



Zooplankton biomass and ETS activity during the Late Winter Bloom in the Canary Islands waters: Vertical migration and active flux

María Couret Huertas

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Santiago Hernández León Laia Armengol Bové

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El presente trabajo fue dirigido por: Santiago Hernández León, Instituto de Oceanografía y Cambio Global (IOCAG), Universidad de Las Palmas de Gran Canaria.

Laia Armengol Bové, Instituto de Oceanografía y Cambio Global (IOCAG), Universidad de Las Palmas de Gran Canaria.

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#### Abstract

Studies about carbon active flux due to diel migrants in the Canary Current are scarce. The wide range of physical structures such as upwelling zones, filaments, mesoscale eddies, and large oligotrophic areas makes this area unique. We measured zooplankton biomass and the electron transfer system (ETS) enzymatic activity as a proxy for zooplankton metabolism in areas of different productivities from the oligotrophic waters off La Palma Island (Canary Islands) to the upwelling zone off Northwest Africa during the late winter bloom. Biomass values and specific ETS activities varied between 355±136 and 695±186 mg prot m<sup>-2</sup>, and 10.6±2.6 and  $26.3\pm3.2 \mu IO_2$  mg prot<sup>-1</sup> h<sup>-1</sup> in oligotrophic waters and productive areas, respectively. Migrant biomass was also measured by sampling during day and night using a WP-2 in the 0-200 m depth water column. Respiratory and total active flux (including respiration, mortality and excretion flux) were assessed using published empirical relationships. Values obtained in upwelling areas were 8.9  $\pm$ 4.9 mgC m<sup>-2</sup> d<sup>-1</sup>, while in oligotrophic waters were 6.4±1.1 mgC m<sup>-2</sup> d<sup>-1</sup>. The results showed the importance of diel vertical migrations in the downward transport of carbon in the biological pump. As a first approach, we also measured <sup>210</sup>Po activity concentration in zooplankton and water samples. Higher values were found in the large size fraction (>1000 µm organisms).

## Key words

Zooplankton, respiration, active flux, ETS, <sup>210</sup>Po concentration.

#### Introduction

The biological pump is the mechanism that drives the carbon flux inside the ocean through interactions between the components of the pelagic ecosystem (Longhurst, 1989). Phytoplankton in the epipelagic layer uptake inorganic carbon transforming it into particulate organic carbon (Steinberg et al., 2002). Then, particulate organic carbon is transported downwards by three different mechanisms: (1) the sinking of organic matter through the water column, the so-called passive or gravitational flux (Carlson et al., 1994; Mestre et al., 2018), (2) the physical mixing of dissolved and particulate organic matter, and (3) the active flux, also known as migratory flux, referring the active transport of organic matter by organisms, especially zooplankton and micronekton, to the deepest areas of the ocean (Longhurst and Harrison, 1988). Carbon leaving the euphotic zone and reaching the mesopelagic layer, via marine food webs (Archibald et al., 2019), remains at depth for timescales ranging from months to several vears (Bode et al., 2018; Koppelmann et al., 2000; Lampitt et al., 2008; Minutoli and Guglielmo, 2009; Siegel et al., 2014). The pump efficiency depends on food web processes (Ducklow et al., 2001) and the depth of carbon remineralization. Considering the processes transporting carbon to the interior of the ocean, it is undeniable that the ocean acts as a natural sink of CO<sub>2</sub> through marine food webs (Archibald et al., 2019).

Organisms performing diel vertical migrations (DMVs), the synchronized movements up and down in the water column over a daily cycle (Brierley, 2014), are the main responsible for the active flux. Migrant organisms remain at depth during daylight hours and move near the surface at night in order to feed, returning to depth before dawn (Bianchi et al., 2013; Lampert, 1989; Steinberg et al., 2002). The behavior of migrant organisms were the subject of debate for a long time (Han and Straškraba, 1998). Some authors hypothesized light intensity or metabolic advantages as main

causes of migrations. However, Gliwicz (1986) examined non-migratory and migratory populations of the copepod *Cyclops abyssorum* reporting that diel vertical migrations just happened when predatory fish were present. Dawidowicz and Loose (1992) also showed predation avoidance as the main reason for migration. Moreover, several authors supported the predator-avoidance hypothesis as the ultimate cause of DVM behavior in marine environment (see Graeme, 2003). Zooplankton migrants are primarily large copepods and euphausiids (Hernández-León et al., 2019a). Their migratory scope depends on external and internal factors such as water temperature (McLaren, 1974), light, predation, and food availability (Simoncelli et al., 2019), as well as oxygen minimum zones, and body mass (Teuber et al., 2013). The amplitude of their movements and vertical distribution vary between species and ontogenetic stages of the same species (Lampert, 1989). Zooplankton may either migrate up and down together in a narrow band or may be sharply stratified in deep waters during daylight, but spread throughout the entire water column at night (Lampert, 1989).

