Biochemical composition and condition of *Crassostrea gigas* (Thunberg, 1793) in relation to integrated multi-trophic aquaculture (IMTA) feed sources

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Agrar- und Umweltwissenschaftliche Fakultät

Rostock, 2015

Directory

I - Abstract

Offshore aquaculture has the potential to satisfy some of the rising demand for animal protein and, using an integrated multi-trophic aquaculture (IMTA) approach, to do so in a sustainable, ecologically friendly and economically valuable way. This approach relies heavily on the bio-extraction capacity of filter-feeding organisms like the Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), to turn excess particles of higher trophic cultured organism like fish, into valuable biomass. This study investigates the incorporation of IMTA related feeds by *C. gigas* based on changes in its biochemical composition. To accomplish this objective, a laboratory based feeding experiment was conducted, using five prepared diets. These consisted of a commercial turbot feed, turbot feces and the microalgae *Tetraselmis chui* and were mixed to simulate different open water feeding scenarios.

Oysters feeding on high energy turbot feed, as part of their diet, managed to significantly increase their condition index (CI), while condition in the other groups remained unaffected. Lower carbon-to-nitrogen (C/N) ratio in oysters feeding solely on low energy turbo feces implied a depletion of energy reserves after the experiment, whereas oysters feeding on a mixture of turbot feed and feces showed no such signs. Changes in fatty acid (FA) composition of oysters were particularly apparent in animals feeding on turbot feed. Composition and abundance of saturated and mono-unsaturated FA changed, in part, in relation to diet composition. Changes are believed to be the result of biosynthesis of non-methylene-interrupted (NMI) FA.

The results presented in this study indicate that *C. gigas* will incorporate particulate fish waste and are even able to increase their condition while doing so. This suggests that they will be able to absorb excess nutrients released from aquaculture sites and become another valuable product of an IMTA system.

II - Zusammenfassung

Um einen Beitrag zur Versorgung der Menschheit mit ausreichend Protein zu leisten, wird die wachsende Aquakulturindustrie in Zukunft immer höhere Erträge mit einem wachsenden Umweltbewusstsein der Verbraucher vereinen müssen. Die offshore Aquakultur, kombiniert mit einem integrierten multi-trophen System Design (IMTA), hat das Potential einen Teil der steigenden Nachfrage auf eine nachhaltige, ökologisch vertretbare und ökonomische Art und Weise zu bedienen. Dieser Ansatz basiert auf den extraktiven Eigenschaften von filtrierenden Organismen wie der Pazifischen Auster, *Crassostrea gigas* (Thunberg, 1793). Diese nehmen partikuläre Stoffe aus der Aquakultur von Fischen auf und bauen sie zu einem hochwertigen Produkt um. Diese Arbeit untersucht den Einbau von solchen IMTA bezogenen Partikeln durch *C. gigas* anhand ihrer biochemischen Zusammensetzung. Zu diesem Zweck wurde ein Laborfutterversuch durchgeführt. Dazu wurden fünf Futtermittel aus Kombinationen von einem kommerziellen Steinbutt (*Scophthalmus maximus*) -Futter, Steinbutt-Fäzes sowie der Mikroalge *Tetraselmis chui* hergestellt.

Austern die, als Teil ihrer Diät, energetisch hochwertiges Steinbutt Futter erhielten, zeigten nach dem Experiment einen signifikant besseren Condition Index (CI), während andere Gruppen keine Veränderung zeigten. Ein niedrigeres Kohlenstoff zu Stickstoff Verhältnis der Austern, die nur energetisch weniger wertvollen Steinbutt Fäzes erhielten, ließen auf den Abbau von Energiereserven in diesen Versuchstieren schließen. Austern die eine Mischung aus Steinbutt-Futter und -Fäzes erhielten, zeigten diese Anzeichen nicht. Veränderungen in der Zusammensetzung von Fettsäuren (FA) waren besonders ausgeprägt in Austern, die Steinbutt-Futter erhielten. Insbesondere die Zusammensetzung und Konzentration gesättigter und einfach ungesättigter Fettsäuren veränderten sich basierend auf dem Futter. Diese Veränderungen werden als Teil der Biosynthese von nicht-Methylen unterbrochene (NMI) Fettsäuren angesehen.

Die hier präsentierten Ergebnisse weisen auf eine Aufnahme von partikulären Abfallstoffen aus der Aquakultur durch *C. gigas* hin. Diese waren unter diesen Bedingungen sogar dazu in der Lage ihren CI zu erhöhen. Dies lässt darauf schließen, dass sie die überflüssigen, von einer Fischzucht ausgehenden, Nährstoffe aufnehmen und in ein weiteres wertvolles Produkt umwandeln

III - Acknowledgments

I would like to express my gratitude to my advisors Dr. Adrian Bischoff-Lang and Prof. Dr. Bela H. Buck for their advice and support as well as for providing me with all necessary equipment and facilities. I especially want to thank Dr. Bernadette Pogoda, whose expertise, knowledge and careful attention contributed immensely to this work.

All the results presented in this thesis have been made possible by the continuous kind support, help and advice from the staff and colleagues of the AWI. I am particularly grateful to Dr. Martin Graeve for divulging his knowledge and experience of fatty acid measurements to me and making the analysis in his lab possible.

IV - Abbreviations and Acronyms

AFDW	Ash Free Dry Weight
Chl *a*	Chlorophyll *a*
CI	Condition Index
C/N	Carbon-to-Nitrogen
DO	Dissolved Oxygen
DW	Dry Weight
FA	Fatty Acid
GC	Gas Chromatography
HUFA	Highly Unsaturated Fatty Acids
IMTA	Integrated-Multi-Trophic Aquaculture
KH	Carbonate Hardness
MUFA	Mono-Unsaturated Fatty Acids
NMI	Non-Methylene-Interrupted
PL	Phospholipids
PUFA	Polyunsaturated Fatty Acids
RAS	Recirculation Aquaculture System
SD	Standard Deviation
SFA	Saturated Fatty Acids
TAG	Triacylglycerols
TDW	Tissue Dry Weight
TPM	Total Particulate Matter
WE	Wax Ester
WW	Wet Weight

V - List of Figures and Captions

VI - List of Tables and Captions

1 Introduction

Fish and seafood are a valuable nutritional component in many regions of the world, especially in densely populated areas where overall protein intake is low. Fish accounted for 16.7 % of all animal protein consumed in 2010 and 6.5 % of all protein. For 2.9 bn people it accounts for 20 % of animal protein while the remaining 4.3 bn consume approximately 15 %. Yields of marine capture fisheries have been stagnating since the late 1990s with a slight peak of 86.6 million tonnes (excluding anchoveta catches) in 2012. At the same time fishing fleets get larger and catch per unit effort rises continuously. Production from freshwater fisheries has been steadily increasing in the same time span, but still only accounts for less than 13 % of global capture production (FAO, 2014a).

These catch numbers are in large parts not sustainable and have caused close to one third of fish stocks to be classified as overfished beyond biologically sustainable levels and nearly two thirds as fully fished. Only a few areas in the Pacific Ocean still show growing catches. In the time span between 1960 and 2012 per capita fish consumption has doubled from 9.9 kg per year to 19.2 kg, creating a constantly growing demand for more production (FAO, 2014a).

World aquaculture production, which has seen substantial growth to a world total of 90.4 million tonnes of live weight in 2012, has started to compensate the increased demand for fish and seafood products. Recent aquaculture production was comprised of 66.6 million tonnes of fish, crustaceans and molluscs and 23.8 million tonnes of aquatic algae. China accounted for roughly 60 % of this output while all of Asia contributed up to 85 % to the global production (FAO, 2014a).

This increase in production has mostly been achieved by an intensification of culture systems, which, in turn, has led to a variety of well documented ecological and socio-economic problems (Wu, 1995; Dierberg and Kiattisimkul, 1996; Naylor et al., 1998, 2000; Tovar et al., 2000). They have been outlined as follows:

(1) Water pollution from aquaculture effluents
(2) Inefficient utilization of fish meal and other natural resources
(3) Excessive use of drugs and antibiotics
(4) Spread of diseases from culture organisms to native populations

(5) Negative effects on biodiversity caused by escape of non-native species introduced for aquaculture
(6) Conflicts with other resource users

(Boyd, 2003)

One strategy to reduce negative impacts are recirculation aquaculture systems (RAS) to control and optimize growing conditions and reduce resource usage (Piedrahita, 2003). Another approach is to adjust aquaculture feeds in order to reduce fish waste and excretion. This can be achieved by providing optimal feed sizes for different stages of growth, reducing the sinking rate of feed and improving its overall digestibility to reduce the excretion of nitrogen and phosphorous (Wu, 1995; Piedrahita, 2003). Feed research also focuses on reducing fish meal content to relieve pressure on wild fish stocks (Naylor et al., 2000). Policy approaches include mandated treatment of farm effluents, limiting allowed concentrations of particulate and dissolved waste products and limiting the number of issued licenses (Tacon and Forster, 2003). At the same time Chinese aquaculture has employed a balanced multi-species ecosystem approach for millennia. Enabling the development of the largest fresh water aquaculture sector in the world by utilizing species that feed at different levels of the food web to sustainably maximize yield and minimize the ecological foot print (McVey et al., 2002).

This multi-trophic approach to sustainable aquaculture has been rediscovered in the last two decades and is being referred to as integrated multi-trophic aquaculture (IMTA) (Barrington et al., 2009; Troell et al., 2009; Chopin et al., 2010). It is defined as co-culturing fed species (e.g. finfish or crustaceans) in proximity to extractive species, which assimilate inorganic (e.g. by seaweeds) and organic (e.g. by suspension and deposit-feeders) nutrients released by the fed culture for their own growth (Chopin, 2012).

This promising approach is mitigating some of the negative ecological effects of aquaculture on the environment (Culley et al., 1981; Ghaly et al., 2005). To date, IMTA systems at or near commercial scale already exist in Canada, Chile, China, Ireland, South Africa, UK, Northern Ireland and the United States, while research is being conducted in several other countries (Barrington et al., 2009).

Although the integration of multiple trophic levels might offer a solution to decrease the ecological footprint and increase the economic yield of aquaculture operations, there are

still many open questions regarding stakeholder interests, sites selection as well as how to best set up and stock such multi-trophic farms.

Aquaculture in Germany, except for mussel culture, is a highly regulated and restricted fringe activity (Rosenthal and Hilge, 2000) producing only 25.000 tonnes in 2013. Although the sector has seen an overall growth of 4.2 % since 2012, the mussel industry, its only mariculture, has shrunk by 27.4 % in the same time (Statistisches Bundesamt, 2014).

Commercial offshore aquaculture installations do not yet exist in Germany as technical challenges and required initial investments are high and the regulatory framework is not yet clearly developed (Buck and Krause, 2013). Another challenge of operating in Germanys exclusive economic zone is the high number of stakeholders involved (Buck et al., 2004). Various studies and projects have been conducted to evaluate candidate species biology, culture techniques, system designs, economic potential, integrated coastal zone management (ICZM), the current and necessary regulatory framework and promising synergistic effects with offshore wind farms (Buck, 2004; Buck et al., 2008; Michler-Cieluch, 2010; Pogoda, 2012).

As the first of its kind in Germany, the project "Aquaculture site selection for sustainable and multifunctional exploitation of marine areas in intensively used waters using the example of the North Sea" (Offshore Site Selection, OSS) draws from all of this accumulated knowledge and builds on it. It brings together different stakeholders and research institutes to establish selection criteria for possible offshore IMTA sites, aiming at a balanced nutrient budget. The Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI) coordinates this project and also develops nutrient budgets between fed and extractive IMTA candidate species such as turbot, *Scophthalmus maximus*, European seabass, *Dicentrachus larbrax*, sugar kelp, *Saccharina latissima* , dulse, *Palmaria palmata*, blue mussel, *Mytilus edulis* and the Pacific oyster, *Crassostrea gigas*. All of these candidate species are commonly found and cultured in temperate European waters.

C. gigas has already been shown to be well suited to the environmental conditions presented by offshore cultivation by Pogoda (2012). The next step is to investigate the incorporation of nutrients, in the form of particulate organic waste leaving the fish cages, by these filter feeding organisms. Recent research has shown that bivalves are able to

utilise particles associated with fish farm effluents in their diet (Reid et al., 2010; MacDonald et al., 2011) and therefore act as biomechanical filters and possibly improve the economic value of fish farms (Lefebvre et al., 2000). Many of these studies focus on salmon aquaculture in combination with mussels (*M. edulis*) and evaluate the feeding on waste particles based on traditional methods, such as absorption efficiency or filtration rates.

Another approach is to monitor the changes in FA composition. These are traditionally used as trophic markers in marine food webs to trace trophic pathways and to establish predator-prey relationships (Kharlamenko et al., 1995; Stübing et al., 2003; Budge et al., 2007; Xu and Yang, 2007; Iverson, 2009). However, only few studies have so far utilised changes in the FA profile of tissue to study the incorporation of certain particle types by *C. gigas* (Handå et al., 2012).

