Unionid metabolic responses to food availability

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Abstract

The metabolic activity of benthic invertebrates, including unionid mussels, influences the oxygen fluxes and physicochemical characteristics in aquatic systems. Unionid oxygen consumption rate (OC) and its dependency on food availability is understudied. In laboratory conditions, the OC of *Anodonta anatina* and *Unio pictorum* is quantified in response to food availability—0.05, 6.0 and 12.0 mg of Ash Free Dry Mass of *Chlorella vulgaris* l^{-1}—and dry soft-tissue mass (DM) at 19 ± 1 °C. Following a 5-hour feeding-period, the OC during a 2-hour digestion-period increases with algal concentration and DM in both species. The mean OC DM^{-1} increases with the algal concentration from 0.56 to 0.64 and 0.68 mg O_2 g^{-1} h^{-1} by *A. anatina* and from 0.16 to 0.19 and 0.22 mg O_2 g^{-1} h^{-1} by *U. pictorum*, with a significant difference between the two species. The *A. anatina* OC DM^{-1} decreases with the increasing DM. Digestion significantly contributes to unionid metabolism. The interspecific differences on OC depend on food availability and are potentially influenced by feeding behaviour. The pressure of cultural eutrophication on unionid metabolic activity and its ecological implications in benthic systems require further examination.

Keywords: digestion, freshwater mussels, opening behaviour, oxygen consumption, phytoplankton.

1. Introduction

The metabolic activity of aquatic invertebrates is driven by internal and external factors such as the body mass, ambient temperature and food availability (Newell *et al.*, 1976). In freshwater mussels of the Unionida order, known as unionids, the body mass's positive correlation to the metabolic activity as well as the species-specific differences have been long demonstrated (Ornatowski, 1967). According to the metabolic theory of ecology, the mass-specific metabolic rate is higher in smaller-bodied animals in comparison to larger-bodied animals within a trophic level under similar temperatures (Brown *et al.*, 2004).

The ambient temperature is crucial in determining the metabolic activity in ectothermic organisms (Clarke and Fraser, 2004). Previous research has shown the positive correlation between unionid respiration rate, oxygen consumption and temperature (Huebner, 1982; Ravera and Sprocati, 1997; Pusch *et al.*, 2001; Lurman *et al.*, 2014b, 2014a; Ganser *et al.*, 2015). Further to its temperature dependency, unionid oxygen consumption is influenced by oxygen availability with mussel activity

and size-, species- and life-stage-specific differences (Massabuau *et al.*, 1991; Polhill and Dimock, 1996; Ravera and Sprocati, 1997; Chen *et al.*, 2001; Lurman *et al.*, 2014a, 2014b). Species differences were, for example, shown in a 25 % higher oxygen consumption rate per dry soft-tissue mass by *A. anatina* compared to *U. tumidus* at 19 °C (Pusch *et al.*, 2001). It is not clear whether this difference is species- or genera-specific and further research, among different species, is required. Additionally, behaviours such as filtration, opening, locomotion and borrowing affect oxygen consumption (Pusch *et al.*, 2001; Lurman *et al.*, 2014a, 2014b). Food availability has been studied in relation to filtration and clearance (Pusch *et al.*, 2001; Bontes *et al.*, 2007; Tuttle-Raycraft *et al.*, 2017) and it has been demonstrated that at constant temperatures and an increasing food availability, the filtration volume required for meeting the animal's metabolic needs decreases, resulting in valve closure (Riisgård and Randløv, 1981). While the increasing food availability is associated with decreasing clearance rates (Bontes *et al.*, 2007; Tuttle-Raycraft *et al.*, 2017), the direct influence of food availability on unionid metabolism and digestion activity remain understudied.

Previous research on unionid digestion showed that digestion time ranges between 13 ± 6 hours and is influenced by food availability (Bril *et al.*, 2014). Whilst the enzymatic activity of digestion was recently studied (Sauey *et al.*, 2015, 2016), the importance of food concentration to digestion associated oxygen consumption has not been addressed. Nonetheless in their research on influence of the seston concentration to unionid respiration, Pusch *et al.* (2001) showed positive linear relationships between seston concentration and retention rate by *Anodonta anatina* and *Unio tumidus*, a positive linear relationship between clearance and respiration rates by *A. anatina* and a negative linear relationship between the clearance rate and respiration by *U. tumidus*. This diverging pattern indicates that individual processes contributing to the metabolic activity may respond differently to food availability while being subject to species-specific differences, affecting the ecosystem processes such as nutrient cycling dynamics (Atkinson and Vaughn, 2015).

Freshwater ecosystems are subject to anthropogenic pressures resulting in nutrient loading, increased primary productivity and eutrophic conditions (Hamilton *et al.*, 2018). Understanding the influence of eutrophication on abundant benthic invertebrates such as unionids and the individual metabolic processes—i.e. opening, filtration, locomotion and digestion—is crucial for predicting ecological interactions. These processes are expected to alter the physicochemical environment in the sediment-water interface. This is particularly important in shallow stratified lentic ecosystems where the benthic processes have a proportionally greater influence on the overlying waters.

The aim of this study is to examine the unionid oxygen consumption rate during digestion based on food availability and to understand the potential implications for freshwater systems. Due to the varying metabolic responses previously reported by Pusch *et al.* (2001) between the *Anodonta* and

Unio families, *A. anatina* and *U. pictorum* are selected for examining species-specific differences in oxygen consumption associated with digestion.

For conducting accurate assessments of unionids' metabolic responses, the accurate biometric indices, such as soft-tissue biomass, need to be considered. Linear relationships between shell biometrics, shown in this study, have been previously documented for native European unionids by Aldridge (1999). Nonetheless, population-specific differences may result from the varying environmental conditions to which the source populations are exposed (Zając *et al.*, 2018). Consequently, biometric readings of shell size and soft tissue mass are recorded for the populations used in this study and are presented in the first section of the results and the discussion. Linear regression models of biometrics relating to the body mass of the populations assessed are then used for examining the following hypothesis:

H1. Oxygen consumption rate significantly increases with the (i) mussel's dry soft-tissue mass (DM), (ii) algal concentration, (iii) mussel opening during the feeding-period and (iv) mussel opening during the digestion-period in both unionid species

H2. *A. anatina* has a significantly higher oxygen consumption rate per DM unit than *U. pictorum*

H3. The oxygen consumption per DM unit significantly decreases with specimens DM in both species

2. Methods

2.1 Specimen collection

Mussels were collected from the sites shown in Table 1 and any attached flora and fauna were removed from the shells. To prevent contamination and kill larvae of zebra mussels, *Dreissena polymorpha*, found in Mapperley reservoir, the shells of *U. pictorum* collected from the reservoir were cleaned with 5 % acetic acid (CH3COOH) (DiVittorio *et al.*, 2012; Davis *et al.*, 2015).

