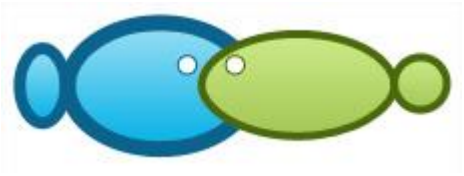

Fully controlled experimental recirculating aquaculture system (RAS) for experimental studies with mussels (*Mytilus edulis*-like), focusing on temperature and salinity regimes

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Abstract. Recirculating aquaculture systems (RAS) allow controlled cultivation of bivalves. RAS offer adjustable environmental conditions such as water temperature, salinity and stable water quality. In such systems, microalgae for mussel feeding are unfortunately filtered out by the common RAS techniques in use (regular biofilter, protein skimmer). We designed a new pilot RAS with an airlift biofilter and tested it with *Mytilus edulis*-like mussels under different temperature and salinity regimes, presenting a standardized technique for long term mussel studies under controlled conditions. In the first trial (12 weeks under feeding conditions), we found a significant decrease (3–43%) of the mussel condition indices (CI) with increasing temperatures (5-25°C). In a second trial (8 weeks), we verified earlier results under doubled feeding (twice a day). Significant differences were found between the temperatures 5°C to 20°C and 10°C to 20°C, however, no significant influence was seen at the tested salinities (10 PSU, 15 PSU, 20 PSU).We demonstrate that the presented small-scale RAS, including the part time use of an airlift biofilter, enabled long-time availability of introduced microalgae feed under experimental conditions, with filter feeders (*M. edulis*-like), excluding erratic influences of inadequate feeding during long term experiments with mussels and possibly other bivalves.
Key Words: blue mussel, condition index, temperature, bivalves, biofilter.

Introduction. Blue mussels (*Mytilus edulis*) are the favored consumed bivalve in Europe and cultivated for human consumption in aquaculture. Filtration of water suspended particles and plankton enables *M. edulis* nutrition (Storch & Welsch 2004) and growth. In the Baltic Sea, *M. edulis* and *M. trossulus* sympatric occur, which are considered as *M. edulis*-like (Stuckas et al 2017). The quantity of filtered seawater amounts 5-15 L (individual day)$^{-1}$, and is influenced, apart from feed concentration itself, mainly by salinity and temperature of the surrounding seawater (Gosling 2003). Nowadays, bivalve cultures are kept in the natural sea, flow-through systems or in recirculating aquaculture systems (RAS). In flow-through systems the introduced algae feed is removed by constant water exchange. There have been several studies upon bivalves in RAS, but such systems are not yet in commercial use (Blanco & Kamermans 2015; Joaquim et al 2014; Magnesen & Jacobsen 2012; Merino et al 2009; Pfeiffer & Rusch 2000; Suantika et al 2000; Xiongfei et al 2005).

Several advantages for the cultivation in RAS were identified, such as a stable water quality and a higher concentration of microbiota (Blancheton et al 2013), an increased biomass production, growth and survival at comparable costs compared to flow-through systems. Temperature control of the water further enabled independency from season and weather condition (Dunning et al 1998; Martins et al 2010; Besson et al 2014). The highest disadvantage of RAS was identified in the removal of introduced feed and the adjustment of predefined concentrations of microalgae, influenced by RAS techniques, e.g. regular biofilter and/or protein skimmer (Kamermans et al 2016). The method is costly, and contrasts the effort to mimic nature conditions in RAS such as the long-time availability of suspended feed for the bivalves to guarantee best feed supply.

Harbach & Palm (2017) presented an experimental setup to study *M. edulis* artificially contaminated with *Vibrio parahaemolyticus* under controlled conditions to estimate the influence of temperature and salinity upon feed uptake and persistence. This study showed the accumulation of nitrogen metabolites in the process water (+0.28 mg L^{-1} NO_2; +7.99 mg L^{-1} NO_3; +1.12 mg L^{-1} NH_4), over an experimental time of only 72h, revealing the need of a biological filter to maintain the water quality. Harbach & Palm (2018) demonstrated in a further developed system design, including a biological airlift filter, over a period of 8 months, an adequate range of water quality. The present study applied a similar methodology, however, with an additional part time airlift biofilter, at different combinations of temperatures and salinities, commonly found in the Western Baltic Sea. The blue mussel were kept over two periods, 12 (first trial) and 8 (second trial) weeks.

The purpose of the present study was to verify the results of Hiebenthal et al (2012), who tested *M. edulis*-like mussels at similar temperatures, but a higher salinity regime (our study: 10, 15, and 20 PSU; Hiebenthal et al 2012: 15, 25 and 35 PSU), under static conditions and by using natural Baltic Sea water. We expected a decrease of the condition index (CI) with increasing temperature (4-25°C, Hiebenthal et al 2012), and no significant influence of the salinity. However, we applied controlled water conditions, a larger water volume (10x) and mussel sampling size (5x). Through part time addition of an airlift biofilter, we kept water parameters stable, excluded any external influences and suggest a more standardized technology. Future possibilities to apply the presented cultivation technique for bivalves under RAS conditions are discussed.

