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Internship at Riasearch - Effect of fasting on energy and protein losses of ongrowing *Penaeus vannamei*.

Ana Margarida Monteiro

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Dissertação de Mestrado apresentada à
Faculdade de Ciências da Universidade do Porto
em Recursos Biológicos Aquáticos

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Mestrado em Recursos Biológicos Aquáticos

Departamento de Biologia

2019

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

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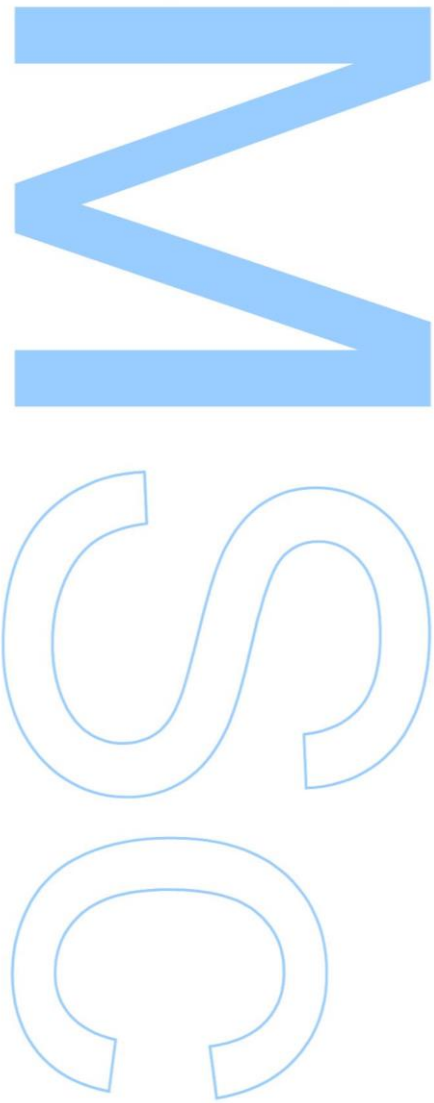

Acknowledgments

Throughout this year, I have received a great deal of support and encouragement.

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I owe a special thanks to Renata Serradeiro. Without her, I would not have had the opportunity to know what it is like to work at a research company. I am proud to say I did my internship at Riasearch, Lda.

This internship allowed me to make friends and, if life has taught me one thing, it is that there is nothing more important than working in a good work environment, where people help one another. Adriana, Bruna, and Francisco: without them, this whole experience would not have been so fulfilling.

To my friends Ana, Bárbara, Bruna, who is also my cousin, Carolina, Débora, Joana and Mafalda, who were always by my side, I hope we can grow old, grey and wrinkly together because what we have is worth keeping.

To Carla Azevedo, who was always there to listen to my worries and to give me encouragement when I needed it the most. I am grateful for her presence in my life.

To my family, especially my parents and grandfather: thank you for the love and affection, and for always believing in me. I am lucky to have you. I will always be your little girl.

Abstract

Within the master's degree in Biologic Aquatic Resources, there was the possibility to carry out an internship at Riasearch, Lda., located in Ribeira de Pardelhas, Murtosa. The main focus of this aquaculture research company is contract research, scientific and technical consulting services as well as recirculation aquaculture system (RAS) technology counseling. Riasearch's team is specialized in marine fish and crustacean health, nutrition, growth performance, and production systems research trials. The trials are done on different species, such as *Sparus aurata* and *Dicentrarchus labrax* but the company main focus is *Penaeus vannamei*. Recently, a greenhouse was added to Riasearch facilities in order to perform trials with halophytes, as *Salicornia ramosissima*.

The internship started on the October 15^{th} of 2018 and ended on the July 1^{st} of 2019. During this time, I was involved in the daily routines of the company, such as feeding, cleaning of the tanks, water-parameters measuring, maintenance of recirculating aquaculture system (RAS), I also accompanied the experimental trials done with whiteleg shrimp and gilthead sea bream as well as participated in some of the samplings done on these trials.

At Riasearch, I also had the opportunity to conduct a research trial with *P. vannamei*. In this trial, shrimp were put into fasting for a known period of time, with the aim of determining this species effects of fasting on energy and protein losses. The data obtained on the trial is going to be posteriorly analyzed by Riasearch in order to enrich Lupatsch et al. (2008) model on protein and energy requirements in *P. vannamei*.

This trial was divided into two parts. In the first part of the trial, twenty-four shrimps of four different weight classes (9.62g; 13.78g; 17.45g; 21.62g) were distributed in twelve tanks and starved for nineteen days. In the second part of the trial, twelve shrimps of two different classes (15.10g; 17.45g) were accommodated in twelve tanks and starved for eighteen day. The weight class of 17.45g was done on both parts of the trial because there was accentuated mortality in the first part of the trial due to shrimps jumping out of the tanks. For both trials, the initial and final whole-body composition were analyzed to determine the nutrient losses during starvation.

This internship allowed me to apply the knowledge acquired under this master's degree, to better understand the functioning and the management of an aquaculture research company and to perfect practices of fish and shrimp handling and management.

Keywords: *Penaeus vannamei*, whiteleg shrimp, fasting, starvation, basal metabolism, activity costs

Resumo

No âmbito do mestrado em Recursos Biológicos Aquáticos surgiu a oportunidade de fazer um estágio na empresa Riasearch, Lda., localizada na Ribeira de Pardelhas, Murtosa. O principal foco desta empresa de pesquisa na área da aquacultura é a investigação por contrato, consultoria científica e técnica em aquacultura e em tecnologia de sistemas de recirculação de água.

A equipa constituinte desta empresa é especializada em ensaios de peixe e camarão na área da saúde, nutrição, performance e sistemas de produção. Os ensaios são feitos em diferentes espécies, tais como em *Sparus aurata* e *Dicentrarchus labrax,* mas o foco da empresa está direcionado para o camarão-de-patas-brancas, *Penaeus vannamei.* Recentemente, uma estufa foi instalada para realizar ensaios com plantas halófitas, bem como em *Salicornia ramosissima.*

O estágio teve início a 15 de outubro de 2018 e terminou a 1 de julho de 2019 e durante esse tempo estive envolvida nas rotinas diárias da empresa, tal como alimentação, limpeza dos tanques, medição dos parâmetros da água, manutenção do sistema de recirculação de água, acompanhei os ensaios feitos com dourada e camarão-de-patas-brancas e participei nas amostragens relativas a estes ensaios.

Na Riasearch tive também a oportunidade de realizar um ensaio com *P. vannamei*. Neste ensaio os camarões jejuaram por um período de tempo conhecido, com o intuito de determinar os efeitos do jejum nas perdas de energia e proteína da espécie. Os dados obtidos neste ensaio serão posteriormente analisados pela Riasearch para enriquecer o modelo das necessidades proteicas e energéticas de *P. vannamei*, criado por Lupatsch et al. (2008).

O ensaio em questão foi dividido em duas partes. Na primeira parte do ensaio, vinte e quatro camarões de quatro classes de peso diferentes (9.62g; 13.78g; 17.45g; 21.62g) foram postos em jejum por dezanove dias e distribuídos por doze tanques. Na segunda parte do ensaio, doze camarões de duas classes de peso diferentes (15.10g; 17.45g) foram postos em jejum por dezoito dias e acomodados em doze tanques. A classe de peso de 17.45g foi utilizada em ambos as partes do ensaio porque o valor da mortalidade foi acentuado devido aos camarões saltarem para fora dos tanques. A composição do camarão inteiro foi analisada, no início e no final de cada ensaio, com vista à determinação das perdas em nutrientes.

Este estágio permitiu aplicar o conhecimento adquirido neste mestrado, perceber o funcionamento e gestão de uma empresa de investigação em aquacultura e aperfeiçoar práticas de manuseamento de peixe e camarão.

Palavras-chave: *Penaeus vannamei*, camarão-de-patas-brancas, jejum, metabolismo basal

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Abbreviations

BW: Bodyweight

CO_2: Carbon Dioxide

COG: Costs of Growth

DE: Digestible Energy

DO: Dissolved Oxygen

e^-: electron

EEZ: Exclusive Economic Zone

GE: Gross Energy

GIH: Gonad-Inhibiting Hormone

GnRH: Gonadotropin-Releasing Hormone

GV: Germinal Vesicle

H^+: Hydrogen Ion

HCl: Hydrochloric Acid

HOBr: Hypobromous Acid

IMTA: Integrated Multi-Trophic Aquaculture

MSY: Maximum Sustainable Yield

N: Nitrogen

NH_3: Ammonia

NO_2^-: Nitrite

NO_3^-: Nitrate

O_3: Ozone

BrO^-: Hypobromite

ORP: Oxidation-Reduction Probe

PL: Postlarvae

RAS: Recirculating Aquaculture System

SCADA: Supervisory Control and Data Acquisition

SDGs: Sustainable Development Goals

UV: Ultraviolet

VIH: Vitellogenesis-Inhibiting Hormone

ΔU: Internal Energy

1. Introduction

1.1. Aquaculture: an overview

Historically, aquatic animals such as fish, crustaceans, and mollusks, have been a major source of nutrients to humans. These are important sources of nutrients such as calcium, iron, iodine and vitamins A, B and D. For this reason, they can positively impact the health of its consumers (FAO, 2015). However, the percentage of fish stocks overexploited (stocks less abundant than their maximum sustainable yield-MSY) increased over the years due to intensive capture, compromising the prosperity and sustainability of marine fish resources and fish availability for human consumption. The exponential growth of the worlds' population and the increase of fish consumption per capita (Figure 1) led to a steady increase in fish captures over the years (FAO, 2018).

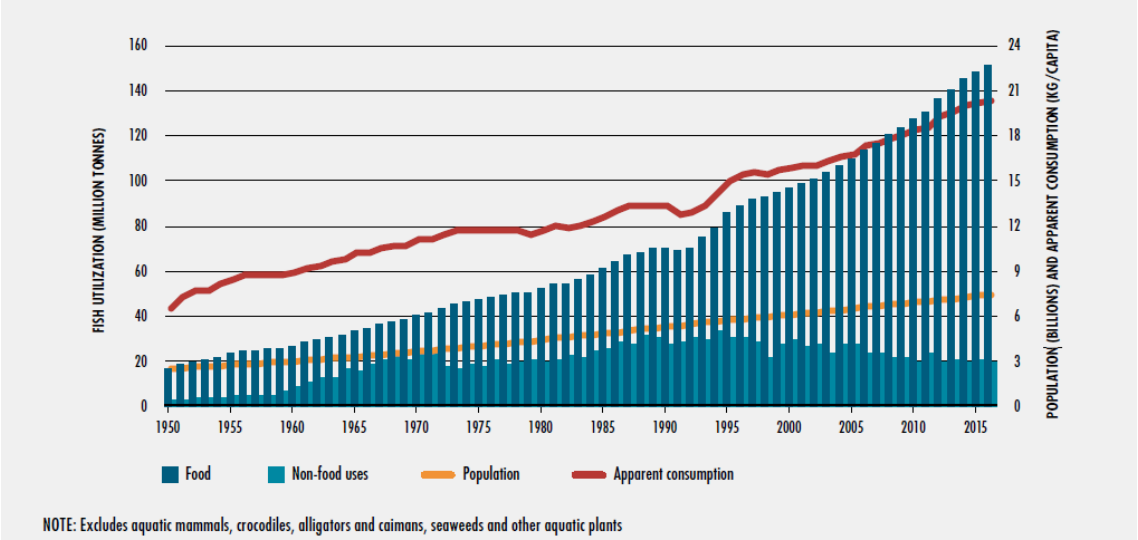

Figure 1: World fish utilization and apparent consumption (FAO, 2018).

