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Research Article

Biomass Composition of Blue Mussels, *Mytilus edulis*, is Affected by Living Site and Species of Ingested Microalgae

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We have investigated changes in specific contents of protein, glycogen and lipid, and fatty acids of blue mussels, *Mytilus edulis*, under different conditions in the field and in laboratory feeding experiments using different microalgae. Specific contents of glycogen and lipid increased in mussels relocated to net bags at a location in Kerteminde Bay (Great Belt, Denmark) in contrast to mussels relocated to a location in Sallingsund (Limfjorden, Denmark). The polyunsaturated fatty acid, eicosapentaenoic acid, reached 3 times higher values in the mussels in Kerteminde Bay. Mussels fed pure cultures of *Cryptocodinium cohnii*, which is rich in the polyunsaturated fatty acid, docosahexaenoic acid, and glycogen, gained the highest specific contents of this fatty acid and glycogen. Mussels feeding on the most protein rich of the microalgae, *Bracteacoccus* sp., gained the highest protein contents. The specific glycogen content of the mussels was influenced by their “condition” (body dry weight/shell length ratio) while specific protein and lipid contents were not. Starvation affected mainly the specific glycogen content. These results show that biomass composition of blue mussels is affected by living site and local phytoplankton species and that the fatty acids composition of mussels reflects the content of fatty acids in the diet.

1. Introduction

The growth rate of filter feeding blue mussels, *Mytilus edulis*, is mainly determined by the concentration of suspended phytoplankton in the water [1, 2] while the biomass produced by the mussels can be of a variable biochemical composition. Seasonal dependent variations in biomass composition of blue mussels are well described [3–7] and spawning occurs at the expense of stored glycogen and lipid [8]. Also the availability and composition of phytoplankton species in the diet influence biomass composition of mussels [9]. Thus, the biochemical composition of *M. galloprovincialis* has been observed to depend on living site in the Mediterranean Sea [10–12]. Starvation will also result in changes of biomass composition of mussels because maintenance metabolism is likely to cause food reserves within different biomass components to be metabolized at unequal specific rates.

Mussels acquire proteins, lipids, carbohydrates, and other components from phytoplankton and use these compounds to build their own biomass. The polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are some of the nutritionally most valuable components of marine biomasses. The PUFA content and composition in mussels vary with season and living site [10–12] and can be affected by the PUFA content in the diet [9, 13]. PUFAs are synthesized by phytoplankton and accumulated in the lipids of marine suspension feeders (“grazers”) and their predators, a process known as trophic upgrading [14–16]. The efficiency by which biomass components are retained during trophic upgrading depends largely on the number of steps in the food chain the compounds are passed through [17]. Mussels being primary consumers represent the shortest possible route of biomass compounds from phytoplankton to fishable or cultivable marine resources.

PUFAs are essential constituents of feed for marine fish and are also beneficial for human health. Fish oil and fish meal are the major sources for EPA and DHA but the resources are limited and future demands for these products can be difficult to meet [17]. DHA and other PUFAs are also produced industrially in cultures of heterotrophic microalgae but only to a smaller amount [18]. Mussels are potential sources for PUFAs and other compounds of marine origin and mussel meal have already to some extent been used successfully to partly replace fish meal in feed for rainbow trout [19] and poultry [20]. Variability in the biochemical composition of mussels may however result in variability of nutritional value and flavour [12]. Taylor and Savage [13] and Ventrella et al. [9] therefore suggested that harvesting of New Zealand green-lipped mussels, *Perna canaliculus*, and *Mytilus galloprovincialis* should be coordinated to periods when PUFA contents in the animals are maximal.

The present study is an integrated part of the research project MarBioShell (2008–2012) concerning growth and the potential for line farming of *Mytilus edulis* in Limfjorden and the Great Belt (Denmark) [2]. In these areas, *M. edulis* grows from settlement in spring to approximately 30 mm in shell length in November. To reach the traditional consumer size of at least 45 mm, the mussels need about 18 months because of the Danish winter period with weight loss and subsequent regrowth during the following season. Therefore, the MarBioShell project suggests a new approach of farming of 30 mm “minimussels” for human consumption on lines or nets based on only one growth season. The “minimussels” are produced without labour intensive sorting of mussels during the growth seasons, while the expected byproduction of undersized mussels may be used for other purposes such as poultry or fish feed [9, 13] or recycling of nutrients in eutrophicated areas [21]. Because the nutritional value of farmed *M. edulis* may be optimized by well-planned harvesting periods, it is important to know how the biomass composition of mussels is affected by the environment. In this study we have surveyed how biomass composition and accumulation of fatty acids, proteins, and glycogen in *M. edulis* are affected by living site, microalgal diet, starvation, and the “condition” of the mussels.

2. Materials and Methods

2.1. Growth of Mussels in the Field. *Mytilus edulis* were collected in Kerteminde Bay (Great Belt) and randomly transferred to net bags in Kerteminde Bay or Salling Sund (Limfjorden), both locations in Denmark. Net bags were regularly collected from both locations and size and biomass composition of the mussels in the net bags, each containing 10 individuals, were analyzed. The two experimental locations are described in details in [2].

2.2. Growth of Mussels in the Laboratory. Blue mussels, *Mytilus edulis*, were collected in Great Belt and stored in aerated tanks with a continuous supply of fresh sea water. Feeding experiments were performed in 15.4 L aquaria with a flow-through of biofiltered seawater (20 psu) of 11 h^{-1} .

Algae were added from a reservoir at a constant rate and 4 air stones ensured aeration and mixing. One week prior the feeding, mussels were placed in the experimental aquarium for acclimatization. Mussels were removed randomly at regular intervals for measurements of shell length, dry weight of soft parts, and for biochemical analysis.

2.3. Determination of Filtration Rates. Steady-state filtration rates were determined as described in, for example, [22]

$$F = \frac{P \times C_c - Fl \times C_a}{n \times C_a}, \quad (1)$$

where F is the filtration rate (1 h^{-1} individual $^{-1}$), P and Fl are rates of addition of algal suspension to the aquarium and flow-through of particle free seawater (1 h^{-1}), respectively, n is the number of mussels, and C_c and C_a are algal concentrations in the added suspension and steady-state algal concentration in the mussel aquarium (1 l^{-1}), respectively.

Filtration rates were also measured regularly by the clearance method [23]. The algal addition and flow-through were temporarily stopped while the reduction in the number of algal cells as a function of time was followed using an electronic particle counter (Elzone 5380). Clearance rate equals filtration rate when all particles are 100% efficiently retained by the mussels and was determined from

$$F = \frac{V \times b}{n}, \quad (2)$$

where V is water volume (L), n is the number of actively filter-feeding mussels, and b is the slope of regression line in a semi-ln plot of algal concentration versus time.

2.4. Determination of Growth Rates. Actual growth rates were estimated as changes of dry weight of soft parts divided by the growth period

$$G_{\text{act}} = \frac{W - W_0}{\Delta t}, \quad (3)$$

where G_{act} is the actual growth rate (mg day^{-1}), W_0 and W are dry weights of soft parts of mussels at the start and end of the experiment (mg), respectively, and Δt is the duration of the experiment (days). Weight specific growth rates were estimated from

$$\mu = \frac{\ln(W/W_0)}{\Delta t} \times 100, \quad (4)$$

where μ is the weight specific growth rate ($\% \text{ day}^{-1}$).

2.5. Condition Index. The “condition” of the mussels was defined by a condition index

$$CI = \frac{W}{L^3}, \quad (5)$$

where CI is the condition index (mg cm^{-3}) and L is the shell length (cm).

