxes.

| Study area | Depth sediment trap (m) | POC flux mg m⁻² d⁻¹ | CaCO3 flux mg m⁻² d⁻¹ | C-CaCO3 mg m⁻² d⁻¹ | PIC/POC ratios | Reference |
|------------|-------------------------|----------------------|------------------------|---------------------|----------------|----------|
| Panama Bight (5°22′N, 85°35′W) | 2,590 | 11.20 | 184.20 | 22.10 | 1.97 | Honjo (1982)45 |
| Station P (501 N; 1451 W) | 3,800 | 10.50 | 67.90 | 8.15 | 0.78 | Honjo (1984)46 |
| Arabian sea (14.51 N; 651 W) | 2,800 | 1.20 | 11.82 | 1.42 | 1.18 | Nair et al.47 |
| Equatorial Pacific (121° S; 135°W) | 3,600 | 0.70 | 16.00 | 1.92 | 2.74 | Honjo et al.48 |
| Bay of Bengal (131 N; 841E) | 2,300 | 6.70 | 42.13 | 5.06 | 0.75 | Beukhout et al.49 |
| Humboldt current (30°S 73°11′W) | 3,700 | 7.50 | 94.70 | 11.36 | 1.61 | Hebbeln et al.50 |
| Humboldt current (30°S 73°11′W) | 2,300 | 7.50 | 82.39 | 9.89 | 1.69 | Hebbeln et al.50 |
| Subantarctic zone (53°02′S; 174°44′W) | 1981 | 2.70 | 18.60 | 2.23 | 0.83 | Honjo et al.50 |
| Subantarctic zone (60°17′S; 170°03′W) | 1,003 | 63.00 | 35.10 | 4.21 | 0.67 | Honjo et al.50 |
| West Sargasso Sea (31°50′N, 64°10′W) | 2,394 | 4.40 | 49.80 | 5.98 | 1.36 | Ramaswamy et al.50 |
| West Sargasso Sea (31°50′N, 64°10′W) | 1,500 | 1.70 | 21.00 | 2.52 | 1.48 | González et al.51 |
| East China Sea slope (25°11′N;122°58′E) | 1,340–1,588 | 128.60 | 214.20 | 25.70 | 0.20 | Hung et al.52 |
| Humboldt current (30°S 73°11′W) | 2,300 | 7.00 | 61.00 | 7.32 | 1.05 | González et al.51 |
| Arabian Sea (16°18′N; 60°30′E) | 1,200 | 14.80 | 106.80 | 12.82 | 0.87 | Ramaswamy et al.50 |
| Bay of Bengal (13°09′N 84°20′E) | 2,282 | 8.50 | 39.50 | 4.74 | 0.56 | Ramaswamy et al.50 |
| Arabian Sea (03°34′N 77°46′E) | 2,394 | 4.40 | 49.80 | 5.98 | 1.36 | Ramaswamy et al.50 |
| Bay of Bengal (05°01′N 87°09′E) | 3,010 | 5.70 | 37.80 | 4.54 | 0.80 | Ramaswamy et al.50 |
| Humboldt current (30°S 73°11′W) | 2,300 | 4.74 (0.86*) | 48.12 (1.64*) | 5.77 (0.19*) | 1.22 (0.22*) | This Study |
| Humboldt current (37°05′S 74°50′W) | 1,000 | 15.60 (3.10*) | 51.62 (0.81*) | 6.19 (0.097*) | 0.39 (0.031*) | This Study |
| Humboldt current (37°05′S 74°50′W) | 2,300 | 21.36 (0.86*) | 44.54 (0.32*) | 5.34 (0.038*) | 0.25 (0.044*) | This Study |
| Humboldt current (37°05′S 74°50′W) | 1,000–2,300 | 19.65 (1.51*) | 46.66 (0.47*) | 5.60 (0.056*) | 0.28 (0.037*) | This Study |