Table 1 Location and date of collection of the mussels

Species	Number of specimens	Site and Grid reference	Date of collection	Mean length (mm)	Standard deviation (mm)
U. pictorum	30	Mapperley reservoir, Ilkeston SK 43478 43600	11 Jan 2018	89.0	9.9
A. anatina	45	Markeaton brook, Derby SK 33973 37293	23 July 2018	62.7	7.4

2.2 Unionid biometrics

Two data sets on unionid biometrics were collected. The first data set consists of the shell length, width, height and hinge size correlations of *A. anatina* and *U. pictorum* specimens previously

collected from the sites shown in Table 1. To identify the strongest metric in explaining biometric variations, a correlation matrix was developed with the bivariate data analysed by Pearson correlation test, using the "cor" function in R 3.2.2 (R Core Team, 2016). To obtain the DM, the specimens' soft tissue was removed and dried overnight, or until stable in a "Gallenkamp (Sanyo/Weiss) Hot Box Size 2 Oven" at 105 °C, and the DM was weighted on a "Kern ABJ-NM/ABS-N" scale with a readability of 0.1 mg. The organic matter content was then combusted in a "Carbolite ELF 11/14" muffle furnace at 550 °C for 4 hours. The samples were re-weighted to obtain the Ash Mass and calculate the Ash Free Dry Mass (AFDM). Linear models (LM) were used to model the DM and AFDM dependent on shell length (Fig. 2). The models utilised a quadratic power form to reflect the non-proportional increase of DM in relation to length, observed in data exploration. The model assumptions were checked using standard residual diagnostics (Zuur *et al.*, 2009).

The second data set involved the readings of the shell length, volume and mass of the living specimens used in the experiments (Table 1), and the relationships were modelled by LM. The mass and volume LM dependent on shell length utilised a quadratic power form. Model assumptions were checked using standard residual diagnostics (Zuur *et al.*, 2009); one overly influential point was removed from the Mass LM which corrected a heteroscedasticity issue.

2.3 Experimental design

The experiments were conducted between the 8th and the 15th of August 2018 and started between 09:00 and 10:00 am by placing each mussel in a 200 ml beaker, in tanks with base dimensions of 37 * 21 cm, (surface area of 0.077 m^2). The tanks were filled with 10 l of dechlorinated tap water and placed in a water bath maintaining the temperature at 19 ± 1 °C.

Chlorella vulgaris (strain CCAP 211/74) was cultured in shaking batch cultures in Jaworski's Medium at 18 °C with light provided by Arcadia T5 Tropical Pro 54W 46" (FO54T5) between 06:00 am and midnight. Before each experiment, *C. vulgaris* was added to the tank resulting in the following concentrations: 0.05, 6.0 and 12.0 mg AFDM *C. vulgaris* l^{-1}; the algal distribution was homogenised by a 5-minute aeration-period through an air stone. The algal concentration was measured spectrophotometrically and calculated by previously developed standard curves. Following that, every hour, spectrophotometric readings were taken and the *C. vulgaris* concentration was replenished to the targeted concentration (0.05, 6.0 and 12.0 mg AFDM l^{-1}) followed by a 5-minute aeration-period. The 0.05 mg AFDM l^{-1} was below the detectable limit of the spectrophotometric threshold and 0.05 mg AFDM l^{-1} were added on an hourly basis.

For the experiments, all specimens, following a 24-hour starvation-period, were exposed to the aforementioned algal concentrations during a 5-hour feeding-period. The opening behaviour of the mussels was recorded at the start of the experiment and on hourly intervals. To ensure that filtration

and ingestion were taking place during the feeding-period, the production of faeces and pseudofaeces by each specimen were recorded. Following the 5-hour feeding-period, 24 specimens from each treatment (2 species and 3 algal concentrations) were placed separately in 1 l sealed glass jars filled with dechlorinated tap water (n = 144) along with 6 controls for each treatment without mussel (n = 36), and placed in the water bath (Fig. 1). This is referred to as the digestion-period, during which the opening behaviour of the mussels was noted every 30 minutes. At the end of the 2-hour digestion-period, after mechanical mixing, the temperature, pH, oxygen concentration and saturation were recorded, as shown in Fig. 1d. These values were subtracted by the mean of the six randomly selected water samples tested at the beginning of the experiment on that day.

A semi-quantitative approach was applied to the opening behaviour of the mussels during the feeding-period and digestion-period in the glass jars. An opening score of 0 was given for closed valves, 1 for semi-open valves with no visible siphons, and 2 for widely open valves and extended siphons indicating filtration. Each specimen's mean score was calculated for the feeding and the digestion-periods.