The main outcome of these up-and-down movements is the increase of organic matter transported to the deep sea (Romero-Romero et al., 2019). Carbon exported to deep layers is achieved by several processes such as respiration (Longhurst et al., 1990), excretion (Steinberg et al., 2002), gut flux (Angel, 1989), and mortality at depth (Zhang and Dam, 1997). Consequently, active flux becomes relevant within marine biogeochemical cycles influencing carbon distribution (Putzeys et al., 2011). This transport mediated by zooplankton DVM below the euphotic layer increases the global export flux by 14% (Archibald et al., 2019), and may even be 20% of the export flux compared to the sedimentation rates or gravitational flux (Longhurst, 1989; Longhurst et al., 1990).

In fact, total active flux (including respiration and estimated mortality, excretion, and gut flux) by zooplankton and micronekton account for about 25% of total flux (passive and active) in oligotrophic zones (Hernández-León et al., 2019a). DVM export carbon downwards mainly due to respiration, regarded to be an ubiquitous process (Filella et al., 2018; Martinez, 1991; Minutoli and Guglielmo, 2009; Packard et al., 2015; Schalk, 2007). Then, the accurate determination of respiration rates is, therefore, essential to these studies of energy flow through marine ecosystems (Cammen et al., 2007; Filella et al., 2018), and help tracing energy pathways and carbon fluxes (Ikeda, 1985; Putzeys and Hernández-León, 2005). The respiration rate is usually measured analyzing the enzymatic activity of the electron transfer system (ETS) activity as a proxy for respiration in the mesopelagic zone, as it can be correlated to *in vivo* respiration (Owens and King, 1975), and used as an estimator of zooplankton respiration rates (Hernández-León and Gómez, 1996).

Zooplankton biomass in subtropical waters varies within the annual cycle and during the so-called late winter bloom, organisms burst due to an increase in productivity due to mixing during winter (Hernández-León et al., 2004; Troupin et al., 2010). In spring the seasonal thermocline is reestablished remaining through the summer and autumn, restricting the injection of nutrients into the euphotic zone, and therefore limiting primary production (Hernández-León et al., 1987). Knowledge of the variability of the carbon pump during these contrasting periods is of paramount importance to relate climatology (cold or warm years) and the functioning of this downward export. Transport of organic matter from the ocean surface to depth is being altered due to changes in the carbon cycle as a consequence of climate change (Brun et al., 2019). Ocean properties and, consequently, carbon flux is also changing but the effects remain poorly understood.

Thus, monitoring carbon flux is of importance to account for changes in the ability of the ocean to uptake anthropogenic carbon. Time-series in the ocean are needed to estimate the effect of a changing ocean to its capacity to transport and store carbon. In this sense, the so-called "Radial Profunda de Canarias" project (RAPROCAN) seeks to study those changes in the Canary Current. Carbon transport due to DVMs is being estimated during cruises carried out two times per year. So, we measured zooplankton biomass and estimated respiration in oligotrophic and eutrophic waters. The goal was to evaluate downward transport of carbon during the RAPROCAN 1903 cruise performed during the most productive season of the annual cycle in the Canary Current (Hernández-León et al., 2007). In order to estimate respiration, we performed four different transects in areas with different productivities, measuring the enzymatic activity of the electron transfer system (ETS) and migrant biomass as the night minus day biomass in the upper 200 m water column. The objective of this study was to obtain zooplankton biomass and ETS activities during the late winter bloom of 2019 as a main component of the time-series, as well as to estimate total active flux due to migrant zooplankton biomass.

Additionally, we measured the concentration of <sup>210</sup>Po in zooplankton samples. Zooplankton take up large amounts of <sup>210</sup>Po, concentrating, biomagnifying, and recycling unassimilated <sup>210</sup>Po back into the oceanic system as fecal pellets and molts (Uddin et al., 2018). Thus it is of interest to measure <sup>210</sup>Po activity concentration as a fraction of this tracer will remain in the body of organisms and accumulated up in the food web. Moreover, few studies have focused in the concentration of <sup>210</sup>Po in zooplankton (Carvalho, 2011; Fowler, 2011; Strady et al., 2015a), and there are no such kind of studies in the Canary Current area. Here, we hypothesized that areas of higher productivity will display higher amounts of <sup>210</sup>Po in zooplankton.

#### **Materials and methods**

Sampling took place from 28th February to 9th March, 2019 on board the R/V Ángeles Alvariño sailing from the northwest of La Palma Island (Canary Islands) to the Western Sahara coast (Northwest Africa) (Fig. 1). Vertical profiles of temperature, salinity, and chlorophyll *a* were obtained using a CTD (Seabird 911plus) mounted on a General Oceanic rosette sampler equipped with 12 liter Niskin bottles.

