BIOCHEMICAL COMPOSITION (PROTEINS, LIPIDS AND CARBOHYDRATES) OF THE SCYPHOZOAN JELLYFISH, *CHRYSAORA HYSOSCELLA*, FROM THE NORTHERN BENGUELA.

Research Report Done By Michelle Madondo
and

Submitted to the Department of Fisheries and Aquatic Sciences, Faculty of Agriculture and Natural Resources, University of Namibia in partial fulfilment of the requirement for the award of a Bachelor of Science Degree in Fisheries and Aquatic Sciences

2010

Supervisors:

Mr. S. Mafwila (University of Namibia)

Mr. I. Kauvee (Nacoma Project)

Professor Mark gibbons (University of Western Cape)

Research Title

Biochemical composition (proteins, lipids and carbohydrates) of the scyphozoan jellyfish, *Chrysaora hysoscella*, from the northern Benguela.

Figure 1. ***Chrysaora hysoscella***

Declaration

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Mr. I. Kauvee, Mr. S. Mafwila and externally by Professor Mark Gibbons. The work has not been presented elsewhere for the award of a degree. All the sources have been duly and appropriately acknowledged.

Candidate signature_____ Date_____

Michelle Madondo: 200743091

Certification

This is to certify that this report has been examined and approved for the award of a degree of Bachelor of Science in Fisheries and Aquatic Sciences of the University of Namibia.

Supervisor_____

Head of Department_____

Acknowledgements

I want to thank God for giving me the strength to do this work which sometimes was challenging but His grace and wisdom sustained me to carry out this work successfully. I would also like to acknowledge the following:

My supervisors Mr. Sam Mafwila, Mr I. Kauvee and Professor M. Gibbons for all their input and committed involvement throughout the whole research process,

The department of Fisheries and Aquatic Sciences of the University of Namibia for their logistical input and support throughout the whole research period,

The National Marine Information and Research Centre (NatMIRC) for all their assistance with sampling and laboratory work and for being very helpful in allowing me to utilise their equipment and participate in their research surveys to collect samples,

The biology department of the University of Namibia for assistance with their freeze dryer without which this research would not have been possible; Special mention to Dr. Julies for her assistance during laboratory biochemical analyses,

The biochemistry department from the University of Namibia for assisting me in carrying out all biochemical laboratory work with precision. Special mention goes to Ms. Serine for her direction in determining the biochemical composition and advice which was paramount for successful laboratory work,

The laboratory technicians from the fisheries department for all their assistance. Thank you for understanding and being patient during all the late nights working in the laboratory.

Finally I would like to thank the University of Namibia in collaboration with the Norwegian Government for awarding me a scholarship to pursue my studies. God bless you all!

Dedication

This research is dedicated to my mother and father, whose hard work and dedication has truly been an inspiration to me, and to my younger brother, Michael. You have been a source of love, strength and motivation to strive for excellence in every work. I love you and continually thank God for you.

TABLE OF CONTENTS

List of Abbreviations

1.	NatMIRC	National Marine Information and Research Centre
2.	UNAM	University of Namibia
3.	MFMR	Ministry of Fisheries and Marine Resources
4.	WW	Wet weight
5.	DW	Dry Weight
6.	AW	Ash weight
7.	AFDW	Ash –free dry weight
8.	R-B	Distilled water and reagent blank

List of Figures

LIST OF TABLES

Abstract

As global fisheries begin to decline, medusae appear to be coming to the fore. Jellyfish biomass (12.2 million tonnes, MT) now exceeds the biomass of the once- abundant fish (3.6 MT) and in order to establish what role jellyfish play within the altered system, one has to look at the biochemical composition.

Specimens were collected aboard R.V. Welwitschia during a National Marine Information and research centre environmental survey in July 2010. A total of 12 undamaged individuals were collected from the trawls, 7 of which had visible gonads. Of all the individuals collected, with and without visible gonads, the average bell diameter of *Chrysaora hysoscella* individuals was 43 cm and the whole wet weight of individuals ranged from 1094g to 8151g in weight. The individuals contained an average of 96% water in their body mass. Of the dry weight matter (3.99% of whole weight), 63.8% was ash content by mass and 36.2% was the percentage ash-free dry weight. A standard curve of Bovine Serum Albumin (BSA) was used to determine protein concentration. Lipids were determined gravimetrically and a standard curve of D-Glucose was used to determine total carbohydrates. Biochemical assays exhibit earlier documented patterns of low carbohydrates (mean 4.64 mg/g DW), intermediate lipids (mean 33.62 mg/g DW) and high protein (111.7mg/g DW) content although there was a high level of variability. In *Chrysaora hysoscella* from the Benguela, mean contents as a percentage of wet mass were 0.0058% carbohydrates, 0.043% lipids and 0.18% protein.

Biochemical composition of these now abundant species in the Northern Benguela is essential in order to incorporate jellyfish in ecosystem models in an effort to achieve the more holistic Ecosystem-Based Fisheries Management to manage the resource more sustainably.

CHAPTER ONE - INTRODUCTION

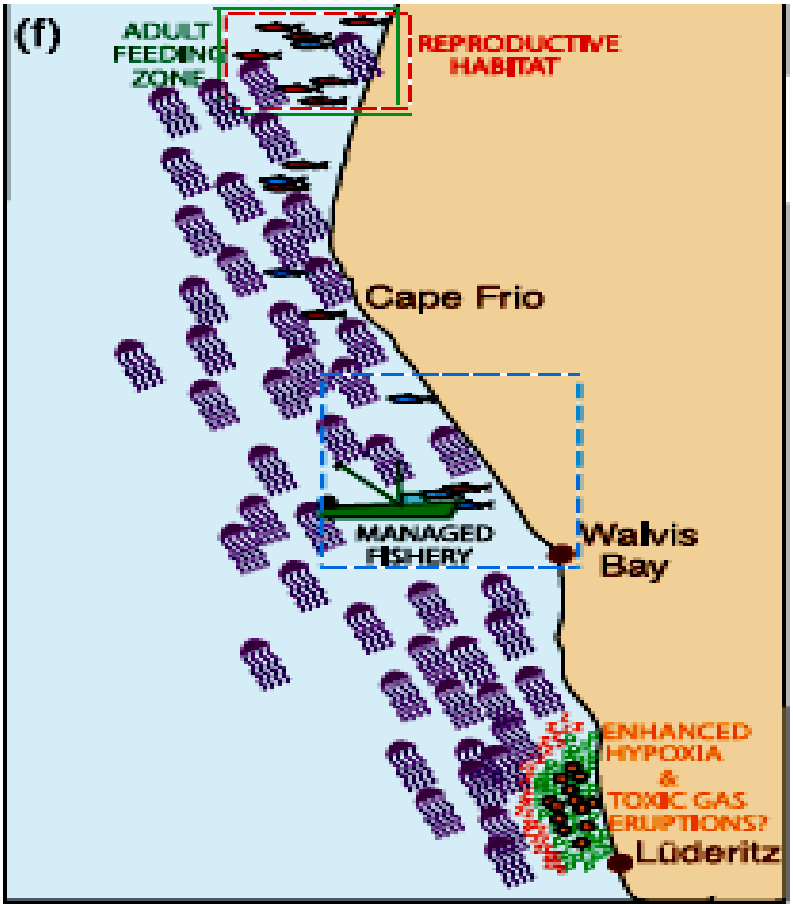


Figure 2. A schematic diagram of the hypothesized regime shift of an increase in jellyfish (purple structures) in the study area; the northern Benguela; as a result of fishing pressure (green boat) (Lynam *et al*., 2006)

Background information

Marine ecosystems, of which the Benguela is a part, appear to be prime examples of complex adaptive systems. According to Bakun and Weeks (2006) it is safe to say that something durable and significant is being done to the ecosystem that goes beyond the simple removal of the fish that are landed. Widespread calls for implementation of ecosystem-based fishery management (EBFM) which recognises the need for a holistic, ecological management approach now emanate. With the increase of jellyfish in Namibian waters, their morphometric and biochemical studies can be very useful in estimating their biomass and energy density. Estimation of the supply of organic matter to the seabed through dead and decaying medusae can then be done, thus considering the direct and indirect ecosystem effects of fishing operations.

General Introduction

The Benguela System

In addition to being one of the four major eastern boundary upwelling systems of the world, the Benguela current is the most significant hydrographic feature in the Southeast Atlantic (Pages *et a.l,* 1992). It originates at the subtropical convergence and is driven by the anticyclone gyre in the Southeast Atlantic, flowing from 34º S to 17 º S. It is bounded at both equator ward and pole ward ends by warm water regimes: to the north we find the tropical waters of Angola, and in the south the warm Agulhas current is dominating. The Benguela ecosystem can be divided into two subsystems, the Northern Benguela (South Angola and Namibia) and the Southern Benguela (South Namibia and South Africa) separated by the permanent upwelling cell off Luderitz; the strongest in the world.

The Northern Benguela Current features the strongest sustained coastal upwelling of any of the 'classical' eastern ocean coastal upwelling zones of the world's oceans, resulting in very high local rates of primary organic productivity. The above-mentioned anticyclone South Atlantic gyre in combination with the equator ward wind flow pattern, gives rise to a series of coastal upwelling centres that characterise the Benguela ecosystem where winds blowing

parallel to the land mass generate Ekman transport that moves water out from the coast and water that flows out perpendicularly to the coast is replenished by upwelling water. The system is one of the world's most productive regions (Pages *et al.*, 1992) as the upwelling makes the nutrients from regeneration processes in deep water available to plants in the euphotic zone.

Effect of fisheries

The Northern Benguela is a highly productive eastern boundary ecosystem fertilised by upwelling, nutrient-rich waters. Historically the region supported large stocks of fish, including sardines (*Sardinops sagax*) and anchovies (*Engraulis encrasicolis*), but heavy fishing pressure has reduced stocks, and total landings have fallen from around 17 MT in the late 1970s to just 1 MT (Lynam *et al*, 2006). Sardines were dominant in the northern Benguela from 1950 to 1975. In the mid- 1960s, the sardine biomass in the Northern Benguela is estimated to have been about 10 million metric tons; catches were at annual levels of about 1.5 million tons (Boyer 1996). Then, under very heavy fishery exploitation, the sardine resource abruptly collapsed in the mid 1970s. Up to the present time, both biomass and catches of sardines have consistently been at levels not exceeding a tenth of those that were typical earlier, and recently have fallen even more, nearly disappearing entirely in some years. This is in spite of the establishment of modern fishery resource management procedures (Hutchings *et al*, 2009).

As global fisheries begin to decline, medusae appear to be coming to the fore (Buecher et al, 2001). Prior to this period of heavy exploitation, large jellyfish (Scyphozoa and Hydrozoa) were not prominent in the Benguela ecosystem: reports of extensive plankton sampling in the 1950s and 1960s do not mention large jellyfish, although numerous small gelatinous species like ctenophores were observed. The exploitable fish species seem to have been largely replaced at that position by a combination of 'jelly predators' (primarily the medusas *Aequorea forskalea, aequoreidae and Chrysaora hysoscella, pelagiidae*) of little fishery interest (Lynam *et al*. 2006). Since the 1990s, reports of these jellyfish have been ever-increasing, particularly because of the nuisance they now cause to fishing (bursting trawl nets, spoiling catches), power generation (blocking power station coolant intakes) and diamond mining (blocking alluvial sediment suction).

Marsh Youngbluth (2001), a jellyfish researcher is quoted to have said that "in some locations jellyfish may be filling ecological niches formerly occupied by now overfished creatures." Jellyfish feed on the same kinds of prey as adult and young fish, therefore if fish are removed from the equation; jellyfish are likely to move in". In their research paper, Lynam *et al* (2006) reported that "by sampling sea life in a heavily fished region off the Namibian coast, total jellyfish biomass has overtaken that of fish, following intense fishing in the area in the last few decades". Jellyfish biomass (12.2 million tonnes, MT) now exceeds the biomass of the once- abundant fish (3.6 MT). This is a profound ecosystem change, with possible consequences, from carbon cycling to inhibiting fish stock recovery.

Problem statement

The northern Benguela has seen a dramatic change in forage fish abundance, with sardines and anchovies declining and being replaced by gobies, horse mackerel and jellyfish. The unexploited zooplanktivores (medusae) have in turn inflicted increased rates of predation mortality on pelagic early life stages of sardines and other fish species (they prey on fish eggs and fish larvae). Increases in jellyfish therefore results in the starvation of top predators of pelagic communities. Consequently, sedimentation of primary producers (phytoplankton) also increases and may eventually lead to sulphur and methane events/ eruptions which alter trophic flows in the system (Bakun and Weeks, 2004). This would act to further exclude grazing organisms from the zone, including preventing sardines from re-establishing the earlier feeding migration to the vicinity of the Luderitz zone, thus helping to keep the system durably trapped in its altered state.

However, to establish what role jellyfish play within the altered system, one has to look at the biochemical composition, but unfortunately there is no data. Recent investigations of the zooplankton in the Benguela system have established that the gelatinous plankton, and more specially the cnidarians, makes up the least known groups in the system (Pages *et al*, 1992). Since jellyfish are no longer considered as trophic dead ends but instead important members of the pelagic communities (Lucas, 2008), one of the ultimate aims in jellyfish research is to incorporate them into ecosystems models used to predict population dynamics and ecosystem effects in an effort to move o Ecosystem Based fisheries Management (EBFM). However

such efforts usually suffer from insufficient information on jellyfish biomass and biology. Construction of bioenergetic models for species that prey on jellyfish, and trophic dynamic models that incorporate jellyfish maybe problematic due to lack of information on their proximate composition and energy density. This jellyfish plays an important role in structuring pelagic ecosystems, directly through predatory pressure on the zooplankton community and indirectly through reduction of herbivorous grazing pressure which may result in large phytoplankton blooms. It is also economically important because it preys on, or competes with larvae of commercial fisheries; therefore, it is imperative that data on the species become available.

Justification

Knowledge of the biochemical and elemental composition of organisms is essential in order to calculate biomass, quantify the transfer of energy through the pelagic food web, and estimate the supply of organic matter to the deep seabed through dead and decaying medusae. But despite this unquestionable need for data emphasized by the calls for implementation of ecosystem-based fishery management (EBFM); which recognises the need for a holistic, ecological management approach; there have been comparatively few morphometric and biochemical studies on jellyfish over the few years (despite their increase in biomass) and data for jellyfish of the Benguela system is extremely rare.

Although the last two decades have seen an increase in research on medusae, our understanding of their role in marine ecosystems is still poor (Buecher *et al*, 2001). There is need for more research on these conspicuous components of coastal and ocean pelagic marine ecosystems as lack of this knowledge prevents their accurate incorporation into models of ecosystem functioning, such as Ecopath.

Research purpose and specific Objectives

The purpose of this study is to provide baseline data for future biochemical composition studies on jellyfish of the Northern Benguela. In addition to merely determining the biochemical composition (proteins, lipids, and carbohydrates) of the jellyfish *Chrysaora hysoscella*, this project endeavours to establish if there is any relationship between gonad weight, bell diameter, whole weight (wet and dry weight), and the biochemical components.

The project will attempt to attain the following objectives:

- Establish the species biochemical composition (protein, lipid, carbohydrate) of the scyphozoan jellyfish species *C. hysoscella*

- Relate the size (bell diameter, wet weight, dry weight, ash weight and ash-free dry weight) to its biochemical composition.

- Relate the gonad weight (wet weight, dry weight, ash weight and ash-free dry weight) to its biochemical composition.

Research Question and hypotheses

Questions:

Is there any significant linear relationship between biochemical composition of *C. hysoscella* and the organism's bell diameter, wet weight, dry weight, ash weight and ash-free dry weight?