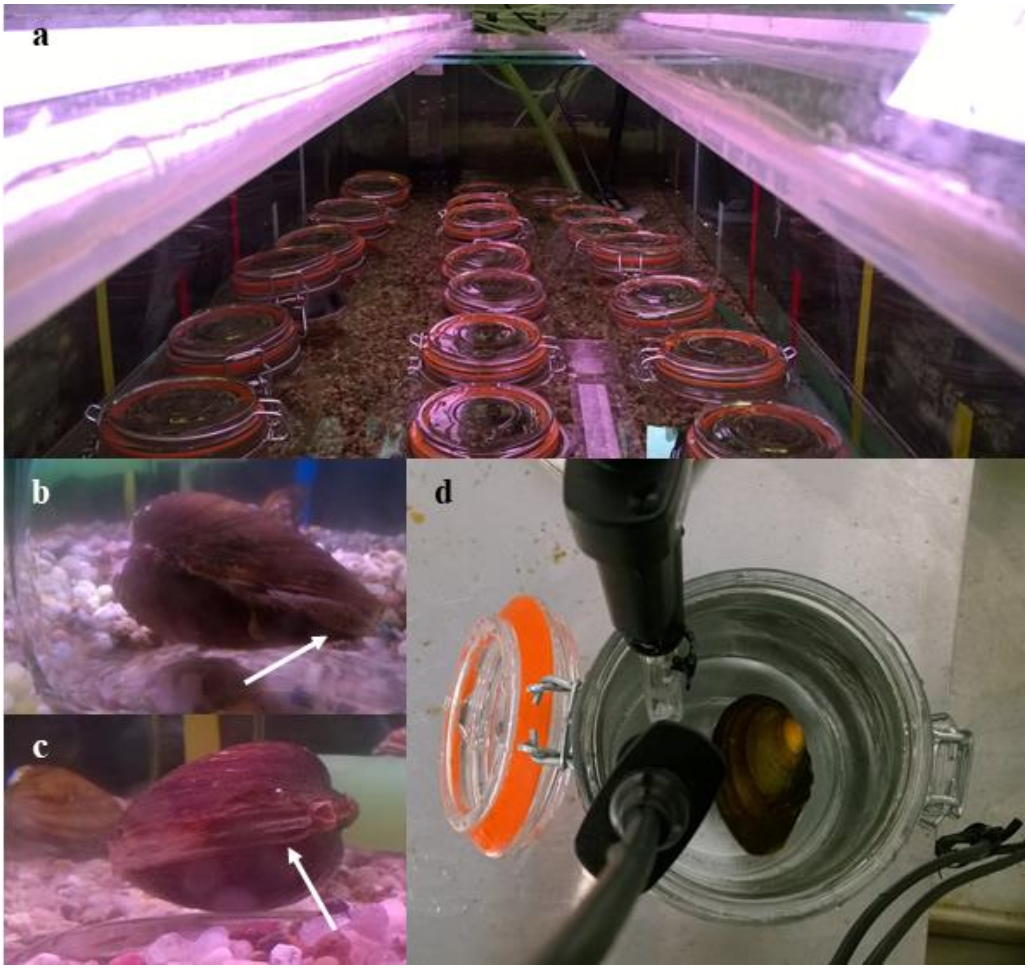

Fig. 1 (**a**) Experimental design of with the 1 l sealed glass jars in the water bath during the digestion-period. Characteristic siphon positioning behaviour of open *U. pictorum* specimens with the white arrow indicating the (**b**) outward and (**c**) inward facing siphon. (**d**) The O_2, pH and temperature readings at the end of *an A. anatina* experiment.

LMs were used to model 'oxygen consumption rate' and 'oxygen consumption rate per DM' dependent on algal concentration, and opening score dependent on algal concentration, mussel species, DM and opening behaviour. Model assumptions were checked using the standard residual diagnostics (Zuur *et al.*, 2009) and one overly influential point obtained by a *U. pictorum* specimen that died shortly after the last experiment was removed. To identify the factors significantly affecting the oxygen consumption rate, the minimum adequate model was selected using the "drop1" function. Comparison of the different models was based on the Akaike Information Criterion (AIC). For the multidimensional LM on oxygen consumption rate the recorded parameters—unionid species, DM, algal concentration, mean opening score during feeding, mean opening score during digestion and temperature—as well as the two-way interaction effects between species, DM and algal concentration were tested. For the multidimensional LM on oxygen consumption rate per DM of *A. anatina*, no interaction effects could be tested without compromising the power of the model. For avoiding collinearity, pH was not included as a factor in these multidimensional models (Appendix Table 5) due to its correlation with oxygen consumption (Fig. 5c) resultant by carbon dioxide production and carbonic acid formation (Harned and Davis, 1943).

3. Results

3.1 Biometrics

The correlation coefficients showed a significant positive relationship between all the biometric indicators measured: length, height, width and hinge ($p < 0.001$; Table 2). The lowest coefficient was 0.84 in *A. anatina* (n = 42) and 0.93 in *U. pictorum* (n = 27). The shell length showed the strongest positive correlation with the other three metrics in both species (Table 2).

The quadratic LMs describing the DM (Fig. 2a) and AFDM (Fig. 2a) dependency on shell length were significant for both unionid species with an increase of both metrics with shell length ($p < 0.001$ in all models; Appendix Table 1). The DM was comparable between the two species while the AFDM indicated a higher organic content in *U. pictorum* compared to *A. anatina*. This appeared to be more prominent in larger specimens. This was confirmed by the data that showed a mean percentage combustion of organic matter of 85.8 % with a Standard Deviation (SD) of 5.14 for *U. pictorum* and 74.8 % with a SD of 7.27 for *A. anatina*. The percentage of organic soft-tissue content (AFDM / DM) of *A. anatina* decreased significantly with the increasing DM ($p > 0.001$; Fig. 2c; Appendix Table 1). The organic content of *U. pictorum* was not influenced by the mussel's DM; no significant model is found.

Table 2 Pearson correlation matrices between the biometrics—length, height, width and hinge—in *A. anatina* (n = 42) and *U. pictorum* (n = 27); all the correlations are significant with a $p < 0.001$.

A. anatina	**Length**	**Height**	**Width**	**Hinge**
Length	1	0.97	0.95	0.88
Height	-	1	0.93	0.84
Width	-	-	1	0.86
Hinge	-	-	-	1

U. pictorum	**Length**	**Height**	**Width**	**Hinge**
Length	1	0.97	0.98	0.97
Height	-	1	0.96	0.93
Width	-	-	1	0.97
Hinge	-	-	-	1

