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Comparing the response of the brown shrimp *Crangon crangon* (Linnaeus, 1758) to prolonged deprivation of food in two seasons

Cláudia Filipa Devesa André Moreira

Dissertação

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Cláudia Filipa Devesa André Moreira

Orientador: Prof. António Paulo Carvalho

Co-orientador: Dra. Joana Campos

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To my family and friends who were deprived of me for so long.

To all my friends that help me achieve.

Thank you all, you are amazing!

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Abstract

Crustaceans often undergo periods of starvation, due to natural food shortage or physiological aspects. During these periods several metabolic and behavioral changes can occur. This study evaluates how the brown shrimp *Crangon crangon* (L.) responds to prolonged deprivation of food in two seasons of the year, and how this species mobilizes its energetic reserves. Shrimps caught in June (summer) and October (autumn) 2010 in Minho estuary (North of Portugal) were placed in individual cages in experimental aquaria and kept in starvation until the last shrimp died or was sacrificed (six shrimps per aquarium every week). The caloric content, total lipids and total proteins, and the oxygen consumption rate were compared between seasons, sacrificed and naturally dead shrimps, and weeks of starvation. Summer shrimps were proven to be better prepared to endure stressful situations than those caught in autumn: they survived 2.5 times longer, had a higher Fulton's condition factor and higher caloric, lipid and protein content at the beginning of the experiments. During the first week of starvation the percentage of total proteins decreased significantly and stabilized in the next four weeks to decrease again abruptly in the fifth week. The percentage of total lipids only started to decrease after four weeks. This suggests that, on one hand, *C. crangon* probably uses stored proteins as a first energetic recourse and after that carbohydrates and eventually lipids, but at much lesser extent; and on the other hand, that after four weeks under starvation a critical point is reached when structural components might be mobilized to pay for maintenance costs.

Keywords: Energy reserves, *Crangon crangon*, starvation, biochemical analysis, oxygen consumption rate.

Resumo

Os crustáceos frequentemente enfrentam períodos de jejum devido à escassez natural de alimento ou a processos fisiológicos. Durante estes períodos podem ocorrer diferentes alterações metabólicas e comportamentais. Este estudo avalia como o camarão mouro *Crangon crangon* (L.) reage a períodos prolongados de jejum em duas épocas do ano e como esta espécie mobiliza as suas reservas energéticas. Os camarões foram capturados em Junho (verão) e Outubro (outono) de 2010 no estuário do Rio Minho (Norte de Portugal), mantidos individualmente em jejum nos aquários experimentais até que o último camarão morreu ou foi sacrificado (seis camarões por aquário todas as semanas). Os resultados referentes ao conteúdo calórico, lípidos totais, proteínas totais assim como o consumo de oxigénio foram comparados entre estações do ano, entre animais sacrificados e mortos naturalmente e entre semanas de jejum. Verificou-se que os camarões de verão se encontravam em melhores condições para enfrentar situações de stress do que os camarões capturados no outono: sobreviveram mais tempo, tinham um índice de condição de Fulton mais elevado e conteúdo calórico, lípidos e proteínas totais mais elevados no início das experiências. Durante a primeira semana de jejum a percentagem de proteínas totais diminui significativamente estabilizando nas seguintes até voltar a descer abruptamente na quinta semana. A percentagem de lípidos totais apenas decresceu na quarta semana de jejum. Estes dados sugerem que, por um lado as proteínas são a principal reserva energética desta espécie, seguidas de hidratos de carbono e só muito eventualmente lípidos; por outro lado depois de quatro semanas de jejum é atingido um ponto crítico a partir do qual os componentes estruturais provavelmente começam a ser mobilizados para suportar os custos de manutenção corporal.

Palavras-chave: Reservas energéticas, *Crangon crangon*, jejum, análises bioquímicas, consumo de oxigénio.

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List of abbreviations

DEB - Dynamic Energy Budget

UV - Ultraviolet

WW - Wet weight

TL - Total length

DW - Dry weight

O_2 - Oxygen

IRMS - Isotope-Ratio Mass Spectrometer

N - Total nitrogen

K - Fulton's condition factor

ANOVA - Analysis of variance

O:N - Oxygen consumed to nitrogen excreted

Introduction

Chapter 1: Species description

The brown shrimp *Crangon crangon* (Linnaeus, 1758) is a highly abundant epibenthic crustacean along European coastal waters from Norway to Morocco and throughout the Mediterranean and Black Seas (fig. 1). *Crangon crangon* inhabits mainly soft bottom (sandy, sandy-mud and muddy substrata) estuarine and marine shallow areas, including coastal lagoons, with preference for grain sizes between 125 and 710 µm (Pinn & Ansell 1993), although it may occur at depths of 20 to 90 m (Al-Adhub & Naylor 1975), especially during winter (Hinz et al. 2004, for more details see Campos & Van der Veer 2008).

Crangon crangon is often very abundant and hence it must represent an important component of the ecosystem. Due to its high abundance it forms an extensive food source for a large range of predators including fish, crustaceans and wading birds (Pihl 1985, Henderson et al. 1992, Del Norte-Campos & Temming 1994, Walter & Becker 1997). In turn it preys heavily upon several benthic species such as bivalve spat and juvenile plaice (Pihl & Rosenberg 1984, Van der Veer et al. 1991, Ansell & Gibson 1993, Van der Veer et al. 1998, Oh et al. 2001, Amara & Paul 2003, Campos & Van der Veer 2008). Besides this ecological role, the species is a valuable fishery resource in the North Sea, with annual catches of up to 37.000 t and a peak landing value of more than 100 million Euro (ICES 2009, Perger & Temming 2012); in south Europe it is only subjected to a small scale fishery in the Adriatic Sea.

With such large geographic distribution and being a species that spends most of the life cycle in estuarine environments *C. crangon* is exposed to great range of abiotic conditions and food availability. Brown shrimp is a euryhaline species occurring at salinities between 0 and 35 ups (Mees 1994, Mouny et al. 2000) and commonly found in waters of relatively low salinity (1-5 ups) (Havinga 1930, Boddeke 1976). Juveniles are often found in the upper reaches of estuaries, in nearly fresh water. As the shrimp mature, they move to water of higher salinity. This out-migration from low salinity water

is related to the reproduction season (Siegfried 1989, Perger & Temming 2012). *Crangon crangon* can survive at temperatures between 6 and 30ºC (Lloyd & Yonge 1947, Abbott & Perkins 1977, Jeffery & Revill 2002) with a temperature tolerance range of 0 to 30ºC and optimal temperature of 23ºC (Freitas et al. 2007). At lower temperatures, as during severe winters, brown shrimp prefers high salinity and hence show a tendency to migrate to offshore waters. This shows that there is a temperature-salinity interaction in the migration of this species, besides that of reproduction season.

Figure 1: Distribution of the brown shrimp *Crangon crangon* (after J. Campos 2009)

Chapter 2: Availability of food

Crangon crangon is characterized as a trophic generalist, an omnivorous species and even a carnivorous opportunist. Its diet includes both meiofauna and endobenthic macrofauna and consist of infaunal organisms like bivalves, cumaceans, foraminifereans, harpacticoids, nematodes, oligochaetes, epifaunal organisms as amphipods, isopods and gastropods, and demersal organisms such as mysids, shrimps and fishes.

Shrimps are mostly nocturnal using an ambush strategy to catch their prey and rarely actively search or pursue them. Experiments in aquaria show that the feeding activity is affected by the animal's physiological condition, such as the reproductive and/or moulting stage. When comparing immature females with mature ovigerous females the stomach content declines (Oh et al. 2001). The same way, during premoult and postmoult less food is ingested than during the intermoult period.

Potential prey items change with increasing shrimp size: juvenile shrimps eat mainly meiofauna changing their diet to macro-fauna items when they reach a total length over 20mm (Pihl & Rosenberg 1984, Gee 1987, Campos & Van der Veer 2008).

Across the brown shrimp's geographic range, which includes temperate (in the southern limit) and cold areas (in the northern limit), warmer periods alternate with colder seasons and condition the systems' productivity, and hence its food availability. Being trophic generalists and opportunistic means that fluctuations in preys' availability are reflected in a seasonality of the brown shrimp's diet. Moreover, not only the prey species vary seasonally; also their quantity varies. Environmental changes in temperature and food conditions will affect the energy available for the different physiological processes and will determine rates of growth and reproduction.

Besides environmental changes in food availability, crustaceans undergo periods of natural starvation due to moulting, a process where the animals shed their exoskeleton and grow. The moulting process involves a series of stages; the intermoult period i.e. between two consecutive moults, lasts for several days during which the animal presents its natural feeding behaviour, feeding actively; prior to moulting, feeding declines until it stops completely during moulting. Finally, feeding begins again in postmoult after the animal has an exoskeleton rigid enough to support the weight of the animal and handle food (Phlippen et al. 2000). However, this process requires a large amount of energy and can take up to 2 or 3 days (Lipcius & Herrnkind 1982, Robertson et al. 1987, Chan et al. 1988) until the animal is ready to feed again.

Chapter 3: Energetic reserves

The ingested food is usually metabolized by the animals to pay for all the metabolic needs, from somatic maintenance to growth, maturity and reproduction. Priority is always given to the organism's maintenance. During periods of food abundance, however, animals can mobilize the remaining of the ingested food which was not used in growth and reproduction to build up reserves. In turn, these reserves can be later mobilized to ensure a continuous supply of essential compounds for metabolism when the animal is starving. This means that after a period of food abundance, the nutritional status of the animals can be close to optimal i.e. presence of plenty of energetic reserves to assure its capacity to cope with a period of food shortage.

The energy flow through an individual and the allocation of the energy to all metabolic needs including growth and reproduction in relation to environmental conditions and food intake can be explained using the Dynamic Energy Budget (DEB) theory (Kooijman 2009). This theory assumes that the ingested food is directly assimilated into reserves which are used to fuel all metabolic needs of an individual: a fixed fraction *k* of the energy utilised from the reserves is spent on growth and somatic maintenance and the rest on maturity and reproduction. Priority is always given to somatic maintenance and if the energy utilisation rate from the reserves is no longer sufficient to pay for somatic maintenance the individual dies (Van der Meer 2006). Reserves are only stored when a surplus of food is available. These reserves can be mobilized by several paths, stored in several organs and in different compounds. When the animal is starving, because of moulting in the case of crustaceans, or because food is not available, its metabolism must rely on the stored reserves.