2.6. Cultivation of Microalgae. *Cryptocodinium cohnii* (CCMP 316) was obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences, USA and grown heterotrophically in a medium containing 30 g L⁻¹ sea salt (Red Sea salt), 0.01 g L⁻¹ FeCl₃, 0.05 g L⁻¹ (NH₄)₂SO₄, 0.6 mM K₂HPO₄, 330 mM D-glucose, and 55 mM L-glutamic acid, vitamins, and trace metals, pH 6.8–7 as described in [24, 25]. Continuous flow cultures were grown under phosphate limitation in a 3 l Applikon bioreactor at 29°C [25].

Rhodomonas salina (supplied from University of Gothenburg, Sweden) and a unicellular chlorophyte, *Bracteacoccus* sp. isolated in our laboratory, were grown phototrophically in repeated batch cultures at 20–25°C in 20 l bottles containing 15 l of sea water (20 psu) supplemented with 0.8 mM NaNO₃, 0.09 mM Na₂EDTA, 0.36 mM H₃BO₃, 1 mM NaH₂PO₄, 0.003 mM FeCl₃, 0.001 mM MnCl₂, 10 µM ZnCl₂, 5.6 µM CoCl₂, 5.3 µM CuSO₄, 0.5 µM (NH₄)₆Mo₇O₂₄, 0.5 µM thiamine dichloride, 0.15 µM biotin, and 0.004 µM cobalamin. The cultures were continuously illuminated by 8 fluorescent light tubes. Every day were 5 l of algal culture replaced by fresh medium. Aeration and mixing were carried out by injection of compressed air.

Galdieria sulphuraria was grown heterotrophically in a batch culture in an orbital shaking incubator at 42°C in 2 l conical flasks containing 300 or 400 mL of a modified medium [26], in which the glucose concentration was increased to 20 g L⁻¹. Cultures were harvested when they reached a stationary phase because of nitrogen limitation at a cell density of 3×10^8 cells mL⁻¹. The cells were separated from the medium by centrifugation and resuspended in seawater before being fed to mussels.

2.7. Dry Weights of Mussels and Microalgae. Mean dry weight of soft parts of mussels was quantified based on the measurements of at least 10 individuals after drying at 90°C for 24 h.

Dry biomass concentrations of microalgal cultures were measured in quintuplicate after the filtration of culture onto predried glass-fiber filters followed by drying overnight at 90°C. The dry weight of individual cells was estimated by comparisons of dry biomass concentration to cell numbers.

2.8. Analysis of Fatty Acids. Lipids were extracted from mussel or algal biomass based on the method of Bligh and Dyer [27] and transesterification of fatty acids were carried out as described in [25]. The formed fatty acid methyl esters (FAMES) were analyzed using GC-FID with helium as carrier gas. The FAMES were separated on a 30 m × 0.32 mm × 0.25 µm Agilent Technologies DB-23 capillary column using the following column temperature program: the initial temperature of 50°C was increased to 175°C at 25°C min⁻¹, maintained constant for 1 min, increased to 240°C at 4°C min⁻¹, and maintained at this temperature for 5 min. Peaks in chromatograms were identified and quantified after comparisons to reference FAMES. Nonadecanoic acid and heptadecane were used as internal standards.

2.9. Analysis of Starch and Glycogen. Glycogen and starch were extracted from mussels or microalgae by sonication (Braun Labsonic L) of biomass for 2 min. The extracts were digested enzymatically and the released glucose was quantified using HPLC as described in [25].

2.10. Analysis of Protein. Aliquots of 20–35 mg lyophilized algal or mussel biomass were resuspended in 1 mL 0.5 M NaOH, sonicated (Braun Labsonic L) for 40 s while cooled on ice, shaken for 24 h at 30°C, and centrifuged for 15 min at 15000 g [25]. Proteins in the supernatant were quantified using the bicinchoninic acid assay [28]. Bovine serum albumin was used as a standard.

3. Results

3.1. Location. Figure 1 compares growth and changes of biomass composition of *Mytilus edulis* after transfer to net bags in Kerteminde Bay and Salling Sund. The mussels grew at average rates of 6.0 mg day⁻¹ in Kerteminde Bay and 4.5 mg day⁻¹ in Salling Sund (Table 1) over periods of time of 70 and 55 days, respectively. The biomass composition of the mussels from the two locations developed differently. The mussels in Salling Sund maintained a relatively constant biomass composition, although these mussels had been moved from one location to another, while the mussels in Kerteminde Bay tripled their specific glycogen content and doubled their specific contents of fatty acids in 70 days (Figure 1). Eicosapentaenoic acid (EPA) in particular, but also other fatty acids, was present in concentrations several times higher in mussels relocated to Kerteminde Bay compared to mussels relocated to Salling Sund at the latest samplings.

3.2. Microalgal Food Source. In order to investigate how the biochemical composition of *Mytilus edulis* may reflect the biochemical composition of the microalgal diet, groups of mussels were in controlled laboratory experiments fed different species of microalgae with distinct differences in biochemical composition (Table 2). Figure 2 shows growth and changes of biomass composition in *M. edulis* fed the dinoflagellate *Cryptocodinium cohnii*, an alga with particular high contents of docosahexaenoic acid (DHA) and starch. For a period of 21 days, the mussels feeding on *C. cohnii* grew at a rate of 5.8 mg day⁻¹ (Table 1). During this period, their specific contents of glycogen and lipid increased approximately 5 and 1.5 times, respectively, while the specific protein content decreased. In the same period, the specific DHA content was more than doubled while the specific content of EPA, which is absent in *C. cohnii*, decreased by 50%. Also the specific contents of palmitoleic and myristic acid, the two saturated fatty acids that were present in the highest concentration in *C. cohnii*, increased in the mussels.

A group of *Mytilus edulis* was also fed pure cultures of *Bracteacoccus* sp. (Figure 3), a microalga characterized by relatively low lipid content, absence of DHA, only a low EPA content, but with the highest protein content of the microalgae used as feed (Table 2). The most prominent