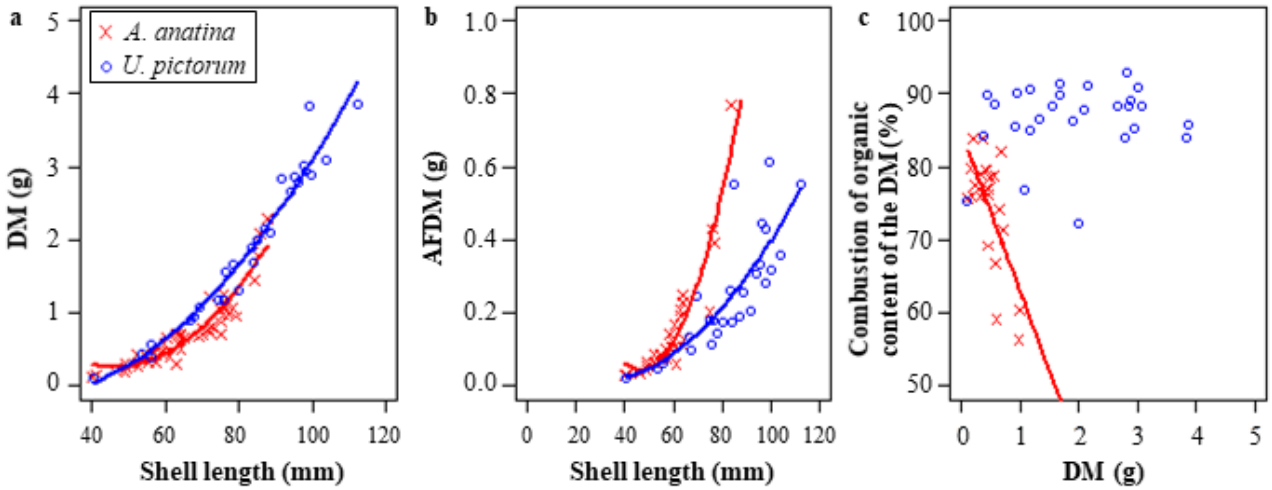

Fig. 2 The quadratic LMs describing DM (**a**) and AFDM (**b**) dependent on shell length and the LM describing % organic matter content of DM combusted dependent on specimens' DM (**c**). Model details: *A. anatina* DM: intercept (inter.) 2.31, DM coef. -0.089, DM^2 coef. $9.66 * 10^{-4}$, $p < 0.001$, residual standard error (res. SE) 0.157, F-statistic (F) 195.3, 48 DF, adjusted R^2 (adj. R^2) 0.886. *A. anatina* AFDM: inter. 0.99, AFDM coef. -0.041, $AFDM^2$ coef. $4.36 * 10^{-4}$, $p < 0.001$, res. SE 0.238, F 85.71, 26 DF, adj. R^2 0.858, *U. pictorum* DM: inter. -0.021, DM coef. -0.020, DM^2 coef. $5.06 * 10^{-4}$, $p < 0.001$, res. SE 0.580, F 246.2, 24 DF, adj. R^2 0.950, *U. pictorum* AFDM: inter. 0.061, AFDM coef. -0.004, $AFDM^2$ coef. $7.46 * 10^{-5}$, $p < 0.001$, res. SE 0.096, F of 24.5 on 24 DF and an adj. R^2 of 0.644, and *A. anatina* % organic content: inter. 84.8, DM coef. -21.731, $p < 0.001$, res. SE 5.47, F of 19.5 on 23 DF and an adj. R^2 of 0.435. Further model details provided in Appendix Table 1.

There was a significant relationship between the shell length and the wet mass of the living specimens, including the shell, for both species ($p < 0.001$) with *A. anatina* showing a greater volume per unit of length than *U. pictorum* (Fig. 3a; Appendix Table 2). This could potentially be due to the ratio of the soft-tissue to shell mass, which might be lower for *A. anatina*. A similar pattern was shown by the significant quadratic LMs ($p < 0.001$) describing volume dependent on shell length in both species (Fig. 3b). The LMs of body mass dependent on volume were significant in both species ($p < 0.001$) and overlapped at the volume of around 20 cm^3 (Fig. 3c). The steeper slope of the *U. pictorum* model resulted in higher volume:mass ratio at volumes higher than 20 cm^3 and a lower ratio at volumes lower than 20 cm^3.

