Bernadette Pogoda

Farming the High Seas: Biological performance of the offshore cultivated oysters *Ostrea edulis* and *Crassostrea gigas* in the North Sea.

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Biological performance of the offshore cultivated oysters *Ostrea edulis* and *Crassostrea gigas* in the North Sea.

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Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.)

Marine Zoologie

Fachbereich Biologie/Chemie

Universität Bremen

März 2012

Cover pictures: Stefan Albrecht, Scandinavian Fishing Year Book, Willapa Bay oysters

DEDICATED TO MY FAMILY

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Declaration/Erklärung

ABBREVIATIONS

BSH	Bundesamt für Seeschifffahrt und Hydrographie
BD	Butendiek
C	carbon
Chl *a*	chlorophyll *a*
CI	condition index
d	day
DM	dry mass
DN	Dornumer Nacken
DOD	Deutsches Ozeanographisches Datenzentrum
FAME	fatty acid methyl ester
FAO	Food & Agriculture Organization of the United Nations
H	hydrogen
HE	Helgoland
JW	Juister Watt
KB	Kaiserbalje
MHW	mean high water
MUDAB	Marine Umwelt Datenbank (Marine Environmental Data Base)
NG	Nordergründe
n	number of individuals
N	nitrogen
NL	neutral lipids
nmi	nautical mile
NO_x	nitrate+nitrite
OSPAR	Oslo-Paris Commission
Pheo *a*	phaeophytin *a*
PL	phospholipids
POC	particulate organic carbon
PON	particulate organic nitrogen
SE	sterol esters
SL	shell length
ST	sterols
TAG	triacylglycerols
TFA	total fatty acids
TFAlc	total fatty alcohols
TL	total lipid
UK	United Kingdom
WA	Wurster Arm
WE	wax ester

Summary

Aquaculture production plays an increasingly important role to meet the global demand for aquatic products and expands continuously. Most mariculture organisms are produced in coastal areas, where space is scarce and stakeholder conflicts exist. For extensive cultures farming off the coast at offshore sites could be a solution to eliminate these problems and facilitate further expansion of environmentally friendly and sustainable aquaculture. The aim of this study was to examine the biological adequacy of two candidate species for such an offshore ostreiculture, the European flat oyster *Ostrea edulis* and the Pacific oyster *Crassostrea gigas*. Research focused on growth, condition and survival. Oyster spat of both species was transferred to different offshore sites in the North Sea in 2004 and 2007 and cultivated for six month during growing season. Samples were taken every six to eight weeks from April to October and growth rates, condition indices and survival rates were measured. To enable a deeper insight in oyster condition, elemental (carbon, nitrogen) and biochemical compositions (protein, glycogen and lipid levels) as well as lipid class and fatty acid compositions were investigated. Furthermore, the macroparasitic infestation of oysters at offshore cultivation sites and for comparison, at nearshore wild banks, was investigated. Low infestation rates or even a complete absence of such parasites would be a major advantage for shellfish production activities and would therefore play an important role in the list of site-selection criteria for a certain region.

Results showed that both oyster species grow successfully in high-energy offshore environments. Mean growth rates were similar to those measured in individuals from coastal habitats (wild banks and cultures) and the condition index revealed normal seasonal variations in both species. Survival rates of both species were extremely high. However, at one single site a high mortality rate was observed for *O. edulis* at the end of the experiment. Differences in the increase of shell length and dry mass were observed between sites and size classes. Taking these results into account site selection criteria for different offshore locations are presented.

Biochemical results showed an increase in glycogen from spring to early summer for both species, which is related to high food abundance during spring phytoplankton bloom. However, during summer, glycogen storage revealed clear differences between *O. edulis* and *C. gigas*: glycogen levels decreased substantially in *C. gigas*, eventually owing to reproductive activity only of the Pacific oyster. Total protein did not show significant seasonal variations, while lipids were accumulated during growing season, more pronounced in *C. gigas*, and decreased in autumn. Phospholipids and triacylglycerols were the main lipid classes in both species. Fatty acids resembled the compositions of nearshore grown oysters.

This indicates that oysters exhibited a natural biological performance during offshore cultivation. Additionally, no macroparasites have been found in oysters from offshore cultivation sites, which provides a high-potential advantage for aquaculture activities in the open ocean.

It can be summarized that oysters are successful candidates for offshore aquaculture operations, e.g. as a multi-use of offshore wind farming areas. As oysters are bioextractive organisms, oyster cultivation as an essential component of integrated multi-trophic aquaculture (IMTA) approaches in offshore areas is recommended in particular.

Key findings

I Growth performance and condition of offshore-cultivated oysters

- Both oyster species, *Ostrea edulis* and *Crassostrea gigas*, achieved growth rates similar to those of oysters in nearshore wild habitats of temperate regions.
- Both oyster species, *O. edulis* and *C. gigas* exhibited an excellent physiological condition during offshore cultivation.
- Even juvenile oysters in poor condition at the beginning of the experiments strongly improved their condition during offshore cultivation and succeeded excellent condition at the end of the cultivation period.
- The severely endangered native oyster species, *O. edulis*, is still well adapted to environmental conditions of the North Sea.
- Offshore cultivated oysters showed high survival rates (>96%).

II Biochemical aspects of condition of offshore-cultivated oysters

- Both oyster species, *O. edulis* and *C. gigas*, accumulated mainly glycogen in periods of high food availability (spring).
- *O. edulis* accumulated glycogen until autumn, while in *C. gigas* glycogen levels decreased in summer.
- Both oyster species accumulated lipids during the growing season, in *C. gigas* this was clearly more pronounced. Lipid levels decreased in autumn.
- Seasonal variations in proteins were significantly lower than those of lipids and glycogen ($p < 0.001$), suggesting a minor relevance of proteins for energy storage.
- Seasonal variations in biochemical composition of *C. gigas* indicated early reproductive activity for this species.
- Both oyster species, *O. edulis* and *C. gigas*, exhibited an excellent physiological condition during offshore cultivation, indicated by PL:TAG ratios of around 1.

- *De novo* synthesized non-methylene-interrupted fatty acids were present in both oyster species and indicated good nutritional conditions during offshore cultivation.

III Macroparasite data of offshore-cultivated oysters and of oysters and mussels from nearshore habitats

- Offshore-cultivated oysters were free of macroparasites.
- Investigated Pacific oysters from nearshore sites in the eastern Wadden Sea showed significantly higher infestation rates by the parasitic copepod *Mytilicola orientalis* than reported from the northern Wadden Sea.
- Infestation rates of *M. orientalis* decrease geographically from east to west along the German coast. The distribution of *M. orientalis* follows the invasion pattern of its main host, the Pacific oyster *C. gigas*.
- Blue mussels (*Mytilus edulis*) of the eastern Wadden Sea were not only infested by *M. intestinalis* but also by *M. orientalis*, which has not been reported for the Wadden Sea before.
- Parasite richness was higher in *C. gigas* than in *M. edulis*.

IV Economic potential of offshore oyster cultivation in the North Sea

- First estimations on the economic viability of oyster cultivation in the North Sea revealed positive results.

Zusammenfassung

Aufgrund der ständig steigenden Nachfrage an Fisch und Meeresfrüchten und einer Stagnation der Fischereierträge in den letzten Jahrzehnten, wächst die Bedeutung der Aquakultur beständig. Der Bereich Aquakultur ist darüberhinaus der weltweit am schnellsten wachsende Sektor der Nahrungsmittelherstellung. Die Produktion mariner Organismen beschränkt sich jedoch traditionell auf geschützte Küstengebiete, die einerseits nur begrenzten Raum bieten und andererseits einer Vielzahl von Nutzungskonflikten unterliegen. Dies führt häufig zu einer ökologischen Überbelastung der empfindlichen Küstenökosysteme, auch durch die Aquakultur. Die Entwicklung umweltschonender Produktionsmethoden in der Aquakultur und deren Verlagerung in küstenferne Meeresgebiete (offshore) erlauben die Verknüpfung von nachhaltiger Produktion und erheblicher Expansion. Die Verwirklichung von Offshore-Aquakultur wird deshalb aktuell als vielversprechender Lösungsansatz diskutiert.

Ziel dieser Dissertation war die Überprüfung der biologischen Eignung der Europäischen Auster *Ostrea edulis* und der Pazifischen Auster *Crassostrea gigas* für die Offshore-Aquakultur. Im Fokus der Untersuchungen standen Wachstum, Kondition und Überlebensfähigkeit der beiden Austernarten in exponierten Seegebieten in der Nordsee. In den Jahren 2004 und 2007 wurden junge Austern für eine Wachstumsperiode von April bis Oktober an verschiedenen Offshore-Standorten in der Deutschen Bucht kultiviert. Eine Probennahme erfolgte alle sechs bis acht Wochen, um Wachstumsraten, Konditionsindices und Mortalität zu bestimmen. Um fundierte Aussagen zur Kondition zu ermöglichen wurden zusätzlich die Elementarzusammensetzung (Kohlenstoff, Stickstoff), die biochemische Zusammensetzung (Proteine, Glycogen, Lipide) sowie Lipidklassen und Fettsäurekomposition analysiert. Zusätzlich wurde die Belastung durch Makroparasiten der offshore-kultivierten Austern und zum Vergleich auch von Austern aus natürlichen Küstenhabitaten untersucht. Niedrige Befallsraten oder ein Fehlen von Makroparasiten in der Offshore-Aquakultur wären ein erheblicher Vorteil und stellen deshalb ein wichtiges Kriterium für das in der Aquakultur zentrale Thema der Standortwahl dar.

Die Ergebnisse der Untersuchungen zeigen, dass *O. edulis* und *C. gigas* an exponierten Offshore-Standorten in der Nordsee erfolgreich wachsen. Die erreichten Wachstumsraten entsprechen Werten aus Küstengebieten temperierter Regionen (Wildbänke und Austernkulturen) und belegen damit ein natürliches Wachstum der untersuchten Austern in Offshore-Regionen. Der Konditionsindex beider Arten zeigte ebenfalls mit Küstenpopulationen vergleichbare Werte mit den erwartungsgemäßen saisonalen Schwankungen. Die Mortalität der offshore-kultivierten Austern lag extrem niedrig bei unter 5%. Einzig an einem Standort wurde für die Europäische Auster eine hohe Mortalität gemessen. Auch Längenzuwachs und Gewichtszunahme der Austern zeigten Unterschiede zwischen den einzelnen Standorten auf. Unter Berücksichtigung der jeweils charakteristischen Umweltbedingungen an den vier Offshore-Standorten werden entsprechende Kriterien zur Standortauswahl diskutiert.

Die biochemischen Analysen ergaben für beide Austernarten einen deutlichen Anstieg an Glycogen bis zum Frühsommer, ausgelöst durch eine hohe Nahrungsverfügbarkeit während der Phytoplanktonblüte im Frühjahr. Deutlich unterschiedliche Strategien im Auf- und Abbau von Energiereserven zeigten sich im Sommer: Während die Europäische Auster weiterhin Glycogen akkumulierte, nahm der Glycogengehalt der Pazifischen Austern erheblich ab und deutete auf eine Reproduktionsaktivität dieser Art hin. Der Proteingehalt zeigte in beiden Austernarten keine bedeutsamen saisonalen Schwankungen. Der Lipidgehalt stieg in der Wachstumsperiode an, in der Pazifischen Auster mehr als in der Europäischen, und nahm in beiden Arten im Herbst wieder ab. Phospholipide und Triacylglyceride wurden in beiden Austernarten als Hauptlipidklassen nachgewiesen und entsprachen, wie auch die analysierten Fettsäuren, der Zusammensetzung von Austern aus Küstengebieten. Im Gegensatz zu Austern aus Küstenhabitaten waren beide Austernarten an den untersuchten Offshore-Standorten frei von Makroparasiten - ein erheblicher Standortvorteil für die Aquakultur in Offshore-Regionen.

Die abschließende Gesamtanalyse und Bewertung ergibt eine sehr gute Eignung der beiden untersuchten Austernarten *Ostrea edulis* und *Crassostrea gigas* für die Offshore-Aquakultur. Im Besonderen bietet sich die integrierte Nutzung von Offshore-Windpark-Flächen für Aquakulturvorhaben mit Austern an.

Umweltschonende und nachhaltige Konzepte für die Aquakultur finden sich heute in der Verbindung intensiver und extensiver Aquakulturorganismen und werden so auch für den Offshore-Einsatz realisiert. Als filtrierende Organismen eignen sich Austern dementsprechend hervorragend als essentielle Komponente für eine integrierte multi-trophische Aquakultur (IMTA).

Outline of publications

The following overview outlines the three first author publications included in this PhD thesis. The idea for this study originated from the feasibility study "Open ocean aquaculture and offshore wind farms" of Prof. Dr. Buck. I developed the general concept of the study with logistic advice and scientific guidance from my supervisors Prof. Dr. Hagen and Prof. Dr. Buck. All analyses were carried out in the laboratories of the University of Bremen and the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven, Germany.

I. B. Pogoda, B.H. Buck, W. Hagen

Growth performance and condition of oysters (*Crassostrea gigas* and *Ostrea edulis*) farmed in an offshore environment (North Sea, Germany)

Experimental design and sampling were realized by myself with assistance from Prof. Dr. Buck. Analyses were performed by myself. I wrote the manuscript with scientific and editorial advice by Prof. Dr. Buck and Prof. Dr. Hagen. The article is published in Aquaculture (2011).

Aquaculture 319:484-492, DOI 10.1016/j.aquaculture.2011.07.017

II. B. Pogoda, B.H. Buck, R. Saborowski, W. Hagen

Biochemical and elemental composition of the offshore cultivated oysters *Ostrea edulis* and *Crassostrea gigas*

I developed the concept and experimental design for this study. Sampling was conducted by myself with help from Prof. Dr. Buck. Analyses were performed by myself. R. Saborowski provided ideas for the interpretation of the data and contributed to the manuscript. I wrote the manuscript with scientific and editorial advice by Prof. Dr. Buck and Prof. Dr. Hagen. The manuscript was submitted to Marine Biology.

III. B. Pogoda, S. Jungblut, B.H. Buck, W. Hagen

Infestations of oysters and mussels by mytilicolid copepods: differences between wild habitats and two offshore cultivation sites in the German Bight

I developed the idea and concept for this study. I shared field work as well as experimental and analytical work with S. Jungblut. Based on the concept I wrote the manuscript on my own. Prof. Dr. Buck and Prof. Dr. Hagen gave scientific and editorial advice. The manuscript was submitted to the Journal of Applied Ichthyology.

1. General introduction and objectives

1.1 Environmentally friendly and sustainable aquaculture

Aquaculture is the fastest growing sector of food production worldwide. Seafood production in aquaculture increased from 1 million tons in 1950 to over 50 million tons in 2009. The great majority of these products comes from the Asian-Pacific region but aquaculture production is increasing worldwide (FAO 2012). Until today, marine aquaculture production is realized exclusively in sheltered regions of coastal waters (Burbridge et al. 2001, FAO 2009). But in coastal areas space is scarce: stakeholder conflicts – harbours and shipping, sediment extraction and disposal, fisheries, tourism, coastal and environmental protection, etc. – exist and increase continuously (Wirtz et al. 2002). In many nearshore areas, the ecological carrying capacity sets limitations to the massive expansion of intensive aquaculture activities (Troell et al. 2009). These forms of seafood production create and generate environmental stress caused by chemicals, eutrophication and by negative impacts on benthic communities (McElwee 1998). Until a decade ago, aquaculture has often been described as a sustainable alternative to conventional fishery of wild stocks and was therefore discussed as an approach to successfully prevent further overfishing and stock depletion. Today we know that e.g. the culture of carnivorous species depends on large amounts of protein, which are partly fed as fish meal or fish oil (Naylor et al. 2000, Roth et al. 2002). Therefore, it does not diminish pressure on wild stocks and does not seem to be a sustainable alternative.

Evidently, environmental sustainability is of tremendous global interest. It is by now a major task with a world population exceeding six billion people and still rapidly increasing. We urgently need approaches to promote new technologies that raise living standards on the one hand and yet reduce human impact on the environment on the other hand (Sachs 2007). Environmentally friendly and sustainable aquaculture should provide such technologies that satisfy the growing demand for seafood products, while reducing the intense pressure that threatens our marine ecosystems.

1.2 Offshore aquaculture and multi-use of offshore wind farms

A promising option for such ecologically sensitive aquaculture is the development of offshore aquaculture. It is defined by its location in a marine environment fully exposed to all kinds of harsh oceanographic conditions (Ryan 2005) and furthermore, by its distance from the coast: ≥6 nautical miles (nmi) (Buck 2007). Extensive cultivation methods and culture species with modest service needs are basic and profitable criteria of environmentally friendly and sustainable aquaculture (Naylor et al. 2000). Furthermore, they offer the chance to move to offshore areas, away from socioeconomic conflicts and coastal pollution (Krause et al. 2003). Ideal candidates for these forms of offshore mariculture are, inter alia, several filter-feeding shellfish species, such as oysters and mussels (Gibbs 2004). They feed on plankton particles and do not require artificial feeding, which avoids eutrophication problems generated by excessive feeding, often typical for aquaculture facilities (Garen et al. 2004, Ferreira et al. 2009). Filter-feeding bivalves are essential bio-extractive organisms and can even improve the water quality at the culture site (Ferreira et al. 2009, Rose et al. 2010). As those organisms produce pseudo-feces, which are sinking to the ground, also shellfish cultures do have an impact on the surrounding environment. However, shellfish aquaculture is described as an example for extensive and environmentally friendly production and has become very attractive (Gibbs 2004).

Higher wind speeds in offshore regions and coastal stakeholder conflicts also enhance growing interests in offshore wind farming. Worldwide wind farms in offshore areas are being planned or already under construction (e.g. Gierloff-Emden 2002, Breton & Moe 2009, OWE 2012). Wind farm sites and foundations offer the opportunity of parallel utilization. Today, sharing of e.g. windmill groundings to attach culture cages, as well as combined maintenance vessels are being discussed and planned (Buck et al. 2004). The "multi-use" of offshore wind farms for aquaculture installations could facilitate the further expansion of environmentally friendly and sustainable aquaculture. Those concepts for a sustainable seafood production in offshore areas show an enormous economic potential (Buck et al. 2008). Although offshore cultivation of oysters is mentioned and recommended in various publications (Delmendo

1998, Goulletquer 1998, Handley & Jeffs 2003, Chávez-Villalba et al. 2010), to date no studies exist that offer oyster cultivation data under "real" offshore conditions as defined by Ryan (2005) and Buck (2007) for open ocean aquaculture.

1.3 Offshore aquaculture in the North Sea

A feasibility study on open ocean aquaculture by Buck (2002) identified the most suitable candidates for the North Sea as sugar kelp (*Laminaria saccharina/Saccharina latissima*), dulse (*Palmaria palmata*), the blue mussel (*Mytilus edulis*) and two oyster species, the European flat oyster (*Ostrea edulis*) and the Pacific oyster (*Crassostrea gigas*). While data on the success of offshore cultivation exist for seaweed and mussels (e.g. Langan 2001, Langan & Horton 2003, Buck & Buchholz 2004, Brenner et al. 2009, Buck et al. 2010), data on species performance of oysters are urgently needed to develop methodologies for the assessment of its environmental and economic viability (Buck et al. 2008).

Oyster cultivation has a long tradition in many coastal regions of the world. Over centuries, different forms of oyster cultures were developed, like all other forms of marine aquaculture, only in nearshore areas (Burbridge et al. 2001). Besides fishery on wild oyster banks, today the great majority of oysters is produced in extensive cultures with little assistance by the grower (semi-culture) (see chapter 2.2). This and the fact that oysters represent high-value products and realize high prices on the market, compared to other shellfish (Troell et al. 2009, FAO 2011), makes them ideal candidates for open ocean aquaculture.

The biological performance of the European oyster *Ostrea edulis* in the North Sea is of special interest. It has become extinct in the German Wadden Sea and in Belgian Waters due to overexploitation, disease and cold winters. A high demand for this indigenous species still exists on the European oyster market (Newkirk et al. 1995). The introduction of the Pacific oyster intended to substitute declining stocks of the European oyster (Reise 1998). As it is known to be a robust and fast-growing species, it seems ideal for offshore cultivation trials (Newkirk et al. 1995, Matthiessen 2001).

1.4 Objectives

This is the first study focusing on the biological suitability of oysters for offshore aquaculture. The research was carried out to gain knowledge on growth performance and fitness of oysters cultivated at highly exposed locations – like offshore areas of the North Sea.

The aims were to investigate:

(i) if oysters grow successfully in offshore regions by assessing growth rates of shell and meat. *(Publication I)*

(ii) species-specific performances and differences between cultivation sites. *(Publication I)*

(iii) the oysters´ condition, defined by condition index, biochemical composition and survival rate. *(Publication I & II)*

(iv) species-specific strategies of energy storage – glycogen vs. lipids. *(Publication II)*

(v) the oysters´ condition in relation to parasitic burden of offshore cultivated oysters and oysters from coastal wild habitats. *(Publication III)*

Answering these questions will help to assess if oysters can be suggested as potential candidates for open ocean aquaculture operations in the near future. Therefore, aquaculture facilities were installed at four different sites in the German Bight. Juveniles of the European oyster *Ostrea edulis* as well as of the Pacific oyster *Crassostrea gigas* were reared over two complete growing seasons at three sites in 2004 and at one site in 2007. Oysters were sampled every six to eight weeks and growth, elemental and biochemical composition as well as infestation by macroparasites were determined in the laboratories of the University of Bremen and the Alfred Wegener Institute for Polar and Marine Research.

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2. Research background

2.1 Biology of the candidates *Ostrea edulis* and *Crassostrea gigas*

Taxonomy: Mollusca/Bivalvia/Pteriomorpha/Ostreoida/Ostreidae:
Ostrea edulis, Crassostrea gigas

The European oyster *Ostrea edulis* (syn. European flat oyster, Flat oyster, Edible oyster, Belon oyster) is the native oyster species of European waters (Fig. 1A). It naturally occurred from the Norwegian Sea in the North, around Ireland and Britain, the Iberian Peninsula to the Mediterranean Sea in the South (Kennedy & Roberts 1999, Laing et al. 2006). Interestingly, natural banks of European oysters also occurred in deeper waters and offshore, down to 50 m, e.g. in the North Sea and the eastern Channel (Haelters & Kerckhof 2009). Today this species is extinct along the German and Belgian coast and *Ostrea edulis* beds are under threat and/or decline in all the regions where they occur. This was caused by a combination of unsustainable harvest rates in the last two centuries, the impact of severe winters and of *Bonamia* disease (e.g. Montes 1990, McArdle et al. 1991, Wehrmann et al. 2000, Laing et al. 2006). *O. edulis* was also believed to be extinct in the Dutch Wadden Sea since 1940, although a small number was found in 1992 (Dankers et al. 1999). Natural populations of the European oyster only exist in the Danish Limfjord (Løfstedt 2010) and around Ireland (Smyth & Roberts 2010). Therefore, *O. edulis* was nominated for inclusion on the OSPAR (Oslo-Paris-Commission) list with particular reference to global and regional importance, rarity, decline, role as a keystone species, sensitivity and threat, and as a priority for OSPAR Region II – Greater North Sea, Northeast Atlantic (OSPAR 2009).

To compensate for the decline of the European oysters, the Pacific oyster *Crassostrea gigas* (Fig. 1B) has been introduced to various coastal areas of the North Sea through aquaculture activities since the 1960´s. By now, stable populations have established in the wild (Andrews 1980, Chew 1990, Ruesink et al. 2005, Diederich 2006, Markert et al. 2010). The Pacific oyster is the most important species in global oyster cultivation. As it grows fast, is tolerant to a wide range of environmental conditions and also resistant to the parasite *Bonamia ostreae* (Linehan et al. 1999), today it accounts for over 90% of produced oysters worldwide (FAO 2012a). Still, the cultivation of flat oysters, e.g. the European oyster, is highly profitable, as it is considered a premium quality product and achieves at least double the price of Pacific oysters on the market (Mitchell et al. 2000).

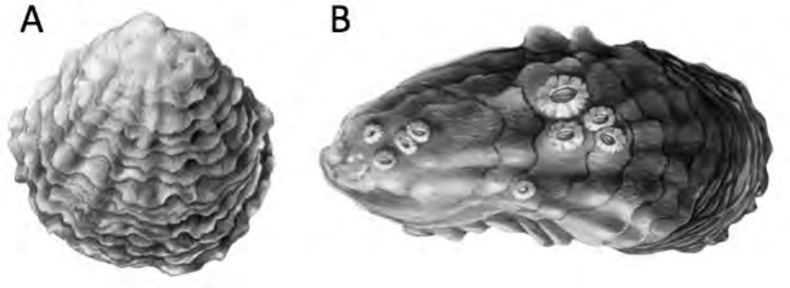

Figure 1: Shell morphology of the European oyster *O. edulis* (A) and the Pacific oyster *C. gigas* (B).
(Scandinavian Fishing Year Book)

The Pacific oyster *Crassostrea gigas* (syn. Japanese oyster, Pacific oyster, Pacific cupped oyster, Pacific rock oyster) naturally occurred in coastal waters of Japan, Korea and in parts of South East Asia (Fig. 1B). Its natural distribution spans over a wide temperature range from Sakhalin Island, Russia in the North to Hong Kong and Taiwan in the South (Matthiessen 2001). Already centuries ago humans transported this species to many areas around the globe for shellfish production purposes. First records are known from the Portuguese, who brought it to southern Europe in the 16th century. Later, Pacific oysters were established in the USA, around South America and South Africa and also imported to Australia, from where they spread to New Zealand (Chew 1990, Carlton 1999, Wolff & Reise 2002). Pacific oysters were also introduced to different regions in Europe for aquaculture purposes: to Portugal, France, Great Britain, the Netherlands and Germany (Walne & Helm 1979, Chew 1990, Grizel & Héral 1991, Reise 1998, Drinkwaard 1999). In most areas, where

C. gigas was introduced, spatfalls occurred and wild populations established with further natural dispersion (Quayle 1969, Andrews 1980, Chew 1990, Ayres 1991, Eno et al. 1997, Wehrmann et al. 2000, Escapa et al. 2004, Robinson et al. 2005, Ruesink et al. 2005). Today it shows a wide distribution in temperate regions of the world (FAO 2011).