Material and Method. *M. edulis*-like originated from a longline mariculture inside Kiel Fjord in the western Baltic Sea. 2 weeks prior to the start of the experiment, test individuals were transferred into a small-scale recirculation system and adapted to the temperature and salinity combinations (Figure 1). Adaptation occured according to the formula of Baur & Rapp (2003) (temperature difference (°C) x 3 = adaptation time (days)). The average length of the test individuals was 2.8 cm. Mussels were selected randomly with an initial stocking density of 100 *M. edulis*-like specimens per unit. Surfaces of individuals were cleaned from adhered materials prior to the start of the experiment. The German Animal Welfare Act does not affect mussels, so the experiments followed the rules of the 3-R-principle.

The experiments were set up in aquaria (60 x 30 x 30 cm) filled with 40 L artificial sea water (ASW, from "Tropic Marin"). Temperature was regulated by placing the aquaria in water filled wells (80 x 40 x 40 cm) connected to cooling unit. Aquaria were covered and a rotary pump guaranteed water movement and oxygen supply. Salinity and temperature were kept at the chosen levels (±1 PSU/±1°C). The seven selected combinations of temperatures and salinities tested in triplicates are given in Table 1.

To validate the results of the first trial the influence of the temperature on the CI was reexamined in a single factor second trial (Table 1). Therefore the resulting temperatures of the first trial, 5°C, 10°C and 20°C, each at 15 PSU, were chosen and again examined under identical conditions in triplicates for 8 weeks.

Table 1

Studied temperatures and salinity combinations (triplicates) tested for 12 weeks

Temperature (°C)	*Salinity (PSU)*		
	10	*15*	*20*
5		**√**	
10		**√**	
15	√	√	√
20		**√**	
25		√	

PSU- Practical Salinity Units; Bold indicates repetitively examined combinations (repetitive trial of 8 weeks).

To prevent accumulation of toxic metabolites (NO_2, NO_3, NH_4) inside the recirculating water, a biological water treatment was applied by an airlift with a filter foam sponge driven by an air pump, so a water flow of 80 L h^{-1} (2 x total water volume h^{-1}) was generated. A possible influence of the filtering technique upon the feed availability to the mussels within the experimental setup was analyzed prior to the experiments. The aim was to achieve the best feed availability (time and distribution in the water column) to the mussels. Low variation coefficient of the subsamples over time was expected, demonstrating an even distribution of the feed particles within the water column, whereas a high variation coefficient was associated with an uneven distribution of feed particles. Two variations were tested: airlift operating for 6 h and airlift not operating. 25 samples were taken over the entire period.

Mussels in each aquarium were fed over 12 weeks once day^{-1} (9 a.m.) with a microalgae suspension of 40,000 cell mL^{-1} (4.0x104 cell mL^{-1}) in the final water body (Trial 1). During Trial 2, mussels were fed for 8 weeks twice day^{-1} (9 a.m. and 3 p.m.). The suspension consisted half of *Phaedactylum tricornutum* 20,000 cell mL^{-1} (2.0x104 cell mL^{-1}) and half of *Tetraselmis suecica* 20,000 cell mL^{-1} (2.0x104 cell mL^{-1}). The concentration of algae cells mL^{-1} was determined by using a hemocytometer ("Neubauer improved"). The lower detection limit of the hemocytometer amounted 2,500 cell mL^{-1}.

Water parameters, temperature, salinity, oxygen content, pH, ammonia (NH_4), nitrite (NO_2) and nitrate (NO_3), were measured in intervals. Temperature, salinity and pH were measured daily. Ammonia, nitrite and nitrate were measured weekly by using wet chemical tests (JBL) adapted to photometrical measurement (HachLange DR 2800). NO_2, NO_3 and NH_4 limits were set to 3% of the lethal concentration (LC50) values (7 days) for *M. edulis* (Gregor 2008). Limits were set to 1.69 mg L^{-1} for NO_2, 300 mg L^{-1} for NO_3 and 2.66 mg L^{-1} for NH_4. To comply with the limits water exchange was executed as necessary.

20 specimens of mussels were sampled randomly from each treatment (Table 1) in monthly intervals (Trial 1 and Trial 2). In compliance with Bustnesj & Erikstadk (1990) and Mallet & Carver (1995) each mussel was individually examined according to the following procedure: *M. edulis* posterior adductor muscle was cut, the mussel was opened and prepared apart from the shells. The shell weight was documented and the *M. edulis* soft tissue was treated 48 h at 60°C to record the dry weight.

The condition index (CI) was calculated after the formula of Walne (1976):

$$CI = (\text{dry weight}/\text{shell weight}) * 100$$

With the help of CI comparisons of *M. edulis* general physiological condition are possible, which determine their fitness (Hiebenthal et al 2012). Statistical analyses were conducted using ANOVA with permutation test, a function of R-language version for Windows 3.1.0 (R Core Team).

