Development of general condition and flesh water content of long-time starved *Mytilus edulis*-like under experimental conditions

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Abstract. Mussels of the family Mytilidae are known for the two commercial important species in Europe, *Mytilus edulis* (Linneaus, 1758) (blue mussel) and *Mytilus galloprovincialis* (Lamarck, 1819) (Mediterranean mussel). To guarantee adequate production and quality in aquaculture general condition of the stock is routinely monitored, either by shell growth rate, soft body weight or condition index (CI). This study tested over 218 days (about 8 months) the starvation capability of blue mussels from the Baltic Sea, a low salinity environment, under fully controlled conditions. Because these mussels, genetically, represent two different species, *M. edulis* and *M. trossulus* (Gould, 1850), we examined a combination of both, as common in the western Baltic Sea. During experimental time the water temperature was kept at 15°C (±1°C) and 15 PSU (±1 PSU). Test animals physiology was verified by standard procedures, e.g. by CI, shell length growth rate and flesh water content. *M. edulis*-like mussel starvation capacity was high, 93 of 100 tested animals survived the entire 8 months. The CI decreased from initial 20.0 to 5.2 (loss of 74%) as well as the soft body weight retrospectively from initial 1.95 to 0.54 g (loss of 72%). The water content of the soft body increased from initial 95% to 98.4%. Regarding the total length of the mussels a decrease was found which ranged between the initial stocking sizes of the mussels (3.28 cm (±0.58 cm). We herewith demonstrate first results of the correlation of decreasing general condition, determined by CI and flesh weight, and an increase of flesh water content of blue mussels.

Key Words: aquaculture, condition, growth, flesh water content, starvation.

Introduction. In the family of *Mytilidae* Rafinesque, 1815 there are two commercially important species in Europe, *Mytilus edulis* (Linneaus, 1758) also known as blue mussel and *M. galloprovincialis* (Lamarck, 1819), also known as Mediterranean mussel. Inside the Baltic Sea, *M. edulis* is sympatric with *Mytilus trossulus* (Gould, 1850), both considered as *M. edulis*-like (Stuckas et al 2017). These species are exploited by fisheries and/or onshore aquaculture, seeking for qualitatively best mussel conditions for commercialization. Such specimens should have a high flesh content, condition index (CI), large size and consequently a high biomass (Župan & Šarić 2014).

Mussel's general condition so far is determined by random sampling of stock animals or tagged animals to collect representative data of the population and to determine either the condition index (CI), also known as fitness, or growth. The latter is often used by the aquaculture producers to identify steady production areas. However, this factor does not include other parameters such as shell proportion, flesh weight as well as flesh water content. Shell growth has been used as a sufficient estimate of the biomass and total flesh production (Hibbert 1977; Rodhouse et al 1984; Ross & Lima 1994; Thórarinsdóttir & Jóhannesson 1996; Ravera & Sprocati 1997; Deval 2001). Contradictory, the shell growth rate was found insufficient because of the shell variables (Seed 1968; Thórarinsdóttir & Jóhannesson 1996; Rueda & Urban 1998; Gimin et al 2004). This was supported by Riisgård & Randløv (1981) who recorded that *M. edulis* suffering malnutrition showed an extent growth of shells. Consequently, though the growth rate is still used in the literature, the reason for the conflicting evidence has not been studied.

The condition of blue mussels is influenced by a variety of factors; the most important is food availability (Župan & Šarić 2014). Variations of the CI are affected by food availability, shell size, environmental factors, seasons as well as reproductive stage (Gosling 2003). For the Kiel Fjord in the Baltic Sea, cultivation above 15 PSU, below 10°C and under presence of the required nutrients positively influenced the CI and was best suitable for *M. edulis*-like cultivation in this area (Harbach in prep.). These results are in accordance with Hiebenthal et al (2012) who demonstrated a continuous decrease of the CI and shell increment at a temperature range from 4 to 25°C. This was suggested to be a result of the low physiological costs under low water temperatures due to poikilothermy. *M. edulis* can sustain longer starvation periods, maintain their shell size but must generate their energy from their own tissue during these periods (Dare & Edwards 1975; Riisgård & Randløv 1981; Riisgård & Larsen 2014; Riisgård et al 2014a, b).