Is there any significant linear relationship between biochemical composition of *C. hysoscella* and the organism's gonadal wet weight, dry weight, ash weight and ash-free dry weight?

Is there any significant difference between gonadal biochemical composition and body tissue biochemical composition?

Research hypotheses:

H_{10}: There is a significant linear relationship between jellyfish size (Bell diameter, WW, DW, AW, and AFDW) and the biochemical composition (proteins, lipids, carbohydrates) of *C. hysoscella*

H_{12}: There is a significant linear relationship between gonad size (WW, DW, AW, and AFDW) and the biochemical composition (proteins, lipids, carbohydrates) of *C. hysoscella*

H_{13}: There is a significant difference between gonadal biochemical composition and body tissue biochemical composition.

Literature Review

Following early collapses of pelagic fish stocks (in the1960s), reports of the large and conspicuous jellyfish *C. hysoscella* (mean umbrella diameter,~ 27cm) became increasingly common (Lynam *et al*, 2006). *C. hysoscella* is one of the two large, widely distributed and abundant species in the Northern Benguela ecosystem (Buecher et al 2001). According to Brierley *et al.* (2001), the jellyfish *C. hysoscella* and *Aequorea aequorea* occur in very high numerical densities in the northern Benguela ecosystem off Namibia.

With regard to biochemical content, the typical gelatinous zooplankton trend of low carbohydrate, intermediate lipid and high protein is observed (Lucas, 2008). Proteins are thought to be the main storage product in gelatinous zooplankton and lipids comprising mainly of phospholipids have a more structural role. According to studies done on *Periphylla periphylla* from the Gulf of Mexico by Cathy H. Lucas, (2008), the following summary of biochemical composition of whole medusae shows the ranges of biochemical composition assays of jellyfish:

Table 1. Lucas (2008) Results for *P. periphylla* from the Gulf of Mexico

Parameter	**N (sample size)**	**Range**	**Mean (SD)**
Total Proteins			
mg/g WW (wet weight)	21	0.85-6.74	3.45 (1.52)
mg/g DW (dry weight)	21	34.14-108.21	63.71 (17.18)
Total lipids			
mg/g WW (wet weight)	21	0.23-2.74	1.14 (0.65)
mg/g DW (dry weight)	21	1.53-48.12	20.57 (8.42)
Total carbohydrates			
mg/g WW (wet weight)	21	0.14-1.03	0.49 (0.23)
mg/g DW (dry weight)	21	5.06-14.45	8.99 (2.34)

Typically jellyfish have high water (~95%) and high mineral ash (~70%) contents.

The lack of information on the energy density of jellyfish is partly caused by methodological problems associated with the high salt content, high water content (95-98% wet mass) and extremely low energy density of this group (Doyle *et al.,* 2007).

According to Doyle *et al.* (2007), it takes 82-410 hours to dry approximately 1g (±1.0g) jellyfish samples to constant mass, with the length of time required being largely determined by the initial wet mass of the specimen and the amount of wet sample exposed to air. Their studies on jellyfish species from the Dingle Harbour, Ireland showed that mean water contents for whole jellyfish were 95.8, 96.1 and 96.2% of wet mass for *C. capillata, R.octopus* and *C. hysoscella* respectively. There were also significant differences in composition between component tissues in *C. hysoscella* with gonads having lower water content than both the bell and oral arms.

Studies on *C. capillata* dried samples showed that protein was by far the largest fraction of organic matter present and dried samples contained only minor amounts of lipids and carbohydrates. Doyle *et al* (2007) study reveals that the protein content for the three different

species (*C. capillata*, *R. octopus* and *C. hysoscella)* ranged from 10.1% to 22.6% dry matter for whole specimens and varied significantly between component tissues. Both the gonads and oral arms had far greater protein content than did the bell component (mean ± S.D. expressed as %DM: gonads = 28.4 ±3.9; oral arms = 29.8 ±3.1; bell 7.9 ±1.5).

CHAPTER TWO – MATERIALS AND METHODS

(a) (b) (c)

Figure 3 (*a, b, c).* Student collecting jellyfish samples from a boat using a scoop net

Research Materials

Refer to the appendix (Appendix 1) for the list of materials used in this research.

Research Methodology

Samples were collected from the Walvis Bay Harbour using a small boat and scoop net, and others aboard the research vessel R.V. Welwitschia using a Carmen trawl net (Appendix 2). A total of 26 samples were collected from the harbour and 12 specimens from the trawls. Only 7 specimens from the trawl samples had visible gonads. All were females.

Collection method

Using a scoop net, jellyfish were individually scooped out of the water upon encounter and immediately washed in a 2L bucket of filtered sea-water. The process was repeated in a second bucket to make sure that all parasites, Hyperiid amphipods were removed from the specimens. *Hyperia medusarum*, the common parasite would otherwise affect the biochemical composition results if unremoved. Afterward each jellyfish was individually bagged in clearly labelled plastics and processed in the laboratory within 3hours upon collection. In the wet laboratory at the Ministry of Fisheries and Marine resources, Swakopmund, each individual was weighed on an electronic balance to determine the wet weight, and measured across the bell for its bell diameter using a measuring tape. The jellyfish were frozen at -10˚C in a blast freezer. Ideal storage is at -20˚C but due to lack of space in the available freezer at that low temperature at NatMIRC, samples were preserved at -10˚C.

Aboard the vessel, undamaged individuals were carefully selected from the trawl landings and washed as described above. Immediately, all measurements were taken at the wet lab area. Gonads were dissected off those individuals that had visible gonads, weighed and individually bagged in labelled pre-weighed plastic bags. Each specimen was also weighed, measured across the bell and individually bagged in a pre-weighed and labelled plastic bag. Samples were kept in the freezer until the survey was complete and moved to NatMIRC.

All specimens were then transported in cooler boxes on crushed ice (except gonads that were small enough to be transported in a portable freezer set at -20˚C) to the University of Namibia were they were kept at a constant temperature of -20˚C until biochemical analyses were done.

Laboratory Procedures

All the data in this report is for individuals collected from the trawls. The individuals collected from the harbour were used for trial testing in order to test previously applied methodology and modify it where necessary in order to adapt it accordingly for the species in question and get accurate results.

Wet weight determination

Using an electronic scale, all individuals were weighed soon after collection and results recorded.

Dry weight determination

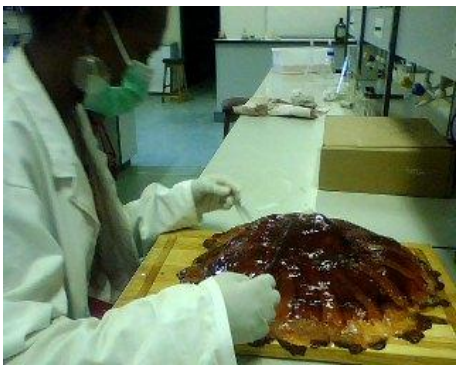


Figure 4. Student cutting off samples for oven drying

Individuals were place in an oven in pre- ashed and pre-weighed crucibles and samples were allowed to dry at 70ºC in the oven. Their weight was monitored for 24 hours or until a constant weight was obtained. If the weight of the sample remained unchanged it meant that the dry weight had been achieved. The dry weight (DW, g) was determined by subtracting the crucible weight from the combined weight of the crucible and the dry matter. The water was determined by subtracting the dried sample weight from the wet sample weight for each specimen.

Ash and Ash-free Dry weight determination

Dried and/or ground samples were incinerated at 550^{o}C for 24h in the furnace and then cooled in a dessicator to room temperature. The ash weight (AW, g) was determined by subtracting the crucible weight from the combined weight of the crucible and the ash. Ash-free dry weight (AFDW, g) was the dry-weight minus the ash weight. Determination of % ash content was done by dividing the weight of the ash by the initial dry weight multiplied by

100. For % ash-free content, the weight of the ash-free content was divided by the initial dry weight and multiplied by 100.

Preparation of material for biochemical composition analyses

The material was defrosted (10min) and the tissue (body tissue/gonadal) was placed in pre-weighed, pre ashed crucibles. Material was left in a somehow frozen state to overcome exploding of material due to high salt content. The material was placed in an Alpha 1-2 D_{Plus} freeze dryer until a totally dry powder was obtained. Body tissue took an average of 72hrs and gonads an average of 48hrs to get completely dry. Material was pulverized in a crucible using a mortar and pestle (Figure 4). The powder was easily obtained for gonadal tissue but since umbrella tissue remains skin like, it was pulverised for longer until it was uniform in appearance.

Figure 5. Student pulverising dried material

Biochemical procedures

All glassware that was used in assays was acid washed in 50% HNO_3 and rinsed with distilled water.

Total protein

The technique employed is that adopted by Lucas (1994). It is a modification of the Lowry *et al.,* (1951) method based on the reaction of protein with copper in alkali, followed by reduction of Folin- Ciocalteau reagent.

Using 5g of dried sample gave absorbance readings that were too high and could not be read using the spectrophotometer available. Some modifications were done and 1g of sample was used. After as series of trials, adding 160ml of 1N NaOH gave readings within the standard curve absorbance range. Therefore 160 ml of 1N NaOH was added to 1g of dried sample and the dilution factor was taken into consideration during calculations.

1g of ground dry material was weighed using a Sartorius Analytical Balance into plastic beaker. 160ml of 1N NaOH was added and the mixture homogenised using a blender for 2min (Figure 5). 2ml subsamples were pipette into glass testubes and protein analysis carried out in triplicate.

Figure 6. Student homogenising dried material in 1N NaOH

Reagents

Soln A:	Potassium Sodium Tartrate	2g
	Na_2CO_3	100g
	1N NaOH	500ml
	Distilled water	up to 1L

Soln B:	Potassium Sodium Tartrate	2g
	$CuSO_4.5H_2O$	1g
	1N NaOH	10ml
	Distilled water	90ml

Soln C: Folin – Ciocalteau reagent

1:15 (v/v) F-CR to distilled water

- The 2ml subsamples were heated for 30min at 56˚C in a water bath to dissolve protein
- Each 2ml subsample was diluted duplicate up to 5ml in distilled water
- 0.9ml Soln. A. was added, the solution mixed and warmed to 50˚C for 10min
- After it had been cooled to room temperature, 0.1ml Soln. B was added. a pale lilac colour was observed when mixed.
- The mixture was left to stand at room temperature for 10min before rapidly adding 3ml. Soln. C and stirring on a Vortex mixer. Soln. C was diluted immediately prior to use. A royal blue colour was produced.

- The boiling tubes were covered with parafilm and aluminium foil (to prevent fading of colour) and warmed to 50˚C for 10min (Figure 6).

Figure 7. Testubes covered in parafilm and aluminium foil

- Once cooled to room temperature, samples were transferred to cuvettes and absorbances read at 650nm on a 'Genesys 20' spectrophotometer.
- Total protein was expressed as mg per g dry weight (DW) tissue.
- A distilled water and reagent blank was prepared in the same way as above. Protein concentration was calculated using a standard curve of Bovine Serum Albumin (BSA) V, prepared from a stock solution of BSA (V) and 0.9% w/v KCl, made up to 100µg ml^{-1} concentration.
- The dilution factor was accounted for using the formula;

$$\text{Real concentration} = \frac{Reference\ concentration\ x\ Sample\ Absorbance}{Absorbance\ of\ reference\ concentration}$$

The reference concentration was the stock solutions concentration, 100µg ml^{-1}

Protein Standard Curve

A standard curve was used to determine the composition of protein in each sample. Serial dilutions of 10, 20, 40, 60, 80,100µg/ml were made from the stock solution Bovine Serum Albumin, (BSA) V and 0.9% w/v KCl made up to 100µg/ml concentration. A distilled water and reagent blank (R-B) was also prepared and used to zero the spectrophotometer used in taking absorbance readings. Three replicates were made for each dilution and absorbance read three times for each dilution. Averages were then used to plot a single standard curve of absorbance against concentration in µg/ml (Appendix 3).

Total lipids

Total lipids was first extracted according to Bligh and Dyer (1959) and determined gravimetrically. The method has been used by Lucas (1994) and is chosen here because of its simplicity widespread use on zooplankton.

Some gonadal material was less than the stipulated 5g therefore solvent volumes were adjusted for each sample size to maintain the same proportions according Honeycutt *et al.* (1995).

Lipid Extraction

The samples were homogenised for 2min using a blender with chloroform and methanol in the proportion of 1g tissue: 1ml chloroform: 2ml methanol. An additional equivalent amount of chloroform was added and the mixture was homogenised for another 30 seconds. Deionised water (1ml: 1g tissue) was then added and the mixture homogenised again for another 30 seconds. The final mixture proportion was 1g tissue: 2ml chloroform: 2ml methanol: 1ml deionised water.

The mixture was then filtered through Whatman No.1 filter paper (Figure 7), and the remaining tissue was homogenised for 2min with 1ml chloroform: 1g tissue. After filtering the mixture again, the combined filtrate was transferred to centrifuge tube and the extract purified according to Lucas (2008).

Figure 8. Lipid extraction using the Bligh and Dyer (1959) method

Lipid purification

Figure 9. Lipid purification (Lucas, 2008). Figure shows a distinct upper phase and lower phase solution containing lipids.

Reagents:

2:1 v/v chloroform-methanol

0.05N KCl

Upper-phase solution – 8 parts $CHCl_3$

4 parts CH_3OH

3 parts KCl

- 1ml of 0.05N KCl was added to bring about dissociation of bound acid, i.e. lipid which would otherwise remain in aqueous phase.
- Samples were centrifuged for 10min.
- The upper aqueous phase containing non-lipid contaminants including amino acids was removed
- The washing process with KCl was repeated. Resulting lower phase was washed with upper-phase solution to further purify the lipid extract, and centrifuged again for 10min

- The upper phase was again removed and the lower phase containing lipids was dissolved in chloroform.
- Mixture was transferred into pre-weighed, acid washed glass vials.
- Vials were left in a dark fume cupboard until the solvent had totally evaporated (approximately 30hours). Tare weight of vials was subtracted from the weight after drying to determine lipid content.

Total carbohydrates

The most common method for the determination of simple sugars and their derivatives in zooplankton is the colorimetric phenol-sulphuric acid method of Dubois *et al*. (1956) the method is suitable for gelatinous material because of its sensitivity to micro-quantities of sugar and has been employed by Arai *et al.* (1989) on *A. victoria.* A modification of the method will be used which is based on a condensation reaction producing a stable orange precipitate when phenol and conc. sulphuric acid are added. Modified method was employed by Lucas (1994) on *A. aurita* and (2008) on *P. periphylla.*

Some modifications were done for this study as using 5g of dried sample gave absorbance readings that were too high to be read by the 'Genesys 20' spectrophotometer that was used. Some modifications were done and 1g of sample was used. After as series of trials, adding 100ml of distilled water gave readings within the standard curve absorbance range. Therefore 100 ml of distilled water were added to 1g of dried sample and the dilution factor was taken into consideration during calculations.

1g of ground dry material was weighed into a plastic beaker using an Analytical Balance. 100ml of distilled water was added and the mixture homogenised using a blender for 2min. Using a pipette 2ml subsamples were obtained and carbohydrate analysis done in triplicate.

Reagents

5% w/v phenol

5g l^{-1} hydrazine sulphate in conc. H_2SO_4

- 0.4ml phenol was rapidly added followed by 2ml hydrazine sulphate to each 2ml homogenate subsample. An orange precipitate was observed

(a) *(b)*

Figure 10 *(a, b).* An orange precipitate was observed

- The samples were mixed using a Vortex mixer and covered with parafilm and aluminium foil and left to stand at room temperature for 30min.