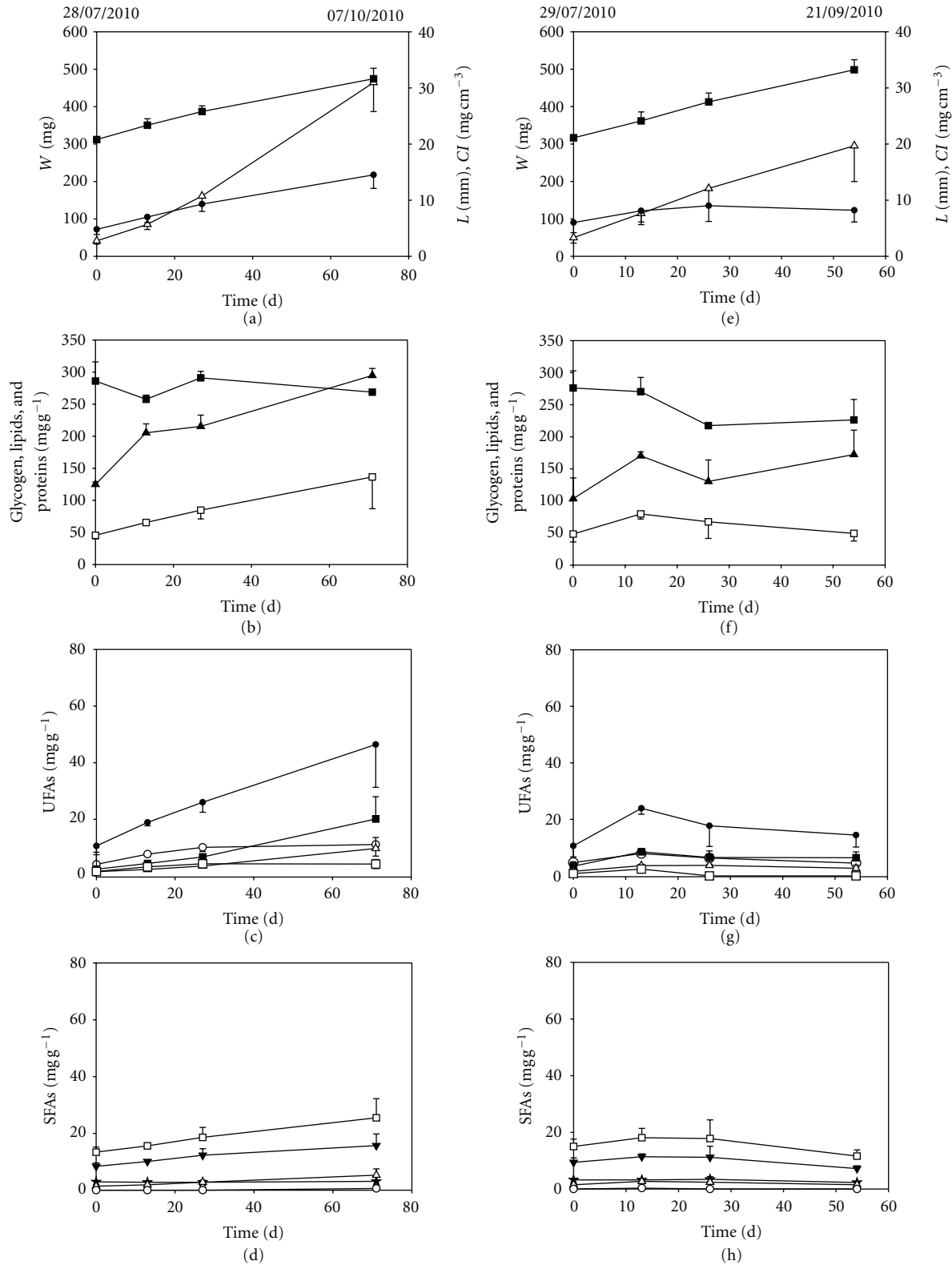


FIGURE 1: *Mytilus edulis* kept in net bags in Kerteminde Bay (a–d) and Salling Sund (e–h) in the year 2010 at $16.0 \pm 3.5^{\circ}\text{C}$ and 14.2 ± 3.4 psu and $19.0 \pm 1.9^{\circ}\text{C}$ and 29.4 ± 1.0 psu, respectively. (a and e) condition index (CI, ●), shell length (L, ■), and dry weight of soft parts (W, △); (b and f) glycogen (▲), protein (■), and lipid (□) contents; (c and g) sum of unsaturated fatty acids (UFAs, ●) and specific concentrations of DHA (○), EPA (■), sum of oleic, linoleic, and α-linolenic acids (□), and palmitoleic acid (△); (d and h) sum of saturated fatty acids (SFAs, □) and specific concentrations of stearic (★), palmitic (▼), myristic (△), and lauric acids (○). Numbers of mussels used for analyses are indicated in Table 1. Data points are mean values + or – S.D.

TABLE 1: *Mytilus edulis*. Start and end dry weight of soft parts (W_0 and W_t), mean filtration rates (F), steady-state concentrations of microalgal cells (C), actual measured growth rates (G_{act}), and weight specific growth rates (μ) during laboratory and field experiments. Number of mussels (n) used for individual measurements of shell length (L), dry weight of soft parts (W), and for biochemical analysis (B). Values indicate means \pm S.D.

Location/feed source	t Days	n $L/W/B$	W_0 mg	W_t mg	F $\text{mL min}^{-1} \text{ ind.}^{-1}$	C cells mL^{-1}	G_{act} mg day^{-1}	μ $\% \text{ day}^{-1}$
Kerteminde Bay July 28–Oct. 7	71	10/10/10	41.1 ± 8.6	464.4 ± 77.7	—	—	6.0	3.3
Sallingsund July 29–Sept. 21	54	10/10/10	49.7 ± 14.5	295.2 ± 95.6	—	—	4.5	3.1
Kerteminde Bay Nov. 2010–Mar. 2011	106	†	202.2 ± 17.1	147.2 ± 42.9	—	—	−0.5	−0.3
<i>Cryptocodinium cohnii</i>	21	15/15/10	154.3 ± 34.8	275.6 ± 71.9	8.6 ± 5.3	3330 ± 3710	5.8	2.8
<i>Bracteacoccus</i> sp.	28	10/10/10	151.6 ± 34.1	161.7 ± 22.4	17.0 ± 8.5	2450 ± 830	0.4	0.2
<i>Bracteacoccus</i> sp.*	28	10/10/10	34.4 ± 5.0	71.1 ± 16.5	10.1 ± 5.1	4430 ± 1900	1.3	2.6
<i>Rhodomonas salina</i>	14	25/5/4	66.3 ± 20.3	124.2 ± 29.5	29.4 ± 13.3	5130 ± 1520	4.1	4.1
<i>Galdieria sulphuraria</i>	34	20/20/4	64.1 ± 9.6	69.4 ± 18.1	22.7 ± 2.7	5500 ± 1880	0.2	0.2
None (starvation)	56	10/10/10	124.7 ± 20.8	108.4 ± 16.9	—	0	−0.4	−0.3

† Number of mussels used for measurements of shell length, dry weight of soft parts, and for biochemical analysis is indicated in Figure 5.

* Mussels starved for 56 days prior to feeding experiment.

TABLE 2: Cell dry weight and specific concentrations of protein, starch, lipid, and dominating fatty acids in phosphorous limited heterotrophic continuous flow culture of *Cryptocodinium cohnii* and phototrophic repeated batch cultures of *Rhodomonas salina* and *Bracteacoccus* sp. Values indicate means \pm S.D.

	Unit	<i>C. cohnii</i>	<i>Bracteacoccus</i> sp.	<i>R. salina</i>
Dry weight	g cell^{-1}	1.9×10^{-9}	6.5×10^{-11}	1.3×10^{-10}
Protein	mg g^{-1}	120.4 ± 13.7	223.9 ± 35.8	146.5 ± 5.9
Starch	mg g^{-1}	437.8 ± 49.4	251.0 ± 15.4	233.8 ± 15.3
Lipid	mg g^{-1}	139.4 ± 8.4	75.2 ± 20.6	174.2 ± 4.1
Lauric acid	mg g^{-1}	5.3 ± 2.9	6.3 ± 0.5	20.8 ± 0.7
Myristic acid	mg g^{-1}	19.7 ± 2.4	0.0	0.0
Palmitic acid	mg g^{-1}	24.6 ± 5.8	10.2 ± 2.3	23.2 ± 0.6
Palmitoleic acid	mg g^{-1}	0.8 ± 0.1	2.2 ± 0.4	3.0 ± 0.1
Stearic acid	mg g^{-1}	3.7 ± 0.8	2.6 ± 0.1	9.5 ± 0.2
Oleic acid	mg g^{-1}	8.1 ± 2.3	10.3 ± 2.3	7.8 ± 0.2
Linoleic acid	mg g^{-1}	0.0	3.9 ± 0.9	13.7 ± 0.3
α -linolenic acid	mg g^{-1}	0.0	5.3 ± 1.6	16.7 ± 0.3
Eicosapentaenoic acid	mg g^{-1}	0.0	1.8 ± 0.1	4.7 ± 0.1
Docosahexaenoic acid	mg g^{-1}	36.2 ± 13.2	0.0	3.0 ± 0.1

change in biomass composition of these mussels during the 28-day feeding period was an increase in specific protein content and a decrease in specific glycogen content. The content of neither DHA nor EPA changed markedly. However, mussels feeding on *Bracteacoccus* sp. grew slowly as compared to mussels suspended in net bags in the sea (Figure 1) or mussels feeding on *Cryptocodinium cohnii* (Figure 2). Although the weight specific growth rate was more than 10 times higher in a group of mussels that had been starved prior to the feeding experiment (Table 1), this alga probably cannot meet all nutritional needs of *M. edulis*.