Determination of an animal's reserves

The hepatopancreas is the mainstorage organ in decapod crustaceans (Gibson & Barker 1979, Sánchez-Paz et al. 2007, Comoglio et al. 2008). Yet, on one hand, this organ is not exclusively used for storage and has other functions like secretion of digestive enzymes; and on the other hand, important reserves are also located in the crustaceans' epidermis, sub epithelial connective and muscle tissues (Stevenson 1985), which are part of the animal structure. Therefore, the estimation of the amount of stored reserves cannot be made directly.

Artificially induced fasting and starvation experiments might be the only way to determine the energy reserves and to enlighten the metabolic routes (in hierarchical order) used during fasting. These experiments have been used to describe the biochemical and physiological adaptation as well as to determine the energetic requirements of several crustaceans (Guderley et al. 2003, Comoglio et al. 2005, Comoglio et al. 2008, Zhang et al. 2009). In these experiments it is assumed that a well fed animal will have plenty of reserves, while an animal in low nutritional condition (low weight, low energy content) will have its reserves depleted. The amount of reserves is then estimated by comparing the decrease in weight or in physiological condition during a period of fasting or starvation.

Animals have different strategies to cope with the lack of food. The hepatopancreas mainly accumulates lipids (Yepiz-Plascencia et al. 2000; Luvizotto-Santos et al. 2003) and to a lesser degree glycogen (Verri et al. 2001) which, in some crustaceans, are used during short-term food shortage such as moult (Sánchez-Paz et al. 2007). Besides the class of reserves mobilized, also the sequence of substrates used varies considerably (Sánchez-Paz et al. 2007). Although proteins are considered as the main reserve compound in most crustaceans (New 1976, Comoglio et al. 2005, Comoglio et al. 2008), it has been suggested that *C. crangon* uses glycogen as a first resource and protein as a last (Cuzon & Ceccaldi 1973). Yet previous studies on this last species had serious flaws because the authors did not accounted for cannibalism and other possible origins of food like bacterial and microalgae productivity within the aquaria system.

Objectives

The aim of this work was to study the response of the brown shrimp *Crangon crangon* to prolonged starvation in two distinct seasons: summer, when energetic reserves were assumed to be higher, and autumn. For that, starvation experiments were performed with shrimps from Minho estuary, north of Portugal. The energy reserves of the species were estimated directly by calorimetric analysis and the sequence of mobilized compounds were estimated directly by biochemical analysis of total lipids and total proteins, and indirectly through the oxygen consumption rate determination.

Material and Methods

Starvation experiment

Crangon crangon were collected at Coura saltmarsh (fig. 2) within Minho estuary in the beginning of summer (June 2010; 18.4ºC and 17.4 ups) for experiment I and in the beginning of fall (October 2010; 17.2ºC and 31.5 ups) for experiment II, with a 1m beam trawl (5mm mesh size) (fig. 3). Shrimps were transferred to a maintenance aquarium and gradually adapted to a closed circulation system of salt water at 29 ±1ups, with temperature controlled at 20 ±1ºC, and under artificial photoperiod (12/12h) for acclimation to laboratory conditions. Animals were maintained with *ad libitum* food to acclimate for a week.

Figure 2: Upper – Minho River estuary in the North of Portugal; Lower – detail of Coura saltmarsh (Google maps)

Figure 3: Beam-trawl used to collect the shrimps for the experiments

After the acclimation period, 374 female shrimps, 180 for experiment I and 194 for experiment II, were randomly selected, measured (total length, from the tip of the scaphocerite to the end of the telson, to the nearest 0.5mm), weighed (wet weight, to the nearest 0.0001g), placed in individual cages (10 x 10 x 17cm) inside the experimental aquaria (see table 1 for total length and wet weight of the animals) and kept without food for 2 days to purge faeces and pseudofaeces. In each experiment, 4 aquaria (8 in total) were used (A to D in experiment I, and E to H in experiment II) and each aquarium was completed with 50 shrimps; exception were the aquaria D which had only 30 shrimps, and aquarium E which had 44 shrimps. These two aquaria were kept in the same conditions but were used exclusively to determine the oxygen consumption rate. Placing the animals in individual cages enabled to monitor each individual separately and eliminated the possibility of cannibalism (fig. 4).

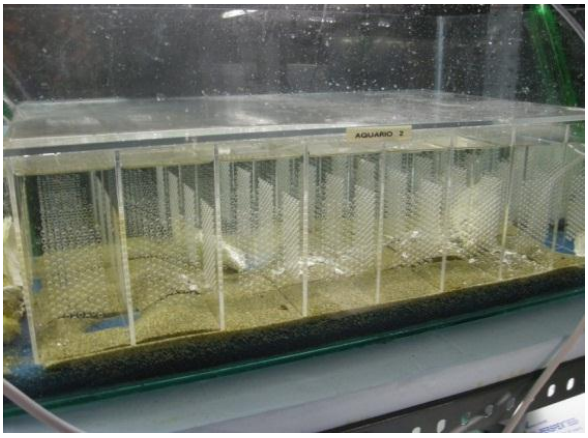


Figure 4: Individual cages for the shrimps

The experimental aquaria were kept in a closed circulation system, isolated and in total darkness so that no algae or microorganisms could grow and be used as food. The aquaria had a 2cm layer of sediment, which was previously burned (580ºC, 8h) to remove all organic matter. Water parameters such as temperature, salinity, ammonia and nitrites were determined daily. Temperature and salinity were maintained similar in the four aquaria (table 1) at 20.2 ±0.11ºC and 30.2 ±0.08 ups (mean ±se) respectively, with no differences between aquaria. The concentration of ammonia presented a mean (±se) of 0.32 ±0.03mg.L^{-1} with no significant differences between aquaria ($p>0.05$). Water was changed twice a week with UV treated water; partial changes were made whenever it was necessary to adjust parameters.

Total length (TL) and wet weight (WW) of the animals at the beginning of the trials are shown in table 1. The animals in aquaria D and E were intentionally larger, so that the oxygen consumption rate could be measured. For these shrimps the total length was significantly larger in experiment II (ANOVA: $F=55.35$, $p<0.0001$), with values ranging from 31.1 and 41.5mm, while in the experiment I the values ranged between 31.2 and 36.5mm. Concerning the wet weight, shrimps from experiment II were also heavier than the ones in experiment I (ANOVA: $F=46.62$, $p<0.0001$), with the weight varying between 0.3124 and 0.4516g in experiment II and 0.2488 and 0.3995g in experiment I. Along the starvation time, TL and WW of the individuals used in experiment I did not change significantly, this is, shrimps used for the oxygen measurements in the first week had similar mean TL and WW as the ones used in the sixth week ($p>0.05$). In the experiment II, the shrimps' size did not differ between the weeks ($p>0.05$). However, the shrimps used in the third week measurements were lighter than the ones used in the first and second week (ANOVA: $F=5.83$, $p=0.0022$).

All observed animals in the other 3 aquaria in the experiment I had similar mean total length and wet weight (table 1). In one aquarium of experiment II (H) the mean total length and wet weight were significantly smaller ($F=676.26$, $p<0.0001$ and $F=7.55$, $p=0.0008$ respectively for TL and WW). Also animals used in experiment II were significantly larger and heavier than the ones of experiment I (table 1).

Every day, besides registering the temperature and salinity, shrimps were checked for dead animals or exuvia, while every week six animals were sacrificed. In all aquaria, animals were kept in starvation until the last animal was sacrificed or died (maximum 49 days). Sex of the individuals was confirmed in the exuvia based on the endopodite of the first and second pairs of pleopods; exuvia was then returned to the animal to prevent mineral disequilibria. Dead and sacrificed individuals were measured, weighed

and frozen for later biochemical analyses. In the end, all shrimps were dried at 60ºC for two days, weighed (dry weight, DW) and then macerated for the biochemical analysis.

Table 1: Number of shrimps and their mean (±se) total length (TL) and wet weight (WW); mean (±se) temperature and salinity; and duration of both experiments; ANOVA results with significant differences in bold

	Experiment	Aquarium A/E	Aquarium B/F	Aquarium C/G	Aquarium D/H	ANOVA Between aquaria	ANOVA between experiments
TL (mm)	I	27.1 ±0.16	26.9 ±0.16	26.9 ±0.15	33.9 ±0.21	0.54, p≥0.05*	622.85, **p<0.0001***
	II	38.8 ±0.23	30.4 ±0.13	30.3 ±0.10	29.5 ±0.17	12.45, **p<0.0001***	
WW (g)	I	0.1654 ±0.004	0.1620 ±0.004	0.1674 ±0.005	0.3093 ±0.007	0.39, p≥0.05*	31.71, **p<0.0001***
	II	0.4008 ±0.006	0.1961 ±0.004	0.1874 ±0.004	0.1726 ±0.004	7.55, **p<0.05***	
TºC	I	20.4 ±0.18	20.0 ±0.18	20.6 ±0.18	19.8 ±0.26	3.42, **p<0.05**	0.68, p≥0.05
	II	20.3 ±0.31	20.2 ±0.33	19.7 ±0.04	19.5 ±0.32	1.57, p≥0.05	
Salinity (ups)	I	29.9 ±0.17	30.1 ±0.15	30.5 ±0.13	30.3 ±0.14	2.33, p≥0.05	4.03, p≥0.05
	II	30.1 ±0.14	30.0 ±0.14	29.7 ±0.16	29.8 ±0.12	1.91, p≥0.05	
Number of animals	I	50	50	50	30**		
	II	44**	50	50	50		
Time of starvation (days)	I	36	36	36	49		
	II	21	14	14	8		

*ANOVA of only 3 aquaria, i.e. excluding the one used in the oxygen consumption rate.
** Aquaria used for the measurements of the oxygen consumption rate

Oxygen consumption rate

Animals from aquaria D and E were used for the determination of the oxygen consumption rate. In the first week O_2 consumption was determined in three consecutive days (every 24h, at the same time), and afterwards measurements were made once a week. A number of shrimps (four and five, respectively in experiment I and II) were randomly selected, weighed and transferred to air tight containers, with substrate. An extra container without any shrimp was used as control. After 15 minutes of acclimation, the oxygen concentration was measured continuously for 45 minutes.