Zooplankton was sampled at 16 stations using a double WP-2 net (UNESCO, 1968) equipped with a 200 µm mesh size (Table 1). Vertical hauls were made from 200 meters depth to surface during day and night. One of the samples from the double net was stored in formalin (4%) for later taxonomic studies, and the second sample was used for measuring protein content and ETS activity. The latter sample was size fractionated into 200, 500 and 1000 µm sieves, frozen in liquid nitrogen, and stored at - 80°C until analysis. In the laboratory, samples were homogenized for ETS activity analysis according to Kenner and Ahmed (1975), with the modifications introduced by Gómez et al. (1996) for zooplankton samples. Protein content was determined using bovine serum albumin (BSA) as the standard following the method given by Lowry et al. (1951) and modified by Rutter et al. (1968). Zooplankton protein content was converted to dry weight using the ratio of 2.49 given by Hernández-León et al. (2019b) in subtropical waters, and dry weight was converted to carbon units assuming carbon content of 40% of dry weight (Dam and Peterson, 1993).

The difference between night and day biomass in the upper 200 m layer was assumed as the migrant biomass (Hernández-León et al., 2019a). However, stations were sampled only during day or night, so migrant biomass was estimated using adjacent stations except for stations 611 and 610 because it was not possible to sample during night. Migrant biomass in stations 7, 4, and 10 was not estimated due to the lack

of nighttime hauls. In station 10, the 500-1000  $\mu$ m size-fraction was lost during analysis, so an average value of that size fraction across T1 was performed to estimate zooplankton biomass. Station MIOCEAN was sampled during the 18<sup>th</sup> and 19<sup>th</sup> February 2019 following the same procedure explained above. We added this station to this study as a reference because it was sampled during night and daytime.

Community ETS respiration in zooplankton was determined using the specific ETS activity multiplied by biomass, while respiratory flux was estimated using the equation given by Hernández-León et al. (submitted) relating migrant biomass and respiratory flux. The latter flux is only a component of active flux, so zooplankton mortality, and excretion were also assessed (Hernández-León et al., 2019a). Mortality was estimated from growth assuming steady-state conditions in the mesopelagic zone (growth=mortality) and using the equation of Ikeda and Motoda (1978). These authors related respiration and growth, and assumed gross growth (growth/ingestion) and assimilation efficiencies of 30 and 70%, respectively (G=0.75 R). Excretion was assessed using the values of Steinberg et al. (2002) making up 24% of the respired plus excreted carbon. Gut flux as gut passage time in zooplankton is normally less than an 1 hour (Dam and Peterson, 1988), and therefore we assumed that fecal pellets are released in the epipelagic zone, and thus, included as passive flux. We are aware that this is not completely true as large copepods and euphausiids have longer gut passage times. However, because of the uncertainty in this transport and in order to be conservative in our estimations we did not add this flux (Hernández-León et al., 2019a).

<sup>210</sup>Po activity concentration in zooplankton was determined analyzing selected samples collected around the Canary Islands waters (Fig. 1). Zooplankton samples were obtained in 5 stations (see Table 1) using a WP-2 net from 200 meters depth to surface. Samples were size-fractionated, frozen in liquid nitrogen, and stored at -80°C until

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analysis. In the laboratory, each fraction was dried in an oven at 60°C for 24 hours. Dry weight of size-fractionated samples was then measured. The dry mass varied between 0.5 to 0.8 g. After the addition of <sup>209</sup>Po as a yield tracer, samples were digested with concentrated hydrofluoric acid (HF), HNO<sub>3</sub>, and H<sub>2</sub>O<sub>2</sub>. The resulting solution was evaporated and dried, and the residue was repeatedly dissolved with concentrated HCl. Final residue was dissolved with 2M HCl and ascorbic acid was added to the solution in order to reduce ferric ions and avoid its interference in <sup>210</sup>Po deposition (Macklin et al., 2014). Finally, the polonium was spontaneously plated onto a silver disc while being stirring continually overnight and measured by alpha spectrometry (Kim et al., 2017; Tejera et al., 2019). Also, seawater was collected at 25 m depth and <sup>210</sup>Po activity concentration was determined by alpha spectrometry (Canberra Model PD-450.18 AM). The activity concentration of the <sup>210</sup>Po was calculated using the following expression (Romero et al., 2003):

$$A_{210} = A_{209} \frac{N_{210}}{N_{209}} \frac{1}{m} \tag{1}$$

where A<sub>209</sub> is the certificated activity of the tracer (<sup>209</sup>Po), N<sub>210</sub> and N<sub>209</sub> are <sup>210</sup>Po and <sup>209</sup>Po net count numbers, respectively (total number of counts less background counts of the isotope), and m is the sample mass.

