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UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Relationship between Respiration and Electron Transfer System (ETS) in zooplankton

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Este trabajo se presenta por la alumna Amelia Iguanira López López para la obtención del título de Graduada en Ciencias del Mar por la Universidad de Las Palmas de Gran Canaria, y es dirigido por el Dr D. Santiago Hernández León del Instituto de Oceanografía y Cambio Global (IOCAG).

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Abstract

Respiration of zooplanktonic organisms is an significant fraction of the global carbon cycle. However, it estimation in order to obtain the data required in oceanography is still a problem. In this work, we studied respiration rates in laboratory and field experiments. Laboratory experiments using *Daphnia spp*. showed a significant decrease of respiration rates during starvation. In addition, we measured the gut fluorescence and enzymatic activity (electron transfer system, ETS). The former did not show the expected decrease probably due to the volume of the incubators. The relationship between respiration and ETS presented the classical variability ranging between 0.5 and 1 as observed in previous works. Copepod respiration rates were measured during RAPROCAN 1504 cruise around the Canary Islands. These organisms showed a high variability in oxygen consumption during incubation. Our study suggests that duration experiments to calibrate enzyme activities to obtain physiological rates should be considered. We suggest the use of experiments covering the metabolic scope of organisms under study. Taking this into consideration, our values of the relationship between respiration and ETS activity is close to a value of 1. Lower ratios could underestimate respiration in the ocean.

Introduction

The ocean plays an important role in the global carbon cycle. Atmospheric carbon dioxide enters into the natural cycle of oceans and remains there for a variable period of time. The photosynthetic organisms start the biological pump capturing the carbon dioxide and producing organic matter and oxygen. This mechanism transport carbon dioxide from the ocean surface to the deep layers. Heterotrophic organisms, or consumers, take the organic matter from other organisms and use it as fuel for their metabolism. These organisms respire the metabolized carbon and return carbon dioxide to the environment.

Understanding the mechanisms linked to respiration in the ocean are a fundamental step to study the carbon flux through the different communities. Therefore, the knowledge of oxygen consumption is essential for the study of the involved processes in production and energy transfer in the ocean due to the different components of marine ecosystems.

The methods used to estimate respiration in heterotrophic organisms, specially in zooplanktonic organisms, are based on the measurement of oxygen consumption. The balance method (Omori and Ikeda, 1984) is normally used and consist in the incubation of zooplanktonic organisms in glass bottles filled with filtered seawater for several hours. During incubation, dissolved oxygen in experimental bottles (with organisms) decreases respect to control bottles (without organisms). The difference between both is the organisms respiration. This technique needs an intensive work on board oceanographic vessels and consume time, limiting the number of estimations. Because of this, Curl and Sandberg (1961) and Packard (1969) proposed the measurement of the enzymatic activities responsible of cellular respiration as an index to be used in oceanography.

The measurement of the electron transfer system activity (ETS) was developed by Packard (1975) and it is also routinely used in oceanography. The ETS is the last enzymatic complex which takes part in the production of ATP and uses the oxygen as the last electron acceptor. However, the relationship between respiration and ETS presents an important variability. Hernández-León and Gómez (1996) observed that the

relationship between respiration and ETS activity presented the classic variability between 0.5 and 1 related to the previous feeding history. These authors also found that during the winter bloom (relatively high primary production) the R/ETS ratio was around 1, while out of the bloom, in a more oligotrophic situation, the values were near 0.5. Besides, this variability could also be explained because of the methology used to assess respiration rates using the classical balance method and the enzymatic response at different time intervals. Long or short incubations could modify this ratio as ETS activity is more conservative than respiration.

In the present work, we studied respiration in the cladoceran *Daphnia* spp. and copepods, its relationship with the gut fluorescence (GF) and the enzymatic activity of the ETS. The main goal was to study the variability in respiration rate and the enzyme activity in experiments of short duration (8 hours). Specifically, we focused in the relationship between high respiration rates during the first hours of incubation and its enzymatic response.

Material and methods

Laboratory experiments

The cladocerans used for experiments were incubated for 12 hours in a beaker of 2 liters at a temperature of 17.8ºC ± 0.1 in saturation of food with the algae *Ankistrodesmus*. Its density was counted with a Thoma device in an inverted microscope, taking an aliquot of 10 µl of the same culture (Table I). After the feeding period, 15 organisms were frozen in liquid nitrogen in order to obtain the time zero in each experiment for enzyme activity.

To measure respiration, the organisms previously incubated in food saturation were incubated in filtered water (0.2 µm) in vials of 5 ml equipped with optodes. Incubations were carried out in triplicate for each incubation time, in the dark, and at 18ºC. The incubation lasted for approximately 8 hours. At different intervals, samples were frozen in liquid nitrogen for later analysis of ETS and gut fluorescence (GF). To obtain the values of respiration we designed an application using the statistical software R. The values of the first half an hour were discarded as it is the time required for stabilization of oxygen inside the incubators. Then, the slopes of experiment and control bottles were subtracted in order to obtain respiration every 0.5 h.

ETS analyses were carried out following the method proposed by Packard (1971). The homogenate was centrifuged at 4000 rpm and 0ºC during 10 minutes. An aliquot of the homogenate was used for the incubation at 18ºC, in darkness, in the presence of NADH, NADPH, succinate (SS) and INT as an artificial electron acceptor. After 20 minutes, the incubation was stopped by a quench solution. The ETS activity was estimated spectrophotometrically at 490 nm with a turbidity base-line of 750 nm.