The containers consisted of glass chambers into which an HQd meter with a LDO101 probe was inserted. This probe monitored the environmental data every 30s transmitting that information to a pen drive where it was stored for later treatment. Consumed oxygen was taken as being the net difference between the measurements at the start and the end. Water in the containers came from the experimental aquarium. Measurements were made at the same temperature as in the experimental aquaria. The shrimps' survival to starvation enabled to follow the oxygen consumption along six and four weeks respectively in the experiments I and II.

Biochemical analysis

Six individuals from each aquarium except D and E (the oxygen consumption study aquaria) were sacrificed, measured and weighed every week; at day zero also 6 individuals from the 3 aquaria were sacrificed to be used as initial estimates. About 3 sacrificed shrimps were used for caloric content determination in cal.DWg^{-1}, in a bomb calorimeter (PARR 1261); other 3 sacrificed shrimps were used to determine total proteins and total lipids, respectively in an Isotope-Ratio Mass Spectrometer (IRMS), which determines the total nitrogen (N), and with a Spinreact® commercial kit. The same procedure was followed for the naturally dead animals. In the end, in total for both experiments, 145 individuals were analyzed for caloric content, 30 of them were naturally dead shrimps; 143 animals for total proteins and total lipids, from which 87 were naturally dead.

The determination of total proteins requires only a small amount of sample (approximately 0.5 mg), so the remaining was used for the total lipids' quantification. This means that data on total proteins and total lipids corresponds to the same individual. The samples were first homogenized in a chloroform-methanol-water (2:2:1) mixture and extraction of lipids was performed according to Sanchés-Paz et al. (2007) protocol, using a ratio of 20 v/w of buffer A and 20 v/w of the chloroform-methanol-water mix. For the calculus of total protein from total N, the standard factor of 6.25 was used (Mariotti et al. 2008). Both, total proteins and total lipids were presented as percentage of DW.

Data treatment

The Fulton's condition factor (K) was determined dividing the body mass (WW) by the cube of the body length (TL), assuming that the individual has an isometric growth. The Fulton's Condition factor was calculated in the beginning and in the end of both experiments. This morphological index allows quantifying the health of an individual and comparing the morphometric data with the biochemical information.

ANOVA tests were applied using Systat 13.0 software. Comparisons were made between experiments, between aquaria, between observation weeks (pooling data from the same week of starvation) and considering each aquarium as a replicate. Whenever differences were detected a Tukey's comparison test was applied. Assuming that naturally dead animals were in lower condition than sacrificed ones, their results were analyzed separately and compared between each other. When comparing across time, at the start or beginning means at day "zero", first week means "at the end of the first week" and the same for the following weeks.

Results

Natural mortality

Shrimps were sacrificed every 7 days for biochemical analysis in both experiments. Natural mortality in the experiment I started to occur after 6 days in starvation, except in the aquarium A, where dead animals were found on the second day. In the experiment II, shrimps started to die after 2 or 3 days in all aquaria. Half of the shrimps were dead after 18 to 31 days and after 3 to 12 days, respectively in the experiments I and II (table 2). Maximum duration of the starvation trials was observed in the experiment I (49 days), allowing observing temporal trends across 6 weeks. In contrast, in the experiment II, the maximum duration was 21 days in the oxygen consumption study aquarium enabling comparisons across 4 weeks. In the other aquaria, shrimps survived up to 14 days, and hence only the results from the first two weeks can be compared.

Table 2: Time till half of the shrimps were dead and maximum time in starvation in each aquarium

Experiment	Aquaria	Time in starvation (days)	Time till 50% mortality (days)
I	A	36	22
	B	36	20
	C	36	18
	D*	49	31
II	E*	21	12
	F	14	6
	G	14	7
	H	8	3

* Shrimps from these aquaria were used in the oxygen consumption study and were not sacrificed

Oxygen consumption

The oxygen consumption rate differed significantly between the two experiments (ANOVA: F=9.06, p=0.0035). Higher rates were found in the experiment II, 0.79 ±0.14 versus 0.29 ±0.05mg $O_2.g^{-1}.h^{-1}$ in experiment I (fig. 5).

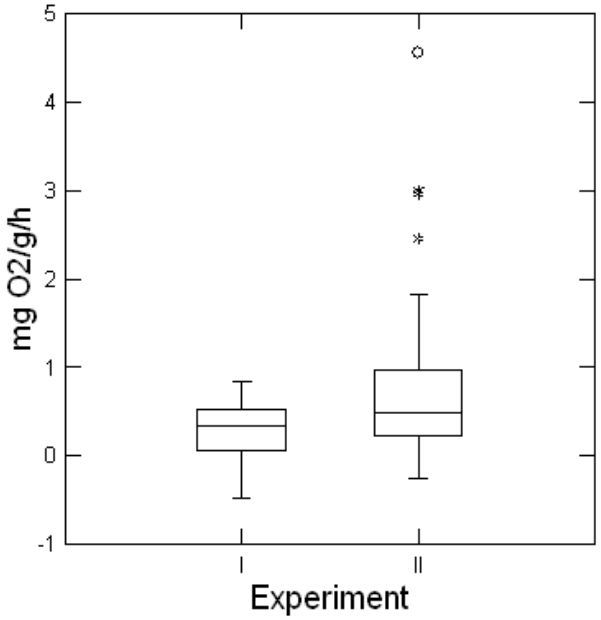


Figure 5: Oxygen consumption rate (mg $O_2.g^{-1}.h^{-1}$) in both experiments (* and ° are outliers)

The oxygen consumption rate was significantly different between the weeks of starvation in the experiment I (ANOVA: F=4.46, p=0.0037). These differences were found between the second, third and fourth weeks and the fifth week (table 3, fig. 6). The highest mean of oxygen consumption was found in the third week and the lowest in the fifth week, respectively 0.45 ±0.06 and 0.12 ±0.03mg $O_2.g^{-1}.h^{-1}$. In the experiment II, the rate of consumption was similar along the weeks (p>0.05), with a maximum mean of 1.08 ±0.31mg $O_2.g^{-1}.h^{-1}$ in the second week and a minimum of 0.48mg $O_2.g^{-1}.h^{-1}$ in the fourth week (fig. 7).

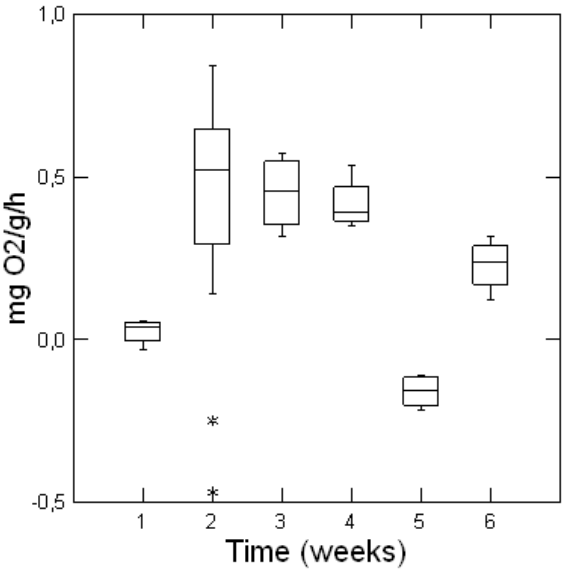


Table 3: Tukey's pairwise comparisons results on the oxygen consumption rate in the experiment I

week	week	p
2	5	0.0053
3	5	0.0292
4	5	0.0444

Figure 6: Oxygen consumption rate (mg $O_2.g^{-1}.h^{-1}$) per week in the experiment I (* are outliers)

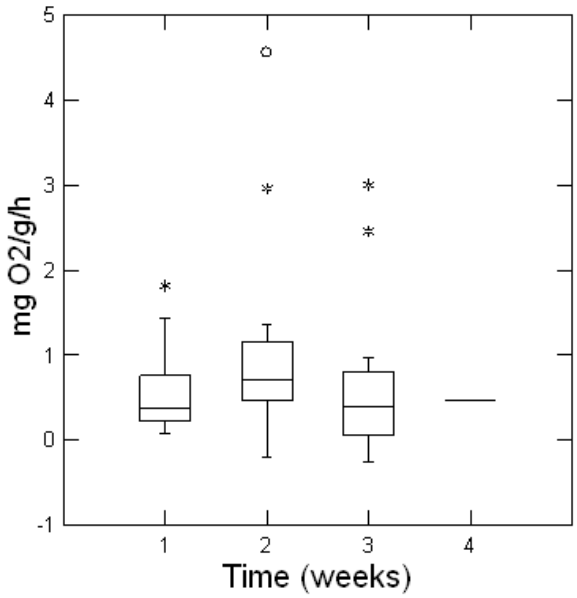


Figure 7: Oxygen consumption rate (mg $O_2.g^{-1}.h^{-1}$) per week in the experiment II; only one shrimp was available in the fourth week (* and ° are outliers)

No clear trend was found relating the oxygen consumption rate with the animals' weight and total length besides a tendency of the larger and heavier experiment II shrimps to present higher rates (fig. 8 and 9).