Since *Mytilus edulis* grew poorly on *Bracteacoccus* sp., a group of mussels were also fed *Rhodomonas salina* (Figure 4), a microalga previously recognized as a suitable food source for *M. edulis* [1]. *R. salina* has a low DHA content compared to *Cryptocodinium cohnii* and also a relatively low EPA content (Table 2). The mussels grew at a rate of 4.1 mg day^{-1}

but major changes in lipid or glycogen contents or fatty acids composition did not occur, although specific contents of linoleic and α -linolenic acids, the dominating unsaturated fatty acids in *R. salina* (Table 2), increased slightly in the mussels fed this microalga (Figure 4). Lastly, a group of blue mussels was fed *Galdieria sulphuraria*, a unicellular rhodophyte that uses starch as its main carbon storage compound. *M. edulis* feeding on *G. sulphuraria* had the lowest growth rates (Table 1), and major changes of their biomass composition were not observed over a 34-day feeding period.

3.3. Starvation and “Condition”. Figure 5 shows changes of biomass composition of *Mytilus edulis* during a 105-day winter period in net bags in Kerteminde Bay as well as during a 56-day starvation period in the laboratory.

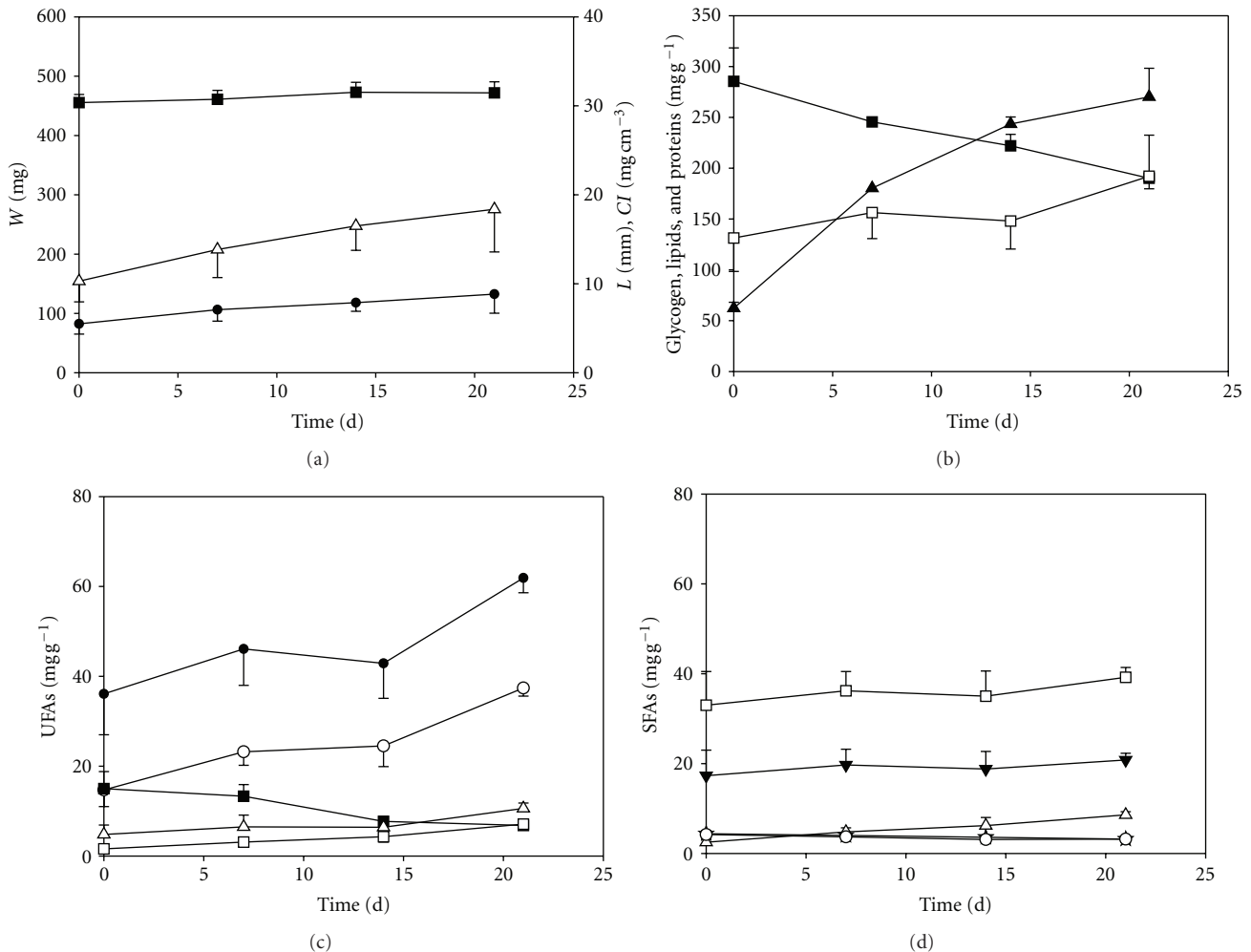


FIGURE 2: *Mytilus edulis* kept under laboratory conditions at $11.3 \pm 1.1^\circ\text{C}$ and 14.2 ± 0.9 psu and fed *Cryptothecodinium cohnii*. (a) Condition index (CI, ●), shell length (L, ■), and dry weight of soft parts (W, △); (b) glycogen (▲), protein (■), and lipid (□) contents; (c) sum of unsaturated fatty acids (UFAs, ●) and specific concentrations of DHA (○), EPA (■), sum of oleic, linoleic, and α-linolenic acids (□), and palmitoleic acid (△); (d) sum of saturated fatty acids (SFAs, □) and specific concentrations of stearic (★), palmitic (▼), myristic (△), and lauric (○) acids. Numbers of mussels used for analyses are indicated in Table 1. Data points are mean values + or - S.D.

The mussels in Kerteminde Bay lost weight during the winter period, mainly due to a selectively decrease in their glycogen and lipid contents, resulting in an increase of the specific protein content. The specific contents of all fatty acids, except oleic acid, decreased. Mussels starved in the laboratory had negative growth rates (Table 1), and the specific contents of glycogen and protein decreased, but starvation did neither result in a decrease of specific contents of lipids nor polyunsaturated fatty acids (PUFAs).

In all the mussels analyzed in this study, specific glycogen and lipid contents varied from 50 to 300 mg g^{-1} and 50 to 230 mg g^{-1} , respectively, and were thus more variable than the specific protein content that varied between 150 and 330 mg g^{-1} . Comparisons between biomass composition and condition index (CI) of all the mussels revealed a strong correlation between CI and specific glycogen content (Figure 6(a), coefficient of determination, $r^2 = 0.7$) but neither between CI and specific lipid content (Figure 6(b),

$r^2 = 0.0$) nor between CI and specific protein content (Figure 6(c), $r^2 = 0.1$).

4. Discussion

The biomass composition of *Mytilus edulis* is variable and the composition of proteins, glycogen, and lipids is affected by the location where it lives and the actually available amount and composition of phytoplankton which vary during the season. The present study has shown that the content of polyunsaturated fatty acids (PUFAs) in *M. edulis* reflects the content of these fatty acids in their diet while the specific glycogen content is closely correlated with the "condition" of the mussels. Starvation affects predominantly the specific content of glycogen whereas also lipid reserves are metabolized during winter starvation in the field.