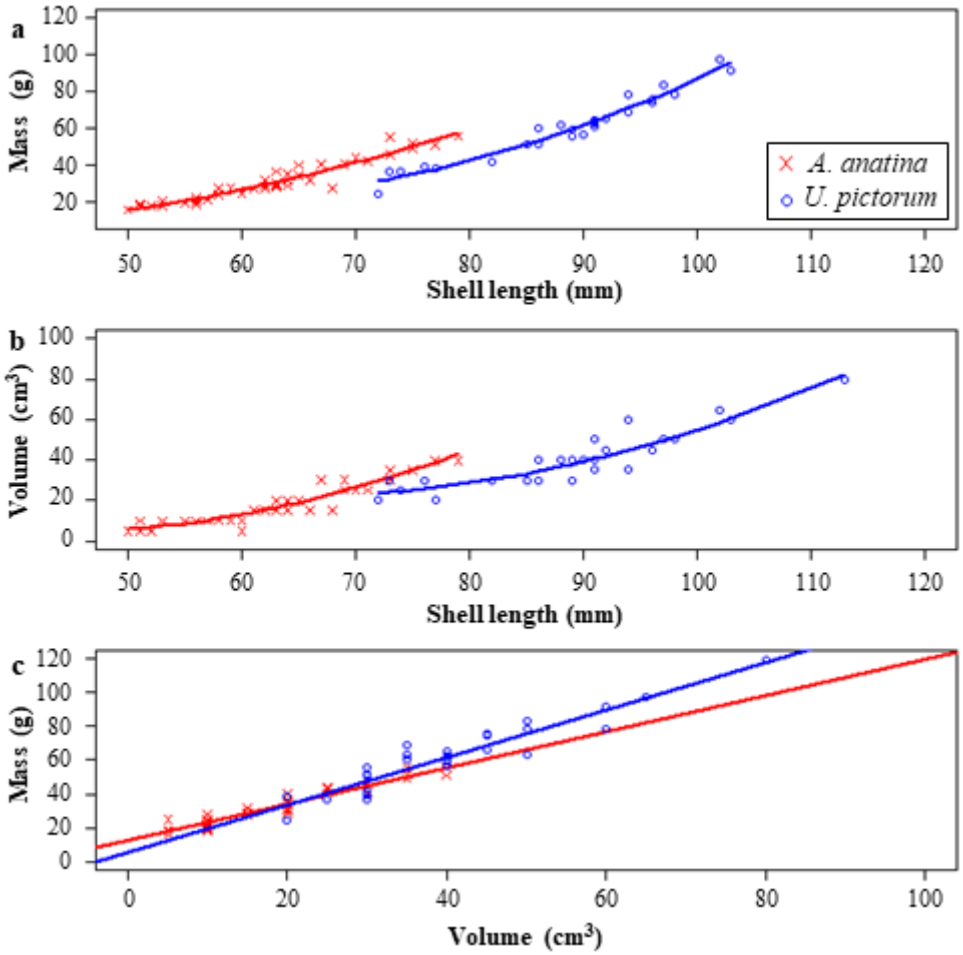

Fig. 3 Quadratic LM of the living specimens' wet mass including the shell length (**a**) and the volume (**b**) dependent on shell length, as well as of mass dependent on the volume (**c**). Model details: *A. anatina* Mass-Length: inter. 14.5, length coef. -0.87, length^2 coef. 0.018, $p < 0.001$, res. SE 3.20, F 234.4, 41 DF, adj. R^2 0.92, *U. pictorum* Mass-Length: inter. 109.5, length coef. -3.28, length^2 coef. 0.031, $p < 0.001$, res. SE 3.87, F 255.1, 23 DF, adj. R^2 0.95, *A. anatina* Volume-Length: inter. 52.8, Length coef. -2.35, Length^2 coef. 0.028, $p < 0.001$, res. SE 3.26, F 175.4, 41 DF, adj. R^2 0.89, *U. pictorum* Volume-Length: inter. 117.6, length coef. -3.05, length^2 coef. 0.024, $p < 0.001$, res. SE 5.78, F 64.1, 24 DF, adj. R^2 0.83, *A. anatina* Mass-Volume: inter. 12.4, volume coef. 1.07, $p < 0.001$, res. SE 3.23, F 457.7, 42 DF, adj. R^2 0.91, *U. pictorum* Mass-Volume: inter. 5.79, volume coef. 1.40, $p < 0.001$, res. SE 7.04, F 202.1, 25 DF, adj. R^2 0.89. Further model details provided in Appendix Table 2.

3.2 Production of biodeposition and opening behaviour

Faeces production started within an hour after feeding and became more prominent with time in both species (Fig. 4). At 3 hours the recorded percentage of mussels that produced faeces, regardless of treatment, ranged between 38.5 and 56.7 %, and at 5 hours, right before the digestion-period, ranged between 53.8 and 90.0 %. The percentage of mussels that produced faeces within 5 hours increased with the *C. vulgaris* concentration in both species with *A. anatina* showing for each of the concentrations a higher percentage than *U. pictorum*. In ascending order of *C. vulgaris* concentration—0.05, 6.0 and 12.0 mg AFDM l^{-1}— the percentage of specimens that produced faeces increased from 63.3 to 76.7 and 90.0 % by *A. anatina* and from 53.8 to 69.2 and 73.1 % by *U.*

pictorum. Production of pseudofaeces was recorded for only 6 specimens of *A. anatina* exposed to 12.0 mg AFDM *C. vulgaris* l^{-1}, that also produced faeces, in both the feeding- and digestion-periods. In both species, the opening behaviour during the feeding-period was characterised by a majority of open and semi-open mussels (Fig. 4b). Only 1 mussel out of 144 experiments had a mean opening score of 0, constantly closed, and 15 mussels had a mean opening score lower than 1. Similarly, during the digestion-period, the mussels were predominantly open with only 2 specimens having a mean opening score of 0 and 10 specimens a mean score lower than 1 (Fig. 4c). During both the feeding- and digestion-periods, *A. anatina* showed in all algal concentrations a higher opening rate than *U. pictorum* (Fig. 4b, c).

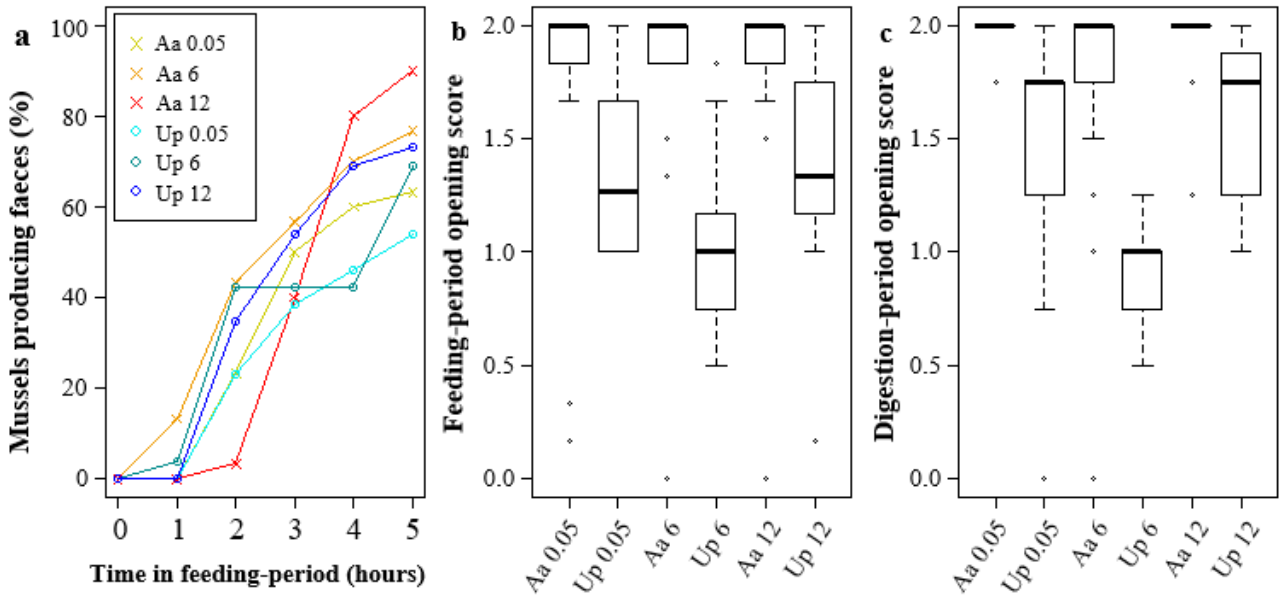

Fig. 4 Percentage of mussels that produced faeces during the 5-hour feeding-period for each treatment (A). Mean opening score, out of 2, per specimen during the feeding-period (B) and digestion-period (C); the rectangles show the 1st and 3rd quartile and the bold horizontal line indicates the median. Aa – *A. anatina*, Up – *U. pictorum*. The number next to the species label indicates the algal concentration in *C. vulgaris* in mg AFDM l^{-1}.