Over time, fishing methods evolved to better serve humans and capturing fish became easier and more efficient, but also more environmentally threatening. Bottom trawling is an example of a destructive fishing method. It involves using heavy nets that are dragged across the ocean floor. When the nets are dragged in the seafloor everything in the path made by the boat that carries the net is destructed or disturbed.

The United Nations Sustainable Development Goals (SDGs) aim to regulate harvesting, cease overfishing and restore stocks to levels that can achieve the MSY in the briefest time possible (FAO, 2018).
Aquaculture is the controlled production of aquatic organisms in freshwater, brackish water, and saltwater, in both coastal and inland areas (FAO, 2015) and may contribute to attaining these goals. Indeed, in 2016, the aquaculture contribution for human

consumption surpassed the contribution of capture fisheries for human consumption (Figure 2; FAO, 2018).

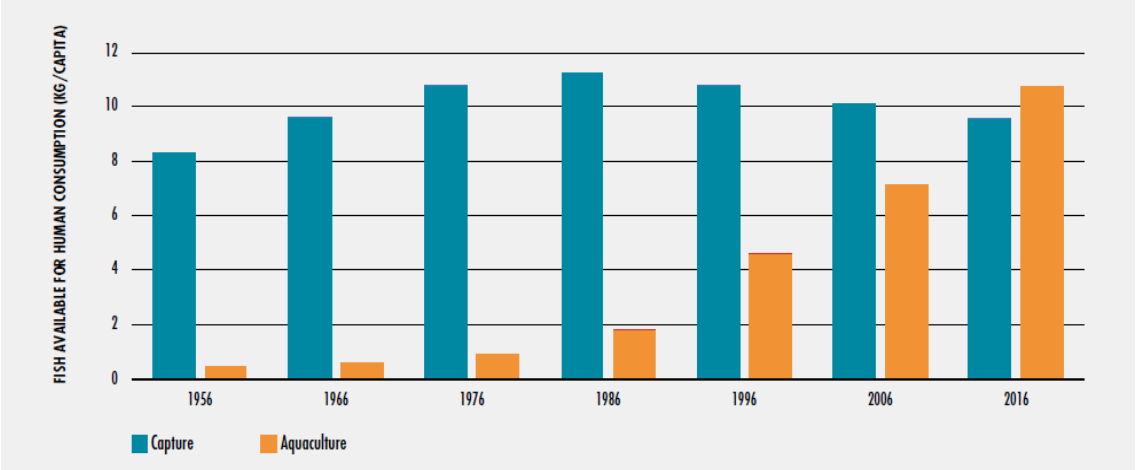

Figure 2: Relative contribution of aquaculture and capture fisheries to fish available for human consumption (FAO, 2018).

Aquaculture production still has a highly uneven distribution and unbalanced development status between countries and regions within countries. For the last two decades, the Asian continent has been the major aquaculture producer, being responsible for 89.4% of the world's total production in 2016. Despite the extensive expansion of this industry, the European aquaculture production shares have slightly decreased and represented 3.7% of the global production in 2016 (FAO, 2018). European countries have to compete with countries where aquaculture production is cheaper because labor costs are lower and production technologies are less advanced (DGRM, 2014). In Europe, Norway is the biggest producer, having produced 1.7% of the global production and 45.9% of the European production, in 2016 (FAO, 2018).

In Europe, the most produced fish species are the Atlantic salmon (*Salmo salar* Linnaeus 1758), European seabass (*Dicentrarchus labrax* Linnaeus, 1758), and gilthead seabream (*Sparus aurata* Linnaeus, 1758). Nowadays, due to their popularity, these species have saturated the market and their profitability has decreased. To overcome this problem, other species such as meagre (*Argyrosomus hololepidotus* Lacepède, 1801), greater amberjack (*Seriola dumerili* Risso, 1810), pikeperch (*Sander lucioperca* Linnaeus,1758), Atlantic halibut (*Hippoglossus hippoglossus* Linnaeus, 1758), wreckfish (*Polyprion americanus* Bloch & Schneider, 1801) are being studied in order to ensure a sustainable aquaculture expansion and diversify the offer to consumers (Mylonas, et al., 2017).

Despite the small representation of Europe in global aquaculture production, this continent is characterized by its qualified labor, educational and investigation institutions that optimize the existence of high technology standards (DGRM, 2014).

1.2. Aquaculture in Portugal

Portugal is a country with a deep connection to the sea and fisheries have an important socio-cultural impact, although not contributing much to the country's economy. Portugal is the first in the European ranking in terms of fish consumption per capita, reaching 61.5kg per year (Phys, 2018). Portugal's Exclusive Economic Zone (EEZ) is the 20^{th} largest EEZ in the world, considered biologically rich, giving fisheries and aquaculture a great potential to develop. In Portugal, aquaculture represents a complement to the traditional methods of obtaining fish, but it is still not as relevant as capture fisheries. Although, fisheries are facing growing difficulties, due to limitations established by the government on captures (DGRM, 2014).

Portuguese marine and brackish aquaculture production started in inland coastal areas, estuaries, and coastal lagoons, on extensive systems in deactivated salt industry infrastructures, to reduce investment costs. In the seventies *Mugilidae* spp., with a low commercial value, was the most cultivated group, with 80% of the national production; in the eighties, inland aquaculture companies increased, being rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) the most produced species, and bivalves that were produced in marine and brackish waters; in the nineties, marine aquaculture improved and some of the extensive systems evolved to semi-intensive and intensive systems, with the introduction of species such as European seabass, gilthead seabream and, most recently, turbot (*Scophthalmus maximus* Linnaeus, 1758) and Senegalese sole (*Solea senegalensis* Kaup, 1858) (DGRM, 2014). This improvement occurred due to funds received when Portugal joined the European Union, in 1986. Nevertheless, this sector in Portugal is majorly composed of small, familiar businesses (DGRM, 2014; INE, 2019).

In 2017, 12 549 tons of fish were produced by aquaculture in Portugal, resulting in an income of 83,2 million euros. Comparing to 2016, there was growth not only in quantity (+11,5%) but in economic profit too (+10,6%) (INE, 2019). Currently, the most significant fish species produced in semi-intensive and intensive systems are turbot (2745 tones),

gilthead seabream (1038 tones), European seabass (700 tones), rainbow trout (655 tones) and Senegalese sole (151 tones) (INE, 2019).

2. *Penaeus vannamei*

2.1. Species characteristics

Penaeus vannamei (Boone, 1931), also known as whiteleg shrimp, is a valuable species due to its characteristic taste quality (Fu et al., 2013).

This species is native from the Eastern Pacific coast from Sonora, Mexico in the North, through Central and South America as far South as Tumbes in Peru, in tropical marine habitats where water temperature is usually above 20°C, but is produced at least in twenty-seven countries (FAO, 2019; Fofonoff et al., 2018).

This crustacean species has great plasticity which allows it to survive under diverse ranges of salinity (0.5-45ppt) and temperature (subtropical-tropical). Although it can survive under extreme salinity values, the species is comfortable between 7 and 34ppt but thrives best when salinity is around 10 to 15ppt (FAO, 2019; Fofonoff et al., 2018). Another advantageous characteristic of this species is the fact that it can be produced at high densities, greater than the ones achievable with *Penaeus monodon* (Fabricius, 1798) in Asia (Briggs et al., 2004).

In terms of coloration, this species assumes a white or bluish color (Figure 3). The cells responsible for this change are the chromatophores that, when contracted, confer a white color but when they expand a blue color appears. Despite this, color is not a reliable characteristic for identification since it can vary inside the species with growth, environment, and disease.

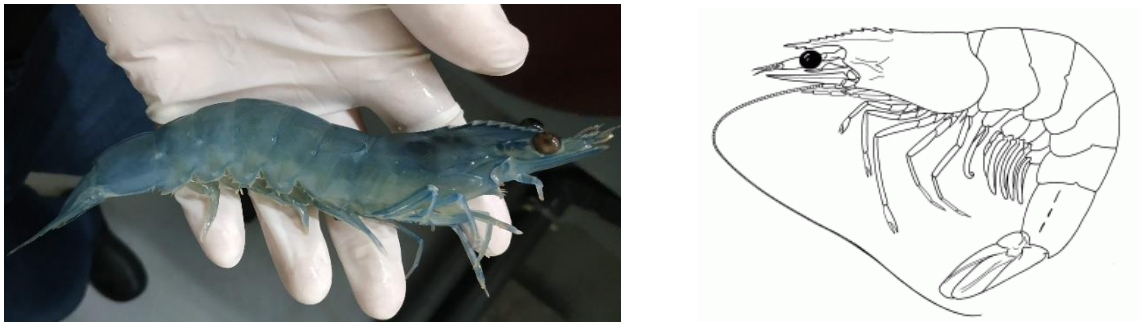

Figure 3: Ongrowing *Penaeus vannamei* specimen, at the left; At the right, a scheme of *Penaeus vannamei* (FAO).

2.2. *Penaeus vannamei* reproduction

Adult males and females live and reproduce in the ocean, when in the wild. Males reach maturity from 20g and females from 28g onwards (at the age of 6/7 months). After mating, females weighing 30-45g spawn 100 000 to 250 000 eggs, with 0.22mm in diameter. Approximately 16 hours after spawning and fertilization, eggs will hatch into non-feeding nauplii (reserves on the egg yolk sack), the first stage larvae, and are positively phototactic. Nauplii develop into protozoa, mysis and early postlarvae (other larval stages) which already eat phytoplankton and zooplankton. Tidal currents carry these larvae to the shore as they develop into postlarvae (PL) and change their eating habits from planktonic to benthonic, starting to feed on benthic detritus, bivalves, worms, and crustaceans. In the coastal area, they become juveniles, sub-adults and then adults. When adults, they return to open sea to complete maturation and reproduce, completing its life cycle (FAO, 2019; Fofonoff et al., 2018; Dugassa et al., 2018). Commercial maturation of female penaeids relies almost exclusively on the technique of unilateral eyestalk ablation (Figure 4). This happens because the vitellogenesis-inhibiting hormone (VIH), also known as gonad-inhibiting hormone (GIH), is synthesized predominantly in the X-organ/sinus gland complex, located in eyestalks. When the eyestalk is removed, VIH synthesis is diminished and GnRH (Gonadotropin-releasing hormone) activates the calcium signaling pathways, leading to the increase of intracellular calcium ion, stimulating folliculogenesis, estradiol and progesterone production in the ovary. Progesterone then induces the maturation of oocytes in stage 1 into stage 4 oocytes (Uawisetwathana, U. et al., 2011; Kannan et al., 2015).