O. edulis and *C. gigas* occur naturally in marine and estuarine coastal waters, but both show preferences for fully saline conditions (Laing et al. 2006). Oyster larvae are able to attach to almost any hard substrate, usually rocks, in sheltered waters. But *C. gigas* also occurs on muddy or sandy bottoms, where they tend to settle on adult specimens or other bivalves and form reef structures. In contrast, natural oyster beds of *O. edulis* are usually not found on muddy sediments (OSPAR 2009). The presence of high quantities of silt in the water can block the digestive and respiratory tracts and lead to death (Partridge 1981, Laing et al. 2006). While *C. gigas* is preferentially found in the intertidal and shallow subtidal zone down to 3 m depth, *O. edulis* beds also occur in deeper waters down to 50 m depth.

Describing the shell morphology of oysters, the upper (right) valve is flat and smaller than the arched lower (left) valve. *O. edulis* reaches a maximum size of 10-12 cm and is characterized by a rather round shape (Fig. 1A). Coloration is variable: grey, brown and shades of green are common. *C. gigas* shows an elongate, oval shape (Fig. 1B), often very irregular and usually larger than *O. edulis* with an average size of 15-20 cm and a maximum size of up to 40 cm (NIMPIS 2012). In general, shape depends on growing conditions, substrate and space. Oysters growing in dense reef structures on soft bottoms are elongate and compact. Those growing on hard substrates are rather roundish. Coloration of *C. gigas* is also variable: whitish grey, shades of blue and purple streaks radiating around the umbo (Nehring 2006).

For both species minimum temperatures in summer and winter are important and appear to be the main limiting factor for reproduction, spawning and recruitment. If temperatures are low, spawning does not occur every year. Summer temperature should reach 15°C to allow spawning of *O. edulis* and *C. gigas* (Chávez-Villalba et al. 2002, NIMPIS 2012). In temperate regions Pacific oysters reach the first reproductive period in summer one year after settling (Goulletquer 1997, Reise 1998, Matthiessen 2001, Nehring 2006), European oysters usually one year later (Newkirk et al. 1995, Matthiessen 2001). Oysters are hermaphrodites and they change sexes during their reproductive lifespan, depending on environmental and physiological conditions (Fabioux et al. 2005). However, European and Pacific oysters exhibit different reproductive strategies: *O. edulis* is characterized by internal fertilization and produces circa 100,000 eggs per spawning season. Eggs remain in the mantle cavity for 10-14 days and are then released as planktonic veliger larvae (Newkirk et al. 1995). Pacific oysters produce 50-100 million eggs per spawning season. Spawning is followed by external fertilization and development of planktonic larvae (Walne 1974). The larval phase of both oyster species will last 18-30 days, depending on the water temperature (Walne 1974, Wehrmann et al. 2000, Fabioux et al. 2005).

Growth also depends on temperature and of course on food availability. In temperate regions of the northern hemisphere the growing season spans from April until late September (Korringa 1952) as long as water temperature is above 10°C (Matthiessen 2001). Hydrodynamic currents provide oysters with food and oxygen and remove waste products. Since filtration rates and food availability increase with flow rate to a certain level (Walne 1979), oysters are expected to grow better at cultivation sites with stronger water currents (Valero 2006). The Pacific oyster is classified as a rapid grower, and normally reaches market size in 2-3 years. In regions of high food availability it even reaches market size within one year (Matthiessen 2001). In contrast, the European oyster is known to show slower development and growth. It reaches market size in 3-4 years. Despite that, interest in the production of *O. edulis* remains intense, stimulated by higher prices and a strong demand for this species in Europe (Newkirk et al. 1995).

2.2 Oyster cultivation and techniques

Oyster cultivation for human consumption has a long tradition. Several different techniques were developed over the centuries (Newkirk et al. 1995). Fisheries on wild oyster banks are still operated in South Korea, Ireland, the USA and Mexico. They constantly achieve about 150,000 t per year (FAO

2012a). By far the major part of global oyster production comes from aquaculture, which showed a constant increase from 1.25 million t in 1990 to 4.3 million t in 2009 (FAO 2012a). In general, oyster cultivation begins with seed production: suitable substrates (cultch) are offered to oyster larvae for settlement (captage). Commercial hatcheries gain in importance and most of the seed oysters are produced there (Draver pers. comm.). Spawning is induced by rearing adult oysters in warm water (>20°C). Larvae are then fed cultured algae, preferably diatoms of the genus *Chaetocerus* and *Thalassiosira*, and flagellates of the genus *Isochrysis*, until settlement (FAO 2012b). The following culture of oyster spat is referred to as nursery stage. Juvenile oysters are reared in temperate water (>12°C) and are continuously fed cultured algae. Different size classes are sold as oyster spat or seed (Newkirk et al. 1995). Large spat is cost-intensive, but small spat is extremely sensitive and requires small mesh sizes for grow-out, which can result in excessive fouling and restricted water flow (Newkirk et al. 1995). A minimum size of >20 mm is recommended, but not always available for all oyster species (Newkirk et al. 1995, Matthiessen 2001, Pogoda et al. 2011, Draver pers. comm.). In the following grow-out period oysters are cultivated in marine or estuarine waters until they reach their species-dependent market-size: 65-75 mm for *O. edulis*, >75 mm for *C. gigas* (Muise et al. 1986, FAO 2012b).

Various culture techniques are used and will be briefly described. *On-bottom culture*: seed oysters are released on wild oyster beds or suitable firm substrate with or without protection fences and experience natural growing conditions with no further husbandry. *Off-bottom culture*: seed is kept in perforated trays or mesh bags (poches) attached to racks or piles in the intertidal or low subtidal (FAO 2012b). Cultures in the intertidal fall dry with low tide and oysters are exposed to the air up to several hours. Other forms of off-bottom cultures are suspended cultures or floating cultures. *Suspended cultures*: strings or wires with attached spat or seed kept in mesh bags or cages (oyster lanterns, lantern nets) hanging from rafts or long-lines in deeper waters of the subtidal (FAO 2012b). Suspended cultures have some advantages: high growth rates, easy access to the stock, exclusion of predators and well-shaped oysters (Newkirk et al. 1995, Matthiessen 2001). *Floating cultures*: early stages of seed are kept in wooden-frame trays provided with buoyancy devices (FAO 2012b). During the grow-out period oysters are sorted and moved regularly to prevent clumping. Nets have to be cleaned from bio-fouling. Depending on oyster species and food availability/nutritional situation, grow-out periods comprise 2-3 years. Especially in France, additional final steps are common: oysters are reared in special tanks or basins to develop a special savor (*affinage*) and conditioned to close their shells, when they fall dry (*trompage*). Finally oysters are cleaned from sediment or silt in pure seawater basins if necessary (*depuration*).

2.3 Offshore environments and the situation in the German Bight

Offshore regions are exposed to extreme wind forces, high wave actions and strong currents and are therefore characterized as high energy environments (Buck 2007). Although some authors use the term "offshore" for all kinds of exposed areas or locations beyond the coast, in this study we follow the definitions of Ryan (2005) and Buck (2007): "offshore" are exposed areas ≥6 nmi beyond the coastline.

Offshore or open ocean aquaculture describes the move of aquaculture operations from sheltered nearshore areas to more exposed environments with corresponding risks (Troell et al. 2009). Wind and hydrodynamic action in these areas generate mechanical stress on organisms as well as on technical equipment, installations and all kinds of operations (e.g. vessel maneuvering, maintenance activities, diving). Requirements on cultivation facilities and personnel are high and cost-intensive. However, offshore areas are still attractive as they offer various advantageous aspects: Due to the strong exchange and dilution effects of water masses in these high-energy environments, the water quality, a key element in aquaculture operations, is regarded to be superior (Takayanagi 1998, BSH 2006). Furthermore, constant salinities and moderate temperature variations in offshore regions offer stable conditions for cultivated organisms. Although offshore areas are known to offer lower plankton concentrations as compared to coastal regions, the higher hydrodynamic flow offshore may compensate for that limitation. As the North Sea in general shows symptoms of eutrophication and is classified as a hyper-nutrified system, food supply in offshore regions is regarded to be sufficient (Ducrotoy et al. 2000). The combination of current speed and food supply could provide high food availability at offshore sites (Pogoda et al. 2011).

The coastline of the German Bight is utilized by numerous stakeholders and mainly used as natural reserves, as recreational areas, for shipping or a combination of these purposes. Therefore, locations for marine aquaculture installations in sheltered nearshore areas are extremely limited. Several types of stakeholders also exist in offshore areas of the German Bight, such as fisheries, underwater cables and pipelines or offshore wind farms. However, off the coast spatial conflicts are not as complex as along coastal areas (Buck 2007). In addition, the actual development and establishment of offshore wind-farming in the German Bight enhances opportunities to integrate the interests of two stakeholders for the same marine area. Wind-mill groundings allow secure installations for cultivation cages or longlines, and the space between wind mills may offer additional areas for aquaculture facilities (Buck et al. 2008).

In summary, the offshore environment of the German Bight could provide promising sites for large-scale offshore aquaculture operations.

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CHAPTER I

GROWTH PERFORMANCE AND CONDITION OF OYSTERS (*CRASSOSTREA GIGAS* AND *OSTREA EDULIS*) FARMED IN AN OFFSHORE ENVIRONMENT (NORTH SEA, GERMANY)

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published in *Aquaculture* (2011)

DOI 10.1016/j.aquaculture.2011.07.017

Aquaculture 319 (2011) 484–492

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Growth performance and condition of oysters (*Crassostrea gigas* and *Ostrea edulis*) farmed in an offshore environment (North Sea, Germany)

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ARTICLE INFO

Article history:
Received 4 March 2011
Received in revised form 12 July 2011
Accepted 14 July 2011
Available online 23 July 2011

Keywords:
Offshore aquaculture
Crassostrea gigas
Ostrea edulis
Multi-use
Site selection

ABSTRACT

Aquaculture production plays an increasingly important role to meet the global demand for aquatic products and expands continuously. Most mariculture organisms are produced in coastal areas, where space is scarce and user conflicts exist. For extensive cultures farming off the coast at offshore sites could be a solution to eliminate these problems and facilitate further expansion of environmentally sustainable aquaculture. The aim of this study was to examine the biological adequacy of two candidate species for ostreiculture, the Pacific oyster *Crassostrea gigas* and the European flat oyster *Ostrea edulis*. Growth rates, condition and mortality in offshore environments were investigated by transferring oyster spat of both species to 4 different sites in 2004 and 2007. Samples were taken every six to eight weeks from April to October and length, width, height and dry mass were measured as well as the Condition Index (CI = dry mass meat · 100/dry mass shell) was calculated. Results show that both oyster species grow successfully in a high-energy environment. Mean growth rates are similar to those measured in individuals from coastal habitats (wild banks and cultures) and the CI shows seasonal variation in both species. The survival rate for both species was >99% in 2007. However, in the previous trial in 2004 a high mortality rate was observed for *O. edulis* at one single site at the end of the experiment. Differences were observed in the increase of shell length and dry mass between sites and size classes. Taking these results into account site-selection criteria for different offshore locations are presented. We conclude that offshore cultivation of oysters will be successful if site-selection criteria are examined carefully when choosing a location for offshore aquaculture.

1. Introduction

Aquaculture is the fastest-growing sector of food production worldwide (FAO, 2009). The production of marine organisms mainly takes place in sheltered areas or embayment of the coastal sea (Buck et al., 2003; Firestone et al., 2004). However, in coastal areas space for all kinds of utilization is scarce, thus user conflicts exist and permanently increase (Buck et al., 2004; Wirtz et al., 2002). Intensive seafood production (e.g. fish or shrimp) often generates environmental stress caused by chemicals, eutrophication and impacts on benthic communities (McElwee, 1998). Therefore, in most nearshore areas the ecological carrying capacity sets limitations to massive expansions of intensive aquaculture activities (Troell et al., 2009). Extensive cultivation methods and extractive culture species with modest service needs offer the chance to move to offshore areas, away from socio-economic conflicts and coastal pollution (Krause et al., 2003) and still have an economic potential (Buck et al., 2010). Offshore or open-ocean aquaculture stands for the move of aquaculture operations from sheltered nearshore areas to more exposed environments where high wave action and strong currents exist (Ryan, 2005). However, due to the strong exchange and dilution effects of the water column in these high-energy environments, the water quality, the major element in aquaculture operations, is regarded to be very good (BSH 2006; Takayanagi, 1998).

Less user conflicts and higher wind speeds in offshore regions also enhance growing interests in offshore wind farming. Worldwide wind farms in offshore areas are being planned or already under construction (Gierloff-Emden, 2002). Following the multi-use concept of the Alfred Wegener Institute for Polar and Marine Research (AWI) and the Institute for Marine Resources (IMARE) foundations of offshore wind turbines can be used for additional purposes. Therefore, aquaculture installations can be attached to or installed between turbines within a wind farm (e.g. Buck & Krause 2011; Buck et al., 2008). Following these concepts of multifunctional use, sustainable seafood production shows an enormous economic potential in offshore areas (Buck et al., 2008).

Shellfish aquaculture, particularly oyster and mussel cultivation, is a good example for extensive production and has become very attractive (Gibbs, 2004). These candidates do not require artificial feeding

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doi:10.1016/j.aquaculture.2011.07.017

Fig. 1. Map of the German Bight showing all test sites: *Butendiek* (BD), *Helgoland* (HE), *Wurster Arm* (WA), and *Nordergründe* (NG).

(Ferreira et al. 2009; Garen et al., 2004) and as essential bio-extractive organisms can even improve water quality in marine systems (Ferreira et al., 2009; Rose et al., 2010). A feasibility study on open-ocean aquaculture by Buck (2002) identified the following extractive candidates as most suitable for the North Sea: sugar kelp (*Laminaria saccharina/Saccharina latissima*), dulse (*Palmaria palmata*), blue mussel (*Mytilus edulis*) and two oyster species, the Pacific oyster (*Crassostrea gigas*) and the European flat oyster (*Ostrea edulis*). As oysters represent high-value products and realize high prices on the market, compared to other shellfish (FAO 2011; Troell et al., 2009), these organisms seem to be ideal candidates for offshore aquaculture. However, techniques for offshore cultivation of oysters have not been developed yet, as over centuries, different forms of oyster cultures were only developed in coastal areas (Burbridge et al., 2001).

Consequently, no information is available on growth performance and fitness of oysters farmed in offshore areas, which experience high-energy environments. From the biological perspective the present study aims to assess whether offshore oyster cultivation offers a promising potential regarding growth performance in rough conditions.

This is the first study focusing on cultivation and subsequent performance characteristics of the Pacific oyster (*C. gigas*) and the European flat oyster (*O. edulis*), exposed to offshore farming conditions. In two experiments (2004 and 2007) we examined growth performance, condition and survival of oysters and analyzed site-specific criteria for the cultivation at offshore sites in the North Sea (Germany).

2. Materials and methods

2.1. Origin of test animals and description of study sites

Juvenile oysters were obtained from commercial hatcheries. For the sampling season 2004 *O. edulis* was produced at the Danish Shellfish Centre (Denmark) in September 2003 (observed as veliger) and *C. gigas* at Guernsey Seafarms (UK) in July 2003. For the sampling season 2007 oysters of *O. edulis* were produced in April 2006 (observed as veliger) at Bømlo Skjell A. Musling (Norway). *C. gigas* spat (produced in July 2006) was obtained from Guernsey Seafarms as well. Initial size classes (shell length) of juvenile oysters were 10–20 mm in 2004 and 20–30 mm in 2007. For the cultivation under offshore conditions juveniles of both species were transferred to the test sites in April 2004 (preliminary experiment for site selection) as well as in April 2007 (main experiment).

In the preliminary experiment oysters were cultivated in three different areas of the German Bight (Fig. 1). These study sites are characterized by various hydrographic features, such as depth, condition of the sea bottom, wave exposure, current velocity and significant wave heights (BSH 2011; Mittelstaedt et al., 1983), which are shown in Table 1. The site-specific conditions of these areas were chosen to fulfill the offshore-criteria in terms of a high-energy environment following the definition of Ryan (2005). Two test sites were set up within the areas of projected wind farms to underline the idea of the multiple-use concept. Furthermore, accessibility to sample and service the sites was also taken into account but of secondary relevance.

The test site *Butendiek* (BD) was located 15 nautical miles (nmi) west of the northern tip of the North Frisian island of Sylt (54° 59,1´ N; 007° 54,4´ E). Water depth was approximately 16 m at mean high water (MHW). This site was chosen because it is located within the area of the planned wind farm "Butendiek". The rounded metal piles of a former research platform of the Federal Maritime and Hydrographic Agency (Buck et al. 2008) provided an adequate fixed position within an offshore (high-energy) environment, and were used as a holding device for the oysters. A permit for the utilization of the study site was obtained in March 2004. Cultures were mounted at 4 m depth (high tide). The test site *Helgoland* (HE) was set up northeast of the island of Helgoland (54° 11,4´ N; 007° 53,0´ E). Water depth at site was approximately 6 m at MHW. Cultures were mounted to a metal frame at 4 m depth of water (high tide). The metal frame was moored on the seabed and co-used as a test site for the settlement of *Mytilus edulis* post-larvae. Despite the fact that this test site was about 150 m off the island of Helgoland, it can be considered as an offshore site due to the typical conditions that characterize this location (strong currents, high waves, good water quality, deep enough for submerged cultures (Barnaby, 2006)) and the fact that Helgoland is defined as an offshore island at all. The test site *Wurster Arm* (WA) was located in the outer Weser estuary (53° 40,7´ N; 008° 24,5´ E). Water depth at the site was approximately 8.5 m at MHW. Cultures were mounted at 1.5 m depth below a navigation buoy of the Water and Shipping Agency (WSA) Bremerhaven and were therefore independent of tidal influence. The main experiment started in 2007 and cultivation was carried out at a

Table 1
Site-specific conditions and classification of the four test sites.

Test site	Code	Year	Distance to coast [nmi]	Classification	Depth at MHW [m]	Sediment type at sea bottom	Sediment load of water column	Significant wave height [m] [a]	Wave exposure	Current velocity [m/s] [a,b]	Max. daily tidal current velocity [m/s] [a]
Butendiek	BD	2004	17	Offshore	14,8	Sand	Low	0.4–5.1	Exposed	0.2–0.6	0.32
Helgoland	HE	2004	25	Semi-offshore	6,0	Sand	Low	0.5–4.0	Exposed	0.1–0.7	0.30
Wurster Arm	WA	2004	3	Nearshore	8,5	Mud (clay)	High	0.2–1.8	Moderate	0.3–0.7	0.45
Nordergründe	NG	2007	9	Offshore	11,5	Sand	Moderate	0.5–5.0	Exposed	0.2–0.8	0.34

nmi: nautical mile, MHW: mean high water.
[a] BSH 2011.
[b] Mittelstaedt et al. 1983.

forth test site called *Nordergründe* (NG), which was located in the outer Weser estuary (53° 51,0′ N; 008° 04,0′ E), approximately 17 nmi northwest of Bremerhaven. It was situated in an official research testing area (Buck, 2007), which was established for offshore aquaculture as a multi-use in offshore wind farms. The planned offshore wind farm "Nordergründe" (Energiekontor, 2011) is located about one nmi off the test site. Experimental oysters were permanently submerged at all four testing sites.

2.2. Experimental design and sampling

In temperate regions both oyster species show an inactive period during winter and start feeding in spring (Løfstedt, 2010; Matthiessen, 2001). Therefore, our experiments were conducted over one complete growing season (first cultivation year) from April to October (Matthiessen, 2001; Robert et al., 1991; Walne & Mann, 1975). Due to the sensitivity of oysters in their early juvenile stage, the first year of cultivation can be considered as the most critical period (Newkirk et al., 1995). Therefore, the present study focused on this period to elucidate survival and robustness of the two species.

The oysters were reared in small oyster lanterns (60 cm length, 16 cm diameter, 4 compartments arranged in vertical levels, mesh size 10×10 mm), which were specifically produced for offshore aquaculture experiments (Fig. 2A). In both experiments 600 individuals were reared in each lantern (150 per compartment, two compartments with *O. edulis* and two with *C. gigas*), respectively. In the preliminary experiment (2004) two lanterns were installed at each site, attached to a metal frame, which acted as a rigid holding device. In the main experiment (2007) two lanterns were fixed to rigid steel rings, which were welded to large offshore marker buoys (Fig. 2B). These test buoys were constructed specifically for offshore aquaculture research on shellfish candidates (e.g. *C. gigas, O. edulis, Mytilus edulis*) (Brenner et al., 2007). Samples (80 individuals of each species) were taken by SCUBA-divers in June, August and October in 2004 and 2007. To ease sampling and maintenance and to avoid the loss of test animals the complete lantern was detached from the holding device and transferred to a zodiac nearby. Each lantern was then opened and oysters collected. In contrast to usual procedures in aquaculture the animals were not sorted into different size classes. Maintenance was performed by cleaning the lantern nets from biofouling and by removing empty shells. Oyster lanterns were returned to the holding device right after sampling to avoid stress for the animals. Sampled oysters were carefully stored in containers and transferred to the research vessel.

2.3. Analysis of growth, condition index and mortality

On board the research vessel length, width and height were measured to the closest 0.1 mm, before animals were stored deep-frozen (−80 °C) and transferred to the lab facilities at the AWI in Bremerhaven as well as to the University in Bremen. Empty shells were counted to estimate mortality rates. To determine dry mass (DM) and condition index (CI) soft tissue and shell of the test animals were freeze-dried (48 h, LYOVAC GT2, Leybold-Heraeus) and weighed (R200D, Sartorius). CI was then calculated by using Eq. (1), as suggested by Davenport & Chen (1987) and Walne & Mann (1975):

$$\mathrm{CI} = \mathrm{DM\ meat\ (g)} \cdot 100 / \mathrm{DM\ shell\ (g)} \quad (1)$$

Selecting a condition index that is based on dry mass is expected to be quite accurate as it is independent of the high variability of intervalval fluid in oysters (Beninger & Lucas, 1984).

2.4. Environmental parameters

Oceanographic parameters (current velocities, wave heights) were extracted from the Operational Circulation Model BSHcmod (BSH, 2011; Dick, 2001). Data describing temperature, salinity, chlorophyll *a*, phaeophytin *a*, nitrate and nitrite (NO_X), particulate organic carbon (POC), and particulate organic nitrogen (PON) were obtained from the Marine Environmental Data Base (MUDAB, BSH, 2009) and Brenner (2009).

2.5. Statistical analysis

Means, standard deviations, standard errors of the mean (mean ± SE) and confidence intervals of growth rates and condition indices were calculated using MS-Excel software. Differences in growth and condition index between sites and species were tested by *t*-test (MS-Excel software) and considered to be significant when $p<0.05$.

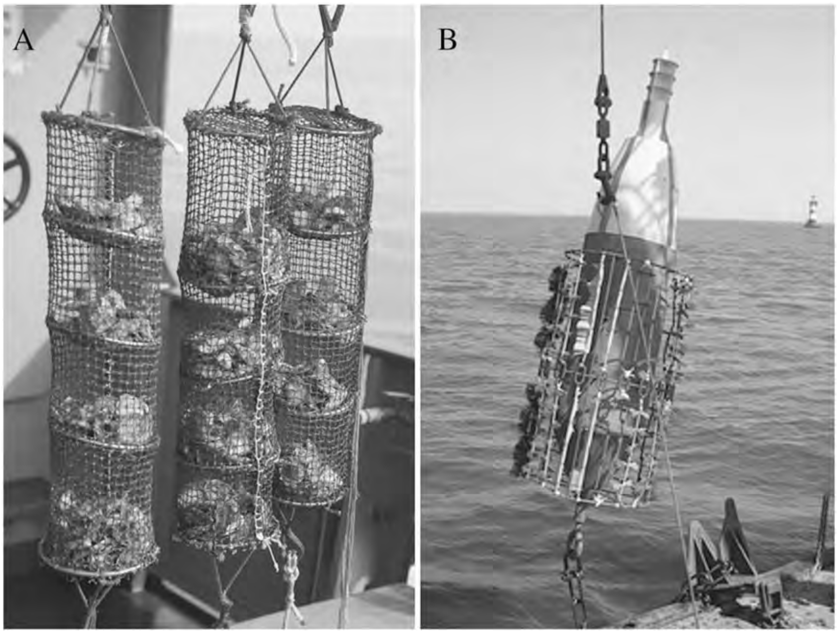

Fig. 2. (A) Oyster lanterns containing juveniles of *Ostrea edulis* and *Crassostrea gigas*. (B) Marker buoy, modified for offshore aquaculture research. Steel rings were used as rigid attachment device for the fixation of oyster lanterns.