Table 3. POC, CaCO3, C-CaCO3 (mgC m⁻² d⁻¹) and PIC/POC ratios estimated from deep sediment traps (range 1,000–3,700 m depth) deployment in different areas of the world oceans. C-CaCO3 correspond to inorganic carbon obtained from CaCO3 bulk. (*) contribution of the FPAs to the carbon and carbonate flux in the CQ and CC site in the HCS.
For the CQ site, fluxes of calcium carbonate and POC into sediment traps below 2,300 m were correlated 
\( y = 6.889x + 15.417, n = 127; R^2 = 0.51 \) (Fig. 7a), which is consistent with published data for various geographic 
regions3,14,44–53, like the PIC/POC ratios (Table 3). Some of these oceanic and coastal areas (such as the CQ site) 
exhibit relatively low productivity1 that favours the export of CaCO3 (mean 48.1 mg m\(^{-2}\) d\(^{-1}\)) over POC (mean 
4.7 mg m\(^{-2}\) d\(^{-1}\)) (Fig. 5a,c) and possibly carbonate-driven POC export as the main ballast mechanism, which 
would explain the high PIC/POC ratio (>1) observed in this site. Thus, biominerals increase substantially sinking 
velocity because of their high densities and may serve to protect organic matter from degradation during transit 
to the ocean floor53–55. Conversely, POC and CaCO3 at CC, were not significantly correlated 
\( y = 1.090x + 27.56, n = 30; R^2 = 0.16 \) (Fig. 7b), and the PIC/POC ratio was lower (<1) than the CQ site, which may be attributable to 
a very productive upwelling centre that supports massive diatom blooms1 and favours the export of both CaCO3 
(mean 46.7 mg m\(^{-2}\) d\(^{-1}\)) and POC (mean 19.7 mg m\(^{-2}\) d\(^{-1}\)) (Fig. 6a,c) with perhaps silicate-driven POC export as 
the main ballast mechanism.

In the HCS, an average appendicularian abundance is estimated at 21.6 ind. m\(^{-3}\)27,56 or 1,078 ind. m\(^{-2}\) integrated 
down to 50 m depth (see www.st.nmfs.noaa.gov/copepod/atlas). On average, one appendicularian can pro-
duce between 7.3 and 10.1 FPa h\(^{-1}\)27,57 or an average of 198.6 FPa d\(^{-1}\) or 72,500 FPa y\(^{-1}\) (Supplementary Table S4).

We estimated a total area of the HCS off Chile of 910,000 km\(^2\), assuming a total latitudinal length of 2,600 km 
between 18.5° and 41.5°S and a width of 350 km58. Because most appendicularians occur within the upper 50 m 
of the water column59,60, an estimate of \(1.08 \times 10^{19}\) appendicularians within this upper layer would produce 
7.12 \times 10^{19} FPa y\(^{-1}\) along the whole HCS. Moreover, considering the size-spectrum of FPa found at both study 
sites (Fig. 2), we assumed that ~90% of the FPa produced are small FPa (<100 \(\mu\)m of diameter) and 10% large.

As the small FPa had an average biovolume and carbon content of 0.26 mg C mm\(^3\) and 0.000364 mg C, respec-
tively, while equivalent values for a large FPa were 0.041 mg C mm\(^3\) and 0.0015 mg C (Fig. 3a and Supplementary 
Table 4). Finally, we estimated that \(3.4 \times 10^{16}\) mg C y\(^{-1}\) or 0.034 Gt C would be released annually as FPa in the 
upper 50 m water column of the HCS (Supplementary Table S4).

Given that the FPa vertical flux averaged 1.04 mg C m\(^{-2}\) d\(^{-1}\) (Figs 5b, 6b), we can estimate that 345.5 \times 10^{6} kg 
carbon (or 0.000345 Gt carbon) are exported annually down to 2,300 m depth in the HCS, which represents 1% 
of the standing stock of the FPa-mediated carbon export from the upper 50 m stratum could reach the ocean floor along the HCS off Chile.

This is in line with reports suggesting that <3% of the generated upper production reaches bathypelagic 
depths (>1,000 m) in the deep sea62, mainly due to the highly complex and variable food web in the water col-
umn63 that removes most of the sinking particles, such as the FPa.

Our non-linear FPa-size derived carbon to volume ratio estimated an average POC flux in the HCS off Chile to be 
345 kton C y\(^{-1}\), twice as much the estimated with the linear model (172 kton C y\(^{-1}\)). This study only includes the HCS 
off Chile, a very productive ecosystem that might differs from the rest of the global oceans (in food supply quality and 
quantity). Nevertheless, a widespread characteristic of the model is its non-linear, FPa-size derived nature, that we re-
commends to be considered for future estimated where FPa are key vehicles of PIC and/or POC carbon export.
Methods

Analysis of total POC and CaCO₃. Samples were obtained from sediment traps (SMT 230, Salzgitter Electronic, Kiel, equipped with 20 sample bottles) deployed at 2,300 m depth ~150 km off the CQ site (30°S, off Coquimbo city), at the CC site (36°S, off Concepción city); the trap was initially deployed down to 1,000 m depth (2005–2006), but later moved down to 2,300 m depth (2006–2009). The water depths in the study sites were 4,700 m for CQ and 4,300 m for CC (Table 1). Sample collection cups were changed between 7 to 18 days in the CQ and 19 days for the CC site (Table 1). Before deployment, each collection cup was filled with a hypersaline NaCl–seawater solution (38–40 g kg⁻¹, prepared with water collected at 2000 m and 1% (v/v) saturated HgCl₂) to retard bacterial activity in the trap material. After recovery, the samples were poisoned again with HgCl₂ (0.5 mL/100 mL of seawater) and stored at 4°C. In the laboratory, a fraction of the samples was split into aliquots by a rotary liquid splitter. The splitting procedure, sample preparation, and analysis were previously described.