Biomass of each size fraction was determined as protein content using the method of Lowry *et al.* (1951) or the method of Peterson (1983) for samples with very low protein content and using bovine serum albumine (BSA) as standards.

For the measurement of fluorescence, we used the method proposed by Nemoto (1968) and Mack and Bohrer (1976). An aliquot of the homogenate used for ETS measurement was introduced in a test tube with 10 ml of 90% acetone and kept at -20ºC during 24 hours. Pigments were measured using a Turner Design fluorometer, previously calibrated with pure chlorophyll (Yentsch and Menzel, 1963), before and after acidification with 10% HCl. Pigments were calculated using the equations given by Strickland and Parson (1972) slightly modified to:

$$\text{Chlorophyll } a = k \cdot (F_0\text{-Fa}) \cdot \text{Individual}^{-1}$$

$$\text{Pheopigments} = k \cdot (R \cdot \text{Fa}\text{-}F_0) \cdot \text{Individual}^{-1}$$

where k is the instrument calibration constant, F_0 and F_a are the fluorescence readings before and after acidification and R is the acidification coefficient. The overall concentration of pigments refers to chlorophyll a and pheopigments expressed as ng pigments·ind^{-1}.

Field experiments

Copepods were collected in six oceanographic stations on board the research vessel "Ángeles Alvariño" around the Canary Islands from 6th to 16th April, 2015. Capture of organisms was carried out using a double WP-2 net equipped with a 100 µm mesh net, from 200 m depth to the surface at about 40 m·minute^{-1}. Three experiments were carried out with organisms of sizes between 100 and 200 µm and three other with copepods between 500 and 1000 µm. The organisms were picked up with a Pasteur pipette and

were incubated for triplicate in the dark at 18ºC for a total of 4 hours on seawater filtered through 0.2 µm.

To measure respiration, was used a precision polarographic oxygen electrode (VWR DO210). Once experiments were completed the difference between the oxygen content in the control and experimental bottles was measured in order to obtain the respiration rate. These data were standardized for the protein weight of incubated organisms. Similar to the experiments carried out in the laboratory we graphed the results using the statistical program STATISTICA 7.

Results

Laboratory experiments

Pigment content in the different experiments decreased during incubation (Fig. 1). However, this decrease was weak and variable (see Fig. 1b and d), and, in general, we did not observe a clear decrease as expected (Fig. 1f).

By opposite, respiration rates showed a significant decrease during the first four hours of the experiment. The initial oxygen consumption ranged between 0.9 and 1.4 $\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$ decreasing in all experiments reaching values of 0.27±0.01, $\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$ after 3-4 h (Table II, Fig. 2). Therefore, the initial respiration was between 3 and 5 times the standard rates obtained after four hours.

The electron transfer system activity (ETS) did not show high values at the beginning of the experiments as observed for respiration (Fig. 2). This activity showed values in the range 0.2-0.5 $\mu l \cdot O_2 \cdot ind^{-1} \cdot h^{-1}$, being the average in the order of 0.36±0.04 (see Table II)

As expected from respiration values, the ratio R/ETS showed a clear decline from the beginning of incubation stabilizing after the first four hours (Fig. 3). The values of the ratio decreased to an average value of 0.29±0.05 (see table II) after the first four hours of incubation.

Field experiments

Small organisms (100-200 µm) showed relatively high values of gut pigment content (mean value of 197.2±106.2 ng pigments/mg protein, table III, Fig. 4a), while larger copepods (500-1000 µm) showed lower average values (93.9±38.1 ng pigments/mg of protein, table III, Fig. 4b). Small organisms showed a high variability in the gut content during incubation. However, the 500-1000 µm organisms showed an unexpected increase. (Fig. 4).

Respiration in both size classes showed a decreasing respiration during incubation as expected. Average values for the 100-200 µm fraction was 0.58±0.28 µlO_2·mg protein·h^{-1} (table III, Fig. 4C), while for the larger animals (500-1000 µm) was lower as also expected (0.31±0.06 µlO_2·mg protein·h^{-1}, table III, Fig. 4d).

Discussion

Respiration rates in our experiments with cladocerans decreased during incubation similar to other studies (Nival et al., 1974; Mayzaud, 1976; Skjoldal et al., 1984; Blažka et al., 1982; Abou, 1984; Ikeda, 1977; Tsuda, 1994; Thor, 2003). In the field experiments, copepods did not show this pronounced decrease probably due to the fact that they were not previously acclimated in food saturation. The results in cladocerans is in agreement with authors who argue that the decrease in metabolic rates after the start of incubation is due to food shortage rather than stress produced by the handling of organisms (Ikeda, 1977; Ikeda and Skjoldal 1980).

Our average respiration for all experiments with *Daphnia* over 8 hours (0.32 ± 0.39 µlO2·ind^{-1}·h^{-1}) matched the values found by Valenciano (2010). In her experiments, standard metabolic rate was reached after 3.5 hours (mean value 0-3.5 h: 0.41 ± 0.07 µlO2·ind^{-1}·h^{-1}). In our study, this average value was quite similar (0.40 ± 0.10 µlO2·ind^{-1}·h^{-1}). After four hours until the end of the experiments, respiration rate was 0.27 ± 0.01 µlO2·ind^{-1}·h^{-1} (table I), while Valenciano (2010) obtained a rate of 0.29 ± 0.11 µlO2·ind^{-1}·h^{-1}. For copepods, we observed a high variability in the respiration rates for the six experiments (average total value of 0.44 ± 0.23 µlO2·mg protein-$^{-1}$·h^{-1}). Valenciano (2010) also found a decrease in respiration rates during one hour, although the number of experiments performed by this author was quite short.