- Samples were transferred to cuvettes and absorbance read at 490nm on a 'Genesys 20' spectrophotometer.
- Sugar content was expressed as mg sugar per g dry weight (DW) tissue
- A distilled water and reagent blank was prepared in the same way as above.
- Sugar content was calculated using a standard curve of D-glucose, prepared from a stock of 50µg ml^{-1} and real concentration calculated using the formula stated above.

Carbohydrate Standard curve

A standard curve was used to determine the composition carbohydrates in each specimen sample. Serial dilutions made from a stock solution of 50µg/ml D-glucose were prepared to concentrations of 10, 20, 30, 40 and 50µg/ml. A distilled water and reagent blank (R-B) was

also prepared and used to zero the spectrophotometer where absorbance was read at 490nm. . 3 replicates were made for each dilution and absorbance read three times for each dilution. Averages were then used to plot a single standard curve of absorbance against concentration in µg/ml (Appendix 4).

CHAPTER THREE – LABORATORY AND STATISTICAL RESULTS

Figure 11. Student carrying out biochemical tests

Figure 12. Student reading Absorbance values from a 'Genesys 20' Spectrophotometer

Laboratory Results

Mean values for Dry Weight, Water content, Ash weight and Ash-free dry weight from whole individual results (Appendix 9. Table 13a.)

Table 2. Size and weight results

Parameter	**Minimum**	**Maximum**	**Mean ± s.d**
Bell diameter (cm)	28	62	43.1 ± 10.6
Wet weight (g)	1094	8151	4221 ± 2649.9
Dry Weight (DW) (g)	39.7	448.9	183.6 ± 13701
% DW	3.1	5.5	3.9 ± 0.7
Water content (g)	1054.3	8150.8	4185.5 ± 2682.8
% Water	94.5	96.9	96.01 ± 0.7
Ash weight (g)	26.3	315.7	116.4 ± 88.4
% Ash weight	50.9	71.6	63.8 ± 5.8
Whole Ash-free DW (g)	13.3	175.8	67.2 ± 53.7
% Ash-free dry weight	28.4	49.1	36.2 ± 5.8

Whole jellyfish had a high water (96.01%) and mineral ash content (63.8%).

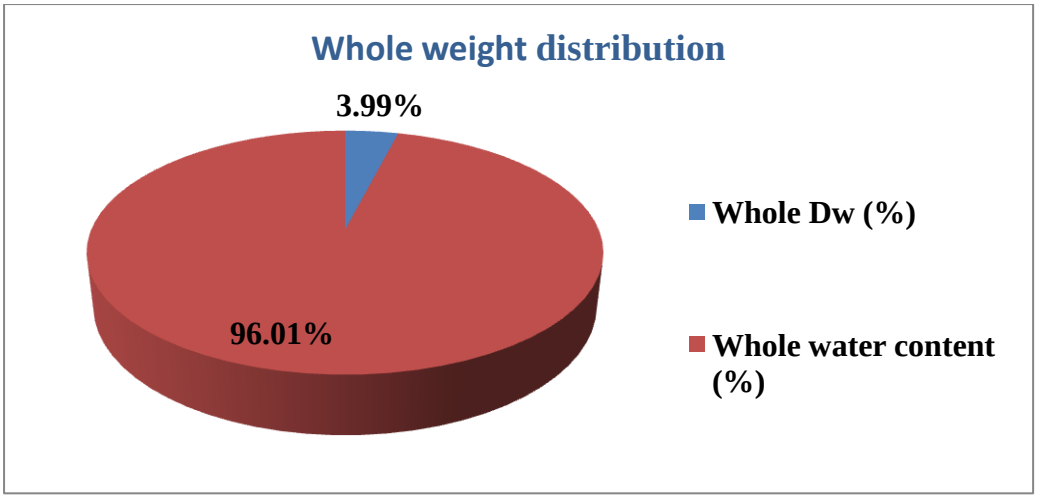


Figure 13. Percentage composition of water and dry matter in wet mass

C. hysoscella, like other jellyfish species, has a high water content (96.01%) and little dry matter (3.99%) in its body composition by mass.

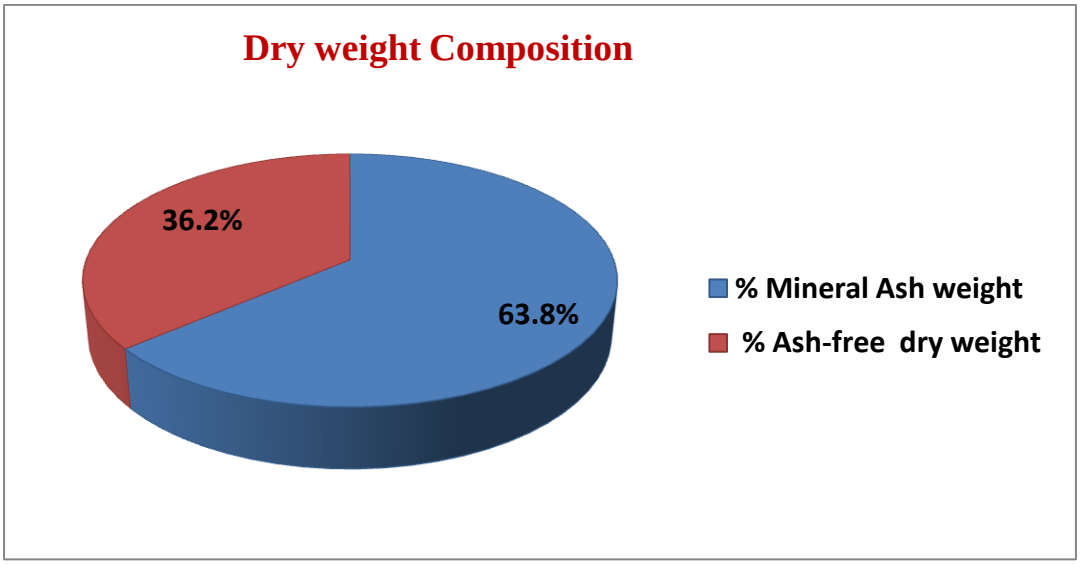


Figure 14. Percentage composition of Ash and Ash-free matter in dry mass

Of the small amount of dry matter in the jellyfish, (3.99%), most of it is mineral ash which makes up 63.8% on average. Therefore, the biochemical parameters measured (proteins, lipids and carbohydrates) are composed within the lower 36.2% ash-free dry matter by mass of total dry weight of each individual.

Biochemical composition results

Table 3. Mean Protein, Lipids and Carbohydrate Results for whole jellyfish (refer to Appendix 8. Table 9a for each specimen's results)

Parameter	**Sample size (N)**	**Range**	**Mean ± SD**
Total Protein			
mg/g DW	12	15.2 mg/g DW - 253.5 mg/g DW	111.7 ± 81.26
% DW composition	12	1.5 %-7.9 %	4.6 ± 2.85
% WW composition	12	0.05%- 0.35%	0.18 ± 0.11
Total Lipids			
mg/g DW	12	4.94 mg/g DW - 91.08 mg/g DW	33.62 ± 31.26
% DW composition	12	0.32%-7.91 %	1.51 ± 2.08
% WW composition	12	0.01%- 0.11%	0.043 ± 0.03
Total Carbohydrates			
mg/g DW	12	0.30mg/g DW - 8.67mg/g DW	4.64 ± 3.179
% DW composition	12	0.03 %-7.91 %	0.79± 2.245
% WW composition	12	0.0009%- 0.0.0160%	0.0058 ± 0.004

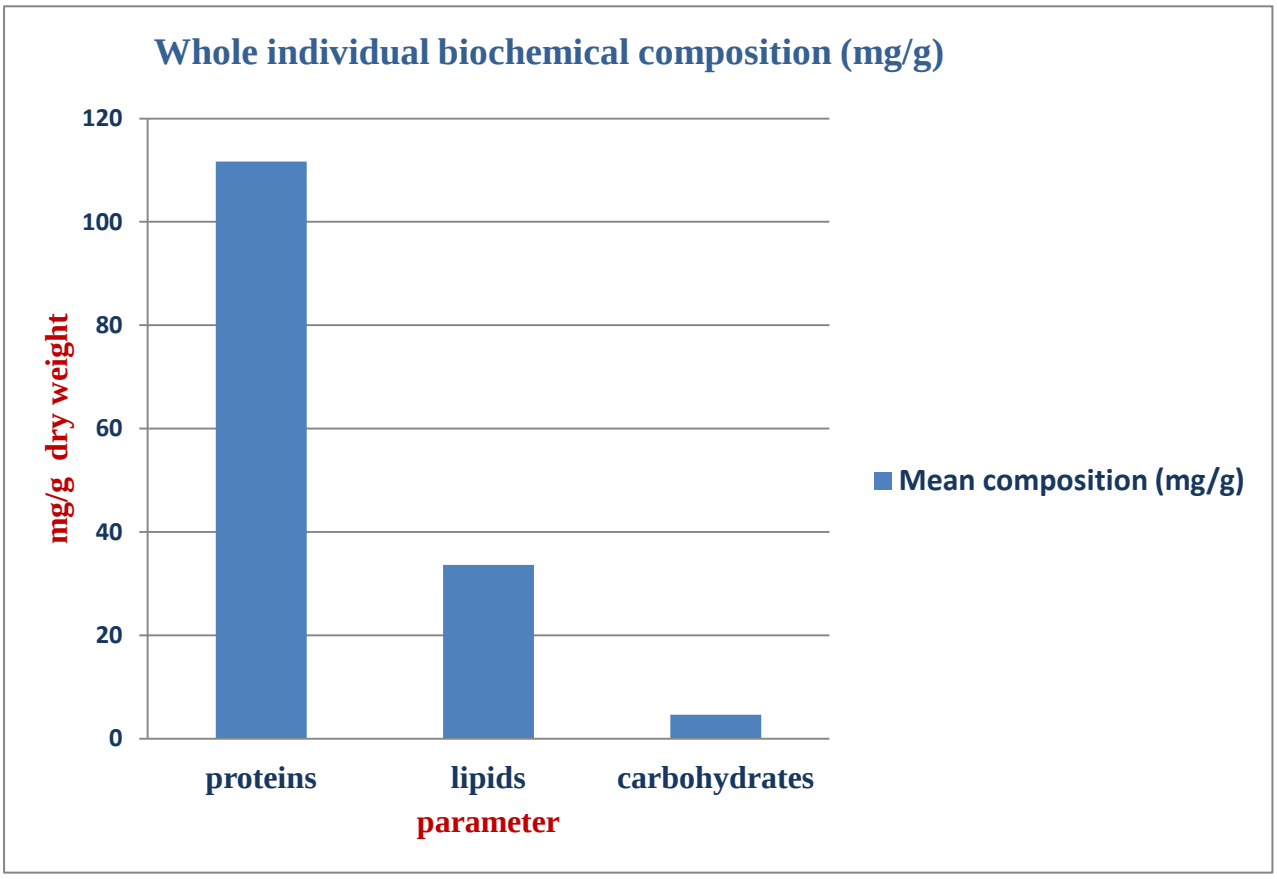


Figure 15. Comparison of biochemical composition for all collected individuals

Results show the trend of high protein, intermediate lipid and low carbohydrate content .The maximum protein lipid and carbohydrate composition was 253.5 mg/g DW, 91.08 mg/g DW and 8.670 mg/g DW respectively. Mean percentage composition of the total wet weight (0.18%; 0.043%; 0.0058% for protein, lipids and carbohydrates respectively) is much lower than the respective mean percentage composition of total dry weight (4.6%; 1.51%; and 0.79%).

Separate Component Results (Gonadal against body tissue)

Table 4. Gonadal against body tissue biochemical composition in mg/g of dry weight

Biochemical component	**Mean ± SD mg/g DW composition**	
	Gonads	**Body Tissue**
Proteins	127.6 ± 60.82	34.7 ± 25.93
Lipids	44.9 ± 28.20	6.5 ± 5.85
carbohydrates	6.007 ± 0.373	1.129 ± 0.793

The composition of gonads for all the three assays (proteins, lipids and carbohydrates) is higher than that of the body tissue. However, the trend of high protein, intermediate lipid and low carbohydrates is evident for both gonadal and body tissue composition.

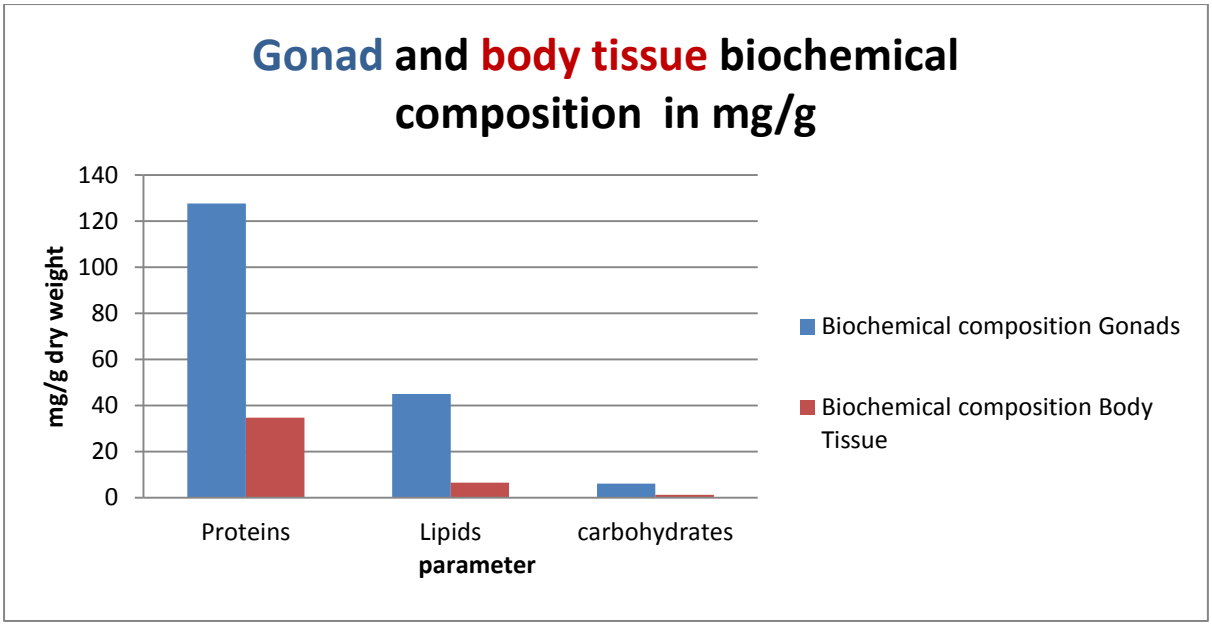


Figure 16 . Body tissue and gonadal biochemical composition in mg/g DW

Table 5. Contrasting mean percentage component results (Refer to Appendix 10. Table 15a. for individual values)

Parameter	**Mean ± SD % composition**	
	Gonads	**Body tissue**
Water content	91.32 ± 3.97	96.03 ± 0.83
Dry weight	8.68 ± 3.97	3.97 ± 0.83
Ash weight	41.95 ± 6.91	66.44 ± 6.19
Ash-free Dry weight	58.05 ± 6.91	33.56 ± 6.19
Protein	12.76 ± 6.08	3.48 ± 2.59
Lipids	4.49 ± 2.82	0.65 ± 0.59
carbohydrates	0.60 ± 0.04	0.11 ± 0.08

Gonads have less water content and less mineral ash content than body tissue does. Consequently, gonadal mean percentage dry weight and ash-free dry weight is higher than that of body tissue. Protein, lipid and carbohydrate mean percentage content is also higher in gonadal tissue than in the body tissue which was a combination of the bell and oral arms.