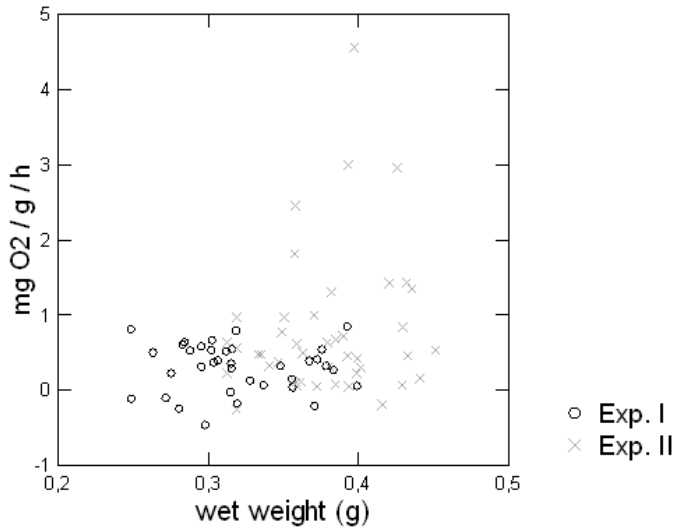


Figure 8: Oxygen consumption rate (mg O_2.g^{-1}.h^{-1}) in relation to the wet weight (g) of the shrimps in both experiments

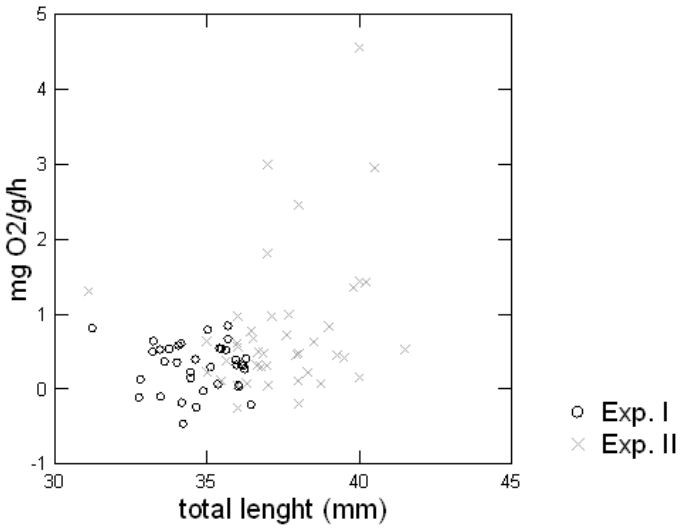


Figure 9: Oxygen consumption rate (mg O_2.g^{-1}.h^{-1}) in relation to the total length (mm) of the shrimps in both experiments

Dry weight, percentage of water and Fulton's Condition factor

The DW and the percentage of water presented a very large individual variability and hence did not differed significantly between the sacrificed and the naturally dead shrimps ($p>0.05$), though the naturally dead had higher mean DW (fig. 10) and the sacrificed shrimps had higher percentage of water (fig. 11). However, between the two experiments only the mean water content of the sacrificed shrimps presented

differences (ANOVA: F=13.55, p=0.0004), being higher and ranging from 55.4 to 95.6% in the experiment I, while in the experiment II ranged from 72.5 to 92.3% (fig. 11).

The mean DW of the naturally dead and sacrificed shrimps in both experiments is shown in the figure 10 and in the table 4.

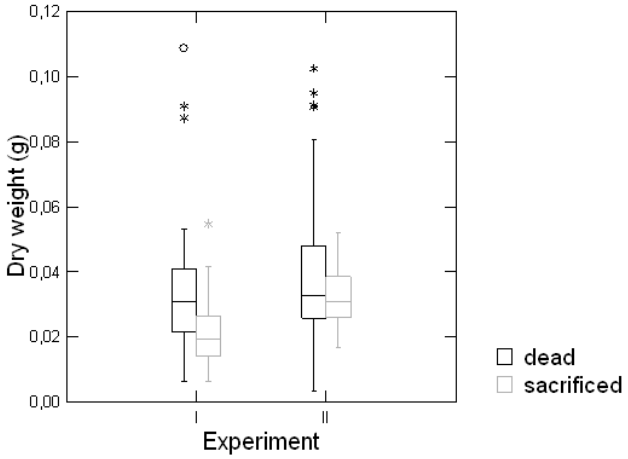


Table 4: Mean (±se) dry weight (DW, g) of the naturally dead and sacrificed shrimps in both experiments

	Exp. I	Exp. II
Naturally dead	0.0343 ±0.0036	0.0394 ±0.0024
Sacrificed	0.0206 ±0.0011	0.0325 ±0.0017

Figure 10: Mean dry weight (DW, g) of the naturally dead and sacrificed shrimps in both experiments (* and ° are outliers)

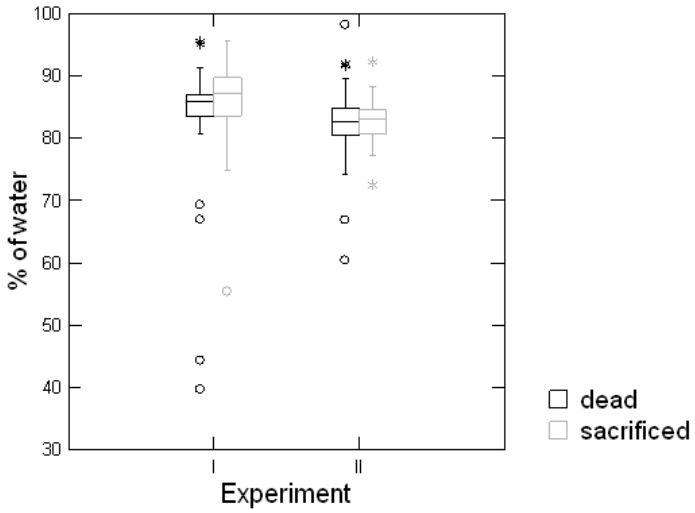


Figure 11: Percentage of water of the naturally dead and sacrificed shrimps in both experiments (* and ° are outliers)

Differences in DW of the sacrificed shrimps between aquaria were significant (ANOVA: F=8.04, p<0.0001), but mainly between the aquaria from experiment I (A, B and C) and those from experiment II (F and G) (fig. 12, table 5). Yet, while in most cases the sacrificed shrimps in the experiment I had less DW, in the experiment II the sacrificed shrimps' DW was close to that of the naturally dead (table 4). For these naturally dead

shrimps, differences were also observed between aquaria (ANOVA: F=10.90, p<0.0001), namely between aquarium E and all other. Regarding the water content, statistical differences were found between aquaria for the sacrificed shrimps (ANOVA: F=3.00, p=0.0147), namely between aquarium G and aquaria A and C (p<0.05). For the naturally dead individuals, no difference was observed (p>0.05) (fig. 13).

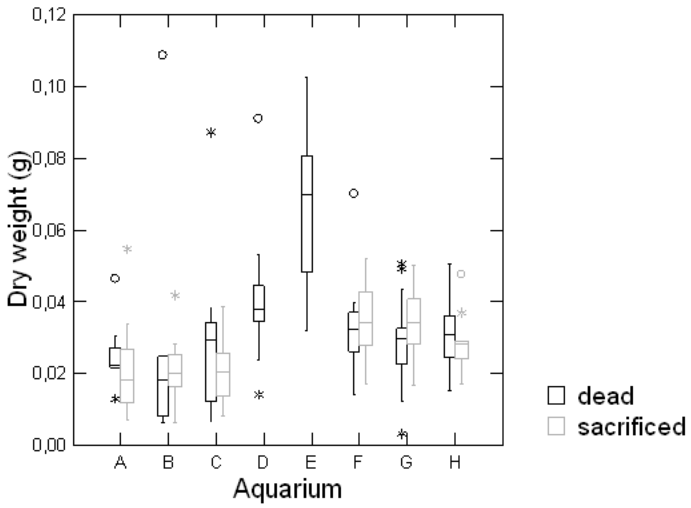


Figure 12: Mean dry weight (DW, g) of the naturally dead and sacrificed shrimps per aquarium (* and ° are outliers)

Table 5: Tukey's pairwise comparisons results on the dry weight (DW)

Aquarium	Aquarium	p
A	F	0.0002
	G	0.0006
B	F	0.0024
	G	0.0049
C	F	0.0013
	G	0.0027

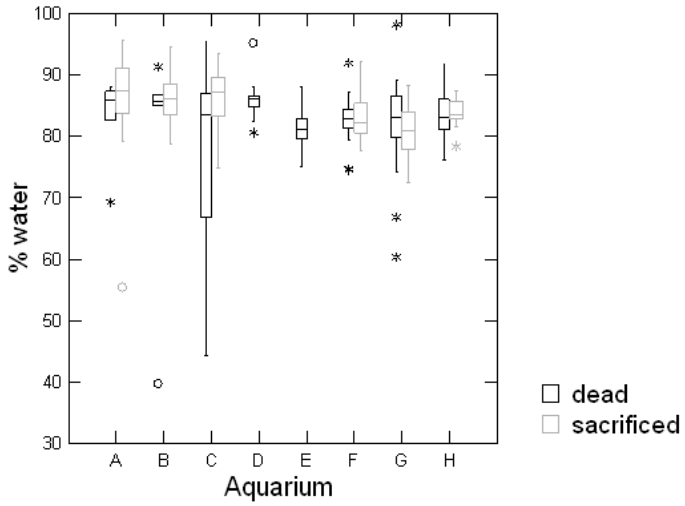


Figure 13: Mean percentage of water of the naturally dead and sacrificed shrimps per aquarium (* and ° are outliers)

Comparing along time confirmed that the sacrificed shrimps in the experiment I had significantly lower mean DW and higher percentage of water than those that naturally died (ANOVA: $F=19.80$, $p<0.0001$; $F=4.82$, $p=0.0304$, respectively). No difference was found in the DW and water content of the naturally dead shrimps along the starvation time ($p>0.05$); in contrast, the DW of the sacrificed shrimps decreased significantly between the first and the fourth and fifth weeks, and between the second and the fourth and following weeks (ANOVA: $F=5.91$, $p=0.0013$), while their water content increased between the first and fifth weeks and between the second and the third, fourth and fifth weeks ($p<0.05$, table 6) (fig. 14). In the experiment II, despite an increasing trend in DW of the naturally dead shrimps, there was no significant difference across the starvation weeks ($p>0.05$). Also, no significant difference between weeks was found in the water percentage of both sacrificed and naturally dead shrimps ($p>0.05$) (fig. 15).