The uncertain associated to this activity is (Romero et al., 2003):

$$u(A_{210}) = A_{209} \sqrt{\frac{1}{N_{210}} + \frac{1}{N_{209}} + \left(\frac{u_m}{m}\right)^2 + \left(\frac{u_{209}}{A_{209}}\right)^2}$$
(2)

Polonium concentration factor (CF) was calculated by dividing zooplankton <sup>210</sup>Po activity (dry weight) between <sup>210</sup>Po activity in seawater.

## Results

#### Study area and hydrological structure

We divided the study area into different sections: Transect 1 (T1) was carried out from the most oligotrophic zone in the northwestern area off La Palma Island to the coastal zone off Northwest Africa (Fig. 1). Transect 2 (T2) was the most northern transect sampled, and transects 3 and 4 (T3 and T4) were located south of the Canarian Archipelago, being T4 the most southern transect (Fig. 1).



**Fig.1**. Location of transects 1 (T1), 2 (T2), 3 (T3), and 4 (T4) showing the different stations sampled. Stations sampled during daylight are represented as yellow triangles and stations sampled during night are represented as green squares. Station MIOCEAN (M) is represented as black dot.

Vertical profiles of temperature in T1 showed a wide mixed layer characterized by an homogeneous distribution of temperature through the first 150 meters depth. By opposite, the eastern area showed a narrower mixed layer as expected from a coastal upwelling. Temperature along the first 150 meters depth in T2 was slightly lower

compared to T1 eastern area. T3 and T4 showed similar temperature distribution. Higher surface temperature were observed in open waters, decreasing as we approached the African coast (Fig. 3). Salinity profiles along the different transects showed similar patterns, characterized by small variations between 36.25 and 36.75. Two high salinity cores (36.5) were observed in T2, at both sides of station 307. Additionally, T4 presented a maximum salinity area (36.75) between stations 611 and 610.

T1 showed a deep chlorophyll maximum (DCM) around 50-80 m depth in the western area. This zone is considered oligotrophic (0.2-0.4 mg Chl*a* m<sup>-3</sup>), but station 17 displayed a relatively higher chlorophyll concentration (0.6 mg m<sup>-3</sup>). On the other hand, the eastern area showed higher chlorophyll concentrations (around 0.8 mg m<sup>-3</sup>). T2, T3 and T4, as expected, showed maximum chlorophyll values (up to 2.4 mg m<sup>-3</sup> in T4), due to their proximity to the coastal upwelling zone. We classified stations according to chlorophyll values in the upper 200 meters depth. So, we divided them in oligotrophic (stations 23, 21, 19, 17, 15, 13, 307 and 610) and productive (stations 10, 7, 4, 910, 907, 310, 309 and 611). Oligotrophic stations were those displaying chlorophyll values lower than 0.5 mg chl-*a* m<sup>-3</sup>, and productive those stations showing values above 0.5 mg chl-*a* m<sup>-3</sup>.

#### Table 1.

Stations location, depth, haul depth, date and time of sampling. Station numbers with asterisk stands for stations where <sup>210</sup>Po was measured.

Station	Latitude	Longitude	Depth	Haul depth	Day	Time
			(m)	(m)	(dd/mm/yy)	
24*	29 10.00 N	18 29.78 W	4261	200	28/2/19	18:27
23	29 10.11 N	18 14.15 W	3962	200	28/2/19	23:15
21	29 10.00 N	17 39.26 W	3804	200	1/3/19	7:44
19	29 10.00 N	16 54.82 W	3887	200	1/3/19	17:40
18*	29 10.00 N	16 34.10 W	3756	200	1/3/19	22:09
17	29 10.00 N	16 11.87 W	3707	200	2/3/19	3:25
15	29 10.00 N	15 30.04 W	3662	200	2/3/19	12:25
15*	29 10.00 N	15 30.04 W	3662	200	2/3/19	12:25
13	29 01.00 N	14 43.78 W	3561	200	2/3/19	22:40
10	28 48.14 N	13 42.31 W	898	200	3/3/19	10:40
7	28 42.53 N	13 12.37 W	1080	200	3/3/19	16:40
4	28 35.44 N	12 44.26 W	154	120	3/3/19	21:24
910	30 14.26 N	12 07.88 W	2109	200	4/3/19	12:50
909*	30 04.75 N	11 50.96 W	2165	200	4/3/19	16:30
907	29 46.43 N	11 22.03 W	2072	200	4/3/19	23:30
310	27 21.47 N	15 07.79 W	2815	200	6/3/19	14:00
309	27 13.74 N	14 57.97 W	2801	200	6/3/19	17:14
307	26 56.42 N	14 36.09 W	2145	200	7/3/19	0:25
303*	26 40.27 N	14 15.60 W	621	200	7/3/19	8:16
610	25 37.25 N	16 19.85 W	2126	200	8/3/19	9:05
611	25 40.04 N	16 24.08 W	2708	200	8/3/19	11:10



**Fig. 2.** Vertical profiles of a) temperature (°C); b) practical salinity; and c) chlorophyll (mg Chl*a* m<sup>-3</sup>) along Transect 1 (T1).