This thesis builds on previous work by combining multiple methods for evaluating the incorporation of IMTA related feed sources by *C. gigas* to further investigate its suitability as extractive component in such a system. Changes in the FA profile, C/N ratio, energy value and condition of *C. gigas* will be measured and interpreted in the light of the following hypotheses:

(1) Elemental composition of *C. gigas* is related to diet composition.
(2) Feeding on feed sources with a high energy content will result in better condition of oysters.
(3) Feeding of *C. gigas* on feed sources with a high nutritional value will result in a higher energy content of oysters.
(4) Incorporation of different IMTA related feed sources can be evaluated based on changes in FA composition of *C. gigas.*

2 Background

2.1 Biology of *Crassostrea gigas*

Oysters are part of the phylum Mollusca (Table 1), which contains approximately 50,000 species, and are found in the intertidal zone of waters around the world and down to a depth of 30 m. They are omnivorous filter feeders and draw their food from the surrounding water column. Oysters of the genus *Crassostrea* are native to inter- and subtidal zones of warm and brackish waters in the western Pacific Ocean but can tolerate and even thrive in a wide range of salinities and temperatures (Matthiessen, 2001).

Fig. 1 - Pacific "cupped" oyster (*Crassostrea gigas*) with slightly opened valve. Photo by: Maximilian Schupp, 2015

As it is part of the Bivalvia, the oyster's body is held between two calciferous shells, the left and right valve. Within the group of the cupped oysters, the left valve is deeply cupped and holds its body while the right valve acts like a lid (Fig. 1). The two valves are connected through a tough ligament at the pointed end. This beak is the oldest part of the shell and the oyster grows outward from this point. Internal organs are arranged around the central adductor mussel, which compresses the ligament when closed (Fig. 2) (Boghen, 1995; Spencer, 2002).

Largest organs are the paired ctenidia (gills). They serve at the same time for gas exchange and food particle collection. Ciliated tracts pump water through the inhalant cavity, over the pore structure of the gills and out of the exhalant cavity. Particles that get trapped on the gill filaments are bound in mucus and transported to the mouth via ciliated

food grooves. There they are sorted by the labial palps. Rejected particles are carried to the ventral margin for disposal as pseudofaeces (Spencer, 2002; Ward and Shumway, 2004).

Table 1 - Commercially farmed genera of oysters and their taxonomical classification starting at phylum and excluding subclass (Ostreidae), order (Ostreoida) and superfamily (Ostreoidea)

Phylum	Class	Family	Subfamily	Genus	Species
Mollusca					
	Bivalvia				
		Ostreidae			
			Ostreinae		
				Ostrea	*O. edulis*
					O. chilensis
			Striostreinae		
				Saccostrea	*S. cucullata*
					S. commercialis
			Crassostreinae		
				Crassostrea	*C. gigas*
					C. virginica
					C. rhizophorae
					C. madrasensis
					C. iredalei
					C. corteziensis
					C. gasar

Mechanism and pathways of preferential particle retention and ingestion in suspension feeding bivalves have been widely discussed and are extensively reviewed by Ward and Shumway (2004). Four different pathways for particle selection were proposed (Fig. **3**):

(1) Preferential retention by the ctenidia
(2) Selection and preferential transport on the ctenidia
(3) Selection by the labial palps
(4) Selection in the stomach

Feed particles carried into the mouth and through the oesophagus are sorted by size on a system of ciliated grooves and ridges and mixed with digestive enzymes throughout the posterior and anterior chamber of the stomach. Particles are further broken down by a crystalline style, a gelatinous rod housed in a blind sac attached to the wall of the stomach. It is rotated by the motion of surrounding cilia and continuously breaks down algal cells or other particles. This grinding motion results in it being constantly worn down and being reformed in its sac. Partially digested and broken down particles are transported to the

digestive gland where they are phagocytosed and intracellular digestion takes place. Undigested particles pass to the lower intestine where they are formed into faeces ribbons. These are shed through the anus into the exhalant cavity for disposal (Spencer, 2002).

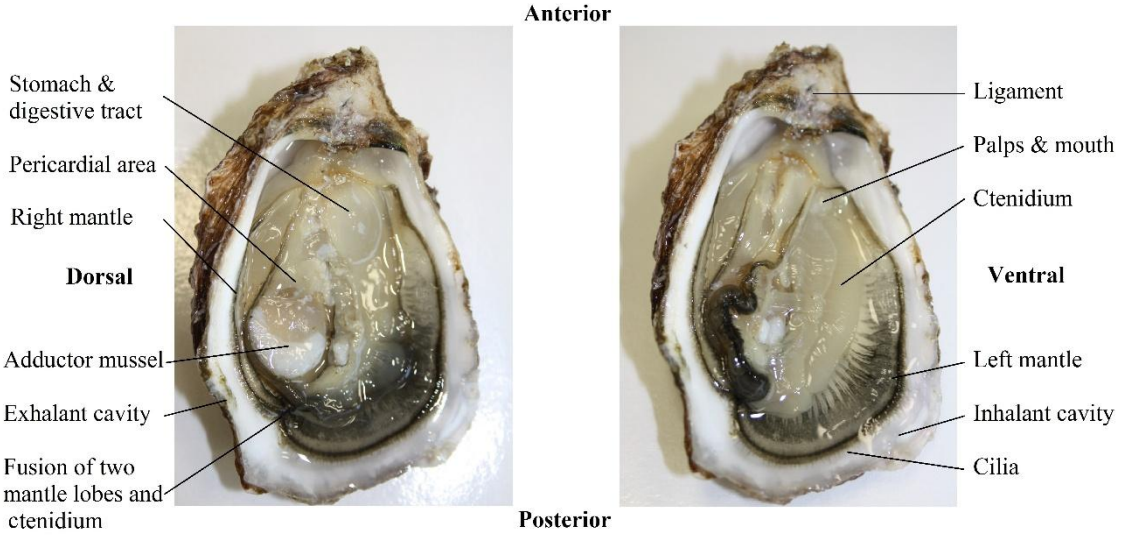

Fig. 2 - Anatomy of *Crassostrea gigas*. Right valve removed, ligament and adductor mussel separated. Ctenidium exposed (right). Photo by: Maximilian Schupp, 2015

Cupped oysters are dioecious (i.e. have two distinctly different sexes) and reach sexual maturity usually after their first year. They are alternating hermaphrodites, meaning they change sexes on an annual cycle. This cycle may be affected by environmental conditions, such as salinity, temperature and food availability. For example environments with a high abundance of food tend to produce higher female to male ratios. The bivalve reproductive system is comprised of two gonads that open into the exhalant cavity. Gonad tissue in the Pacific oyster has usually a creamy white colour regardless of sex and grade of maturity. When gonads have ripened, spawning is induced at threshold temperatures of 20 °C, which is usually from June to July in its natural habitats. Especially during low tides the remaining water body is quickly heated by the sun. Once other oysters detect sperm in the water they start spawning themselves to guarantee successful fertilization (Boghen, 1995; Spencer, 2002).

Spermatozoa are released from males via the exhalant cavity, while the eggs traverse the widened ostia of the gills. They then pass out of the inhalant cavity into the water column where fertilization occurs. Female oysters can produce between 10 and 100 million eggs per year, which are continuously expelled throughout the spawning period. Each egg is sized 50 - 80 µm and carries enough lipid reserves for the larva to sustain itself for between 8 - 14 days, until active feeding can succeed (Matthiessen, 2001; Spencer, 2002).

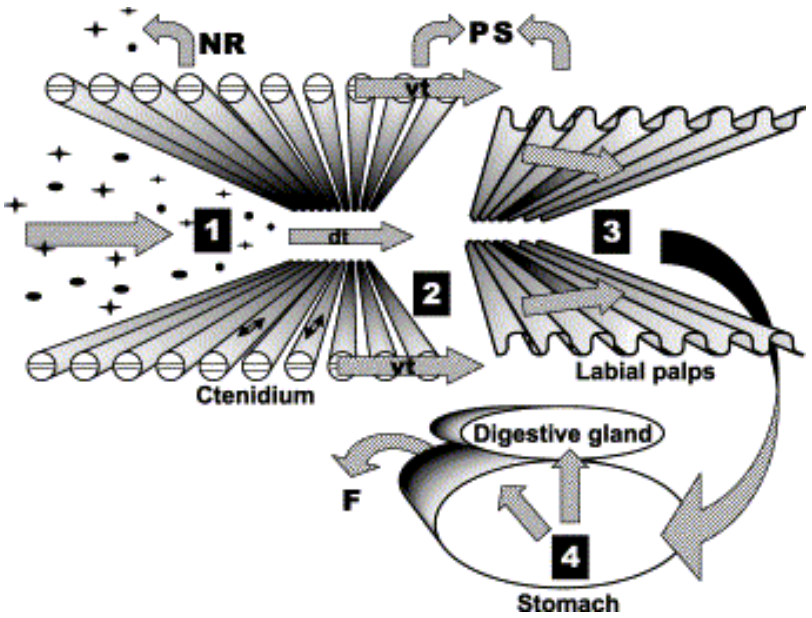

Fig. 3 - Possibilities for particle selection in lamellibranchiate bivalves in order of possible occurrence during ingestion and digestion. (1) Preferential retention of some particles on ctenidium and resulting non-retention (NR) of others; (2) differential transport on the ctenidium and rejection as pseudofaeces (PS); (3) selection by labial palps and further rejection as pseudofaeces; (4) selection in the digestive tract with unabsorbed material being ejected as feces (F). Not all species demonstrate all sorting loci. [1]

Oyster larvae complete three distinct stages during the veliger stage life span: The straight-hinged, the umboed and the eyed larval stage. They spend this time actively swimming in the water column but are nonetheless distributed over a wide area by ocean currents. After two to three weeks, during the last larval stage, animals are approximately 280 - 370 µm large and have developed an exploratory foot and an eye spot, which help in selecting a clear, hard surface for settling. Once a surface has been selected it begins permanently cementing its left valve to it, using a cement gland in the foot. During the following two to three days the metamorphosis takes place and internal organs undergo a change to suit the new sessile life style. Especially the gill structure quickly grows in complexity to allow active filtration of food particles to provide enough energy for the juvenile phase. Oysters are now called spat (Boghen, 1995; Spencer, 2002).

Since most larvae die during the settling process, the so called spatfall, due to a lack of suitable substrate, most recruitment for aquaculture takes place during this phase by simply providing collectors as artificial substrate (Boghen, 1995; Spencer, 2002).

[1] Reprinted from Journal of Experimental Marine Biology and Ecology, Volume 300, Issues 1-2, Ward, J.E., Shumway, S.E., Separating the grain from the chaff: particle selection in suspension-and deposit-feeding bivalves, Pages 83-130, 2004, with permission from Elsevier.

With this profile and its prolific reproduction especially *C. gigas* has been spread to many temperate waters around the globe (Spencer, 2002). Worldwide oyster landings in 2012 amounted to 4.7 million tonnes with a market value of USD 3.9 bn. While many species of oysters are commercially farmed *Crassostrea* spp. makes up almost 4.0 million tonnes of that amount (

Table **2**) with China producing more than 80 %. Other important producers of *Crassostrea* spp. include the Republic of Korea, Japan and France (FAO, 2014b).

Table 2 - Principal producers (excluding China) of *Crassostrea* spp. by main species (*C. gigas*, *C. virginica*) and world total production (including China). F = FAO estimate (FAO, 2014b)

			2009	2010	2011	2012
Species	Common name	Country	Thousand tonnes			
Crassostrea gigas	Pacific/ Japanese Oyster	Korea, Rep. of	240.911	267.776	281.022	284.856
		Japan	210.188	200.298	165.910	161.116
		France	103.467	95.000 F	83.548	82.000 F
		USA	38.910	29.169	29.718	31.529
		Taiwan	21.882	36.056	34.643	26.923
		World total	648.720	652.669	618.596	608.688
Crassostrea virginica	American Cupped Oyster	Canada	3.077	3.463	3.380	4.026
		USA	90.000 F	108.279	67.975	99.047
		World total	93.077	111.742	71.355	103.073
Crassostrea spp.		World total	3.536.330	3.677.591	3.770.466	3.982.361

2.2 Lipids and fatty acids

Lipids are a heterogeneous group of substances with a wide variety of properties and physiological uses. They do not readily dissolve or mix with water, show a variety of structures and can therefore serve many purposes from cell membranes to buoyancy regulation or energy storage. Most lipids fit into the categories of triacylglycerols (TAG), wax esters (WE), phospholipids (PL) or sterols. TAGs usually consist of up to three FA esterified to the sn-1,-2, and -3 positions of an L-glycerol molecule. Saturated or monounsaturated FAs are usually bound to the sn-1 and sn-3 positions while polyunsaturated fatty acids (PUFA) are preferentially bound to the sn-2 position. WEs consist of one FA esterified to one usually saturated or monounsaturated fatty alcohol. PLs are structurally very similar to the TAGs with the exception that they have a phosphate group and sometimes other molecules bound to the sn-3 position of their L-glycerol. This gives them one distinctively polar and one nonpolar end and makes them the principal component of almost all biological membranes (Pond, 1998). Sterols form a different category. They can be found esterified to a FA or non-esterified in cell plasma and membranes (Sargent et al., 2002).