Statistical Results

Data was entered into Microsoft Excel worksheets and then loaded into GENSTAT 7.1 statistical software for all statistical analyses (refer to Appendix 11 for all Analysis of Variance (ANOVA) probability values).

Clear linear relationships were found between whole individual size (WW, DW, AW, and AFDW) with lipid and carbohydrate composition ($p < 0.05$). These clear linear relationships suggest that the above-mentioned size- weight measurements, which are cheap and easy to measure, may provide a good proxy for jellyfish lipid composition were data is lacking. However there were no significant linear relationships between each of the above-mentioned morphometric measurements and protein composition ($p > 0.05$).

There were also clear linear relationships found between gonad size (WW, DW, AW, and AFDW) and lipid composition (Regression ANOVA: $F_{1, 10}$ <0.001, p< 0.05). However there were no significant linear relationships between each of the gonadal size-weight measurements and protein or carbohydrate composition (p > 0.05) except for gonad wet weight and carbohydrate content (Regression ANOVA: $F_{1, 10}$ = 0.012, p< 0.05). These results may have been because there was a high degree of variation in the data.

Importantly the statistical results highlight significant differences in biochemical composition between the separated body components. In the 7 medusae used for separate component analysis, there were significant differences between gonad tissue and body tissue (p< 0.05).

The body tissue (which includes bell and oral arm tissue) had approximately 2 times less dry and ash-free matter and approximately 5 times less protein, lipids and carbohydrate percentage composition relative to component mass compared to the gonad tissue (Table 5). In essence, this statistical data reiterates the importance for collecting biochemical composition data for the species associated body component tissue (Tierney *et al*., 2002).

Comparing the percentage composition, protein was by far the largest fraction of organic matter present in *C. hysoscella* dried samples, which contained intermediate and minor

amounts of lipids and carbohydrates respectively. The protein ranged from 1.5% to 7.9% DW for whole specimens (Table 2) and varied significantly between component tissues. (ANOVA: F_{12}=0.003, p<0.05). The gonads had far greater protein content than did the body tissue (mean ± S.D expressed as % DW: gonads = 12.76 ± 6.08, body tissue = 3.48 ± 2.59) (Table 6). This is mainly because protein is the main storage product in *C. hysoscella.*

The total lipid content ranged from 0.32% to 7.91% DW for whole specimens (Table 2) and varied significantly between component tissues. (ANOVA: F_{12}=0.004, p<0.05). The gonads had far greater lipid content than did the body tissue (mean ± S.D expressed as % DW: gonads = 4.49 ± 2.82, body tissue = 0.65 ± 0.59) (Table 6). The relatively smaller amount of lipids is because they have a more structural role in cells and are not the main storage product.

The total carbohydrate content ranged from 0.03 %-7.91 % DW for whole specimens (Table 2) and varied significantly between component tissues. (ANOVA: $F_{12}<0.001$, $p<0.05$). The gonads had far greater carbohydrate content than did the body tissue (mean ± S.D expressed as % DW: gonads = 0.60 ± 0.04, body tissue = 0.11 ± 0.08) (Table 6). Carbohydrates are the lowest in composition mainly because the diet of jellyfish in the aquatic ecosystem offers very little carbohydrates as they are carnivorous.

In addition to that, gonad sizes also varied significantly ($p< 0.05$) from body tissue sizes;

- wet weight; ANOVA: $F_{1,10} =0.010$,
- dry weight ANOVA: $F_{1,10}=0.010$,
- ash weight ANOVA: $F_{1,10} <0.001$,
- ash-free dry weight ANOVA: $F_{1,10} <0.001$.

CHAPTER FOUR –DISCUSSION AND CONCLUSION

Figure 17. The bell of *C. hysoscella*

Discussion

A total of 12 *C. hysoscella* undamaged medusae were captured on the cruise ranging in size from 28 to 62cm bell diameter. The catch comprised of 5 immature (no visible gonads) and 7 female individuals with visible gonads. Mean water content for whole jellyfish ranged from 94.47% to 96.94% (mean ±S.D 96.01 ± 0.7%). This is similar to a previously published mean value of 96.2% for *C. hysoscella* from a jetty in Dingle Harbour, Ireland (Doyle *et al*., 2007).

Dry mass for whole medusae (N=12) ranged from 3.06 % to 5.53% of wet weight (mean ± S.D= 3.99 ±0.7). Ash-free dry weight varied between 28.43% and 49.11% of dry weight (mean ±S.D = 36.21 ±5.8). The dry mass is almost similar to that of Doyle *et al*. (2007) for the same species in Ireland (mean 3.8%) and within close proximity to previously published values for different species of jellyfish (mean 5.49%, range 1.12% – 10.53% for *P. periphylla* from the Gulf of Mexico (Lucas, 2008); mean 3.24 ± 0.2%, range 2- 3.9% for *P. periphylla* from the Norwegian fjords (Youngblouth & Bamstedt, 2001); and mean 4.92 ± 0.28% for *Atolla wyvillei* (Clarke *et al*., 1992). The high ash contents 50.89% - 71.57% (mean 63.8%) is typical of all gelatinous plankton and is similar to that of a related species *C. capillata* from the Dingle Harbour, Ireland which had a mean of 67.8% (Doyle *et al*., 2007). The ash values are also comparable with results obtained for a different species *P. periphylla* from the Gulf of Mexico whose values ranged between 65 – 75% (Lucas, 2008). These high ash content results however, are biased by 'water of hydration' which is water retained during drying at 70 ˚C and lost during ignition at 550 ˚C (Lucas, 2008).

Although proximate analysis is frequently used to quantify the biochemical composition of a wide range of taxa, estimates for jellyfish (and other gelatinous plankton) have previously been problematic due to the low energy density of samples, uneven distribution of inorganic matter in dried samples and the fact that some residual/bound 'water of hydration' always

remains after the drying process (Clarke *et al*., 1992; Lucas, 1994; Arai, 1997). These results can be considered to be close to the composition of jellyfish without accounting for the bound water of hydration.

The estimates of biochemical composition for proteins, lipids and carbohydrates ranged from 15.23 to 253.5 mg/g DW, 4.940 to 91.08 mg/g DW and 0.3005 to 8.670 mg/g DW respectively. These results are comparable to previously published estimates obtained for biochemical composition of a jellyfish species, *P. periphylla* from the Gulf of Mexico; which ranged between 34.14 to 108.21mg/g DW protein; 1.53 to 48.12 mg/g DW lipids; 5.06 to 14.45 mg/g DW carbohydrates (Lucas, 2008).

Jellyfish may have up to 58 times less energy per gram of wet mass than herring flesh (Doyle *et al*., 2007). These biochemical composition estimates confirm that jellyfish have a very low nutritional content and this low biochemical composition is a combination of (i) the high mineral ash content so that the compositions per g of dry mass are low and (ii) the high water content which means that low biochemical compositions per g of dry mass translate to even lower relative compositions on a wet mass basis.

The findings of this study confirm the salient findings of Doyle *et al*. (2007) suggesting that the difference in biochemical composition between the different jellyfish tissues may have some bearing on the foraging decisions of jellyfish predators. The species' parasite *Hyperia medusarum* (Buecher *et al*., 2001) was found in very large quantities in the gonadal tissue. The preferred food for these Hyperiid amphipods that are facultative parasites of jellyfish is the gonads and *vis-a-vis* the confirmed higher nutritional content in gonads compared to other jellyfish components, this research supports the assertion that variation in the quality of prey tissues may alter feeding behaviour (Doyle *et al*., 2007). Considering an approximately 5 fold difference in the biochemical composition (proteins, lipids and carbohydrates) of *C. hysoscella*, it would be interesting to observe if the new predominant prey species (Utne-

palm *et al*., 2010) and recently discovered predator for jellyfish, *Sufflogobius bibarbatus* (commonly known as the bearded goby) selectively targets the more nutritious gonads.

Conclusion

The biochemical composition (total proteins, total lipids and total carbohydrates) of whole fresh medusae (28 to 62cm bell diameter) shows the typical gelatinous zooplankton trend (Lucas, 2008) of low carbohydrates (mean 4.64 mg/g DW), intermediate lipids (mean 33.62 mg/g DW) and high protein (111.7mg/g DW) content was observed although there was a high level of variability. In *C. hysoscella* from the Benguela, mean contents as a percentage of wet mass were 0.18% protein, 0.043% lipids and 0.0058% carbohydrates. These values are approximately 5 times lower than those for *A. wyvillei* from the Southern Ocean which according to Lucas (2008) are similar to those reported for a variety of coastal and shallow water species and the few published values for mesopelagic and deep sea species.

Recommendations

For this research, the size distribution of species was biased toward larger jellyfish; I would recommend that any following work take into account the need to include even small sized jellyfish as this may have an effect on the mean biochemical composition and statistical

results. Given more time, subsequent studies with larger sample sizes should be carried out so that the statistical results are undoubtedly a true representation of the population.

Furthermore, component studies where all three components namely the bell, oral arms and gonad tissue are analysed separately should be done. Combining them was effective for gonadal studies but separating them would further help to compare them as distinct components as there could be significant differences between bell and oral arm composition.

Finally, for this study protein and carbohydrate assays were done using 1g of dried tissue which may played a role in the high level of variation for protein and carbohydrate results. I would recommend that a study be done to find out the effect of dried sample size used on the biochemical composition. A build up on what has been currently established in this research should be done to come up with the best research methodology that works for the species, *C. hysoscella* of the Benguela ecosystem.

Addition to knowledge

Although the total organics of *C. hysoscella* are overally very low, the species has relatively a high protein, very low carbohydrate and intermediate lipid content. In the event of the jellyfish having visible gonads, the organism's gonadal tissue had significantly higher biochemical composition than the body tissue. These jellyfish can be safely included among the least nutritious organisms in the aquatic environment in terms of protein, lipid and carbohydrate content. This may explain why for a long time they have been thought to be trophic dead ends as only a few known organisms to date (for example the leather back turtles) had been found to feed on jellyfish. With their recent increase in biomass, jellyfish play potentially major controlling roles in marine ecosystems and marine ecosystem managers and modellers cannot afford to ignore them. This research is a timely response to the unquestionable need for data.

References

- Arai, M.N., 1997. *A Functional Biology of Scyphozoa*. Chapman & hall: London

- Bakun, A. and Weeks, S. J. (2006). Adverse feedback sequences in exploited marine systems: are deliberate interruptive actions warranted? *Fish and Fisheries*. **7**: 316–333

- Brierley, A.S., Axelsen, B. E., Beucher, Sparks, C. A. J., Boyer, H., and Gibbons, M. J. 2001. Acoustic observations of jellyfish in the Namibian Benguela. *Marine Ecology Progress.* **210**: 55-66

- Buecher, E., Sparks, C., Brierley, A., Boyer, B., and Gibbons, M. 2001. Biometry and size distribution of *Chrysaora hysoscella* (Cnidaria, Scyphozoa) and *Aequorea aequorea* (Cnidaria, Hydrozoa) off Namibia with some notes on their parasite Hyperia medusarum. *Plankton research.* **23**(10): 1073-1080

- Clarke, A., Holmes, L. J. and Gore, D. J., 1992. Proximate and elemental composition of gelatinous zooplankton from the Southern-Ocean. *Experimental Marine biology and Ecology*. **155**: 55-68

- Doyle, T.K., Houghton, J.D.R. McDevitt, R. Davenport J. And Hays. G. C. 2007. The Energy Density of Jellyfish: Estimates from Bomb Calorimetry and Proximate – Composition. *Experimental Marine Biology and Ecology*. **343**: 239-252

- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956.Calorimetric method for determination of sugars and related substances. *Analytical Chemistry*. **28**: 350 - 356

- Honeycutt. M. E., McFarland, A.and McCant, D. D. 1995. A Comparison of Three Lipid Extraction Methods. *U.S Army Engineer Waterways Experiment Station.* **1**: 35-42

- Lowry, O.H., Rosbrough, N.J., Farr, A.L. and Randall R.J. 1951. Protein measurement with the bolin phenol method. *Biology and Chemistry*. **193**:265-275

- Lucas, C.H., 1994. Biochemical composition of *Aurelia aurita* in relation to age and sexual maturity. *Experimental Marine Biology and Ecology*. **183**:179–192

- Lucas, C.H., 2008. Biochemical composition of the mesopelagic coronate jellyfish *Periphylla periphylla* from the Gulf of Mexico. *Marine and Biological Association of the United Kingdom.* **86**: 77-81

- Lynam, C. P. Gibbons, M.J. Axelsen, C.A.J., Sparks, J., Coetzee, B.G. Heywood, and Brierley, A.S. 2006. Jellyfish overtake fish in a heavily fished ecosystem. *Current Biology*. **16**(13): 492-494

- Pages, F., Gili, J., & Bouillon, J. (1992). Medusae (Hydrozoa, Scyphozoa, Cubozoa) of the Benguela Current (Southeast Atlantic). *Scientia Marina*. **56**(1): 1-64

- Petersen, P.L, Honig, M.B., Ryan, P.G. and Underhill, L.G. (2009). Seabird bycatch in the pelagic longline fishery off Southern Africa. *African Journal of Marine Science*. **31**(2): 191–204

- Tierney, M., Hindell, M. A.and Goldsworthy, S. 2002. Energy content of mesopelagic fish from Macquarie Island. *Antarctica science*. **14**: 225–230

- Utne- Palm, A. C., Salvanes, A. G. V., Currie, B., Kaartvedt, S., Braithwaite, V. A., Nilsson, G. E., Stecyk J. A. W., Hundt, M., Van der Bank, M., Flynn, B., Sandvik, G. K., Klevjer, T. A., Sweetman, A. K., Bruchert, V., Pittman, K., Peard, R. K., Lunde, I. G., Strandabo, R. A. U. and Gibbons, M. J. 2010. Trophic Structure and Community Stability in an Overfished Ecosystem. Retrieved on July 25 2010 from http://www.sciencemag.org/vol-329

- Youngblouth M. J., and Bamstedt U. (2001) Distribution, Abundance, Behaviour and Metabolism of *Periphylla periphylla, a* mesopelagic coronate medusae in a Norwegian Fjord. *Hydrobiologia*. **453**: 221-333

APPENDIX

APPENDIX LIST OF CONTENTS

Appendix

1. Materials

- Alpha 1-2 D plus Freeze dryer
- Crucibles
- Folin-Ciocalteau reagent
- Potassium sodium tartrate
- Water bath
- NaOH
- $CuSO_4.5H2O$
- Vortex mixer
- Parafilm and aluminium foil
- Cuvettes
- Genesys 20 spectrophotometer
- Philips blender (Glass rod homogeniser is ideal)
- Bovine Serum Albumin (BSA)
- KCl
- Phenol
- Pipettes
- Hydrazine Sulphate
- H_2SO_4
- Jellyfish tissue powder
- D-glucose
- Chloroform
- Methanol
- Baird and Tatlock autobench Centrifuge
- Oven
- Desiccator
- Sartorius Electronic Analytic balance
- Muffle furnace
- Dissecting kit
- Boat and skipper
- Plankton net
- 25l buckets and cooler boxes
- 25L containers
- Measuring tape and field balance
- Plastic bags, waterproof stickers and permanent markers
- Blotting paper
- Whatman No.1 filter paper

2. Trawl Sampling Sheet (July 2010 Oceanographic Monitoring & Luderitz sampling cruise aboard R.V Welwitschia (MFMR; NatMIRC).