Results. A lower total feed availability was found in the turned off airlift tested systems (-52.4% over 360 minutes), compared to the turned on airlift tested systems when. Feed availability was reduced by 61.9% over the first 120 minutes and by 35.9% on the next period up to 360 minutes, with a turned on airlift.

The feed particle distribution showed a variation coefficient of 56.4% (120 min) and 72.6% (360 min) under running airlift, while a variation coefficient of 32.6% (120 min) and 39.8% (360 min) was found under airlift turned off (Table 2, Table 3). The highest variation coefficients under running airlift showed a temporarily absence of particles inside the water column.

According to these results, best performance was achieved with the airlift turned off for two hours after application of the feed, offering a highly stable feed concentration and supply. During this period, each mussel filtrated a presumed volume of 4.8 L water (15°C=40±1.5 mL min^{-1}) (Riisgård 1991). By stopping the airlift only for 2 h after feeding, the variation coefficient of the observed algae concentrations was reduced to a minimum, 32.6% after 2 h and 39.8% after 6 h. This contrasted a higher variation coefficient with the running airlift, demonstrating uneven feed availability throughout the experiment.

Table 2

Feed availability (microalgae) inside the system with a running airlift

Time (min)	*Tetraselmis sp. (cells mL^{-1})*	*Phaeodactylum sp. (cells mL^{-1})*	*Sum (cells mL^{-1})*	*Variation coefficient (%)*
5	5,000	12,500	17,500	20.2
10	5,000	10,000	15,000	10.9
15	2,500	7,500	10,000	27.0
20	2,500	7,500	10,000	28.6
25	n.d.	n.d.	n.d.	28.6
30	2,500	10,000	12,500	25.1
35	5,000	10,000	15,000	22.7
40	n.d.	5,000	5,000	34.5
50	n.d.	2,500	2,500	47.2
60	n.d.	5,000	5,000	50.8
75	n.d.	7,500	7,500	50.0
90	n.d.	2,500	2,500	56.4
105	n.d.	n.d.	n.d.	56.4
120	n.d.	n.d.	n.d.	56.4
135	n.d.	2,500	2,500	61.5
150	n.d.	2,500	2,500	65.8
165	n.d.	n.d.	n.d.	65.8
180	n.d.	2,500	2,500	69.3
210	2,500	2,500	5,000	69.1
240	n.d.	10,000	10,000	66.0
255	2,500	2,500	5,000	65.9
270	n.d.	n.d.	n.d.	65.9
300	n.d.	2,500	2,500	68.4
330	2,500	n.d.	2,500	70.6
360	n.d.	2,500	2,500	72.6

min-minutes; n.d.-not detected.

Table 3

Feed availability (microalgae) inside the system with airlift turned off

Time (min)	*Tetraselmis sp. (cells mL^{-1})*	*Phaeodactylum sp. (cells mL^{-1})*	*Sum (cells mL^{-1})*	*Variation coefficient (%)*
5	n.d.	7,500	7,500	47.1
10	n.d.	12,500	12,500	35.4
15	7,500	7,500	15,000	32.7
20	2,500	7,500	10,000	28.7
25	2,500	17,500	20,000	37.0
30	n.d.	17,500	17,500	34.0
35	n.d.	15,000	15,000	30.8
40	n.d.	12,500	12,500	29.2
50	2,500	7,500	10,000	29.6
60	n.d.	n.d.	n.d.	29.6
75	n.d.	7,500	7,500	32.6
90	n.d.	7,500	7,500	34.6
105	2,500	10,000	12,500	33.0
120	n.d.	17,500	17,500	32.6
135	2,500	7,500	10,000	32.3
150	n.d.	7,500	7,500	33.7
165	2,500	n.d.	2,500	40.2
180	n.d.	12,500	12,500	38.7
210	2,500	2,500	5,000	41.2
240	2,500	10,000	12,500	39.9
255	2,500	7,500	10,000	39.2
270	5,000	10,000	15,000	38.2
300	2,500	2,500	5,000	40.2
330	2,500	7,500	10,000	39.5
360	2,500	5,000	7,500	39.8

min-minutes; n.d.-not detected.