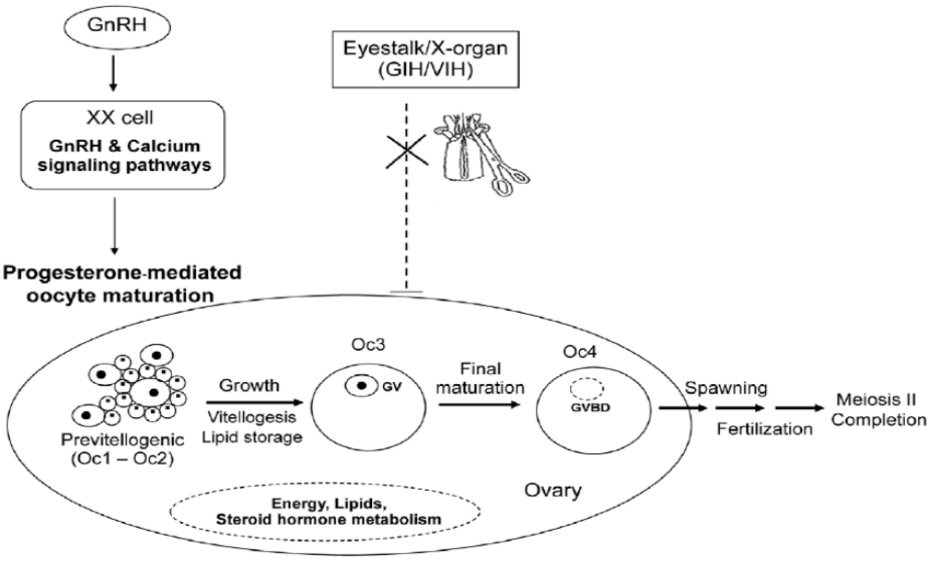

Figure 4: Eyestalk ablation and induction on the maturation of female gonads. GnRH: gonadotropin-releasing hormone receptor; GIH/VIH: gonad/vitellogenesis inhibiting hormones; GV: germinal vesicle; GVBD: germinal vesicle breakdown; XX cell is an unknown cell in shrimp but is correspondent to the pituitary gonadotrope cell in other organisms (Uawisetwathana, U. et al., 2011).

2.3. *Penaeus vannamei* aquaculture

Shrimp is one of the main groups of species captured by bottom trawling and tropical shrimp trawl fisheries have the highest discard rate among other fishing methods, due to bycatches (Kelleher, 2005). This is one of the reasons why it is necessary to develop other alternatives that help to prevent environmental degradation, such as aquaculture.

P. vannamei is the most produced species of crustacean in aquaculture (FAO, 2018), and its production has increased over the years (Figure 5), maintaining a higher value than most aquaculture species (Figure 6).

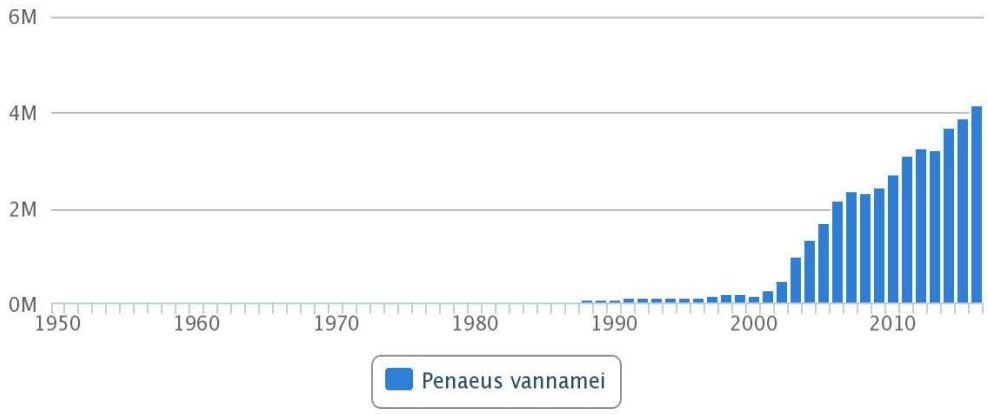

Figure 5: Aquaculture production (in tons) of *Penaeus vannamei (*FAO, 2019).

A-6 World aquaculture production of fish, crustaceans, molluscs, etc., by principal species in 2016
Production mondiale de l'aquaculture de poissons, crustacés, mollusques, etc., par espèces principales en 2016
Producción mundial de acuicultura de peces, crustáceos, moluscos, etc., por especies principales en 2016
Q = t
V = USD 1 000

Species Espèce Especie		2008	2009	2010	2011	2012	2013	2014	2015	2016
World total Total mondial Total mundial	**Q**	**52 914 703**	**55 690 829**	**58 962 053**	**61 796 344**	**66 443 023**	**70 154 486**	**73 666 433**	**76 053 701**	**80 030 862**
	V	**106 929 854**	**114 930 842**	**133 936 529**	**159 921 940**	**178 162 244**	**201 415 262**	**220 584 367**	**216 315 250**	**231 584 120**
Ctenopharyngodon idellus	Q	3 797 977	4 184 455	4 362 251	4 659 697	5 017 622	5 228 327	5 538 992	5 839 349	6 068 015
	V	5 358 788	6 082 195	6 865 968	8 607 842	10 063 505	11 339 823	12 695 245	13 223 974	13 908 964
Hypophthalmichthys molitrix	Q	3 792 346	4 100 488	4 099 666	4 130 258	4 193 252	4 598 429	4 967 866	5 124 258	5 300 736
	V	5 261 209	5 905 631	6 465 272	7 607 764	8 331 155	9 665 700	11 000 284	11 177 291	11 663 866
Cyprinus carpio	Q	2 974 495	3 145 844	3 420 657	3 496 865	3 752 683	3 968 053	4 160 957	4 329 150	4 556 622
	V	4 016 400	4 509 516	5 265 740	6 266 789	7 162 864	8 012 720	8 888 678	8 977 733	9 546 788
Ruditapes philippinarum	Q	3 110 042	3 249 381	3 604 708	3 676 537	3 774 606	3 887 250	4 014 204	4 049 229	4 228 594
	V	3 186 782	3 456 385	4 125 902	4 971 724	5 467 451	6 133 905	6 631 611	6 569 648	6 959 821
Oreochromis niloticus	Q	2 061 577	2 240 349	2 537 445	2 809 802	3 259 825	3 424 404	3 676 911	3 953 211	4 199 567
	V	2 960 858	3 655 419	4 410 049	5 343 629	6 459 283	7 131 375	7 560 797	7 636 312	7 913 664
Penaeus vannamei	Q	2 304 558	2 444 776	2 688 233	3 089 293	3 238 382	3 210 098	3 696 903	3 881 293	4 155 827
	V	9 862 544	10 472 115	12 641 134	16 440 753	17 983 959	20 380 125	23 570 542	23 111 621	24 404 810

Figure 6: Aquaculture production (Q in tons) and value (V in USDx1000) of aquatic species including *Penaeus vannamei* (FAO, 2018).

China is the major producer of *P. vannamei*, with 1 672 287 tons produced in 2017 and the main consumers are the United States, European Union and Japan (Fu et al., 2013; Figure 7).

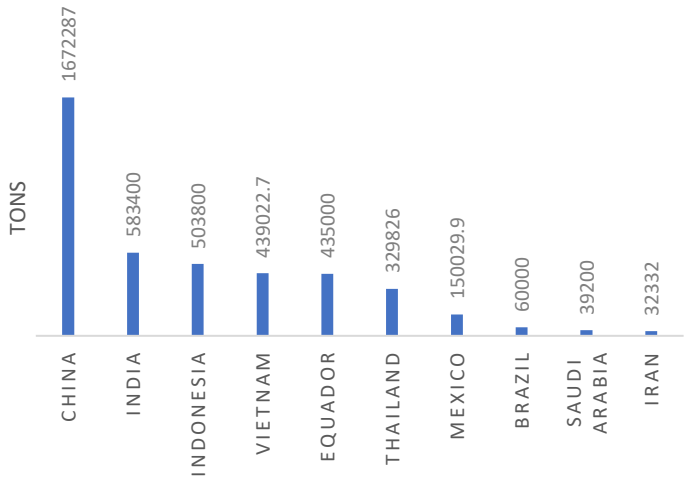

Figure 7: Main aquaculture producer countries of *Penaeus vannamei*, in 2017 (FAO FIGIS, 2019).

The interest in whiteleg shrimp has led to the necessity of improving the scientific knowledge on the species biology in order to perfect its production, optimize its costs and improve the wellbeing of the animals.

P. vannamei requires a relatively low dietary protein level (20-35%) when comparing with other species such as *Penaeus monodon* (40-42%), which contributes to the reduction of the production costs (Wyban et al., 1991). A research work made with whiteleg shrimp to test the possibility of substituting fish meal by plant protein sources lead to the conclusion that this species only needs 6% of fish meal to develop normally. Indeed, when comparing *P. vannamei* performance on diets with 6, 10 and 15% of fish meal there were no differences in weight gain and specific growth rate (Suárez, et al., 2009).

3. Bioenergetics

In aquaculture, feeds may represent 50% or more of the production costs (FAO, 2009). Because of that, it is of utmost importance to feed the animals with an adequate amount of feed in order to minimize the feed lost and, consequently, optimize production costs and guaranty maximum growth performances. The uneaten feed is an issue for the profitability of the aquaculture as it is impossible to recover this feed, representing an additional cost due to feed lost and an increase of organic matter wastes which contributes to environmental degradation (Bureau et al., 2008). If water from the tanks is released directly to the environment, these nutrients loads may cause environmental changes. To ease this problem, since the major source of wastes derives essentially from biological and dietary origins, it is important to improve and formulate specific feeds for each species (Bureau et al., 2010). The development of robust feeding guides and nutrition models to predict growth and feed requirements is of maximum importance, helping to manage feed, reducing the wastes and improving the growth performance of the animals.

Nutritional models consider all the species energetic retentions and expenditures, leading to better feed formulation and administration. These are highly applicable to aquaculture and can help to predict nutrition excretion, estimate the amount of feed necessary to a specific production, improve the performance of the produced species by improving feeding strategies and, consequently, reduce the production costs. (Tedeschi et al., 2005).