All lipids contain FA as their building blocks. They are, in their most basic form, hydrocarbon chains with an acid group (carboxyl, COOH) at one end and a methylene end (MH_3) at the other. Chains can vary widely between 2 and 80 carbon atoms in length, but most common FA show a length of from 14 to 24 carbon atoms. Double bonds can occur within the chain and define so called unsaturated FA. These are usually interrupted by a methylene group, but in certain cases non-methylene-interrupted (NMI) FA occur. FA are commonly named after the length of the carbon chain, followed by the amount of double bonds and the position of the first double bond, counting from the methylene end (Pond, 1998; Iverson, 2009). They are commonly classified as saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA) and highly-unsaturated fatty acids (HUFA).

Organisms are able to biosynthesize certain FA by elongating chain lengths and desaturation of bonds (Fig. 4) but are limited by biochemical constraints based on phylogenetic group and species (Iverson, 2009). These constraints tend to increase with the complexity of the synthesizing organism, culminating in vertebrates (Cook and McMaster, 2002). Most organisms are able to elongate and desaturate short-chain SFA

by adding a new methylene group to the end of the chain or removing a hydrogen atom to form a double bond between carbon atoms, respectively (Cook and McMaster, 2002).

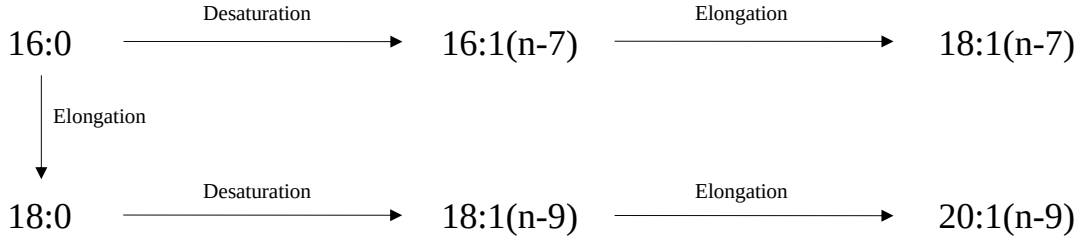

Fig. 4 - Portrayal of possible *de-novo* biosynthesis pathways of short-chain mono-unsaturated fatty acids (Cook and McMaster, 2002; Monroig et al., 2013).

Due to the constraints of FA biosynthesis, they are often not completely broken down like proteins and carbohydrates after ingestion, and are absorbed by tissue as building blocks for new lipid molecules instead. These are deposited in several locations in the organism. As a result of this storage and incorporation, FA can bioaccumulate through trophic food webs and certain groups of FA can be traced back to their origin (Iverson, 2009).

To identify these groups an understanding of the biosynthesis pathways within the target organism as well the changes in FA composition must be gained.

3 Materials and methods

3.1 Specimen

Approximately 420 diploid Pacific oysters in the size class of 6 - 8 cm were procured from Dittmeyer's Austern Compagnie GmbH on the island of Sylt in September of 2014. Oysters were shipped via express carrier packed with wood and damp straw and arrived within 24 hours at the AWI in Bremerhaven, were inspected for deceased individuals and cleansed from epibionts and silt immediately upon arrival. They were then split up into two contingents and held in aerated 150 L tanks with a hydraulic retention time of 1 h and a constant temperature of 15°C where they could acclimate for two weeks to water temperature and salinity of the experimental system. System water was refilled approximately every 2 months with natural seawater from the island of Helgoland. It is treated by removing solids in a sedimentation tank and removing ammonia using two biofilters.

3.2 Experimental setup

A 28 day laboratory based feeding experiment with daily feedings was conducted at the AWI in Bremerhaven in November of 2014. A 30 tank shelving unit (Aquatic Habitats, Z Hab System) with a tank size of 9 L each, set within a climate controlled room (15°C ambient temperature), was used as the experimental system.

Five different diets were defined and produced (see chapter 3.4) to resemble multiple open water feeding scenarios. Five tanks per diet were stocked with six oysters each, three days prior to the start of the experiment. Additionally, one tank per diet was left empty as a control for feed concentration within the natural seawater. Tanks were randomly distributed on the shelves according to Fig. 5, to reduce the possible impact of tank parameters. All tanks were aerated using a diaphragm compressor, set on the top left of the shelf, connected to 6/8 mm flexible PVC air hose. Stable airflow and uniform water circulation in all tanks was achieved by adjusting hose clamps along the length of the aeration system.

Fig. 5 - Distribution of diets within the shelving unit (FD (feed), FS (feces), FF (feed and feces), M (feces, feed and microalgae) and C (*T. chui*). Empty control tanks for each diet are marked as 0.

One of the constraints on the effectiveness of extractive organisms in open water multi-trophic aquaculture is the temporal variability of emitted particle plumes (Cranford et al., 2013). For this reason a batch experiment was chosen over of a flow-through design with constant food supply. Oysters were fed a fixed amount of their respective diets of suspended particulate matter every day at 9:00 am and then had subsequently 23 hours to filter the water before the tanks were emptied, washed with sea water and filled again. This simulates a daily feeding at an aquaculture site and the following nutrient and particle influx. The amount of feed per tank was based on feed and tissue carbon content. Daily feed amounts were calculated based on literature values and adjusted to correspond to approximately 2 % of tissue carbon content and augment natural seston in the system (Handå et al., 2012).

3.3 Determination of dry weight

Feeding amounts for the experiment were based on mean carbon content of the tissue. For the determination of the tissue dry weight (TDW), it is usually required to open the oyster for drying and weighing the soft tissue. To circumvent this fact, a model for predicting approximate TDW was developed. Length, width, height and total wet weight (WW) of 30 oysters of the stock were measured. TDW was then determined by opening the same 30 individuals and freeze-drying (CHRIST, Alpha 1-4 LSC & Vacuubrand, RZ 6) the tissue for 24 – 48 h in pre-weighed (Sartorius LE4202S) 15 mL Falcon tubes. TDW was then calculated by subtracting the weight of the empty tube from that of the dried tube. To adapt feeding amount, approximate TDW of experimental oysters was estimated based on correlations between TDW of measured oysters and their external dimensions.

Instead of calculating the volume of oysters based on their geometric shape, a different methodology, based on the weight of the water displaced by the oyster, was used. The volume was determined by placing a pre-weighed 1 L measuring cylinder on a laboratory scale (Sartorius LE 4202S), filling it with de-ionized water of a known temperature to the 500 mL mark and noting the weight. After adding the oyster the displaced water above the 500 mL mark was pipetted from the cylinder. The oyster was then removed and excess water allowed to drip off into the cylinder. Care was taken to ensure the oyster shell remained closed during this procedure. The volume was then calculated by dividing the weight of the displaced water by the density of the water at the current temperature.

3.4 Feed

Three different nutrient solutions, with differing concentrations of suspended solids, were produced: (1) turbot feed, (2) turbot feces and (3) microalgae. Diet components were compiled according to Table 3.

Table 3 – Diet compilation. Included components are marked by ● for every diet and constitute equal parts of overall carbon supply in mixed diets (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)).

	Diets				
	FD	FS	FF	M	C
Turbot feed	●		●	●	
Turbot feces		●	●	●	
Tetraselmis sp				●	●

Turbot feed (Skretting, R7 Europa 15F) was freeze dried and ground using a blade grinder (Retsch GM 200) on 4000 rpm in 10 second bursts with 30 second cooling periods until evenly crushed. A stock solution with a suspended solids concentration of 10 g/L was produced with 0.7 µm filtered seawater. Solutions were frozen in aliquots at -80°C and thawed when feed was prepared.

Turbot feces were collected prior to the start of the experiment in a RAS at the AWI in Bremerhaven. The turbots had been fed the same feed which was used in this experiment. Feces were collected at daily intervals with a fine sieve and pipetted out of the culture tank. They were subsequently centrifuged at 4000 rpm (Eppendorf, 5810 R) for 5 minutes and the supernatant discarded to reduce water content. Afterwards, feces were frozen at -80°C before freeze drying for 24 - 48 hours, and then finely grinding them with mortar and pestle. Freeze dried and ground feces were used to create a stock solution with a suspended solids concentration of 10 g/L with 0.7 µm filtered seawater (Whatman GF/F 45 mm filters).

Tetraselmis chui (Strain: PLY492) was chosen as microalgal component for the different diets and as control diet due to its biochemical composition. It has a high lipid content and high concentrations of essential FA and sterols. Juvenile eastern oysters, *Crassostrea virginica*, have shown higher growth rates fed on a *T. chui* diet than with widely used shellfish diets based on *Isochrysis sp.* (Strain: T-ISO) (Wikfors et al., 1996). Its amino acid composition has been compared to *Crassostrea gigas* larvae and has shown similar or greater concentrations which implies a high protein quality as feed (Brown and Jeffrey, 1992). *T. chui* is a unicellular marine flagellate with an elliptical shape that is 12 - 16 µm

long and 7 - 10 µm broad (Fig. 6). It is most commonly found in tide pools and estuaries across Europe and North America (Hori et al., 1986).

Fig. 6 - *Tetraselmis chui*, live sample Roscoff Culture Collection, strain 128. © F. Jouenne [2]

Live algae were procured from the UK (VariconAqua Solutions) as Tetraselmis 3600 concentrate with a listed cell count of 720 million per mL and a dry weight of 18 %. The oily concentrate was diluted in a ratio of 1:1000 with filtered artificial sea water for better handling. Both the concentrate and the dilution were stored at 4°C until feed solutions were prepared.

Based on pre-determined carbon contents and the estimated oyster TDW in each tank, diets were mixed from the stock solutions according to Table 4.

Table 4 - Amounts of stock solution mixed daily for every diet (measured using 50 mL measuring pipette) to create different feeds (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) and final dose fed per tank and day

Diet components [mL]	Stock solutions			Dose per tank
	Feed solution	Feces solution	Algae solution	
FD	70.0			11.5
FS		188.0		31.0
FF	38.0	94.0		21.5
C			163.0	60.5
M	23.0	62.5	121.0	34.5

[2] This work is licensed under a Creative Commons Attribution-Share Alike 3.0 License. Content accessed: 13.04.2015 URL: http://planktonnet.awi.de/index.php?contenttype=image_details &itemid=55878#

3.5 Sampling

Tissue samples (n = 30) for CI, FA composition, C/N ratio and energy value were taken out of the overall stock of oysters at the start of the experiment (T_0). Nutrient concentrations, salinity, pH, O_2 concentration and carbonate hardness (KH) were routinely measured twice a week in the seawater tanks (Table 5) by staff from the AWI. Data were provided by courtesy of Mr. Fredy Moraleda. Daily checks of dissolved oxygen (DO), salinity and temperature (WTW multi 340i) in two random oyster tanks showed no deviation from the norm.

Particle load in total particulate matter (TPM) and C/N ratio were determined every day after feeding for every diet by filtering 500 mL of water out of the control tanks over pre-washed, pre-ashed (500°C for 4 h, ThermoConcept muffle furnace) and pre-weighed GF/F filters (Whatman 25 mm). 500 mL of water out of the control tanks for the diets M and C were filtered over pre-washed GF/F filters to determine Chlorophyll *a* (Chl *a*) concentrations. All parameters were also measured in the seawater, used for the experiment, to determine background levels.

Table 5 - Mean biophysical water parameters over the experimental period. SD = standard deviation.

	$NH4^+$ [mg/L]	$NO2^-$ [mg/L]	$NO3^-$ [mg/L]	Temp. [°C]	Sal. [PSU]	pH	O2 [mg/L]	KH
Mean	0.11	0.03	28.7	15.8	33.6	8.01	9.56	6.0
SD	0.02	0.01	11.9	0.2	0.7	0.03	0.86	0.3

3.6 Measurements

3.6.1 Chlorophyll a

Chl *a* concentration was measured using the fluorometric method. Chl *a* fluoresces in the red spectrum after it is excited by blue wavelengths. The fluorescence by phaeopigments is accounted for and corrected by acidifying the sample and thereby converting the Chl *a* into phaeopigment and subtracting the values.

Frozen (-20°C) GF/F filters are thawed in a dark room and 8 mL of acetone (90 %) are added before homogenizing the sample using ultrasound. The sample is stored in ice during this process. After homogenizing the sample it is stored at 4°C for 2 h to extract chlorophyll. The sample is then centrifuged at 5°C for 10 minutes (5000 rpm, Sigma 3K-1) and fluorescence in the supernatant liquid is measured against an acetone blank

(cuvette thickness: 1 cm). Subsequently it is acidified by adding two drops of 1 N HCl and fluorescence of phaeopigments is measured after 30 seconds.

$$\frac{Fm}{Fm-1} * (F - Fa) * Ks * \frac{v}{V} \quad (1)$$

Chl *a* concentration in µg/L is determined using equation (1) with Fm = 2.14, Ks = 0.9624, F = fluorescence before acidification, Fa = fluorescence after acidification, v = volume of acetone and V = volume of filtrated water.

3.6.2 Carbon-to-nitrogen ratio

The C/N ratio was determined using a EURO EA 3000 CN-Element Analyser. At a furnace temperature of 1010°C and in an atmosphere of concentrated oxygen the sample is spontaneously oxidized. The gaseous oxidization products are carried through a catalyst column by the carrier gas helium. The resulting mix of gasses consists of nitric oxides, carbon dioxide, unburned oxygen and water. It passes through a copper reduction column, which binds the oxygen, leaving nitrogen. A condensate separator removes residual moisture from the mixture before remaining gases are separated by a gas chromatography (GC) column and detected by a thermal conductivity detector.