Analysis of the gut fluorescence showed different values for *Daphnia* and copepods as expected. In *Daphnia*, the concentration of cells per milliliter was $1.5 \cdot 10^6$-$5 \cdot 10^5$ cells/ml^{-1} during 12 hours of incubation in food saturation with the algae *Ankistrodesmus*. However, other authors such as Valenciano (2010) incubated in a final concentration of cells between $1.8 \cdot 10^5$ and $3 \cdot 10^5$ cells/ml^{-1} during 24 hours. According to Simčič and Brancelj (1997) and Yebra (2001), food availability was enough to obtain food saturating conditions, so the organisms used in our laboratory study were potentially under saturate food conditions at the beginning of experiments. After eight hours, the gut fluorescence for all experiments was 123.4 ± 88.6 ng pigments·ind^{-1}. However, Valenciano (2010) obtained values of pigments in the range of 5.1-78.9 ng pigments ind^{-1}. This difference could be explained by the volume of incubators. While our incubations were carried out in a quite small volume (5 ml), Valenciano (2010) used a volume of 300 ml. This difference suggests that in our small volume incubator, organisms could preserve pigments in the gut because they are able to ingest their own fecal pellets, maintaining a level of pigments in the relatively large tract. Conversely, the use of larger volumes implies the difficulty to find these pellets as these are more diluted. This fact deserves further research.

In copepods, gut fluorescence showed average values of 145.5 ± 91.1 ng pigments·mg^{-1}. Hernández-León et al. (2002) obtained an average value of 50.9 ± 61.0 ng pigments·mg^{-1} protein for total zooplankton (>200 µm). However, Hernández-León et al (2004) found higher values, approximately between 500 and 3000 ng pigments·mg^{-1} protein for in 100-200 µm and 500 and 1500 ng pigments·mg^{-1} protein for 500-1000 µm. These data suggest that herbivorous feeding in our copepods used for experiments was low as it is expected in oceanic waters of the Canary Current.

Enzyme activity measured in *Daphnia*. showed quite constant values while respiration rates decreased during the experiments. The average for all experiments was 0.35 ± 0.04 µlO2·ind^{-1}·h^{-1}. These data are similar to the one found by Valenciano (2010) who observed also quite constant values along her experiments. The ratio between respiration and the enzyme activity decreased as observed in previous studies. This ratio showed values between 0.5 and 1.0 as expected. These results coincide with that found by Hernández-León and Gómez (1996) and many other authors working in the relationship between physiological rates and enzyme activities (e.g., Herrera et al,

2011; Fernández-Urruzola et al., 2011). However, our findings raise the problem of the experiment duration to determine the R/ETS relationship. After four hours this relationship remained constant, but during the first hours of the experiment the organisms showed the values in which organisms move daily. Long experiments show R/ETS values predicting standard metabolism, while short ones give conversion factors representing better the metabolic scope of zooplankton approaching the R/ETS value of 1.Conservative values using R/ETS from long experiments may be underestimating the respiration in the ocean when they are obtained from enzyme measurements.

Conclusions

Respiration rates showed a decline throughout the incubation period. The gut fluorescence content in *Daphnia spp.* showed high values, not decreasing as expected, probably due to the small volume used for experiments. The ETS activity measured in *Daphnia* showed a near-constant behavior as respiration rates decreased. The ratio between respiration and ETS showed a range between 0.5 and 1.0 as expected. However, this work raised the use of long or short duration experiments to calibrate the enzyme activity to be used in oceanography. Short experiments are suggested in order to account for the metabolic scope of organisms.

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Tables and Figures

Table I. Average of values of respiration ($\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$), ETS ($\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$), R/ETS, and gut fluorescence (ng pigments$\cdot ind^{-1}$) during first four hours and from four to eight hours.

	RESPIRATION		ETS		R/ETS		GF	
EXP.	**0-4 h**	**4-8 h**	**0-4 h**	**4-8 h**	**0-4 h**	**4-8 h**	**0-4 h**	**4-8 h**
1	0.27 ± 0.24	0.26 ± 0.40	0.30 ± 0.09	0.32 ± 0.11	0.95 ± 0.82	0.34 ± 0.12	131.11 ±52.15	103.11 ±27.7
2	0.53 ± 0.50	0.29 ± 0.41	0.34 ± 0.13	0.33 ± 0.12	1.33 ± 1.38	0.25 ± 0.14	150.36 ±42.70	100.16 ±37.5
3	0.39 ± 0.32	0.27 ± 0.38	0.31 ± 0.09	0.39 ± 0.19	1.70 ± 2.54	0.31 ± 0.10	6.82 ± 7.44	4.15 ± 4.09
4	0.47 ± 0.44	0.27 ± 0.42	0.40 ± 0.08	0.33 ± 0.08	1.20 ± 1.40	0.33 ± 0.07	296.87 ±85.89	175.17 ±68.3
5	0.34 ± 0.32	0.25 ± 0.34	0.40 ± 0.09	0.42 ± 0.11	0.80 ± 0.83	0.23 ± 0.24	121.39 ±24.24	76.11 ±23.23
Total Exp	0.4 ± 0.10	0.27 ± 0.01	0.35 ± 0.05	0.36 ± 0.04	1.20 ± 0.35	0.29 ± 0.05	141.31 ±103.5	91.74 ±61.39

Table II. Experimental conditions, including date, density of food (cell/ml), temperature of acclimation and incubation.