3. Results

The results of the preliminary experiment acted as the basis to determine the site for the main experiment: The site *Nordergründe* (NG) was chosen as it represents an offshore aquaculture location class 4 (Ryan, 2005) plus a similar situation as the site *Butendiek* (BD) but was easier to access due to its shorter distance from the city of Bremerhaven. Additionally, NG is situated within the official testing area mentioned above.

3.1. Environmental conditions

Environmental conditions at the test sites are displayed in Table 2. Temperature measurements showed comparable conditions at all three test sites in 2004. In spring and autumn 2007 temperature was slightly lower than in spring and autumn 2004. Salinity ranged between 29.4 and 33.6 at the offshore sites and was lower at the nearshore site (WA): Depending on the direction of the tidal current it ranged between 11.8 and 22.1. Chlorophyll and nutrient concentrations characterizing the suitability for shellfish cultivation at the sites were extracted from the Marine Environmental Data Base (BSH, 2009) and Brenner (2009) and are also shown in Table 2.

3.2. Growth

The average length of *O. edulis* was 11.2 ± 0.2 mm at the beginning of the preliminary experiment (2004). At the end of the experiment it reached 27.9 ± 0.4 mm at WA, 38.6 ± 0.5 mm at HE, and 42.0 ± 0.9 mm at BD. The average length of *C. gigas* at the beginning of the experiment was 22.9 ± 0.2 mm. At the end of the experiment it reached an average length of 29.0 ± 0.6 mm at WA, 47.1 ± 0.9 mm near HE, and 50.5 ± 1.1 mm at BD. *C. gigas* and *O. edulis* showed similar growth patterns in terms of shell length. Growth of young oysters was moderate during spring 2004 and increased in summer and autumn. Compared to the sites *Butendiek* (BD) and *Helgoland* (HE) a significantly slower growth ($p<0.01$) was observed for both species at the site *Wurster Arm* (WA) (Fig. 3A-B).

At the beginning of the main experiment (2007) the average length of *O. edulis* was 38.5 ± 0.5 mm and 24.7 ± 0.5 mm of *C. gigas*. At the end of the experiment the average length of *O. edulis* was 58.9 ± 0.8 mm and 55.3 ± 1.1 mm of *C. gigas*, respectively. Both species showed rapid growth in spring, which slowed down in summer and increased again during autumn. Seasonal differences of growth rates were more pronounced in *C. gigas* than in *O. edulis*, which showed a rather constant increase in shell length (Fig. 3C). Daily growth rates at the different sites (and years) are shown in Table 3. They were very similar for both species at sites BD (*C. gigas* 0.17 mm/d, *O. edulis* 0.18 mm/d), HE (*C. gigas* 0.15 mm/d, *O. edulis* 0.16 mm/d), and in the same range at NG (*C. gigas* 0.19 mm/d, *O. edulis* 0.13 mm/d). Only at site WA growth rates were significantly lower (*C. gigas* 0.02 mm/d, *O. edulis* 0.06 mm/d) ($p<0.01$). Both species showed considerable intraspecific variability in growth success at all sites. To highlight

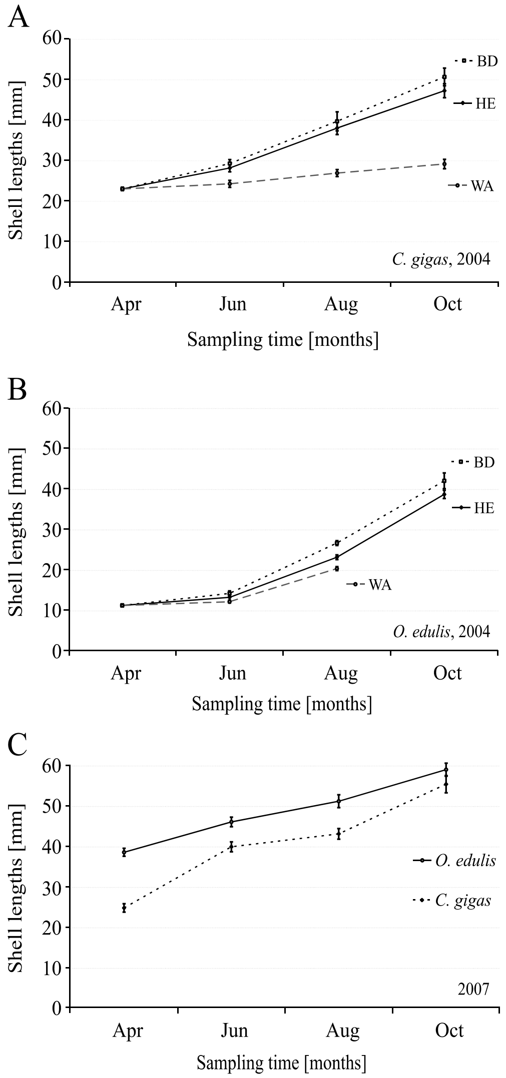

Fig. 3. Shell growth of *C. gigas* (A) and *O. edulis* (B) at test sites: *Butendiek* (BD), *Helgoland* (HE) and *Wurster Arm* (WA) during the preliminary experiment (2004). $n \approx 50$ per species, sampling date and site. (C) Shell growth of *O. edulis* and *C. gigas* during the main experiment (2007) at test site *Nordergründe* (NG). $n \approx 80$ per species and sampling date. Graphs displayed include the mean increase in shell length (mm) ± confidence interval.

Table 2
Environmental parameters at the four test sites.

Test site		Temperature [°C]	Salinity	Chl *a* [μg/l]	Pheo *a* [μg/l]	POC [μg/l]	PON [μg/l]	NO_X [μmol/l]
BD	April	7.2	32.9	17.0	1.0	512.2	88.7	31.5
	June	14.3	33.1	21.2	15.6	155.7	923.4	4.8
	August	17.9	31.6	5.3	8.2	76.8	504.2	2.3
	October	13.7	31.9	9.4	3.0	22.3	134.2	26.5
HE	April	8.1	33.6	10.8	n. d.	486.3	67.8	30.5
	June	14.1	33.5	21.2	n. d.	n. d.	n. d.	4.5
	August	18.3	31.5	6.3	n. d.	n. d.	n. d.	0.7
	October	14.0	32.9	3.7	n. d.	20.1	112.9	6.3
WA	April	10.5	18.4	9.7	6.3	801.5	92.3	30.2
	June	16.6	11.8	28.2	10.3	226.1	1015.8	n. d.
	August	21.1	22.1	10.1	2.5	100.8	368.6	n. d.
	October	12.9	19.7	7.2	5.7	24.2	78.2	11.6
NG	April	6.0	31.8	6.0	0.9	1045.8	118.5	38.2
	June	18.9	29.9	13.7	22.8	176.3	1518.6	7.7
	August	18.0	32.1	8.8	14.2	87.0	456.6	7.9
	October	11.1	29.4	6.3	3.2	18.5	130.0	7.1

BD: *Butendiek*, HE: *Helgoland*, WA: *Wurster Arm*, NG: *Nordergründe*, Chl *a*: Chlorophyll *a*, Pheo *a*: Phaeophytin *a*, POC: particulate organic carbon, PON: particulate organic nitrogen, NO_X: nitrate + nitrite, n.d.: no data available.

Table 3
Growth rates of *Crassostrea gigas* and *Ostrea edulis* presented as daily increase of shell length (SL) and dry mass (DM) at the four test sites.

Test site	Code	Year	Increase of SL/d [mm]		Increase of DM/d [mg]	
			C. gigas	*O. edulis*	*C. gigas*	*O. edulis*
Butendiek	BD	2004	0.17	0.18	2.31	1.14
Helgoland	HE	2004	0.15	0.16	1.93	0.95
Wurster Arm	WA	2004	0.02	0.06	0.86	0.25
Nordergründe	NG	2007	0.19	0.13	2.33	4.57

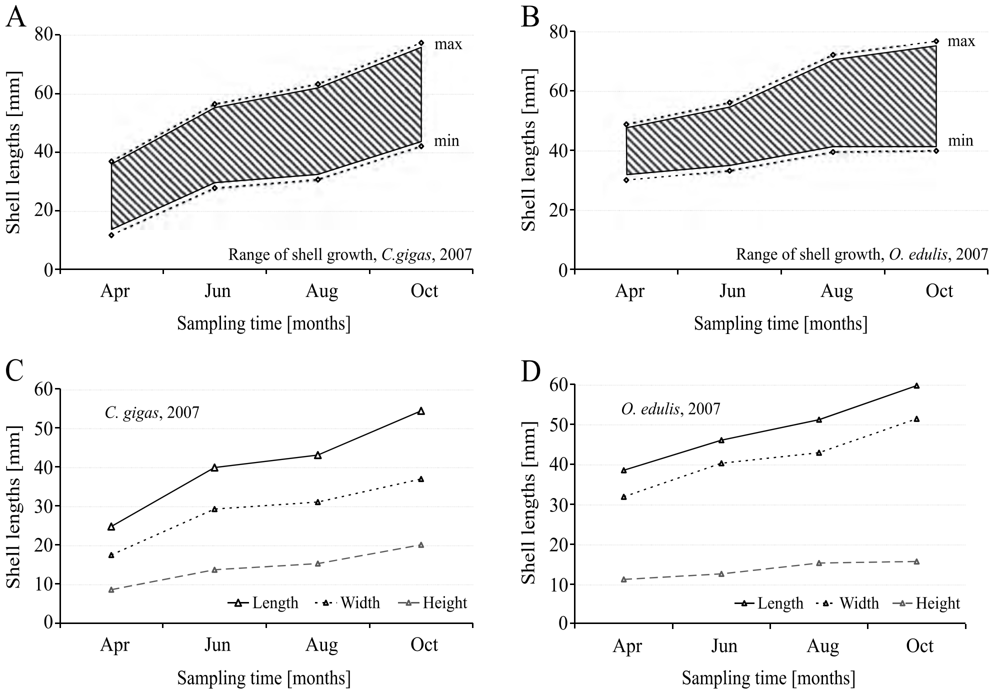

Fig. 4. Growth range (minimum and maximum growth of shell length) of *C. gigas* (A) and *O. edulis* (B) during the main experiment (2007). Species-specific shell growth (length, width, thickness) of *C. gigas* (C) and *O. edulis* (D) in the main experiment (2007).

these differences the range of shell lengths of *C. gigas* and *O. edulis* in the experiment 2007 is presented in Fig. 4A–B. The two oyster species showed clear differences in terms of shell length, width, height and absolute size because of their species-specific morphology (Newkirk et al., 1995; Walne, 1979). *O. edulis* is rather round, whereas *C. gigas* shows a more elongated morphology (Fig. 4C-D).

3.3. Dry mass, condition index and mortality

Dry mass of both species increased during cultivation time at all sites in 2004 as well as in 2007 (Fig. 5A–C). In 2004 low growth rates in terms of increasing dry mass were observed for both species at site WA (*C. gigas* 0.86 mg/d, *O. edulis* 0.25 mg/d) and significantly higher rates ($p<0.01$) at HE (*C. gigas* 1.93 mg/d, *O. edulis* 0.95 mg/d), and BD (*C. gigas* 2.31 mg/d, *O. edulis* 1.14 mg/d) (Table 3). In 2007 both species showed a similar increase in dry mass during springtime. Afterwards different performances were observed for the two species: *O. edulis* individuals grew slightly slower in summer than in spring but growth increased again in autumn. *C. gigas* individuals showed no length changes during summer, however, marginal increases in dry mass in during autumn were observed (Fig. 5C). *C. gigas* obtained the highest daily increase in dry mass at sites BD and NG (same growth rates in 2004 and 2007). Clearly lower increases in dry mass were observed in 2004 for individuals of *O. edulis*. In the main experiment of 2007 (site NG) growth rates of *O. edulis* (4.57 mg/d) were almost two times higher than those of *C. gigas* (2.33 mg/d) (Table 3).

Condition index (CI) of the Pacific oysters ranged between 3.4 ± 0.12 and 11.8 ± 0.37 at all sites (Fig. 5E). Corresponding values for the European oysters ranged between 1.8 ± 0.02 and 4.8 ± 0.13 in the preliminary experiment (2004) and were higher (between 5.2 ± 0.26 and 7.2 ± 0.22) in the main experiment (2007) (Fig. 5F). In the preliminary experiment values for both species increased during cultivation at sites BD and HE. *C. gigas* obtained the highest CI at BD (10.0 ± 0.31) and similar characteristics at HE but with lower values (7.7 ± 0.29) at the end of the cultivation period. Data for *O. edulis* show similar inclines for BD and HE with maximum values (4.8 ± 0.13 and 4.6 ± 0.07) at the end of the cultivation period. Only at WA a different pattern was observed: In June *C. gigas* obtained values similar to those at BD and HE. Subsequently the CI remained constant and after August even a slight decrease in CI was observed. *O. edulis* obtained values similar to those at BD and HE in June and August at WA but no values exist for October due to mortality after the sampling in August. In the main experiment *C. gigas* showed a high CI after the spring period (11.8 ± 0.37) which decreased during the rest of the sampling season with faster rates in summer and slightly slower rates in autumn (Fig. 5E). *O. edulis* also showed the highest CI after the spring period (7.2 ± 0.2) and a decrease during summer. In contrast to *C. gigas* a slight increase was observed for *O. edulis* in autumn (Fig. 5F).

In 2004 mortality was low (<3.2%) for both species at sites BD and HE. However, at site WA *O. edulis* individuals died after August. In 2007 (NG), mortality was low (<1%) for both species during the entire sampling period (Fig. 5D).

4. Discussion

Offshore cultivation of oysters is mentioned and recommended in various publications (e.g. Chávez-Villalba et al., 2010; Delmendo, 1998; Goulletquer, 1998; Handley & Jeffs, 2003), however, none of these publications give attention to “real” offshore conditions as defined by Ryan (2005) and Buck (2007) for open-ocean or offshore aquaculture, namely a high-energy environment with strong currents and high waves. Therefore, no data on offshore farming of oysters among those conditions were available so far to allow comparison of the results of our test animals.

In this study we investigated the biological response of Pacific and European oysters in high-energy environments in terms of growth performance, condition and survival rates.

4.1. Growth

The results of the length increase in this study show that both *C. gigas* and *O. edulis* can grow successfully at exposed conditions in an offshore environment. *O. edulis* and *C. gigas* were reared outside their natural coastal habitat, which is normally located on seabeds of the

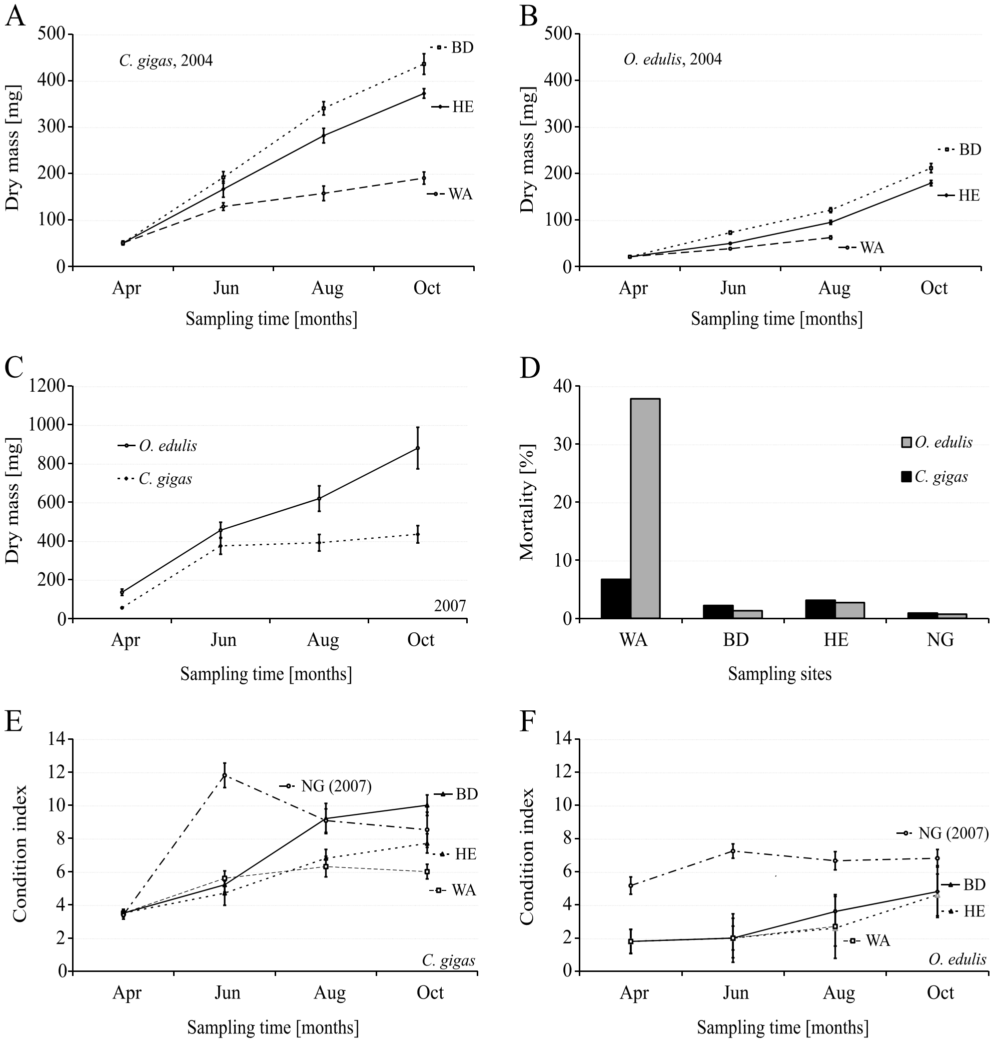

Fig. 5. Dry mass of *C. gigas* (A) and *O. edulis* (B) at test sites: *Butendiek* (BD), *Helgoland* (HE) and *Wurster Arm* (WA) during the preliminary experiment (2004). (C) Dry mass of *O. edulis* and *C. gigas* during the main experiment (2007) at test site *Nordergründe* (NG). Graphs present the mean increase in shell length (mm) ± confidence interval. Mortality (D) and condition index of *C. gigas* (E) and *O. edulis* (F) in 2004 and 2007 at the different test sites. $n \approx 50$ per species, sampling date and site. (F) Please note: Lines WA, HE and BD are overlapping from April to June, and Lines WA and HE from June to August. Line WA ends in August.

coastal sea. The animals obtained positive growth rates in terms of shell length and dry mass. However, the duration of the project did not allow the farming of oysters to market size. Nevertheless, this time period allows an estimation of oyster growth over a certain life span. Accepting the lack of growth data of offshore grown oysters, mean growth rates are still comparable to those measured of individuals from nearshore coastal areas (Diederich, 2006; Robert et al., 1991, 1993; Schmidt et al., 2008; Valero, 2006; Walne & Mann, 1975). Following Valero (2006) there is evidence that juvenile oysters show high length growth in their first year which means that they primarily invest in shell development before flesh biomass starts to increase. In the second and third year they change to higher growth of their body mass. Oysters of this study still grew in the first year of the typical oyster cultivation period. They showed an accelerated length increment, which in turn underlines the projected development.

Seasonal differences in growth performance were observed for the Pacific oyster in the main experiment. The European oyster showed a more constant increase in shell length and dry mass at the same site (NG). The experimental set-up at NG was only conducted in 2007 and no data of other seasons at the same site were available for comparison. However, we assume a higher ability of dietary assimilation, when food availability is low in summer (Rick et al., 2006) and a better adaptation of the native European oyster to this region (Matthiessen, 2001; Newkirk et al., 1995) instead of its counterpart, the introduced Pacific oyster (Andrews, 1980; Chew, 1990; Ruesink et al., 2005).

Oysters of the present study produced an esthetic shell and achieved their species-specific morphology, which plays an important role for their market value in Europe (Matthiessen, 2001). Only oysters grown at *Wurster Arm* (WA) developed a different shape: Thicker shells and a very compact appearance indicate that animals strongly invested in shell growth to withstand the high currents as well as to prevent shell abrasion (Newkirk et al., 1995).

Mean growth rates in terms of shell length and dry mass strongly depended on the different culture sites. Both oyster species preferred the same sites: At the sites *Butendiek* (BD) and *Nordergründe* (NG), both species showed the highest growth rates. As the Pacific oyster showed very similar daily increases in dry mass at both sites, we assume that the sites BD and NG offer similar good conditions for cultivation. However, the European oyster performed differently: Daily increase in dry mass was four times higher at NG (main experiment, 2007) than at BD (preliminary experiment, 2004). We assume that the reason for this different growth development originates in the condition of the spat regarding its larval source. In the preliminary experiment of 2004 European oysters were obtained from the Danish Shellfish Centre (DSC), which started land-based cultivation of *O. edulis* in 2002. Løfstedt (2010) indicated poor conditions for some of the first generations of oyster spat produced at DSC, thus, data on growth rates and mortality of *O. edulis* in the preliminary experiment have to be interpreted with caution. This could explain the deviating results for the European oyster at sites BD and NG. As *O. edulis* of DSC shows positive growth rates in 2004, the question arises, if growth rates could have been even higher with spat animals, which had optimum condition initially. We conclude that *O. edulis* individuals used for the preliminary cultivation experiment in 2004 were in poor condition and too small to move off the coast. After Buck (2002) only those organisms are appropriate for offshore cultivation that are healthy and comprise a certain size class, which is characterized by sufficient robustness. According to typical size classes used in commercial aquaculture farms, larger *O. edulis* spat from a Norwegian producer was used in the main experiment (Buck, 2002; Matthiessen, 2001; Newkirk et al., 1995; Draver, pers. comm.). These animals yielded higher growth rates, even exceeding those of Pacific oysters. The better condition of these animals is reflected in the higher condition index of European oysters in the main experiment.

4.2. Condition

The condition index of various oyster species such as *O. edulis*, *C. gigas*, *C. virginica* etc. is commonly used to evaluate the effects of the surrounding environment on these organisms (Rheault & Rice, 1996). It is an adequate parameter to describe the commercial quality and physiological state (health) of bivalve molluscs (Dridi et al., 2007). In shellfish production, the most commonly applied condition index (CI) is the ratio of flesh mass to shell mass (Davenport & Chen, 1987). The data on the condition of both oyster species support the positive results observed for the growth performance at offshore sites. Condition index values for the Pacific oyster indicate good condition (Linehan et al., 1999) in both experiments. Values for the European oyster also indicate good condition in the 2007 experiment. In contrast, condition indices were quite low at the beginning of the preliminary experiment in April 2004, confirming the poor condition of the DSC oyster spat. But the CI increased with cultivation time and the European oyster showed a better condition (Linehan et al., 1999; Walne & Mann, 1975) at the end of the experiment in October 2004. Apparently, *O. edulis* was capable of an excellent recovery at sites BE and HE. The condition index showed seasonal variations for both species. Similar variations have also been reported for cultured oysters in coastal areas of Western Europe (Abad et al., 1995; Linehan et al., 1999; Ruiz et al., 1992; Soletchnik et al., 2006) and South Australia (Li et al., 2009). These variations are generated by changing food supply and in adult individuals by gametogenesis and spawning (Li et al. 2009; Soletchnik et al., 2006). In summer 2007 only slow growth in terms of dry mass was observed for the Pacific oyster. This resulted in a decline of the CI, which has also been reported for late summer/autumn in South Australia (Li et al., 2009). Similarly, a slight decline of the CI could be observed for the European oyster. Whereas the CI of *O. edulis* showed a little increase again after August, *C. gigas* did not recover. This is another indication for a better adaptation of the European oyster to this region.

4.3. Survival rates

Survival rates for both oyster species (>99% in 2007) were very high and appropriate for the culture of marine organisms and are rarely achieved in commercial oyster aquaculture production (Chávez-Villalba et al., 2010; Costil et al., 2005; Soletchnik et al., 2006; Valero, 2006). High survival or low mortality rates of Pacific oysters have also been reported for wild banks in the German Wadden Sea (Diederich, 2006; Schmidt et al., 2008) and for oyster cultures in South Australia (Li et al., 2009). However, in 2004 a high mortality rate was only observed for *O. edulis* at one single site: At site WA, animals died some time after the sampling period in August. This mass die-off could have been caused by the high sediment-load in the water column at this site (BSH, 2011), since natural *O. edulis* oyster beds are never found on muddy sediments (OSPAR, 2009). The presence of high quantities of silt in the water can block the digestive and respiratory tracts, causes stress to the animal and results in the death of the oyster after a certain time (Laing et al., 2005; Partridge, 1981). Additionally, due to the short distance of this site to the port of Bremerhaven, urban sewage or contaminants could have affected the water quality. In general, high mortality of oysters can be correlated to energy expenditure during spawning, high water temperatures in summer as well as the result of multiple stressors (e.g. low salinity, low dissolved oxygen, pathogens) creating physiological pressure (Costil et al., 2005; Soletchnik et al., 2006).

The high survival rates presented in this study indicate that water conditions at the other offshore cultivation sites have no negative impact on the survival of the tested oyster species. This is supported by the fact that natural banks of the European oysters also occurred in deeper waters and offshore down to 50 m, e.g. in the North Sea and the eastern English Channel (OSPAR, 2009).

4.4. Site-specific performance

In the experiments of 2004 and 2007 oysters were cultivated at four different sites. Due to the varying conditions from site to site, differences in growth performance, condition and survival were observed. To evaluate these sites regarding site selection criteria, biotic and abiotic parameters (e.g. temperature, salinity, nutritional situation) and their impact on the overall condition of the candidates were examined.