For the analysis of the tissue samples 500 - 800 µg of freeze dried and ground sample were weighed (Sartorius MSA2.7S-000-DM) into 5x9 mm tin cups (HEKAtech GmbH). Tin cups were folded three times taking care not to spill any of the sample and placed in a microtiter plate sealed with Parafilm tape and placed in a desiccator until analysis. All tools were cleaned with 96 % ethanol before and after every step, letting excess ethanol vaporize before further usage.

Frozen filters were first thawed and, after adding three drops of 0.1 N HCl to remove carbonate, dried for at least 24 h at 60°C and stored in a desiccator. They were then gently folded using blunt tweezers and placed in large tin cups (8x8x15 mm, Elementar, #22137420). The cup was then folded around the filter and formed into a small roll which is then placed in a pill press and pressed into shape. Should the package not be tightly sealed or be ripped in places it is placed in a second cup and the process is repeated. The

pressed capsules are stored in a sealed microtiter plate in a desiccator. All utensils and surfaces that come into contact with sample, filter or cups are cleaned with acetone or ethanol.

The large tin cups had to be washed before their use to remove organic materials. This was done by filling a 250 mL Schott flask half full with tin cups and covering them with chloroform. The flask was left to rest for 10 minutes, while occasionally gently rolling the flask to remove any air bubbles. The filters were decanted after 10 minutes or drained over a funnel. The process was repeated once with acetone. Afterwards, about 50 cups were placed in a petri dish washed with acetone, placed in a cold drying oven and brought up to 90 - 100°C for at least 2 h. The cooled petri dish was covered with parafilm and stored in a desiccator until use.

Blanks and standards are measured before every set of analyses. Weighed standards contain between 0.2 and 1.2 mg of acetanilide. After 35 measurements an unknown standard is measured to test the system.

C/N ratio was determined in triplicate for three oysters from all tanks with at least three remaining individuals as well as 10 oysters from the start of the experiment.

3.6.3 Condition index

CI in marine bivalves are commonly used as indicators for overall physiological state and may for example indicate physiological stress such as environmental pollution or parasitism (Brown and Hartwick, 1988; Pridmore et al., 1990; Mercado-Silva, 2005). The chosen CI correlates TDW to shell DW (2). It may be used when salinity is not inhibiting oyster growth and primary factors affecting oyster condition are water temperature and available food (Brown and Hartwick, 1988).

$$CI = \frac{tissue\ dry\ weight}{shell\ weight} * 100 \qquad (2)$$

This index was also chosen for its convenience, reliability and compatibility with planned analyses since most indices require the cooked meat weight, altering the biochemical composition of tissue in the process, or the volume of the shell (Lucas and Beninger, 1985; Davenport and Chen, 1987). It is also reliable due to its independence of the high

variability of intervalval fluid in oysters (Beninger and Lucas, 1984). TDW was determined by opening the oyster and letting fluids but no tissue drain out and then flash freezing the remaining tissue by submerging the oyster in liquid nitrogen to preserve the biochemical composition. Frozen tissue was cut with a scalpel and scraped into 15 mL pre-weighed (Sartorius LE4202S) Falcon tubes, taking care not to include shell parts or silt deposits incorporated into the shell. Samples were then stored at -80°C until freeze drying for 24 – 48 h (CHRIST, Alpha 1-4 LSC & Vacuubrand, RZ 6). Tubes with dried samples were weighed again and TDW was determined by subtracting DW from WW. Empty shells were dried on numbered trays at 60°C for 48 h to remove residual moisture before they were weighed.

3.6.4 Energy value

Dry tissue energy value of samples was determined by using a Parr calorimeter 6100 and the oxygen bomb type 1108. The Parr 6100 is an isoperibol calorimeter and operates by igniting and burning a known quantity of a sample under a pressurized oxygen atmosphere inside a high-strength, high nickel stainless steel bomb submerged in a bucket filled with deionized water. The water contains a stirrer and thermistor which continuously measures the temperature of the water during the burning process. If the sample is fully burned up after ignition, all released heat energy is transferred to the surrounding water and causes a temperature increase. After a state of equilibrium is reached the released energy is calculated based on the measured temperature increase as well as correction factors for various parameters.

Energy value was determined in triplicate for three oysters from all tanks with at least three remaining individuals as well as ten oysters from the start of the experiment. Values are recorded as MJ/kg DW. Due to the small amount of material available per sample, gelatine capsules (Feinbiochemica Heidelberg, Gelatine Cups Nr. 0) were used as accelerant. The mean caloric value of the gelatine capsules was determined beforehand and deducted from the overall measured energy release.

To prepare the calorimeter for operation the motor of the stirrer is warmed up after starting the device, the pressurized oxygen valve is opened and the bucket is filled with 2000 g (± 0.5 g) of deionized water. The oxygen bomb was prepared by lightly coating the seal with deionized water and tying a fuse (length, 10.00 cm) to the wire (length, 6.35 cm). Approximately 20 - 25 mg of finely ground and freeze dried sample was weighed

(Sartorius Quintix224-1S) into a pre-weighed gelatine capsule. The filled and closed capsule was then laid into the crucible and on top of the fuse to ensure continuous contact during ignition and to prevent misfires. After tightly closing the bomb and the gas outlet valve, the oxygen tube is connected to the inlet valve of the bomb and the filling process is started on the calorimeter (Oxygen: N48, 99.9 %). The pressurized bomb is then gently lowered into the bucket using a pair of pliers and the fuse is armed by plugging the wires into the contact sockets on top off the bomb. After the measurement is complete the bomb is removed from the bucket and the pressure is slowly released by opening the gas outlet valve. The crucible and bomb are washed with deionized water and dried off before and after every measurement. The bucket is refilled to replace water losses during the removal of the bomb.

3.6.5 Fatty acid composition

FA content and compilation was measured using gas chromatography (GC) (Agilent Technologies 6890 Network GC System, Agilent J&W GC Columns 122-3232). Approximately 20 mg of freeze dried and ground sample was weighed (Sartorius 1602 MP8/8-1) into 12 mL glass centrifuge vials and 100 µl of 23:0 standard (Sigma-Aldrich, Methyl tricosanoate) were added. 2 mL of Dichloromethane/Methanol (2:1) were added before submerging the sample in an ultrasonic bath (Sonorex Digital 10p) for 10 minutes. This process was repeated twice, taking care of always fully submerging the sample and washing down any ground sample from the walls of the vial. The sample was then centrifuged at approximately 2000 rpm for 10 minutes (Hettich EBA 8S,40 %-setting) and the supernatant siphoned out into 8 mL vials using expendable 2 mL glass Pasteur pipettes. Solvents were evaporated under a nitrogen atmosphere while the sample was heated. 3 mL of the Dichloromethane/Methanol mixture were added and the vials swayed until all residue was resolved to create the raw lipid extract. The raw lipid extract was stored at 4°C until further processing.

For derivatization 1.5 mL of raw lipid extract were pipetted into 12 mL vials (checked for seal tightness) and the solvents evaporated. 250 µl of cyclohexane and 1 mL of derivatization reagent (3 % H_2SO_4 in Methanol) were added. The samples were than heated to 80°C for 4 hours. After the samples had cooled down to room temperature, 4 mL of deionized water and 2 mL of cyclohexane were added and samples were shaken for 30 seconds. After the two liquid phases have completely separated, the upper phase

was siphoned off into 8 mL vials using disposable Pasteur pipettes. This process was repeated two times to remove any remaining impurities. The cyclohexane was then evaporated and the residue solved in 250 µl of Cyclohexane before being transferred into GC-Vials with inserts and stored at 4°C until measuring.

During the measurement process three vials of cyclohexane, a copepod standard and a commercial PUFA-3 standard (MATREAYA LLC, Catalog No. 1177) were measured before every 10 samples to check the column. Peaks were detected and peak area calculated using the ChemStation (B.04.01 SP1) software. The peaks were identified by comparison to the known peaks and retention times in the two standards. This was done using MS Excel, matching every retention time to closest retention time measured in the standards.

All glass wares (expendable and reusable) were pre-treated by washing with lukewarm water and air-drying before being submerged in a bubble free decontamination solution for 24 hours. Afterwards the glass wares are rinsed with water before being washed in a dish washer once with detergent and once only with water. They are then soaked in a Milli-Q bath for another 24 hours. Following the soak the glass wares are dried at 200°C in a muffle furnace for 2 hours and ashed at 500°C for 5 hours. A cover of aluminium foil keeps particles from accumulating during storage periods.

3.7 Statistical analysis

Arithmetic mean and standard deviations (SD) were calculated using equations (3) and (4) embedded into MS Excel 2013. All statistical analyses were conducted using Sigma Plot Build 12.5.0.38. Normality was tested using the Shapiro-Wilk test. Non-normally distributed data was analysed using the Kruskal-Wallis One Way ANOVA on Ranks and specific differences detected using Dunn's Methods for multiple comparisons versus a control group and in pairs.

$$\bar{x} = \frac{\sum x_i}{n} \tag{3}$$

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}} \tag{4}$$

Tests were conducted using the p-value 0.05 as threshold for significance. Tests deviating from these norms are specified in the following.

4 Results

4.1 Mortality

Due to mortality during the experiment the duration of 28 days was reduced to 23 days. This was done to preserve at least three tanks per diet with at least 3 remaining oysters each for analyses. Dead individuals were removed daily, frozen and replaced with oysters from the same stock and approximately mean volume to retain a comparable filtration capacity for every tank. Newly added oysters were separated from original oysters by their placement in the tanks. Only oysters present in the system since the start were used for analysis.

The overall mortality after 23 days is comparatively displayed in

Fig**. 7**. There was no significant difference in mortality between treatments (ANOVA, p = 0.125). Pearson Product Moment Correlation shows a positive correlation coefficient of 0.533 (p = 0.006) for the height of the tank in the shelving unit and the mortality per tank. A positive correlation coefficient implies that the values tend to increase together. The three tanks where too few individuals remained (n ≤ 2) due to mortality, where omitted from all analyses.

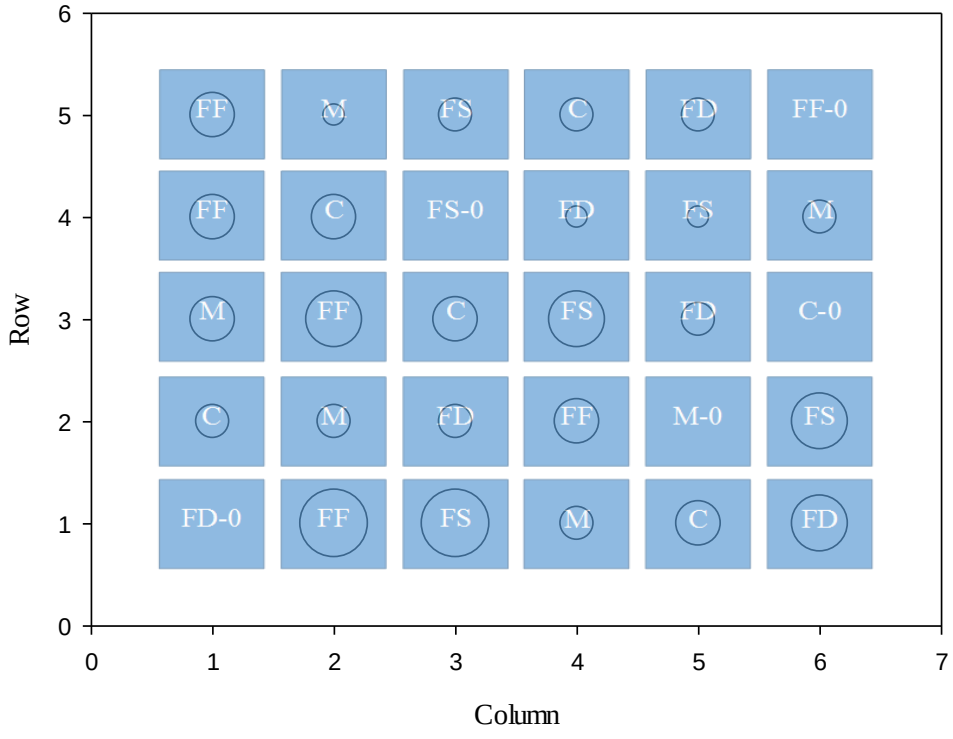

Fig. 7 - Ball plot comparing remaining starting oysters in every tank of every treatment (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)), arranged by their spatial positions (columns and rows) in the shelving unit. Blank spaces represent empty control tanks. Ball size increases with number of remaining starting oysters.

4.2 Determination of dry weight

The external parameters width, height, length and total WW were plotted against TDW and a linear regression was performed to fit a linear model to the data. As shown in Fig. 8, no coefficient of determination higher than 0.48 was achieved for any of the initially measured external parameters.