Table 4

Water parameters and mortality of the aquaria tanks during the first trial

	T(°C)	*PSU*	*pH*	*NO_2*	*NO_3*	*NH_4*	*Mort.*
A1-A3 Ø/min/max T (°C)	10.5/10/11	15/14/16	7.3/6.7/8	0.8/0/1.6	39/1/79	1.4/0.4/2.4	27
B1-B3 Ø/min/max	19.8/19/21	15/14/16	7.3/6.5/8	0.8/0/1.6	55/4/114	1.3/0.4/2.5	19
C1-C3 Ø/min/max	5.3/4.2/6	15/14/16	7.4/6.8/7.8	0,8/0/1.6	30/1/68	1.4/0.4/2.6	18
D1-D3 Ø/min/max	15/14/16	10/9/11	7.5/6.8/8	0.8/0/1.6	60/3/119	1.2/0.2/2.5	19
E1-E3 Ø/min/max	15/14.3/16	15/14/16	7.2/6.2/8.2	0.6/0.1/1	23/9/47	1.1/0.6/1.7	12
F1-F3 Ø/min/max	24.6/24/25.4	15/14/16	7.1/6,4/7.8	0.6/0.1/1.1	48/5/99	1.1/0.5/2	258
G1-G3 ø/min/max	15/14.3/15.9	20/19/21	7.1/6.1/7.9	0.6/0.2/1.1	30/5/62	1.4/0.8/2	14

max-maximum; min-minimum; PSU-practical salinity units; T-Temperature; Ø-mean.

Table 5

Water parameters and mortality of the aquaria tanks during the second trial

	T(°C)	*PSU*	*pH*	*NO_2*	*NO_3*	*NH_4*	*Mort.*
A1-A3 Ø/min/max T (°C)	10.6/10/11	10/10/11	7.1/6.1/8	0.1/0/0.4	6/1/16	1/0.6/1.5	6
B1-B3 Ø/min/max	19.6/19/20.9	15/15/16	7.3/6.7/7.9	0.1/0/0.2	4/1/9	1.2/0.8/2.4	3
D1-D3 ø/min/maxMort.	4.9/4/6	15/14/16	7.5/7/8.2	0.4/0/0.9	14/1/28	1.4/0.7/2.1	3

max-maximum; min-minimum; Mort.-mortality; PSU-practical salinity units; T-temperature; Ø: mean.

Water parameters during the trials ranged within the intended limits. Minimum and maximum, mortality are presented in Table 4 (first trial) and Table 5 (second trial).

Influence of the salinity on blue mussel physiology was studied for 3 months. Initial CI values at 15 PSU and 20 PSU were remarkable lower than at 10 PSU. At all tested salinity levels the temperature was 15°C (±1°C). The found trends were: a decreasing CI from an initial value of 17.8 to 11.6 at salinity values of 10 PSU and an almost stable CI at an initial value of 7.0 to 7.1 and at an initial value of 7.0 to 7.2 at 15 and 20 PSU, respectively (Figure 1).

The influence of the temperature on the physiology of *M. edulis* was studied for three (Trial 1) and two (Trial 2) months. The pooled condition indices (n=240) and correlated standard deviations during the first 12 weeks are given in Figure 1. Due to high mortalities at 25°C this part of the experiment was finished ahead of schedule after one month. The initial CI values of mussels at 15°C and 25°C were lower compared to the other temperatures. In general, with the same feed availability, a decrease of the condition indices was found with an increase of the temperature (5–25°C). Significant CI differences were found for various temperatures. At 5°C average CI decreased from an initial value of 17.8 to 17.2 (-3.4%), at 10°C the average CI decreased from an initial value of 17.8 to 14.8 (-16.9%), at 15°C the average CI remained stable, between 7.0 and 7.1, at 20°C the average CI was reduced from an initial value of 17.8 to 9.4 (-47.2%) and at 25°C the average CI decreased from an initial value of 7.0 to 4.0, after only one month (-42.9%).

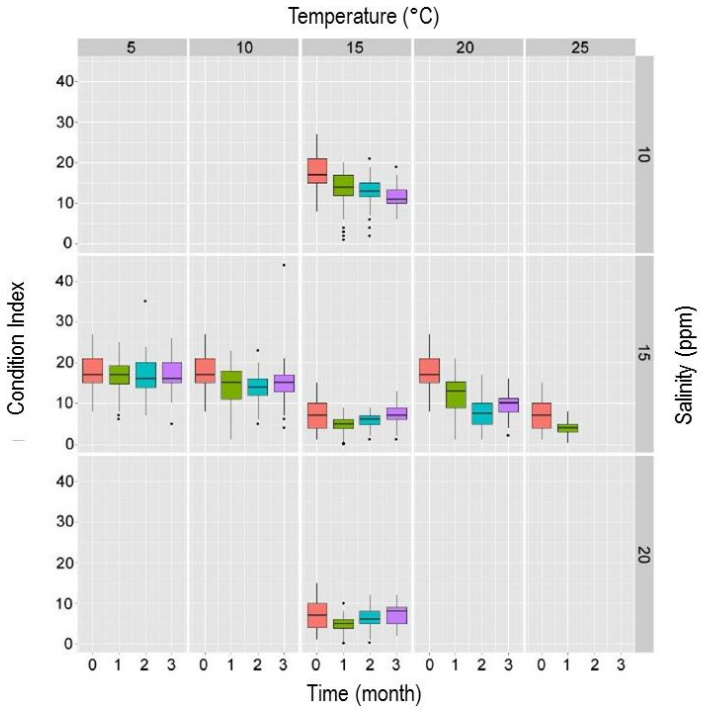

Figure 1. First trial CI and standard deviations of blue mussels (n=20) over 12 weeks.