The present study for the first time investigates the starvation capacity of blue mussels for 218 days, about 8 months, under fully controlled experimental conditions in a low salinity environment. Because the mussels were obtained from the Kiel Fjord, Baltic Sea, a possible composite of the two species *M. edulis* L. and *M. trossulus* (*M. edulis*-like according to Stuckas et al 2017). We evaluated the total length, total weight, soft body weight, CI and flesh water content, enabling comparisons to be made to earlier studies. Consequences for the marketing of blue mussels and the quality control at this location are discussed.

Material and Method. Blue mussels were obtained from an Aquaculture facility (longline culture) in the Baltic Sea (Kiel Fjord, Germany) during the season 2012. Blue mussels of this region are considered *Mytilus edulis*-like, a composite of *M. edulis* L. and *M. trossulus* (Stuckas et al 2017). Both species occur sympatric at this sampled location, with no mosaic-like structure but a pattern of interspecific flow of genes. Because of constant interaction the composition percentages also change constantly.

The mean length of the mussels was 3.28 cm (±0.58 cm). Animals were adapted to an artificial recirculation system (adopted from Harbach & Palm (2017) with additional filter system) 14 days prior to the start of the experiment, and 100 *M. edulis*-like were randomly stocked. Shell surfaces were cleaned (byssus, adhered material) prior to the start of the experiment. The aquarium (60 cm x 30 cm x 30 cm) was filled with 40 liter artificial sea water (15±1 g L^{-1}) with passive temperature adjustment to 15±1°C by placing it in one fresh water filled well. A single rotary pump (300 L h^{-1}) guaranteed in the aquarium continuous water movement and oxygen supply to the mussels, surplus an biological filter (airlift) driven by air pump reduced nitrogen metabolites (NO_2, NH_4). Important water parameters, like temperature, pH and salinity were measured daily. Nitrogen metabolites were measured once per week. Limits of the nitrogen metabolites were determined according to laboratory experiments on blue mussels by Gregor (2008). The chronic LC50-values (7 days) of this study were reduced to safety values of 3%. So, NO_2 limits were set at 1.69 mg L^{-1}, NO_3 limits at 300 mg L^{-1} and NH_4 limits at 2.66 mg L^{-1}. All water parameters ranged within the given limits. Loss of evaporated water was compensated once per week by adding small amounts of freshwater into the aquarium.

Random sampling (n = 10) of test individuals was conducted at the beginning, after a period of 3 months and then in once a month intervals. The shell length was measured by a variable caliper (precision > 1 mm) by scaling the distance between the posterior and anterior margins of the shell, giving the mean values (n = 10) with correlating standard deviations. After cutting the posterior adductor muscle the mussel was opened and the soft body prepared apart from the shells. The soft body weight was recorded. Then the soft body and the shells were dried at 60°C for 48 h to document the dry weight, following Bustnes & Erikstad (1990) and Mallet & Carver (1995). By weighing the soft body dry weight, the flesh water content and the CI according to Walne (1976) was determined. The CI uses the dry weight and the shell weight of mussel samples to enable comparisons of the general physiological condition, also known as fitness

(Hiebenthal et al 2012). The CI formula reads as follows: dry weight/shell weight * 100 = CI (Walne 1976).

Observations of the mussel behavior were performed once a day throughout the experiment. Mussels do not underlie the contemporary German Animal Welfare Act. Nevertheless the experiment followed the rules of the 3-R-principle for the handling of laboratory animals.

Results. This study presents the influence of starvation upon the general condition of mussels in a definite Baltic Sea environment. The evaluation of the production site and the harvest time is essential to guarantee the best product quality in aquaculture. We are aware that the chosen individuals of *Mytilus edulis*-like in the region of the Kiel Fjord represent a composite of the species *M. edulis* and *M. trossulus* (Stuckas et al 2017). A direct comparison of this Baltic population of *M. edulis*-like to non-Baltic populations of *M. edulis* and/or *M. trossulus* is not possible (Stuckas et al 2017), requiring separate studies from the different regions.