3. Standard Curves

Absorbances read using a Genesys 20 spectrophotometer

- **Protein**
  Using a standard curve of Bovine Serum Albumin (BSA) V, prepared from a stock solution of BSA (V) and 0.9% w/v KCl, made up to 100µg ml^{-1} concentration.

Table 2.a. Serial Dilutions and Mean Absorbencies for BSA stock solution

Concentration (µg/ml)	Mean Absorbance
R-B	-0.056
10	0.034
20	0.066
40	0.123

60	0.187
80	0.236
100	0.294

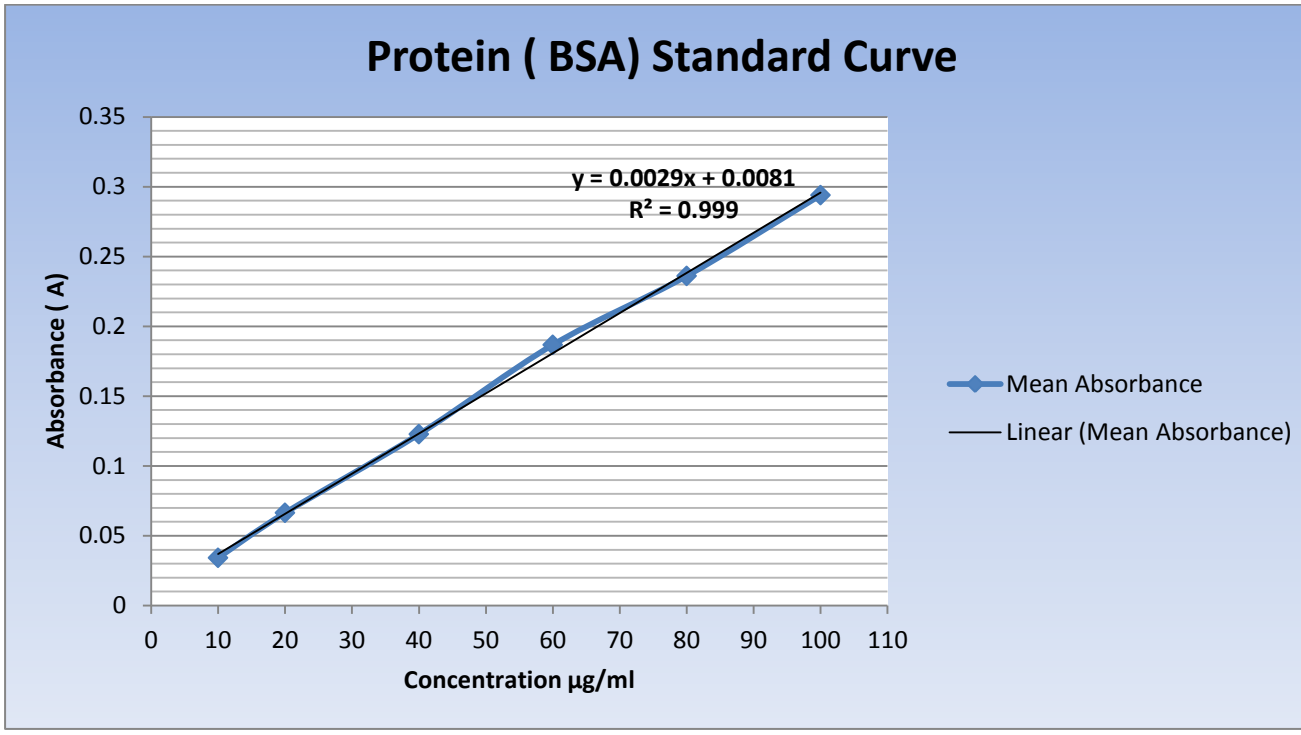


Figure 18a. Protein Standard Curve

4. Carbohydrates

Using a standard curve of D-glucose, prepared from a stock of 50µg ml^{-1}.

Table 3a. Serial dilution concentrations and mean absorbance (A)

Concentration (µg/ml)	Mean zeroed Absorbance (A)
R-B	0.269
5	0.016
10	0.023
20	0.044
40	0.102
50	0.142

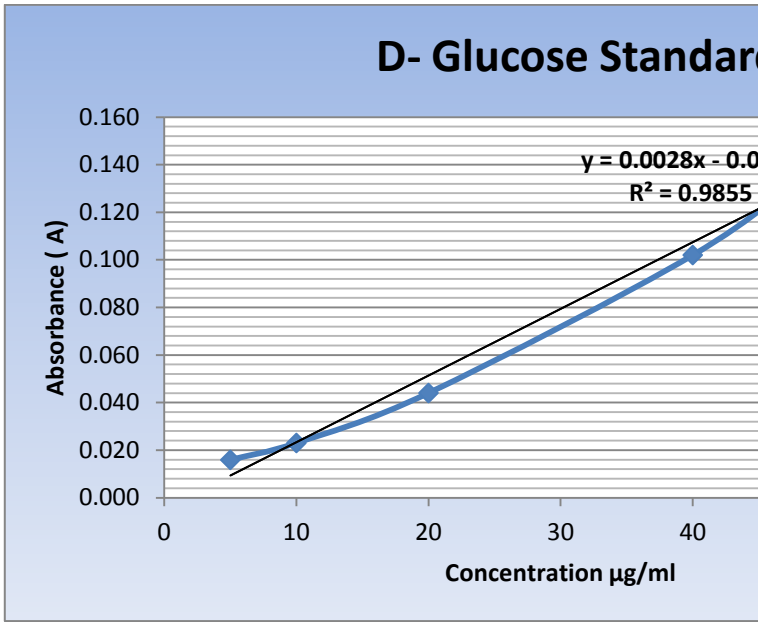


Figure 19a. Carbohydrate Standard curve

5. Raw Data For Separated Components

Table 4a. Mean absorbance readings for proteins (Jellyfish without gonads = Tr.A – Tr.E; Jellyfish with removed gonads = Tr.G1 - TrG7; Gonads = G1 – G7

Specimen ID	Absorbance				Absorbance				Absorbance				Overall Mean (A)
	Trial 1	Trial 1	Trial 1	Mean	Trial 2	Trial 2	Trial 2	Mean	Trial 3	Trial 3	Trial 3	Mean	
Tr.A	0.259	0.255	0.261	0.258	0.24	0.239	0.24	0.240	0.166	0.168	0.164	0.166	0.221
Tr.B	0.221	0.218	0.219	0.219	0.234	0.235	0.236	0.235	0.243	0.248	0.244	0.245	0.233
Tr.C	0.049	0.052	0.056	0.052	0.049	0.052	0.056	0.052	0.056	0.057	0.056	0.056	0.053
Tr.D	0.049	0.051	0.048	0.049	0.046	0.041	0.043	0.043	0.048	0.045	0.045	0.046	0.046
Tr.E	0.048	0.055	0.046	0.050	0.036	0.038	0.036	0.037	0.042	0.053	0.049	0.048	0.045
G1	0.294	0.293	0.293	0.293	0.445	0.444	0.443	0.444	0.143	0.142	0.142	0.142	0.293
G2	0.491	0.491	0.49	0.491	0.641	0.64	0.643	0.641	0.544	0.548	0.549	0.547	0.560

G3	0.091	0.092	0.092	0.092	0.412	0.419	0.409	0.413	0.395	0.39	0.389	0.391	0.299
G4	0.502	0.499	0.498	0.500	0.569	0.57	0.57	0.570	0.428	0.43	0.429	0.429	0.499
G5	0.167	0.165	0.166	0.166	0.154	0.152	0.152	0.153	0.167	0.167	0.168	0.167	0.162
G6	0.758	0.771	0.766	0.765	0.562	0.565	0.564	0.564	0.503	0.495	0.499	0.499	0.609
G7	0.212	0.211	0.209	0.211	0.146	0.149	0.15	0.148	0.253	0.251	0.248	0.251	0.203
Tr G1	0.104	0.11	0.118	0.111	0.024	0.022	0.022	0.023	0.021	0.023	0.024	0.023	0.052
Tr G2	0.491	0.49	0.49	0.490	0.06	0.06	0.061	0.060	0.007	0.006	0.006	0.006	0.186
Tr G3	0.051	0.042	0.045	0.046	0.024	0.026	0.026	0.025	0.624	0.622	0.615	0.620	0.231
Tr G4	0.035	0.031	0.034	0.033	0.075	0.075	0.075	0.075	0.024	0.022	0.023	0.023	0.044
Tr G5	0.071	0.072	0.072	0.072	0.077	0.079	0.076	0.077	0.054	0.053	0.058	0.055	0.068
Tr G6	0.046	0.043	0.035	0.041	0.026	0.024	0.025	0.025	0.038	0.045	0.046	0.043	0.036
Tr G7	0.109	0.109	0.109	0.109	0.125	0.124	0.124	0.124	0.063	0.063	0.064	0.063	0.099

- **Protein Results for Combined components**

Table 5a. Protein Results for Combined components

I.D	Gonad content mg/g	Gonad DW (g)	Total gonadal content (mg)	Body tissue content mg/g	Body tissue DW (g)	Total body tissue content (mg)	mg/g DW, whole biochemical content	Total content (mg)	Total content (g)	Jellyfish DW (g)
Tr G1	99.7	6.2	615.4	17.7	156.5	2768.3	117.4	3383.7	3.38	162.69
Tr G2	190.4	6.0	1137.9	63.2	68.5	4323.1	253.5	5460.9	5.46	74.43
Tr G3	101.6	5.4	547.3	78.4	173.1	13577.5	180.0	14124.8	14.12	178.52
Tr G4	169.9	26.9	4572.2	14.9	269.3	4009.5	184.8	8581.7	8.58	296.19
Tr G5	55.1	38.2	2106.0	23.1	279.0	6454.2	78.2	8560.2	8.56	317.27
Tr G6	207.2	74.93	15526.0	12.4	283.0	3508.2	219.6	19034.1	19.03	357.93
Tr G7	69.1	17.4	1203.0	33.6	431.5	14480.9	102.7	15683.9	15.68	448.90

6. Carbohydrate Absorbance Readings

Table 6a. Carbohydrate Absorbance Readings

Specimen ID	Absorbance				Absorbance				Absorbance				Overall Mean (A)
	Trial 1	Trial 1	Trial 1	Zeroed Mean	Trial 2	Trial 2	Trial 2	Zeroed Mean	Trial 3	Trial 3	Trial 3	Zeroed Mean	
Tr.A	0.559	0.56	0.559	0.480	0.507	0.514	0.51	0.431	0.517	0.524	0.518	0.441	0.45
Tr.B	0.294	0.293	0.293	0.214	0.47	0.47	0.471	0.391	0.299	0.3	0.298	0.220	0.28
Tr.C	1.432	1.445	1.42	1.163	0.62	0.62	0.62	0.351	0.569	0.568	0.569	0.300	0.60
Tr.D	0.314	0.313	0.313	0.234	0.288	0.288	0.288	0.209	0.242	0.242	0.242	0.163	0.20
Tr.E	0.166	0.166	0.166	0.087	0.147	0.147	0.147	0.068	0.18	0.181	0.179	0.101	0.09
G1	1.813	1.814	1.816	1.545	2.128	2.131	2.127	1.860	1.752	1.753	1.753	1.484	1.63
G2	1.823	1.825	1.826	1.556	2.057	2.059	2.06	1.790	1.704	1.7	1.701	1.433	1.59
G3	1.947	1.949	1.95	1.680	2.227	2.226	2.226	1.957	2.02	2.021	2.021	1.752	1.80
G4	1.927	1.92	1.926	1.655	2.338	2.351	2.343	2.075	2.178	2.179	2.18	1.910	1.88
G5	1.857	1.859	1.859	1.589	1.78	1.786	1.787	1.515	1.988	1.991	1.987	1.720	1.61
G6	1.883	1.882	1.882	1.803	1.86	1.858	1.859	1.780	1.68	1.679	1.68	1.601	1.73
G7	1.929	1.928	1.93	1.660	1.918	1.92	1.92	1.650	2.079	2.08	2.08	1.811	1.71
Tr G1	0.437	0.439	0.44	0.170	0.593	0.593	0.594	0.324	0.453	0.454	0.471	0.190	0.23
Tr G2	0.66	0.661	0.661	0.392	0.626	0.626	0.627	0.357	0.682	0.684	0.686	0.415	0.39
Tr G3	0.393	0.397	0.394	0.126	0.372	0.372	0.373	0.103	0.386	0.387	0.387	0.118	0.12
Tr G4	0.66	0.67	0.664	0.396	0.637	0.645	0.644	0.373	0.575	0.575	0.572	0.305	0.36
Tr G5	0.622	0.625	0.652	0.364	0.672	0.673	0.673	0.404	0.466	0.466	0.466	0.197	0.32
Tr G6	0.175	0.175	0.174	0.096	0.146	0.145	0.146	0.067	0.15	0.149	0.15	0.071	0.08
Tr G7	1.347	1.351	1.357	1.083	0.792	0.797	0.793	0.525	0.926	0.928	0.927	0.658	0.76

- **Carbohydrates Results for Combined components**

Table 7a. Combined components results for carbohydrates

I.D	Gonad content mg/g	Gonad DW (g)	Total gonadal content (mg)	Body tissue content mg/g	Body tissue DW (g)	Total body tissue content (mg)	mg/g DW, whole biochemical content	Total content (mg)	Total content (g)	Jellyfish DW (g)
Tr G1	5.7	6.2	35.41	0.8	156.5	125.71	6.54	161.1	0.161	162.6
Tr G2	5.6	6.0	33.52	1.4	68.5	93.52	6.97	127.0	0.127	74.4
Tr G3	6.3	5.4	34.06	0.4	173.1	70.45	6.73	104.5	0.105	178.5
Tr G4	6.6	26.9	178.18	1.3	269.3	339.33	7.88	517.5	0.518	296.1
Tr G5	5.7	38.2	216.42	1.1	279.0	315.95	6.79	532.4	0.532	317.2
Tr G6	6.1	74.9	455.89	0.3	283.0	77.39	6.36	533.3	0.533	357.9
Tr G7	6.0	17.4	104.60	2.7	431.5	1147.44	8.67	1252.0	1.252	448.9

7. Lipid Gravimetric analysis Results

Table 8a. Lipid Laboratory results

ID	Tare weight	Final weight	Lipid content	total sample content mg	mg/g DW	whole DW (g)	Whole DW (mg)	Total lipids (mg)	% DW of lipids	whole (g
Tr.A	9.7347	9.7914	0.0567	56.7	11.34	39.66	39658.41	449.73	1.134	
Tr.B	9.4848	9.5155	0.0307	30.7	6.14	91.83	91832.04	563.85	0.614	
Tr.C	9.4157	9.456	0.040	40.3	8.06	52.83	52830.18	425.81	0.806	
Tr.D	9.6149	9.6396	0.0247	24.7	4.94	95.84	95838.05	473.44	0.494	
Tr.E	9.5636	9.628	0.0644	64.4	12.88	87.32	87318.33	1124.66	1.288	