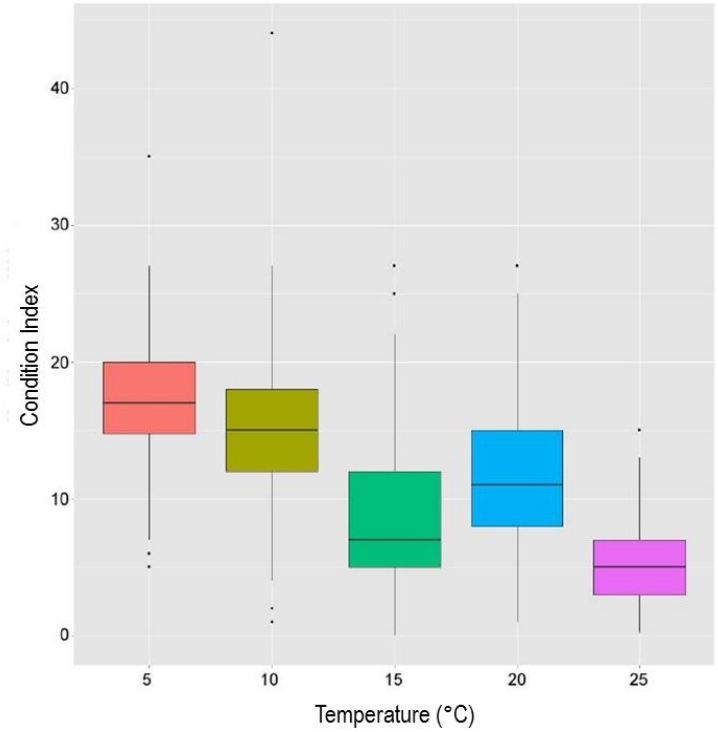

Figure 2. Pooled condition indices (12 weeks/25°C: 4 weeks) with standard deviations of the temperatures 5°C, 10°C, 15°C, 20°C and 25°C.

Testing a possible influence of temperature on the *M. edulis* physiology showed a similar result for the repetitive trials, during a two months period. Significant differences were found between the CI values at the temperatures 5°C to 20°C and 10°C to 20°C, and between the individual samplings (0-2) at 5°C, 10°C and 20°C temperature (Figure 3). CI at 5°C increased from an initial value of 15.2 to19.6 (+29%), remained stable at an initial value of 15.2 to 15.4 at 10°C and decreased from an initial value of 15.2 to 12.6 (-17.1%) at 20°C.

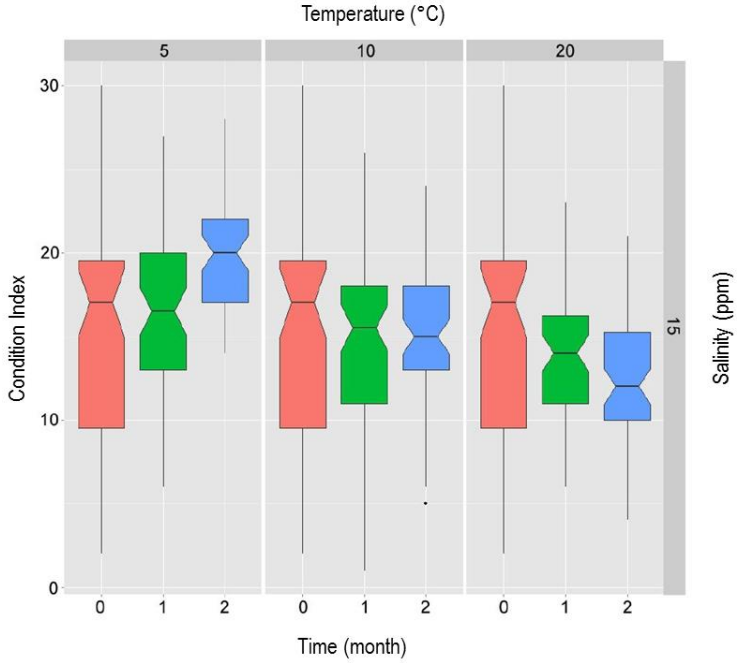

Figure 3. Repetitive trial development of CI at 5°C, 10°C and 20°C at 15 PSU over 8 weeks.

Discussion. This study demonstrates for the first time, that our new designed experimental RAS enabled long-time availability of microalgae feed inside a fully recirculating system, under cultivation of filter feeders, in this case *M. edulis*-like (Bivalvia). At the same time, the water parameters kept stable, demonstrating a functioning biofilter. The results of this RAS system for future experimental cultivation of bivalves are thus promising.