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Transect 3 (T3), and Transect 4 (T4)

#### Zooplankton biomass and respiration.

Organisms of the large size fraction showed higher biomass that intermediate and small-size organisms (Kruskal-Wallis ANOVA, Tukey Test, p<0.01). Larger biomass was found close to productive areas as expected (Kruskal-Wallis ANOVA, p<0.05). Values observed varied between 174 and 577 mg prot m<sup>-2</sup> in T1, and 404 and 856 mg prot m<sup>-2</sup> in T2, T3, and T4 (Fig. 4). However, zooplankton biomass in station 17 was remarkable high considering its location in oligotrophic waters (577 mg prot m<sup>-2</sup>). The most abundant fraction (>1000  $\mu$ m) coincided with the chlorophyll peak found in that station. The maximum zooplankton biomass (856 mg prot m<sup>-2</sup>) was found in the northern transect (station 907, Table 2).

Specific ETS activities for each size fraction (Fig. 5) showed maximum values in the smaller sizes (Kruskal-Wallis ANOVA, Test de Mann-Whitney, p<0.01). Higher values were observed in stations close to the upwelling area (Fig. 5b) compared to values observed in transect T1 (Fig. 5a). Community ETS activity also showed higher values close to upwelling influenced zone (Kruskal-Wallis ANOVA, p<0.01) (Fig. 6). The highest community ETS activity value took place in station 611 coinciding to high values of biomass and specific ETS activities.

Zooplankton migrant biomass showed values between 92.4 and 353.8 mg C m<sup>-2</sup> (Table 4, Fig. 7). Larger migrant biomass was observed between stations 910 and 907 (T2) and, in general, coinciding with areas displaying high chlorophyll values (stations 19-17 and 910-907). Our estimations of migrant biomass were similar to values in station MIOCEAN of 269 mg C m<sup>-2</sup> (Table 3).

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**Fig. 4.** Zooplankton biomass (mg prot  $m^{-2}$ ) in the upper 200 m depth at A) T1 and B) T2, T3 and T4. Stations marked with an asterisk were sampled during night.





**Fig. 5.** Specific ETS activities ( $\mu$ L O<sub>2</sub> mg prot <sup>-1</sup> h <sup>-1</sup>) for each zooplankton size fraction. Average specific ETS activities ( $\mu$ L O<sub>2</sub> mg prot <sup>-1</sup> h <sup>-1</sup>) for A) T1 and B) T2, T3 and T4 are represented as blue dots (±SD).

#### Table 2.

Zooplankton total biomass, specific ETS activities, and community ETS activity at the different sampled stations. Mean values of each transect are shown in bold.

				Community ETS	
Station	Total biomass	ETS activity	SD	Activities	SD
	(mg prot m <sup>-2</sup> )	( $\mu$ lO <sub>2</sub> mg prot <sup>-1</sup> h <sup>-1</sup> )		$(ml O_2 m^{-2} h^{-1})$	
23	314.5	11.6	4.7	1.1	0.7
21	221.7	10.6	4.4	0.6	0.4
19	303.9	12.5	2.5	1.2	0.5
17	577.8	8.7	4.6	1.1	0.3
15	174.3	5.2	2.8	0.3	0.3
13	341.1	12.2	5.1	1.4	0.8
10	358.6	9.1	5.5	1.1	0.6
7	562.4	12.2	3.2	2.2	0.3
4	343.3	13.7	2.0	1.7	1.5
Average T1	355±136	10.6	2.6	1.2	0.5
910	500.8	20.8	8.5	2.9	1.0
907	856.1	24.0	15.7	3.4	1.7
Average T2	678±251	22.4	2.3	3.1	0.3
310	404.0	19.3	16.1	2.3	1.4
309	557.6	17.4	9.3	3.1	1.3
307	630.0	17.0	4.3	3.0	2.2
Average T3	530 ± 115	17.9	1.2	2.8	0.4
610	575.8	28.6	7.4	5.5	4.7
611	814.4	24.1	7.9	6.3	1.8
Average T4	695 ± 186	26.3	3.2	5.9	0.6

#### Table 3.

Zooplankton migrant biomass, respiration, mortality and excretion fluxes, and total active flux along Transects 1 (stations 23 through 13), Transect 2 (stations 910 - 907) and Transect 3 (stations 310 - 307). Average values are shown in bold.