Table 6: Tukey's pairwise comparisons results on the dry weight (DW, g) and percentage of water (%) in the experiment I

DW			% of water		
week	**week**	**p**	**week**	**week**	**p**
1	4	0.0101	1	5	0.0344
	5	0.0006	2	3	0.0173
2	4	0.0064		4	0.0411
	5	0.0003		5	0.0006

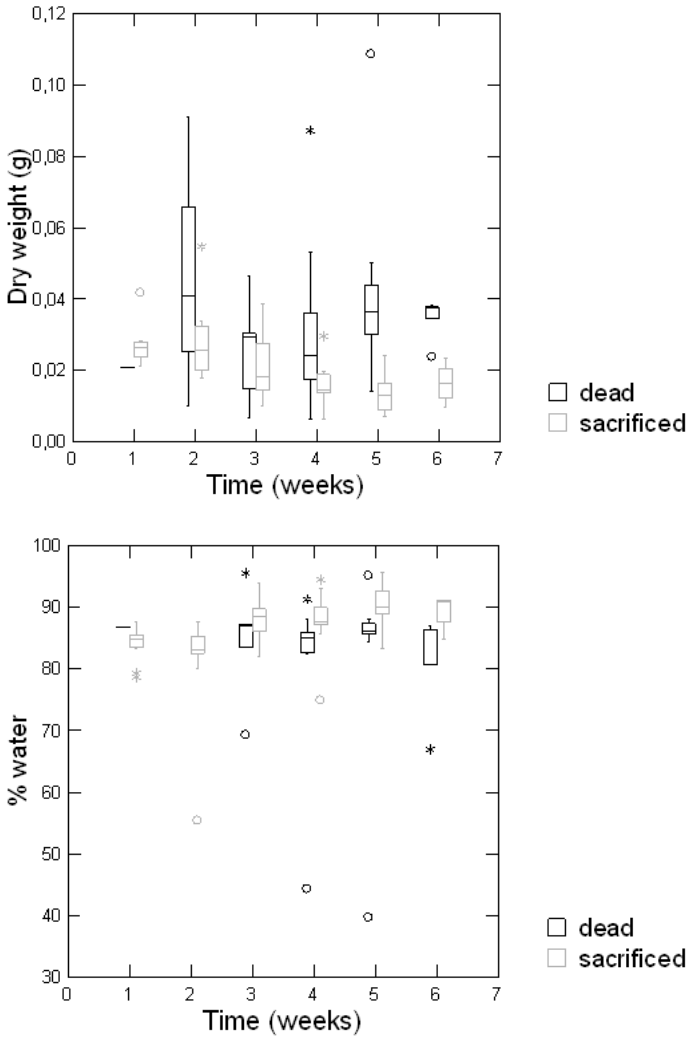


Figure 14: Dry weight (DW, g, upper) and water content (%, lower) along the weeks of starvation in the experiment I (* and ° are outliers)

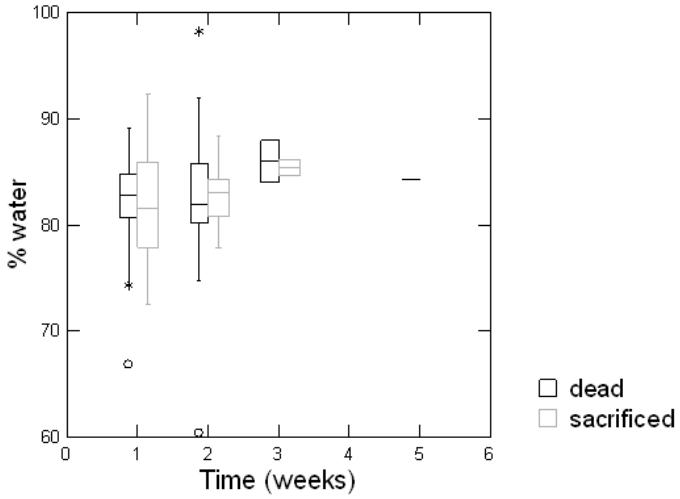


Figure 15: Percentage of water per week in the experiment II (* and ° are outliers)

Fulton's Condition factor

At the beginning of the experiment I, all shrimps had about 20% higher K (table 7) than that of the ones used in the experiment II (ANOVA: F=60.38, p<0.0001; F=119.67, p<0.0001, respectively for sacrificed and naturally dead shrimps) (fig. 16). Differences in the initial K of the sacrificed and naturally dead shrimps were found mainly between the aquaria from experiment I (A, B and C) and the aquaria from experiment II (F, G and H) (fig. 17).

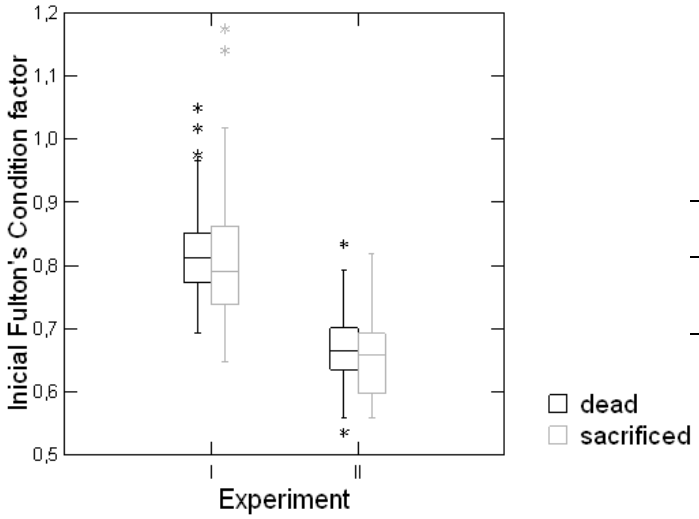


Table 7: Mean (±se) Fulton's condition factor of the naturally dead and sacrificed shrimps at the beginning of the experiments

	Exp. I	Exp. II
Naturally dead	0.82 ±0.014	0.67 ±0.007
Sacrificed	0.81 ±0.013	0.66 ±0.011

Figure 16: Initial Fulton's condition factor of the shrimps in both experiments (* are outliers)

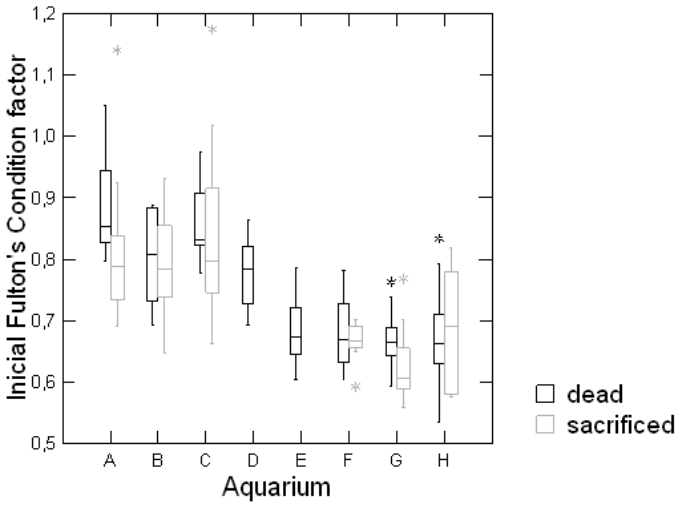


Figure 17: Initial Fulton's condition factor of the shrimps in each aquarium (* are outliers)

At the end of both experiments, K of sacrificed shrimps was significantly different from that of the naturally dead (ANOVA: F=18.87, p<0.0001). In the experiment II, values of

K ranged between 0.51 and 0.78 and between 0.37 and 0.87, respectively for the sacrificed shrimps and for the naturally dead (table 8). However, while in the experiment I mean final K was higher for the sacrificed shrimps, in the experiment II these shrimps had a lower final K than naturally dead shrimps (fig. 18a).

Final K also significantly differed between aquaria (ANOVA: $F=16.32$, $p<0.0001$), with the exception of aquaria B and H (fig. 18b). Despite a great individual variability, shrimps that naturally died in all aquaria were in a similar final condition ($p>0.05$).

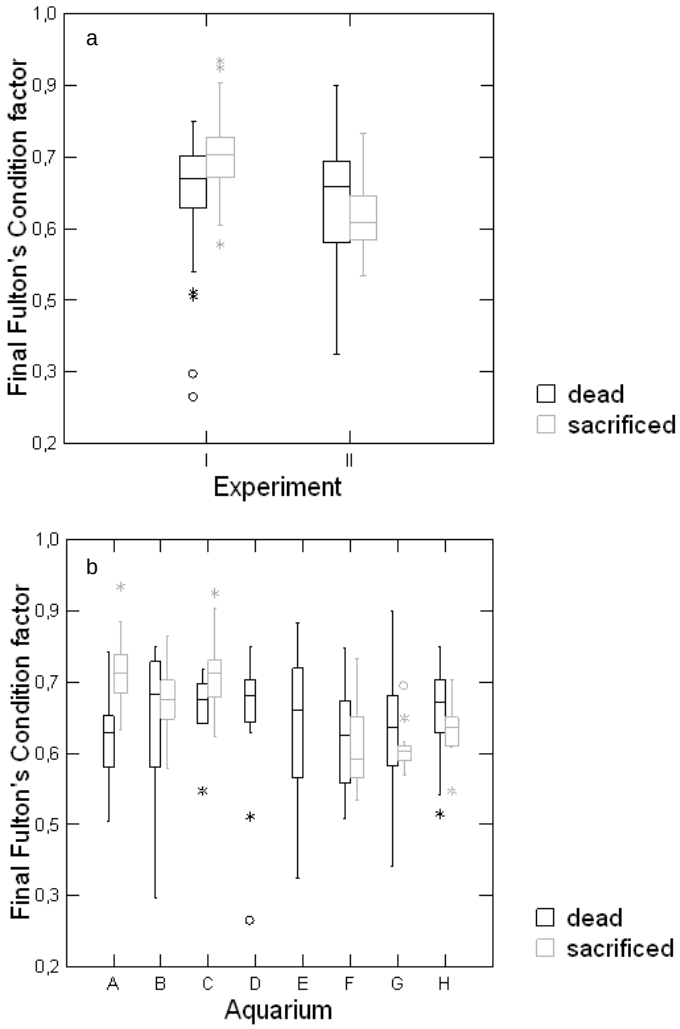


Figure 18: Final Fulton's Condition factor of the shrimps in both experiments (a) and in each aquarium (b) (* and ° are outliers)

Table 8: Mean (±se) Fulton's condition factor of the naturally dead and sacrificed shrimps in the end of the experiments

	Exp. I	**Exp. II**
Naturally dead	0.62 ±0.032	0.65 ±0.013
Sacrificed	0.74 ±0.008	0.62 ±0.011

Over the time differences started to appear. Shrimps that were sacrificed had higher values of final K than those that naturally died (ANOVA: F=18.72, p<0.0001). For the sacrificed shrimps, differences were clear between the first and the fourth, fifth and sixth weeks (p<0.05, table 9) (fig. 19). The highest condition was found in the sacrificed shrimps' first week in experiment I (0.91). In the experiment II, no statistical difference was found across time for both sacrificed and naturally dead shrimps (p>0.05).