Bioenergetics is the study of the balance between dietary energy supply, expenditures, and gain, and requires a knowledge of the processes by which the energy is transformed in living organisms. For a complete definition of the energetic requirements, it is necessary to study all the compartments of the energy and the balance between catabolism, as fuel, and anabolism, as storage of energy in tissues (Cho et al., 1995). Bioenergetic models are used to stimulate growth and predict feed consumption (Figure 8).

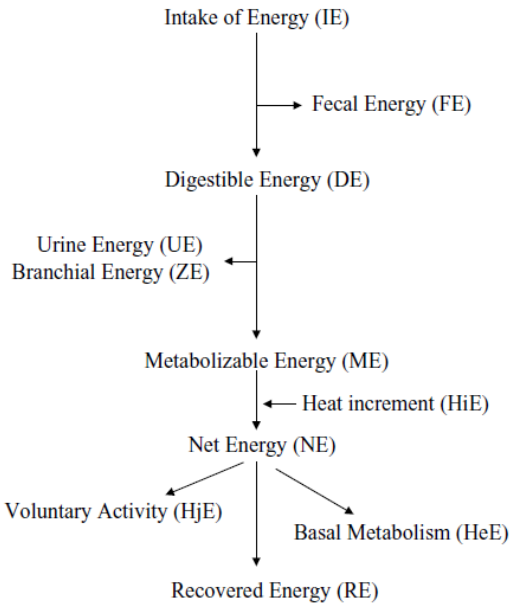

Figure 8: Energy partitioning scheme and nomenclature (NAS-NRC, 1981).

The intake of energy (IE) represents the animal's intake of gross energy (GE) (Cho et al., 1995), where GE is the total energy content of a feed, obtained from combustion in a bomb calorimeter (Hall et al., 2013).

Fecal energy is the portion of the energy that is lost through feces by resisting digestion, representing the portion of GE that is excreted through feces (Bureau, 2002). Feces are not just composed of undigested food but also mucosa cells, residues of microflora, digestive enzymes and digestive secretions (Cho et al., 1995).

Digestible energy (DE) can be described as the difference between the GE of the feed ingested (feed intake) and the fecal energy lost (Cho et al., 1995).

Urinary (UE) and branchial (ZE) energy losses are described as the energy spent on excreting ammonia, and to a lesser extent on urea, through the kidneys (UE) and through the gills (ZE) (Bureau, 2002).

Metabolizable energy (ME) is the physiological available energy value of a diet to the fish (Cho et al., 1995).

Heat increment of feeding (HiE) is the energy expenditure due to feeding to the increase of the metabolic rate as a consequence of the extra work required for the ingestion, digestion and metabolic utilization of the components of the feed (Cho et al., 1995; Bureau et al., 2002).

Net energy (NE) is the available energy for maintaining life processes and growth (Cho et al., 1995).

Basal metabolism (HeE) energy is the energy spent on basal metabolism which is known as the minimum rate of metabolic activity needed to keep the structure and function of the body tissues of an animal (Cho et al., 1995; Bureau et al., 2002).

Voluntary activity (HjE) energy is the portion of energy spent on resting and minor voluntary activity (Cho et al., 1995; Bureau et al., 2002).

Recovered energy (RE) is the difference between the GE of the body at the beginning and the GE of the body at the end of a period of time (Bureau et al., 2002).

Fasting energy losses has been considering a good estimate of HeE. For that, measuring the carcass energy loses during fasting is a common method to determine the fasting heat production (HeF) and consequently the HeE (Bureau, 2002). Under this context, a fasting trial was done during this internship to estimate the fasting energy and protein losses of ongrowing *P. vannamei* shrimp.

4. Internship at Riasearch

4.1. Company and internship introduction

The internship at the company - Riasearch, Unipessoal, Lda. was done between October 15th of 2018 and July 1st of 2019. During this time, I was involved in the daily routines of the company, maintenance of the recirculation aquaculture system (RAS) and accompanied the experimental trials done with whiteleg shrimp and gilthead seabream.

I also had the opportunity to conduct a research trial with *P. vannamei* at Riasearch. In this trial, shrimp were subjected to fasting for a known period of time, with the aim of determining the effects of fasting on energy and protein losses on this species.

Riasearch, Unipessoal, Lda. was founded in 2016 and is located in Cais da Ribeira de Pardelhas, Murtosa, Aveiro, Portugal. The core business of this company is contract research and scientific and technical consulting services for the aquaculture sector and RAS technology, developing an innovative service in the field of aquaculture. Its team is specialized in conducting research trials in the fields of crustaceans and fish health, nutrition, growth performance and production systems. Trials are performed with different species (with the main focus on *P. vannamei*) and on different experimental conditions. In order to develop research projects conducted in its facilities, Riasearch collaborates with a broad network of scientific industrial partnerships.

4.2. Facilities

This company is divided into four main spaces: the office and laboratory area, the water treatment area, the trial room, and the outdoor area.

The water treatment area is the place where water from the experimental systems is collected, treated and monitored, with the aid of machines such as pumps, that recirculate the water in the systems, heat exchangers, that maintain the water temperature stable and filters, that filter the suspended solids of the water.

Facilities hold three independent RAS and the two largest ones can be combined, leading to a system with 55 identical tanks (Figure 9).

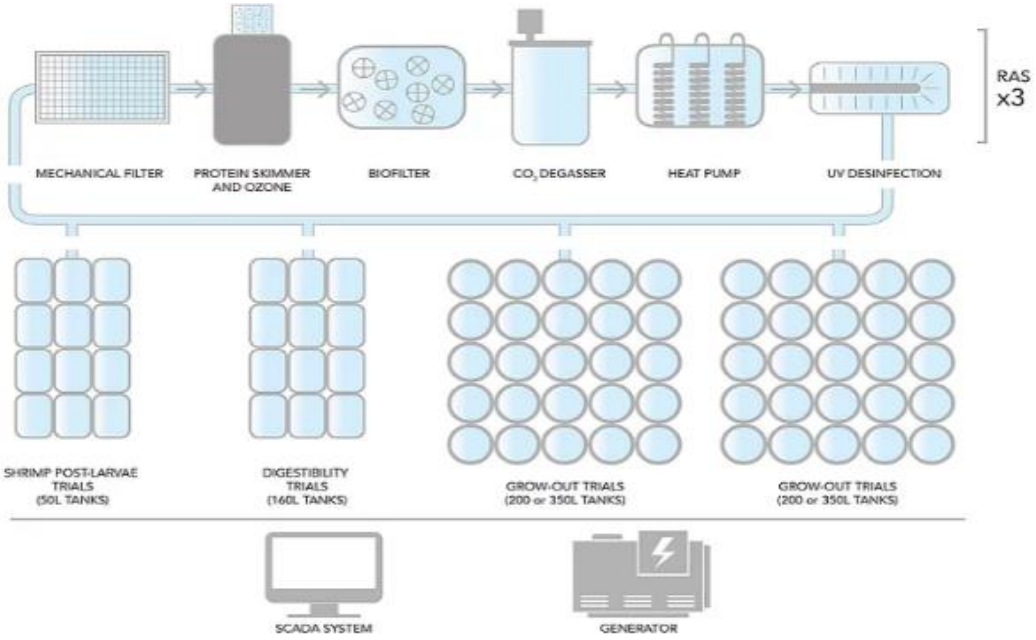

Figure 9: Riasearch RAS and experimental facilities. Source: https://www.riasearch.pt/facilities

At the trial room, there are fifty-five tanks with a capacity of 350L and twelve tanks that hold up to 160L, grouped in two recirculating water systems, to conduct experimental trials with crustaceans/fish.

Each RAS system is equipped with an individual system of treatment of water, including a mechanical filter, a biofilter, a carbon dioxide (CO_2) degasser, and a protein skimmer. The research facilities are monitored by SCADA, an automated system that controls oxygen levels, pumps and temperature and it's connected to a 24-hour alarm.

Outdoor facilities comprehend 9 culture tanks of 500L for Integrated Multi-Trophic Aquaculture (IMTA). The greenhouse is located outdoors and is used for halophyte (such as *Salicornia ramosissima,* J. Woods) and RAS trials. The RAS system used for the trials is composed of twelve tanks of 50L, used to conduct trials with shrimp postlarvae, and four tanks of 600L each, used to stock animals.

4.3. Recirculating Aquaculture System

Although aquaculture has benefits, as preventing wild populations overexploitation and, thus, avoid extinction of aquatic species, there are some concerns to its sustainability, such as the release of wastes in effluents that may result in nutrient enrichment and, possibly, cause eutrophication of the receiving environment (Bureau et al., 2010).

Decomposition of solid wastes, as feces and uneaten food, may result in oxygen depletion and ammonia toxicity. When the nitrification process occurs, ammonia (NH_3) is converted into nitrite (NO_2^-) and nitrite into nitrate (NO_3^-). Although nitrate is the most oxidized form of nitrogen, when its concentration rises in the presence of other essential nutrient factors, environmental problems such as algae blooms, which can lead to eutrophication, may occur.

Net-pens are an open system, and, for that reason, there is a high degree of interaction between the cage and the environment. This means that wastes, such as feces, metabolic products, and uneaten feed, are directly discharged in the water body. The higher the density of production, the higher the environmental impact because wastes, being organic matter, contribute to environmental enrichment (Cao et al., 2007).

Conventional net-pen and raceways aquaculture systems have barriers such as the lack of space, the competition with other industries for new sites, the limited availability of fresh water and the pollution (Badiola et al., 2012).

Earthen ponds represent other concerns. To harvest, sometimes the pond is drained, and the water is released in the effluent and even though the harvest is done without the pond being drained, the pond may need to be repaired and that requires draining. The pond water has organic wastes, potentially harmful for the receiving waterbody (Boyd et al., 2000; Boyd, 2001).

RAS are expensive to build and operate but the advantages are great: control of water quality and temperature, independence of the weather conditions, low discharged water volume and space required. Moreover, as RAS allows a high level of water quality control, the risks of bacterial and parasitic infections are reduced, and the particulate and soluble waste production are decreased, making these systems environmentally

sustainable (Michaud, 2007). The nutrients removed from the water can be used to fertilize agriculture crops or to produce biogas (Bregnballe, 2015).

RAS includes different devices to remove or convert toxic into non-toxic products the fish wastes produce, as solid wastes, ammonium, and carbon dioxide. Solid wastes (fecal matter and uneaten feed) can be divided into three categories: settleable, suspended and dissolved solids.

At Riasearch, settleable solid wastes are removed from the tanks through net filtration or through a siphon pump. These are the easiest portion of solid wastes to remove and they should be discharged as soon as possible. Suspended solids are too small to easily settle to the bottom of the water column, contrarily to what happens with settleable solids.