Biochemical compositions of *Mytilus edulis* have in earlier studies shown considerable variation. Dare and

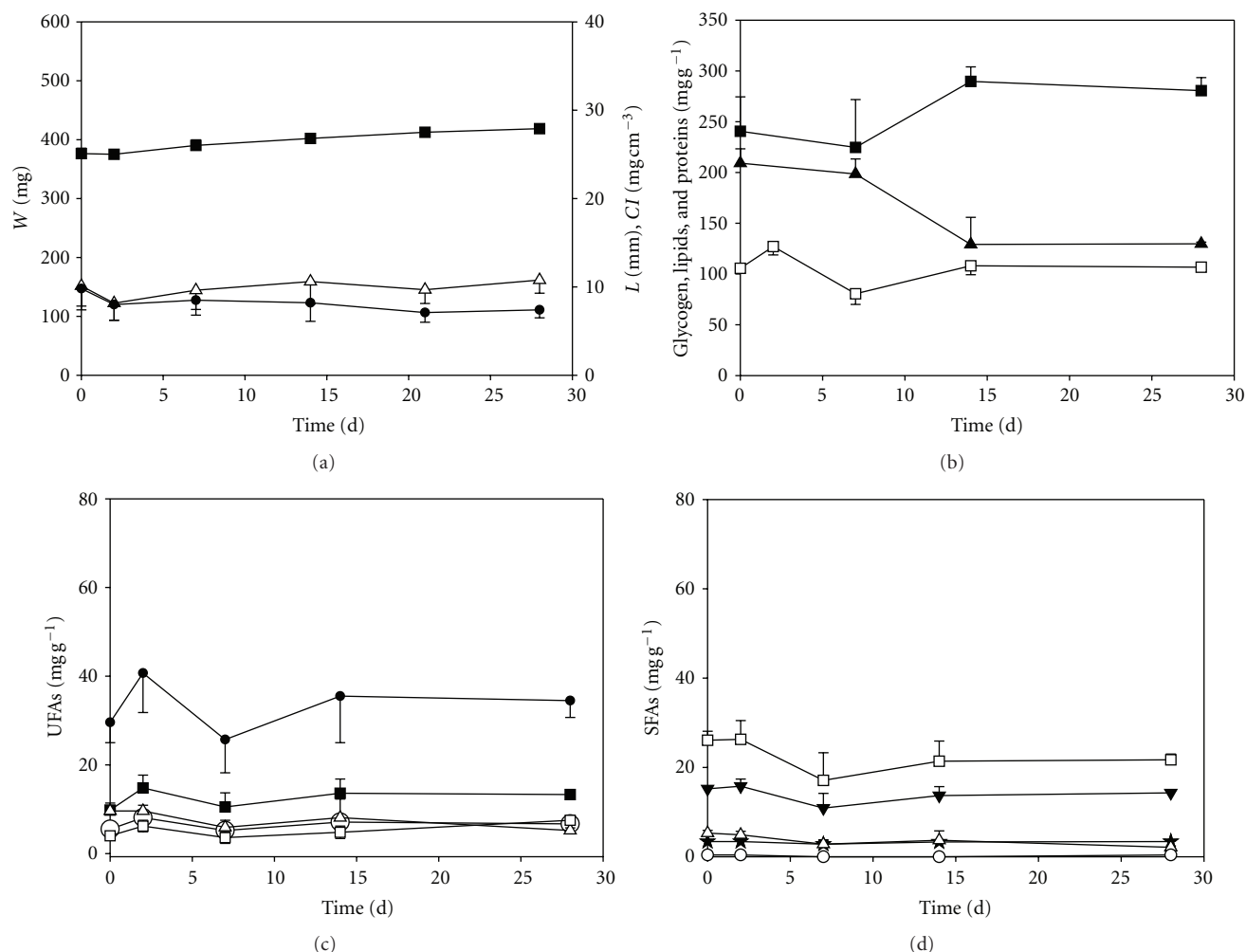


FIGURE 3: *Mytilus edulis* kept under laboratory conditions at $8.2 \pm 2.0^\circ\text{C}$ and 17.7 ± 4.2 psu and fed *Bracteacoccus* sp. (a) Condition index (CI, ●), shell length (L, ■), and dry weight of soft parts (W, △); (b) glycogen (▲), protein (■), and lipid (□) contents; (c) sum of unsaturated fatty acids (UFAs, ●) and specific concentrations of DHA (○), EPA (■), sum of oleic, linoleic, and α-linolenic acids (□), and palmitoleic acid (△); (d) sum of saturated fatty acids (SFAs, □) and specific concentrations of stearic (★), palmitic (▼), myristic (△), and lauric (○) acids. Numbers of mussels used for analyses are indicated in Table 1. Data points are mean values + or – S.D.

Edwards [29] and Pieters et al. [30] found specific protein contents between 400 and 700 mg g⁻¹ in *M. edulis*, while we found that proteins accounted for only 250 to 300 mg g⁻¹ in mussels grown in net bags in the sea (Figure 1) as well as in the laboratory (Figures 2–4). In net bag mussels from Kerteminde Bay (Figure 1) as well as mussels feeding on *Cryptocodinium cohnii* in the laboratory (Figure 2), specific glycogen and lipid contents reached approximately 300 and 100 mg g⁻¹, respectively. The specific glycogen and lipid contents were high in these mussels, and similar contents have been found in earlier studies [29, 31].

The biomass composition of *Mytilus edulis* can be affected by the environment over periods of just a few weeks under natural conditions (Figure 1). The specific contents of glycogen and lipids increased rapidly in mussels in Kerteminde Bay after they were transferred to net bags. The increase in lipid content was related to an increase in specific contents of eicosapentaenoic acid (EPA), docosahexaenoic

acid (DHA), and saturated fatty acids, and EPA ended up being the dominating fatty acid. In contrast, we did not observe larger changes in biochemical composition of mussels transferred from Kerteminde Bay to net bags in Salling Sund although both groups of mussels were collected at same time and place. Rapid changes of fatty acid profiles have also been observed in *M. galloprovincialis* [10]. Three weeks after individual mussels had been transferred to new locations, the fatty acid profiles of these mussels closely resembled the fatty acid profiles of the local mussels. The accumulation of EPA in mussels in Kerteminde Bay (Figure 1) may reflect that EPA-rich phytoplankton species; for example, diatoms [32] have been more abundant in Kerteminde Bay than in Salling Sund, where the mussels may have fed on less PUFA-rich phytoplankton species.

In order to investigate how fast fatty acid profiles can be affected by the fatty acid composition of the phytoplankton species in their diet, *Cryptocodinium cohnii* was selected