Table 9: Tukey's pairwise comparisons results on the Fulton's condition factor in the experiment I

week	week	p
1	4	0.0001
	5	0.0255
	6	0.0461

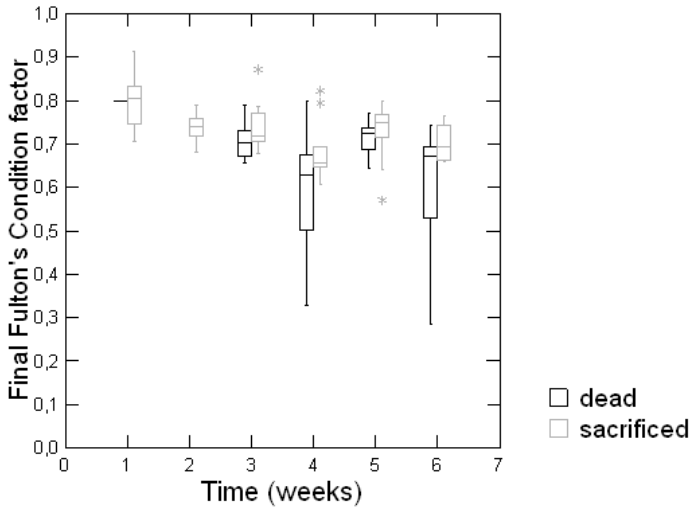


Figure 19: Mean Fulton's condition factor of the shrimps along the weeks of starvation in the experiment I (* are outliers)

Total lipids, total proteins and caloric content

For the quantification of the total lipids and total proteins, 143 shrimps were analyzed, but 10 lipids and 5 proteins' values were discharged because the results were not reliable. Of the 145 shrimps analyzed for the caloric content only 72 produced reliable results, because some of the shrimps were lighter than the minimum required by the

calorimeter (0.150g DW), weighing less than 0.100g. For the lighter individuals an excipient of fishmeal (with a known and stable energy content of 5777cal.g^{-1}) was used to make up for the weight, but nevertheless in several cases the equipment was not capable of detecting the low levels of energy produced in the combustion. Therefore, several errors and some outliers were obtained and only a small set of data provided reliable information.

The percentage of total lipids and the caloric content of the sacrificed and the naturally dead shrimps did not differ considering the data from both experiments ($p>0.05$), while differences were found between the percentage of total proteins for the naturally dead shrimps in the experiment I and of the naturally dead and sacrificed shrimps in the experiment II (ANOVA: $F=37.81$, $p<0.0001$). However, in both experiments, the sacrificed shrimps presented mean values of lipids near half of the naturally dead's levels (table 10), ranging from 0.004 to 1.593% in the experiment I and from 0.060 to 1.470% in the experiment II, while for the naturally dead shrimps the percentage ranged between 0.024 and 2.886% in the experiment I, and 0.013 and 4.265% in the experiment II (fig. 20).

Regarding the percentage of total proteins, naturally dead shrimps from the experiment II showed the highest values (table 11), ranging from 4.2 to 66.2% (fig. 21). In the experiment I the percentage varied between 13.6 and 47.4%. For the sacrificed shrimps, differences were not significant between experiments, and total proteins ranged between 33.4 and 66.4% in the experiment II and 34.3 and 58.4% in the experiment I.

The mean caloric content of the sacrificed shrimps varied between 774 and 6273cal.g^{-1} in the experiment I and 1143 and 4394cal.g^{-1} in the experiment II. The naturally dead shrimps presented mean values between 1634 and 2334cal.g^{-1} in the experiment I and 196 and 4060cal.g^{-1} in the experiment II (fig. 22).

Table 10: Mean (±se) percentage of total lipids (%) of the naturally dead and sacrificed shrimps in both experiments

	Exp. I	**Exp. II**
Naturally dead	1.060 ±0.155	0.869 ±0.114
Sacrificed	0.602 ±0.059	0.567 ±0.109

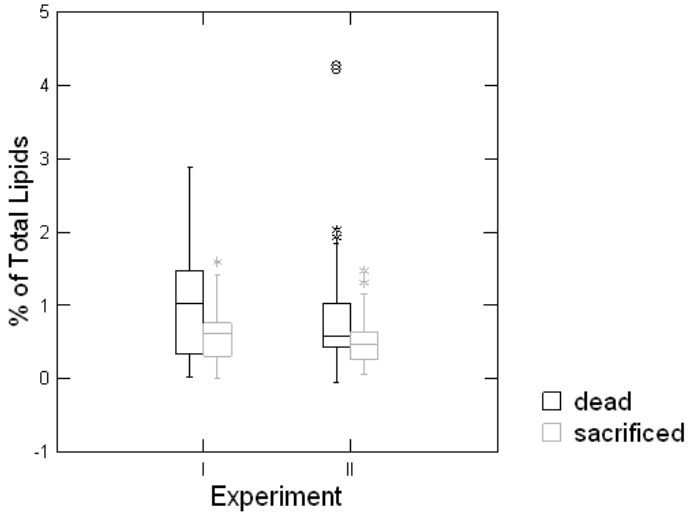


Figure 20: Percentage of total lipids of the naturally dead and sacrificed shrimps in both experiments (* and ° are outliers)

Table 11: Mean (±se) percentage of total proteins (%) of the naturally dead and sacrificed shrimps in both experiments

	Exp. I	**Exp. II**
Naturally dead	31.2 ±1.55	46.8 ±1.66
Sacrificed	48.8 ±0.89	51.3 ±1.91

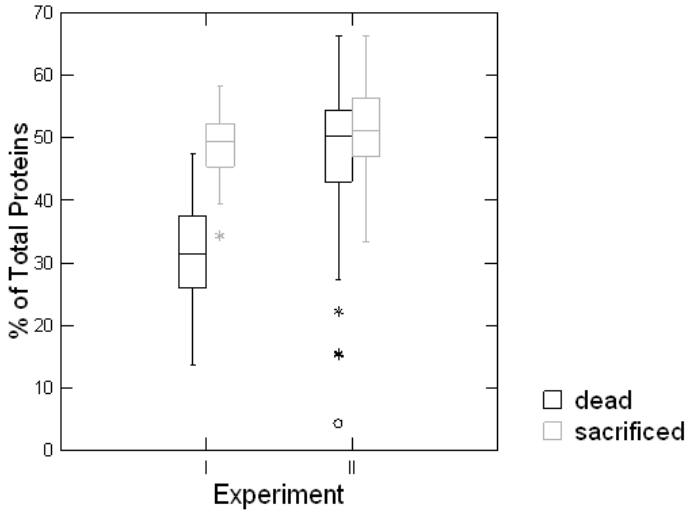


Figure 21: Percentage of total proteins of the naturally dead and sacrificed shrimps in both experiments (* and ° are outliers)

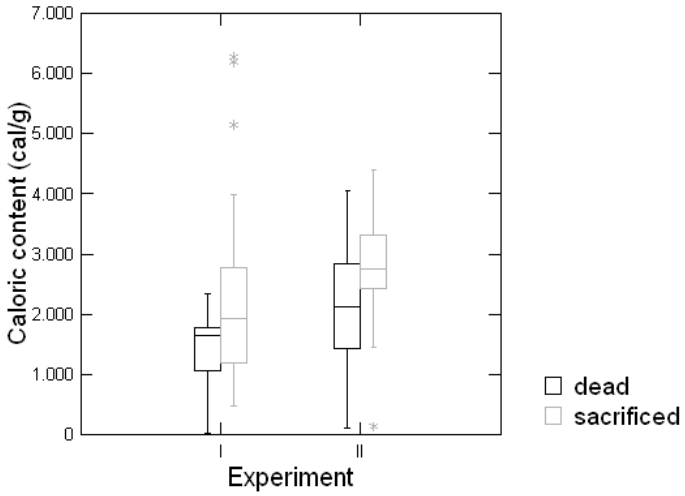
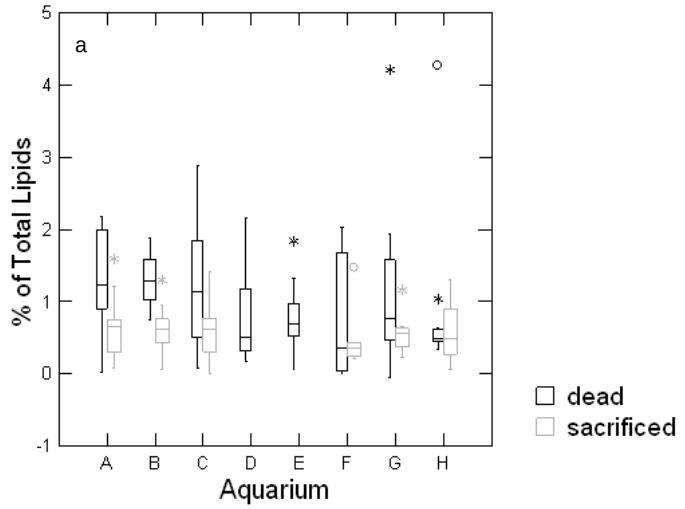


Figure 22: Mean caloric content (cal.g^{-1}) of the naturally dead and sacrificed shrimps in both experiments (* are outliers)

In both experiments, results of total lipids (fig. 23a) and total proteins (fig. 23b) of both sacrificed and naturally dead shrimps were not significantly different between aquaria ($p>0.05$). Regarding the caloric content, differences were only detected in the sacrificed shrimps from aquaria A and C (experiment I) ($p<0.05$) (fig. 24).