At Riasearch, outlet water from the experimental systems is pumped to the wastewater reservoirs and then to the screen filtration system, composed of a propylene filter housed in a specific container where water flows through to retain the suspended solids (Figure 10, left). Filter mesh size is 25µm for the postlarvae trials and 50µm for juveniles and adult trials.

After the mechanical filtration, the water passes through a protein skimmer for dissolved solids removal. Due to the particle size, dissolved solids cannot be removed using traditional mechanisms such as screen filters but resorting to a process called protein skimming (Figure 10, right). This consists of injecting pressurized air and ozone (O_3) in a closed column of water with a venturi injector (Figure 11), forming bubbles. Solid wastes adhere to the surface of the bubbles leading to the formation of foam on the top of the water column. The foam, containing the dissolved organic compounds, is then collected by a waste drain cup (Losordo et al., 1999).

Ozone is used in RAS to disinfect, remove organic carbon, turbidity, algae, and color of the water. It can effectively inactivate fish pathogens such as bacteria, viruses, fungus, and protozoans but its concentration, contact time, pathogen loads and level of organic matter of the water are factors that affect its efficiency. Higher concentrations of this compound can lead to the production of by-products (reacting with brackish or seawater, ozone may form hypobromous acid, HOBr, and hypobromite ion, BrO^-) that can cause tissue damage, mortality and affect bacterial films of the biofilter (Gonçalves et al., 2011; Summerfelt, 2002). To avoid these side effects, at Riasearch the ozone concentrations are maintained at the recommended levels and are monitored by an ORP.

An ORP, located inside a compartment of the protein skimmer, is used to monitor the concentration of oxidation agents such as ozone in the water. ORP values are measured in millivolts and represent the potential of a substance to release or accept electrons during a chemical reaction. Oxidation is the process of losing electrons while reduction is the gain of electrons. The ORP sensor is composed of a platinum measuring electrode and a reference electrode. Platinum is used because it is an inert metal and, due to its low resistance, will receive or give electrons to the reference electrode, until developing a potential, due to the accretion of charge, which is equal to the ORP value of the sample. In a RAS system, measuring the ORP value of the water is important to determine whether the water is sanitized or not. Higher positive readings are associated with better water quality, higher oxygen concentrations and less organic waste.

Figure 10: Water filtration system used at Riasearch. At the left: suspended solids filtration system; At the right: protein skimmer, to remove dissolved solids. Photographs by Ariana Laranjeira, Riasearch.

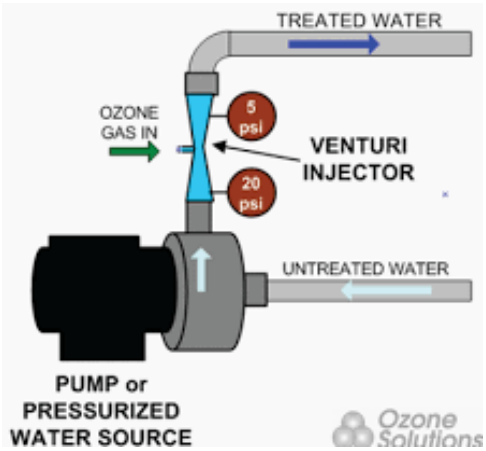

Figure 11: Addition of ozone in water by a venturi injector. Source: https://tinyurl.com/venturiozone

After skimming and ozonation, water flows to the “bio tower”, a biological trickling filter (Figure 12) where ammonia is converted to less toxic compounds by the action of nitrifying bacteria, and where carbon degassing takes place. The biotower is cylindrical and contains a plastic filter medium covered by a biofilm composed of bacteria, namely *Nitrosomonas* spp. and *Nitrobacter* spp., who, by nitrification, convert ammonia (NH_3) into nitrite (NO_2^-) and nitrite into nitrate (NO_3^-), respectively.

Nitrification is an aerobic process and occurs when water is pumped to the top of the trickling filter and, by gravity, it passes down through the plastic medium (EPA, 2002).

Ammonia to nitrite conversion: $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$

Nitrite to nitrate conversion: $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

Nitrification is an important step on RAS because nitrogen compounds, specifically ammonia and nitrites, are highly toxic for aquatic organisms, affecting their growth, feeding, and survival. The biofilter allows the reduction of these elements, contributing to the welfare of the cultured animals. Besides the bacteria, biofilms include other phylogenetic groups such as algae, arthropods, protozoans and such (Pandey et al., 2014).

Figure 12: “Biotower”, where nitrification process and carbon degassing occur. Photograph by Adriana Laranjeira, Riasearch.

After passing through the biotower, water is stored in the clean water reservoirs until it is required for the tanks. Before being directed to the tanks, water passes through a UV system and plate-type heat exchanger. UV, when contacting the water, destroys microorganisms by disintegrating its genetic material (DNA of bacteria, fungus or parasites, or RNA, the genetic material of viruses). As turbidity reduces the effectiveness of UV disinfection, this is one of the last stages of the water treatment, to guarantee its success (Losordo et al., 1999).

The last stage happens with the water passing through the heat exchanger (Figure 13, left) where the water is heated or cooled as required. The inlet water enters the heat exchanger and when in contact with the plates that contain hot/cold water between them, gets warmer/colder and attains the desired temperature for the trial. The two liquids do not have direct contact (Figure 13).

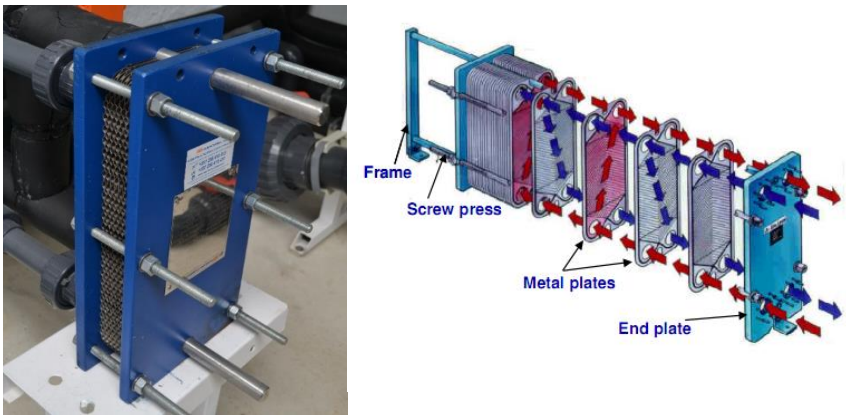

Figure 13: At the left: Riasearch's heat exchanger. Photograph by Adriana Laranjeira, Riasearch.
At the right: : Heat exchanger diagram. Source:
https://heatexchangercleaning.files.wordpress.com/2014/12/exc-4.png

Water for renewals and semi-renewals is brackish (18ppt) and is obtained from a 6m depth borehole near Riasearch. As the concentration of dissolved iron is high, the water passes through an oxidation system, where the air is injected in the water, oxidizing the iron and leading to its precipitation. Then, water passes through a sand filter to remove precipitated iron and other particles. After this process, water is stored in a free-iron water reservoir until new water for the tanks is required. Before being used this water passes through the water treatment steps aforementioned.

Each tank has adequate biomass and water flow so that the oxygen levels stay stable. Although, when oxygen levels decrease an air-blower is automatically activated and the aeration of water is assured by an air diffuser placed in each tank.

4.4. Maintenance routines

Maintenance of water quality was ensured through daily measurements of several water chemical parameters, such as pH, dissolved oxygen, salinity, and temperature. These parameters were measured with a probe (Figure 14), from the outlet water of the tanks. Nitrogenous compounds, such as nitrites and ammonia were measured three times a week with commercial kits, from tanks inlet water.

Figure 14: Hanna probe, used to measure water parameters from tanks. Source: https://www.hanna.pt/produto/hi98194

As Riasearch operates under RAS systems, these measurements are of utmost importance to ensure survival, health and the wellbeing of the animals accommodated in the tanks.

Throughout the fellowship, I was in charge of the daily routines. Firstly, I checked if there were dead animals or animals with altered behavior. I collected the dead animals, recorded the tank number as well as its weight. I also informed the colleagues responsible for the trials, if some changes in the behavior of fish and shrimp were observed. Then, I cleaned the oxidation-reduction, temperature and oxygen probes. Tanks were clean, in order to remove feces and uneaten feed and after that, animals were fed (animals from the experimental trials and stock animals too). I measured water parameters from outlet water (pH, temperature, dissolved oxygen, and salinity) and inlet water (nitrites and ammonia). Lastly, mechanical filters were substituted, and a 5-10% water renewal was done, even if the parameters were comprehended between the stipulated levels by the company.

Heat exchanger cleaning was done once a week and, when pH values were low, sodium bicarbonate was added to the systems' water in order to increase alkalinity and consequently increase the pH value.

4.5. *Penaeus vannamei* and *Sparus aurata* feeding trials

Riasearch conducted experimental trials with *P. vannamei* and *Sparus aurata* and during the internship, I had the opportunity to collaborate in these trials. Due to the confidentiality of the trials, I am not allowed to go on details, but I was involved in all routine procedures including the feeding and maintenance of the experimental systems. I also participated in sampling activities that consisted of weighing (*P. vannamei and S. aurata*), measuring the fork length (*S. aurata*) and, with a sieve, sorting gilthead seabreams juveniles. I also participated in a digestibility trial, that included feces collection in gilthead seabream, in order to determine the digestibility of different feeds.

P. vannamei and *S. aurata* were fed with experimental diets according to a blind experimental design. Gilthead seabreams were fed until apparent satiation while shrimp were fed based on a feeding table, considering the weight of the individuals and the density of the shrimps on the tanks. Feeding is done according to predefined schedules, depending on the trial.

I also participated in a stocking calibration of fish. For that, the sorting of gilthead seabream was made with a plastic sieve (Figure 15). Fish were put inside the sieve that was positioned underwater, to minimize the stress. Then, carefully with the hands, the fish were leaned against the sieve and the bigger fish did not pass through the plastic bars of the sieve. With this process, bigger fish were separated from smaller fish.

Figure 15: Sieve used for *Sparus aurata* sorting. Photograph by Adriana Laranjeira, Riasearch.

For the fish feces collection, 15 fish from each tank were euthanized with a lethal dosage of anesthetic and ventrally opened. Clamps were positioned in the beginning and at the end of the posterior intestine, to prevent feces from different portions of the intestine of getting mixed. Then, the clamp from the end of the intestine was removed and the feces were stripped to a falcon tube. Each falcon tube contained feces from the 15 fishes of the same tank.

4.6. Transport of *Penaeus vannamei* postlarvae

P. vannamei postlarvae present at Riasearch during the internship were purchased from the company Global Blue Technologies located in Texas, United States of America. These were transported by plane to the airport in Lisbon, Portugal, and then by car to Riasearch. The postlarvae were transported in two puncture-resistant polyethylene bags (one inside the other) filled with one-third of filtered seawater and two-thirds with pure oxygen, as shown in Figure 16. Some granules of new and washed activated carbon were added to the water to prevent high ammonia levels during transportation. After sealing, the bags were put into polystyrene boxes and the water temperature was adjusted to 17-18 ºC (by placing ice on the bottom and the top of the box), with the aim of decreasing the postlarvae metabolic rate minimizing waste excretion and oxygen consumption during transportation (Figure 16).