G1	9.592	9.6091	0.017	17.1	17.1	6.17	6170.52	105.52	1.71	
G2	9.5006	9.5204	0.020	19.8	19.8	5.98	5977.46	118.35	1.98	
G3	9.6068	9.6254	0.019	18.	18.6	5.39	5385.81	100.18	1.86	
G4	9.679	10.0289	0.350	349.9	69.98	26.91	26914.32	1883.46	7.00	
G5	9.6972	9.9524	0.255	255.2	51.04	38.22	38220.23	1950.76	5.10	
G6	9.5814	10.0294	0.44	448	89.6	74.93	74925.57	6713.33	8.96	
G7	9.5815	9.8237	0.242	242.2	48.44	17.40	17403.22	843.01	4.84	
Tr G1	9.5524	9.5705	0.018	18.1	3.62	156.51	156514.83	566.58	0.36	
Tr G2	9.4139	9.4226	0.009	8.7	1.74	68.45	68454.85	119.11	0.174	
Tr G3	9.5677	9.5853	0.018	17.6	3.52	173.14	173137.82	609.45	0.35	
Tr G4	9.6845	9.736	0.052	51.9	10.38	269.27	269270.73	2795.03	1.04	
Tr G5	9.6594	9.6951	0.036	35.7	7.14	279.05	279048.99	1992.41	0.71	
Tr G6	9.665	9.6724	0.007	7.4	1.48	283.01	283005.10	418.85	0.15	
Tr G7	9.7957	9.8841	0.088	88.4	17.68	431.49	431491.83	7628.78	1.77	

- **Lipid results for combined components**

Table 9a. Combined component results for lipids

I.D	Gonad content mg/g	Gonad DW (g)	Total gonadal content (mg)	Body tissue content mg/g	Body tissue DW (g)	Total body tissue content (mg)	mg/g DW, whole biochemical content	Total content (mg)	Total content (g)	Jellyfish DW (g)
Tr G1	17.1	6.2	105.5	3.6	156.5	566.6	20.7	672.1	0.67	162.6
Tr G2	19.8	6.0	118.4	1.7	68.5	119.1	21.5	237.5	0.24	74.4
Tr G3	18.6	5.4	100.2	3.5	173.1	609.4	22.1	709.6	0.71	178.5
Tr G4	70.0	26.9	1883.5	10.4	269.3	2795.0	80.4	4678.5	4.68	296.1
Tr G5	51.0	38.2	1950.8	7.1	279.0	1992.4	58.2	3943.2	3.94	317.2
Tr G6	89.6	74.93	6713.3	1.5	283.0	418.8	91.1	7132.2	7.13	357.9
Tr G7	48.4	17.4	843.0	17.7	431.5	7628.8	66.1	8471.8	8.47	448.9

8. Biochemical Composition summary table

Table 10a. Biochemical composition of whole individuals

	Proteins			Lipids			Carbohyd	
I.D	mg/g DW	%DW	%WW	mg/g DW	%DW	%WW	mg/g DW	%DW
Tr.A	75.3	7.5	0.27	11.340	1.134	0.041	1.59	0.16
Tr.B	79.3	7.9	0.35	6.140	0.614	0.027	0.97	0.10
Tr.C	18.2	1.8	0.06	8.060	0.806	0.025	2.13	0.21
Tr.D	15.7	1.6	0.07	4.940	0.494	0.022	0.71	0.07
Tr.E	15.2	1.5	0.05	12.880	1.288	0.040	0.30	0.03
Tr G1	117.4	2.1	0.09	20.720	0.413	0.019	6.54	0.10
Tr G2	253.5	7.3	0.23	21.540	0.319	0.010	6.97	0.17
Tr G3	180.0	7.9	0.31	22.120	0.397	0.016	6.73	0.06
Tr G4	184.8	2.9	0.11	80.360	1.580	0.057	7.88	0.17
Tr G5	78.2	2.7	0.13	58.180	1.243	0.058	6.79	0.17
Tr G6	219.6	7.9	0.25	91.080	7.912	0.094	6.36	7.91
Tr G7	102.7	3.5	0.20	66.120	1.887	0.108	8.67	0.28
Min	15.2	1.5	0.05	4.940	0.319	0.010	0.30	0.03
Max	253.5	7.9	0.35	91.080	7.912	0.108	8.67	7.91
Mean	111.7	4.6	0.18	33.623	1.507	0.043	4.64	0.79
± s.d	81.26	2.85	0.11	31.258	2.080	0.031	3.179	2.245

9. Morphometric , Drying and Ashing Results

Bell diameter (cm)	Whole wet weight (g)	Presence of Gonads (Y/N)	Whole Dry Weight (g)	Whole DW (%)	Whole water content by mass	Whole water content (%)	Whole Ash weight (g)	% DW of Ash weight	Whole Ash-free DW (g)

Table 11a. Jellyfish without gonads

Specimen I.D.	Bell diameter (cm)	On collection whole wet weight (g)	Presence of Gonads (Y/N)	Crucible weight (g)	Sample Wet Weight (g)	Crucible + sample Wet Weight (g)	Crucible + Sample Dry Weight (g)	Crucible + sample Ash Weight (g)	Sample DW (g)	Whole Dry Weight (g)	Whole Dw (%)	Whole water content by mass	Whole water content (%)
Tr.A	28	1094	N	29.8	10.2	40.0	30.2	30.1	0.4	39.7	3.6	1054.3	96.4
Tr.B	37	2091	N	29.1	16.3	45.5	29.8	29.6	0.7	91.8	4.4	1999.2	95.6
Tr.C	32	1692	N	100.1	56.3	156.4	101.9	101.2	1.8	52.8	3.1	1639.2	96.9
Tr.D	37	2163	N	101.4	20.7	122.0	102.3	101.9	0.9	95.8	4.4	2067.2	95.6
Tr.E	39	2820	N	44.4	21.9	66.3	45.1	44.8	0.7	87.3	3.1	2732.7	96.9

Table 12a. Jellyfish with removed gonads

Specimen I.D.	Bell diameter (cm)	On collection whole wet weight (g)	Presence of Gonads (Y/N)	Crucible weight (g)	Sample Wet Weight (g)	Crucible + sample Wet Weight (g)	Crucible + Sample Dry Weight (g)	Crucible + sample Ash Weight (g)	Sample DW (g)	Whole Dry Weight (g)	Whole Dw (%)	Whole water content by mass	Whole water content (%)
Tr.G1	44	3591	Y	100.1	20.5	120.6	101.0	100.7	0.9	156.5	4.4	3434.5	95.6
Tr.G2	39	2343	Y	31.9	20.9	52.8	32.6	32.3	0.6	68.5	2.9	2274.5	97.1
Tr. G3	37	4511	Y	96.0	27.8	123.7	97.0	96.7	1.1	173.1	3.8	4337.9	96.2
Tr. G4	54	8151	Y	45.9	30.9	76.8	46.9	46.5	1.0	269.3	3.3	7881.7	96.7
Tr. G5	58	6795	Y	92.7	40.5	133.1	94.3	93.8	1.7	279.0	4.1	6516.0	95.9
Tr.G6	50	7571	Y	103.3	30.5	133.8	104.4	103.9	1.1	283.0	3.7	7288.0	96.3
Tr.G7	62	7831	Y	99.7	38.4	138.1	101.8	101.2	2.1	431.5	5.5	7399.5	94.5

Table 13a. Gonads

Specimen I.D.	On collection whole wet weight (g)	Crucible weight (g)	Sample Wet Weight (g)	Crucible + sample Wet Weight (g)	Crucible + Sample Dry Weight (g)	Crucible + sample Ash Weight (g)	Sample DW (g)	Whole Dry Weight (g)	Whole DW (%)	Whole water content by mass	Whole water content (%)	Sa A W
G1	85	45.0	3.0	48.0	45.2	45.1	0.2	6.2	7.3	78.8	92.7	
G2	88	30.5	4.7	35.2	30.8	30.6	0.3	6.0	6.8	82.0	93.2	
G3	82	32.2	4.9	37.1	32.5	32.3	0.3	5.4	6.6	76.6	93.4	
G4	296	29.8	12.9	42.7	31.0	30.2	1.2	26.9	9.1	269.1	90.9	
G5	509	45.9	25.3	71.2	47.8	46.7	1.9	38.2	7.5	470.8	92.5	
G6	430	32.8	11.2	44.1	34.8	33.5	2.0	74.9	17.4	355.1	82.6	
G7	286	96.7	15.1	111.8	97.7	97.1	0.9	17.4	6.1	268.6	93.9	

Table 14a. Whole Individuals morphometric and weight results

28	1094	N	39.7	3.63	1054.3	96.37	26.3	66.4	13.3
37	2091	N	91.8	4.39	1999.2	95.61	59.2	64.5	32.6
32	1692	N	52.8	3.12	1639.2	96.88	33.3	63.1	19.5
37	2163	N	95.8	4.43	2067.2	95.57	59.2	61.7	36.7
39	2820	N	87.3	3.10	2732.7	96.90	51.8	59.3	35.5
44	3591	Y	162.7	4.43	3513.3	95.57	113.3	69.6	49.4
39	2343	Y	74.4	3.06	2356.6	96.94	47.9	64.4	26.5
37	4511	Y	178.5	3.89	4414.5	96.11	127.8	71.6	50.8
54	8151	Y	296.2	3.51	8150.8	96.49	172.4	58.2	123.8
58	6795	Y	317.3	4.34	6986.7	95.66	207.4	65.4	109.9
50	7571	Y	357.9	4.47	7643.1	95.53	182.2	50.9	175.8
62	7831	Y	448.9	5.53	7668.1	94.47	315.7	70.3	133.2
28	1094		39.7	3.06	1054.3	94.47	26.3	50.9	13.3
62	8151		448.9	5.53	8150.8	96.94	315.7	71.6	175.8
43.1	4221.1		183.6	3.99	4185.5	96.01	116.4	63.8	67.2
10.6	2649.9		137.1	0.74	2682.8	0.74	88.4	5.8	53.7

10. Component Study Results

Table 15. Gonad vs, body tissue Wet weight (WW), Dry weight (DW), Ash weight (AW) and Ash free dry weight (AFDW) results

Jellyfish ID	WW (g)		DW (g)		AW(g)		AFDW (g)	
	*G	*B	G	B	G	B	G	B
Tr.G1	85	3591	6.171	156.51	2.936	110.331	3.235	46.184
Tr.G2	88	2343	5.977	68.45	2.811	45.102	3.166	23.353
Tr. G3	82	4511	5.386	173.14	2.690	125.080	2.696	48.058
Tr. G4	296	8151	26.914	269.27	8.810	163.559	18.104	105.711
Tr. G5	509	6795	38.220	279.05	15.937	191.434	22.284	87.615
Tr.G6	430	7571	74.926	283.01	24.727	157.432	50.199	125.573
Tr.G7	286	7831	17.403	431.49	7.251	308.486	10.153	123.006
Mean	253.71	5827.57	25.00	237.27	9.31	157.35	15.69	79.93

*G= Gonadal, *B= Body tissue (includes bell and arms)

Table 16a. Percentage composition in separate components (water content (WW), Dry matter (DW), Mineral ash (AW), Ash-free dry matter (AFDW))

Jellyfish ID	WW (%)		DW (%)		AW (%)		AFDW(%)		%Protein		% Lipids		%Carbohydrates	
	*G	*B	G	B	G	B	G	B	G	B	G	B	G	B
Tr.G1	92.741	95.641	7.259	4.359	47.573	70.492	52.427	29.508	9.97	1.717	1.71	0.36	0.574	0.08
Tr.G2	93.207	97.078	6.793	2.922	47.033	65.886	52.967	34.114	19.04	6.32	1.98	0.174	0.561	0.137
Tr. G3	93.432	96.162	6.568	3.838	49.938	72.243	50.062	27.757	10.16	7.84	1.86	0.35	0.632	0.041
Tr. G4	90.907	96.696	9.093	3.304	32.735	60.742	67.265	39.258	16.99	1.49	7.01	1.04	0.662	0.126
Tr. G5	92.491	95.893	7.509	4.107	41.697	68.602	58.303	31.398	5.51	2.31	5.1	0.71	0.566	0.113
Tr.G6	82.575	96.262	17.425	3.738	33.002	55.629	66.998	44.371	20.72	1.24	8.96	0.15	0.608	0.027
Tr.G7	93.915	94.490	6.085	5.510	41.662	71.493	58.338	28.507	6.91	3.36	4.84	1.77	0.601	0.266
Mean	91.32	96.03	8.68	3.97	41.95	66.44	58.05	33.56	12.76	3.48	4.49	0.65	0.60	0.110

*G= Gonadal, *B= Body tissue (includes bell and arms)

11. Statistical Output Results

a. Testing for a linear relationship between individual size (WW, DW, AW and AFDW) and biochemical composition (proteins, lipids, carbohydrates)

b. Testing for a linear relationship between gonad size (WW, DW, AW, AFDW) and biochemical composition (protein, lipids, carbohydrates)

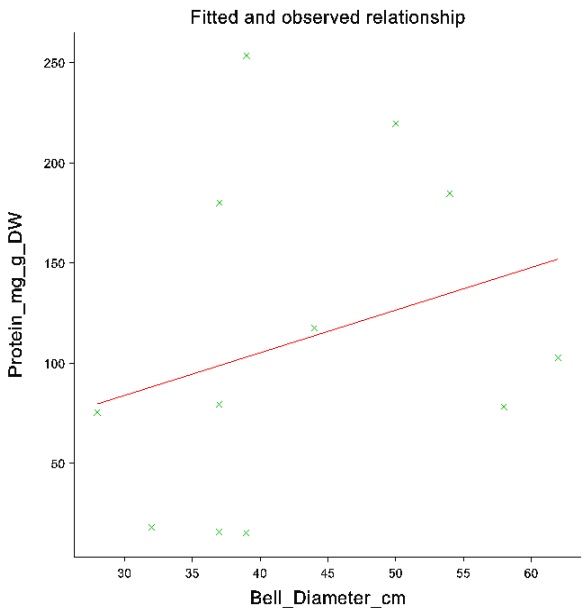
c. Testing for significant differences in gonadal composition and body tissue (bell and oral arms) composition

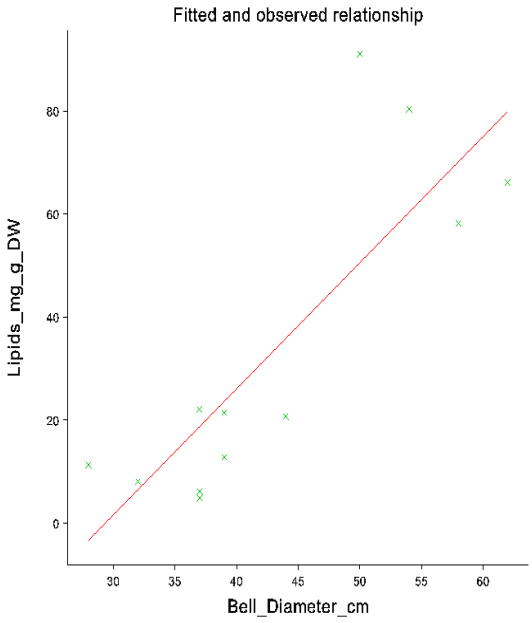
a. Simple linear regression analysis was performed to find out if there are any significant differences between whole individual's size (bell diameter, wet weight, dry weight, ash weight and ash-free dry weight) and the biochemical composition (proteins, lipids, carbohydrates)

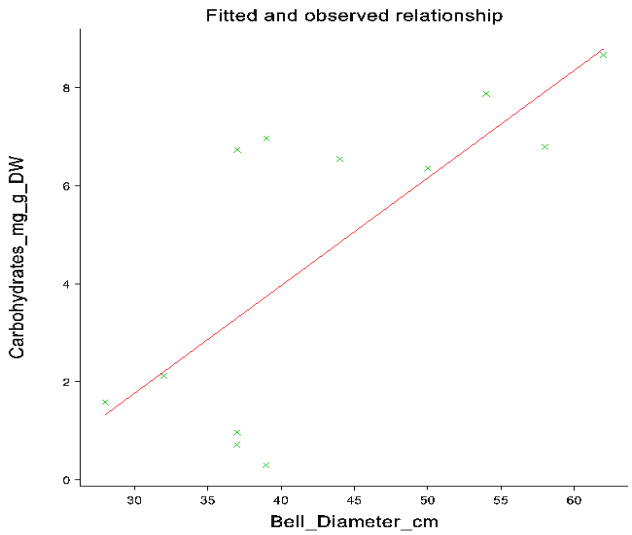
There was no significant linear relationship between bell diameter with protein content, $p>0.05$, $F_{1, 10}=0.381$