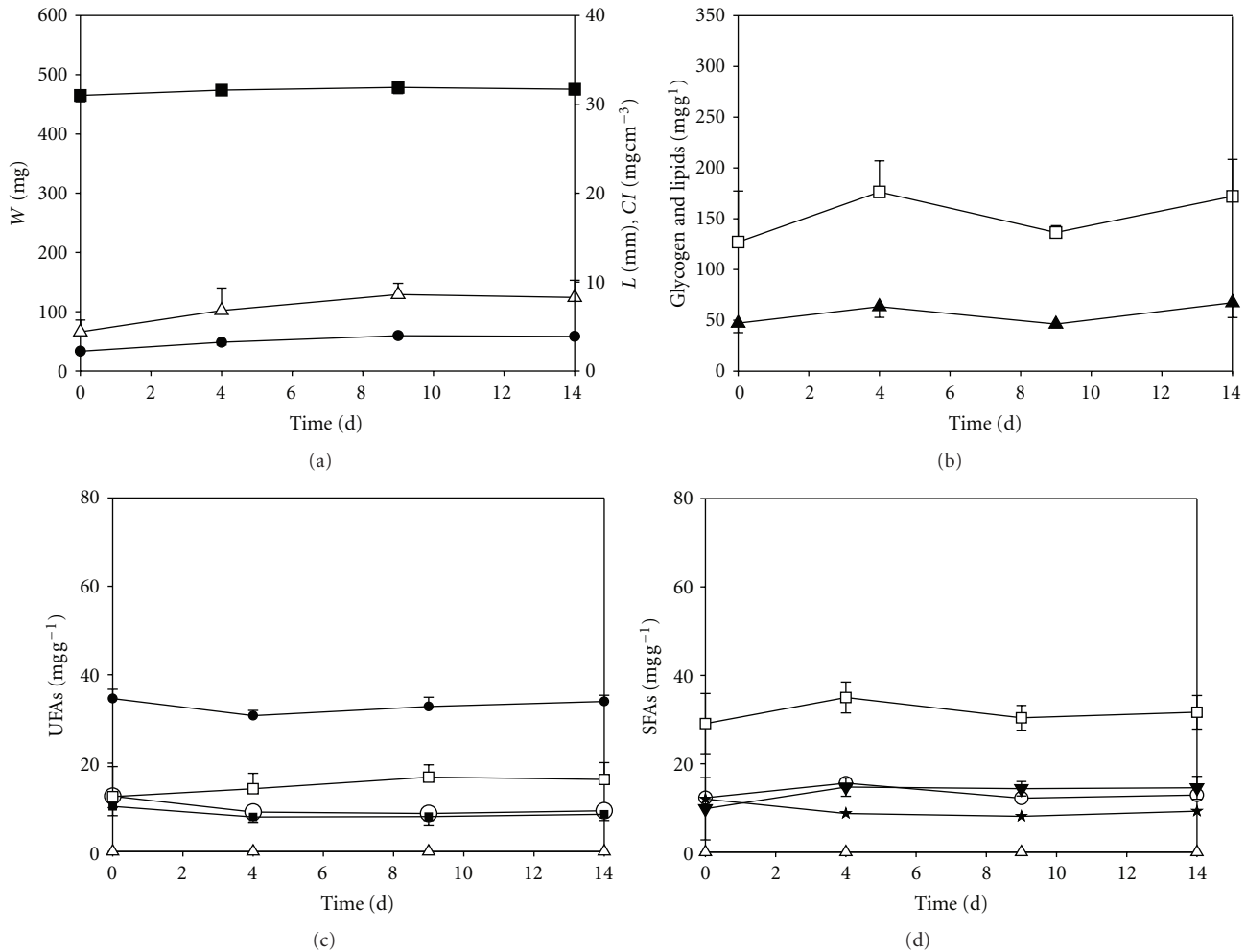


FIGURE 4: *Mytilus edulis* kept under laboratory conditions at $20.4 \pm 1.0^\circ\text{C}$ and 15.8 ± 2.3 psu and fed *Rhodomonas salina*. (a) Condition index (CI, ●), shell length (L, ■), and dry weight of soft parts (W, △); (b) glycogen (▲), and lipid (□) contents; (c) sum of unsaturated fatty acids (UFAs, ●) and specific concentrations of DHA (○), EPA (■), sum of oleic, linoleic, and α-linolenic acids (□), and palmitoleic acid (△); (d) sum of saturated fatty acids (SFAs, □) and specific concentrations of stearic (★), palmitic (▼), myristic (△), and lauric (○) acids. Numbers of mussels used for analyses are indicated in Table 1. Data points are mean values + or - S.D.

as feed alga for a group of mussels (Figure 2) because this dinoflagellate is well suited for investigations of accumulation of DHA. Its specific DHA content is high and other PUFAs are virtually absent [18], and cells of constant biomass composition can be produced in a continuous flow culture [25]. The blue mussels feeding on *C. cohnii* increased their specific content of DHA above levels observed in any other mussels in this study. The saturated fatty acid, myristic acid, that was also present in *C. cohnii* but neither in *Bracteacoccus* sp. nor *Rhodomonas salina*, also accumulated solely in mussels feeding on *C. cohnii*. These mussels also developed the highest glycogen content observed, probably as a consequence of the high specific starch content also found in *C. cohnii* (Table 2). The specific EPA content in the mussels decreased but their total EPA content remained constant. These mussels grew at the highest rate observed in this study, but the new biomass produced was composed of mainly lipid and glycogen.

When mussels were fed pure cultures of *Bracteacoccus* sp., *Rhodomonas salina*, or *Galdieria sulphuraria*, growth rates varied more than one order of magnitude but was positive in all 3 cases, in contrast to the negative growth of starving mussels (Table 1). The mussels have therefore been nourished by all 3 species of microalgae. The most notable changes in biomass composition were the decrease of specific glycogen content and increase of specific protein content seen in the mussels fed *Bracteacoccus* sp. (Figure 3). This microalga has a specific protein content almost twice as high as *C. cohnii* while its specific starch and lipid contents are lower. When mussels were fed *R. salina* or *G. sulphuraria*, changes of specific contents of glycogen or lipid did not occur, but the increase of specific linoleic and α-linolenic acid contents in the mussels fed *R. salina* probably reflects that these are the dominating unsaturated fatty acids in this microalga. Since the biomass compositional changes were different with different microalgal species as feed algae, these