					Total Resp	Total ETS	Total R/ETS	Total GF
Exp	**Date**	**t^a**	**cells/ml**	**t^a inc.**	**0-8 h**	**0-8 h**	**0-8 h**	**0-8 h**
1	19/03/15	17.9	$1.5 \cdot 10^6$	18	0.26 ± 0.35	0.31 ± 0.09	0.71 ± 0.71	120.61 ± 45.99
2	20/03/15	17.9	$1.5 \cdot 10^6$	18	0.38 ± 0.46	0.33 ± 0.12	0.95 ± 1.22	131.54 ± 47.08
3	23/03/15	17.8	$1.5 \cdot 10^6$	18	0.31 ± 0.36	0.34 ± 0.13	1.26 ± 2.17	5.97 ± 6.58
4	24/03/15	17.8	$5 \cdot 10^5$	18	0.35 ± 0.44	0.37 ± 0.09	0.87 ± 1.17	254.54 ± 98.43
5	25/03/15	17.9	$5 \cdot 10^5$	18	0.28 ± 0.34	0.41 ± 0.10	0.58 ± 0.72	104.41 ± 32.36
TOTAL EXPERIMENTS					0.32 ± 0.39	0.35 ± 0.04	0.87 ± 0.26	123.41 ± 88.63

Table III. Comparison of respiration rates ($\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$) obtained by different authors in relation to results obtained in this study.

References	Organisms	Respiration rates	Units	Temperature	Acclimation time
Ikeda (1976)	Subtropical copepods	0.46-2.12	μlO_2 ind^{-1} h^{-1}	22ºC	36 hours
Ikeda (1977b)	*Acartia tonsa*	0.089-0.181	μlO_2 ind^{-1} h^{-1}	22ºC	None
Schmoker and Hernández-León (2003)	Cladoceran (D. Magna)	0.5-4	μlO_2 ind^{-1} h^{-1}	19ºC	24 hours
Li et al. (2004)	Zooplankton	0.64-0.84	μlO_2 ind^{-1} h^{-1}	18-27ºC	None
Present study	*D. magna*	0.2-1.3	μlO_2 ind^{-1} h^{-1}	17-18ºC	12 hours
	Copepods	0.27-0.78	μlO_2 mg protein h^{-1}	17-18ºC	None

Table IV. Average values of respiration rates ($\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$) and gut fluorescence (ng pigments·ind^{-1}) of experiments performed with copepods

COPEPODS EXPERIMENTS		Respiration	GF
		0-3 h	0-3 h
100 µm	1	0.69 ± 0.94	303.22 ± 386.74
	2	0.26 ± 0.29	197.45 ± 105.29
	3	0.78 ± 0.39	90.87 ± 109.99
TOTAL 100 µm		0.58 ± 0.28	197.18 ± 106.18
500 µm	4	0.27 ± 0.32	115.16 ± 150.94
	5	0.38 ± 0.32	49.85 ± 61.26
	6	0.27 ± 0.25	116.59 ± 108.91
TOTAL 500 µm		0.31 ± 0.06	93.87 ± 38.13
TOTAL EXPERIMENTS		0.44 ± 0.23	145.5 ± 91.1