There was a significant linear relationship between bell diameter with lipid content, $p<0.05$, $F_{1, 10}< 0.001$

There was a significant linear relationship between bell diameter with carbohydrate content, $p<0.05$, $F_{1, 10}= 0.007$



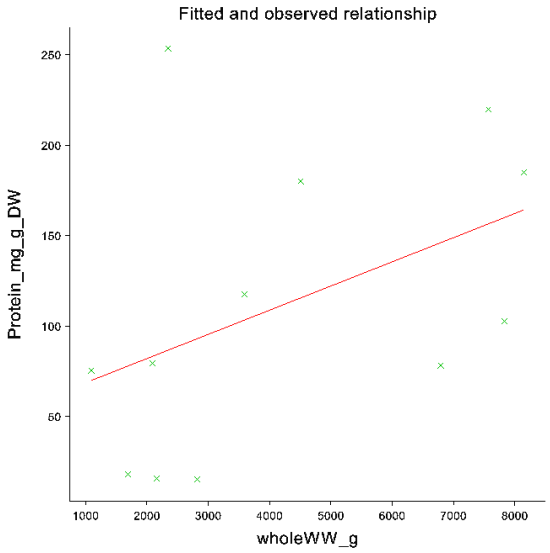


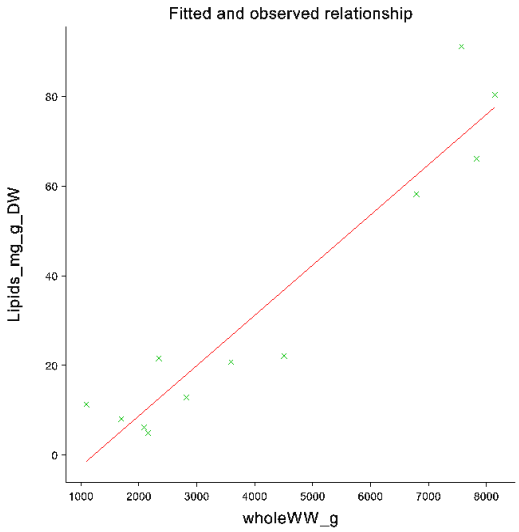


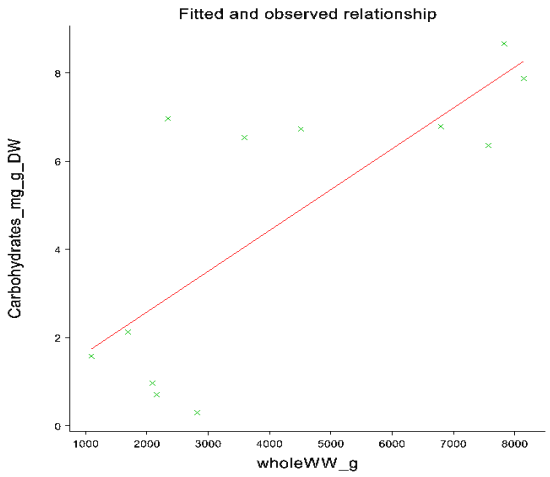
There was no significant linear relationship between whole wet weight and protein content, $p>0.05$, $F_{1, 10}=0.157$

There was a significant linear relationship between whole wet weight and lipid content, $p<0.05$, $F_{1, 10}< 0.001$

There was a significant linear relationship between bell diameter in centimetres with carbohydrate content, $p<0.05$, $F_{1, 10}= 0.003$



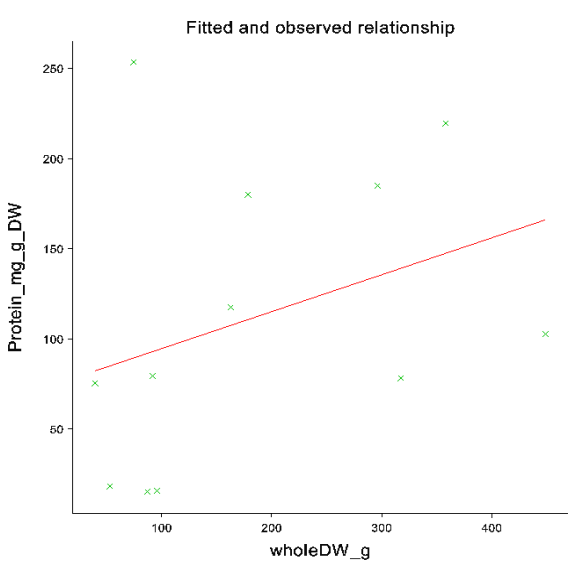


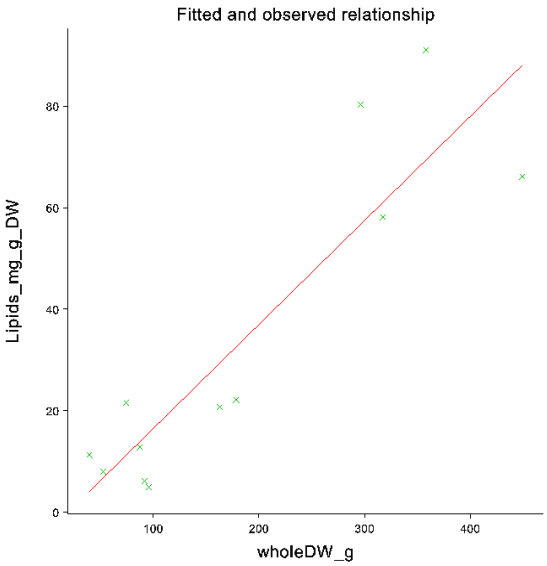


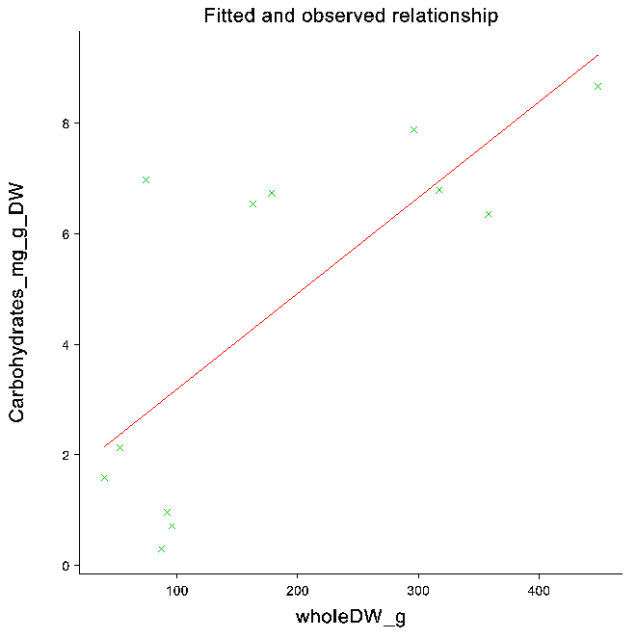
There was no significant linear relationship between whole dry weight and protein content, $p>0.05$, $F_{1, 10}=0.271$

There was a significant linear relationship between whole wet weight and lipid content, $p<0.05$, $F_{1, 10}< 0.001$

There was a significant linear relationship between bell diameter in centimetres with carbohydrate content, $p<0.05$, $F_{1, 10}= 0.005$



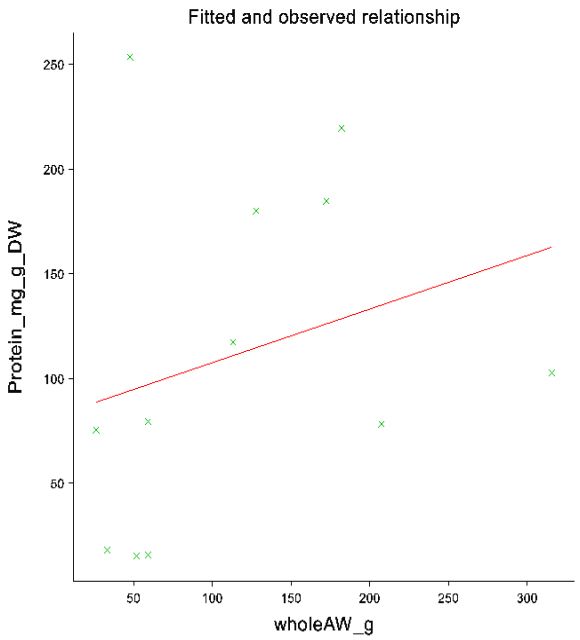


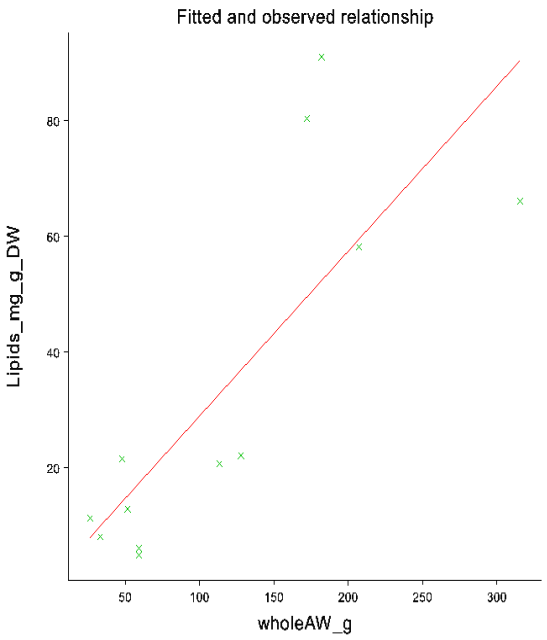


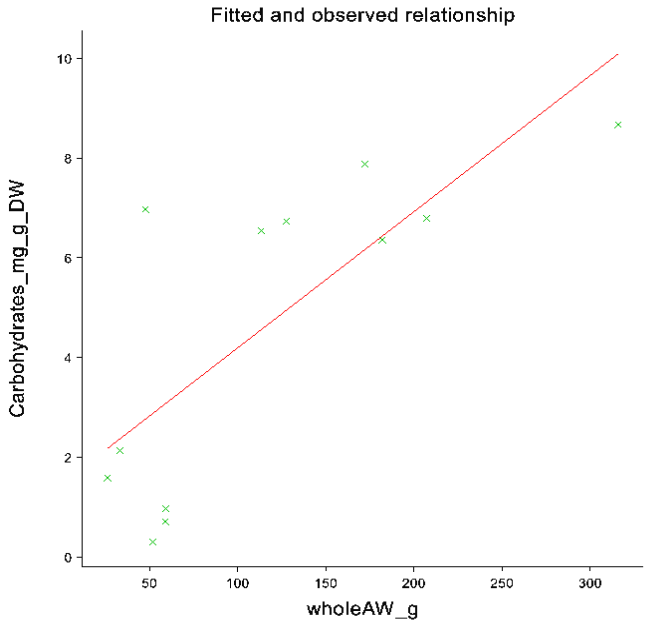
There was no significant linear relationship between whole ash weight and protein content, $p>0.05$, $F_{1, 10}=0.381$

There was a significant linear relationship between whole wet weight and lipid content, $p<0.05$, $F_{1, 10}= 0.002$

There was a significant linear relationship between bell diameter in centimetres with carbohydrate content, $p<0.05$, $F_{1, 10}= 0.004$



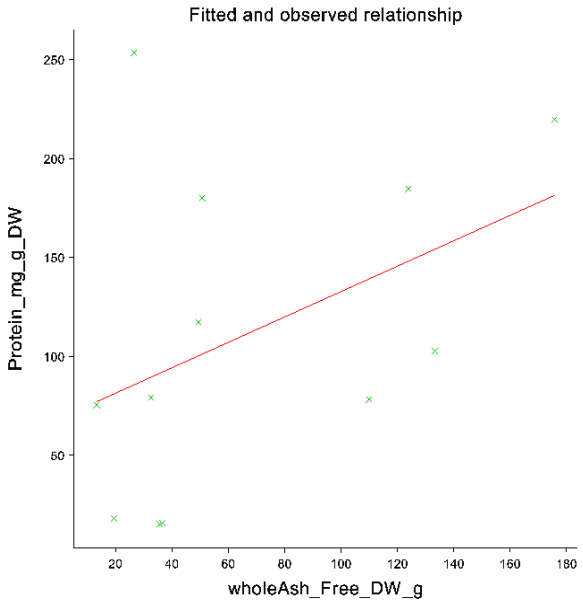


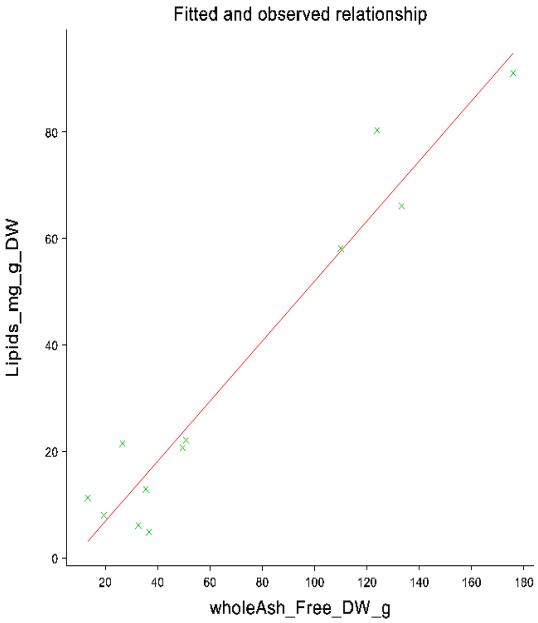


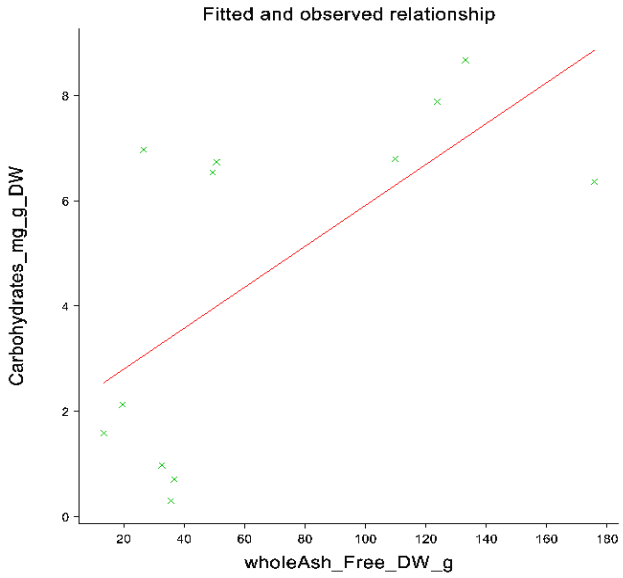
There was no significant linear relationship between whole ash-free dry weight and protein content, $p>0.05$, $F_{1, 10}=0.169$

There was a significant linear relationship between whole wet weight and lipid content, $p<0.05$, $F_{1, 10}<0.001$

There was a significant linear relationship between bell diameter in centimetres with carbohydrate content, $p<0.05$, $F_{1, 10}= 0.020$