Subsequently, particulate organic carbon (POC) analyses were done after removal of CaCO₃ with HCL 2 N, while for total particulate carbon (TPC), part of the sample (e.g. 1/10 to 1/4,096) was filtered (pre-combusted GF/F Whatman) and dried at 50°C. Later, these samples were analysed in a Carlo Erba C/N-analysers using acetonilide as a standard. Finally, the TPC and POC data were converted by a stoichiometric balance for the final determination of the total particulate carbonate ([(TPC-POC) x 8.3333]⁺).

Analysis of POC in FPa. Intact FPa (1,114 units, 528 from CQ and 586 from CC) of various sizes between 50 and 340 μm in diameter were isolated from sediment trap samples that covered most of the study period, and measured (major and minor axes of the FPa ellipsoid shape) by using a stereo microscope (Leica model MZ6). After a gentle wash with de-carbonated mineral water, using petri dish, to maintain the integrity of the FPa and photographed (Nikon Coolpix model 4500) using the stereo-microscope, then they were placed on 23 Whatman GF/F glass fibre filters in batches depending on their size (from 14 large to 142 small FPa). The images were processed in ImageJ software to estimate the volume of each faecal pellet and the total volume of each batch. Finally, subsamples of FPa on GF/F filters were acidified with 0.2 mL (~4 drops with a Pasteur pipette) of 2N HCl to remove particulate inorganic carbon, washed with distilled water to remove excess acid, dried at 50°C, and stored in hermetic plastic bottle with silica gel to await organic carbon (CHN) analysis, as described above. Due to the high variability in the diameters of the FPa used for elemental carbon analysis in the time series data from 1995 and 2004 (Fig. 2), average POCs of 0.26 mg mm⁻³ were used for small FPa (<100 μm diameter) and 0.041 mg mm⁻³ for large FPa (>100 μm diameter).

Total carbonate, coccolith abundance, and coccolithophore composition in FPa. 110 intact FPa (50–340 μm in diameter) were removed from sediment trap samples deployed at CQ (44 FPa pooled in 16 bunches) and CC (66 FPa pooled in 18 bunches) throughout the study period. This was made with the aid of a stereomicroscope (Leica model MZ6) at ×4 magnification and placed in a Petri dish with pre-filtered de-carbonated mineral water to prevent coccolith dissolution. Faecal pellets were washed five to eight times with filtered de-carbonated water and photographed. Images were processed with ImageJ software to estimate faecal pellet size and biovolume (Figs 2, 4) by applying prolate spheroid geometry.

In order to analyse the composition of coccospheres and coccoliths within FPa, 1–10 pellets (number depending on size) were removed from the Petri dish, gently placed in 2.5–5 mL vials containing 2 mL de-carbonated mineral water and sonicated at 50 to 60 Hz for 30 seconds. The contents of the vials were placed in sedimentation chambers and coccospheres and coccoliths identified and counted with an inverted microscope (×1,000 magnification). The coccoliths and coccospheres observed in a known area of the bottom of the chamber were counted and the data extrapolated to the total volume of the faecal pellets. The samples also were examined by scanning electron microscopy to confirm the coccolithophores species identified with light microscopy. Finally, the number of coccoliths per coccolithophore species were transformed to CaCO₃ using conversion factors from the literature,

Statistical analyses. A Kolmogorov-Smirnov test (Minitab software) showed that our data for POC and CaCO₃ fluxes mediated by FPa in the CQ and CC sites were not distributed normally. We therefore used the non-parametric Mann Whitney test for multiple statistical comparisons (confidence limits set at 95%).

Received: 10 July 2018; Accepted: 12 October 2019;
Published online: 11 November 2019