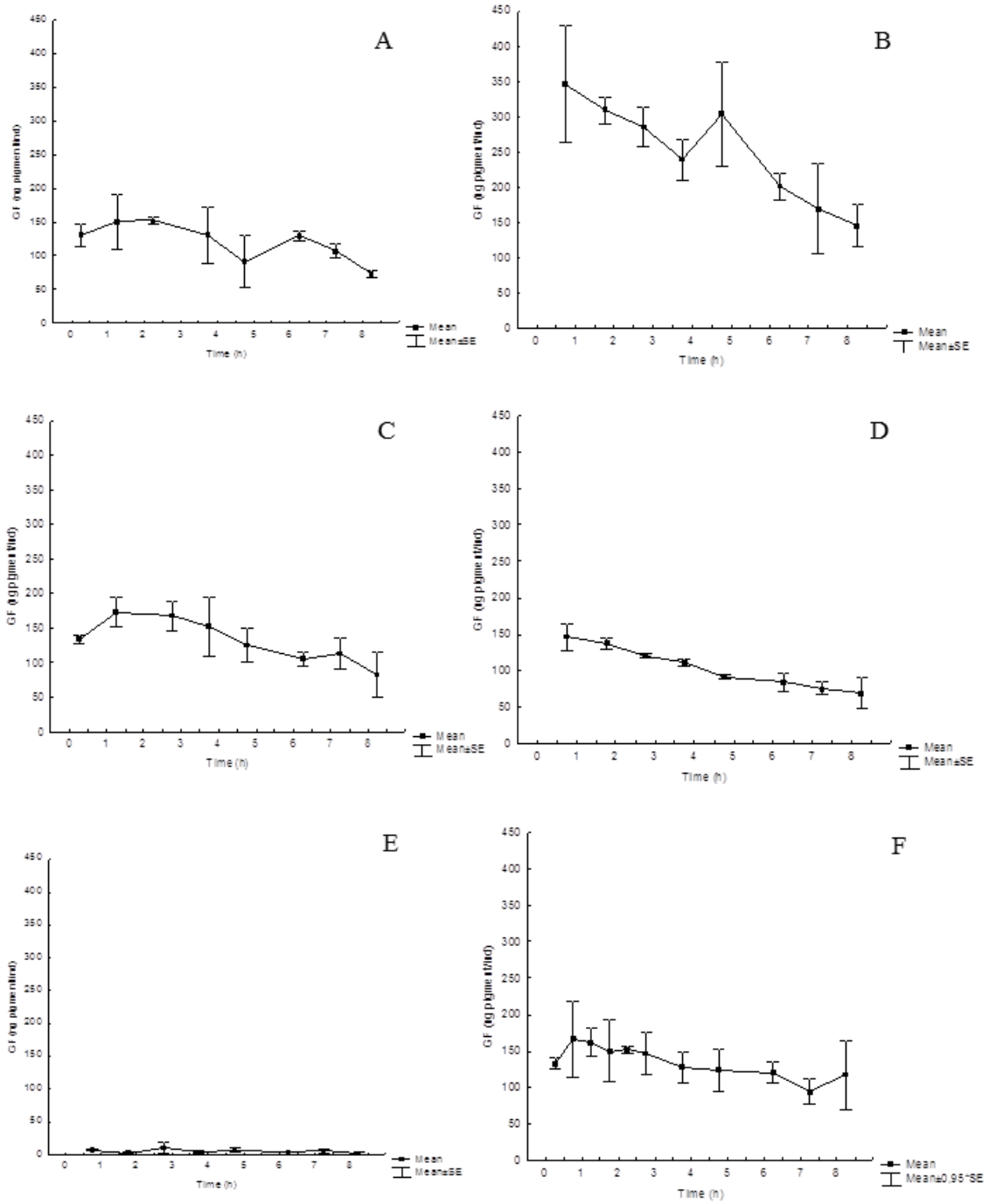

Figure 1. Time-course of gut fluorescence (ng pigments·ind^{-1}) and standard error for the different experiments. Figure 1A corresponds to experiment 1, B for experiment 4, C for the number 2, D for 5, E for 3, and F for 6.