Fitted and observed relationship
Protein_mg_g_DW
wholeAsh_Free_DW_g


Fitted and observed relationship
Lipids_mg_g_DW
wholeAsh_Free_DW_g

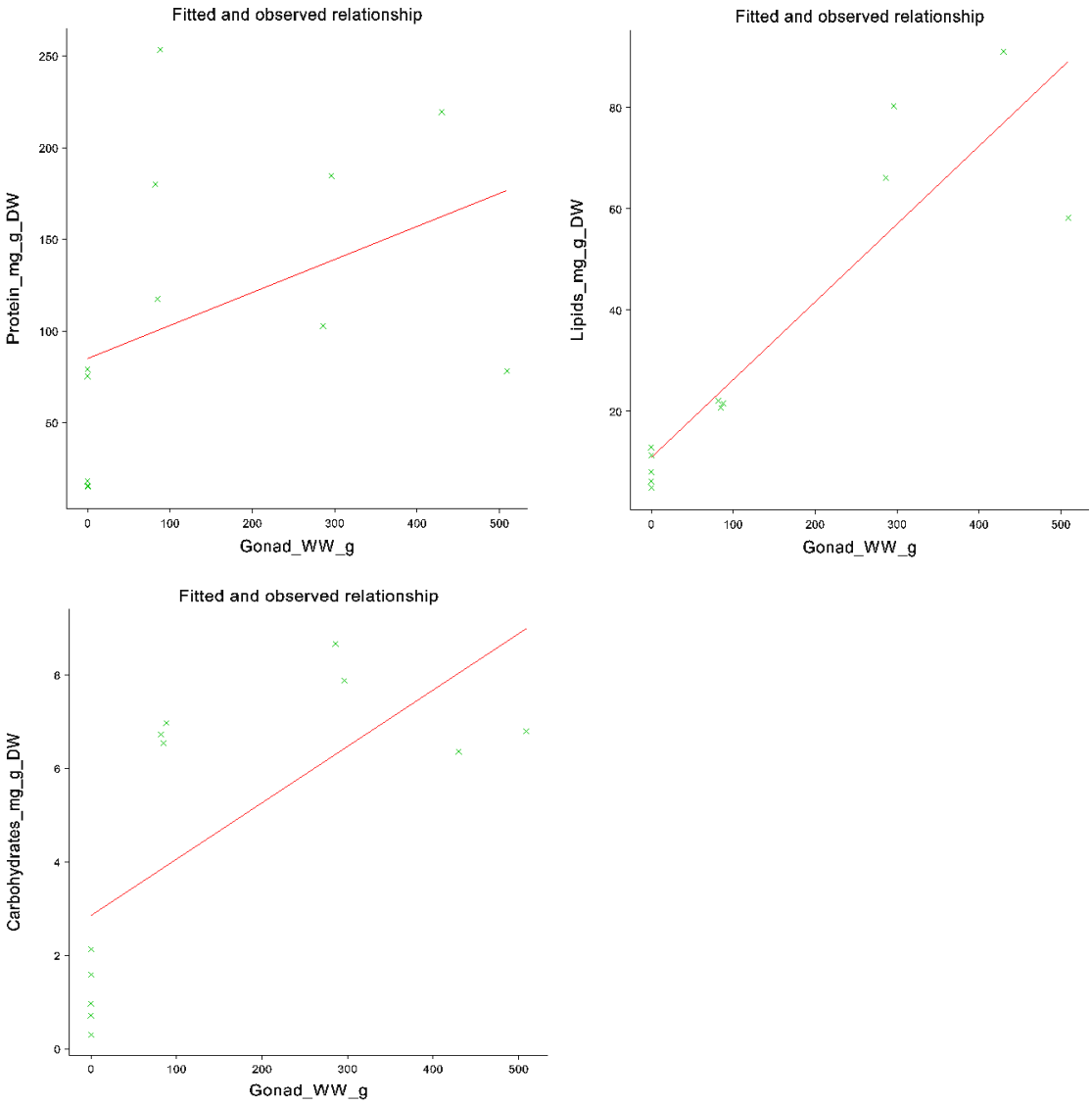

Fitted and observed relationship
Carbohydrates_mg_g_DW
wholeAsh_Free_DW_g

b. Simple linear regression analysis performed to find out if there are any significant differences between gonad size (wet weight, dry weight, ash weight and ash-free dry weight) and the biochemical composition (proteins, lipids, carbohydrates):

There was no significant linear relationship between gonad wet weight and protein content, $p>0.05$, $F_{1, 10}=0.188$

There was a significant linear relationship between gonad wet weight and lipid content, $p<0.05$, $F_{1, 10}< 0.001$

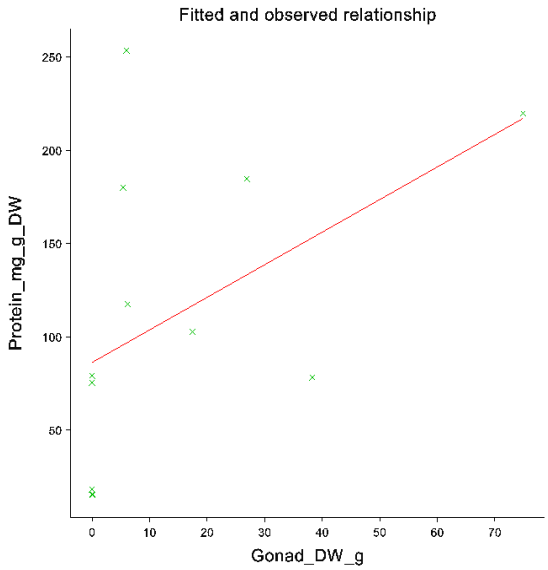
There was a significant linear relationship between gonad wet weight and carbohydrate content, $p<0.05$, $F_{1, 10}= 0.012$

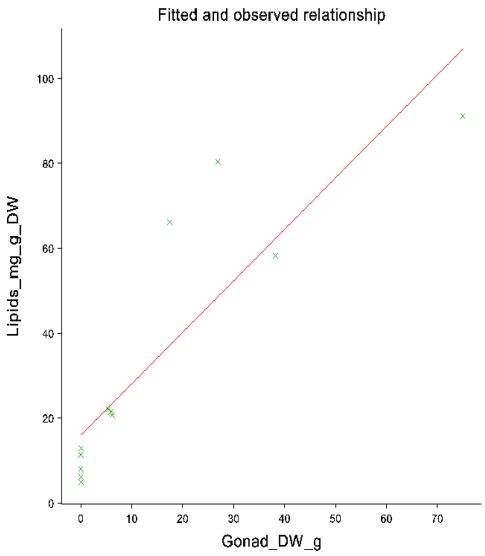


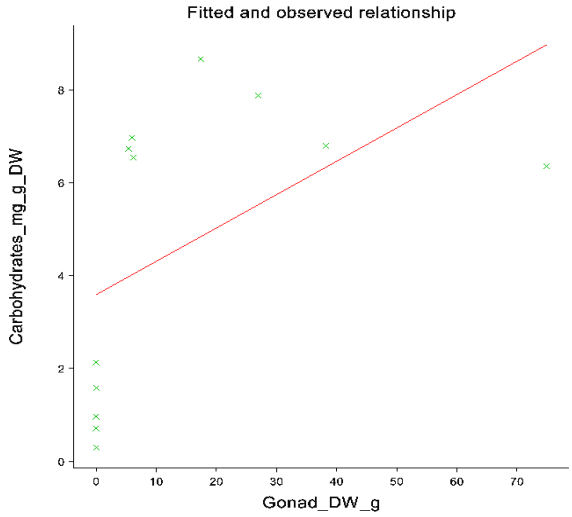
There was no significant linear relationship between gonad dry weight and protein content, $p>0.05$, $F_{1, 10}=0.108$

There was a significant linear relationship between gonad dry weight and lipid content, $p<0.05$, $F_{1, 10}< 0.001$

There was no significant linear relationship between gonad dry weight and carbohydrate content, $p>0.05$, $F_{1, 10}= 0.089$



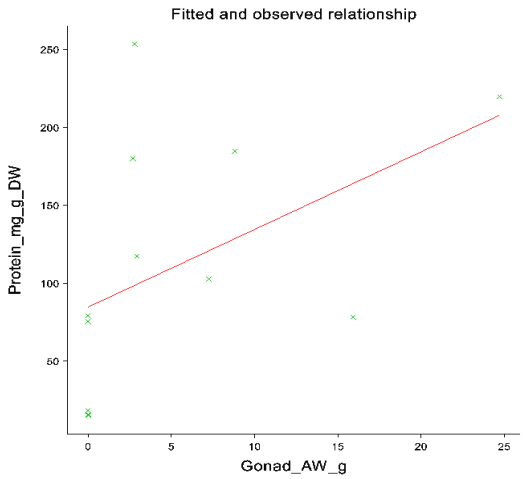


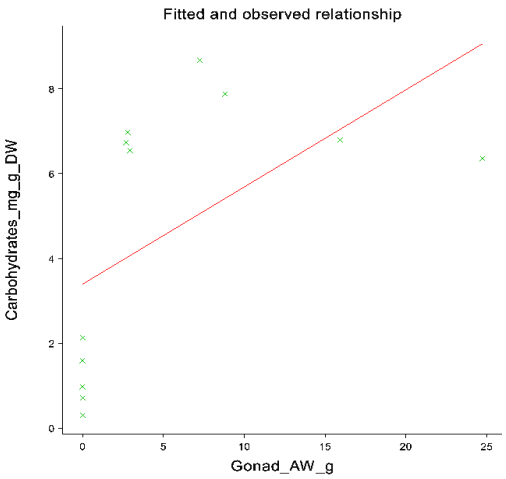


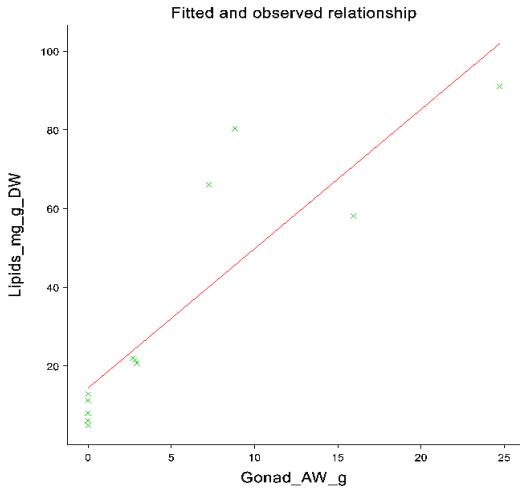
There was no significant linear relationship between gonad ash weight and protein content, $p>0.05$, $F_{1, 10}=0.117$

There was a significant linear relationship between gonad ash weight and lipid content, $p<0.05$, $F_{1, 10}< 0.001$

There was no significant linear relationship between gonad ash weight and carbohydrate content, $p>0.05$, $F_{1, 10}= 0.058$



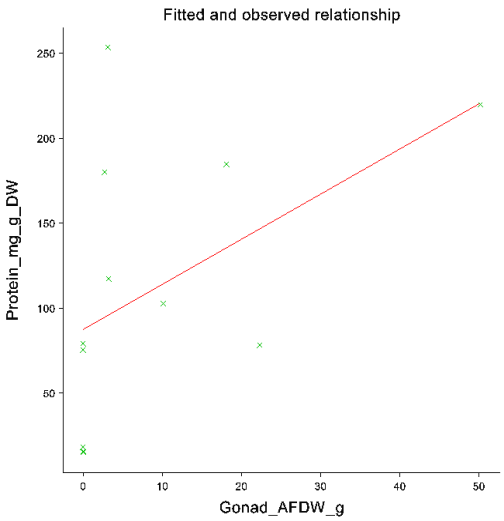


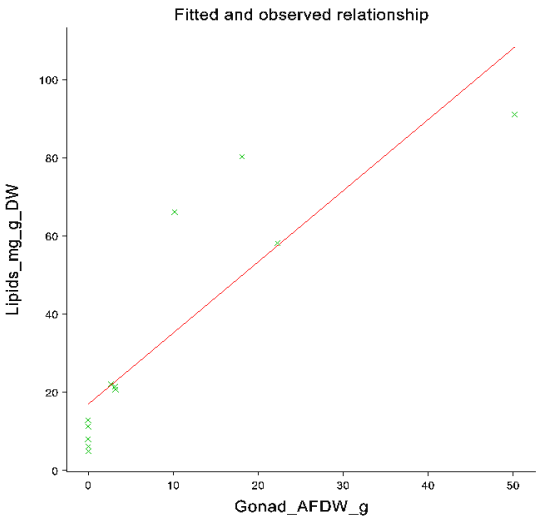


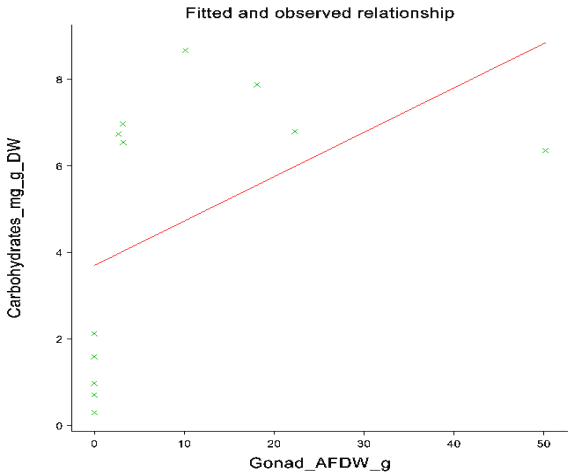
There was no significant linear relationship between gonad ash-free dry weight and protein content, $p>0.05$, $F_{1, 10}=0.106$

There was a significant linear relationship between gonad ash-free dry weight and lipid content, $p<0.05$, $F_{1, 10}< 0.001$

There was no significant linear relationship between gonad ash-free dry weight and carbohydrate content, $p>0.05$, $F_{1, 10}= 0.111$


Fitted and observed relationship
Protein_mg_g_DW
Gonad_AFDW_g
0
10
20
30
40
50
50
100
150
200
250


Fitted and observed relationship
Lipids_mg_g_DW
Gonad_AFDW_g
0
10
20
30
40
50
0
20
40
60
80
100


Fitted and observed relationship
Carbohydrates_mg_g_DW
Gonad_AFDW_g
0
10
20
30
40
50
0
2
4
6
8

c. Performing a One Way Analysis of Variance in a Complete Randomised Design on the gonadal and body tissue data:

There was a significant difference between the percentage water content in gonads and that in the body tissue, $p<0.05$, $F_{12}=0.010$

There was a significant difference between the percentage dry weight of gonads and that of the body tissue, $p<0.05$, $F_{12}=0.010$

There was a significant difference between the percentage ash weight of gonads and that of the body tissue, $p<0.05$, $F_{12}<0.001$

There was a significant difference between the percentage ash-free dry weight of gonads and that of the body tissue, $p<0.05$, $F_{12}<0.001$

There was a significant difference between the percentage protein content in gonads with that in the body tissue, $p<0.05$, $F_{12}=0.003$

There was a significant difference between the percentage lipid content in gonads with that in the body tissue, $p<0.05$, $F_{12}=0.004$

There was a significant difference between the percentage carbohydrate of gonads and that of the body tissue, $p<0.05$, $F_{12}<0.001$

Summary statistics table for gonadal against body tissue composition

Parameter	Gonads	Body tissue	Grand mean ± s.e	l.s.d
% Water content	91.32	96.03	93.68 ± 2.871	3.344
% Dry weight	8.68	3.97	6.32 ± 2.871	3.344
% Ash weight	41.9	66.4	54.2 ± 6.56	7.64
% Ash-free dry weight	58.1	33.6	45.8 ± 6.56	7.64
% Protein	12.8	3.5	8.1 ± 4.68	5.45
% Lipids	4.49	0.65	2.57 ± 2.037	2.372
% carbohydrates	0.601	0.113	0.357 ±0.0620	0.0722