No significant differences in temperature and salinity were detected between the three offshore cultivation sites. The growing season of oysters depends on temperature and naturally proceeds from April until late September in the North Atlantic (Korringa, 1952; Matthiessen, 2001; Walne & Mann, 1975). As *C. gigas* tolerates a wide range of temperatures and cultivated *O. edulis* individuals belong to the well-adapted cold-water population (OSPAR, 2009), no seasonal temperature abnormalities were observed. Additionally, salinity is known to be very stable at offshore sites and both species prefer the marine and fully saline conditions that characterize those areas (Laing et al., 2005).

Oysters are supplied with food and oxygen and relieved from waste products through the action of hydrodynamic currents. Since filtration rate and food availability increase with flow rate (Walne, 1972; 1979), oysters were expected to grow better at cultivation sites with stronger water currents (Valero, 2006). Although offshore areas are known to offer lower plankton concentrations compared to coastal regions (Ducrotoy et al., 2000), the higher hydrodynamic flow offshore can compensate for this limitation. However, the offshore test sites in the present study resemble special conditions as the North Sea shows symptoms of eutrophication. Additionally, turbid areas, like estuaries and their run-offs, can be classified as hyper-nutrified systems where food supply is regarded to be sufficient (Ducrotoy et al., 2000). The combination of current speed and food amount implies high food availability at the offshore sites BD and NG with high current speed. WA, also a site with high currents, deviated from that scheme. As described above, oysters did not show successful growth rates and the shell morphology indicated mechanical stress. Former studies have shown that filtration rates increase with increased flow rates until a maximum value when oysters are unable to remove more particles from the water passing by (Walne, 1979). Apparently, current forces at WA exceeded this maximum. Furthermore, low salinity values at this site could have resulted in physiological stress for the oysters and affected their growth success (Laing et al., 2005).

In the preliminary experiment, test site *Butendiek* (BD) showed the best results. The distribution of nutrients and plankton in the German Bight follows the current system, a counter-clockwise gyre parallel to the coast (Ducrotoy et al., 2000, Mittelstaedt et al., 1983). Hence, nutrients from river run-offs and the coast are transported to the north (BSH, 2004). Subsequently, high plankton production can be observed parallel to the coast of the North Frisian Wadden Sea and around the area of the test site BD (Ducrotoy et al., 2000; Weigelt-Krenz et al., 2010). This situation provides optimal nutritional conditions for the cultivated oysters. Results of similar quality in terms of shell growth were found at test site *Helgoland* (HE). However, the area around Helgoland is located further offshore, outside of the nutrient-rich zone of the German Bight (Weigelt-Krenz et al., 2010). Correspondingly, slightly lower growth rates (dry mass) of the Pacific oyster indicate a different food situation at this site. At site *Wurster Arm* (WA) low salinity and a high sediment load of water originating from the Wadden Sea (BSH, 2004) may explain the low growth success. Poor growth performances of both species and the high mortality of the European oyster lead to the conclusion that this site is not adequate for aquaculture production.

Consequently, for the main experiment in 2007 it was aimed for a cultivation site, which fulfills the described site selection criteria and offers the same quality as site BD. De facto, growth performance, condition and survival of both oyster species showed the expected success at the selected site *Nordergründe* (NG).

5. Conclusions and outlook

This study shows that juvenile oysters (1) exhibit a convincing growth performance at offshore sites, (2) are in strong condition, and (3) cope well with rough conditions. Subsequently, from an oyster farmer perspective, these data attest the suitability of open ocean environments for oyster cultivation and encourage aquaculture activities at offshore sites. Both, *C. gigas* and *O. edulis*, respectively, play a significant contributor to the European economy, especially on the oyster half shell market in France. Generally, the oyster trade is of enormous commercial importance with wholesale average prices for the European oyster commonly being 3 to 5 times higher than for the Pacific oyster. Following Goulletquer (2004), *O. edulis* fills an economic niche as a premium seafood item. Therefore, oysters as high-value products would be potential candidates for offshore cultivation since an economic analysis for mytiliculture by Buck et al. (2010) have calculated higher costs when moving far off the coast.

Our experiments clearly reveal indications that the native species is very well adapted (Matthiessen, 2001; OSPAR, 2009) and shows an adequate growth performance. Therefore, the cultivation of the European flat oyster in the North Sea is recommended in particular.

Assuming appropriate site selection criteria, we conclude that both oyster species, *C. gigas* and *O. edulis*, are successful candidates for offshore cultivation. A multiple-use of potential offshore locations together with other users, e.g. offshore wind farms is promising and synergistic effects may offer technical and financial advantages to oyster farmers.

Future studies will focus on physiological parameters and fitness indicators of oysters cultivated at offshore sites. Additionally, complete grow-out periods during various seasons should be examined to increase knowledge about oyster culture experiments off the coast.

Acknowledgements

We thank the Water and Shipping Authority, namely Mr. Boening and the Captain and Crew of the *MV Bruno Illing* and *MV Mellum* for their support in maintaining the test sites *Wurster Arm* and *Nordergründe*. Thanks are expressed to the captain and crew of the research vessel *Uthörn* for their assistance. We also thank the AWI-diving group for technical support and all divers involved in this project. Thanks go to Dr. Matthias Brenner (IMARE) who provided environmental data from the support program of the *Mytifit*-Project and to Dr. Susanne Tamm from the German Oceanographic Data Centre (DOD) for her help with the databases of the BSH. The research carried out for this manuscript has been funded by the University of Bremen and the Alfred Wegener Institute for Polar and Marine Research and is further a partial fulfillment for a Doctoral degree of the leading author.

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CHAPTER II

BIOCHEMICAL AND ELEMENTAL COMPOSITION OF THE OFFSHORE CULTIVATED OYSTERS *OSTREA EDULIS* AND *CRASSOSTREA GIGAS*

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submitted to *Marine Biology*

Biochemical and elemental composition of the offshore cultivated oysters *Ostrea edulis* and *Crassostrea gigas*

Abstract

Offshore production of seafood is a promising approach to evade numerous specific problems of aquaculture activities in coastal areas. The aim of this study was to investigate the biological performance of oysters, typical nearshore organisms and ambitious aquaculture candidates, in an offshore environment. Juveniles of two oyster species, *Ostrea edulis* and *Crassostrea gigas*, were transferred to an offshore cultivation site in the German North Sea and cultivated from April to October 2007. Samples were taken every six to eight weeks to determine biochemical and elemental compositions: total glycogen, total protein, total lipid as well as lipid classes, fatty acids and CHN ratio. Results show an increase in glycogen from spring to early summer for both species, which is related to high food abundance during spring phytoplankton bloom. During summer, glycogen storage revealed clear differences between *O. edulis* and *C. gigas*, owing to reproductive activity only of the Pacific oyster. Total glycogen ranged between 6% and 23% DM in *O. edulis* and between 5% and 16% DM in *C. gigas*. Total protein did not show significant seasonal variations and ranged between 34% and 41% DM in both species. Total lipids increased during summer, although clearly more pronounced in *C. gigas*, followed by a decrease in both species in autumn. Total lipid levels ranged between 7% and 14% DM in both species. Phospholipids and triacylglycerols were the main lipid classes in both oyster species, followed by sterols. Fatty acid compositions resembled those of nearshore grown oysters. We conclude that offshore-cultivated oysters exhibit a natural biological performance, emphasizing their suitability as offshore aquaculture candidates.

1 Introduction

Marine aquaculture activities cumulate along coastal areas of the oceans. These activities, however, often cause serious and unresolved problems like pollution and coastal destruction due to e.g. intensive culture methods (McElwee 1998). Environmental stress as well as stakeholder conflicts in ocean use (Wirtz et al. 2002, Buck et al. 2004) constrain further expansion of this important sector of food production (FAO 2009). Accordingly, efforts are required to establish alternative, sustainable, and environmentally friendly cultivation methods. A promising but technically ambitious approach is an extensive cultivation of extractive culture species in the open ocean (Delmendo 1998, Buck 2002, Krause et al. 2003, Buck et al. 2004, 2008, Ferreira et al. 2009, Troell et al. 2009, Pogoda et al. 2011). Open ocean or offshore aquaculture transfers aquaculture operations from sheltered near-shore areas to more exposed environments. Structures are exposed to high wave action and strong currents (Ryan 2005, Pogoda et al. 2011). However, excellent water quality is a great advantage over coastal areas, which is crucial for the performance and health of cultured species (Takayanagi 1998, BSH 2006).

Filter-feeding shellfish species such as oysters are suitable candidates for extensive cultivation, as they do not require supplemental feeding (Garen et al. 2004, Gibbs 2004). Moreover, they can even improve water quality as essential bio-extractive organisms (Ferreira et al. 2009, Rose et al. 2010). Oysters, furthermore, represent high-value products. Compared to other shellfish species they yield high prices on the market (Troell et al. 2009, FAO 2011) and are therefore ideal candidates for cost-intensive offshore aquaculture.

Several studies were carried out on seasonal changes of the biochemical composition of oysters (Ruiz et al. 1992, Robert et al. 1993, Abad et al. 1995, Linehan et al. 1999, Costil et al. 2005, Soletchnik et al. 2006, Dridi et al. 2007, Li et al. 2009). However, no information is available about the biological performance and fitness of oysters grown under offshore farming conditions. Therefore, the present study aims at assessing the performance of oysters from a biochemical and ecophysiological point of view. We cultivated two species of oysters, the European flat oyster (*Ostrea edulis*) and the Pacific oyster

(*Crassostrea gigas*), in an experimental offshore culture system in the North Sea (Pogoda et al. 2011). The oysters were raised over an entire growing season and analyzed for C, H, N contents, total lipid contents, lipid class and fatty acid compositions as well as glycogen and protein contents. The data were evaluated with regard to the biological performance of *Ostrea edulis* and *Crassostrea gigas* in the North Sea and their suitability for offshore aquaculture.

2 Material & methods

2.1 Study site and experimental design

The European oyster and the Pacific oyster were cultivated at the test site *Nordergründe* (NG) in the German Bight (53° 51,0´ N; 008° 04,0´ E, Fig. 1) This site was previously established as a test site for offshore aquaculture activities (Buck 2007) and fulfills the offshore-criteria in terms of a high-energy environment according to Ryan (2005).

Figure 1: Map of the German Bight showing the offshore cultivation site *Nordergründe* (NG) in the North Sea.

In temperate regions both species of oysters are inactive during the winter period but start feeding in spring (Matthiessen 2001, Løfstedt 2010). Therefore, this study was conducted over one complete growing season from April to

October 2007. Juvenile oysters of 25-40 mm size (Pogoda et al. 2011) were obtained from commercial hatcheries. *Bonamia*-free oyster spat of *Ostrea edulis* was bought from Bømlo Skell A. Musling (Norway) and *Crassostrea gigas* spat from Guernsey Seafarms (UK). Both species were transferred to the test site in April 2007. The oysters were reared in small oyster lanterns, which were specifically developed for offshore aquaculture experiments as described in Pogoda et al. (2011). Oysters were permanently submerged and independent from tidal water level changes. Samples (80 individuals of each species) were taken by SCUBA-divers in April, June, August and October. Details of the sampling procedure and maintenance are described in Pogoda et al. (2011).

2.2 Environmental parameters

Data for temperature, salinity, chlorophyll (a, b, $c_{1,2}$, phaeophytin) and carotenoids, nitrate and nitrite (NO_X), particulate organic carbon (POC), and particulate organic nitrogen (PON) as well as phosphate and ammonium concentrations were obtained from the Marine Environment Reporting System (BSH 2009) and Brenner (2009). These data are presented in detail by Pogoda et al. (2011).

2.3 Biochemical analysis

The oysters were opened carefully and the entire soft body was removed from the shell. The soft body was not separated into organs or body parts to avoid leakage of inter- and intracellular fluids (Whyte et al. 1990). After freezing at -80°C and lyophilization (Leybold-Heraeus Lyovac GT2) for 48 h oyster dry mass was determined using a Sartorius micro-balance (R200D).

Lipid extraction and gravimetric determination of total lipids were performed after the method of Folch et al. (1957) modified by Hagen (2000). The samples were first homogenized in dichloromethane:methanol (2:1/v:v, Braun Biotech International, Potter S) and then treated with an ultrasonic cell disruptor (Bandelin electronic, UW 2070). Finally, the extract was washed with an aqueous KCl solution (0.88%) to remove proteinous compounds.

Lipid classes were separated and analyzed by high-performance thin-layer chromatography (HPTLC) flame ionization detection (TLC-FID) with an Iatroscan Laboratories model Mk-5 TLC/FID analyzer (Fraser et al. 1985).

Samples were run in triplicate. Calibration was affected with single compound standards.

Fatty acids were analyzed after Kattner & Fricke (1986). Subsamples (500 µg) of the total lipid extracts were hydrolyzed and fatty acids converted to methyl ester derivatives (FAMEs). The reaction was performed in 1 ml methanol containing 3% concentrated sulfuric acid and 250 µl hexane at 80°C for 4 h. 2 ml of aqua bidest. were added to the reaction mixture after cooling and FAMEs were extracted three times with 1 ml hexane. Separation of fatty acids was carried out with a gas chromatograph (HP 6890A), which was equipped with a DB-FFAP column (30 m length, 0.249 mm diameter, 0.25 µm film thickness). The carrier gas was helium and the device operated with a temperature programme. FAMEs and fatty alcohols were detected by flame ionization and identified by comparing the retention times with those of known standards.

For carbohydrate (glycogen and glucose) and protein determination freeze-dried soft tissue of oysters was ground by mortar and pestle into a fine powder under liquid nitrogen. Glycogen and glucose were determined after Keppler & Decker (1984) and Kunst et al. (1984) as modified for use in microplates by Saborowski & Buchholz (1996). Frozen and lyophilized tissue powder (20-30 mg) was transferred into reaction cups, placed on ice, and homogenized in 1 ml aqua bidest. by ultrasonication (Branson Sonifier B15). Immediately after homogenization the extracts were heated for 10 min at 95°C (Eppendorf Thermomixer Comfort) to destroy glycogen-degrading enzymes. Subsamples were taken for glycogen and glucose determination. The subsamples for glycogen determination were incubated for 2 h at 40°C in 500 µl 0.1 acetate buffer (pH 4.8) with 20 µl amyloglucosidase to hydrolyze glycogen to glucose (Boehringer). After centrifugation (10 min, 15000 g, 4°C), supernatants of both subsamples were used for the determination of glucose performed with a commercial glucose UV test kit (Boehringer, No. 10716251035). 10 µl of sample and 300 µl of buffer solution 1 (test kit) were transferred into mircoplates. The optical density of the samples was read at 340 nm in a microplate reader (Thermoscientific, Multiscan FC, Software Scan it 2.5.1). These values served as background values in the subsequent calculation of the glucose content. Hexokinase suspension (dilution 1:5 in aqua bidest.) was added to each sample and the plate was incubated for 30 min at 25°C before the optical density was read again at 340 nm. All samples were run in triplicate and the standards in parallel yielding 0, 2.5, 5.0, 7.5 and 10.0 µg of glucose per well.

Protein was analyzed after Lowry et al. (1951). Frozen tissue powder (400-500 µg) was homogenized on ice in 1 ml aqua bidest. by ultrasonication (Branson Sonifier B15). The dye reaction was performed with the commercial test kit DC Protein Assay Kit II (BIORAD, 500-0112). Bovine serum albumin served as standard.

Carbon (C), nitrogen (N) and hydrogen (H) were measured in an elemental analyzer (VarioMicroCube, Elementar), using sulfanilamid as standard.

2.4 Statistical analysis

Means, standard deviations, standard errors of the mean (mean ± SE) and confidence intervals (mean ± CI) of biochemical and elemental compositions were calculated and tested for normal distribution with MS-Excel software. Differences in total lipid, glycogen and protein as well as lipid class and fatty acid compositions between seasons and species were tested with the Student´s *t*-test (MS-Excel software) and considered to be significant when $p < 0.05$. Additionally two-way ANOVA was performed using GraphPad Prism 5.0.4.

3 Results

Elemental composition

Carbon, the main elemental component of the soft body, increased in both oyster species during summer and decreased slightly in autumn (Table 1). Mean carbon values of *O. edulis* increased from 29.1 ± 1.6% in April to 37.6 ± 0.5% in June and to 39.4 ± 0.7% in August. In October they dropped to 31.4 ± 1.2%. Mean carbon values of *C. gigas* increased from 26.9 ± 0.7% in April to 38.8 ± 0.7% in June. The values stayed constant until August (38.0 ± 1.3%) and dropped to 34.9 ± 1.6% in October. Nitrogen contents of *O. edulis* remained at a constant level of 7.5% in spring and summer but slightly rose to 9.9 ± 0.9% in autumn. *C. gigas* showed a continuous increase in nitrogen over the cultivation period. It rose from 7.1 ± 0.2% in April to 8.7 ± 0.2% in June to

9.0 ± 0.3% in August and to 13.8 ± 2.4% in October (Table 1). The share of hydrogen remained constant at around 5% for both oyster species (Table 1).

Table 1: Seasonal variation of elemental composition of *Ostrea edulis* and *Crassostrea gigas* at an offshore cultivation site (n = 20 per species and sampling date). C: carbohydrates, H: hydrogen, N: nitrogen.

Ostrea edulis	%C	%N	%H	C/N
April	29.1 ± 1.6	7.2 ± 0.5	4.9 ± 0.3	4.0
June	37.6 ± 0.5	7.9 ± 0.4	6.1 ± 0.1	4.8
August	39.4 ± 0.7	7.6 ± 0.3	6.1 ± 0.1	5.2
October	31.4 ± 1.2	9.9 ± 0.9	5.1 ± 0.2	3.2
Crassostrea gigas				
April	26.9 ± 0.7	7.1 ± 0.2	4.8 ± 0.2	3.8
June	38.8 ± 0.7	8.7 ± 0.2	6.3 ± 0.1	4.4
August	38.0 ± 1.3	9.0 ± 0.3	5.9 ± 0.2	4.2
October	34.9 ± 1.6	13.8 ± 2.4	5.7 ± 0.2	2.5

Resulting C/N ratios of *O. edulis* and *C. gigas* are shown in Fig. 2. In *O. edulis* it increased from April (4.0) to August (5.2) but significantly dropped (3.3) in October ($p < 0.05$). C/N ratios of *C. gigas* started at 3.8 in April and increased to 4.4 in June followed by a slight decrease to 4.2 in August. Thereafter, it dropped significantly to 2.5 in October ($p < 0.05$).

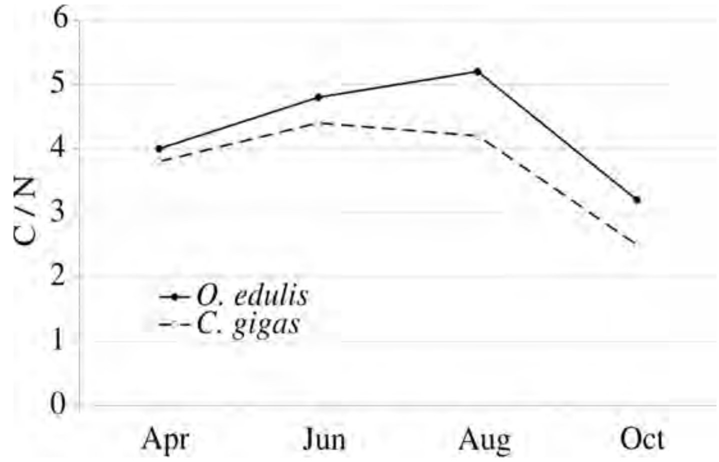

Figure 2: Seasonal variation of C/N ratio in soft tissue of *O. edulis* and *C. gigas*. C: carbohydrates, N: nitrogen.

Biochemical composition

Total lipid

Lipid levels of the European oyster *Ostrea edulis* ranged between 6.9 ± 0.5% DM in spring and a maximum of 9.4 ± 1.3% DM in early summer (Fig. 3A). These values showed no significant differences during the growing season. Lipid contents of the Pacific oyster *Crassostrea gigas* were slightly higher than those of *O. edulis*. These increased from 8.0 ± 0.8% DM in spring to 11.3 ± 1.1% DM in early summer and reached a maximum of 14.4 ± 1.4% DM in late summer (Fig. 3B). The differences over the season were statistically significant ($p < 0.0001$) (Table 2).

Lipid class composition

In both species triacylglycerols (TAG) served as main storage lipids throughout the growing season. The relative amount of TAG in *O. edulis* ranged between 18.5 ± 6.3% TL in spring and 40.1 ± 3.1% TL in autumn. In *C. gigas* lipid contents ranged between 3.3 ± 2.8% TL in spring and 42.2 ± 6.6% TL in late summer (Fig. 3A&B, Table 2). Due to the applied analytical method wax ester and sterol ester could not be separated and are combined in one group. Fatty alcohols, which usually derive from wax esters, appeared regularly. These are, however, no typical compounds in oysters. The relative amounts of the wax esters/sterol esters were similar in both species. However, the periods of highest accumulation differed. The amounts ranged between 5.4 ± 2.5% TL and 7.9 ± 1.2% TL in *O. edulis* (minimum in spring, maximum in late summer) and 5.9 ± 3.2% TL and 9.5 ± 1.2% TL in *C. gigas* (minimum in autumn, maximum in late summer). Sterols showed highest values in spring (*O. edulis* 17.8 ± 2.6%, *C. gigas* 22.7 ± 1.5%) and lower but constant levels of around 10% during the rest of the growing season. Only in *C. gigas* a slight increase to 15.4 ± 2.1% was observed in late autumn. Phospholipids formed the main polar lipid component in both species and ranged between 40 and 60% of total lipid (Table 2). The low share of free fatty acids confirms the high quality of the samples (no autolysis).

Table 2: Seasonal and growth–induced development of dry mass (DM) (n=60), total protein (n=15), total glycogen (n=15), total lipid (n=30) and lipid class composition (n=15) of European and Pacific oysters from offshore cultivation in 2007 (n per species and sampling month). Values ± confidence interval. FFA: free fatty acids, TAG: triacylglycerols, SE/WE: sterol esters/wax esters, ST: sterols, PL: phospholipids.

	Ostrea edulis				***Crassostrea gigas***			
	Apr	Jun	Aug	Oct	Apr	Jun	Aug	Oct
Dry mass [mg]	134 ± 16	455 ± 41	619 ± 65	879 ± 97	54 ± 3	374 ± 43	390 ± 42	434 ± 44
Total protein [% DM]	35.6 ± 1.3	38.9 ± 0.7	35.7 ± 1.2	35.0 ± 0.7	33.4 ± 1.3	40.5 ± 0.7	36.8 ± 0.6	35.5 ± 0.7
Total glycogen [% DM]	6.6 ± 2.5	21.1 ± 1.7	23.2 ± 1.5	9.9 ± 2.2	4.6 ± 1.0	16.5 ± 1.7	4.2 ± 1.6	7.2 ± 2.4
Total lipid [% DM]	6.9 ± 0.5	9.4 ± 1.3	8.9 ± 1.3	7.7 ± 0.8	8.0 ± 0.8	11.3 ± 1.1	14.4 ± 1.4	8.3 ± 0.9
FFA	2.8 ± 0.6	0.8 ± 0.8	0.9 ± 0.3	0.5 ± 0.4	3.5 ± 1.5	0.2 ± 0.4	0	0.4 ± 0.8
TAG	18.5 ± 2.3	34.4 ± 2.1	36.6 ± 1.2	40.1 ± 1.6	3.3 ± 1.4	37.6 ± 3.7	42.2 ± 2.8	26.1 ± 3.7
SE/WE*	5.4 ± 0.9	6.2 ± 0.8	7.9 ± 0.5	6.7 ± 0.6	8.7 ± 1.5	7.7 ± 0.6	9.5 ± 0.5	5.9 ± 2.4
ST	17.8 ± 1.0	10.8 ± 0.9	10.6 ± 0.5	10.1 ± 0.5	22.7 ± 0.8	9.9 ± 1.2	8.7 ± 1.1	15.4 ± 1.6
PL	55.5 ± 2.4	47.8 ± 2.0	44.0 ± 1.4	42.6 ± 1.4	61.8 ± 2.6	44.6 ± 3.0	39.6 ± 2.1	52.2 ± 4.2
PL/NL	3.0	1.4	1.2	1.1	18.7	1.2	0.9	2.0

*not separated by applied method

Fatty acid composition

Seasonal and growth induced changes in the fatty acid compositions are shown in Table 3. The typical membrane components 16:0, 20:5(n-3) and 22:6(n-3) predominated the fatty acid compositions of both species (>12% each). Fatty alcohols were present as 14:0- and 16:0-alcohols. In the neutral lipid fraction 15 important fatty acids (maximum values ≥ 2% of total fatty acids (TFA)) were identified. Two C22 dienoic acids appeared: 22:2i and 22:2j, which were identified by mass spectrometry as 22:2 Δ7,13 and 22:2 Δ7,15, respectively.