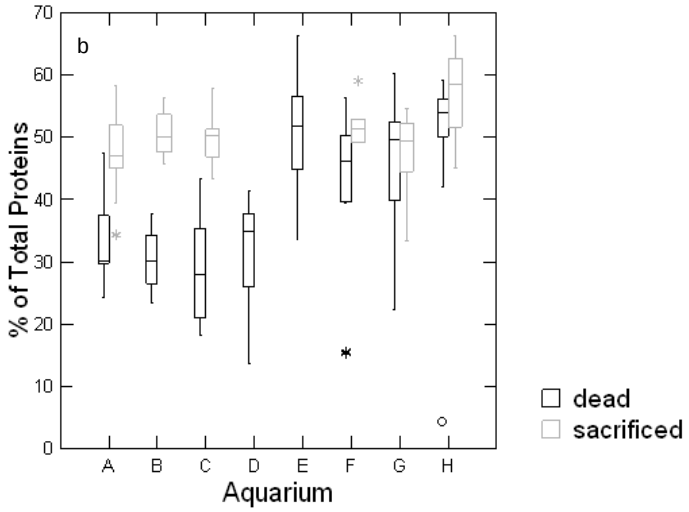


Figure 23: Mean percentage of total lipids (a) and total proteins (b) of the naturally dead and sacrificed shrimps per aquarium (* and ° are outliers)

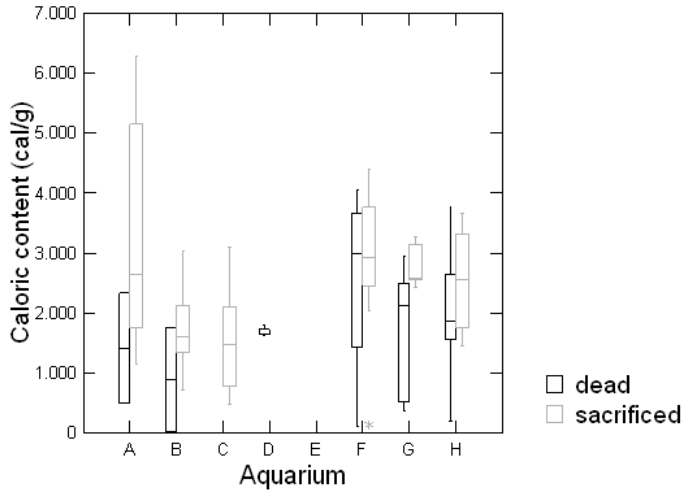


Figure 24: Mean caloric content (cal.g^{-1}) of the naturally dead and sacrificed shrimps per aquarium (* are outliers)

Differences between aquaria were found in the total proteins of the naturally dead individuals (ANOVA: F=6.06, p<0.0001) (fig. 23b, table 12). Despite not significant (p>0.05), the mean caloric content was higher in shrimps from experiment II than in those from experiment I, for both naturally dead and sacrificed shrimps (table 13).

Table 12: Tukey's pairwise comparisons results on the total proteins

Aquarium	Aquarium	p
E	B	0.0397
	C	0.0031
	D	0.0003
H	C	0.0146
	D	0.0049
G	D	0.0427

Table 13: Mean (±se) caloric content (cal.g^{-1}) of the naturally dead and sacrificed shrimps in both experiments

	Exp. I	Exp. II
Naturally dead	1419 ±269.7	2041 ±255.6
Sacrificed	2311 ±309.0	2734 ±235.5

Along the starvation time, data for the percentage of total lipids presented statistical differences between naturally dead and sacrificed shrimps in the experiment I (ANOVA: F=10.03, p=0.0024), the first with higher values (fig. 25). The percentage of total lipids of the sacrificed and naturally dead shrimps varied along the time of starvation though with no significant difference (p>0.05): mean percentage of total lipids rose till the fourth week and then decreased abruptly, especially in the naturally dead shrimps. The highest value was found in the third week in the experiment I (2.900%) and in the first week in the experiment II (4.270%), both for the naturally dead shrimps.

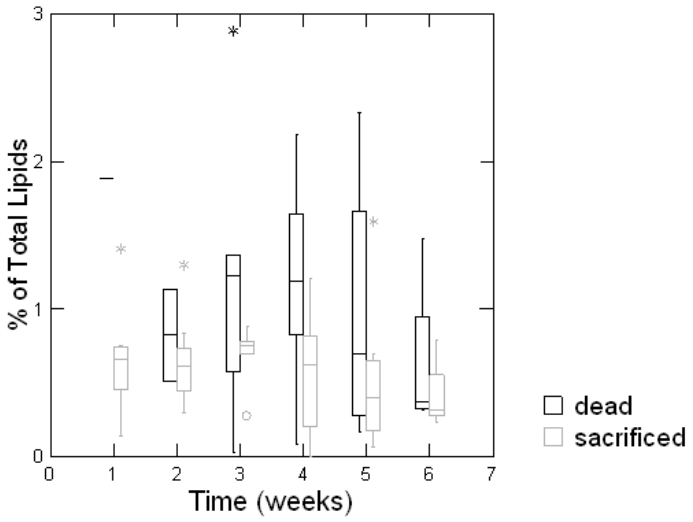


Figure 25: Percentage of total lipids of the shrimps along the weeks of starvation in the experiment I (* and ° are outliers)

The percentage of total proteins varied along the time of starvation, being higher for the shrimps that were sacrificed than for those that naturally died (ANOVA: $F=109.93$, $p<0.0001$).

Regarding the experiment I, significant differences were found between weeks for both the sacrificed shrimps (ANOVA: $F=12.81$, $p<0.0001$) and the naturally dead (ANOVA: $F=4.09$, $p=0.0084$). The main differences were obtained between all weeks and the final 6th week ($p<0.05$) (fig. 26). There were also differences between the first week and the second, fourth and fifth weeks (table 14). For the naturally dead individuals the differences were observed between the second week and the third and fifth weeks (table 15). Regarding the experiment II, no statistical differences were found between weeks, though sacrificed shrimps had always higher mean percentage of total proteins.

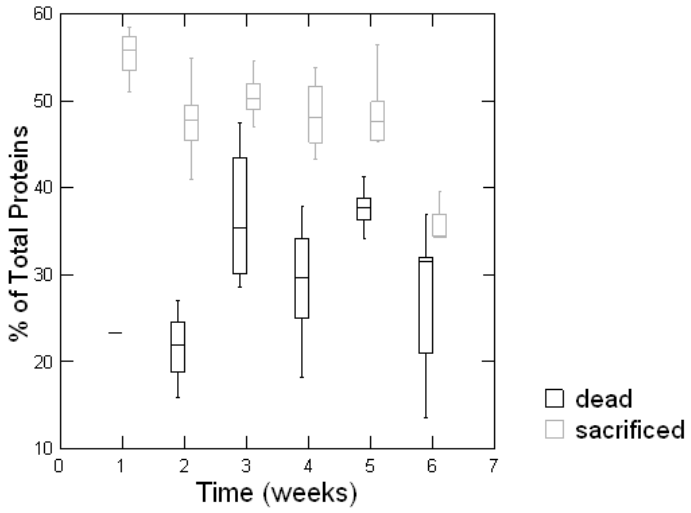


Figure 26: Percentage of total proteins of the shrimps along the weeks of starvation in the experiment I

Table 14: Tukey's pairwise comparisons results on the total proteins of the sacrificed shrimps in the experiment I

week	week	p
1	2	0.0028
	4	0.0077
	5	0.0089
	6	0.0000
2	6	0.0004
3	6	0.0000
4	6	0.0002
5	6	0.0002

Table 15: Tukey's pairwise comparisons results on the total proteins of the naturally dead shrimps in the experiment I

week	week	p
2	3	0.0456
	5	0.0221

Despite a decrease across time in the mean caloric content of the sacrificed shrimps in the experiment I, the difference was not significant ($p>0.05$) (fig. 27). Also the energy in the first week was higher in the experiment I than in the experiment II (table 16). Yet data was insufficient for a statistical sound analysis.

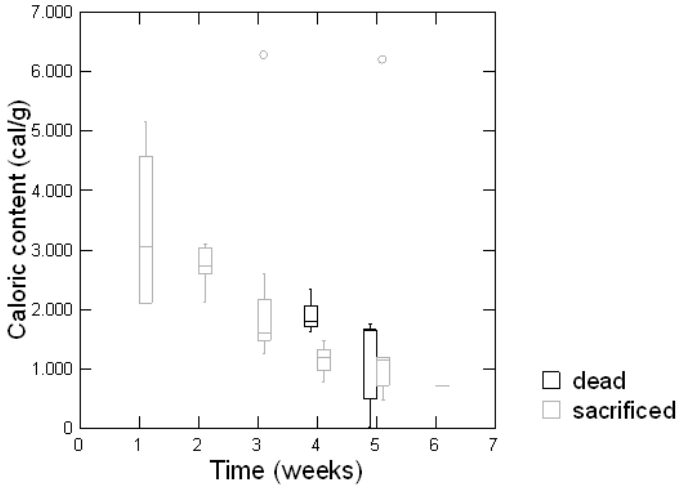


Figure 27: Caloric content (cal.g^{-1}) of the shrimps along the weeks of starvation in the experiment I (° are outliers)

Table 16: Mean (±se) caloric content (cal.g^{-1}) per week of the sacrificed shrimps in both experiments

week	Exp. I	Exp. II
1	3343 ±747.6	2842 ±329.3
2	2722 ±143.0	2626 ±352.7
3	2354 ±673.2	
4	1146 ±204.6	
5	1949 ±1069.8	
6	719	

Discussion

This study shows that shrimps from different seasons are differently prepared to cope with starvation being in better condition during summer than during autumn. It further brings new enlightenments on how *Crangon crangon* endures long periods of starvation allowing us to support that this species uses primarily proteins, followed probably by carbohydrates and lipids (though these might not be used at all), like other species of marine decapods, namely *Lithodes santolla* (Comoglio et al. 2008). The role of carbohydrates as energetic reserve, however, is still unclear.

Summer versus autumn shrimps

Nutritional deprivation is a natural and frequent condition throughout the life cycle of many aquatic organisms, due to the environmental lack of food or to the animal's physiological condition (Mehner & Wieser 1994, Comoglio et al. 2008). Periods of food shortage are very common especially at temperate and cold areas. Usually, during the warmer spring and summer, productivity is higher and hence preys are more available than in the colder and less productive autumn and winter. The nutritional level of crustaceans is also influenced by their moulting and sexual maturity stages, and by their feeding state and activity level. Survival to these periods is conditioned by the animals' capacity to reduce their nutritional needs and/or by the availability of body reserves to pay for the vital metabolic costs of maintenance (Comoglio et al. 2005, Vinagre et al. 2007).