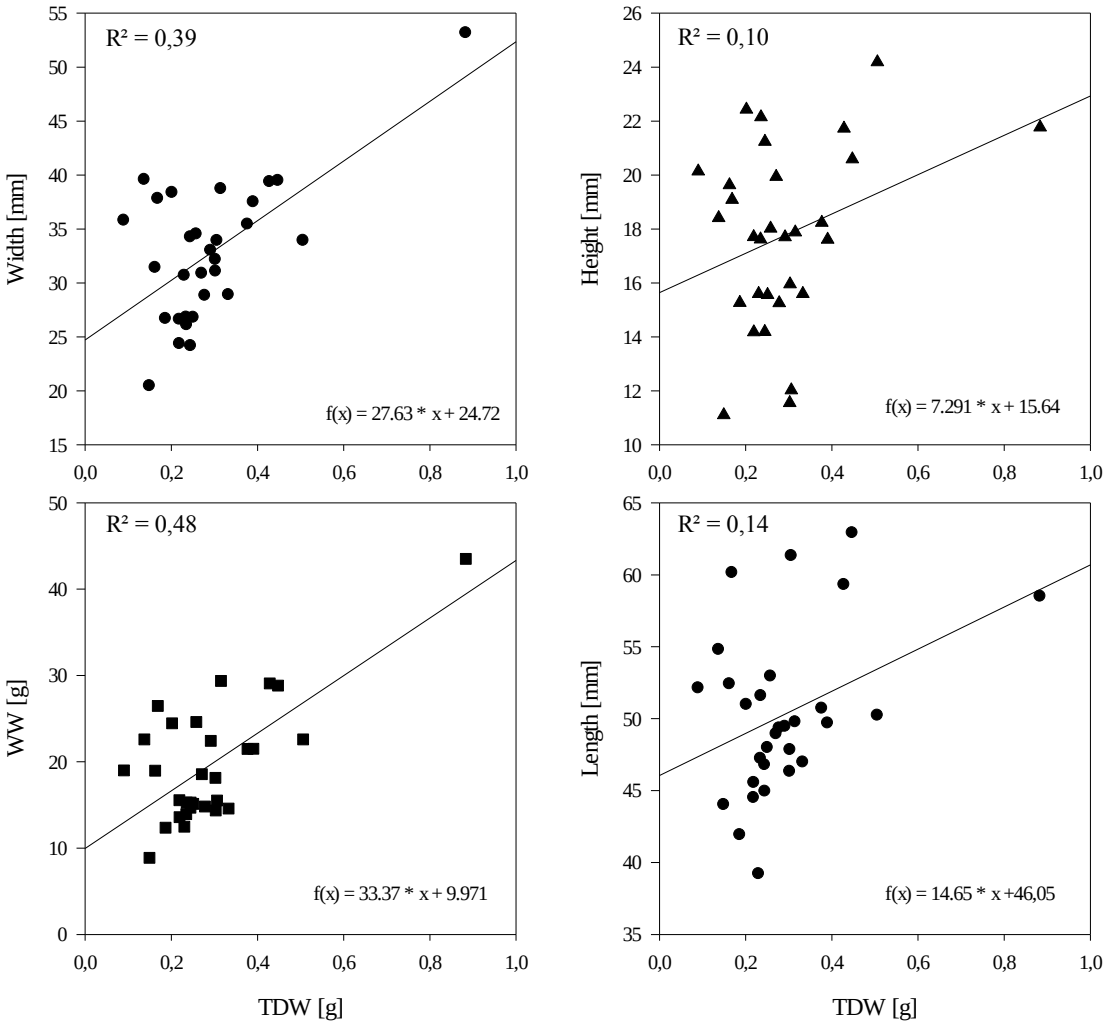

Fig. 8 - Comparison of coefficients of determination for linear regression models for width, height, wet weight (WW) and length to tissue dry weight (TDW)

Cluster Analysis was performed to fit different models to slightly different size classes but no correlation could be found. A higher coefficient of determination (Fig. 9) was achieved by comparing the total volume of the oysters to their TDW (n = 15) after freeze drying.

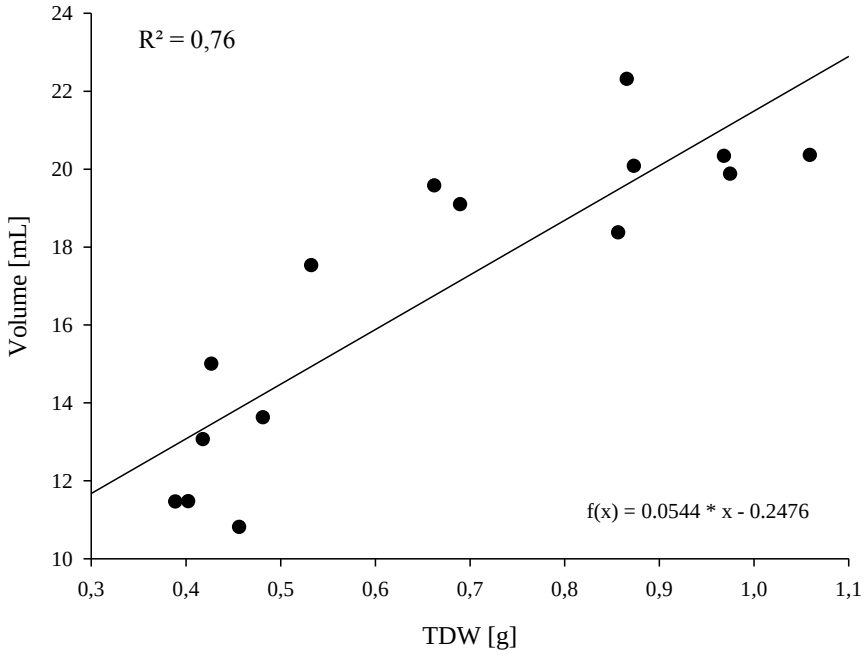

Fig. 9 - Linear regression model for volume to tissue dry weight (TDW) with f(x) = 0.0544 * x -0.2476

Estimated TDW of the stocked oysters based on Fig. 9 was approximately 1.3 g while analysis of a subsample (n = 30) of the same stock later showed an actual mean TDW of 0.32 ± 0.08 g.

Oyster from different treatment groups are from this point on referred to by the designation of the diet they received: FD (turbot feed), FS (turbot feces), FS (turbot feed and feces), C (*T. chui*) and M (a mix of all components).

4.3 Feed composition

Mean carbon content of a subsample (n = 3) of oyster tissue, feces, fish feed and algae were determined before the start of the experiment to be 345 µg/mg, 180 µg/mg, 480 µg/mg and 493 µg/mg of DW respectively. Mean volume of stocked oysters at the start of the experiment was 29.8 ± 2.7 mL (n = 150). Mean particulate matter supplied by the different diets ranged from 2.7 mg/L in the FS group to 1.3 mg/L for group C (Table 6) with large variances.

Table 6 - Mean total particulate matter (TPM) in mg/L in all feeds (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) and in the seawater (SW) over the duration of the experiment (n = 10) Background TPM of the seawater was subtracted from feed TPM to calculate added particulates. SD = standard deviation.

TPM [mg/L]	Feeds					
	FD	FS	FF	M	C	SW
Mean	2.380	2.683	2.015	2.600	1.250	1.927
SD	1.039	1.342	1.258	1.535	1.473	0.776

Background particle load of the used seawater (1.9 mg/L) was subtracted from all determined total particle loads to obtain the particle load resulting from the added diets. Chl *a* concentrations were highest in the feed supplied to group C (5.2 µg/L) and lowest in the base seawater (0.3 µg/L) (Table 7).

Table 7 - Mean Chl *a* concentration in µg/L in feeds M, C and base concentration in the seawater (SW) (n = 10). SD = standard deviation.

Chl *a* [µg/L]	Feeds		
	M	C	SW
Mean	1.698	5.223	0.253
SD	0.646	0.636	0.233

Supplied carbon concentrations were highest in the FD and FS diets and lowest in the C diet (Table 8). Nitrogen concentrations showed a similar but less distinct trend with the highest and lowest concentrations found in FD and C. The C/N ratio for the diets FD, FS and FF were approximately 9.3. M and C diets had ratios of 7.2 and 6.2, respectively. Lowest values for all three parameters were measured in the seawater.

Table 8 - Mean Carbon (C) and Nitrogen (N) amounts in µg/L for every diet (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) and the used seawater (SW) (n = 10). Mean C/N ratio of every diet and the seawater. SD = standard deviation.

	Feeds:					
C [µg/L]	FD	FS	FF	M	C	SW
Mean	1508.21	1477.27	1292.15	1130.79	669.18	315.61
SD	663.09	592.12	454.00	425.83	527.05	147.48
N [µg/L]						
Mean	175.66	154.97	154.46	157.69	106.29	55.62
SD	91.18	58.94	59.73	52.64	78.94	25.94
C/N						
Mean	9.33	9.28	8.52	7.21	6.22	5.74
SD	2.58	1.93	1.04	1.05	0.80	0.70

After accounting for actual mean TDW of the used oysters and mean carbon concentrations supplied by each diet, the percentage of carbon to TDW supplied to the oysters per day could be calculated. Actual feed supply for the diets FD and FS was 2.0 % of tissue carbon content per day, followed by FF with 1.8 % and M and C with 1.5 % and 0.9 %, respectively.

4.4 Condition index

The mean CI was 1.4 ± 0.4 for T_0 oysters (n = 30). At the end of the experiment mean CI for every group was higher and highest in FD (Table 9). Data was not normally distributed ($p > 0.05$) but still showed significant differences between treatment groups and T_0.

Table 9 - Mean condition index and n for every group (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)). SD = standard deviation.

	Oysters fed with:					
Condition index	FD	FS	FF	M	C	T_0
Mean	2.0	1.5	1.9	1.9	1.8	1.4
SD	0.8	0.4	0.9	0.6	0.5	0.4
n	14	19	21	13	17	30

Condition of oysters fed the FD, FF and Mix diets was significantly better with medians of 1.7, 1.7 and 1.6, respectively (ANOVA on Ranks, $p \leq 0.05$). Oyster feeding on FS and C showed no significant difference in medians (Fig. 10) compared to the starting point ($p > 0.05$) (Fig. 10).

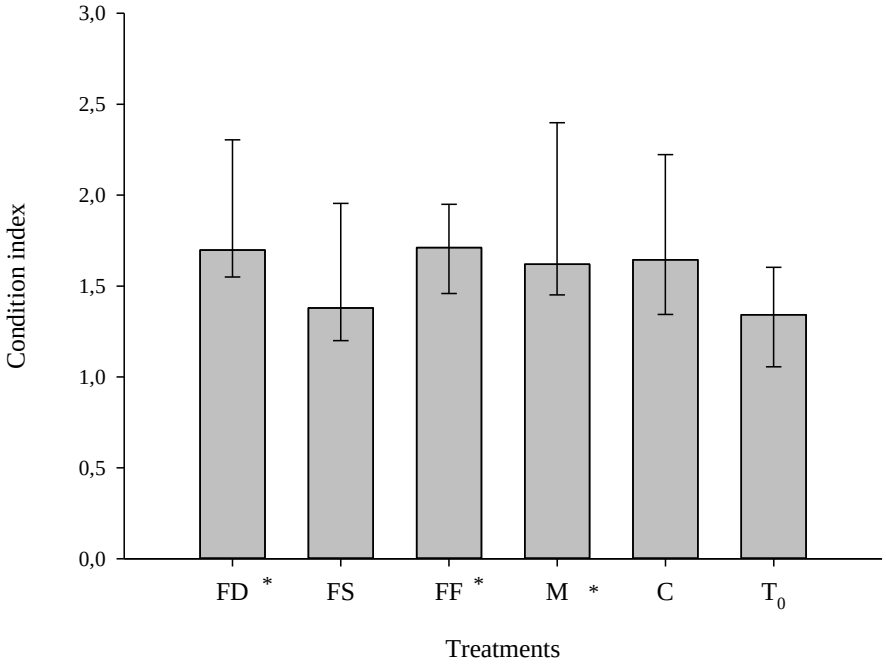

Fig. 10 - Median CI of all groups (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) and the starting point T_0 with 25th and 75th percentile displayed as error bars. Groups marked with * showed significant differences to the start (T_0).

When T_0 is excluded from the comparison no significant difference is found in condition between treatments (ANOVA on Ranks, $p = 0.371$).

4.5 Energy value

Mean energy values for *T. chui*, turbot feed and turbot feces were 17.1 ± 0.2 MJ/kg, 23.1 ± 0.9 MJ/kg and 8.5 ± 1.9 MJ/kg, respectively (n = 3). Mean energy values for oyster tissue from all treatment groups had large standard deviations (Table 10) and data were not normally distributed (Shapiro-Wilk, $p < 0.05$). All values ranged between 18.7 and 16.1 MJ/kg DW.

Table 10 - Mean energy value for all groups (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) and the starting point T_0 in MJ/kg. SD = standard deviation.