	Migrant	Respiratory	Mortality	Excretion	Total Active
Station	biomass	flux	flux	flux	flux
	(mgC m <sup>-2</sup> )	$(mgC m^{-2} d^{-1})$	$(mgC m^{-2} d^{-1})$	$(mgC m^{-2} d^{-1})$	$(mgC m^{-2} d^{-1})$
23-21	92.4	1.7	1.3	0.4	3.5
19-17	273.8	4.9	3.6	1.2	9.7
15-13	166.1	3.0	2.3	0.7	6.1
Average T1	177.1 ± 90.7	3.2 ± 1.6	2.4 ± 1.2	0.8 ± 0.4	6.4 ± 1.1
910-907	353.8	6.3	4.7	1.5	12.4
310-307	148.6	2.7	2.1	0.7	5.4
Average T2, T3	251.2 ± 145.1	4.5 ± 2.5	3.4 ± 1.9	1.1 ± 0.6	8.9 ± 4.9
MIOCEAN	269.8	4.8	3.6	1.2	9.6

#### Table 4.

Comparison of migrant biomass and respiratory flux in the oceanic waters of Canary Islands during

different seasons.

Migrant biomass	Respiratory flux	Location	Season	Source
(mg C m <sup>-2</sup> )	$(mg C m^{-2} d^{-1})$			
148-353	2.73-6.25	North Canary Islands, Cape	Late Winter Bloom	This study
		Ghir and North Africa Upwelling		
261	1.92-4.29	Gran Canaria Island	Late Winter Bloom	Hernández-León et al. 2001
560.6	8.28	South Gran Canaria	Agost	Yebra et al. 2005
		(Inside anticyconic eddy)		
252.8	1.85	South Gran Canaria	Agost	Yebra et al. 2005
		(Outside anticyconic eddy)		
204.43 ± 99.90	$0.81 \pm 0.38$	North Gran Canaria	March	Putzeys et al. 2011
		(Subtropical NA gyre)		
266 ± 128	$3.4 \pm 1.9$	North Gran Canaria	March - June	Ariza et al. 2015



Fig. 6. Community ETS activity (ml  $O_2$  m  $^{-2}$  h  $^{-1}$ ) for transects A) T1 and B) T2, T3 and T4. Black lines stands for SD.

#### Total active flux

Total active flux was assessed as the sum of respiratory, mortality, and excretion fluxes. Active flux varied between  $6.4\pm1.1$  and  $8.9\pm4.9$  mg C m<sup>-2</sup> d<sup>-1</sup> in oligotrophic and productive areas, respectively. This flux was slightly higher coinciding with areas of higher migrant biomass (Table 3). Estimated active flux in this study needs to be considered as a proxy of real active flux, as day and night hauls were not performed in the same stations. However, our results showed similar values as in MIOCEAN station which was sampled during day and nighttime.

## <sup>210</sup>Po in sea water and marine organisms

Radionuclide activity concentrations determined in sea water and zooplankton samples are shown in Table 5. Seawater <sup>210</sup>Po slightly oscillated from  $0.0025 \pm 0.0001$ and  $0.0086 \pm 0.0003$  Bq kg<sup>-1</sup>. By opposite, <sup>210</sup>Po activity concentration in zooplankton samples varied between 213 ± 9 and 419 ± 15 Bq kg<sup>-1</sup>. In station 18 and 303, the large size fraction displayed the highest values (419 ± 15 and 340 ± 12 Bq kg<sup>-1</sup>, respectively) while station 24 displayed the lowest <sup>210</sup>Po concentration activity (213 ± 9 Bq kg<sup>-1</sup>). The Spearman correlation matrix of radionuclides was calculated and is summarized in Table 6. Correlation coefficients were considered statistically significant at p<0.05. Strong positive correlations were observed between biomass and <sup>210</sup>Po (Figure 8). Finally, we also found a significant difference between zooplankton concentration factor in oligotrophic waters and productive areas (p=0.005).



**Fig. 7.** Migrant biomass (mg C m<sup>-2</sup>) for A) stations in T1 and B) stations 910-907 (T2), station 310-307 (T3) and station MIOCEAN.

#### Table 5.

<sup>210</sup> Po concentration and concentration factor (CF) of zooplankton samples in wet weight (Bq kg<sup>-1</sup>)

Station	Size	<sup>210</sup> Po	CF	
	(μm)	(Bq kg <sup>-1</sup> )		
24	> 200	213 ± 9	1.7E+04	
SW <sub>24</sub>		0.0025 ± 0.0001		
18	> 1000	419 ± 15	2.4E+04	
18	200 - 1000	264 ± 9	1.5E+04	
18**	> 200	340 ± 12	2.0E+04	
SW <sub>18</sub>		0.0035 ± 0.0001		
15	> 200	232 ± 2	3.3E-03	
SW <sub>15</sub>	0.0033 ± 0.0002			
909	> 200	272 ± 10	1.6E+04	
SW <sub>909</sub>	0.0034 ± 0.0002			
303	> 1000	340 ± 12	7.9E+03	
303	200 - 1000	271 ± 10	6.3E+03	
303**	> 200	307 ± 11	7.2E+03	
SW <sub>303</sub>		0.0086 ± 0.0003		

SW stands for seawater and \*\* for weight average.