In this study, shrimps collected in two different seasons showed different capacity to endure prolonged food deprivation: summer shrimps were proved to be more resistant to starvation than animals from autumn, since they survived almost 2.5 times longer than autumn shrimps which only lasted about 20 days. Autumn shrimps were bigger than those from summer and hence the longer survival of summer shrimps cannot be attributed to differences in size. In contrast, summer shrimps had a better body condition, evaluated by the Fulton's condition factor, at the beginning of the trials, and hence a longer survival to starvation could then be expected. Moreover, the final

condition of summer shrimps that naturally died was approximated to that of autumn shrimps at the beginning of the experiment. The better condition of summer shrimps was confirmed by their higher caloric content at the beginning of the study, suggesting that they had higher energetic reserves. In fact, the percentage of total proteins was slightly higher in summer shrimps, while their lipid content was almost double of that of autumn shrimps. This suggests that part of the initial energetic reserves of summer shrimps might correspond to proteins and to a lesser extent, if ever, also lipids, though other compounds, namely carbohydrates, can also be important. Moreover, the oxygen consumption rate was higher in the autumn shrimps suggesting that they started earlier to metabolize lipids, probably structural ones, than summer shrimps.

Why are summer shrimps more resistant to starvation?

In fact, it was expected that summer shrimps would have more energetic reserves than autumn ones. Due to the increase of temperature in spring and summer the Minho estuary productivity must be higher in these seasons and hence shrimps' prey availability must also be higher, maybe with a time lag. Higher food abundance can then be reflected in the predators' better nutritional status. Consequently, the opportunity of building up reserves is probably higher in warmer months. Yet, with the exception of late summer and severe droughts events (Sousa et al 2007, 2008), no information exists on the abundance of benthic fauna in the area along an annual cycle to confirm this general trend observed in other European estuaries (see for example Reiss & Kroncke 2005). Despite the previewed better nutritional condition of summer shrimps, such a low condition of autumn shrimps was not expected. These shrimps would have to survive to the winter when food is even scarcer and such low capacity to cope with starvation suggests that winter mortality is high.

Another energy allocation which might explain differences between summer and autumn shrimps is reproduction. During the 2010 monthly surveys, several ovigerous females were found in July, after the collection of the experimental shrimps, and October, contemporaneous of the experimental shrimps. Therefore, the lower energetic reserves and Fulton's condition factor of autumn shrimps can then be due to a recent utilization of reserves to produce and release the eggs - all observed shrimps were females. In contrast, summer shrimps could still be immature or not belonged to the reproductive pool.

Reserve compounds and their sequence of use under prolonged starvation

Separating the study of shrimps that died naturally from those that were sacrificed along the starvation period, and hence, were still capable to endure starvation highlighted some critical points. This is the case of the body condition at death: naturally dead shrimps from both seasons had a similar final condition, suggesting a condition level under which life is no longer possible. Sacrificed shrimps presented more energetic reserves than those that naturally died, confirming that they still had reserves to survive longer. Eventually more information could also highlight an energetic critical value under which life would not be possible but the data was insufficient for this conclusion. The percentage of proteins was also higher in the sacrificed shrimps while the percentage of total lipids was larger in the naturally dead. Since production or accumulation of lipids was not possible due to starvation, this means that the quantity of lipids probably did not differ between sacrificed and naturally dead shrimps, though due to the consume of other compounds like proteins, their relative quantity differed. Therefore, this suggests that proteins are used as energetic storage compounds which are mobilized during fasting, maybe along carbohydrates, and lipids are more structural. Moreover, sacrificed shrimps had a very consistent percentage of total lipids of about 0.600 to 0.570% for summer and autumn, respectively, indicating that in fact lipids might be structural and not used much during fasting. In most crustaceans, the main reserve compound is protein (New 1976, Comoglio et al. 2005, Comoglio et al. 2008). However, previous studies suggest the use of glycogen by the brown shrimp as a first reserve compound and protein as a last (Cuzon & Ceccaldi 1973) which was not confirmed in this work. Carbohydrates like glycogen might be used, but proteins were confirmed to be a primary reserve compound.

A remark must be made about the Fulton's condition factor: despite being broadly used in fish studies (Stevenson & Woods 2006), it is based on the wet weight, a parameter which can easily be biased. From the moment the samples are defrosted till being weighed the time can differ greatly and depending on the weather conditions shrimps can dry faster. Also the first shrimps to be weighed might still have extra water. Therefore, this index might not be the best factor to apply to small crustaceans. Moreover, despite the loss of body weight (Steffens 1989) as a result of the consumption of accumulated endogenous energy reserves to pay for essential metabolic processes, this loss of organic matter can be compensated with the uptake

of water; this way no loss of (wet) weight might be detected (Dall 1974, Wilcox & Jeffries 1976). Even so, summer sacrificed shrimps had a higher percentage of water than those from autumn. This then suggests that they consumed more reserves and replaced those compounds with water, so that the body volume and internal turgidity were maintained.

The metabolism of crustaceans is characterized by high intra and inter-specific variability which makes it difficult to determine which reserves are used and in what order (Oliveira et al. 2003). During nutritional stress, reserves and their order of utilization varies according to the species, recent feeding history, diet composition and length of fast (Clifford & Brick 1983, Vinagre & Da Silva 2002, Vinagre et al. 2007). Some authors refer that the crustaceans' metabolism is primarily based in glycogen and fatty acids; in contrast, decreased levels of protein have been noted during fasting in several marine decapods (Welsh 1975, Barclay et al. 1983, Dall & Smith 1986, Wen et al. 2006, Comoglio et al. 2008, Zhang et al. 2009).
In the present research, some evidence of a primary use of proteins followed by carbohydrates and/or lipids, these if ever, as reserve compounds in *Crangon crangon* under prolonged starvation was obtained, although further investigations are still required. The amount of proteins stored in the hepatopancreas is probably very small, and lasts only for a short time; therefore, under prolonged fasting, the shrimps have to use other compounds, namely carbohydrates and eventually lipids. In this work, the evidences were obtained directly (following the percentage of total proteins and lipids over starvation time) and indirectly (analyzing temporal trends in the oxygen consumption rate).

The percentage of total proteins and total lipids was followed by the analysis of sacrificed and naturally dead shrimps from summer and autumn subjected to a prolonged period of starvation. Only carbohydrates and the mineral component were not analyzed. The percentage information, however, is not straightforward: for example, the fact that the mean percentage of lipids increased up to the fourth week does not mean that lipids were being produced but, on the contrary, that probably other compounds were being consumed; the net result would then be an increase in the lipids portion at the expense of a decrease of the other compounds' portion. In fact, proteins (percentage) decreased up to the fourth week, though not entirely consistent. This was probably the reserve compound in use to pay for the maintenance costs of

the shrimps. However, further research is required to clarify if carbohydrates like glycogen, as suggested by Cuzon & Ceccaldi (1973), were consumed as well.

The oxygen consumption rate can further give an insight of the type of reserve compound is being used. It is assumed that catabolizing a 1g of proteins requires 0.94L of oxygen and 1g of lipids requires 2.04L of oxygen (Mayzaud & Conover 1988, Comoglio et al. 2005). So it was expected that small amounts of oxygen would be consumed in the first weeks due to a primary consume of proteins, with an increase to the next ones, referring to the consume of lipids, which was confirmed by the data obtained in summer – the autumn shrimps did not last enough to show it - supporting that shrimps use proteins as a first resource of energy under stress conditions. Comoglio et al. (2005), working with the false southern king crab *Paralomis granulosa*, found the same tendency. In the fifth week of the experiment I the oxygen consumption rate was below 0mg $O_2.g^{-1}.h^{-1}$. This means that oxygen was being produced and not consumed. The only production possible was by microorganisms that could be present in the substratum or the probe was uncalibrated. In the last week of starvation, the oxygen consumption rate decreased again probably because the energetic reserves depleted and the animals started to mobilize the structural proteins. The present work, however, contradicts the decrease in the oxygen consumption under starvation period observed by other authors in *Carcinus maenas* (Wallace 1973) and *C. crangon* (Regnault 1981).

The atomic O:N ratio is commonly used as an index of substrates' use in metabolism (Mayzaud & Conover 1988, Carvalho & Phan 1997, Comoglio et al. 2005). This index relates the consumption of oxygen with the excretion of ammonia. Greater values of O:N correspond to an increase in lipid and carbohydrates catabolism. However, in this work it was not possible to calculate this ratio because ammonia was only quantified in the aquaria and not in the individual oxygen measurements. This could then be an improvement in a future research.

Finally, the fourth week of starvation seems to be a critical point for most of the studied parameters, which suggests that after this period the animal has no reserves left and starts to metabolize structural compounds. In fact, consumption of structure is not restricted to the hepatopancreas tissues (Lawrence 1976) and this may explain the higher lipid percentages after some weeks of starvation, as Comoglio et al. (2005) also suggested for *P. granulosa*. Besides the steep decrease in condition and the trends on

the lipids and proteins' content up to the fourth week, the oxygen consumption rate increased from the first week to the second, stabilizing in the next weeks and decreasing after the fourth week, supporting the fact that in a first phase shrimps used proteins, switching to other compounds, probably carbohydrates, until about the fourth week when they start to consume structural lipids and proteins as last resources.

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Appendix

Table 17: Summary of the results in both experiments

Variables	Experiment I	Experiment II	
Inicial K	Lower for sacrificed shrimps	Similar between sacrificed and naturally dead shrimps	Higher in exp I
Final K	Lower for naturally dead shrimps	Similar between sacrificed and naturally dead shrimps	Higher in exp I
DW	Lower for sacrificed shrimps	Similar between sacrificed and naturally dead shrimps	Higher in exp II
Energy	Lower for the naturally dead shrimps	Lower for the naturally dead shrimps	Higher in exp II
% of Total Lipids	Lower for sacrificed shrimps	Lower for sacrificed shrimps	Higher in exp I
% of Total Protein	Lower for naturally dead shrimps	Lower for naturally dead shrimps	Higher in exp II
% of Water	Lower for naturally dead shrimps	Similar between sacrificed and naturally dead shrimps	Higher in exp I
Oxygen consumption rate			Higher in exp II