Energy value [MJ/kg]	Oysters fed with:					
	FD	FS	FF	M	C	T_0
Mean	18.7	18.5	16.1	18.2	17.7	17.7
SD	2.0	1.7	3.1	2.2	2.8	2.1
n	15	12	12	12	13	10

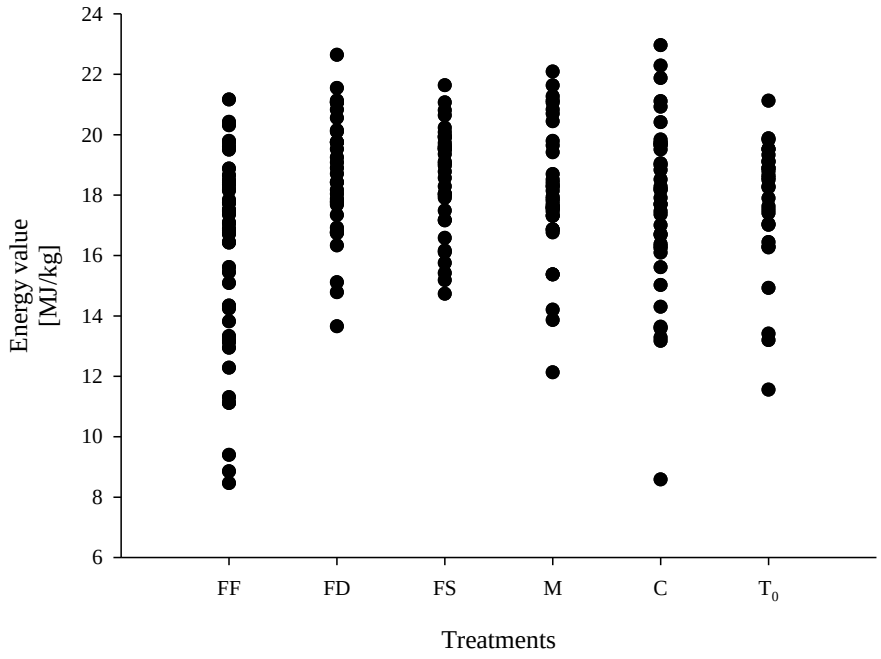

Fig. 11 - Comparison of the distribution of energy values of all treatments groups (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) and the starting point T_0.

Differences between treatment groups and T_0 were not significantly different (Kruskal-Wallis ANOVA on Ranks, $p > 0.05$) while all groups but C showed significantly higher energy values than FF ($p < 0.05$).

Variance inside the treatment groups (Fig. 11) and standard deviation was largest in FF with 3.1 MJ/kg (Table 10). Oxygen supply to the calorimeter had to be exchanged after measurement 28, after which point the variance was smaller (Fig. 12).

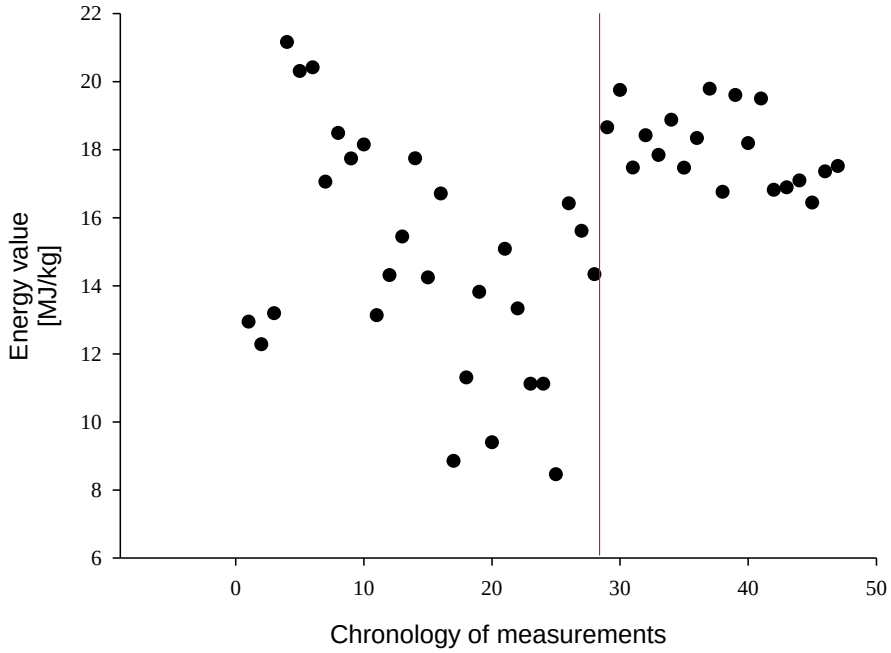

Fig. 12 - Energy values for all measurements from the FF (feed and feces) group in chronological order. Exchange of the oxygen supply is marked with a vertical line after measurement 28.

When the first 28 measurements are excluded from the comparison, the new resulting mean and standard deviation, 18.0 ± 1.1 MJ/kg (n = 6), are no longer significantly lower than any of the other groups (Kruskal-Wallis ANOVA on Ranks, p = 0.192).

4.6 Carbon-to-nitrogen ratio

Mean C/N ratio of the three feed components *T. chui*, turbot feed and turbot feces were 5.81 ± 1.34, 5.57 ± 0.69 and 13.50 ± 0.67, respectively. C/N ratio of oyster tissue is displayed in Table 11. Data was not normally distributed (Shapiro-Wilk, $p < 0.05$) but showed significant differences between treatment groups and T_0 (ANOVA on Ranks, $p \leq 0.001$).

Table 11 - Mean C/N ratio for all groups (FD (feed), FS (feces), FF (Feed and Feces), M (Mix of all components) and C (*T. chui*)) and the starting point T_0. SD = standard deviation.

	Oysters fed with:					
C/N ratio	FD	FS	FF	M	C	T_0
Mean	4.05	3.71	4.07	3.87	3.68	4.10
SD	0.26	0.19	0.37	0.20	0.37	0.55
n	14	14	15	14	15	10

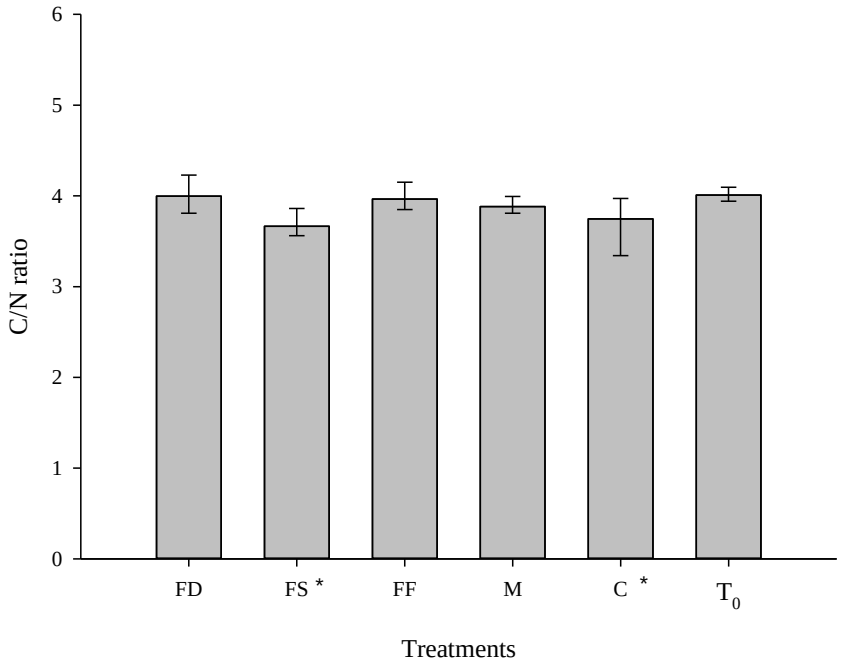

Fig. 13 - Median C/N of all groups (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) and the starting point T_0 with 25th and 75th percentile displayed as error bars. Groups marked with * showed significant differences to the start (T_0).

Comparison versus T_0 showed that C/N ratios of FS and C were significantly lower ($p < 0.05$). After excluding T_0 from the test, significant differences between treatment groups can be shown. All diets except C show higher C/N ratios than the FS group, while FD and FF groups showed higher ratios than the C group (Fig. 13)

4.7 Fatty acid composition

FA composition in percent of total fatty acids (% TFA) for all three diet components as well as oyster groups and T_0 are comparatively displayed in Table 12. Only components that constituted at least 1 % of TFA are displayed and considered in this study. Fractions smaller 1% are grouped together, regardless of their structure, as others. Levels of others are comparable in all three feed components, but differ between oyster groups.

All three components showed distinctively different compositions of the major FA groups (Fig. 14). Turbot feed is characterized by a large proportion of SFA compared to *T. chui* and turbot feces. This large faction is comprised mostly of 16:0, followed by 18:0 and 14:0 FA (Fig. 15). *T. chui*, on the other hand, shows high amounts of PUFA. These are mostly 18:2(n-6), 18:3(n-3) and 18:4(n-3) FA. Turbot feces exceeded the other groups with HUFA, which also showed higher diversity than in the other feed choices and consisted of 20:4(n-6), 20:5(n-3), 22:5(n-3) and 22:6(n-3). 20:5(n-3) and 22:6(n-3) comprised the largest part of this portion with 13.5 % and 9.2 % TFA, respectivly. MUFA composition did not differ much between components (Fig. 16).

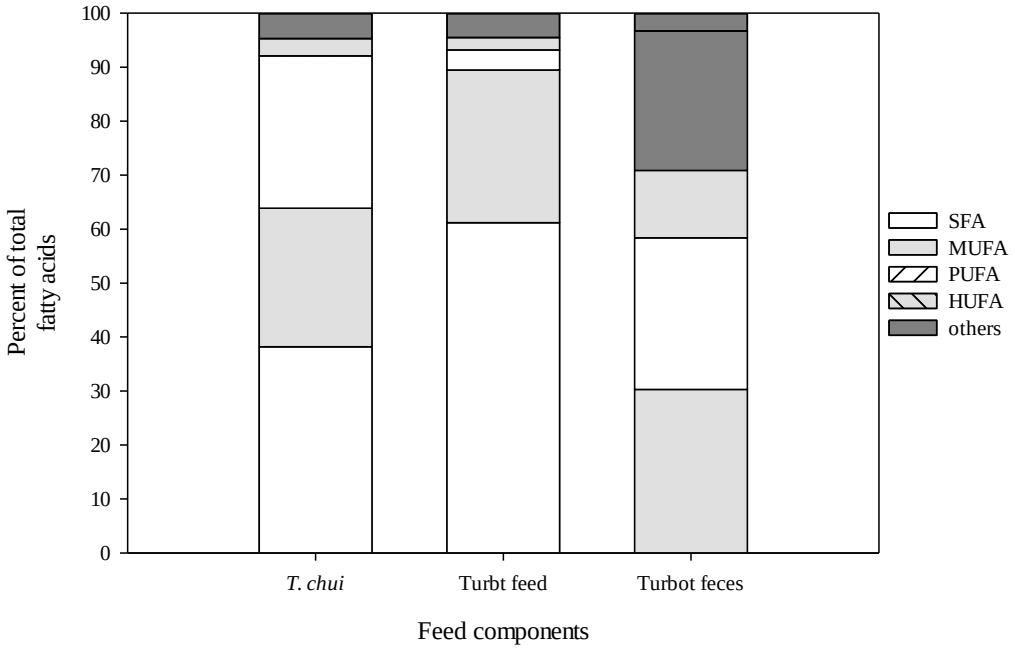

Fig. 14 - Mean percentages of fatty acid classes (saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA) and highly-unsaturated fatty acids (HUFA)) in the three feed components. Fatty acids constituting less than 1 % of TFA are classified as others (n = 3).

Table 12 - Fatty acid composition of feed components, T_0 and oysters out of every group (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*)) after the experiment. Components are displayed as percent of total fatty acids (% TFA) ± SD (standard deviation) and grouped into the classes saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA) and highly unsaturated fatty acids (PUFA). Components accounting for less than 1.0 % of TFA summarised as others. For feed components n = 3 while for oyster tissue n ranges from 10 - 15.