3.3 Oxygen consumption, temperature and pH

The temperature recorded at the end of the digestion-period ranged between 18.3 and 20.5 °C; only a single recording of 20.5 °C is outside the targeted range of 19 ± 1 °C. In ascending order of *C. vulgaris* concentration—0.05, 6.0 and 12.0 mg AFDM l^{-1}— the mean temperature was 18.5, 18.7 and 19.7 °C for *A. anatina* and 18.6, 18.4, 19.4 °C for *U. pictorum* experiments with a SD less than 0.2 °C in all treatments (Fig. 5a). Significant differences were found between the treatments with the oxygen consumption rate per DM unit increasing significantly with temperature in both species and the *A. anatina* rate being significantly higher than the *U. pictorum* (Fig. 5b; Appendix Table 3).

The mean pH recorded at the beginning of the experiments was 8.31 (SD 0.04). The pH recorded at the end of the digestion-period ranged between 8.02 and 8.27, with a mean of 8.14 (SD 0.05). The pH recorded in the control jars at the end of the digestion-period ranged between 8.21 and 8.34, with a mean of 8.28 (SD 0.04). A significant LM described a negative relationship between pH at the end of the digestion-period and oxygen consumption ($p < 0.001$) with no significant difference between the two species (Fig. 5c).

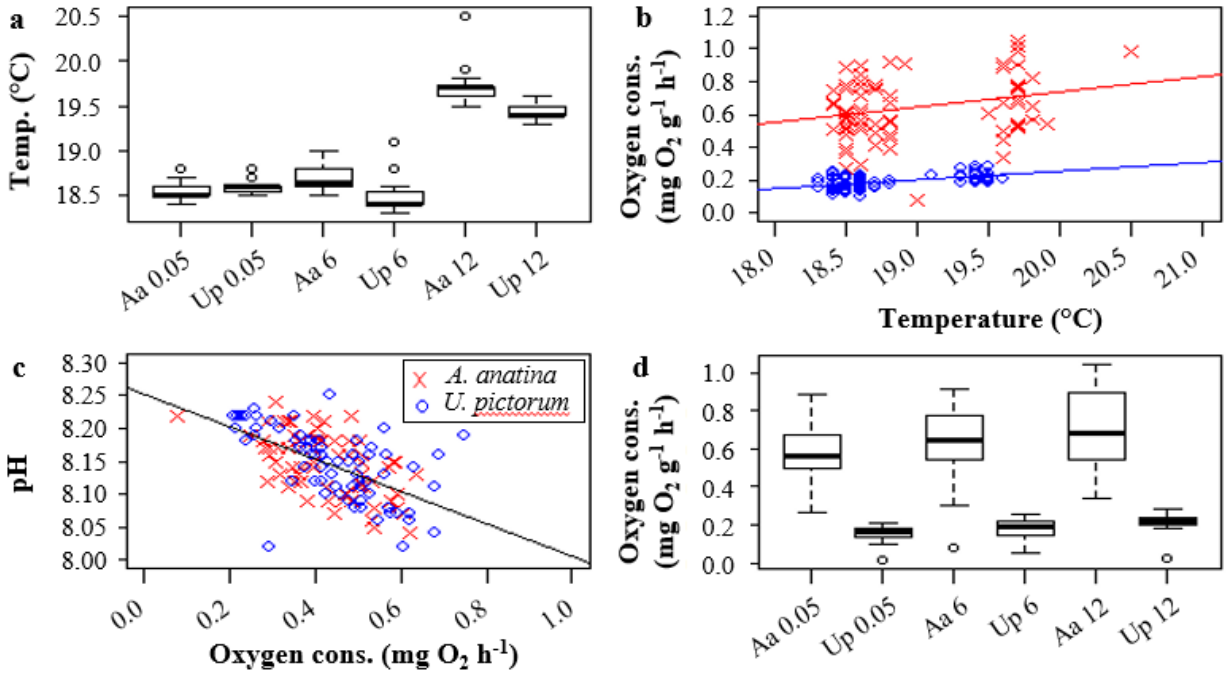

Fig. 5 a Temperature at the end of the 2-hour digestion-period based on mussel species and algal concentrations. Aa – *A. anatina*, Up – *U. pictorum*; the number next to the species label indicates the *C. vulgaris* concentration in mg AFDM l^{-1}. **b** LM of oxygen consumption rate per DM unit during the 2-hour period in the glass jars dependent on temperature. **c** LM of pH dependent on oxygen consumption after the 2-hour digestion-period in the sealed jars. Model details: inter. 8.25, O2 consumption coef. -0.25, res. SE 0.04, 142 DF, adj. R^2 0.354, F 79.55, $p < 0.001$). **d** Oxygen consumption rate per DM unit of *A. anatina* and *U. pictorum* during the 2-hour digestion-period. Further model details provided in Appendix Table 3.

Oxygen consumption rate per DM increased with algal concentration (Fig. 5d). In ascending order of *C. vulgaris* concentration—0.05, 6.0 and 12.0 mg AFDM l^{-1}—the mean oxygen consumption per DM was 0.56 (SD 0.14), 0.64 (SD 0.20) and 0.68 (SD 0.20) mg O_2 g^{-1} for *A. anatina* and 0.16 (SD 0.04), 0.19 (SD 0.05), 0.22 (SD 0.05) for *U. pictorum* (Fig. 5d). Both species showed a significantly higher oxygen consumption at 12.0 mg, compared to 0.05 mg AFDM *C. vulgaris* l^{-1} (*A. anatina* $p = 0.010$, *U. pictorum* $p < 0.001$). *U. pictorum* showed a significant difference between all the three treatments ($p \leq 0.01$; Appendix Table 3).