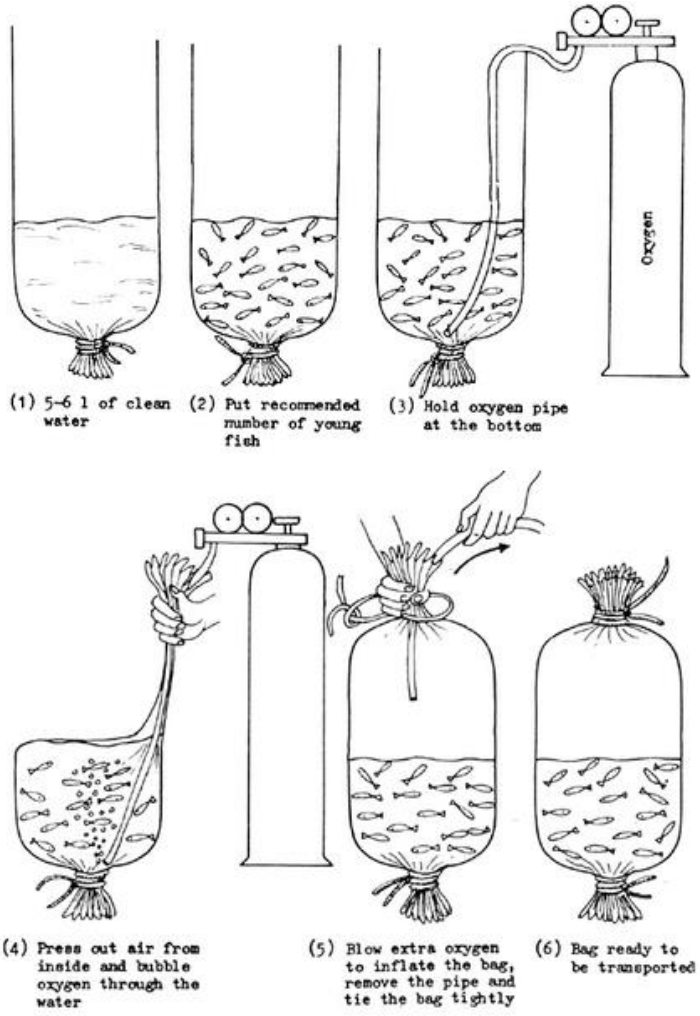

Figure 16: Simplified scheme of the process of filling the polyethylene bags. Source: “The transport of live fish. A review.”, Berka, R.,1986.

4.7. Reception and acclimation of *Penaeus vannamei* postlarvae

At the company, the transportation boxes and the plastic bags were opened and poured into the reception tanks (600L), where the postlarvae would be reared during the acclimation period. With a probe, dissolved oxygen (DO), salinity, pH and temperature, were measured on the tank with water from the transportation bags. Then, system water was slowly added while the parameters were continuously being checked, to make sure that the acclimation was being done correctly.

It is of major importance that the flow rate is adjusted in order to ensure that the temperature does not change faster than 1°C every 15 minutes, the salinity does not increase or decrease more than 1ppt every 15 minutes, and that pH is regulated not faster than 0.1 every 15 minutes (low pH values are due to CO_2 increase, as a consequence of respiration, since the closed bags do not allow gas exchanges).

Acclimation time depends on the difference between the values of transportation and system water parameters. It is finished when transport water attains the same parameter values as the system water.

After the acclimation, postlarvae started being fed on co-feeding of live newly hatched artemia and inert diet, for 3 days (75%-25%; 50%-50%; 25%-75%) and on the fourth day, feeding only on inert diet. From the fourth day on, postlarvae were fed eight times per day, four during the day, by hand, and four during the night, with an automatic feeder (Figure 17).

Figure 17: Hydor automatic feeder. Source: https://reefs.com/2017/09/04/hydor-mixo-ekomixo-automatic-feeders-times-away/

4.8. Transport and acclimation of *Sparus aurata*

Sparus aurata present at Riasearch during the internship were produced at Sonrionansa, S. L., a company based in Cantabria, Spain. Two groups of fish with different sizes were purchased, one group consisted of fish averaging 0.5g of body weight while the second group consisted of fish averaging 3g.

The transport lasted 6 hours and was made by car, in two 500L polypropylene certified deposits. The temperature during the transportation was maintained at 20°C, by an air conditioner system. Medicinal oxygen was diffused on the water at a concentration of 8,5mg/L, conferring water a saturation rate of 120%. Considering the transportation time, no water renewals were necessary.

Acclimation was done directly on the polypropylene deposits, by adding system water. When the water parameters in the deposits met those from the receiving tanks, fish were randomly distributed to the tanks.

5. *Penaeus vannamei* fasting trial

5.1. Aims

Being one of the main produced species in aquaculture, the development of bioenergetic models for *P. vannamei* would bring tremendous benefits to producers and researchers. Indeed, these are almost non-existing, and the few models that have been constructed are based on small data sets and therefore, not very robust. Herein, this study aimed at gathering data on energy and protein losses during fasting of ongrowing *P. vannamei,* crucial for the determination of the fasting energy losses, an integral part of a bioenergetic model. Fasting energy losses are dependent on the water temperature and body weight, and so this fasting energy losses were determined for different weight classes. Quantifying these losses during fasting supply additional data for the enrichment of existing models on *P. vannamei,* such as the one established by Lupatsch et al. (2008). The data obtained is going to be posteriorly analyzed by Riasearch and gathered in a database that the company has been assembling over the last few years with the goal of establishing an optimized and reliable bioenergetic model for *P. vannamei*.

5.2. Materials and methods

5.2.1. Treatments and shrimp rearing

P. vannamei used on the trial were obtained from Shrimp Improvement Systems (Miami, United States of America) in August of 2018 and were maintained at Riasearch (Murtosa, Portugal) for five/six months until reaching the desired body weight. The transportation of the shrimp was done as aforementioned.

The experimental trial was carried out in a RAS system composed of twelve tanks of 50L described earlier (section 4.2). Due to the low availability of tanks (12), the trial was divided into two parts, following the same experimental design. Another reason for this division is the fact that mortality was high due to shrimp jumping out of the tanks, leading to low data on the first part of the trial. To prevent jumps, a plastic mesh was added to the top of the tanks on the second part of the trial.

In both parts of the trial, daily, the water outlets mesh was cleaned, the system water was partially renewed, the exoskeletons were removed from the tanks and parameters such as dissolved oxygen, pH, temperature and salinity were measured with a probe. Nitrites and ammonia levels were measured three times a week with commercial kits. Aeration was made using an air blower connected to diffuser stones, distributed through the system.

In the first part of the trial, twenty-four on-growing *P. vannamei* shrimp were individually weighed and grouped in four weight classes of six shrimps each (Table 1). Shrimp were randomly accommodated in twelve tanks and fasted for nineteen days. Each tank was divided into two similar sections with a plastic mesh to prevent cannibalism and allowing that shrimp from different classes could be allocated in the same tank without any contact (Figure 18). During the experimental period, temperature was maintained at 27.33 ± 0.50°C, dissolved oxygen at 8.42 ± 0.39mg/L, pH at 7.87 ± 0.07, salinity at 20.25 ± 0.38, ammonia at 0mg/L and nitrates at 0.92 ± 0.37mg/L.

In the second part of the trial, twelve on-growing *P. vannamei* shrimp were individually weighed and randomly distributed into two weight classes (Table 1), accommodated in twelve tanks, and fasted for eighteen days (Figure 19). During the experimental period, temperature was maintained at 27.00 ± 0.41°C, dissolved oxygen at 7.96 ± 0.46mg/L, pH at 8.05 ± 0.10, salinity at 21.26 ± 0.21ppt, ammonia at 0mg/L and nitrates at 0.32 ± 0.15mg/L.

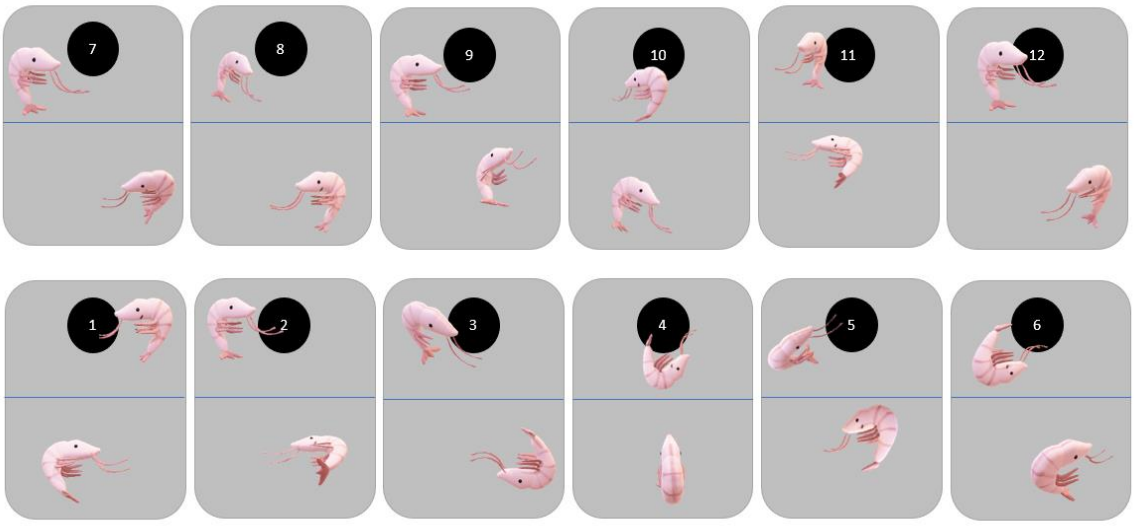

Figure 18: Tank distribution of shrimps on the first part of the trial.