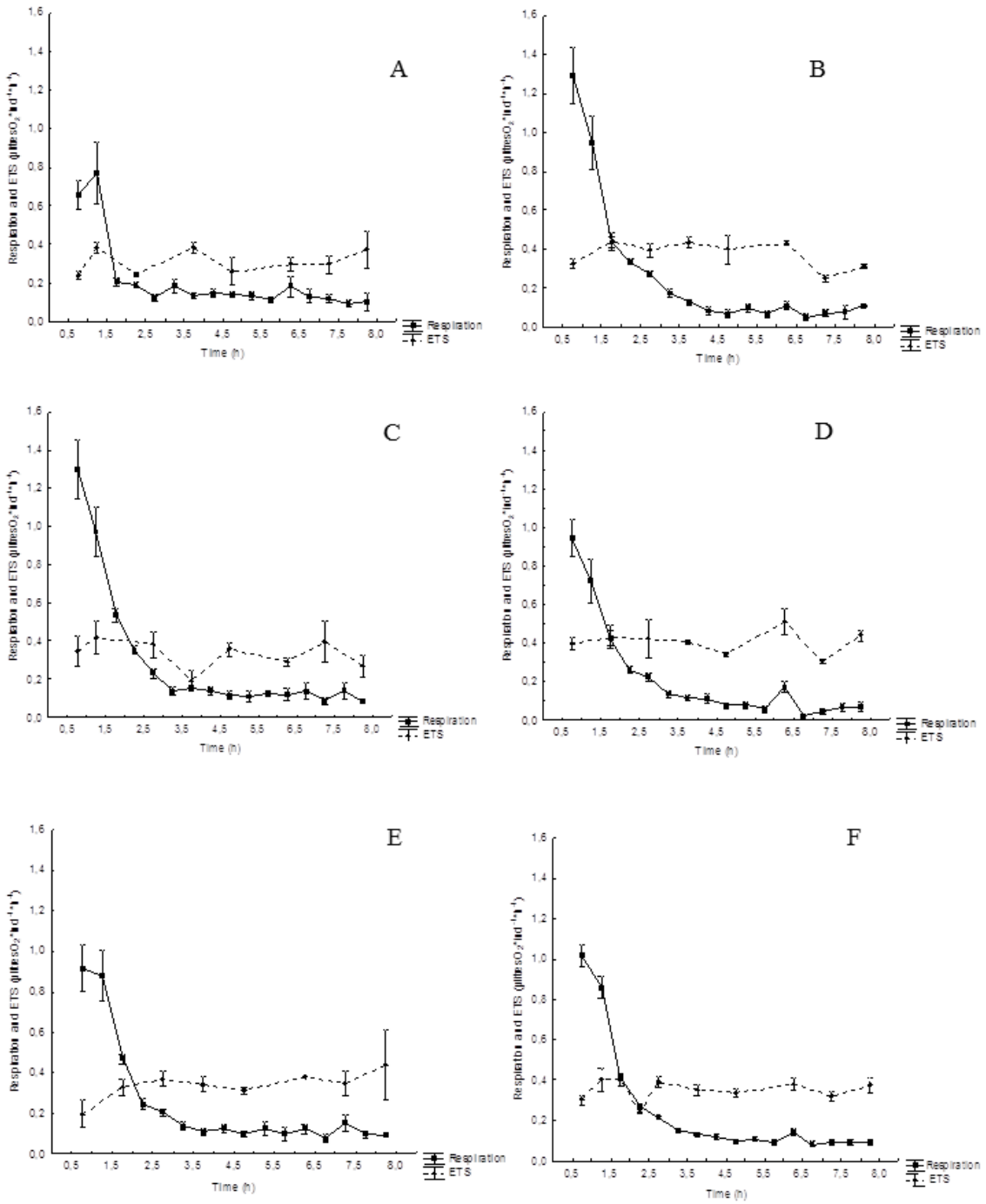

Figure 2. . Time-course of respiration rates ($\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$) and ETS activity ($\mu lO_2 \cdot ind^{-1} \cdot h^{-1}$) for each experiment. Figure 2A corresponds to experiment 1, B for experiment 4, C for the number 2, D for 5, E for 3, and F for 6. Vertical lines are standard error

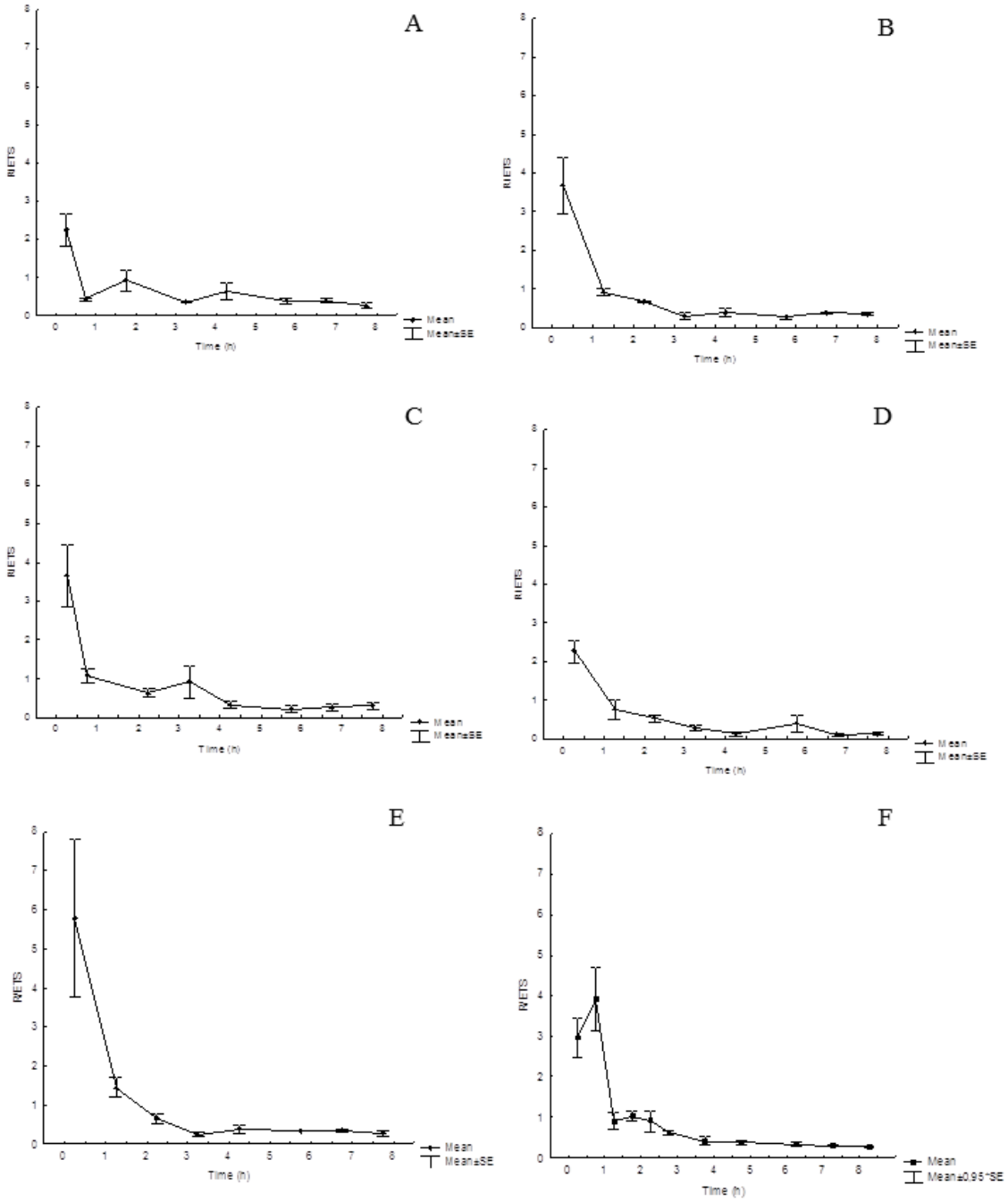

Figure 3. Time-course of the respiration/ETS ratio and standard error for the different experiments. Figure 3A corresponds to experiment 1, B for experiment 4, C for the number 2, D for 5, E for 3, and F for 6.