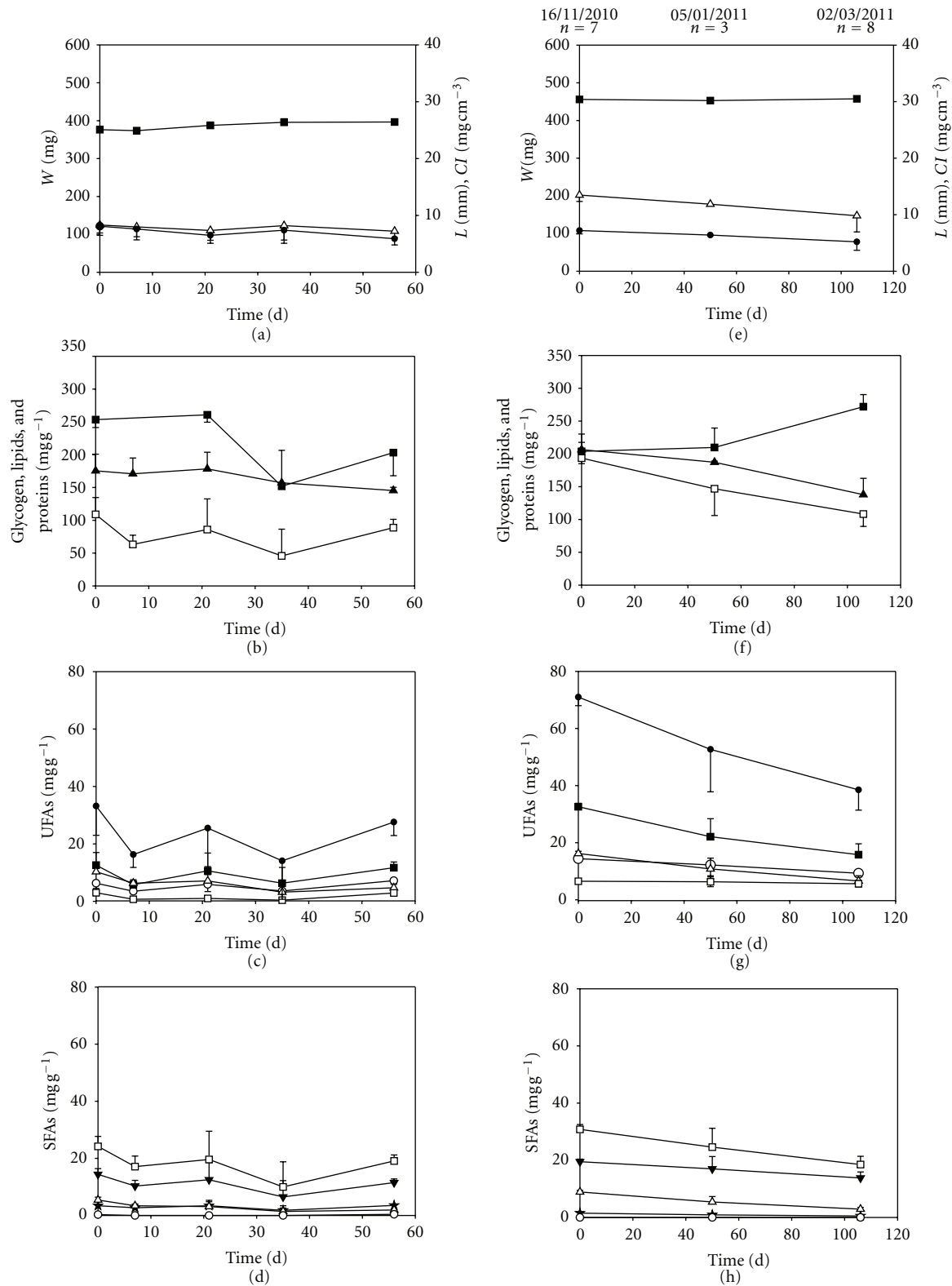


FIGURE 5: *Mytilus edulis* starved for a period of 56 days under laboratory conditions at $9.0 \pm 0.6^\circ\text{C}$ and 19.8 ± 2.3 psu (a–d) and in Kerteminde Bay from November 16, 2010 to March 2, 2011 (e–h), n is number of mussel sampled for analyses. (a and e) condition index (CI, ●), shell length (L, ■), and dry weight of soft parts (W, △); (b & f) glycogen (▲), protein (■), and lipid (□) contents; (c and g) sum of unsaturated fatty acids (UFAs, ●) and specific concentrations of DHA (○), EPA (■), sum of oleic, linoleic, and α-linolenic acids (□), and palmitoleic acid (△); (d and h) sum of saturated fatty acids (SFAs, □) and specific concentrations of stearic (★), palmitic (▼), myristic (△), and lauric (○) acids. Numbers of mussels used for analyses are indicated in Table 1. Data points are mean values + or – S.D.

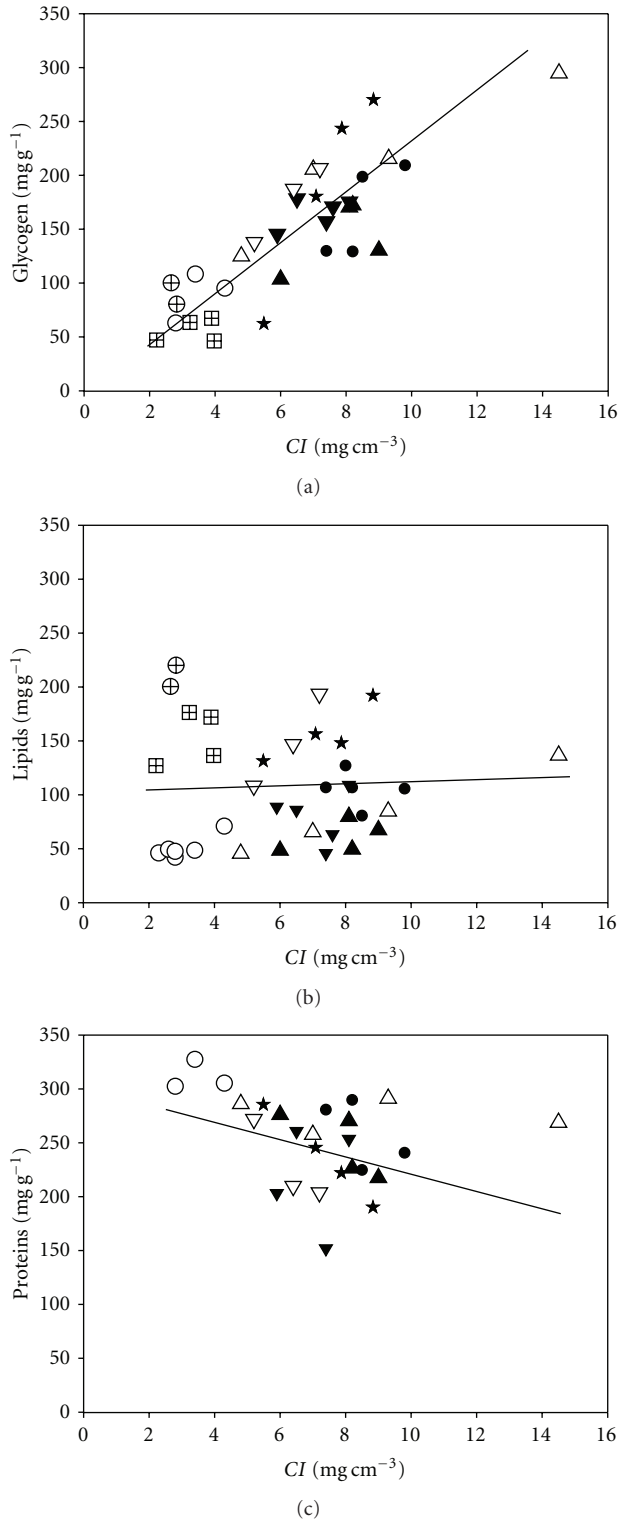


FIGURE 6: *Mytilus edulis*. Specific glycogen (a), lipids (b), and proteins (c) contents as a function of condition index (CI) of mussels from Kerteminde Bay July–October (Δ), Salling Sund (\blacktriangle) July–September, or Kerteminde Bay November–March (∇), and mussels fed *Crypthecodinium cohnii* (\star), *Bracteacoccus* sp. (\bullet), *Rhodomonas salina* (\boxplus), or *Galdieria sulphuraria* (\oplus), previously starved mussels fed *Bracteacoccus* sp. (\circ), and starved mussels (\blacktriangledown).

changes are unlikely explained by artificial conditions in the laboratory. The specific contents of the major biomass constituents in blue mussels, proteins, glycogen, and fatty acids seem therefore to reflect the biochemical composition of their microalgal diet.

Not only can the quality of the food source affect the biomass composition of *Mytilus edulis*. The specific glycogen content shows seasonal changes with maximal values in late summer and fall, and minimal values in spring [3]. We also found decreasing specific glycogen contents in mussels during winter, and also decreasing lipid contents (Figures 5(e)–5(h)). Proteins were better retained by the mussels during winter, resulting in an increasing specific protein content during the winter period. However, when mussels were starved (Figures 5(a)–5(d)), mainly proteins and glycogen were degraded while the lipids and therefore also the PUFAs in the biomass were retained. It has also in earlier studies been seen that starving mussels degrade mainly glycogen and proteins to supply maintenance energy [33]. Therefore, shorter periods with possible food limitation during the productive season will not necessarily result in the degradation of the PUFAs already incorporated into the biomass of the mussels. The missing relationships between the condition index (CI) and specific lipid or protein contents and the strong relationship between CI and specific glycogen content (Figure 6) indicate that glycogen is indeed the most labile of these 3 major biomass components and the main storage compound to be mobilized by starving mussels.

Mussels are potential sources of PUFAs originally synthesized by phytoplankton [9, 13]. Biomass composition and particularly PUFA content are important for the nutritional value, whether the mussels are used for human consumption, as fish meal replacement, or as feed for poultry or fish. It has already been suggested that different mussel species should preferentially be fished when their biomass composition is of the highest quality [9, 13]. Our results support that also the nutritional quality of *Mytilus edulis* will be amendable to optimization via knowledge-based planning of fisheries. *M. edulis* is one of the species of mussels that are also farmed in the sea. Local phytoplankton abundances are of central importance to the productivities of mussel farms [34] and are therefore taken into consideration when farms locations are planned. Since the phytoplankton composition affects the quality of the mussel, it will probably be beneficial also to consider local phytoplankton species composition in relation to the planning of mussel farm locations. To sum up, the biomass composition of *Mytilus edulis* depends on the living site from where the mussels are collected and is affected by at least the dietary composition of the phytoplankton and the condition index of the mussels. Not only the contents of PUFAs and other fatty acids in mussels reflect the composition of fatty acids in the phytoplankton species consumed, but also the protein and glycogen contents seem to be affected by the contents of these compounds in the mussels' diet. Although the specific lipid content was variable, the lipids, including the valuable PUFAs, were among the biomass compounds that showed the strongest resistance to degradation during starvation in *M. edulis*.