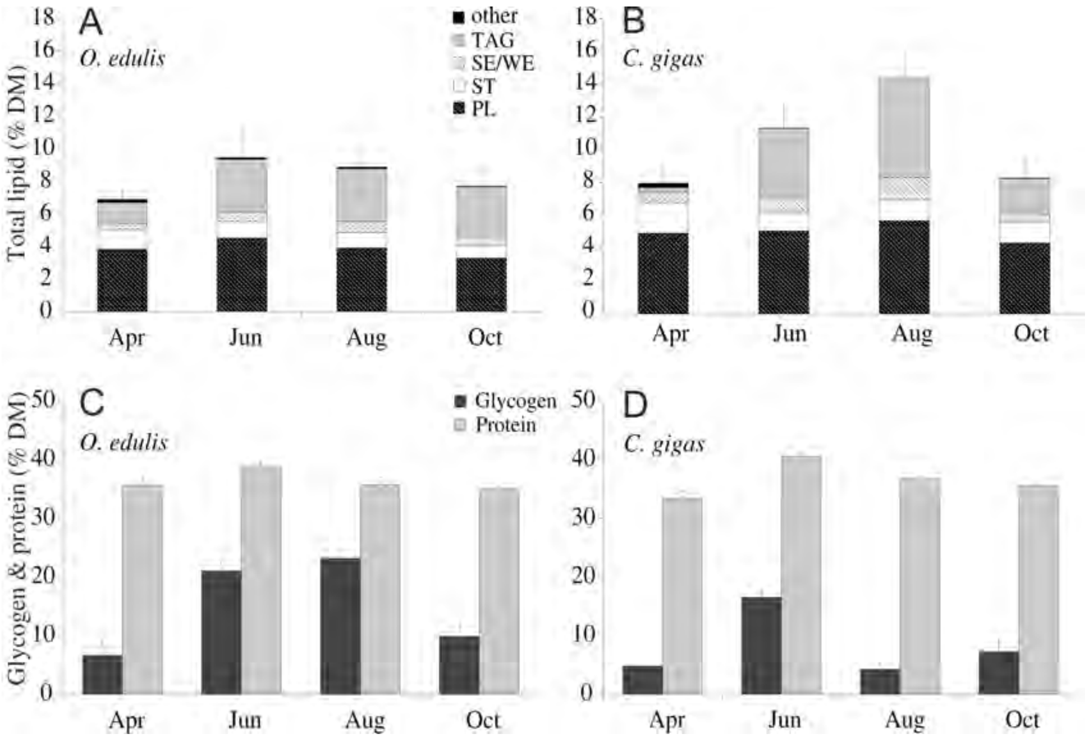

Figure 3: Biochemical composition: Total lipids [% DM] and lipid class composition [% TL] of (A) *O. edulis* and (B) *C. gigas* (n = 30 per species and sampling date). Total glycogen and protein content of (C) *O. edulis* and (D) *C. gigas* (n = 15 per species and sampling date). Columns display means ± confidence interval. TL: total lipid, TAG: triacylglycerols, SE/WE: sterol esters/wax esters, ST: sterols, PL: phospholipids.

Table 3: Seasonal and growth-induced development of fatty acid compositions of European and Pacific oysters from offshore cultivation in 2007 (n = 15 per species and sampling month). TFA: total fatty acids, TFAlc: total fatty alcohols.

Fatty acids [%TFA ± SD]		*Ostrea edulis*				*Crassostrea gigas*			
		Apr	Jun	Aug	Oct	Apr	Jun	Aug	Oct
14:0		2.0 ± 0.4	4.1 ± 0.6	3.7 ± 0.3	3.7 ± 0.2	1.3 ± 0.4	5.0 ± 1.1	6.2 ± 2.7	2.1 ± 0.8
16:0		18.9 ± 2.2	22.5 ± 3.1	21.7 ± 1.4	21.0 ± 0.5	19.1 ± 1.2	20.5 ± 2.0	24.6 ± 3.9	23.5 ± 1.7
16:1(n-7)		1.5 ± 0.2	2.6 ± 0.4	2.4 ± 0.3	2.4 ± 0.2	1.9 ± 0.5	3.3 ± 1.4	5.2 ± 1.2	1.6 ± 0.6
17:0		2.3 ± 0.3	1.8 ± 0.4	1.6 ± 0.2	1.4 ± 0.1	1.9 ± 0.3	1.0 ± 0.1	1.3 ± 0.3	1.9 ± 0.5
17:1		2.5± 2.0	1.0 ± 0.9	1.3 ± 1.4	1.2 ± 1.6	4.1 ± 1.2	0.5 ± 0.8	0.5 ± 0.6	3.0 ± 2.8
18:0		5.6 ± 0.6	5.4 ± 1.1	3.9 ± 2.1	4.8 ± 0.3	0	3.8 ± 0.4	2.2 ± 1.9	1.9 ± 2.5
18:1(n-7)		1.7 ± 0.3	2.9 ± 0.6	2.1 ± 0.3	2.2 ± 0.3	3.9 ± 0.4	5.4 ± 0.5	4.7 ± 0.5	4.4 ± 0.5
18:1(n-9)		3.0 ± 0.2	2.0 ± 0.4	2.3 ± 0.2	2.3 ± 0.2	2.1 ± 0.3	2.0 ± 0.2	1.9 ± 0.4	1.1 ± 0.3
18:4(n-3)		1.6 ± 0.3	1.7 ± 1.1	1.5 ± 0.8	1.9 ± 0.1	0	2.3 ± 0.3	1.3 ± 1.0	0.7 ± 1.0
20:1(n-11)		1.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	2.9 ± 0.3	0.8 ± 0.3	0.7 ± 0.4	1.8 ± 0.5
20:1(n-9)		1.4 ± 0.2	1.4 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	2.1 ± 0.4	1.4 ± 0.2	1.3 ± 0.2	1.9 ± 0.1
20:1(n-7)		3.9 ± 0.2	3.8 ± 1.7	3.4 ± 1.5	3.9 ± 0.3	2.1 ± 0.4	3.6 ± 0.6	1.9 ± 1.3	1.9 ± 1.6
20:4(n-6)		3.9 ± 0.5	2.4 ± 0.3	2.2 ± 0.3	2.0 ± 0.2	4.3 ± 0.6	2.3 ± 0.3	2.0 ± 0.8	3.6 ± 0.9
20:5(n-3)		12.5 ± 0.9	18.0 ± 2.6	19.5 ± 1.1	19.9 ± 0.8	19.5 ± 1.6	22.5 ± 1.2	19.1 ± 1.8	16.4 ± 1.8
22:2(n-x)i	**22:2 Δ7,13**	1.3 ± 0.3	0.5 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	2.1 ± 0.4	0.6 ± 0.2	0.5 ± 0.1	0.9 ± 0.3
22:2(n-x)j	**22:2 Δ7,15**	4.9 ± 0.9	4.0 ± 0.6	3.9 ± 0.5	3.4 ± 0.3	5.6 ± 0.5	3.3 ± 0.4	3.8 ± 0.6	5.8 ± 1.4
22:5(n-3)		1.6 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	2.1 ± 0.2	1.3 ± 0.1	1.3 ± 0.3	1.5 ± 0.3
22:6(n-3)		23.7 ± 1.3	18.8 ± 2.0	21.7 ± 1.7	21.9 ± 1.2	19.3 ± 1.2	15.9 ± 2.2	15.6 ± 5.6	21.3 ± 2.2
Fatty alcohols (%TFAlc)									
14:0 A		18.1 ± 17.6	17.1 ± 15.5	6.7 ± 9.9	5.5 ± 12.6	23.4 ± 11.9	0	18.1 ± 15.0	13. 9 ± 11.4
16:0 A		81.9 ± 17.6	82.9 ± 15.8	83.3 ± 29.4	94.5 ± 12.6	76.6 ± 12.6	100.0 ± 49	81.9 ± 15.9	86.1 ± 12.0

Total glycogen

Glycogen levels of the European oyster showed a different pattern than those of the Pacific oyster (Fig. 3C&D). In *O. edulis* total glycogen (TG) increased from 6.6 ± 2.3% DM in April to 21.1 ± 1.5% DM in June to 23.2 ± 1.3% DM in August, followed by a decrease to 9.9 ± 2.3% DM in October. In *C. gigas* TG increases from 4.8 ± 1.0% in April to 16.5 ± 1.5% DM in June. In August glycogen levels dropped below the spring value (4.2 ± 1.4% DM) but increased again in October (7.2 ± 2.2% DM).

Total protein

Protein contents of the European oyster were, after April, slightly lower than those of the Pacific oyster (Fig. 3C&D). In *O. edulis* total protein showed a small increase from April (35.6 ± 1.3% DM) to June (38.9 ± 0.7% DM) and a subsequent slight decrease in August and October (35.7 ± 1.2% and 35.0 ± 0.7% DM). Pacific oysters showed an increase of total protein from April (33.4 ± 1.3% DM) to June (40.5 ± 0.7% DM) and similar to *O. edulis* a slight decrease in August and October (36.8 ± 0.6% and 35.5 ± 0.7% DM).

4 Discussion

The biochemical and elemental compositions of *Ostrea edulis* and *Crassostrea gigas* during one growing season were analyzed to investigate the nutritional condition of these species under offshore aquaculture conditions. Accumulation and depletion of metabolic energy reserves primarily depend on food quantity and quality, environmental effects on metabolic processes, and reproductive activities (Beninger & Lucas 1984, Whyte et al. 1990, Ruíz et al. 1992). A previous study already described successful growth performances for offshore-cultivated European and Pacific oysters, as individuals of both species significantly increased in shell length and dry mass (Pogoda et al. 2011). The present study focused on seasonal dynamics of the major energy storage products, namely carbohydrates, proteins and lipids, on the compositions of lipid classes and fatty acids as well as on carbon and nitrogen.

Lipids are the most efficient energy sources. They provide much more metabolic energy than the same amount of carbohydrates (glycogen) or proteins. Besides lipids, glycogen is used as energy store, which due to hydrolysis results in a ten times higher mass to reach the same energy yield (Schmidt-Nielsen 1999). Nevertheless, for some groups of benthic animals, including oysters, glycogen is a preferred form of energy reserve, as it has two major advantages: 1) glycogen catabolism is fast providing instant energy; 2) glycogen catabolism can be performed under hypoxic or anoxic conditions. This is particularly important, when oysters keep their shells closed for longer periods (Hummel et al. 1989, Whyte et al. 1990).

It is still controversially discussed, which type of energy deposition is preferred by European and Pacific oysters. Starvation experiments showed that juvenile *O. edulis* and *C. gigas* follow different strategies of energy storage and utilization (Child & Laing 1998). The European oyster preferably relied on lipids, while the Pacific oyster was assumed to utilize proteins based on insignificant reductions in lipid and carbohydrate contents. Other authors identified lipids (Robinson 1992, Ruiz et al. 1992) or glycogen (e.g. Holland & Hannant 1974, Whyte et al. 1990) as main energy reserve in juvenile and adult European and Pacific oysters: e.g. in *O. edulis* glycogen peaked in summer and decreased again in winter. Our study on offshore-cultivated *O. edulis* and *C. gigas* revealed similar seasonal glycogen dynamics with a substantial increase from spring to summer and a decrease in autumn. Variations in the lipid and protein contents were much lower. Accordingly, these data support the hypothesis that both species utilize primarily glycogen as an important energy store during the seasonal cycle.

However, more detailed data analyses revealed distinct differences in lipid and glycogen deposition between both species. *C. gigas* exhibited a significantly stronger lipid accumulation until autumn ($p < 0.001$) as compared to *O. edulis*, while glycogen dropped drastically after accumulation during spring and early summer. Overall, the Pacific oyster showed a more pronounced energy deposition, but in autumn lipid and glycogen reserves were depleted to similar levels as those of the European oyster. Energy utilization in *C. gigas* coincided with reduced shell growth and a decrease in dry mass (Pogoda et al. 2011). This may be explained by reproductive effort, as during summer *C. gigas* >40 mm starts to invest energy in gonad maturation (Costil et al. 2005, Royer et al. 2008). The simultaneous glycogen decrease and lipid increase may indicate the conversion of carbohydrates into lipids during ontogenesis (Whyte et al. 1990, Robinson 1992, De la Parra et al. 2005), as lipid contents of oyster eggs are directly related to survival rates of the lecithotrophic embryonic stages and veliger larvae (Gallager & Mann 1986). In contrast, *O. edulis* does not reproduce in its first year after settlement (Walne 1974, Wilson & Simons 1985, Newkirk et al. 1995). Accordingly data on growth performance (Pogoda et al. 2011) and biochemical composition did not indicate any reproductive activities. *O. edulis* of the same size and age as *C. gigas* continued to accumulate glycogen until late summer and lipid levels remained relatively constant.

Thus, for both species glycogen seems to be an important energy store during periods of high food availability, as it was mainly accumulated during the phytoplankton bloom in spring. However, glycogen accumulation continued in *O. edulis*, while in *C. gigas* carbohydrates were eventually converted to lipids and fuelled reproductive processes.

The elemental composition (C, N) of an organism basically reflects growth and nutritional status (Postel et al. 2000). It gives some hints on physiological condition and indicates shifts in the occurrence of the main organic components, expressed as the ratio between proteins and lipids+carbohydrates in animal tissue (Bayne 2009). The dominance of lipids and carbohydrates is indicated by a C:N ratio beyond 2.9 (Postel et al. 2000). Increasing C/N values from 4.0 to 5.2 confirm the observed accumulation of carbohydrates in *O. edulis*. Interestingly, C/N values of *C. gigas* stay quite constant around 4 even in August, when glycogen contents dropped drastically. This supports the above-mentioned accumulation of lipids and the suggested conversion of carbohydrates to lipids during gametogenesis. In both species a clear decrease of the C:N ratios was observed after August, when lower values of 3.2 (*O. edulis*) and 2.5 (*C. gigas*) indicate the build-up of muscular mass (proteins). These low C/N values reflect decreasing lipid and glycogen levels in the bivalves, due to the utilization of these reserves in autumn, when food supply is limited.

Seasonal variations in lipid class compositions of offshore-cultivated oysters are basically similar to those of nearshore-grown individuals (e.g. Abad et al. 1995, Linehan et al. 1999, De la Parra et al. 2005). Within the lipid classes, triacylglycerols (TAG) and wax esters (WE) are the principal energy reserves of most marine invertebrates (Lee et al. 2006). Energy reserves of *O. edulis* and *C. gigas* are primarily made up of the neutral lipid TAG, as WE are not common in oysters (Ackman 1989). Since gas chromatographic analyses combined with mass spectometry revealed two fatty alcohols we assume that these wax ester moieties originate from small zooplankton specimens ingested by the oysters. WE and sterol esters (SE) are not separated by the applied analytical method (Iatroscan). SE have no energy storage functions but provide important components of membrane lipids (Fraser et al. 1985). We assume that the WE/SE fraction is SE-dominated (e.g. Ackman 1989, Abad et al. 1995) hence, this fraction will not be of major importance for energy storage processes in the oysters. Still, a certain increase of WE/SE in *O. edulis* and *C. gigas* during summer and a subsequent decrease in autumn may point to some seasonal wax ester storage.

TAG serve as short-term energy reserves and they are the main lipid stores in oysters. Together with glycogen, they accumulate during periods of high food availability and are depleted in periods of food paucity. Accordingly, the amount of TAGs is a sensitive indicator of the nutritional condition of an animal (Fraser et al. 1985). It can be expressed as the ratio of phospholipids to triacylglycerols (PL:TAG) or as the ratio of phospholipids to neutral lipids (PL:NL) (Watanabe & Ackman 1974, Abad et al. 1995, Caers et al. 2000). Values ≤1 indicate a good nutritional state and have been observed e.g. in well-fed adults of *O. edulis* and *C. virginica* from the wild (Watanabe & Ackman 1974, Abad et al. 1995). According to this scale, the European and Pacific oysters of the present study showed poor nutritional conditions at the beginning of the experiment in spring (PL:TAG ratios of 3 (*O. edulis*) and 18 (*C. gigas*) respectively). During summer *O. edulis* fed continuously (Pogoda et al. 2011) and PL and TAG levels reached equal proportions of 1 in summer and in autumn, which indicates a good nutritive state. *C. gigas* also clearly improved its condition during summer (PL:TAG ratios of about 1). In contrast to *O. edulis* the condition of *C. gigas* significantly deteriorated in autumn reaching a PL:TAG ratio of about 2. These differences in the seasonal TAG accumulation may be explained by the onset of reproductive activities in *C. gigas*, which was not the case in *O. edulis* (e.g. Newkirk et al. 1995, Royer et al. 2008).

The fatty acid compositions of the European and the Pacific oysters were dominated by 16:0, 20:5(n-3) and 22:6(n-3). They are major components of phospholipids and typical of marine organisms (e.g. Lee et al. 2006). The polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) are important and conservative elements of bio-membranes. Their concentrations remain rather constant and, thus, they are not suitable as direct indicators for the nutritional condition. However, the proportion of phospholipid (n-6) fatty acids, principally 20:4(n-6), can indirectly show the depletion or accumulation of lipid reserves through an increase or decrease, respectively (Child & Laing 1998). Both species of offshore-cultivated oysters showed a significant decrease ($p < 0.001$) in 20:4(n-6) during high food availability from spring to early summer and therefore indicate the accumulation of lipids as energy reserves.

The amount of essential fatty acids can greatly affect growth and condition of oysters (Pazos et al. 1996) and may serve as an indicator for the preferred diet (Soudant et al. 1999, Dalsgaard et al. 2003). Diatoms, for example, are characterized by high concentrations of 16:1(n-7), 18:1(n-7) and 20:5(n-3) fatty acids (Sargent et al. 1987). Dinoflagellates show high amounts of 18:4(n-3), 22:6(n-3) (Sargent et al. 1987, Virtue et al. 1993) and 18:5(n-3) (Mayzaud et al. 1976). In the present study, levels of 22:6(n-3) stayed quite constant in *O. edulis*, but showed a slight decrease in *C. gigas*. Furthermore, 18:4(n-3) and 18:5(n-3) were not present or showed very low concentrations. Accordingly, dinoflagellates were not an important food organism. However, diatom markers increased during spring and early summer in both oyster species and indicate a diet rich in diatoms. During the cultivation experiment, *O. edulis* and *C. gigas* maintained high ratios of (n-3)/(n-6). These increased from 10 to >18 in late summer, which furthermore indicates the excellent physiological condition of both oyster species at the offshore site (Sargent et al. 1990, Pazos et al. 1996, Soudant et al. 1999).

Dienoic fatty acids, also known as non-methylene-interrupted fatty acids (NMI FA), in which more than two methylene residues separate the double bonds, are found in a wide range of marine invertebrates, but especially in molluscs. The most common of these fatty acids are 22:2 Δ7,13 and 22:2 Δ7,15 and their precursors 22:2 Δ5,11 and 22:2 Δ5,13. They are synthesized *de novo* in bivalves by chain elongation, followed by Δ5 desaturation of 18:1(n-9) and 16:1(n-7), respectively (Barnathan 2009). Offshore-cultivated oysters of this study showed two newly synthesized NMI FA (22:2 Δ7,13 and 22:2 Δ7,15) in similar concentrations as nearshore-grown oysters (Abad et al. 1995, Pazos et al. 1996, Soudant et al. 1999). Apparently, the nutritional situation at the offshore site and especially levels of 16:1(n-7) were high enough to allow biosynthesis of these fatty acids (Zhukova 1991, Thompson & Harrison 1992, Soudant et al. 1999).

Summary and conclusions

(1) Biochemical compositions including lipid classes and fatty acid profiles of the studied oysters reflect a similar composition as those of nearshore-grown or wild oysters.
(2) Offshore-cultivated oysters generally exhibited excellent physiological conditions during the growing season, as indicated by their proximate biochemical compositions (protein, glycogen and lipid contents) as well as triacylglycerol and fatty acid profiles.
(3) Both species accumulated glycogen in periods of high food availability.
(4) We postulate that *C. gigas* started to invest energy in maturation in summer, which resulted in low glycogen values and TAG accumulation until autumn. In contrast, *O. edulis* of the same age and size showed no reproductive activities and continued accumulation of glycogen and TAG until autumn.
(5) *O. edulis* and *C. gigas* are suitable candidates for offshore aquaculture operations.

Outlook

Modern concepts for offshore aquaculture rely on sustainable and environmentally friendly cultivation techniques combined with economic viability. Integrated multi-trophic aquaculture (IMTA) reduces eutrophication problems (Troell et al. 2009) and strives for a balanced budget of nutrient input and uptake. Filter-feeding oysters are therefore an ideal bio-extractive component for the realization of IMTA in offshore areas. The rapid development of wind farming projects in many offshore areas of the world (Breton & Moe 2009, OWE 2012) offers the opportunity of parallel utilization for offshore aquaculture operations and financial benefits of such a "multi-use". Today, sharing of e.g. windmill groundings to attach culture cages, as well as combined maintenance vessels are being discussed and established (Buck et al. 2008). Correspondingly, this study clearly qualified both, the European oyster and the Pacific oyster as suitable candidates for such offshore aquaculture operations.

Acknowledgements

We thank the Water and Shipping Authority Bremerhaven, namely Mr Boening and the captain and crew of *MV Bruno Illing* and *MV Mellum* for their support in maintaining the test site *Nordergründe*. Thanks are expressed to the captain and crew of the AWI research vessel *Uthörn* for their assistance. We also thank all divers involved in this project and the AWI diving group for the technical support. Thanks go to Dr. Martin Graeve (AWI) who confirmed fatty acid identifications by mass spectrometry. All research carried out for this manuscript was funded by the University of Bremen and the Alfred Wegener Institute for Polar and Marine Research and is further a partial fulfillment for a doctoral degree of the leading author.

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CHAPTER III

INFESTATIONS OF OYSTERS AND MUSSELS BY MYTILICOLID COPEPODS: DIFFERENCES BETWEEN WILD HABITATS AND TWO OFFSHORE CULTIVATION SITES IN THE GERMAN BIGHT

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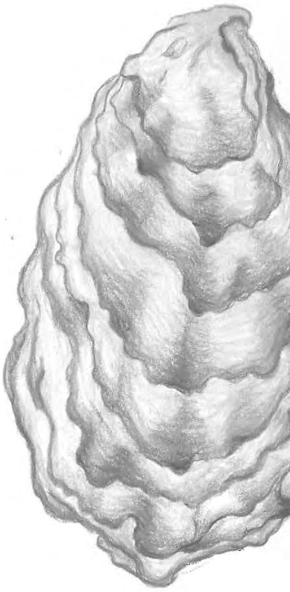
submitted to *Journal of Applied Ichthyology*



Infestations of oysters and mussels by mytilicolid copepods: differences between wild habitats and two offshore cultivation sites in the German Bight

Abstract

Aquaculture related introductions of parasitic copepods may affect condition and commercial value of oysters and mussels. Low infestion rates or even a complete absence of parasites would be a major advantage for shellfish aquaculture and would therefore play an important role as site-selection criterion. The aim of this study was to investigate the macroparasitic infestation of oysters from the southern German Bight focussing on copepods of the genus *Mytilicola*. *Crassostrea gigas*, *Ostrea edulis* and *Mytilus edulis* were collected at 5 locations: 3 nearshore sites in the eastern Wadden Sea and 2 offshore cultivation sites in the German Bight. To reveal seasonal variations one sampling site was investigated in winter and summer. At the nearshore sites, we regularly detected *Mytilicola orientalis* in *C. gigas*. Prevalence ranges between 32.3% and 45.1%, intensity between 3.0 ± 0.6 and 8.2 ± 1.5. Infestation rates of *C. gigas* within the southern German Bight decrease from west to east: Apparently, *M. orientalis* has started its migration along the German coast with retardation but generally follows the invasion route of its main host, the Pacific oyster. Interestingly, we detected not only *M. intestinalis* but also *M. orientalis* as an intestinal parasite in *M. edulis*, which has not been described for this region before. We conclude that *M. orientalis* is flexible in its host infestation. Furthermore, in the eastern Wadden Sea infestation rates of oysters and mussels by copepods are similar. These results deviate from the pattern observed for the northern Wadden Sea in terms of infestation level and host specificity. No macroparasites were found in oysters and mussels from the offshore sites. This absence provides a high-potential advantage for aquaculture activities in the open ocean.

1 Introduction

The Pacific oyster *Crassostrea gigas* was introduced to Dutch tidal backwaters in the 1970s (Andrews 1980, Chew 1990, Ruesink et al. 2005) to substitute depleted stocks of the European oyster *Ostrea edulis* and to reanimate declining oyster fisheries. The further invasion by *C. gigas* within the North Sea region developed from west to northeast along the German coast as a result of the prevailing current regime and the transport of pelagic oyster larvae (Wehrmann et al. 2000). In contrast, the European oyster is considered to be extinct in German waters since it was extremely diminished by overexploitation, diseases and severe winters (Wehrmann et al. 2000). It has not been found along the German coastline for half a century. Populations of *O. edulis* still exist in the Danish Limfjord, in Norway and around Ireland but they are under threat or declining in all these regions (OSPAR 2009). Some former Wadden Sea habitats of the European oyster and even more excessively wild mussel banks of *Mytilus edulis* are now more or less dominated by the invading Pacific oyster (Diederich 2006, Schmidt et al. 2008).