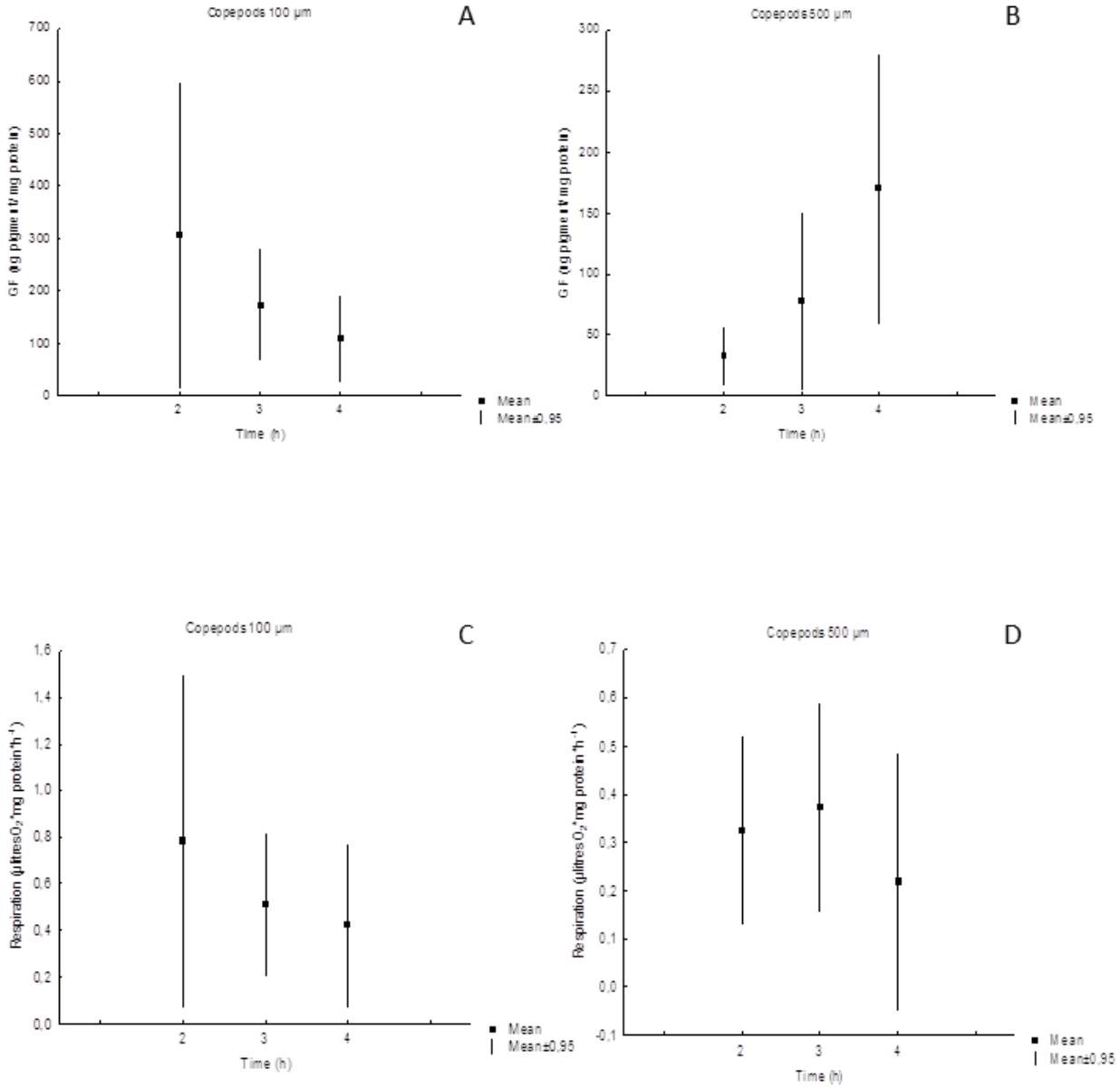

Figure 4. Oxygen consumption and gut fluorescence of copepods in the 100-200 µm (A and C) and 500-1000 µm (B and D) size fractions

VALORACIÓN PERSONAL (PERSONAL ASSESSMENTS)

- **Descripción detallada de las actividades desarrolladas durante la realización del TFT**

Para poder abarcar sobre este punto, es necesario destacar que el TFT se realizó con todos los datos que se obtuvieron de las distintas prácticas realizadas tanto en el laboratorio como a bordo del buque oceanográfico, BIO Ángeles Alvariño. Estos datos fueron tratados de tal forma que son los resultados de los que en este estudio hablamos. Por tanto, se trata de un estudio experimental en el que hemos unido la realización de las prácticas con el Trabajo de fin de título.

Durante la realización del TFT, se realizaron búsquedas de artículos científicos, los cuales fueron leídos para poder encontrar correlación con nuestro estudio y para saber qué diferencia aportaban nuestros experimentos a los ya realizados anteriormente. Además, como ya se ha mencionado anteriormente, se estudiaron casos de respiración tanto en el laboratorio como en el barco.

Comenzando con los experimentos que se realizaron en el laboratorio, es importante señalar que era necesario dejar a los organismos en saturación de alimento, durante un periodo de 12 horas, hasta el día siguiente que era cuando se realizaban las medidas del consumo de oxígeno. Además, mientras los individuos permanecían incubados, se realizaba el contaje del número de células por mililitro que se le suministraba. Una vez acabados los experimentos, se limpiaba todo el material y se ordenaba el lugar de trabajo.