Fatty acids	Feed components				Oyster fed with:				
(% TFA ± SD)	*Tetraselmis sp.*	Turbot feed	Turbot feces	T_0	FD	FS	FF	M	C
SFA									
14:0		7.2 ± 0.4	6.9 ± 0.5	1.2 ± 0.6	2.8 ± 1.1	1.6 ± 0.7	1.9 ± 0.7	1.5 ± 0.6	1.3 ± 0.5
15:0				1.3 ± 0.6	1.7 ± 0.2	1.8 ± 0.3	1.5 ± 0.4	1.3 ± 0.6	1.9 ± 0.1
16:0	38.2 ± 1.3	42.7 ± 0.7	19.5 ± 0.2	28.8 ± 2.8	33.9 ± 4.7	29.7 ± 2.7	32.0 ± 4.3	28.4 ± 3.0	33.8 ± 3.3
18:0		11.3 ± 0.5	3.9 ± 0.1	21.6 ± 12.4	11.3 ± 1.8	12.0 ± 1.6	12.5 ± 2.5	20.9 ± 13.5	14.4 ± 2.2
MUFA									
16:1(n-7)		4.0 ± 0.2	8.0 ± 0.2	1.5 ± 0.3	4.0 ± 1.0	2.3 ± 0.5	2.8 ± 0.7	1.8 ± 0.4	2.0 ± 0.4
18:1(n-9)	13.9 ± 0.2	13.3 ± 0.3	13.2 ± 0.2	5.3 ± 3.5	8.0 ± 2.0	5.7 ± 1.8	6.3 ± 0.9	6.4 ± 3.0	4.7 ± 1.5
18:1(n-7)	4.4 ± 0.1	2.9 ± 0.1	3.4 ± 0.1	4.5 ± 1.2	6.1 ± 1.0	5.1 ± 1.3	6.0 ± 1.8	4.8 ± 1.7	5.7 ± 0.9
20:1(n-11)	5.1 ± 0.2	3.0 ± 0.1		3.6 ± 2.1	4.4 ± 0.5	3.4 ± 1.8	4.8 ± 0.8	3.4 ± 1.8	3.6 ± 1.1
20:1(n-9)	2.3 ± 0.0	3.0 ± 0.1	1.9 ± 0.1	1.7 ± 0.9	2.6 ± 0.4	2.3 ± 0.7	2.9 ± 0.4	1.4 ± 0.7	2.6 ± 0.4
20:1(n-7)				6.5 ± 3.7	6.5 ± 0.8	7.3 ± 0.7	7.7 ± 1.3	4.9 ± 2.8	8.5 ± 0.6
22:1(n-11)		2.1 ± 0.0	1.6 ± 0.0						
22:1(n-7)				1.9 ± 1.1	2.0 ± 0.4	5.9 ± 4.6	2.4 ± 1.0	5.9 ± 4.7	2.6 ± 0.5
24:1(n-9)				1.7 ± 0.3				1.6 ± 0.1	
PUFA									
16:2(n-4)			1.3 ± 0.0						
16:3(n-4)			1.4 ± 0.0	4.2 ± 3.2	5.4 ± 3.2	6.3 ± 3.5	5.4 ± 3.6	3.7 ± 3.5	6.0 ± 3.3
16:4(n-1)			1.5 ± 0.1						
18:2(n-6)	10.6 ± 0.2	3.7 ± 0.1	5.1 ± 0.6	2.4 ± 1.8	1.3 ± 0.3	1.4 ± 0.3	1.7 ± 0.4	2.5 ± 1.9	1.5 ± 0.3
18:3(n-3)	14.1 ± 0.3		1.3 ± 0.0						
18:4(n-3)	3.5 ± 0.2		1.9 ± 0.0						
HUFA									
20:4(n-6)	1.4 ± 0.1		1.2 ± 0.0	2.4 ± 1.5	1.3 ± 0.7	1.8 ± 0.8	1.5 ± 0.8	1.4 ± 1.0	1.9 ± 1.0
20:5(n-3)	1.8 ± 0.1	1.2 ± 0.1	13.5 ± 0.2	4.8 ± 3.1	2.9 ± 1.4	3.4 ± 1.6	3.1 ± 1.6	2.9 ± 2.4	3.1 ± 1.8
22:5(n-3)			1.9 ± 0.1	1.0 ± 0.7	1.0 ± 0.8				1.0 ± 0.5
22:6(n-3)		1.1 ± 0.2	9.2 ± 0.4	5.4 ± 3.7	2.8 ± 1.7	3.5 ± 1.6	3.1 ± 1.8	3.1 ± 2.7	3.2 ± 1.9
others	4.7	4.5	3.3	0.2	3.0	6.5	4.4	4.1	2.6

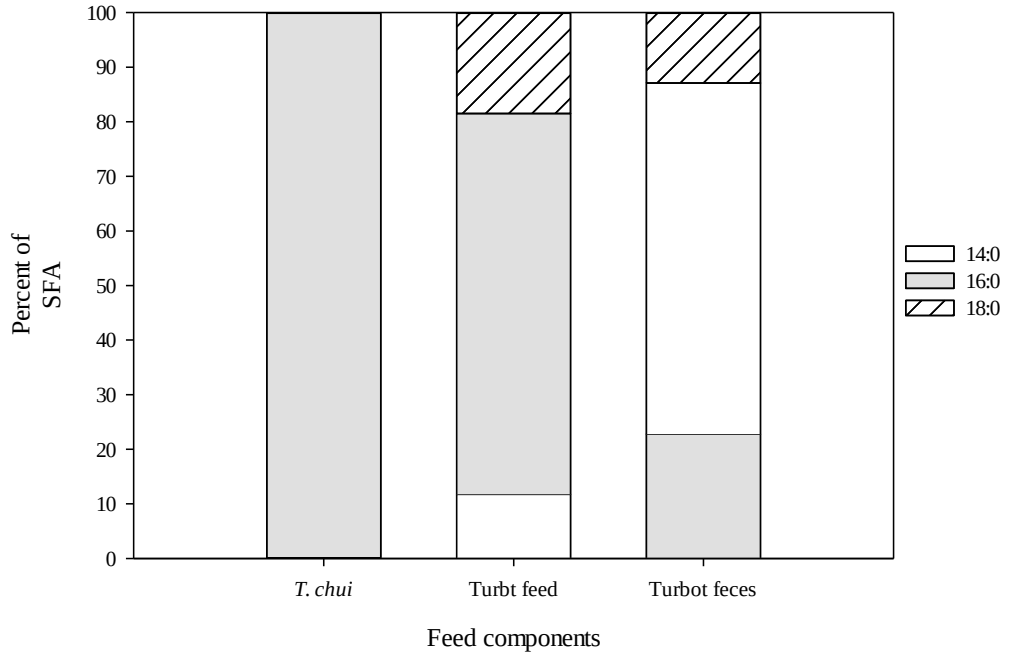

Fig. 15 - Mean percentages of fatty acids in the class saturated fatty acids (SFA) in the three feed components. FA are displayed as percent of the total SFA fraction of the respective component (n = 3).

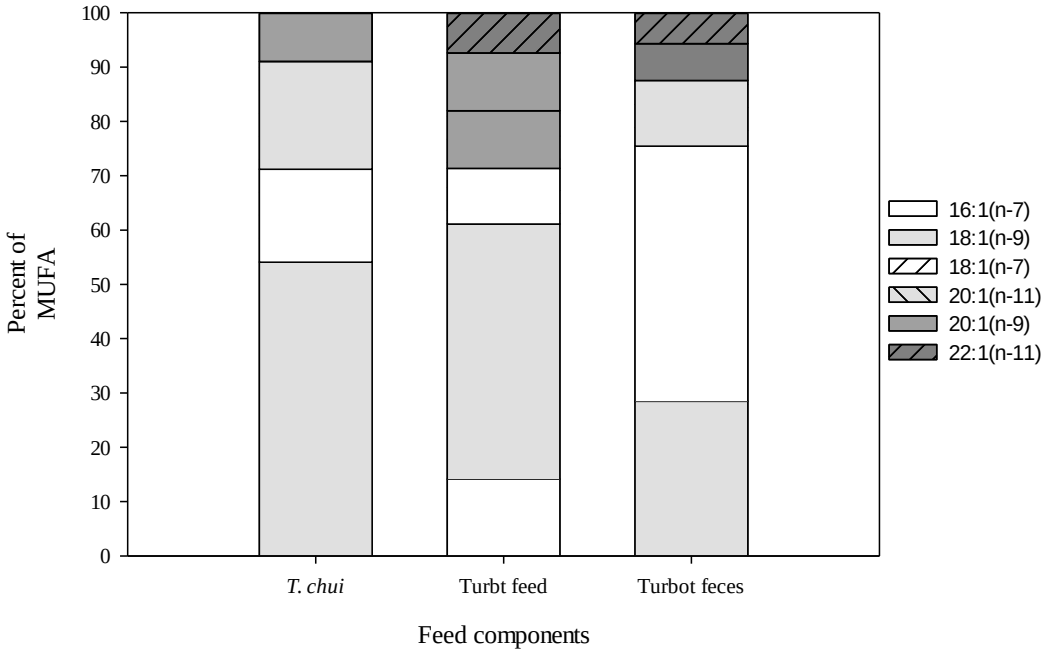

Fig. 16 - Mean percentages of fatty acids in the class mono-unsaturated fatty acids (MUFA) in the three feed components. FA are displayed as percent of the total MUFA fraction of the respective component (n = 3).

Fatty acid composition of oyster tissue before and after the experiment showed larger standard deviations compared to feed components. Distribution of the major FA groups differed very little between the groups and T_0. Proportions of components less than 1 %

were lowest (0.2 %) in the T_0 tissue and highest in FS (6.5 %), with all other groups between 2.6 to 4.4 %.

Most differences in FA composition could be detected in the saturated and mono-unsaturated fatty acids. The SFA of FD showed significantly higher levels of 14:0 as well as lower levels of 18:0 ($p < 0.05$) compared to T_0. Relative 14:0 proportions were also larger than in the control group C and group M.

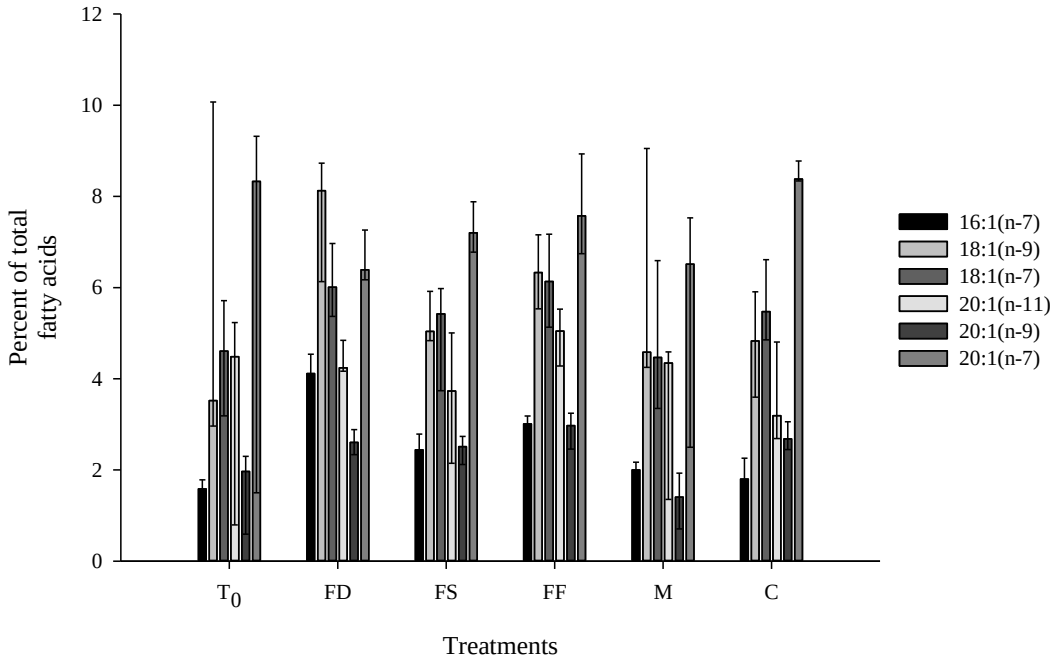

Fig. 17 - Mean percentages (n = 10 - 15) of principal fatty acids in the class mono-unsaturated fatty acids (MUFA) in oyster tissue from the five groups (FD (feed), FS (feces), FF (feed and feces), M (mix of all components) and C (*T. chui*) and the starting point T_0. FA are displayed as percent of total fatty acids.

Many significant differences in proportions of MUFA were detected. These changes occurred in the 16:1, 18:1 and 20:1 isomers (Fig. 17). Levels of 16:1(n-7) were higher in FD and FF compared to T_0 and M while FD was also higher than C. FD also showed higher levels of 18:1(n-9) compared to T_0 and C. In the case of 18:1(n-7), FD and FF again showed higher proportions than at the start. 20:1(n-9) proportions were largest in FD, FF and C.

20:1(n-7) proportions did not differ between the treatment groups and the start. Groups M and FD showed significantly lower values than the control group C.

Significant changes identified in percent of TFA are reflected in changes of absolute FA concentrations. Changes in absolute FA concentrations between T_0 and FD for SFA and

some MUFA are listed in Table 12. Composition of PUFA and HUFA did not differ between groups or T_0.

Table 12 – Mean absolute FA concentrations [µg/mg TDW] ± SD (standard deviation) for oyster tissue from T_0 (n = 6) and FD (n = 9) and feed components (n = 3). Significant differences (t-test for normally distributed data and Mann-Whitney Rank Sum test for non-normally distributed data) ($p < 0.05$) between T_0 and FD are marked with *.

Absolute FA	*T. chui*			Turbot feed			Turbot feces			T_0			FD		
[µg/mg]	Mean	±	SD	Mean	±	SD	Mean	±	SD	mean	±	SD	mean	±	SD
14:0*	0.29	±	0.04	7.13	±	0.41	2.87		0.24	0.09	±	0.02	0.36	±	0.20
15:0	0.07	±	0.00	0.44	±	0.01	0.34	±	0.02	0.21	±	0.02	0.19	±	0.06
16:0	18.72	±	1.00	20.04	±	0.13	17.05	±	0.68	3.68	±	0.46	4.05	±	1.53
18:0*	0.49	±	0.03	3.99	±	0.04	4.52	±	0.15	1.74	±	0.16	1.25	±	0.26
16:1(n-7)*	0.42	±	0.02	8.21	±	0.15	1.59	±	0.11	0.21	±	0.02	0.49	±	0.27
18:1(n-9)*	6.81	±	0.27	13.58	±	0.14	5.33	±	0.26	0.36	±	0.06	0.94	±	0.45
18:1(n-7)*	2.17	±	0.09	3.54	±	0.04	1.17	±	0.06	0.61	±	0.12	0.70	±	0.31
20:1(n-11)*	2.52	±	0.06	0.84	±	0.87	1.20	±	0.05	0.61	±	0.04	0.50	±	0.14
20:1(n-9)	1.12	±	0.04	1.95	±	0.08	1.20	±	0.05	0.28	±	0.04	0.30	±	0.07
20:1(n-7)*				0.25	±	0.00	0.11	±	0.00	1.07	±	0.05	0.71	±	0.14

5 Discussion

The IMTA approach to offshore aquaculture supports a sustainable, ecologically friendly and economically valuable offshore industry (Troell et al., 2009; Chopin et al., 2010) with some potential to satisfy future demands of the global seafood market. Its effectiveness is based, in part, on the supplemental effects of the co-cultured species

The bio-extraction capacity of filter-feeding organisms in such IMTA systems builds on two assumptions: (1) Bivalves will ingest waste particles leaving the fish culture site and (2) they will absorb and incorporate them (Cranford et al., 2013). Feeding responses of different bivalves to fish farm effluents have already been investigated (Lefebvre et al., 2000; Reid et al., 2010) and it was concluded that bivalves will ingest aquaculture by-products. The next step was to investigate the incorporation of these by-products by the bivalves. This incorporation was demonstrated in *Mytilus edulis* by Redmond et al. (2010) and Handå et al. (2012). The assessment of incorporation by oysters, e.g. *Crassostrea gigas*, was still pending. In the present study, a variety of different analyses was chosen to investigate how the biochemical composition of *C. gigas* will change in relation to feeds expected to emanate from an IMTA site.