Since the beginning of the 1980s *Crassostrea gigas* spat was imported to Germany (Meixner 1973, Neudecker 1984) and environmental requirements of the species were investigated along the German coast. Various biotic and abiotic parameters affected fitness and health such as hypoxia, anoxia, symptoms of eutrophication, as well as outbreaks of oyster parasites (Jackson et al. 2001). Wild and farmed oysters are subject to several diseases caused by macro- and microparasites such as copepods, trematodes, nematodes, polychaetes, and protozoans, respectively (e. g. Katkansky & Warner 1968, Montes 1990, Motes & De Paola 1996, Aguirre-Macedo & Kennedy 1999, Carnegie et al. 2000). Among others, two of these parasites, the intestinal mytilicolid copepods *Mytilicola orientalis* and *M. intestinalis* are introduced species in the North Sea: *M. orientalis* was introduced to the southern North Sea in the 1990s together with its main host, the Pacific oyster, whereas its congener *M. intestinalis* was initially introduced to the North Sea in the 1930s and is a long-established common parasite of the indigenous Blue mussel *Mytilus edulis* in this region (e.g. Dethlefsen 1975, Lauckner 1983, Davey 1989, Buck et al. 2005, Thieltges et al. 2008). The concrete effects of *Mytilicola* *orientalis* and *M. intestinalis* on their specific hosts are still under discussion. Low condition indices, low ability of recovery after spawning and mass mortalities in the Netherlands and Germany have been related to infested oysters and mussels (Katkansky et al. 1967, Korringa 1968, Paul 1983). Other investigations reported no effects by mytilicolid infestations (Chew et al. 1965) or only in times of severe environmental conditions (Campbell 1970, Gee et al. 1977).

In the German Wadden Sea (northern and eastern part) high infestations of native mussels *M. edulis* by parasitic copepods of the species *M. intestinalis* have been observed (e.g. Buck et al. 2005, Krakau et al. 2006, Elsner et al. 2010). Interestingly, investigations on the parasite burden at offshore locations in the North Sea could not detect any macroparasites in mussels (Buck et al. 2005, Buck 2007, Brenner 2010). In case of oysters, Pacific oysters in the northern Wadden Sea were not infested by *M. intestinalis* and the infestation of oysters by *M. orientalis* was remote: *M. orientalis* was only found with low prevalence and intensity at one single site close to the Island of Sylt (Elsner et al. 2010). In the eastern part of the Wadden Sea, infestation of oysters with mytilicolid copepods has not been examined so far.

From an economic point of view, today, mussels (*M. edulis* and *M. galloprovincialis*) as well as the Pacific oyster (*C. gigas*) are the most important species in European shellfish production. There is also a respectable market for the European oyster *O. edulis* (FAO 2009). As oysters represent high-value seafood products an aesthetic appearance of shell – especially on the half-shell market – and meat is rather important (FAO 2011). Parasite infestations could reduce harvests and severely deplete local populations. Some macroparasites could also evoke a deteriorated morphological appearance. Understanding the development of infestation patterns is therefore crucial for the successful site-selection in oyster cultivation. In addition to ecological and economic advantages of offshore oyster cultivation (Pogoda et al. 2011) such cultures would further benefit from an absence of parasites, as observed for Blue mussels (Buck et al. 2005, Buck 2007, Brenner 2010).

The aim of this study was to describe presence, infestation levels and host specificity of macroparasites, particularly of two parasitic copepod species (*Mytilicola orientalis* and *M. intestinalis*) in oysters from the eastern Wadden Sea and from two offshore cultivation sites. To demonstrate the general existence of parasitic copepods and other macroparasites in shellfish at the investigated sites the native mussel *M. edulis* was also sampled. As these mussels, originating from sub- and intertidal habitats, are commonly infested by macroparasites, *M. edulis* acts as a useful reference organism. Furthermore, one nearshore site was investigated in winter as well as in summer to reveal potential seasonal variations of the parasite burden in oysters and mussels.

Therefore, we investigated parasite infestations of (1) *C. gigas* and *M. edulis* at three nearshore wild banks located in the eastern Wadden Sea and of (2) *C. gigas*, *O. edulis* and *M. edulis* at two offshore cultivation sites in the German Bight.

2 Material and methods

Sampling sites and investigated bivalves

Examinations on macroparasite infestations were carried out with oysters and mussels from four different sites (Fig. 1): three intertidal nearshore sites (former mussel banks which transformed to wild oyster reefs) and two offshore sites (cultivation experiments). Site 1 *Juister Watt* (JW) is located in the western part of the German East Frisian Wadden Sea near the Island of Juist (53° 38.5´N, 006° 56.5´E). Site 2 *Dornumer Nacken* (DN) is located about 8 nautical miles (nmi) east of site 1, south of the western tip of the Island of Langeoog (53° 41.9´N, 007° 28.1´E), and site 3 *Kaiserbalje* (KB) another 10 nmi further to the east, southeast of Mellum island (53° 38.7´N, 008° 16.0´E). Oysters and mussels collected at these sites originate from bottom habitats, which fall dry several hours per day. The offshore test site *Nordergründe* (NG) (site 4) was located in the outer Weser estuary (53° 51.0´N, 008° 04.0´E), 9 nmi off the coast (offshore classification by Ryan 2005) as part of an official testing area, which was established for the multi-use research of offshore aquaculture within offshore wind farms (Buck 2007, Pogoda et al. 2011). The planned offshore wind farm "Nordergründe" (Energiekontor 2011) will be realized about 1 nmi off the test site. The offshore test site *Butendiek* (BD) (site 5) was located 15 nmi west of the North Frisian island of Sylt (54° 59.1´N, 007° 54.4´E), within the area of the planned wind farm "Butendiek" (Buck et al. 2008). For the offshore cultivation experiments juvenile oysters were obtained from commercial hatcheries (Pogoda et al. 2011).

Figure 1: Map of the German Bight and Wadden Sea with sampling sites: 1 Juister Watt (JW), 2 Dornumer Nacken (DN), 3 Kaiserbalje (KB), 4 Nordergründe (NG), 5 Butendiek (BD).

In total, 296 oysters (*Crassostrea gigas*) of 50-230 mm size were collected from the nearshore sites (Table 1): n = 50 (per sampling site and date) for size class <100 mm, accordant to the size of sampled offshore-cultivated oysters. As bigger oysters were found at nearshore sites, those were also collected but in lower numbers: n≥18 (per sampling site and date, Table 1). *Mytilus edulis* acted only as a reference organism (Buck et al. 2005, Thieltges et al. 2006, Jungblut 2011) and was also collected in lower numbers: n=15 (per sampling site and date). In total, 60 mussels of 40-50 mm size were collected from the nearshore sites (Table 1). Due to the absence of the European oyster along the German coast *O. edulis* samples were only evaluated from the offshore cultivation experiments (Table 1).

Table 1: Information on environmental conditions at sampling sites and on sampling dates. JW: Juister Watt, DN: Dornumer Nacken, KB: Kaiserbalje, NG: Nordergründe, BD: Butendiek.

	Site	Site classification	Salinity range*	Temperature range [°C]*	Sampled individuals [n] *C. gigas*	*O. edulis*	*M. edulis*	Sampling time
1	JW	nearshore	24.3 – 31.4	-1.7 – 23.2	87	-	15	05/2011
2	DN	nearshore	24.6 – 31.2	-1.7 – 22.9	71	-	15	02/2011
3	KB	nearshore	7.8 – 30.8	-1.5 – 23.9	68,70	-	15,15	12/2010,06/2011
4	NG	offshore	23.1 – 33.6	3.1 – 19.6	50	50	15**	10/2007
5	BD	offshore	30.8 – 33.1	2.8 – 18.9	42	50	-	10/2004

* BSH (2011)
** Mussels analyzed by Brenner (2010)

At the offshore sites juvenile oysters (*C. gigas* and *O. edulis*) were transferred to oyster lanterns for cultivation in April 2004 and 2007. At site 4 (NG) oyster lanterns were fixed to rigid steel rings welded to large offshore marker buoys, specifically constructed for offshore aquaculture research on shellfish candidates: *C. gigas*, *O. edulis*, *M. edulis* (Brenner et al. 2007, Pogoda et al. 2011). At site 5 (BD) oyster lanterns were fixed to steel frames attached to metal piles of a former research platform of the Federal Maritime and Hydrographic Agency. Culture plots at sites 4 and 5 were permanently submerged. After a six-month growth period in offshore waters 50 oysters of *C. gigas* and *O. edulis* (per site and species) were recollected for macroparasitic investigations in October 2004 and 2007 (Table 1). Data on macroparasitic infestation of *M. edulis* from offshore site 4 (same sampling date) is found in Brenner (2010).

Analysis of parasite infestation and condition index

Length, width and height of each animal were measured with a calliper to the nearest 0.1 mm. Oysters and mussels were then opened by cutting the adductor muscle. The soft body was separated from the shell and dried on absorbent paper for 10 sec. before weighing (wet mass [WM] of meat). Digestive gland and rectum were inspected separately to detect and extract *Mytilicola* individuals as a whole. Adult individuals can be detected easily due to their size and bright red colour (Elsner et al. 2010). Most of the extracted copepods were stored in alcohol for detailed species determination. Some female individuals with developed nauplii in their egg sacs were reared in seawater to observe larval development. To ease investigations, parts of the soft body (digestive gland with rectum, muscle, gills, connective tissue) were squeezed separately between glass compressoria. Smaller *Mytilicola* individuals and further macroparasites were then identified under the stereomicroscope. Parasite infestation was documented in terms of prevalence and mean intensity. Prevalence is defined as the percentage of infested host individuals within a certain group. Mean intensity (mI) signifies the mean number of parasites living in one host, while *n* of uninfested hosts is excluded.

$$mI = mI_i/mI\sum \tag{1}$$

mI_i = sum of total parasites of species i
$mI\sum$ = sum of total hosts infected with parasites of species i

Shells of oysters and mussels were inspected for the presence of shell-boring polychaetes. Shells were then oven dried for 48 h at 65°C and weighed (dry mass [DM]) to calculate the condition index (CI) according to Davenport & Chen (1987):

$$CI = WM\ meat\ (g) * 100/DM\ shell\ (g) \tag{2}$$

Determination of mytilicolid copepods to species level was performed after Dethlefsen (1985), Gee & Davey (1986) and Elsner et al. (2010). Additional parasite species were identified following Lauckner (1980, 1983), Grizel (1985) and Watermann et al. (1998). For trematodes and shell-boring polychaetes only the prevalence was recorded (Thieltges et al. 2006).

Statistical analysis

Means, standard errors of the mean (mean ± SE) and confidence intervals (mean ± conf. interv.) of condition indices and intensities were calculated using MS-Excel software. Data were also tested for normality with MS-Excel software. Differences between sites were analyzed with two-tailed Mann-Whitney tests using GraphPad Prism 5.0 with significance levels of $p < 0.05$.

3 Results

Individuals of *Crassostrea gigas*, *Ostrea edulis* and *Mytilus edulis* were examined to assess and compare their parasite burdens at nearshore and offshore sites in the German North Sea. The study focused on the dominant macroparasites described for these species: intestinal mytilicolid copepods of the genus *Mytilicola*, namely the species *M. intestinalis* and *M. orientalis*.

At the nearshore sites (1-3), *M. orientalis* was detected in the Pacific oyster *C. gigas* and, interestingly, *M. intestinalis* as well as *M. orientalis* in the Blue mussel *M. edulis*. Furthermore, another parasitic copepod of the genus *Modiolicola*, metacercaria of unidentified trematode species as well as unidentified nematode species and the bristle worm *Polydora ciliata* (Polychaeta) were detected in the Pacific oysters from the nearshore sites. In addition, *Renicola roscovita* (Trematoda) was detected in the Blue mussel as a further macroparasite at the nearshore sites (Table 2). No macroparasites were detected in oysters from offshore cultivation (sites 4-5).

In the Blue mussel *M. edulis* both parasitic copepods *M. intestinalis* and *M. orientalis* (Fig. 2) were detected at all nearshore sites.

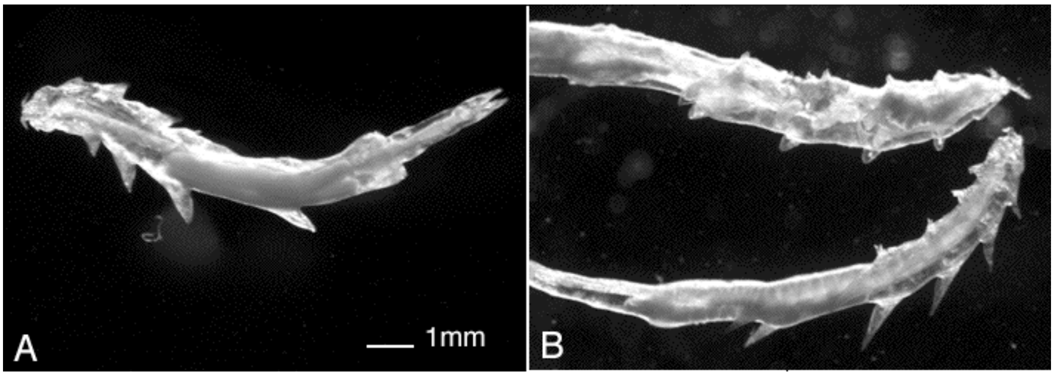


Figure 2: (A) *Mytilicola orientalis* female with sharp dorsal appendages, (B) *Mytilicola intestinalis* female with rounded dorsal appendages (top) and for comparison *M. orientalis* (below).

Prevalence was first calculated for infested mussels that carried only one mytilicolid species (Table 2, Fig. 3A): For *M. intestinalis* prevalence ranged between 13.3% (site 2), 26.7% (site 1) and 33.3% (site 3), for *M. orientalis* between 20.0% (sites 2, 3) and 26.7% (site 1).

As both parasite species were found in some individuals, prevalence was also calculated for mussels infected by *M. intestinalis* and *M. orientalis*: It ranged between 6.7% (sites 1, 3) and 13.3% (site 2). In total, 46.7% (site 2) to 60.0% (sites 1, 3) of Blue mussels were infested by mytilicolid copepods (*M. intestinalis*, *M. orientalis*, or both species). Mean intensity ranged between one and two individuals for both species at all sites (Fig. 3B). No significant differences could be observed. For a seasonal comparison of mytilicolid infestation site 3 was investigated twice: in summer *M. intestinalis* showed a lower prevalence (20.0%) while *M. orientalis* reached a higher prevalence (33.3%) as compared to the first sampling in winter (Fig. 3A). Also deviating from the winter scheme described above, no mussels were infested by both species at the same time in summer and the total prevalence was slightly lower than in winter (53.3%). Mean intensities were one copepod per mussel for both mytilicolid species during summer, also somewhat lower than in winter (Fig. 3B).

Table 2: Prevalence and mean Intensity (mI ± conf. Interv.) of macroparasitic species in oysters (*C. gigas* and *O. edulis*) and mussels (*M. edulis*) at three intertidal nearshore sites (1-3) and two suspended offshore sites (4-5).

				Parasites										
						Copepoda				**Trematoda**		**Polychaeta**	**Nematoda**	
				M. intestinalis		***M. orientalis***		***Modiolicola sp.***		***R. roscovita***	**other**	***P. ciliata***		
Site	**Classification**	**Host**		**P [%]**	**mI**	**P [%]**	**mI**	**P [%]**	**mI**	**P [%]**	**P [%]**	**P [%]**	**P [%]**	**mI**
1 JW	nearshore	*C. gigas*		0	0	37.9	8.2 ± 1.5	0	0	0	48.3	2.3	5.8	1.6 ± 0.5
		M. edulis		26.7	1.4 ± 0.3	26.7	2.0 ± 0.7	0	0	50.1	0	0	0	0
2 DN	nearshore	*C. gigas*		0	0	45.1	4.6 ± 0.8	0	0	0	0	11.3	7.0	2.4 ± 3.1
		M. edulis		13.3	1.3 ± 0.3	20.0	1.8 ± 0.7	0	0	0	0	0	0	0
3 KB	nearshore	*C. gigas*	*winter*	0	0	32.4	2.9 ± 0.6	8.8	1.2 ± 0.4	0	0	0	16.2	1.2 ± 0.6
			summer	0	0	37.1	3.0 ± 0.6	0	0	0	54.3	8.6	4.3	1.0 ± 0.0
		M. edulis	*winter*	33.3	2.0 ± 0.3	20.0	1.5 ± 0.5	0	0	0	0	0	0	0
			summer	20.0	1.0 ± 0.5	33.3	1.0 ± 0.0	0	0	73.3	0	0	0	0
4 NG	offshore	*C. gigas*		0	0	0	0	0	0	0	0	0	0	0
		O. edulis		0	0	0	0	0	0	0	0	0	0	0
5 BD	offshore	*C. gigas*		0	0	0	0	0	0	0	0	0	0	0
		O. edulis		0	0	0	0	0	0	0	0	0	0	0

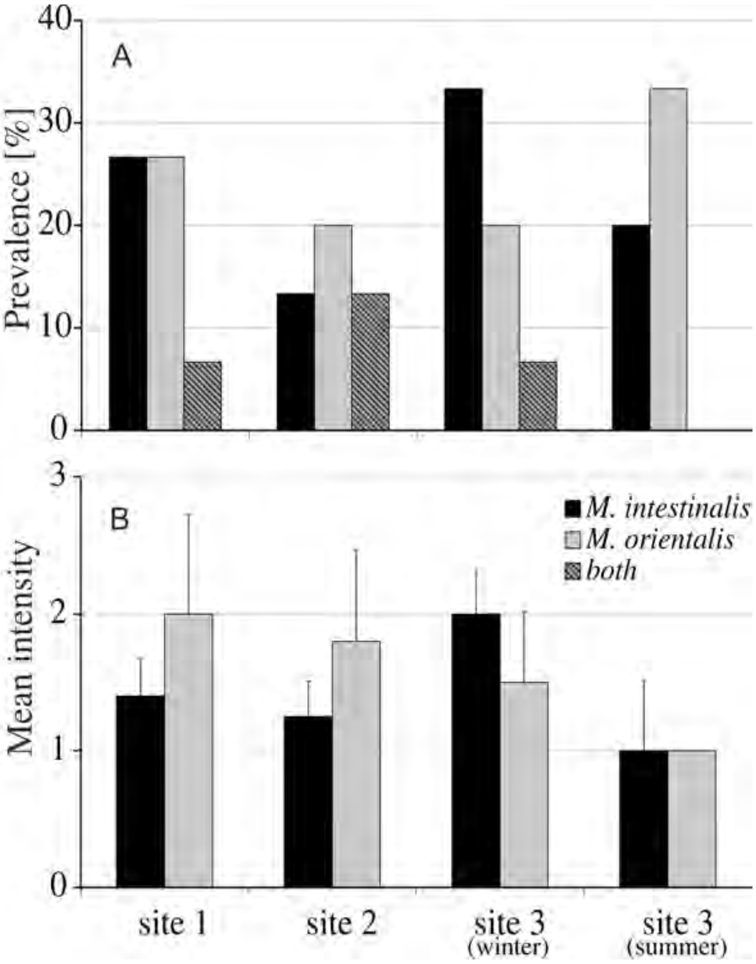


Figure 3: Prevalence (A) and intensity (B) of mytilicolid copepods in the Blue mussel *Mytilus edulis* at nearshore sites (n = 15 per site & sampling). No macroparasites were observed at offshore site NG (Brenner 2010).

The parasitic copepod *M. orientalis* occurred in *C. gigas* at all nearshore sites: Its prevalence ranged between 32.4% (site 3), 37.9% (site 1) and 45.1% (site 2) (Fig 4A). Mean intensity (mI) decreased from 8.2 ± 1.5 in the west (site 1) to 4.6 ± 0.8 in the central eastern Wadden Sea (site 2) and to 3.0 ± 0.6 in the east (site 3) (Table 2, Fig 4B). Significant differences could be found between site 1 and site 2 ($p < 0.05$) and between site 1 and site 3 ($p < 0.05$). The seasonal comparison revealed no significant differences for the parasite infestation in oysters: Summer sampling at site 3 showed a prevalence of 37.1% and the same intensity in winter (three copepods per oyster).

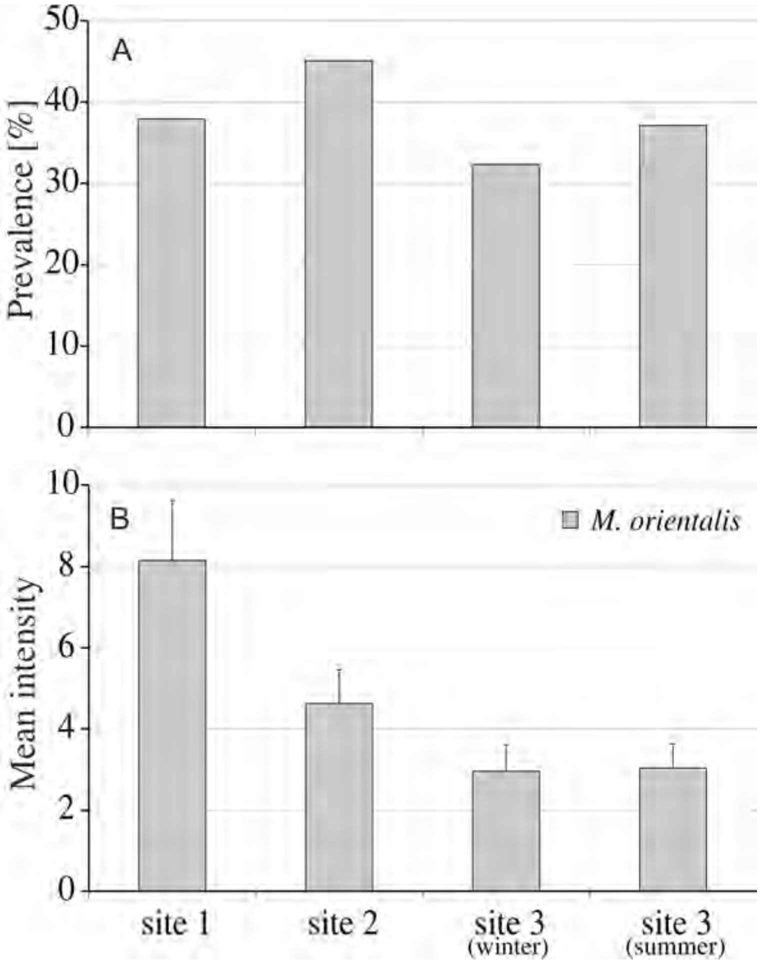


Figure 4: Prevalence (A) and intensity (B) of the mytilicolid copepod *M. intestinalis* in the Pacific oyster *C. gigas* at nearshore sites (n ≈ 70 per site & sampling). No macroparasites were observed at offshore sites (n ≈ 50).

No correlation could be observed between condition index (CI) and parasite infestation (intensity) of the examined oysters (Fig. 5A-C). Non-infested Pacific oysters showed essentially the same CI at all nearshore sites (15.5 ± 4.4 at site 1, 15.7 ± 3.4 at site 2 and 16.1 ± 3.6 at site 3). The CI of infested Pacific oysters ranged between 14.9 ± 4.2 (site 1), 15.1 ± 4.0 (site 2) and 15.8 ± 3.8 (site 3) with no significant differences ($p > 0.05$) between the nearshore sampling sites but a weak tendency of an increasing condition from west to east. In general, mean condition values of infested oysters showed no significant differences ($p > 0.05$) to the CI of not infested oysters.

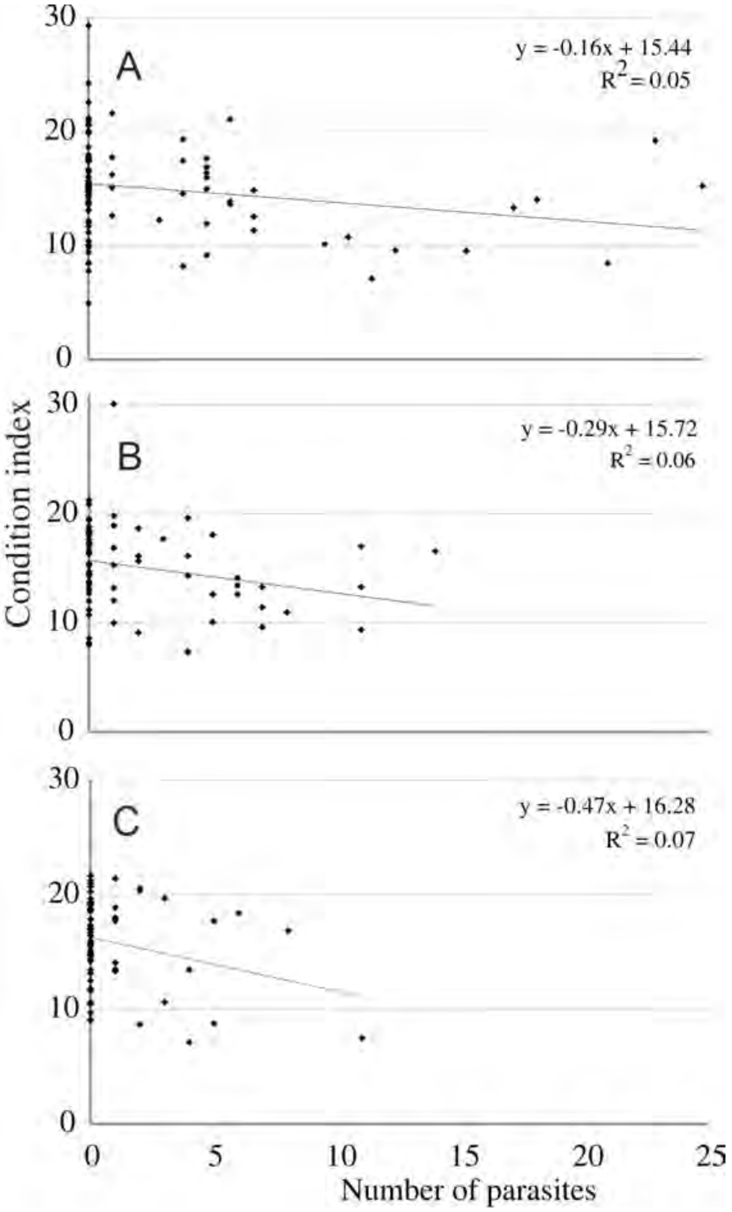


Figure 5: Non-significant relation of condition index and number of parasites in the Pacific oyster *C. gigas* at nearshore sites from west to east: (A) site 1 (JW), (B) site 2 (DN), (C) site 3 (KB, winter) (n≈70 per site & sampling). No macroparasites were observed at offshore sites.