The highest disadvantage of RAS cultivation of bivalves is the removal of predefined feed concentration in the water column by the biofilter or the protein skimmer (Kamermans et al 2016), used to maintain water quality. We found, in accordance to earlier studies (Kamermans et al 2016), an influence of the installed biofilter on the availability of microalgae inside the water column. However, the installed airlift biofilter did not fully eliminate the microalgae. The number of countable microalgae fluctuated instead, and it was not possible to keep the amount of algae feed inside an intended range. Better performance was achieved by shutting down the airlift for two hours directly after addition of the algae feed, as seen in relatively low variation coefficients. This can be explained through the direct filter feeding activity of the mussels immediately after feeding, reducing the amount of left over feed that subsequently can be filtered and removed through the biofilter. The airlift had the advantage that this filter did not completely retain the feed over 360 minutes, allowing a certain amount of algae to pass through unharmed. After 360 minutes, the whole water body had been completely filtered through the airlift filter for approximately 12 times (80 L h^{-1}). Consequently, this high filtration rate provided the required biological treatment for an adequate water quality, without eliminating the feed given to the mussels.

We kept mean value concentrations of microalgae at 6.9x103 cells mL^{-1} under operating airlift and 10.9x103 cells mL^{-1} without the airlift. These values are in accordance to the values determined by Riisgård et al (2011) for *M. edulis* filtration capacity, over extended time periods, ranging between 5x103 and 8x103 (*Rhodomonas salina*) cells mL^{-1}, confirmed also by Pleissner et al (2013), who suggested values ranging from 6x103 to 7x103 (*R. salina*) cells mL^{-1}. Based on these results, the applied feed input during the present study was appropriate. An optimal feeding regime was also seen through the absence of pseudo-faeces or shell closure, indicating adequate mussel health, while the water quality was effectively maintained by the RAS. We herewith used

two algae species, *Tetraselmis suecica* and *Phaedactylum tricornutum*. Aghzar et al (2013) could not detect any nutritional differences for *M. edulis* between diets consisting of the species *P. tricornutum*, *T. suecica*, *Isochrysis galbana* and *Chaetoceros gracilis*, allowing the assumption that the nutritional value of our applied microalgae suspension was appropriate for the *M. edulis*-like feeding. By choosing a feeding regime of once a day during the first trial and twice per day during the second trial, enough feed was theoretically provided to the mussels for 6 and 12 hours, respectively. Consequently, our mussels were not overfed but kept on an adequate nutrient level during the run of the experiments.

Similarly to Hiebenthal et al (2012), the CI continuously decreases from 4 to 25°C. Unfortunately no initial CI data and no exact final CI were given by the authors, requiring interpretation of Figure 3. Hiebenthal et al (2012) found higher CI decreases than the current study: -27% at 10°C, -36% at 15°C, -45% at 20°C, -54% at 25°C. These results are directly comparable to our results, -14% (first trial) and -21% (second trial) at 10°C, -45% (first trial) and -36% (second trial) at 20°C. We recorded stable CI values (7.0 to 7.1) at 15°C, probably due to the effect of a lower initial CI (-61%) of the studied cohort. The same pattern was observed at 25°C after one month.

The mussel size, between 13.3 and 26.5 mm (mean 19.0±2.23 SD), and the genetics of the tested *M. edulis* specimens in the current study can be considered comparable to the characteristics of the specimens from the research study of Hiebenthal et al (2012), also originating from Kiel Fjord (southwestern Kiel Bight). The authors used natural Baltic Sea water in their study and their experiments were conducted in smaller containers (4 L) and at a stocking density of 12 individuals. We used artificial sea water and our experiments were conducted in 40 L containers with 100 individuals. The water quality parameters (N-metabolites) of the single static systems used by Hiebenthal et al (2012) were not mentioned, being maintained by a twice a week water exchange of one-eighth of the total water volume (0.5 L). Their natural sea water was not treated before usage, supplying natural feed of undetermined amount. We therefore can state several advantages of our experimental RAS. The water exchange of one-eighth of the water volume equals a total exchange of 4,200 L over the entire experiment. Through the installation of an airlift as biofilter, the maintenance and thus the human labor costs were very low, and the system was running very stable without causing additional interventions, mussels' performances disturbance, or overall experimental results flaws.

We could demonstrate that the CI of the *M. edulis* increased at lower temperatures (5°C), remained more stable at mid-range temperatures (10°C) and decreased at higher temperatures (25°C), with this adverse effect already beginning at 20°C. The mussels showed best development of the CI under low temperatures, of 5°C. This temperature implies a reduced metabolism in poikilothermic animals (Bayne 1976; Vernberg & Vernberg 1969). The blue mussel-like physiology and feed conversion is adapted to low temperatures. It is important to note that in Schulte (1975), the filtration rates of *M. edulis* at 5°C were found reduced to very low values (350 mL h^{-1}). This would also lead to a reduced feed requirement in comparison to the mussels living at higher water temperatures. According to Coulthard (1929) and Widdows (1973), the temperature optimum for *M. edulis* was determined between 10 and 20°C, with a higher feed uptake rate and possible growth rate, that would also increase the CI. Consequently, the reduced filtration rate and feed uptake at low temperatures is even compensated by a lower metabolic rate, resulting in a better development of the CI, compared with higher temperatures.