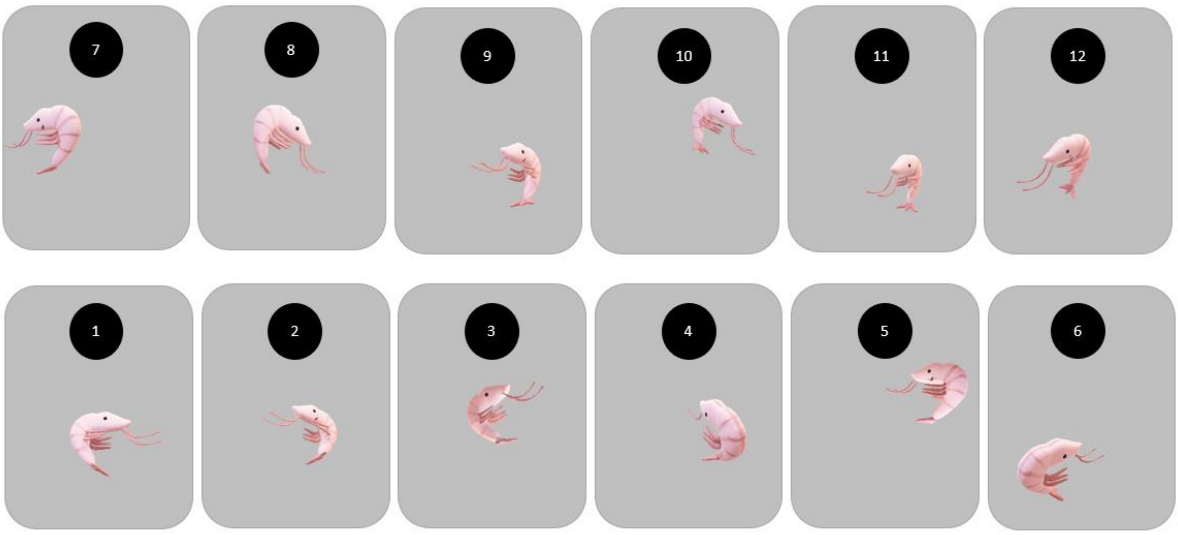

Figure 19: Tank distribution of shrimps on the second part of the trial.

Weight classes of the first part of the trial (mean ± standard deviation) (Figure 18)	Weight classes of the second part of the trial (mean ± standard deviation) (Figure 19)
9.62 ± 0.31 g	15.10 ± 0.22 g
13.78 ± 0.36 g	17.45g ± 0.34 g
17.45 ± 0.38 g	
21.62 ± 0.64 g	

Table 1: Shrimp weight classes used in the first and second part of the trial (mean ± standard deviation).

5.2.2. Sampling

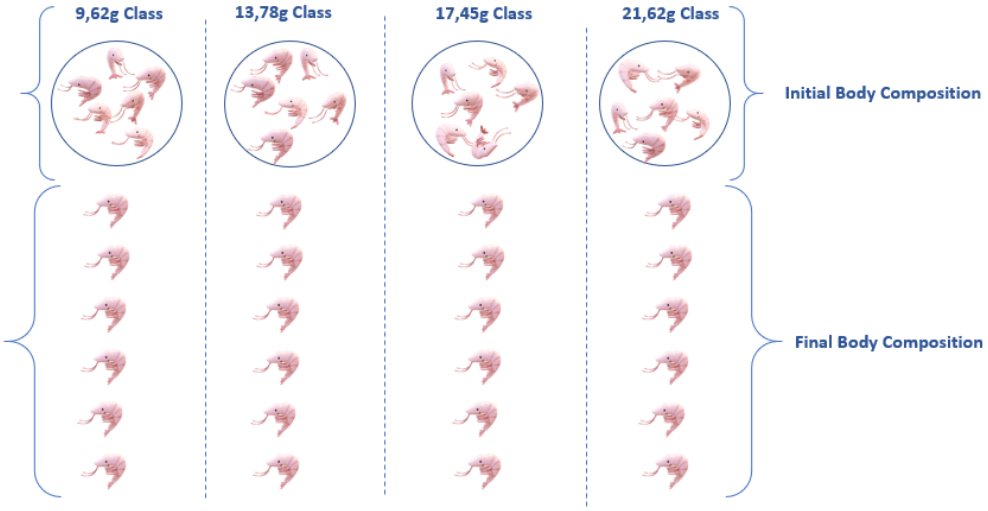

Figure 20: First part of the trial weighing scheme.

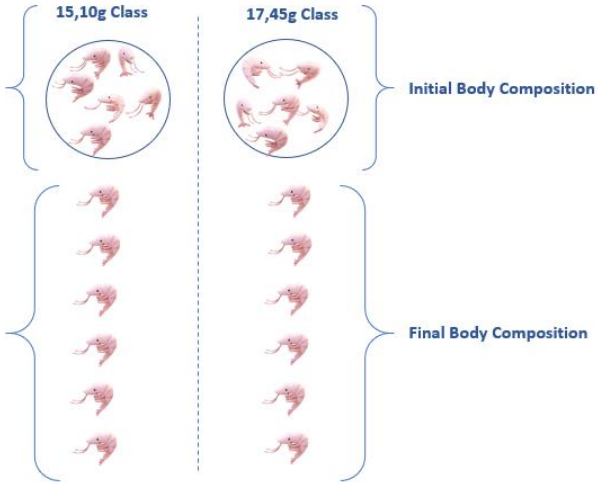

Figure 21: Second part of the trial weighing scheme.

At the beginning of each part of the trial and for initial body composition shrimp were weighed in groups of 6 and frozen. At the end of the first part of the trial, the remaining shrimps from each of the four weight classes were weighed and frozen (Figure 20). At the end of the second part of the trial, the remaining shrimps from each of the two weight classes were weighed and frozen. Before the freezing process, shrimp were washed with freshwater (Figure 21).

5.3. Analyses of the whole-body composition

5.3.1. Dry matter

After defrosting, shrimps were cut (head was separated from the body and the exoskeleton was cut dorsally in order to facilitate the drying process) and placed in identified pre-weighed crucibles. Crucibles plus fresh shrimps were weighed and then went to a stove, at 80ºC, during approximately 4 days, until constant weight (Figure 22). After that, the moisture content was determined by the weight difference of the initial and final weight of shrimps plus crucibles.

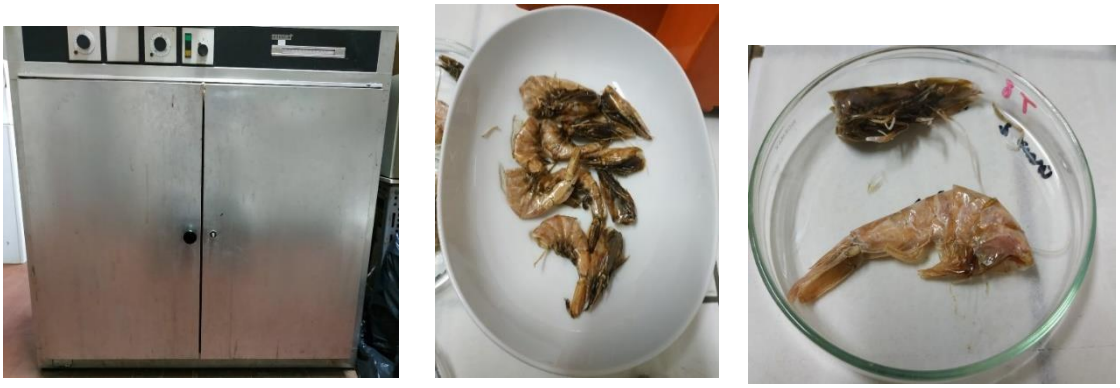

Figure 22: At the left: the stove; In the middle: a group of 6 dried shrimps (initial body composition): At the right: a dried shrimp at the end of the fasting trial.

After dry matter determination, shrimps were powdered and stored in identified falcon tubes for further analysis (Figure 23).

Figure 23: At the left: dry shrimp in a coffee grinder; In the middle: powdered shrimp; At the right: identified falcons containing powdered shrimp.

5.3.2. Crude protein

The protein content of the shrimp samples was determined using the Kjeldahl method after acid digestion using a Kjeldahl digestor (Figure 24, left) and a distillation unit (Figure 24, middle). The crude protein value was obtained by multiplying the nitrogen content of the samples by 6.25, considering that the nitrogen has its origin solely on protein.

Samples were individually weighed in duplicate and added to the distillation tubes, then one Kjeldahl tablet for catalyzation and 5ml of sulfuric acid were added to each tube.

Digestion took 1 hour, with the digestion unit at 450°C. At the end of the digestion, the distillation process was done in a distillation unit. After distillation, nitrogen content was determined by titration (Figure 24, right) with hydrochloric acid (HCl; 0.2N) and methyl red-bromocresol green as the pH indicator.

Figure 24: At the left: a Kjeldahl digestion unit; In the middle: a distillation unit; At the right: a titration unit.

5.3.3. Gross lipid

The gross lipid content was determined with a Soxtec extractor (Figure 25) and petroleum ether as a solvent. Pre-weighed samples were added to a cartridge and then positioned in the extraction unit. Then, approximately 35mL of petroleum ether was added to each extraction cup (weighed and identified previously) placed below each cartridge, on the extractor. Samples went over a boiling process for one hour and then rinsed for two hours until all the lipids content of the samples were collected. After that, the remaining solvent was collected and the extraction cups went to the oven at 100°C, to guarantee that there was no solvent left in the cups. Then, cups were transferred to a desiccator until attaining room temperature. The gross lipid content of each sample was then obtained by the weight difference of the extraction cups before and after the process.

Figure 25: Soxtec extractor and cartridges.

5.3.4. Gross energy

Gross energy was determined using a Parr calorimetric bomb (Figure 26, right), where the sample was combusted after being pelletized with a pellet presser (Figure 26, left), in a pressurized environment rich in oxygen, inside the bomb vessel. The bomb vessel is steeped in 2L water of a bucket and, after the combustion, internal energy (ΔU) is calculated by the change in the bucket water temperature, leading to the energetic value of each sample, in Cal/g of sample.

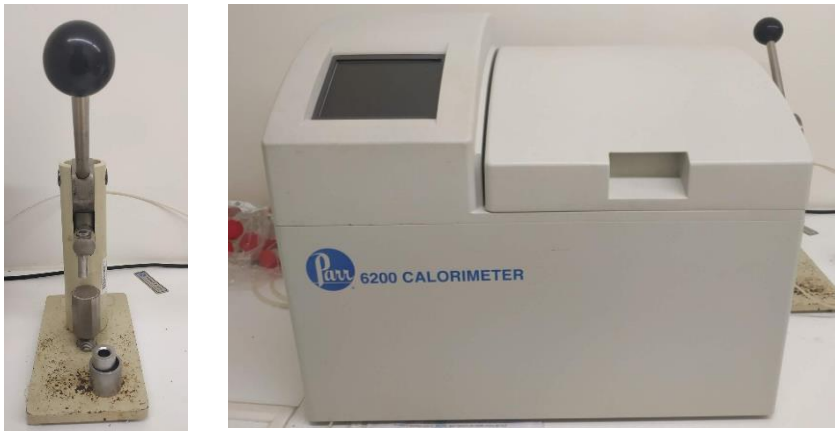

Figure 26: Pellet presser, at the left; Parr calorimetric bomb, at the right.