#### Table 6.

Spearman correlation analysis between <sup>210</sup>Po, biomass, concentration factor (CF) and zooplankton ETS.

	<sup>210</sup> Po	Biomass	<b>CF (10</b> <sup>4</sup> )	ETS comm	sp ETS activity
	(Bq kg <sup>-1</sup> )	(mg protein ·m⁻³)		(µlO₂·h⁻¹·m⁻²)	(µlO₂·mg prot <sup>-1</sup> ·h <sup>-1</sup> )
<sup>210</sup> Po	1				
Biomass	0.82846**	1			
CF (10 <sup>4</sup> )	0.24268 ns	0.25 ns	1		
ETS comm	0.39496 ns	0.56067 ns	-0.5021 ns	1	
sp ETS activity	-0.14226 ns	-0.01667 ns	-0.58333 ns	0.69457 *	1

Correlation is significant at the 0.05. \* p >0.05; \*\* p>0.01; \*\*\* p>0.001. ns stands for non-significant.

# Table 7.

<sup>210</sup>Po concentration in marine organisms in dry weight (Bq kg<sup>-1</sup>).

<sup>210</sup> Po ± SD	Sample	Source	Location
(Bq kg <sup>-1</sup> )	(μm)		
222.4 ± 13.9	> 200	This Study	North Canary
$419.1 \pm 14.9$	200 - 1000		Islands
263.6±9.1	> 1000		
271. 9 ± 9.8	> 200		Cape Ghir
339.7 ± 12.2	200 - 1000		Canary Current
270.6 ± 9.7	> 1000		
242.4 ± 102.9	200 - 500	Strady et al. 2015	Summer,
189.0 ± 57.1	500 - 1000		Golf of Lion
175.2 ± 117.7	1000 - 2000		
274.0 ± 91.8	200 - 500		Winter,
259.8 ± 80.8	500 - 1000		Golf of Lion
262.6 ± 36.7	1000 - 2000		
73.8 ± 52.8	Copepods	Uddin et al. 2018	Kuwait,
			Northern
			Arabian Gulf
214 ± 116	Mesozoplankton	Skwarzec and	Slupsk Trough,
		Bojanowski, 1988	Baltic
55 ± 27	Mesozoplankton		Gdansk Basin,
			Baltic
163 ± 6	Copepods	Carvalho 2011	Porcupine Basin,
218 ± 14	Calanoids		Atlantic Ocean
84 ± 4	Euphausiids		
38 ± 14	Zooplankton mix		



**Fig. 8.** Correlation between zooplankton biomass (mg prot m<sup>-3</sup>) and <sup>210</sup>Po (Bq kg<sup>-1</sup>). Numbers stands for stations, S for small-size organisms and L for large-size organisms.

#### Discussion

In this study, a noticeable difference in physical oceanographic characteristics were observed between oligotrophic and meso- or eutrophic areas. A wide mixed layer was located in the western area of T1. By opposite, stations near the upwelling zone displayed a narrower mixed layer as consequence of the deep water rise. These physical characteristics well reflected the differences between open and coastal upwelling waters. As expected, high chlorophyll values were obtained in the upwelling system. However, we obtained a relative high value of zooplankton biomass in station 17, located in oligotrophic waters, mainly due to the large size fraction. However, this station was sampled during nighttime (see Table 1), so it was also expected to find large values in the large size fraction due to DVM.

On the other hand, near the continental shelf and shallow waters (T1 eastern waters, T2, T3 and T4), interactions between physical properties and chlorophyll are more complex than in the open ocean (Simpson and Sharples, 2012). Consequently, upwelling areas were expected to hold larger zooplankton biomass, as water intrusion pulses close to shore create large phytoplankton events (Reyes-Mendoza et al., 2019). The values found were comparable with the results obtained by Hernández-León et al. (2002) in the coastal transition zone off Northwest Africa, also showing the highest values in the large size fraction and large biomass in productive areas. However, we obtained lower specific ETS activity than Hernández-León et al. (2002) but similar to those obtained by Hernández-León et al. (1987) obtaining higher specific ETS activities in productive areas.

Community respiration rates estimated from ETS measurements and zooplankton biomass in the upper 200 meters depth were considerably higher in T2, T3 and T4, especially in the latter, compared to T1 (Table 2). These differences are in

accordance to the different productivity of the transects. Our results in productive areas are similar to those obtained by Hernández-León et al. (1987) and results of Hernández-León et al. (2002) between Cape Juby and Cape Bojador.