Para finalizar, hablamos de los ensayos realizados a bordo del barco. En estos ensayos medíamos el consumo de oxígeno con un oxímetro diferente, puesto que éste nos daba medidas puntuales. El número de botellas donde se incubaban era menor a las de los estudios en laboratorio, pero de un volumen más grande (250 ml). Además, los organismos eran recién tomados de su medio natural y pasados a las botellas de incubación, por tanto, no conocemos con seguridad si venían de estar en saturaciones de alimento o no. Aunque este dato podríamos conocerlo debido a las condiciones oceanográficas, ya que las pescas para estos estudios fueron lanzadas en áreas más oligotróficas, lejanas al afloramiento.

- **Formación recibida (cursos, programas informáticos, etc.)**

En este apartado podemos destacar que si bien previamente se tenía conocimiento de cómo trabajar en un laboratorio en cuanto a orden, pulcritud y vestimenta, tuve fallos que poco a poco fui corrigiendo. Así mismo, en cuanto a cómo tratar algunos datos, se proporcionó también bastante información nueva. En cuanto al trabajo en el laboratorio, antes de realizar cualquier tipo de análisis, se me explicaba con detalle cómo funcionaban los instrumentos que se iban a utilizar en cada momento.

Además, en el caso de la utilización del oxímetro con el que obtuve los valores de respiración para las *Daphnia spp.*, se me facilitó un disco duro desde el que pude descargarme el software del programa que necesitaba dicho instrumento.

Por otra parte, los datos que proporcionaba el oxímetro señalado anteriormente, fueron tratados con el programa Excel, con el que ya había trabajado. Sin embargo, antes de obtener ningún dato de mis experimentos, mi tutor me proporcionó otros datos con los que tuve que manejar en el programa estadístico R. Esto me ayudó a familiarizarme con esta herramienta que más adelante utilizaría con mis propios datos. Cabe destacar la formación que recibí con otros programas estadísticos, como el STATISTICA, con el cual obtuve los gráficos representados en el trabajo.

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

La integración en el departamento se puede considerar totalmente satisfactoria, puesto que desde el principio los técnicos del laboratorio se mostraron atentos ante cualquier duda que se me pudiera ocurrir en cuanto a la utilización de material, al lavado o simplemente a la colocación del mismo en el laboratorio. Además de ayudarme al traslado de materiales desde los laboratorios de la Facultad hasta los laboratorios de Taliarte, concretamente al IOCAG (Instituto de Oceanografía y Cambio Global). Además, obtuve el apoyo del equipo de investigación que trabaja dentro de esta institución como profesores, estudiantes de doctorado, personal de trabajo del instituto en general, desde el servicio de limpieza por suministrarme las llaves para acceder a los laboratorios hasta el servicio de seguridad por abrirme las puertas del parking y dejarme acceder los días que no se trabajaba en el instituto pero que yo tenía que ir para poder realizar los experimentos.

Considero que he estado totalmente implicada en todas las tareas que he hecho a lo largo del estudio, ya que se me ha explicado cómo hacerlas y si bien en muchas ocasiones he fallado, a las siguientes lo he corregido y eso me ha hecho ganar conocimiento. En cuanto a las relaciones con el personal puedo decir que he aprendido de todos y cada uno de los que me han ayudado de una forma u otra en cada momento.

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

En este apartado se debe ser más objetivo si cabe, ya que, es una forma de mejorar y así ayudar a los futuros estudiantes de TFT.

En primer lugar, sobre los aspectos negativos durante el desarrollo del Trabajo de Fin de Título, al igual que se expuso en la memoria de prácticas externas, podemos decir que lo más destacado de este punto es el hecho de acudir durante días no lectivos hasta los laboratorios de la facultad o de Taliarte, para dar comienzo al experimento. Sin embargo, de este rasgo negativo he concluido en muchas cosas positivas.

En cuanto al aprendizaje positivo durante el desarrollo de este trabajo, cabe destacar sin ninguna duda que el hecho de realizar un trabajo experimental contiene muchos aspectos positivos, puesto que además de ganar en conocimiento, he aprendido a ser paciente, ya que para llegar a obtener unos buenos resultados, realicé muchos ensayos anteriores.

- **Valoración personal del aprendizaje conseguido a lo largo del TFT.**

Como valoración personal puedo decir con certeza que la realización del Trabajo de Fin de Título, junto con las demás asignaturas de la carrera, me ha hecho crecer como persona. No sólo me he enriquecido académicamente después de tanta búsqueda de bibliografía, lecturas, prácticas, trabajos, etc, sino que además en lo personal me ha hecho crecer mucho, implantando un poco en mí una gran virtud de la que carecía, la paciencia.

Para mí el TFT no sólo ha sido una asignatura más de la carrera, ha sido una etapa de mi vida, en donde he vivido momentos buenos y momentos malos. Puesto que lo he pasado mal cuando las cosas que he hecho no me han salido bien, en la que he

aprendido a solventar cada uno de los errores que he cometido, pero también en la que me lo he pasado muy bien, en la que me he enriquecido como bien expresé anteriormente y en donde he conocido a muchas personas buenas las cuales me han brindado su ayuda en cualquier momento que las he necesitado (debo expresar mi agradecimiento haciendo mención especial hacia éstas personas que han sido principalmente Acorayda, Lidia Nieves y Minerva).