To allow a better comparison of oyster size data regarding parasite burden we sorted the oysters into three defined size classes: S (<100 mm), M (100-150 mm) and L (>150 mm) (Table 3). Site 1 (JW) and site 2 (DN) show similar patterns with highest prevalence in size class M, followed by S and L. At site 3 (KB), highest prevalence was observed in size class L, followed by M and S.

Intensity did not show significant differences according to host size, except size class S showed significantly higher intensity at site 1 compared to site 2 ($p < 0.05$) and site 3 ($p < 0.005$). Mussels and offshore-cultivated oysters of both species were not separated into different size classes, all individuals belonged to size class S.

Table 3: Prevalence (P) and mean intensity (mI) of *M. orientalis*-infestations in Pacific oysters from nearshore sites, separated in different size classes.

Site	Size class*	n	P [%]	mI [ind^{-1}]
1 JW	S	54	38.9	9.0 ± 7.2
	M	22	50.0	6.8 ± 6.3
	L	11	9.1	4.0 ± 0.0
2 KB	S	46	43.5	4.2 ± 3.8
	M	13	76.9	5.4 ± 3.2
	L	12	16.7	5.5 ± 0.7
3 DN	S	50	28.0	3.3 ± 3.2
	M	9	33.3	2.7 ± 2.1
	L	9	55.6	2.2 ± 1.3

* Size classes: S < 100 mm, M = 100-150 mm, L > 150 mm.

Larval development of Mytilicola orientalis

Mytilicola orientalis females with completely developed nauplii in their egg sacs were found during the examination of Pacific oysters from site 2. After extracting an intact *M. orientalis* individual from the rectum of the oyster, the outer membrane surrounding the egg sacs broke and swimming nauplius larvae could be observed. A total of 70 nauplii were collected and put in a beaker with artificial seawater, which was exchanged every two days. Larvae were maintained at 15°C and larval development could be observed successfully for two weeks. After passing the first developmental stages (Fig. 6A, B) most larvae died. However, at least one individual reached the infective stage (first copepodite stage). Some individuals of each stage were conserved in alcohol for subsequent examinations.

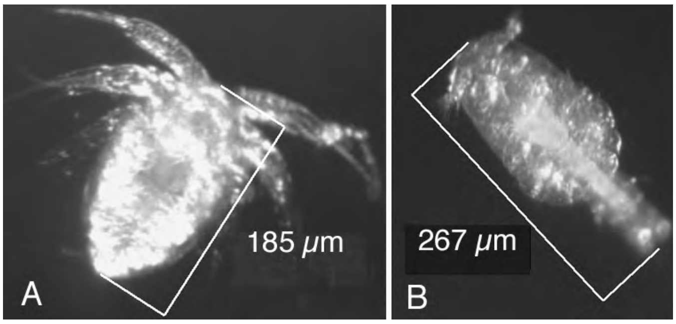


Figure 6: Early larval stages of *M. orientalis*. (A) Nauplius larvae 3h (A) and 72h (B) after hatching and leaving the egg sac.

4 Discussion

High abundances of the Pacific oyster in the Wadden Sea raised the question, if *Crassostrea gigas* could outcompete and replace the native Blue mussel *Mytilus edulis* (Diederich 2006, Schmidt et al. 2008, Markert et al. 2010). For a successful comparison of these species it is important to distinguish between direct competition (e.g. food and space) and indirect competition, for example parasite burdens, which were in the focus of this study. Knowledge on parasite burdens in relation to their geographical variations is a relevant factor, as the absence of macroparasites, for example at offshore locations, is an important (market-related) quality feature in shellfish production (Buck 2007).

The effects of the parasitic copepods *Mytilicola orientalis* and *M. intestinalis* on their specific hosts are still under discussion. Lower condition indices of infested oysters and mussels have been reported, as well as *Mytilus* extinctions in the Netherlands and Germany have been related to infestations with *M. intestinalis* (Katkansky et al. 1967, Korringa 1968). Also, a lower ability of recovery after spawning was observed for mussels (Paul 1983). In contrast, other investigations found no effects in case of an infestation of oysters by *M. orientalis* (Chew et al. 1965) or negative effects were detected only in times of severe environmental conditions (Campbell 1970, Gee et al. 1977): low food supply, severe winters and high infestation rates beyond 25 copepod individuals per host. Lower amounts of parasites could not be related to host size or gonad development, while seasonal cycles and other environmental factors caused greater effects on host condition. Long-term studies suggested that mytilicolid copepods should not be classified as harmful parasites but rather live as commensals in mussels and oysters (Gee & Davey 1986, Davey 1989, Steele & Mulcahy 2001). Our study did not reveal any correlation between infestation rate and condition index of oysters and mussels of the eastern Wadden Sea, which in turn supports the concept of commensalism. At the western site we found three oyster individuals infested by more than 20 mytilicolid copepods and condition indices of host animals ranged between 9 and 20, which is well within the observed range of uninfested oysters. Small and medium size classes showed highest prevalences of *M. orientalis* at sites 1 and 2. In contrast, at site 3 a constant increase of prevalence with increasing size classes was observed. Intensity decreases from west to east in every size class. Within a sampling site, intensities decrease with increasing size class. As recruitments of *Mytilicola* are local and irregular phenomena (Robledo et al. 1994), parasitization can differ over years. Aguirre-Macedo & Kennedy (1999) observed much higher prevalences and intensities in the same size classes in 1995 in the Exe estuary (Great Britain) than in 1996. However, patterns of intensity regarding the oysters´ age classes were similar for both years. Older oysters contained more parasites per individual than younger ones (Aguirre-Macedo & Kennedy 1999), which could not be confirmed for every size class in this study.

Interestingly, recent studies suspected furthermore that heavily infested mussels could provide a competitive advantage for invasive and less infested Pacific oysters, which could enhance their successful distribution along the North Sea coast and eventually the outcompeting of the indigenous mussel (Diederich 2005, Diederich et al. 2005, Krakau et al. 2006, Schmidt et al. 2008, Elsner et al. 2010). However, Markert et al. (2010) did not observe any suppression of *M. edulis* in the eastern Wadden Sea, even if former mussel beds had transformed to *Crassostrea* reefs. Mean abundance of *M. edulis* (individuals/m^2) was even higher within the oyster reef than in the surrounding *Mytilus* bed. The fact that mussel shell size tended to be smaller in oyster reefs was not interpreted as a disadvantage, as mussels in turn benefit from their sheltered position between robust oyster shells, protected from predation by birds (Markert et al. 2010). Additionally, our study did not reveal different infestation levels for oysters and mussels: Parasite burdens in mussels and oysters of the eastern Wadden Sea showed prevalences for *M. orientalis* in *C. gigas* between 32% and 45% at all investigated nearshore sites. Mean intensities ranged between three and eight parasites per host decreasing from west to east. Prevalences of mytilicolid copepods in *M. edulis* ranged between 45% and 60% with intensities around two parasites per host. This is in substantial contrast to studies conducted in the northern Wadden Sea (Krakau et al. 2006, Thieltges et al. 2006, 2008, Elsner et al. 2010), where the infestation with mytilicolid copepods in terms of prevalence and intensity was nine times higher in *M. edulis* than in *C. gigas*. Prevalence of *M. intestinalis* in *M. edulis* was around 90% and the intensity was approx. four parasites per host. In contrast, prevalence and intensity of *M. orientalis* in *C. gigas* were very low: 10% prevalence at one single site (0% at all other sites) and the intensity was two parasites per host (Elsner et al. 2010).

Furthermore, clear host specificity has been observed in the northern Wadden Sea: *M. orientalis* was only found in *C. gigas*, while *M. intestinalis* occurred only in *M. edulis* (Elsner et al. 2010). In contrast, in the eastern Wadden Sea both species, *M. intestinalis* and *M. orientalis*, were found in *M. edulis*. This is the first widespread record of *M. orientalis* in *M. edulis* in Europe. In other regions, e.g. the Pacific east coast and Sea of Japan, *M. orientalis* is known to parasitize on a variety of hosts: *C. gigas*, *Ostrea lurida*, *M. edulis*, *M. californianus*, *M. crassistesta*, *Crepidula fornicata* (Katkansky et al. 1967, Grizel 1985). In Europe, only the Pacific oyster had been infested so far. In experiments, *M. orientalis* had shown a clear preference for infecting *M. edulis* instead of the oyster *O. lurida* (Bradley & Siebert 1978). *M. edulis* has also been the most infected host in Canadian and US waters (Odlaug 1946, Bernard 1969). Stock (1993) was the first scientist who found very few *M. orientalis* individuals in mussels of the East Scheldt, Netherlands. Therefore, it is not surprising that *M. orientalis* has now been detected in *M. edulis* in the North Sea, too.

The question arises, whether *M. orientalis* has only recently started to infest the indigenous Blue mussel in the North Sea region? Or has it been overlooked in former studies that focused on the common parasite of the Blue mussel: *M. intestinalis* (Buck et al. 2005, Krakau et al. 2006, Thieltges et al. 2006, 2008, Brenner 2010)? To distinguish *M. orientalis* from *M. intestinalis* complete animals need to be examined under the stereomicroscope. If mytilicolid copepods are detected in compressed preparations, species determination is usually impossible. Squeezed individuals of *M. orientalis* could have been misidentified and recorded as *M. intestinalis*. In 2004 and 2005 no mytilicolid copepods have been found in Pacific oysters of the northern Wadden Sea and *M. intestinalis* was only detected in *M. edulis* (Krakau et al. 2006, Thieltges et al. 2006). However, oysters and mussels were always examined by compressing the soft body parts between glass plates, as described above. In British waters *M. intestinalis* was reported to infest *C. gigas* (Aguirre-Macedo & Kennedy 1999), but soft parts were also squeezed before examination. In our study only *M. orientalis* was found to infest *C. gigas*. This parasite was mentioned first by Elsner et al. (2010) for the North Sea region. They extracted *Mytilicola* individuals before squeezing and found *M. orientalis* in *C. gigas* but not in *M. edulis*.

On the one hand, the appearance of the Pacific oyster and its parasitic copepod in the North Sea may have initiated the recent infestation of the indigenous Blue mussel with the introduced parasite. On the other hand, introduced Pacific oysters might divert parasite burdens taking pressure off native shellfish species (Krakau et al. 2006). The occurrence of other parasite species in the investigated oysters and mussels support this hypothesis: *C. gigas* was host to at least five different species (metacercariae of trematodes were not identified to species level as well as nematodes), *M. edulis* hosted only three different species. This is also in contrast to findings in the northern Wadden Sea where *C. gigas* was host to only two parasite species, *M. edulis* to nine species. Hence, in our study *M. edulis* exhibited lower parasite richness than *C. gigas* and also lower than that of *M. edulis* from the northern Wadden Sea. But due to the fact that not all parasites could be identified to species level it can only be seen as a trend, which requires additional thorough investigations.

Aquaculture activities along the North Sea coastline form the vector of introduction of several invasive species, e.g. Pacific oysters and their subsequent biogeographic distribution. First settlements of the Pacific oyster in the western part of the German Wadden Sea have been recorded in 1996

(Wehrmann et al. 2000). The further expansion of *C. gigas* is apparently related to natural distribution processes via larval drift (Nehring 2006): pelagic stages of oyster larvae are carried northeast along the German coast as a result of prevailing current systems (Wehrmann et al. 2000). Subsequently, the invasion of *C. gigas* shows a clear northeastward direction beginning in the Netherlands, where spat of Pacific oysters was imported for aquaculture activities from the 1960´s on (Wehrmann et al. 2000, 2009, Brandt et al. 2008, Schmidt et al. 2008). *M. orientalis*, a common parasite of the Pacific oyster, was co-introduced to the southern North Sea in the 1990´s (Elsner et al. 2010). Observed infestation rates of *M. orientalis* in *C. gigas* decreased from west to east: Hence, *M. orientalis* may have started the succession along the German coast with retardation, but essentially follows the invasion pattern of its main host.

From an economic point of view the absence of macroparasites in shellfish products is certainly favourable. Oysters are commonly eaten raw and consumers would dislike the appearance of e.g. parasitic copepods, as they are easy to recognize due to their bright red colour and size (up to 25 mm). This is an issue, as it would result in a serious decrease of the oysters´ value. In contrast, mussels are cooked before consumption and copepods loose their colour and are no longer easy to detect.

Recent studies on macroparasite burdens by Buck et al. (2005) and Brenner (2010) reported a zero infestation of Blue mussels at offshore locations in the North Sea. These observations can also be confirmed for oysters by this study. No macroparasites have been detected in the European and Pacific oyster from the offshore cultivation sites. Absence of trematodes at offshore locations can be explained by their complex life cycle: they usually infest intertidal gastropods as first intermediate hosts (e.g. *Littorina littorea* and *Hydrobia ulva*) (Lauckner 1984, Jensen & Mouritsen 1992, Huxham et al. 2001, Bordalo et al. 2011). Due to the absence of these exclusively coastal organisms the parasite´s life cycle cannot be completed in offshore regions (Buck et al. 2005). Mytilicolid copepods and shell-boring polychaetes (e.g. *Polydora ciliata*) are abundant in inshore waters (Kent 1981, Davey 1989, Ambariyanto & Seed 1991, Thieltges et al. 2006). Their short planktonic larval phase restricts successful dispersion to coastal waters and larvae drifting away from the coast are bound to die in the absence of hosts (e.g. predation and starvation), which are only available at very few selected culture locations (Buck et al. 2005). An experimental study conducted in British waters also showed that young *C. gigas* (< 25 mm) were not infested by *M. intestinalis* and few infestations occurred in individuals around 45 mm (Dare 1981). Furthermore, some parasites are known to occur only in mature molluscs (Thieltges et al. 2006). This implies, that spat oysters even if received from coastal regions with known parasite infestation would not yet be infested when transferred to offshore cultivation sites for grow-out.

Conclusions and outlook

(1) This study emphasizes the commercial advantages of offshore shellfish cultures with regard to parasite burdens: no macroparasites have been found in the Pacific oyster *Crassostrea gigas* and the European oyster *Ostrea edulis* at the offshore sites.

(2) Furthermore, this study shows that the infestation of nearshore oysters and mussels with the parasitic copepod *Mytilicola orientalis* decreases geographically from west to east and therefore, the distribution of *M. orientalis* follows the invasion pattern of its main host, the Pacific oyster *C. gigas*. With regard to the formerly existing distribution gap of the invading Pacific oyster, we assume that the co-invasion of *M. orientalis* along the North Sea coast is not yet completed and abundances might still increase in the northeastern region.

(3) In the eastern Wadden Sea infection levels in oysters and mussels are similar, whereas in the northern Wadden Sea the parasite burden on mussels is much higher than on oysters. We expect that parasite setting in the northern Wadden Sea will shift to similar infection levels as observed in this study for the eastern Wadden Sea region.

(4) *M. orientalis* also infests the native Blue mussel *M. edulis* in the eastern Wadden Sea. Subsequently, the former described host specificity of *M. orientalis* for *C. gigas* is no longer valid for the North Sea.

Following ICES criteria for sustainable and environmentally friendly aquaculture shellfish transfers for aquaculture purposes on regional, national and international scales should be minimized and monitored continuously. These practices fostered the invasion of hitchhiking organisms, e.g. parasitic copepods, in the past (ICES 2010) and will increase environmental and economic problems with introduced marine species in the future (Torchin et al. 2002).

Acknowledgements

We thank Alexandra Markert (Senckenberg, Wilhelmshaven), as site selection and sampling were realized with her helpful advice and the support of the captain and crew of *RV Senckenberg*. We thank the Water and Shipping Agency in Bremerhaven and the captain and crew of *MV Mellum* for their support in maintaining the offshore test site *Nordergründe*. Thanks are expressed to the captain and crew of *RV Uthörn* for their assistance, to the AWI-diving group and all divers involved in this project. We also thank Dr. Matthias Brenner for his support in parasite determination and Steffi Bröhl for providing the photo-stereomicroscope at ZMT in Bremen. The research carried out for this study was funded by the University of Bremen and the Alfred Wegener Institute for Polar and Marine Research and is further a partial fulfillment of a Doctoral degree of the leading author.

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4. Synthesis and perspectives

The marine aquaculture sector is constantly growing, and growing rapidly. A promising opportunity for a large-scale expansion of the aquaculture industry is presented in offshore aquaculture installations, which are observed with increasing attention by researchers, industry and policy (Troell et al. 2009). At the same time, high interests and demands for sustainable and environmentally friendly production processes become more and more evident. In addition to the existing enormous pressure on our marine ecosystems, consumers have started to ask not only for healthy but also environmentally friendly products on their tables. To satisfy these demands in the field of seafood production and specifically in aquaculture innovative cultivation procedures are being developed.

The integrated culture of fed species together with extractive species will contribute to the sustainability of aquaculture (e.g. Chopin et al. 2001, Langan 2004, Troell et al. 2009, FAO 2012). Aquaculture related eutrophication can be efficiently reduced: Organic extractive species, e.g. filter-feeding oysters, as well as inorganic extractive species, such as seaweeds, feed on by-products of intensive cultures, e.g. the cultivation of finfish. Therefore, integrated multi-trophic aquaculture (IMTA) strives for a balanced budget of nutrient input and uptake. Today, experiences from nearshore IMTA are extended to offshore projects, but essential research on the performance of candidate species in offshore cultures is still limited and requires significant expansion (Langan 2004, Troell et al. 2009).

In this study the biological performance of offshore-cultivated Pacific and European oysters was investigated. Young oysters were cultivated from April to October 2004 and 2007 at offshore locations in the German Bight, North Sea. Research focused on growth performance, condition and survival rates of *Crassostrea gigas* and *Ostrea edulis* in these high-energy environments. For a higher resolution of overall condition, elemental and biochemical compositions as well as macroparasitic burden were analyzed.

4.1 Successful growth performance at offshore sites

Results of this study clearly elucidated that both oyster species can grow successfully under exposed conditions in an offshore environment (Fig. 2). *O. edulis* and *C. gigas* obtained positive growth rates in terms of shell length and dry mass outside their natural coastal habitat, which is normally located on the seabed of coastal seas. In general, growth rates were similar to those of oysters from nearshore coastal areas (e.g. Walne & Mann 1975, Robert et al. 1991, 1993, Diederich 2006, Valero 2006, Schmidt et al. 2008). This documents that offshore-cultivated oysters are able to achieve a "normal" or "natural" growth performance.

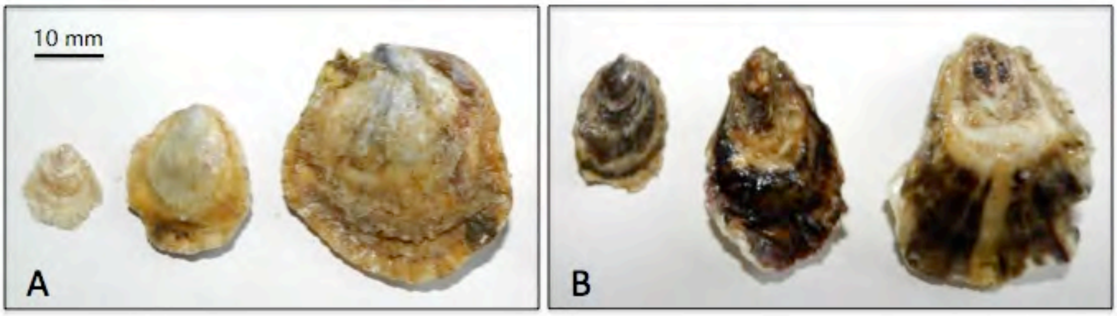

Figure 2: Shell growth of the European oyster *Ostrea edulis* (A) and the Pacific oyster *Crassostrea gigas* (B) during one offshore cultivation season.

Furthermore, oysters of the present study developed their species-specific morphology and produced an "aesthetic" shell, which plays an important role for their market value (Matthiessen 2001) (Fig. 2). Only oysters grown at site *Wurster Arm* (WA), where daily tidal currents and sediment loads are high (Pogoda et al. 2011), developed a different shape (Fig. 3.) Thicker shells and a very compact appearance indicate that these animals strongly invested energy in shell growth to withstand the strong currents as well as to prevent shell abrasion (Newkirk et al. 1995). This emphasizes the importance of a detailed and thorough site selection prior to the start of offshore oyster cultivation or aquaculture operations in general (see Pogoda et al. 2011).

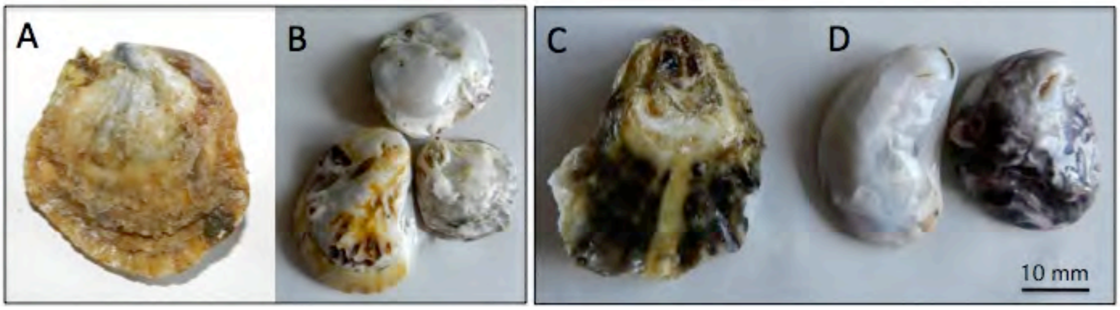

Figure 3: (A) *O. edulis* showing normal shell growth from offshore sites and (B) with strong shell abrasion from site WA (nearshore/exposed), where tidal currents are high. (C) *C. gigas* showing normal shell growth from offshore sites and (D) with strong shell abrasion from site WA.

In general, growth rates varied between culture sites. While three offshore cultivation sites offered good conditions, the fourth site was clearly not suitable for oyster growth (Pogoda et al. 2011). Both oyster species preferred the same sites: Highest growth rates were observed at sites *Butendiek* (BD) and *Nordergründe* (NG). As the Pacific oyster showed very similar increases in dry mass per day at both sites, we assume that these sites offered similar good conditions for cultivation. However, the European oyster performed differently: Increase in dry mass per day was four times higher at NG (2007) than at BD (2004). This may be explained by the small size and inferior condition of the *O. edulis* spat in 2004, when European oysters were obtained from the Danish Shellfish Centre (DSC). Løfstedt (2010) reported that this oyster spat was in a poor condition due to inadequate nutrition. This could be the reason for the deviating results for the European oyster at sites BD and NG. As *O. edulis* of DSC still showed positive growth rates in 2004, the question arises, whether growth rates may have been even higher with spat animals in optimum initial condition. According to typical size classes used in commercial aquaculture farms, larger *O. edulis* spat from a Norwegian producer was used in the experiment of 2007 (Newkirk et al. 1995, Matthiessen 2001, Buck 2002, Draver pers. comm.). These animals yielded significantly higher growth rates ($p < 0.0001$), even beyond those of Pacific oysters. The superior condition of these European oysters is reflected in the higher condition index in 2007 (Pogoda et al. 2011).

4.2 Species-specific strategies reflected in growth and biochemical composition

The European oyster showed constant increases in shell length and dry mass in 2004 and 2007. This implies a high ability of dietary assimilation of the native European oyster, even when food availability is low in summer (Rick et al. 2006) and a good adaptation to this environment (Newkirk et al. 1995, Matthiessen 2001, Laing et al. 2006). In contrast, seasonal variations with reduced growth rates in summer were observed for the Pacific oyster in 2007. Interestingly, this species-specific difference is also clearly reflected in parallel changes of the biochemical compositions of *C. gigas* (Fig. 4).

The biochemical and elemental compositions of *O. edulis* and *C. gigas* during one growing season were analyzed to characterize the nutritional condition and for a better understanding of related energetic processes. Accumulation and depletion of metabolic energy reserves depend primarily on food quantity and quality, environmental effects on metabolic processes, and reproductive activities (Beninger & Lucas 1984, Whyte et al. 1990, Ruíz et al. 1992). Therefore, investigations focused on seasonal dynamics of the major energy storage products, namely carbohydrates, proteins and lipids, on the compositions of lipid classes and fatty acids as well as on carbon and nitrogen.

Lipids provide much more metabolic energy than the same amount of carbohydrates (glycogen) or proteins and are a most efficient energy source. Due to the parallel incorporation of water molecules glycogen storage results in a ten times higher mass to reach the same energy yield (Schmidt-Nielsen 1999). Nevertheless, for oysters glycogen is a convenient form of energy reserve, as it has the advantage of providing instant energy as glycogen catabolism is fast. Furthermore, it can be performed under hypoxic or anoxic conditions. This is particularly important, when oysters keep their shells closed for longer periods (Hummel et al. 1989, Whyte et al. 1990).