5.3.50. Ash

Identified, clean and dry ceramic crucibles were weighed, then, after adding the sample, crucibles plus samples were weighed again and placed in a muffle furnace for 16h at a temperature of 450°C, until samples in the crucibles were reduced to ashes. After that, crucibles plus samples were placed in a desiccator until attaining room temperature. When at room temperature, crucibles plus ashes were weighed and the ash content was determined, after applying a formula.

5.4. Results

After whole-body analyses of the shrimp, data was processed in order to obtain the initial and final percentage of protein, lipid, moisture, ash, energy content, and protein and energy losses/gains after the fasting period (Table 2 and Table 3).

The initial body composition of the shrimps that went through starvation was assumed to be equal to the body composition of the average value of each group sacrificed at the beginning of the two parts of the trial.

The daily nutrients, energy and protein losses/gains of fasting shrimps were calculated for each shrimp, using the geometric mean of its weight.

Weight classes and parts of the trial	Weight (g)	Protein (%FM)	Lipid (%FM)	Humidity (%)	Ash (%FM)	Energy (J/g)
First part of the trial (19 days)						
Weight class of 9.6g						
Initial (N=6)	9.6±0.30	14.20±0.14	2.52	79.6	2.88	19474.62
Final	8.40±0.54	9.99±1.04	3.59±0.40	84.29±1.22	3.05±0.13	17819.20±418.22
Final-Initial	-1.2	-4.21	1.07	4.69	0.17	-1655.42
Weight class of 13.8g						
Initial (N=6)	13.78±0.34	14.63±0.09	3.21	79.58	2.82	20278.35
Final	11.93±0.90	10.39±0.64	2.82±0.20	84.19±0.84	2.81±0.42	19097.25±848.37
Final-Initial	-1.85	-4.24	-0.39	4.61	-0.01	-1181.1
Weight class of 17.5g						
Initial (N=6)	17.57±0.34	13.92±0.07	2.34	80.44	2.64	20267.74
Final	15.67±0.27	11.27±0.43	2.97±0.20	83.29±1.01	2.95±0.18	19045.42±351.18
Final-Initial	-1.9	-2.65	0.63	2.85	0.31	-1222.32
Weight class of 21.6g						
Initial (N=6)	21.62±0.61	15.48±0.15	4.53	78.03	2.93	20553.81
Final	18.7±1.45	11.20±1.37	3.45±1.12	83.47±0.93	2.81±0.33	19417.94±465.07
Final-Initial	-2.92	-4.28	-1.08	5.44	-0.12	-1135.87
Second part of the trial (18 days)						
Weight class of 15.1g						
Initial (N=6)	15.14±0.21	13.47±0.26	2.23	80.44	2.66	20652.91
Final	13.70±0.57	11.42±0.31	2.94±0.28	82.91±0.50	3.17±0.21	17980.17±1131.60
Final-Initial	-1.44	-2.05	0.71	2.47	0.51	-2672.74
Weight class of 17.5g						
Initial (N=6)	17.54±0.28	15.60±0.10	1.89	77.69	2.91	19926.7
Final	16.00±0.37	10.41±2.26	2.80±0.35	83.12±0.31	3.21±0.08	19809.61±508.92
Final-Initial	-1.54	-5.19	0.91	5.43	0.3	-117.09

Table 2: *P. vannamei* weight (g), whole body-composition percentages, and energy content (J/G) before and after the fasting trial. g: grams; FM: fresh matter; J: joules.

Weight classes and parts of the trial	Energy loss (J)/g shrimp/day	Protein loss (mg)/g shrimp/day	Mortality
First part of the trial (19 days)			
Weight class of 9.6g	-85.87±18.00	-3.08±0.66	1
Weight class of 13.8g	-86.91±14.70	-3.26±0.44	0
Weight class of 17.5g	-62.74±11.37	-2.15±0.26	3
Weight class of 21.6g	-102.99±15.55	-3.29±0.85	0
Second part of the trial (18 days)			
Weight class of 15.1g	-73.88±14.67	-1.84±0.39	1
Weight class of 17.5g	-49.16±9.26	-3.55±1.12	1

Table 3: Energy loss (J/g shrimp/day), protein loss (mg/g shrimp/day) and mortality values during the fasting trial on *P. vannamei*. J: joules; g: grams; mg: milligrams.

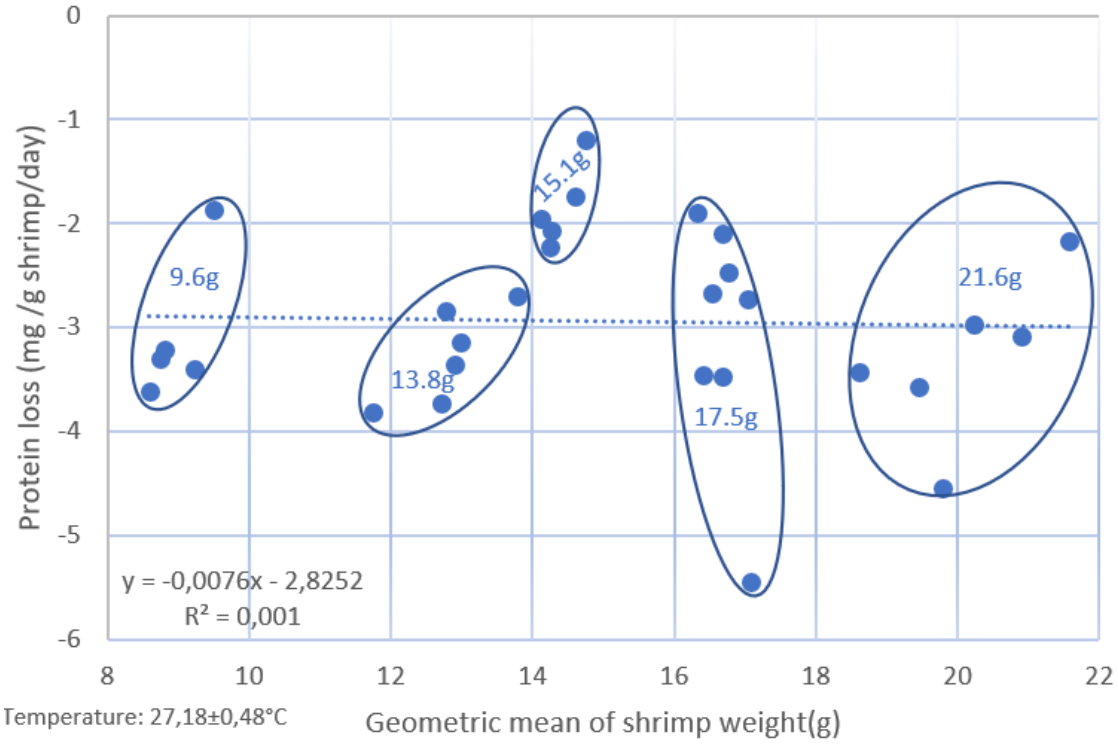

Figure 27: Protein loss (mg/g shrimp/day) in function of the geometric mean of shrimp weight (g)

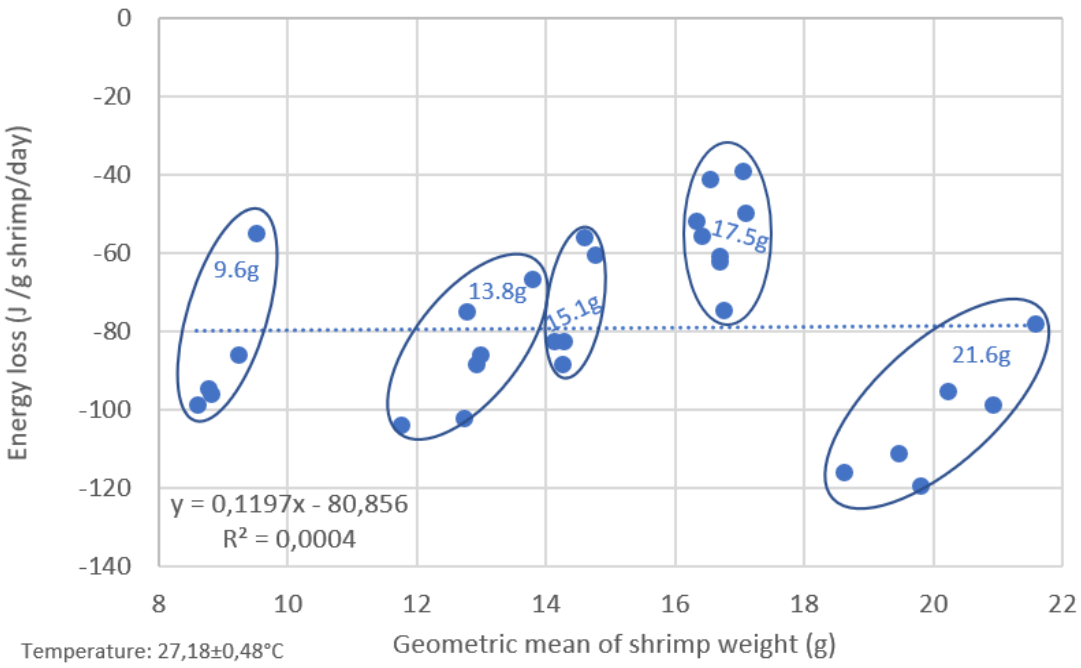

Figure 28: Energy loss (J/g shrimp/day) in function of the geometric mean of shrimp weight (g)

The two graphs presented above (Figure 27 and Figure 28) describe the relation between protein and energy loss in function of the geometric mean of shrimp weight, respectively.

The protein loss (mg/g shrimp/day) is described by the following equation: $y=-0.0132x^2+0.3813x-5.509$ $R^2 = 0.0497$

Where y is the protein loss (mg)/g shrimp/day and x is geometric mean of shrimp weight.

The energy loss (J/g shrimp/day) is described by the following equation: $y=-0.6858x^2+20.297x-220.1$ $R^2 = 0.2103$

Where y is the energy loss (J)/g shrimp/day and x is geometric mean of shrimp weight.

All classes of shrimp after passing through the fasting lost weight, protein, and energy. In terms of lipids the average lipid content decreased in only two weight classes (13.8g and 21.6g). Humidity content increased in all weight classes after the trial, due to starvation.

Ash content increased at the end of the trial because, due to the decrease in protein content, the exoskeleton, rich in ash, represents a higher percentage on the whole-body composition of the shrimp.

On the weight class of 17.5g of the first part of the trial, the mortality was higher. Deaths cause were mainly due to shrimp jumping out of the tanks. Another reason for death was the fact that one shrimp got injured due to being caught on the divisor net of the tank.

Further studies with different weight classes and different temperature conditions should be done, to support the data obtained and to determine if temperature affects protein and energy values on shrimp. Also, a higher number of shrimps must be used on each weight class to obtain more robust results.

For confidentiality reasons, the results are not going to be discussed. Riasearch will analyze them in order to complement and enrich Lupatsch et al. (2008) protein and energy requirements model in *Penaeus vannamei*.

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