The mussels from the Kiel Fjord demonstrated a correlation of the growth and condition factors with the flesh water content. During 218 days, the tested mussels lost about 72% of their soft body weight from 1.95 to 0.54 g. Until day 93, the soft body weight loss was only 3.1% (1.89 g), followed by a strong decrease during the run of the experiment (Table 1).

Table 1

Mean values of the condition index (CI) and water content (WC) (%) with correlating standard deviations (SD), mean total weight (g), mean total length (cm) and soft body weight (g) of examined *M. edulis*-like (n = 10) at the sampling dates

Date	*CI*	*CI SD*	*WC (%)*	*WC SD*	*Total weight (g)*	*Total length (cm)*	*Soft body (g)*
23.01.2012	20.04	6.01	95	1.44	4.55	3.61	1.95
25.04.2012	13.16	3.53	96.6	0.77	5.60	3.85	1.89
23.05.2012	8.77	1.00	97.7	0.26	3.92	3.33	1.21
19.06.2012	9.19	1.94	97.6	0.43	3.88	3.37	1.16
23.07.2012	6.62	2.45	98.1	0.65	2.08	2.78	0.58
28.08.2012	5.17	1.61	98.4	0.52	2.20	2.76	0.54

CI: condition index; SD: standard deviation; WC: water content.

The total shell length did not change during the experiment, with a variation in the range of the initial stocked size range (±0.58 cm). Due to subsampling and measurement of 10 individuals each, after 93 days, the total weight first appeared to increase about 23% but decreased until the end of the experiment to values of about 48% of initial weights. The CI reduced over the experiment in the first 93 days about 66% from 20.04 to 13.16 and in comparison to initial values to values of about 26% from 20.04 to 05.17. The water content of the soft body increased from 95 to 96.6% (+1.6%) during the first 93 days. During the entire experiment the water content increased constantly from 95 to 98.4% (+3.4%). The decrease in CI and soft body weight was associated with an increase of the meat water content (Figure 1).

Mortality of the mussels was low, 93 of 100 tested animals survived the entire 8 months. The surviving mussels regularly changed between phases of closed shells and phases of opened shells. Most common was that mussels observed on the subsequent day had opened their shells after they were seen without filtration activity the day before, and vice versa.