It is still controversially discussed, which type of energy deposition is the preferred by European and Pacific oysters (e.g. Holland & Hannant 1974, Whyte et al. 1990, Robinson 1992, Ruíz et al. 1992, Child & Laing 1998). This study, however, clearly revealed that both species utilized primarily glycogen as energy store during times of high food availability. After the phytoplankton spring bloom, when reduced growth rates were observed for *C. gigas*, glycogen contents were drastically depleted, whereas lipid contents increased. In contrast, *O. edulis* kept on growing and accumulating glycogen, while lipid contents remained relatively constant (Fig. 4). These different strategies may be explained by the earlier maturity of Pacific oysters and the resulting conversion of carbohydrates to lipids to enhance the production of eggs, which are rich in lipid (Gallager & Mann 1986, Whyte et al. 1990, Robinson 1992, De la Parra et al. 2005). In contrast to the European oyster, the Pacific oyster already starts reproductive activities in the first year after settlement, especially when water temperatures rise above 17°C (Walne 1974, Newkirk et al. 1995, Costil et al. 2005, Royer et al. 2008). This was the case in the North Sea during the warm summer of 2007, when water temperatures in the southern German Bight were above 18°C from June until September (Pogoda et al. 2011). Interestingly, elemental compositions of *C. gigas* stayed quite constant, even in August, when glycogen contents dropped drastically. This is in agreement with the accumulation of lipids and the suggested conversion of carbohydrates to lipids during gametogenesis.

4.3 Excellent physiological condition of offshore cultivated oysters

In shellfish production, the condition index of oyster species such as *Ostrea edulis*, *Crassostrea gigas*, *C. virginica* etc. is commonly used to evaluate the effects of the surrounding environment on these organisms (Rheault & Rice 1996). It is an adequate parameter to describe the commercial quality, physiological state and health of bivalve molluscs (Dridi et al. 2007). The most commonly applied condition index (CI) is the ratio of flesh mass to shell mass (e.g. Walne & Mann 1975, Davenport & Chen 1987). Condition indices for both offshore cultivated oyster species support the positive results already observed for the growth performance at offshore sites. CI values for the Pacific oyster indicate a good condition (Linehan et al. 1999) during both experiments. Values for the European oyster also indicate a good condition in the experiment of 2007. In contrast, the condition indices were quite low at the beginning of the preliminary experiment in April 2004, confirming the poor condition of the DSC oyster spat described above (Løfstedt 2010, Pogoda et al.

2011). But the CI increased with cultivation time and European oysters were in good condition (Walne & Mann 1975, Linehan et al. 1999) at the end of the experiment in October 2004. Apparently, *O. edulis* was capable of an excellent recovery at sites *Butendiek* (BD) and *Helgoland* (HE), which furthermore underlines the great potential of successful offshore cultivation.

Seasonal variations in lipid class compositions of offshore-cultivated oysters were essentially similar to those of nearshore-grown individuals (e.g. Abad et al. 1995, Linehan et al. 1999, De la Parra et al. 2005). Triacylglycerols (TAG) are the main lipid stores in the investigated oysters and serve as short-term energy reserves. Together with glycogen, they accumulate during periods of high food availability and are depleted in periods of food paucity. Accordingly, the amount of TAGs is a sensitive indicator of the nutritional condition of an animal (Fraser et al. 1985). It can be expressed as the ratio of phospholipids to triacylglycerols (PL:TAG), whith values ≤ 1 indicating a good nutritional state (Watanabe & Ackman 1974, Abad et al. 1995, Caers et al. 2000). According to this scale, the European and Pacific oysters of the present study showed poor nutritional conditions at the beginning of the experiment in spring 2007 (PL:TAG ratios of 3 for *O. edulis* and 18 for *C. gigas*). During summer *O. edulis* and *C. gigas* clearly improved its condition and PL and TAG levels reached equal proportions of 1 in summer, which indicate well-fed animals (Watanabe & Ackman 1974, Abad et al. 1995, Caers et al. 2000) and excellent growing conditions at offshore cultivation sites.

The amount of essential fatty acids can greatly affect growth and condition of oysters (Pazos et al. 1996) and may serve as an indicator for the preferred diet (Soudant et al. 1999, Dalsgaard et al. 2003). Diatoms, for example, are characterized by high concentrations of 16:1(n-7), 18:1(n-7) and 20:5(n-3) fatty acids (e.g. Sargent et al. 1987), while dinoflagellates show high amounts of 18:4(n-3), 22:6(n-3) (e.g. Sargent et al. 1987, Virtue et al. 1993) and 18:5(n-3) (Mayzaud et al. 1976). The fatty acid compositions of the European and the Pacific oysters were dominated by 16:0, 20:5(n-3) and 22:6(n-3), major components of phospholipids and typical of marine organisms (e.g. Lee et al. 2006). As the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) are conservative elements of biomembranes, they have only a limited value as direct indicators for nutritional condition. However, the proportion of phospholipid (n-6) fatty acids, principally 20:4(n-6), can indirectly show the depletion or accumulation of lipid reserves through an increase or decrease, respectively (Child & Laing 1998). Both species of offshore cultivated oysters showed a significant decrease ($p < 0.001$) in 20:4(n-6) during high food availability from spring to early summer, therefore indicating the accumulation of lipids as energy reserves. Levels of 22:6(n-3) stayed quite constant in *O. edulis*, but showed a slight decrease in *C. gigas*. Furthermore, 18:4(n-3) and 18:5(n-3) were not present or showed very low concentrations. Accordingly, dinoflagellates were not an important food organism. However, diatom markers increased during spring and early summer in both oyster species and suggest a diet rich in diatoms. During the cultivation experiment, *O. edulis* and *C. gigas* maintained high ratios of (n-3)/(n-6). These increased from 10 to >18 in late summer, which furthermore indicates the excellent physiological condition of both offshore-cultivated oyster species (Sargent et al. 1990, Pazos et al. 1996, Soudant et al. 1999).

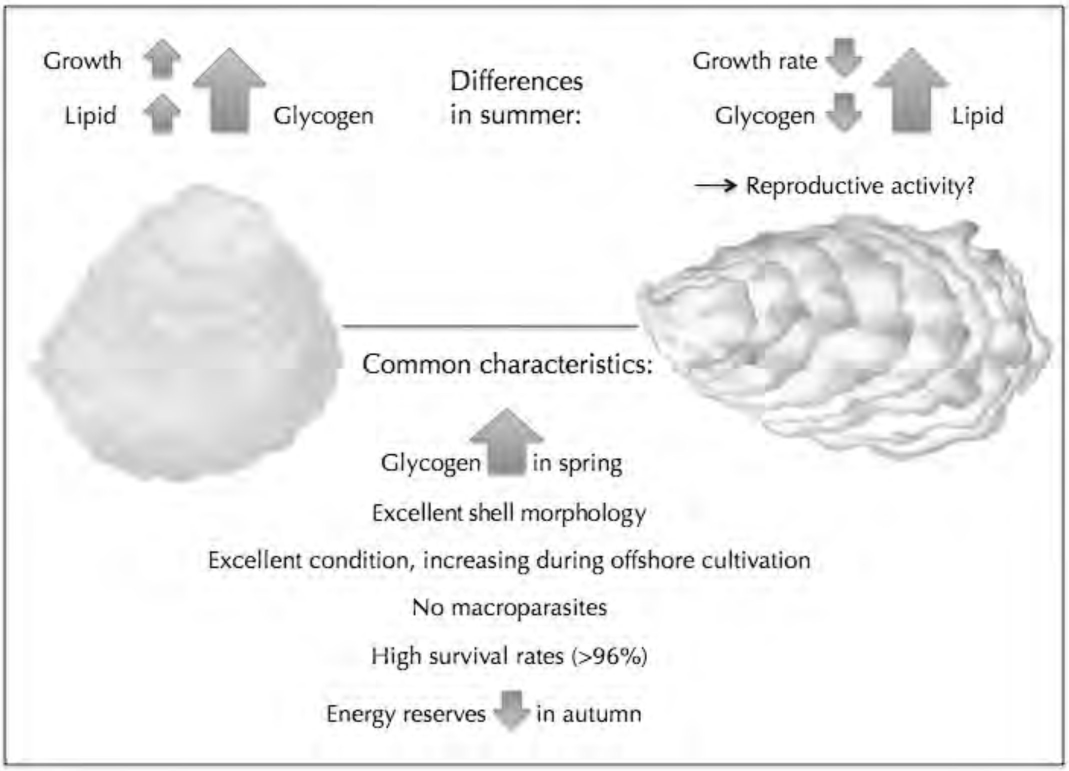

Figure 4: Differences and commonalities of the two oyster species *O. edulis* and *C. gigas* during offshore cultivation.

4.4 Parasite-free offshore oysters and high survival

In general, parasites can affect condition and health of host animals. Three major groups of macroparasites are known to infest oysters: shell-boring polychaetes, trematodes and mytilicolid copepods (e.g. Katkansky et al. 1967, Lauckner 1983, Krakau et al. 2006, Elsner et al. 2010). From an economic point of view the absence of macroparasites in shellfish products is certainly favourable. Oysters are commonly eaten raw and consumers would not accept the appearance of e.g. parasitic copepods, as they are easy to recognize due to their bright red colour and size (up to 25 mm). This is an issue, as it would result in a serious decrease of the oysters´ value. Some macroparasites could also evoke a deteriorated morphological appearance, e.g. shell-boring polychaetes. As oysters represent high-value seafood products, an aesthetic appearance of the shell – especially on the half-shell market – and meat is rather important (FAO 2012). Furthermore, parasite infestations could reduce harvests and severely deplete local populations. Understanding the development of infestation patterns is therefore crucial for the successful site-selection in oyster cultivation.

In this study the macroparasite burden of offshore-cultivated oysters and oysters of wild habitats in coastal areas of the North Sea (eastern Wadden Sea) were investigated. Recent studies on the macroparasite burden of Blue mussels already reported a zero infestation at offshore locations in the North Sea (Buck et al. 2005, Brenner 2010). These observations were also confirmed for oysters by this study. In contrast to regularly infested oysters and mussels from nearshore sites, no macroparasites have been detected in European and Pacific oysters from the offshore cultivation sites (Pogoda et al. subm.). Absence of trematodes at offshore locations can be explained by their complex life cycle: they often infest intertidal gastropods as first intermediate hosts (e.g. *Littorina littorea* and *Hydrobia ulva*) (Jensen & Mouritsen 1992, Huxham et al. 2001, Bordalo et al. 2011). Due to the absence of these exclusively coastal organisms the parasite’s life cycle cannot be completed in offshore regions (Buck et al. 2005). Mytilicolid copepods and shell-boring polychaetes (e.g. *Polydora ciliata*) are abundant in inshore waters (Kent 1981, Davey 1989, Ambariyanto & Seed 1991, Thieltges et al. 2006). However, their short planktonic larval phase

restricts successful dispersion to coastal waters. Larvae drifting away from the coast are bound to die in the absence of hosts due to predation and starvation, which are only available at very few selected offshore culture locations (Buck et al. 2005).
In addition to ecological and economic benefits of offshore oyster cultivation (Pogoda et al. 2011), these results present another commercial advantage of such offshore shellfish cultures.

The combination of successful growth performance and obviously excellent overall condition of offshore cultivated oysters resulted in insignificant mortalities. In contrast to commercial oyster production in nearshore environments, which often suffer from high mortalities (Costil et al. 2005, Soletchnik et al. 2006, Valero 2006, Chávez-Villalba et al. 2010), in this study survival rates for both oyster species were very high (> 96% in 2004, >99% in 2007) and encourage offshore cultivation.

4.5 Economic viability of offshore ostreiculture

Both species, *Crassostrea gigas* and *Ostrea edulis*, contribute significantly to the European economy, especially on the oyster half shell market in France. In total, oysters account for 23% of total shellfish production in the European Union (Ferreira et al. 2009).

As *O. edulis* is one of the most savoury oysters and has become quite rare, it achieves high prices on the market. This oyster trade is of huge commercial importance with wholesale average prices for *O. edulis* commonly being 3 to 5 times higher than for *C. gigas*. Following Goulletquer (2004), *O. edulis* fills an economic niche as a premium seafood item. Experiments of this study clearly revealed indications that the native species is still well adapted (Matthiessen 2001, OSPAR 2009) and shows an adequate growth performance. Therefore, the cultivation of the European flat oyster in the North Sea is recommended in particular.

Culture experts even appreciate varieties of Pacific oysters that show a compact growth, because these resemble the expensive European oysters

(Mathiessen 2001) and are more profitable. In general, the outer appearance of the shell is extremely important for successful marketing. Offshore cultivated European and Pacific oysters of both experiments in this study have an attractive shell appearance, which is an essential qualification for the success of offshore cultures.

But offshore cultivation in general has its challenges, especially concerning the economic viability of such cultures. The long distances from the coast require special vessels for technical installation, maintenance and harvest. Offshore aquaculture in exposed environments has to cope with harsh climatic and hydrographic conditions, such as high wind velocities, high waves and high current speeds. These conditions call for special culturing and harvesting techniques. Consequently, economic analyses for mytiliculture by Buck et al. (2010) yielded higher costs, when moving far off the coast. Costs for oyster production will even exceed those of Blue mussel production owing to extra expenses on oyster spat and cage techniques. Generally, oyster larvae are not abundant in offshore regions of the North Sea and in contrast to mussels (Brenner 2009, Buck 2007), no natural spatfall of oysters will occur in these regions. Spat oysters have to be purchased from commercial hatcheries, which however, is a common phenomenon in ostreiculture. Furthermore, Blue mussels can be cultivated directly at longlines, while oysters have to be reared in cages, oyster lanterns or barrels, which are then installed to longlines.

Buck et al. (2010) postulated that offshore production of consumer mussels is sufficiently profitable even under the assumption of substantial additional costs. Considering the comparatively high market value of oysters, their offshore cultivation may be even more profitable than offshore cultivation of mussels. Mussels present a relatively low margin of profit of about 1.0 €/kg. In contrast, Pacific oysters yield around 4 €/kg profit, costs for seed oysters already included (Ferreira et al. 2009) and European oysters generate even higher profits (Goulletquer 2004). Therefore, oysters, as high value products, are candidates with a high economic potential for offshore cultivation. Studies on the economic viability of oyster cultivation in offshore regions are in preparation (Ebeling pers. comm., own data).

4.6 Ideal candidates for integrated multi-trophic aquaculture (IMTA)

Today, concepts for offshore aquaculture also involve candidate species usually cultured in intensive systems, e.g. finfish (e.g. Benetti et al. 2003, Firestone et al. 2004, Naylor & Burke 2005). Still, sustainable and environmentally friendly cultivation processes need to be established. Environmental impact of offshore aquaculture is discussed controversially. Several studies reported no negative impact for small-scale cultures, while others indicated that released nutrients from offshore farms, especially from larger operations, are transported with passing water masses and may concentrate even far away from farm areas (e.g. Naylor & Burke 2005). Most finfish cages are flow-through systems, where currents transport feed residuals, particulate and dissolved nutrients, to the environment, which results in a reduced water quality (Troell et al. in prep.).

The implementation of integrated multi-trophic aquaculture (IMTA) offers considerable environmental advantages (Chopin et al. 2001, Troell et al. in prep.). An integrated culture of intensively fed species and extractive species considerably reduces waste release of aquaculture facilities. Oysters and mussels filter particulate organic matter (POM), sea cucumber feed on deposits on the sea floor (POM, pseudofaeces of the cultivated bivalves), while seaweeds assimilate dissolved inorganic matter (Fig. 4). As filter-feeders, oysters act as bioextractive organisms and can improve the water quality at culture sites (e.g. Langan 2004, Rose et al. 2010). Therefore, these candidates are ideal components of an integrated multi-trophic aquaculture (Fig. 2).

Several projects aim at the realization of IMTA in offshore aquaculture operations (Langan 2004), but experiences mainly exist for nearshore projects and have to be extrapolated to the special situation in the open ocean (Ferreira et al. 2009, Troell et al. 2009). As already described above, a variety of stakeholders, e.g. shipping and platform operations, are also present in offshore areas. Offshore wind farms constitute a relatively new but rapidly expanding user group. Actual development proposals of offshore IMTA contribute to a wind farm-aquaculture co-use of open ocean space (McVey & Buck 2008). Besides still open questions in the context of legislature and regulatory frames, the viability of offshore IMTA strongly depends on the technological and biological feasibility as well as on the profitability of such commercial operations (Buck & Krause 2012). Selected species should be of high commercial value to be cost-effective and research data on species performance are extremely valuable (Troell et al. in prep.).

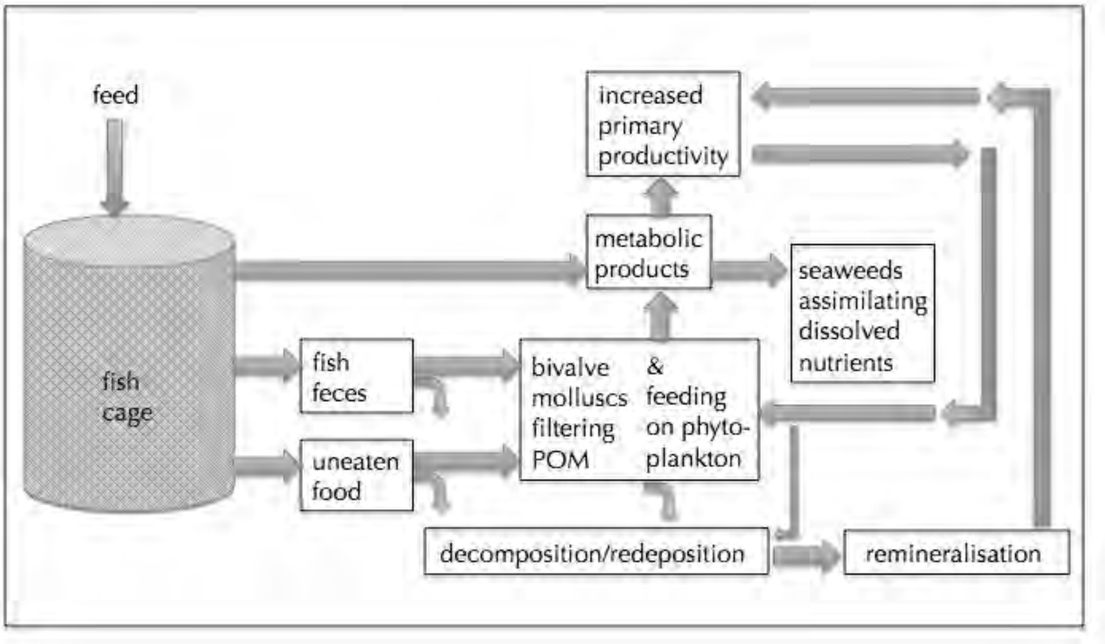

Figure 4: Theoretically balanced nutrient input and uptake of an integrated multi-trophic aquaculture (IMTA) system, showing pathways between fed and extractive species. Suspension/deposit feeders are not included as bottom depth may restrict their performance in offshore aquaculture operations. POM: particulate organic matter.

In this study, a comprehensive data set is presented for two oyster species, *Ostrea edulis* and *Crassostrea gigas*. As oysters are high-value seafood products and both investigated oyster species succeeded excellent biological performances during offshore cultivation, this study qualifies oysters as promising candidates for offshore IMTA operations.

4.7 Restocking of the native European oyster in the wild?

Another topic, which could only be touched within this thesis, is the idea of restocking the European oyster *Ostrea edulis* along the German coastline or in the European Wadden Sea in general. As already mentioned above, there has been a dramatic reduction in native oyster stock abundance throughout Europe since the beginning of the last century (Laing et al. 2006). *O. edulis*, the native oyster species of European waters, is extinct along the German and Belgian coast and *O. edulis* beds are under threat or decline in all the regions, where they naturally occur (OSPAR 2009). The severely depleted state of *O. edulis* in the wild can be explained by overexploitation, a series of severe winters, declining water quality and diseases, e.g. infections with the parasitic protist *Bonamia ostreae* or protozoa, such as *Marteilia refringens*. In addition, the introduction of exotic pests, e.g. competitors like the slipper limpet *Crepidula fornicata* or an introduced oyster drill, the whelk tingle *Urosalpinx cinerea*, has negative impacts on the stocks of the European oyster (Laing et al. 2006).

Feasibility studies on stock restoration revealed the importance of the European oyster for biodiversity enhancement. Furthermore, its commercial viability as a sustainable seafood product became evident (Laing et al. 2006). Successful restocking experiments with *O. edulis* were already conducted in Spain (Perez-Camacho 1987, Guerra 1998), Ireland (Kennedy & Roberts 1999) and Denmark (Dolmer & Hoffmann 2004). Such restoration trials have also been recommended to several areas of the UK and are a conceivable scenario for the German coast. The excellent condition as well as continuous growth of *O. edulis*, even in times of low food availability during summer, indicate that European oysters are still very well adapted to the temperate climate and environmental conditions of the North Sea.

On the one hand, several constraints to restoration have to be considered, e.g. degraded habitats, which are probably less suitable for recruitment. On the other hand, recent developments in water quality improvements as well as the existing knowledge on sustainable management and preservation practices increase the prospects of successful restoration. These aspects should be examined carefully and aim at the development of strategies for the preservation of this endangered species in European waters.

5. Conclusions

The existing multitude of coastal stakeholder conflicts restricts the development of aquaculture activities along the German coastline and is also problematic in most coastal areas of the world. The move of aquaculture operations to offshore areas is discussed as a promising solution with high economic and ecological potential, and offshore aquaculture projects are implemented worldwide. However, it is an ambitious and expensive approach and therefore intense research is essential to minimize risks of failure.

This study focused on the biological performance of two oyster species, the European oyster *Ostrea edulis* and the Pacific oyster *Crassostrea gigas*, cultivated in an offshore environment. Research results provide a comprehensive data set on the suitability of oysters, typical nearshore activated organisms, for offshore aquaculture operations.

Oysters exhibited a convincing growth performance at offshore sites and both species showed an excellent physiological condition at the end of the cultivation periods. Apparently, investigated oyster species cope well with the harsh conditions in offshore regions.

Offshore cultivated oysters were free of macroparasites, which presents a significant ecological and economic advantage of aquaculture sites in the open ocean.

First estimations on the economic viability of offshore oyster cultivation presented positive results: Oysters, as high value seafood products are profitable candidates for offshore cultivation.

Furthermore, as filter-feeders, oysters represent an ideal extractive component of integrated multi-trophic aquaculture (IMTA) systems in offshore areas.

Consequently, from an oyster farmer's perspective, data of this study attest the suitability of open ocean environments for oyster cultivation and encourage aquaculture activities at offshore sites. Applying appropriate site selection criteria, we conclude that both oyster species, *C. gigas* and *O. edulis*, are successful candidates for offshore cultivation. A multiple-use of potential offshore locations together with other stakeholders, e.g. offshore wind farms, is promising and synergistic effects may offer technical and financial advantages for the oyster farmer or IMTA enterprise.

Future studies should focus on complete growth periods during various seasons. This will lead to a better understanding of the performance of oysters in offshore cultivation experiments in the North Sea and off the coast in general.

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Acknowledgements

I am grateful to many people for their long-lasting support and encouragement which enabled the successful completion of this research work. Some of them are gratefully acknowledged here. However, there are many more and to all I want to express a warm and honest: Thank you.

First I would like to thank Prof. Dr. W. Hagen and Prof. Dr. B.H. Buck who provided scientific and financial support to make this work possible. Thank you Willy for the chance to choose this topic, for your understanding and your guidance. Thank you Bela for integrating me into your convincing ideas and thank you for your invaluable support. I very much appreciated your advice during this work - and your humor.

Special thanks go to all my colleagues involved in this study, there is no order – just gratitude:

Matthias Brenner, Markus Geisen, Bela Buck, Hendrik Wessels, Christoph Haacke, Eddie Egert, Tanja Michler-Cieluch, Susanne Spahic – thank you for being a reliable and great diving team! We did a great job out there, and it was fun...

Willy Hagen, Holger Auel, Anna Schukat, Britta Grote, Lena Teuber, Petra Wencke, Britta Meyer-Schlosser, Simon Jungblut, Juliane Kutter and the complete working group Marine Zoology at the University of Bremen, thank you for all the help, for valuable discussions and your support during the last years.

Uli Bathmann, Reinhard Saborowski, Alexandra Markert, Martin Graeve, Sonja Kleinertz, Gesche Krause, Steffi Bröhl, Sabine Strieben, Moni Weiss, Jan Schmidt, Uli Seemann, David Thieltges, Udo Schilling †, Philipp Fischer, Carsten Wanke and all AWI and IMARE colleagues, thank you for your advice and your technical and practical support.

Furthermore, I would like to thank the captains and crew of *RV Uthörn*, *RV Senckenberg* as well as the captains and crews of Water and Shipping Agency vessels *Bruno Illing* and *Mellum* for their everlasting logistic and creative assistance, especially Jörg Böning for realizing our ideas of offshore cage installation. Thank you Susanne Tamm (BSH) for your assistance in DOD-data acquisition.

For logistic support and funding I thank the University of Bremen and the Alfred Wegener Institute for Polar and Marine Research.

Sincere thanks also go to Mark Draver (Guernsey Sea Farms), Ditte Tørring (Danish Skaldyr Center), Eivind Bergtun (Bømlo Skjell), Werner Wosniok (University of Bremen), Michael Ebeling (vTI) and Bine Pöhner (Dittmeyer´s Austern-Compagnie) for answering my questions.

Special thanks go to Anna Schukat, Lena Teuber and Nicola von Berg-Dark for their thorough proofreading, for happy discussions and many, many helpful comments.

And now I would like to thank my family and friends – for everything!

Thank you all for being there.

ERKLÄRUNG

Eidesstattliche Erklärung

(Gem. § 6(5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich die vorliegende Arbeit

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Bernadette Pogoda

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