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References

- [1] I. Clausen and H. U. Riisgård, "Growth, filtration and respiration in the mussel *Mytilus edulis*: no evidence for physiological regulation of the filter-pump to nutritional needs," *Marine Ecology Progress Series*, vol. 141, no. 1–3, pp. 37–45, 1996.
- [2] H. U. Riisgård, K. Lundgreen, and P. S. Larsen, "Field data and growth model for mussels *Mytilus edulis* in Danish waters," *Marine Biology Research*, vol. 8, pp. 683–700, 2012.
- [3] A. de Zwaan and D. I. Zandee, "Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*," *Comparative Biochemistry and Physiology Part A*, vol. 43, no. 1, pp. 53–58, 1972.
- [4] P. A. Gabbott, "Storage cycles in marine bivalve molluscs: an hypothesis concerning the relation between glycogen and gametogenesis," in *Proceedings of the 9th European Marine Biology Symposium*, H. Barnes, Ed., pp. 191–211, Aberdeen University Press, Aberdeen, Scotland, 1975.
- [5] E. Orban, G. Di Lena, T. Nevigato, I. Casini, A. Marzetti, and R. Caproni, "Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites," *Food Chemistry*, vol. 77, no. 1, pp. 57–65, 2002.
- [6] J. Ojea, A. J. Pazos, D. Martínez, S. Novoa, J. L. Sánchez, and M. Abad, "Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle," *Aquaculture*, vol. 238, no. 1–4, pp. 451–468, 2004.
- [7] S. Dridi, M. S. Romdhane, and M. Elcasi, "Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia," *Aquaculture*, vol. 263, no. 1–4, pp. 238–248, 2007.
- [8] D. I. Zandee, J. H. Kluytmans, W. Zurburg, and H. Pieters, "Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis," *Netherlands Journal of Sea Research*, vol. 14, no. 1, pp. 1–29, 1980.
- [9] V. Ventrella, M. Pirini, A. Pagliarani, F. Trombetti, M. P. Manuzzi, and A. R. Borgatti, "Effect of temporal and geographical factors on fatty acid composition of *M. galloprovincialis* from the Adriatic sea," *Comparative Biochemistry and Physiology*, vol. 149, no. 2, pp. 241–250, 2008.
- [10] L. Freitas, M. J. Fernández-Reiriz, and U. Labarta, "Fatty acid profiles of *Mytilus galloprovincialis* (Lmk) mussel of subtidal and rocky shore origin," *Comparative Biochemistry and Physiology*, vol. 132, no. 2, pp. 453–461, 2002.
- [11] L. Freitas, U. Labarta, and M. J. Fernández-Reiriz, "Evolution of fatty acid profiles of subtidal and rocky shore mussel seed (*Mytilus galloprovincialis*, Lmk.). Influence of environmental parameters," *Journal of Experimental Marine Biology and Ecology*, vol. 268, no. 2, pp. 185–204, 2002.
- [12] A. Fuentes, I. Fernández-Segovia, I. Escriche, and J. A. Serra, "Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins," *Food Chemistry*, vol. 112, no. 2, pp. 295–302, 2009.
- [13] A. G. Taylor and C. Savage, "Fatty acid composition of New Zealand green-lipped mussels, *Perna canaliculus*: implications for harvesting for n-3 extracts," *Aquaculture*, vol. 261, no. 1, pp. 430–439, 2006.
- [14] W. C. M. Klein Breteler, M. Koski, and S. Rampen, "Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate," *Marine Ecology Progress Series*, vol. 274, pp. 199–208, 2004.
- [15] W. C. M. Klein Breteler, N. Schogt, M. Baas, S. Schouten, and G. W. Kraay, "Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids," *Marine Biology*, vol. 135, no. 1, pp. 191–198, 1999.
- [16] A. J. Veloza, F. L. E. Chu, and K. W. Tang, "Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod *Acartia tonsa*," *Marine Biology*, vol. 148, no. 4, pp. 779–788, 2006.
- [17] Y. Olsen, "Resources for fish feed in future mariculture," *Aquaculture Environment Interactions*, vol. 1, pp. 187–200, 2011.
- [18] C. Ratledge, "Fatty acid biosynthesis in microorganisms being used for single cell oil production," *Biochimie*, vol. 86, no. 11, pp. 807–815, 2004.
- [19] G. M. Berge and E. Austreng, "Blue mussel in feed for rainbow trout," *Aquaculture*, vol. 81, no. 1, pp. 79–90, 1989.
- [20] L. Jönsson and K. Elwinger, "Mussel meal as a replacement for fish meal in feeds for organic poultry—a pilot short-term study," *Acta Agriculturae Scandinavica A*, vol. 59, no. 1, pp. 22–27, 2009.
- [21] O. Lindahl, "Mussel farming as a tool for re-eutrophication of coastal waters: experiences from Sweden," in *Shellfish Aquaculture and the Environment*, S. E. Shumway, Ed., pp. 217–237, John Wiley & Sons, 2011.
- [22] P. S. Larsen and H. U. Riisgård, "Validation of the flow-through chamber (FTC) and steady-state (SS) methods for clearance rate measurements in bivalves," *Biology Open*, vol. 1, pp. 6–11, 2011.
- [23] J. Coughlan, "The estimation of filtering rate from the clearance of suspensions," *Marine Biology*, vol. 2, no. 4, pp. 356–358, 1969.
- [24] D. Pleissner, R. Wimmer, and N. T. Eriksen, "Quantification of amino acids in fermentation media by isocratic HPLC analysis of their," *Analytical Chemistry*, vol. 83, no. 1, pp. 175–181, 2011.
- [25] D. Pleissner and N. T. Eriksen, "Effects of phosphorous, nitrogen, and carbon limitation on biomass composition in batch and continuous flow cultures of the heterotrophic dinoflagellate *Cryptocodinium cohnii*," *Biotechnology and Bioengineering*, vol. 109, pp. 2005–2016, 2012.
- [26] W. Gross and C. Schnarrenberger, "Heterotrophic growth of two strains of the acido-thermophilic red alga *Galdieria sulphuraria*," *Plant and Cell Physiology*, vol. 36, no. 4, pp. 633–638, 1995.
- [27] E. G. Bligh and W. J. Dyer, "A rapid method of total lipid extraction and purification," *Canadian Journal of Biochemistry and Physiology*, vol. 37, no. 8, pp. 911–917, 1959.
- [28] P. K. Smith, R. I. Krohn, and G. T. Hermanson, "Measurement of protein using bicinchoninic acid," *Analytical Biochemistry*, vol. 150, no. 1, pp. 76–85, 1985.

- [29] P. J. Dare and D. B. Edwards, "Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales," *Journal of Experimental Marine Biology and Ecology*, vol. 18, no. 2, pp. 89–97, 1975.
- [30] H. Pieters, J. H. Kluytmans, W. Zurburg, and D. I. Zandee, "The influence of seasonal changes on energy metabolism in *Mytilus edulis* (L.). I. Growth rate and biochemical composition in relation to environmental parameters and spawning," in *Cyclic Phenomena in Marine Plants and Animals*, E. Naylor and R. G. Hartnoll, Eds., pp. 285–292, Pergamon Press, 1979, Proceedings of the 13 European Marine Biology Symposium Isle of Man.
- [31] İ. Okumuş and H. P. Stirling, "Seasonal variations in the weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs," *Aquaculture*, vol. 159, pp. 249–261, 1998.
- [32] J. K. Volkman, S. W. Jeffrey, P. D. Nichols, G. I. Rogers, and C. D. Garland, "Fatty acid and lipid composition of 10 species of microalgae used in mariculture," *Journal of Experimental Marine Biology and Ecology*, vol. 128, no. 3, pp. 219–240, 1989.
- [33] B. Bayne, "Aspects of the metabolism of *Mytilus edulis* during starvation," *Netherlands Journal of Sea Research*, vol. 7, pp. 399–410, 1973.
- [34] S. C. Ogilvie, A. H. Ross, and D. R. Schiel, "Phytoplankton biomass associated with mussel farms in Beatrix Bay, New Zealand," *Aquaculture*, vol. 181, no. 1-2, pp. 71–80, 2000.