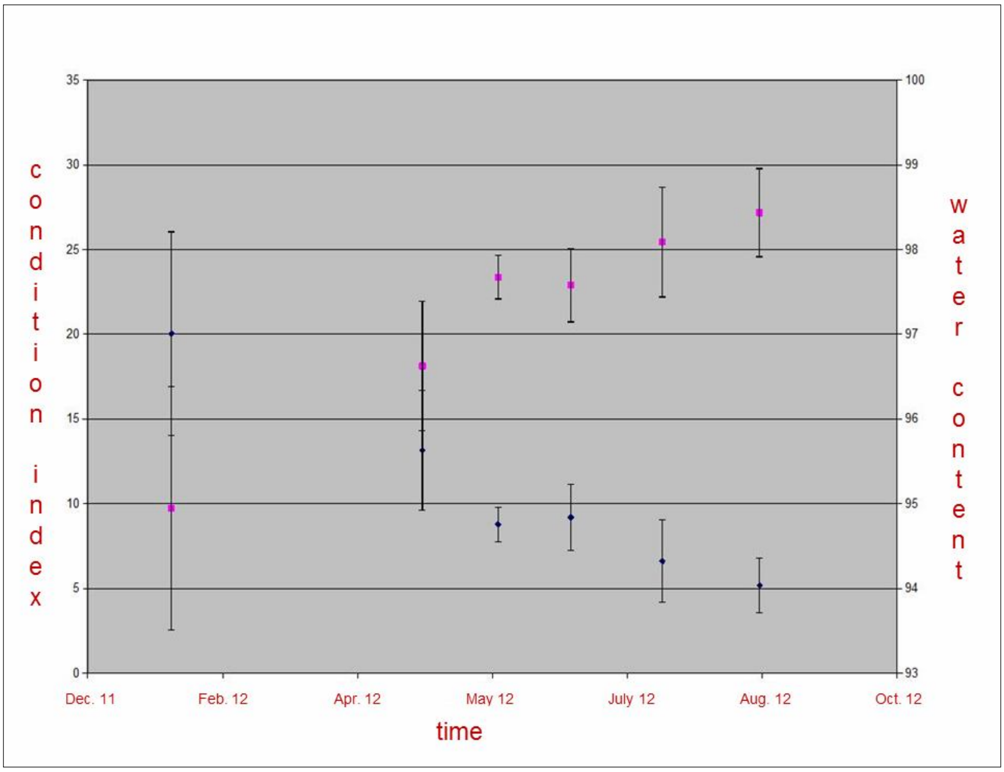

Figure 1. Development of the condition index and the water content with correlating standard deviations of the *M. edulis*-like (n = 10) over the experimental period (blue dots = condition index; magenta dots = water content).

Discussion. *Mytilus edulis* has a high capability to overcome long-time starvation periods. Only few studies have been conducted, mainly for an experimental time of 47-159 days (Dare & Edwards 1975 (90 days); Pleissner et al 2012 (56 days and 105 days); Riisgård et al 2014a, b (106 days); Riisgård & Larsen 2014 (159 days); Riisgård & Randlov 1981 (47 days)). The presented study is the first laboratory study over a long-term starvation period (218 days) of blue mussels. Because the mussels were obtained from a low salinity habitat inside the Baltic Sea where blue mussels are a sympatric composite of the two species *M. edulis* and *M. trossulus*, our results are representative for the blue mussels from this special Baltic Sea location.

So far, four laboratory and two field studies are available, showing decreasing soft body weights as well as decreasing CI with a prolonged starvation period (Riisgård & Randlov 1981 (15°C and 29 PSU); Riisgård & Larson 2014 (12.9±2.6°C and 20 PSU); Riisgård et al 2014a (field study), Riisgård et al 2014b (11°C and 20 PSU); Dare & Edwards 1975 (field study), Pleissner et al 2012 (20 PSU)). The authors demonstrated similar decreasing rates of the soft body weight as well as the CI apart from the shell lengths. Within the period between 47-106 days a loss of 12% (47 d), 13% (56 d), 25% and 19% (90 d), 29% (104 d) and 27% (106 d) soft body weight and a loss of the CI of 25% (56 d), 34% (104 d), 27% (106 d) were observed. However, none of the authors reached a starvation period of more than 159 days. Comparing the loss of the soft body weights (-3.1%) and CI (-66%) during the present study over the first 93 days suggests that there is a high interactive correlation between the CI and water content (%). The body soft water content increased within the first 93 days 1.6%, from 95 to 96.6%. During the further experimental time with a decrease of the CI a further increase of the average water content of finally 3.4% was observed. Consequently, with prolonged starvation, the mussel loose soft body weight (glycogen, protein and lipids according to Pleissner et al 2012 and Dare & Edwards 1975) while adding water to the remaining body tissue. There are only few studies about starvation and its influence upon *M. edulis* general condition. Starvation often occurs at times where primary production is reduced, like during winter periods (Dare & Edwards 1975; Dare 1976). Also, field mussels experience periods, which can be shorter or longer, with lower algal concentrations (Dolmer 2000a, b; Tweddle et al 2005; Nielsen & Maar 2007; Riisgård et al 2007; Saurel et al 2007). Starving *M. edulis* reduces the filtration rate by reducing its valve gape (Lassen et al 2006; Riisgård et al 2003, 2006; Saurel et al 2007). It is hypothesized that the decrease of valve opening reduces the oxygen uptake and with that the animal saves energy to reduce body-weight loss by the help of a reduced metabolism during starvation periods (Riisgård & Larsen 2014). Famme (1980) found in the mantle cavity of closed mussels reduced oxygen levels in comparison to open mussels to levels of 16%. The opening of the mussel valves is induced by minimum algal concentrations (Riisgård & Larsen 2014). Clausen & Riisgård (1996) determined these minimum concentrations, which are equivalent to cover mussel's maintenance, at 814 cells mL^{-1} (*Rhodomonas* sp.). In the case of closure of valves *M. edulis* also can live anaerobically several weeks. But the energy use of glycogen is much more efficient under aerobic conditions (about +94%) (de Zwann & Mathieu 1992; Gosling 2003). Therefore short valve openings between closures (Famme & Kofoed 1980) can be interpreted to save energy also to maintain low oxygen levels within the mantle cavity to stay under more efficient aerobic energy metabolism (Riisgård & Larsen 2014). The mussels during the present study also regularly changed between closed and open shells periods, demonstrating their attempts to feed while minimizing their nutrient demands and metabolic activities.