References
1. Daneri, G. et al. Primary production and community respiration in the Humboldt Current System off Chile and associated oceanic areas. Mar. Ecol. Prog. Ser. 197, 41–49 (2000).
2. Strub, P. T., Mesias, J. M., Montecino, V., Rutllant, J. & Salinas, S. The Sea (eds Robinson, A. R., Brink, K. H.) 273–313 (John Wiley & Sons, Inc. 1998).
3. Kühl, M., Thier, M., Oeser, K., Schrader, T. & Eicken, H. Deep Sea Res. II 56, 3462–3475 (2009).
4. Cannistraro, S., Sala, G. & Giordano, G. J. Plankton Res. 24, 643–660 (2002).
5. Lutz, M., Dunbar, R. & Caldeira, K. Regional variability in the vertical flux of particulate organic carbon in the ocean interior. Global Biogeochem. Cycles 16, 11–15 (2002).
6. Muller-Karger, F. E. et al. The importance of continental margins in the global carbon cycle. Geophys. Res. Lett. 32, 1–4 (2005).
54. Armstrong, R. A., Lee, C., Hedges, J. I., Honjo, S. & Wakeham, S. G. A new, mechanistic model for organic matter carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. Deep-Sea Res. II. 49, 219–236 (2002).
55. Bathmann, U. V., Noji, T. T., Voss, M. & Peinert, R. Copepod fecal pellets: abundance, sedimentation and content at a permanent station in the Norwegian Sea in May/June 1986. Mar. Ecol. Prog. Ser. 38, 45–51 (1987).
56. Aravena, G. & Palma, S. Taxonomic identification of appendicularians collected in the epipelagic waters off northern Chile (Tunicata, Appendicularia). Rev. Chil. Hist. Nat. 75, 307–325 (2002).
57. Taguchi, S. Seasonal study of fecal pellets and discarded houses of Appendicularia in a subtropical inlet, Kaneohe Bay, Hawaii. Est. coastal Shelf Sci. 14, 545–555 (1982).
58. Fuenzalida, R., Schneider, W., Garcés-Vargas, J. & Bravo, L. Satellite altimetry data reveal jet-like dynamics of the Humboldt Current. J. Geophys. Res. 113, C07043 (2008).
59. Fenaux, R. Quelques aspects de la distribution verticale chez les appendiculaires en Méditerranée. Cah. Biol. Mar. 9, 23–29 (1968).
60. Tomita, M., Shiga, N. & Heda, T. Seasonal occurrence and vertical distribution of appendicularians in Toyama Bay, southern Japan Sea. J. Plankton Res. 25, 579–589 (2003).
61. Wakeham S. G. & Lee C. Organic Geochemistry (eds Engel M. H., Macko S. A.) 145–165 (Springer, Boston, 1993).
62. De La Rocha, C. & Passow, U. Factors influencing the sinking of POC and the efficiency of the biological carbon pump. Deep-Sea Res. II. 54, 639–658 (2007).
63. Legendre, L. & Rivkin, R. B. Fluxes of carbon in the upper ocean: regulation by food-web control nodes. Mar. Ecol. Prog. Ser. 242, 95–109 (2002).
64. Lee, C., Hedges, J. I., Wakeham, S. G. & Zhu, N. Effectiveness of various treatments in retarding microbial activity in sediment trap material and their effects on the collection of swimmers. Limnol. Oceanogr. 37, 117–130 (1992).
65. Wefer, G. & Fischer, G. Annual primary production and export flux in the Southern Ocean from sediment trap data. Mar. Chem. 35, 597–613 (1991).
66. Sun, J. & Lui, D. Geometric models for calculating cell biovolume and surface area for phytoplankton. J. Plankton Res. 25, 1331–1346 (2003).
67. Bairbakhish, A. N., Bollmann, J., Sprengel, C. & Thierstein, H. R. Disintegration of aggregates and coccospheres in sediment trap samples. Mar. Micropaleontol. 37, 219–223 (1999).
68. Young, J. R. & Ziveri, P. Calculation of coccolith volume and its use in calibration of carbonate flux estimates. Deep-Sea Res. II. 47, 1679–1700 (2000).

Acknowledgements
We thank the captain and crew of the R/V AGOR Vidal Gormaz for their help during sampling. We are grateful to Mr. Ricardo Silva, who assisted us with sample preparation and observations during the scanning electron microscope analysis at UACH, and Dr. O. Pizarro, M. Marchant, D. Hebbeln and C. Hormazabal, who helped during the deployment and retrieval of the deep sediment trap. The POC and carbonate data for the CQ site were measured at the AWI- Bremerhaven, where our former collaborators, Drs. D. Hebbeln, and M. Marchant, deserve our deep gratitude. Special thanks are due to Dr. Lluisa Cros (ICM, Barcelona, Spain) for her valuable collaboration in methodology for studying coccolithophores and in their identification. Editorial services were provided by Sea Pen Scientific Writing and the English grammar was polished by Dr. R. Thompson. Several projects contributed with funds (partial financial support) at different stages of the time series: FONDAP-COPAS (150100007), FIP (2004-20), Ocean Certain (EU-FP7 N°603773), COPAS-Sur Austral Basal (PFB-31); FONDAP-IDEAL (15150003). EM acknowledges a fellowship provided by CONICYT to conduct graduate studies at the Universidad de Concepción.

Author contributions
H.E.G. was responsible for acquiring funding and for sampling methods and design; E.M. collected the samples and analysed the data; H.E.G. and E.M. co-wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-019-52469-y.

Correspondence and requests for materials should be addressed to H.E.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2019