3.4 Oxygen consumption and body mass

The oxygen consumption rate increased significantly with the DM in both unionid species ($p < 0.001$) and was lower for *U. pictorum* compared to *A. anatina* (Fig. 6a; Appendix Table 4). The factors significantly contributing to the minimum adequate LM of oxygen consumption rate dependent on DM (Fig. 6a) were the DM ($p < 0.001$), *C. vulgaris* concentration ($p < 0.001$), opening behaviour during the digestion-period ($p = 0.006$) and species-DM interaction ($p = 0.018$; Appendix Table 5). The significant contribution of the species-DM interaction indicates that the oxygen consumption by *A. anatina*, shown in Fig. 6a, is significantly higher and increases more steeply with DM than that of *U. pictorum*. The oxygen consumption rate per DM decreases significantly with the DM in *A. anatina* ($p < 0.001$) while no significant relationship is shown for *U. pictorum* (Fig. 6b; Appendix Table 4).

There is a significant difference between the nominal categories of *C. vulgaris* concentration of 0.05 and 6.0 mg l^{-1} ($p < 0.001$) as well as 0.05 and 12.0 mg l^{-1} ($p < 0.001$), and no difference between 6.0 and 12.0 mg l^{-1} ($p = 0.345$). The multidimensional LM of oxygen consumption per DM for *A. anatina* (Fig. 6b) is significantly influenced by DM ($p < 0.001$), *C. vulgaris* concentration ($p < 0.001$) and the opening behaviour in the feeding-period ($p = 0.027$; Appendix Table 5). There is a significant difference between the nominal categories of *C. vulgaris* concentration of 0.05 and 6.0 mg l^{-1} ($p = 0.043$), 0.05 and 12.0 mg l^{-1} ($p < 0.001$), and potentially a marginal difference between 6.0 and 12.0 mg l^{-1} ($p = 0.096$).

The opening behaviour during the digestion-period is significantly influencing ($p = 0.006$) the LM of OC including both unionid species (Appendix Table 5). When the LMs of OC were analysed separately for each species—based on "DM, algal concentration, opening during feeding and opening during digestion"—*A. anatina* showed a significant positive relationship between opening during the feeding-period and oxygen consumption with a gradient of 0.045 ($p = 0.024$); *U. pictorum* showed a marginally significant positive relationship with a gradient of 0.022 ($p = 0.085$). In contrast to that, the oxygen consumption rate per DM of *A. anatina* showed a significant positive relationship with the opening in the feeding-period ($p = 0.027$; Appendix Table 5).

4. Discussion

This study addresses the influence of food availability on the unionid metabolism in *A. anatina* and *U. pictorum*, by using oxygen consumption as an indicator of metabolic activity. Furthermore, it considers the opening behaviour during feeding and digestion. The dependency of oxygen consumption rate and oxygen consumption rate per unit of DM on body mass are also studied. Further to the autecological aspects and the importance at an ecosystem level in high-density mussel beds, the applicability of the study lies in oxygen consumption quantification. This is an essential component for the development of self-sustaining bioremediation models in eutrophic systems, based

on unionid species and abundance. Assessing the risk of inducing anoxic conditions is crucial, especially for managing large-scale projects such as the Mussels for Clean Water Initiative (MuCWI) currently developing in the Delaware River Basin, Pennsylvania, USA (Partnership for the Delaware Estuary, 2019).

Agrell (1948) showed that in eutrophic waters, *A. anatina* (synonym *A. piscinalis*; Lopes-Lima *et al.*, 2017) and *U. pictorum* are more common and are found in higher densities than *A. cygnea* and *U. tumidus*. Additionally, *A. anatina* inhabits a broader range of habitats than *A. cygnea* and *U. pictorum* a broader range of habitats than *U. tumidus* (Killeen *et al.*, 2004; Lopes-Lima *et al.*, 2017). Consequently, the two species assessed in the current investigation are expected to be more effective in dealing with higher phytoplankton concentrations and have a higher bioremediation potential compared to *A. cygnea* and *U. tumidus* in a broader ranger of eutrophic ecosystems. The bioremediation potential of these four species is crucial since they are the only native European unionids with a Least Concern IUCN status (Lopes-Lima *et al.*, 2017; IUCN, 2019) and hence, more likely to be used for habitat restoration projects in Europe.

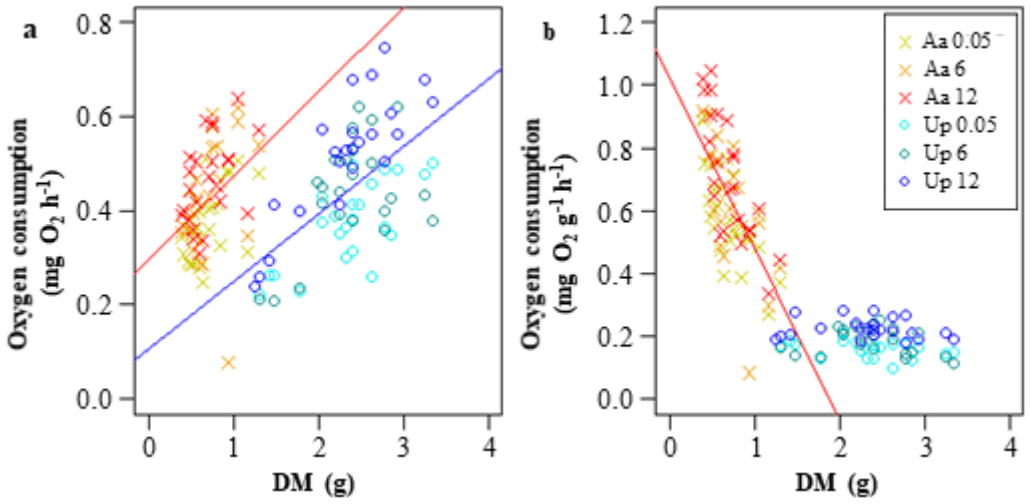

Fig. 6 Oxygen consumption rate dependent on DM (**a**) and oxygen consumption rate per gram of DM dependent on DM (**b**). Aa - *A. anatina*, Up - *U. pictorum*; the number next to the species label indicates the algal concentration in *C. vulgaris* in mg AFDM l^{-1}. Model details: *A. anatina* oxygen consumption: inter. 0.30, DM coef. 0.18, res. SE 0.10, 70 DF, Adj. R^2 0.15, F 13.44, $p < 0.001$, *U. pictorum* oxygen consumption: inter. 0.10, DM coef. 0.143, res. SE 0.10, 67 DF, Adj. R^2 0.34, F 36.12, $p < 0.001$, *A. anatina* oxygen consumption per DM: Inter. 1.03, DM coef. -0.55, res. SE 0.14, 70 DF, Adj. R^2 0.47, F 63.4, $p < 0.001$. Further model details provided in Appendix Table 4.