The influence of the salinity upon the CI of *M. edulis* was studied at 10, 15 and 20 PSU, with better performance at 15 and 20 PSU. Studies about the effect of the salinity upon the blue mussel physiology are scarce. Remane & Schlieper (1971) observed that *M. edulis* showed reduced growth and total length at 4-5 PSU. Livingstone et al (1979) found that a salinity of 20-30 PSU did not influence growth of *M. edulis*, whereas 15 PSU resulted in reduced growth. Hiebenthal et al (2012) found no significant influence of salinity (15, 25, 35 PSU) upon the CI of *M. edulis*. The findings of this study were not significant, but had the same tendencies, showing a negative effect onto the mussel physiology by lower salinities, with a decreasing CI below 10 PSU. Presumably the low

salinity requires higher physiological costs through an increased energy demand for the adaptation of the intracellular concentrations of ions and amino acids (Bayne & Newell 1983). The non-significant difference between 10 and 20 PSU might be an adaptation of the *M. edulis*-like to the specific environmental conditions in the Western Baltic Sea, where the salinity fluctuates within this range.

Conclusions. We conclude that our newly designed RAS enables long-time experiments on the performance of *M. edulis*-like (Bivalvia) under constant water conditions. Through inclusion of an airlift biofilter combined with no filter activity for 2 hours after addition of microalgae feed, the system allowed adequate feeding of the bivalves while maintaining stable and adequate water quality. Such experimental RAS allow studies of the bivalve physiology and a better understanding of mussel growth performance in the field. Another advantage is an experimental setup independently of the location of the laboratory (far from the sea), not causing the risk of improper water characteristics through the water exchange with natural sea water. The system enables reproducible experiments with a minimum of surveillance and labor force.

Acknowledgements. The authors would like to thank Dr. A. Mueller-Belecke for helpful discussions, for critically reading the manuscript and for the continuous support. In addition, we thank the Federal Institute for Risk Assessment and its staff whose cooperation was crucial to the operational implementation. Special thanks to Martin Teterra for the voluntary commitment.

References

Aghzar A., Miñambres M., Alvarez P., Presa P., 2013 A cost-benefit assessment of two multi-species algae diets for juveniles of *Mytilus galloprovincialis*. Thalassas 29:9-16.

Baur W. H., Rapp J., 2003 [Healthy fish: Practical guide to prevent identifying and treating fish diseases]. MVS Medizinverlage Stuttgart, pp. 26-33. [In German].

Bayne B. L., 1976 Marine mussels: their ecology and physiology. Cambridge University Press, New York, pp. 38-120.

Bayne B. L., Newell R. C., 1983 Physiological energetics of marine mollusks. In: The Mollusca. Vol. 4, Physiology, Part 1. Saleuddin A. S. M., Wilbur K. M. (eds), pp. 407-515, Academic Press, New York.

Blanco G. A., Kamermans P., 2015 Optimization of blue mussel (*Mytilus edulis*) seed culture using recirculation aquaculture systems. Aquaculture Research 46(4):977-986.

Blancheton J. P., Attramadal K. J. K., Michaud L., Roque D. E., Vadstein O., 2013 Insight into bacterial population in aquaculture systems and its implication. Aquacultural Engineering 53:30-39.

Besson M., Komen H., Aubin J., de Boer I. J. M., Poelman M., Quillet E., Vancoillie C., Vandeputte M., van Arendonk J. A. M., 2014 Economic values of growth and feed efficiency for fish farming in recirculating aquaculture system with density and nitrogen output limitations: a case study with African catfish (*Clarias gariepinus*). Journal of Animal Science 92:5394–5405.

Bustnesj O., Erikstadk E., 1990 Size selection of common mussels, *Mytilus edulis*, by common eiders, *Somateria mollissima*: energy maximization or shell weight minimization. Canadian Journal of Zoology 8:2280-2283.

Coulthard H. S., 1929 Growth of the sea mussel. Contributions to Canadian Biology and Fisheries 4:123-136.

Dunning R. D., Losordo T. M., Hobbs A. O., 1998 The economics of recirculating tank systems: a spreadsheet for individual analysis. Southern Regional Aquaculture Center Publication No. 456, 8 p.

Gosling E., 2003 Bivalve molluscs. Biology, ecology and culture. Fishing News Books, division of Blackwell Publishing, Oxford, pp. 87-130.