C/N ratio has been widely used to characterise nutritional quality of food sources for decapod crustaceans (Burns and Walker, 2000), aquatic snails (Sheldon and Walker, 1997) and bivalves (Gallager and Mann, 1981). Changes in composition of the organism's tissue reflect changes in nutritional status and growth (Postel et al., 2000) and represent shifts in the accumulation of the three main organic components proteins, lipids and carbohydrates (Bayne, 2009). A composition dominated by lipids and carbohydrates, classical storage molecules, is indicated by a C/N ratio higher than 2.9 (Postel et al., 2000). An increase in the C/N ratio therefore indicates the storage of energy reserves, while a decrease signifies the conversion of energy reserves to metabolic energy or further to muscle mass.

All C/N ratios matched the ranges generally reported for *C. gigas* (Bayne, 2009; Pogoda et al., 2011). No significant increase in CI, i. e. tissue mass to shell weight, was recorded for the groups FS (fed with feces) and C (fed with microalgae). The decrease in the C/N ratio of both groups indicates a depletion of energy reserves in these groups instead of the generation of new protein. Oysters only store lipids in large quantities as reserves during gametogenesis (Dridi et al., 2007), meaning this decrease probably occurred in the

carbohydrate fraction, the glycogen reserves, of the tissue (Deslous-Paoli and Héral, 1988). A depletion of energy reserves without a resulting growth of the muscle mass signifies a limited or inadequate food supply (Pogoda et al., 2013). Based on the low carbon content of the TPM of diet C and its nevertheless high energy value, food supply for this group seems to have been too low.

Gallager and Mann (1981) identified the optimum dietary C/N range for the bivalve *Tapes japonica* as 8.4 - 10.5 with values below or above resulting in lesser growth rates. The C/N ratio of the TPM of all experimental diets of this study, except M (fed with a mix of all components) and C fit into this range (Table 8), implying good nutritional quality based on the C/N ratios. This assumption is consolidated by specific energy values of the feed components turbot feed, turbot feces and *T. chui*, which were used in the different diets. Accordingly, diets containing the high energy turbot feed as a major component (FD (feed), FF (feed and feces) and M (mix)) seemed to provide sufficient nutrition and energy, resulting in significant increases of the CI in FD, FF and M, which is generally used as an indicator for overall physiological state of marine bivalves (Lawrence and Scott, 1982; Rheault and Rice, 1996; Soletchnik et al., 2006; Dridi et al., 2007; Li et al., 2009).

The poor condition of oysters at the beginning of the experiments (Linehan et al., 1999; Pogoda et al., 2011) implies low energy reserves and an overall weak physiological state and is the most likely explanation for the mortalities all groups experienced throughout the experiment. This poor starting condition can be attributed to a variety of possible factors. The most likely one is that the oysters could not replenish the energy reserves expended during the previous spawning season and lost large amounts of their body mass in the process (Deslous-Paoli and Héral, 1988; Dridi et al., 2007). Different feeds had no significant effect on mortality during the experiments, while tank height and mortality showed a weak correlation, implying an unknown stressor.

The depletion of energy reserves of oysters in FS and C implied by the reduced C/N ratio and lacking improvement of their condition is not reflected by their respective energy values. Much like the C/N ratio, the energy value of an organism's tissue is mainly dependent on the distribution and make-up of proteins, lipids and carbohydrates (Brey et al., 1988; Dauvin and Joncourt, 1989). Any changes in the amount or specific make-up of these constituents should, consequently, be reflected in changes of the tissue energy value of the organism (Li et al., 2009). These components have different structures and

densities, which result in different energy values. The lowest of them is 17.58 MJ/kg ash free dry weight (AFDW) for carbohydrates (Wacasey and Atkinson, 1987), which provides a minimum value for possible energy values. Reference energy values in literature are mostly reported in J/g DW or J/g AFDW. The two values can still be compared, since Wacasey and Atkinson (1987) have shown that a linear correlation exists between energy/g DW and % organic content (R = 0.904) of marine bivalves. Energy values reported as per AFDW are slightly (~9 %) higher and display a smaller standard deviation (Brey et al., 1988; Dauvin and Joncourt, 1989). The determined values are similar to values reported for bivalves (18.03 ± 1.41 MJ/kg) (Deslous-Paoli and Héral, 1988; Dauvin and Joncourt, 1989), though the measured standard deviations exceed reported values slightly.

Since oysters feeding on turbot feed, as part of or as their sole diet component, managed to improve their CI significantly, they should also have been able to increase their energy content due to the accumulation of energy reserves (Deslous-Paoli and Héral, 1988). The only significant difference occurred in FF, where measured values (16.1 ± 3.1 MJ/kg) were below the credible minimum value and displayed large variations. As shown in Fig. 12, these differences most likely occurred as results of insufficient oxygen supply. This error led to an underestimation of the real energy value of the FF group, allowing the conclusion that the chosen measurement method is too imprecise to detect increases or decreases in energy content, most likely resulting from short-term changes in the levels of energy reserves (Deslous-Paoli and Héral, 1988). Two additional factors influencing the conclusiveness of the energy value, which are gender and organ specific differences, need to be taken into account for future analyses. These differences would result from variances in the stage of gametogenesis. Females deposit large amounts of lipids into their gonads as energy reserves for the gametes (Dridi et al., 2007). These lipids have a higher energy density than glycogen storage usually used by oysters and result in higher overall energy values for females if no distinction is made between organs (Deslous-Paoli and Héral, 1988).

FA composition can serve as an indicator for the preferred diet of oysters (Soudant et al., 1999), since they incorporate some dietary FA rather than catabolically break them down and reassemble them. Spat of *C. gigas* have previously shown to modify their FA composition to resemble feed composition within 28 days (da Costa et al., 2015). Especially PUFA and HUFA fractions changed to match the supplied feed (Knauer and

Southgate, 1997). Similar changes have been reported in tissue of mature oysters after an experiment lasting eight weeks (Delaporte et al., 2005) and in mature *M. edulis* after 28 days (Handå et al., 2012). The differing composition of these FA classes in the feed components lead to the expectation that incorporation will become apparent based on changes in these groups.

FA composition of T_0 tissue largely coincides with compositions of *C. gigas* reported in literature (Linehan et al., 1999; Soudant et al., 1999; Pogoda et al., 2013), though 15:0 and 18:0 saturated FA (SFA) were more prominent. Most changes occurred in saturated and mono-unsaturated FA. Accordingly, groups feeding on diets containing the largest proportions of turbot feed (FD, FF), which contained the largest amounts of these FA, showed the most differences.

Larger proportions of 16:1(n-7) in both diets compared to the start indicate the assimilation of dietary 16:1(n-7) or the *de-novo* biosynthesis from precursors. Redmond et al. (2010) recently suggested this MUFA as a possible lipid biomarker for assimilation of dietary FA from salmon feed by *M. edulis*.

This biosynthesis pathway has previously been reported in marine molluscs (Guillou et al., 2010; Monroig et al., 2013) and for *C. gigas* larvae (da Costa et al., 2015). Precursor quantities in the tissue were not significantly reduced compared to the start, but turbot feed provided the largest amount of necessary dietary 16:0 and 18:0 to both FD and FF. Incorporation of dietary 16:1(n-7) is less likely than biosynthesis from dietary 16:0, since turbot feces showed higher proportions of it than turbot feed but oysters fed with the FS diet did not show higher levels. The *de-novo* biosynthesis of short-chain SFA from Acetyl-CoA can also not be excluded as a source of precursor FA (Cook and McMaster, 2002).

18:1(n-7) seemed to follow the same pattern in oysters from FD and FF. Dietary 18:1(n-7) content was similar in all diet components but turbot feed showed the largest amount of the saturated FA 18:0, resulting in the synthesis of the MUFA 18:1(n-7) from this precursor. Additionally, the enrichment of 18:1(n-7) in FD and FF could be explained by its synthesis via elongation from already elevated levels of 16:1(n-7) in those groups (Thompson and Harrison, 1992).

Zhukova (1986) and da Costa et al. (2015) previously reported the biosynthesis of these FA as part of the biosynthesis of $20{:}2_{\Delta7,13}$ and $20{:}2_{\Delta7,15}$ NMI FA in marine molluscs. In

this process both 18:1(n-9) and 16:1(n-7) are elongated to a length of 20 and desaturated by the activity of a Δ5 desaturase. Further elongation creates the specific NMI FA (Barnathan, 2009), which were shown in offshore cultivated *C. gigas* (e. g. Pogoda et al., 2013).

NMI FA in *C. gigas* are reported to occur mostly in the phospholipid (PL) fraction of the gills (Kraffe et al., 2004), an organ relying heavily on membranes for its functionality. Klingensmith (1982) partly correlated the biosynthesis of NMI FA with a deficiency in essential FA, which are an important structural component in cell membranes. Especially the occurrence of long-chain PUFA, such as 20:5(n-3) and 22:6(n-3) are commonly used to evaluate the nutritional value of microalgae diets (Spencer, 2002). The PUFA and HUFA fractions of turbot feed are smaller than in the other two components, leading to the assumption that *C. gigas* is actively counteracting this lack by creating the necessary precursors for the biosynthesis of NMI FA. The turnover rate for gill tissue in *C. gigas* is approximately 28 days, while the time for overall turnover is closer to two months (Delaporte et al., 2005) and can take even longer in the winter months when metabolism slows down (Marín Leal et al., 2008). This may imply that measured changes in FA composition after 23 days are attributable to changes in the PL fraction of the gills. Further expected changes could possibly need more time to become apparent. The high proportions of FA smaller than 1 % TFA in all groups, compared to the start, signal those pending changes.

Based in these results, it is assumed that *C. gigas* will feed on waste particles leaving an aquaculture site and the starting hypotheses can be addressed as follows:

(1) Elemental composition of *C. gigas* is related to diet composition.

    Oysters feeding on a limited feed supply or feed of inadequate composition showed lower C/N ratios, resulting from changes in the composition of experimental animals. These changes were most probably caused by the depletion of glycogen based energy reserves. This hypothesis is accepted.

(2) Feeding on feed sources with a high energy content will result in better condition of oysters.

    All oysters managed to increase their CI, while the increase was only significant in oysters receiving the high energy turbot feed as part of their diet. This hypothesis is accepted.

(3) Feeding of *C. gigas* on feed sources with a high nutritional value will result in a higher energy content of oysters.

No significant increase in energy content of oysters feeding on feed with a perceived higher nutritional value was observed in this experiment. It is believed that a more precise measurement method will show the expected results. This hypothesis cannot be accepted based on the offered data.

(4) Incorporation of different IMTA related feed sources can be evaluated based on changes in FA composition of *C. gigas.*

The accumulation of SFA and MUFA in oysters feeding on turbot feed is believed to be the result both of incorporation of dietary FA and *de-novo* biosynthesis from short-chain precursor FA. While both pathways are possible, none can be excluded using the methods chosen in this experiment. This hypothesis cannot be accepted based on evidence from this experiment.

6 Conclusion and outlook

Results presented in this study support the conclusion that *C. gigas* will feed on and incorporate particles produced by a fish aquaculture. Oysters feeding on these managed to increase their condition considerably. Especially changes in the composition of FA based on the supply of dietary FA are promising and it is assumed that, given a longer experimental period, changes in composition of oysters between different experimental diets will become even more distinct. To further research and trace the exact pathways of incorporation of aquaculture waste particles by *C. gigas*, the use of more explicit methods like stable isotope analysis and lipid biomarkers, as well as a sampling scheme allowing distinction between different organs, is recommended.

Based on these findings, the Pacific oyster presents itself as a prime candidate as extractive component for future field trials of an IMTA system. Using the aforementioned methods, tracing the nutrients expelled by cultured fish down the trophic levels should be possible, even under open-ocean conditions.

7 References

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8 Appendix

Eidesstattliche Erklärung gemäß §26 (2) PO

Hiermit erkläre ich, Maximilian Felix Schupp, geb. 06.06.1990 in Lippstadt,

dass ich die vorliegende Arbeit selbstständig und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegt.

Bremerhaven, den 03.05.2015 Maximilian Felix Schupp

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Unterschrift