We obtained similar migrant biomass as Hernández-León (2001), Yebra (2005) and Hernández-León (2019a) (transect 21°N), but our respiratory flux estimation was slightly higher (Table 4) due to a different conversion factor from proteins to dry weight. Differences between our respiratory flux and those obtained by other authors also differ slightly, except for the respiratory flux obtained by Yebra et al. (2005) inside an anticyclonic eddy and Hernández-León (2019a) at the 21°N transect. They showed the effects of mesoscale structures on zooplankton distribution and hence carbon flux.

Migrating zooplankton and the consequently export flux displayed a quite variable role in the biological pump (Putzeys et al., 2011). Active flux was higher in productive areas rather than in oligotrophic because of the large and constant food supply (Hernández-Leon et al., 2019a). On the other hand, active flux estimations have limitations such as biomass underestimation and size-bias (Ariza et al., 2015). Additionally, active flux depends on the processes taken into account to estimate the flux (respiration, excretion, gut or mortality fluxes) and net efficiency. In this sense, active flux values obtained in the northern transect doubled the value found in oligotrophic waters (Table 2).

Our preliminary study of <sup>210</sup>Po in the sampled area cannot be compared with nearby areas because of the lack of data in the Canary Current. However, our results are in good agreement with values obtained by other authors (see Carvalho, 2011; Strady et al., 2015a; Uddin et al., 2018) (Table 6). Larger <sup>210</sup>Po activity concentration was found in large-sized organisms, opposite to results by Strady et al (2015b). They obtained higher <sup>210</sup>Po activity in smaller sized organisms during winter due to different <sup>210</sup>Po

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inputs, geographical variations, or different planktonic community (Skwarzec and Bojanowski, 1988). Here, as a first approach, we estimated the concentration factor in zooplankton (Table 5) which were slightly lower than the ones obtained by Uddin et al. (2018) for copepods, although our results are of the same order of magnitude than those observed by Skwarzec and Bojanowski (1988).

In summary, we found higher zooplankton biomass, specific, and community ETS activities in productive areas. Migrant biomass and total active flux were only slightly higher in productive areas than in oligotrophic waters during the late winter in this area of the Canary Current. This study of active flux due to diel vertical migrants along the RAPROCAN survey also showed the feasibility to use day and night zooplankton samples obtained along the transect to assess active flux in the ocean. Our comparison with the MIOCEAN station showed similar values and this will allow to build a time series of active flux in the near future for this area of the ocean.

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# Descripción detallada de las actividades desarrolladas durante la realización del TFT

Las actividades realizadas durante el TFM se han centrado en el análisis de las muestras tomadas durante la campaña oceanográfica RAPROCAN 1903.

Actividades realizadas previo a la campaña:

- Organización del material necesario para la toma de muestras.
- Transporte y ordenación del material en el barco.
- Análisis de muestras de campañas anteriores como toma de contacto con los análisis a realizar con las muestras tomadas durante la campaña.

Actividades realizadas tras la campaña:

- Análisis de las muestras: ETS, proteínas y taxonomía.
- Análisis de los parámetros físicos con el software ODV.
- Análisis de <sup>210</sup>Po en zooplancton.
- Representación de los datos y comparación de estos con estudios relacionados.

A lo largo de la realización del TFM se ha llevado a cabo la búsqueda y lectura de bibliografía relacionada con el tema de estudio.

#### Formación recibida

Durante la realización del TFM he aprendido como se organiza y se desarrolla una campaña oceanográfica, así como el análisis de las muestras. Escribir la memoria en formato publicación científica ha sido un aprendizaje de alto valor.

Además de aprender nuevos análisis de laboratorio, he tenido la oportunidad de asistir a un curso sobre el software "ZooProcess & Ecotaxa" para la identificación y clasificación de zooplancton. Por otro lado, he podido aprender a representar mapas y variables en el software ODV y mejorar mi capacidad critica.

# Nivel de integración e implicación dentro del departamento y relaciones con el personal.

El nivel de integración e implicación dentro del departamento, en mi opinión, ha sido alto, así como las relaciones con el personal.

# Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFT

El principal aspecto positivo del TFM ha sido tener la oportunidad de participar en una campaña y poder tomar las muestras, pues facilita la compresión de los análisis de las muestras y los datos.

Gracias al TFM he aprendido a trabajar en una línea de investigación que no conocía y que ha sido muy motivante. Además, he recibido ayuda en todo momento y al ambiente de trabajo ha sido bueno.

El único aspecto negativo que destacaría es la falta de tiempo para el desarrollo del TFM.

#### Valoración personal del aprendizaje conseguido a lo largo del TFT

Considero que la autonomía adquirida durante el TFM es el mejor aprendizaje que he podido desarrollar. Tanto organizar el material necesario para la campaña como llevar a cabo el análisis de las muestras y aprender nuevos programas informáticos van a ser útiles para continuar con mi formación académica. Considero que con respecto al TFG he madurado en sentido académico, principalmente a la hora de escribir textos científicos y sintetizar el contenido.