Gregor N., 2008 [Toxicity of various nitrogen metabolites on *Mytilus edulis* L.] BSc Thesis, Humboldt-University of Berlin, pp. 64-67. [In German].
Harbach H., Palm H. W., 2017 Risk reduction of potential *Vibrio parahaemolyticus* transfer via blue mussels (*Mytilus edulis* L.) into the consumer in the Baltic Sea Region. Journal of Food Safety and Food Quality 68:106-111.
Harbach H., Palm H. W., 2018 Development of general condition and flesh water content of long-time starved *Mytilus edulis*-like under experimental conditions. AACL Bioflux 11(2):301-308.
Hiebenthal C., Philipp E. E. R., Eisenhauer A., Wahl M., 2012 Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and *Arctica islandica*. Aquatic Biology 14:289-298.
Joaquim S., Matias D., Matias A. M., Leitão A., Soares F., Cabral M., Chícharo L., Gaspar M. B., 2014 The effect of density in larval rearing of the pullet carpet shell *Venerupis corrugata* (Gmelin, 1791) in a recirculating aquaculture system. Aquaculture Research 47:1055–1066.
Kamermans P., Blanco A., Joaquim S., Matias D., Magnesen T., Nicolas J. L., Petton B., Robert R., 2016 Recirculation nursery systems for bivalves. Aquaculture International 24:827-842.
Livingstone D. R., Widdows J., Fieth P., 1979 Aspects of nitrogen metabolism of the common mussel, *Mytilus edilus*: adaptation to abrupt and fluctuating changes in salinity. Marine Biology 53:41-55.
Magnesen T., Jacobsen A., 2012 Effect of water recirculation on seawater quality and production of scallop (*Pecten maximus*) larvae. Aquacultural Engineering 47:1–6.
Mallet A. L., Carver C. E., 1995 Comparative growth and survival patterns of *Mytilus trossulus* and *Mytilus edulis* in Atlantic Canada. Canadian Journal of Fisheries and Aquatic Sciences Sciences 52:1873-1880.
Martins C. I. M., Eding E. H., Verdegem M. C. J., Heinsbroek L. T. N., Schneider O., Blancheton J. P., d'Orbcastel E. R., Verreth J. A. J., 2010 New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. Aquacultural Engineering 43:83–93.
Merino G., Uribe E., Soria G., von Brand E., 2009 A comparison of larval production of the northern scallop, *Argopecten purpuratus*, in closed and recirculating culture systems. Aquacultural Engineering 40:95–103.
Pfeiffer T. J., Rusch K. A., 2000 An integrated system for microalgal and nursery seed clam culture. Aquacultural Engineering 24:15–31.
Pleissner D., Lundgreen K., Lüskow F., Riisgård H. U., 2013 Fluorometer controlled apparatus (FCA) designed for long duration algal-feeding experiments and environmental effect studies with mussels. Journal of Marine Biology 902152:12.
Remane A., Schlieper C., 1971 [Biology of brackish water]. Schweitzerbart'sche Verlagsbuchhandlung, Stuttgart, 372 p.
Riisgård H. U., 1991 Filtration rate and growth in the blue mussel, *Mytilus edulis* Linneaus 1758: Dependence on algal concentration. Journal of Shellfish Research 10:29-35.
Riisgård H. U., Egede P. P., Saavedra I. B., 2011 Feeding behavior of the mussel, *Mytilus edulis*: New observations, with a mini review of current knowledge. Journal of Marine Biology Article ID312459, https://doi.org/10.1155/2011/312459.
Schulte E. H., 1975 Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. Marine Biology 30:331-341.
Storch V., Welsch U., 2004 [Systematic zoology]. Spektrum Akademischer Verlag, Heidelberg, pp. 191-196. [In German].
Stuckas H., Knöbel L., Schade H., Breusing C., Hinrichsen H. H., Bartel M., Langguth K., Melzner F., 2017 Combining hydrodynamic modelling with genetics: can passive larval drift shape the genetic structure of Baltic *Mytilus* populations? Molecular Ecology 26:2765-2782.
Suantika G., Dhert P., Nurhudah M., Sorgeloos P., 2000 High density production of the rotifer *Brachionus plicatilis* in a recirculation system: consideration of water quality, zootechnical and nutritional aspects. Aquacultural Engineering 21:201–214.

Vernberg F. J., Vernberg W. B., 1969 Thermal influence on invertebrate respiration. Chesapeake Science 10:234-240.
Widdows J., 1973 The effects of temperature on the metabolism and activity of *Mytilus edulis*. Netherlands Journal of Sea Research 7:387-398.
Xiongfei W., Zhidong Z., Deshang L., Kangmei C., Zhuanshang T., Liegang S., Kaichong X., Bailin G., 2005 Closed recirculating system for shrimp-mollusk polyculture. Chinese Journal of Oceanology and Limnology 23:461–468.
*** German Animal Welfare Act, 2020 Bundeministerium der Justiz und für Verbraucherschutz. Tierschutzgesetz, http:// http://www.gesetze-im-internet.de/tierschg/BJNR012770972.html. (Accessed on 23 July 2020).

Received:15 July 2020. Accepted: 21 September 2020. Published online: 05 October 2020.
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How to cite this article:
Harbach H., Palm H. W., 2020 Fully controlled experimental recirculating aquaculture system (RAS) for experimental studies with mussels (*Mytilus edulis*-like), focusing on temperature and salinity regimes. AACL Bioflux 13(5):2694-2704.