Pleissner et al (2012) found that starvation periods directly influence the glycogen content, demonstrating a strong correlation of the CI and specific glycogen content. Another source of energy for *M. edulis* is to metabolize body protein and lipid (Dare & Edwards 1975). Bayne & Thompson (1970) recorded unfed *M. edulis* to lose 57% weight over a period of 90 d at 15°C (±1°C) water temperature and values of 33.4 PSU (mean). *Mytilus edulis*-like specimens from the Baltic Sea lost 48% also at 15°C, however, after over 200 days. This suggests that Baltic Sea mussels are more resilient to prolonged starvation periods. In general, North Atlantic *Mytilus edulis* and Baltic Sea mussels differ also in general growth and size, which might be reflected in the presented smaller body weight loss during the present study.

The mussels increased their water content of the soft body tissue, from an average of 95% (1.85 g of 1.95 g) to 96.6% (1.83 g of 1.89 g) after 93 d and to 98.4% (0.53 g of 0.54 g) after 218 d. A higher water content inside the body tissue reduces the product quality, with importance for the aquaculture producers. Consequently, prolonged starvation periods do not influence the mortality under adequate environmental conditions but reduce the nutritional value of the mussels for the consumer. It seems as if the incorporation of water into soft body tissue is a kind of compensatory measure, and might be a good indicator for the mussel quality. Water content inside the mussels has not yet been allied to monitor mussel quality with respect to the customers. This factor should be monitored in future because it informs about the nutritional status of the mussels, the availability of sufficient food before harvesting, and its general quality.

According to our results as well as the available literature, mussels loose quality as soon as they underlie prolonged starvation periods with decreased natural primary production, as seen in low phytoplankton concentrations. Consequently, harvesting during periods with shorter or longer decreased natural primary production, e.g. during the colder months, should be avoided (Dolmer 2000a, b; Tweedle et al 2005; Nielsen & Maar 2007; Riisgård et al 2007, Saurel et al 2007). This also concerns the *Mytilus edulis*-like from the Kiel Fjord. Aquaculture producers have the possibilities to either monitor the surrounding food availability (density of phytoplankton) or can determine the soft body weight, water contents as well as the CI to assure their best mussel quality. However, the total length of the mussels it determined by the shell length, either by sampling representative specimen or a determined and tagged group of individuals which are continuously sampled over a period of time (Župan & Šarić 2014). This method is fast and easy but inadequate to inform about the real productivity and quality of a mussel culture. According to Davenport & Chen (1987) the CI used in this study is reliable and practical for scientific or commercial purposes. It is more time intensive, but already regular samplings of 10 individuals are sufficient to provide adequate results.

Conclusions. *Mytilus edulis* has a high capability to overcome long-time starvation periods. Starvation often and regularly occurs at times where primary production is reduced, like during winter periods. We herewith conclude that a decreasing CI and soft body weight, caused by metabolism of glycogen, protein and lipids, is correlated with an increase of soft body water content. The presented study is the first laboratory study over a long-term starvation period (218 days) of blue mussels in a low salinity habitat, like the western Baltic Sea. Higher water content inside the body tissues reduces the product quality, highly important for the aquaculture producers. In fact, prolonged starvation periods reduce the nutritional value of the mussels for the consumer. The incorporation of water into soft body tissue seems to be a kind of compensatory measure, and possible might be a good indicator for mussel quality. Water content inside the mussels has not yet been allied to monitor quality. In future this factor should be monitored because it informs about the nutritional status of the mussels, the availability of sufficient food before harvesting, and its general quality.

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