4.1 Unionid biometrics

Some of the linear relationships between shell biometrics, shown in this study, have been previously documented for the species used by Aldridge (1999). Nonetheless, this biometric section provides additional information on the volume, mass and soft-tissue content of the species of interest. This is

necessary for reducing inaccuracies of metabolic activity readings, which may arise from population-specific biometric differences associated with the environmental conditions (Agrell, 1948; Zając *et al.*, 2018).

In the populations examined in this study, shell length showed the strongest positive correlation among the metrics assessed (length, width, height and hinge length) in both species (Table 2) indicating that length is the best metric determining the specimens' volume and mass. Multiple linear regression assumes no multicollinearity in the data, meaning that the independent variables of a model cannot be highly correlated to each other. Consequently, in the shell biometric models the length was selected as the sole independent variable to avoid overfitting of the model and violating the assumption of independence (Zuur *et al.*, 2009). The strong relationships found align with the findings of Aldridge (1999) on the same species and those of Chojnacki *et al.* (2011) on *A. cygnea*. Chojnacki *et al.* (2011) have also found strong relationships between length and girth; this relationship can be examined in further work for *A. anatina* and *U. pictorum*.

The differences in morphology between *A. anatina* and *U. pictorum* indicate that, for direct comparisons of the animals' metabolic responses, a mass-based standardisation is required. The wet mass to volume LMs of the two species (Fig. 3c) overlap at the volume of 19.96 cm^3, giving a mean wet mass of living specimens of 33.80 g for both species. For the selected populations, using specimens of this size (mass and volume) would provide a strong level of standardisation for direct comparisons between the two species in future research. Nonetheless, a stronger indicator than the wet mass of the living specimens would be the soft-tissue mass, DM or the AFDM. In this study, the metabolic responses of mussels were assessed for specimens of various sizes, and therefore, the soft-tissue DM and AFDM are used for standardisation. The quadratic relationship of DM to shell length is stronger than of AFDM for both *A. anatina* and *U. pictorum* (Fig. 2). In combination with the dependency of body mass on the organic matter content of *A. anatina* (Fig. 3c), the oxygen consumption rate was standardised in specimens' DM.

The quadratic increase of DM and AFDM in *A. anatina* and *U. pictorum* aligns with the findings for the unionid *Lampsilis siliquoidea* (Larson *et al.*, 2014), marine bivalves such as *Mytilus edulis*, *Limecola balthica* (synonym: *Macoma balthica*) and *Cardidae spp.*, as well as with other species of marine gastropods, insects and crustaceans (Eklöf *et al.*, 2017). Furthermore, these three marine bivalve species also show a decreasing AFDM to DM percentage with the increasing body mass. Researchers suggest as potential explanation the temporary loss of biologically active tissue due to spawning by larger specimens and the continuation of the production of non-active support tissue in larger specimens characterised by a lower growth (Kautsky, 1982; Eklöf *et al.*, 2017). This could also be the case resulting in the pattern found in this study. The *U. pictorum* specimens that did not show a change in the AFDM to DM percentage were collected in November 2017 and kept frozen. The

specimens used for the oxygen consumption experiments were collected in January 2018 and were reproductively active in February 2018. The *A. anatina* specimens were collected between January and July 2018. In the specimens kept alive for the experiments, all collected in July 2018, no reproduction activity was recorded until they were released in December, so potentially reproduction had taken place before the collection of the specimens.

The limitation of the biomass collection by mechanical separation of the soft-tissue, which might contribute to an underestimation of the biomass measurements (Palmerini and Bianchi, 1994), would not explain the strong pattern observed. Additionally, it would not affect only the larger specimens of *A. anatina*. Since the AFDM to DM percentage may influence the specimens' metabolism and oxygen consumption rate, for future research, specimens smaller than 0.5 g DM could be used for direct comparisons between the two populations.

4.2 Production of biodeposition

The production of biodeposition, faeces and pseudofaeces, was conducted to ensure that filtration and ingestion were taking place during the experiments and that during the digestion-period the mussels' intestinal tract was not empty. The recorded opening and biodeposition production (Fig. 4) indicate that mussels in all treatments had algae passing through their intestinal system. The higher percentage of *A. anatina* producing faeces in all treatments could indicate a higher food uptake and processing than that of *U. pictorum*, but a gut content collection would be necessary for confirming this. No information was found on the digestion-period of the two species assessed. The most relevant data come from Bril *et al.* (2014) who found that the digestion time of *Amblema plicata* and *Lampsilis cardium* ranges between 13 ± 6 hours. Based on this, it is assumed that the mussels were actively digesting while kept in the glass jars, further supported by the production of faeces recorded in the digestion-period.

4.3 Factors contributing to oxygen consumption rate

As hypothesised, oxygen consumption significantly increased with mussel body mass, by using the DM as an indicator, in both *A. anatina* and *U. pictorum*. Similarly, during digestion, oxygen consumption rate per DM significantly increased with food availability in both unionid species. The aforementioned results indicate the importance of individual metabolic responses' contribution to the overall unionid metabolism. The potential contribution of the changing environmental variables, such as the trophic state, influenced by anthropogenic activities on mussels' impact on their environment requires further examination.

Contrary to our hypotheses, no clear trends were found concerning the influence of the opening behaviour during the feeding- and digestion-periods on oxygen consumption. For *U. pictorum*, the

opening behaviour during the feeding-period not significantly contributing to oxygen consumption. For *A. anatina*, the significant contribution opening behaviour during the feeding-period allows to state that the hypothesis is potentially supported, and further research required. Similarly, the contribution of the opening behaviour during the digestion-period in oxygen consumption allows the potential acceptance of the hypothesis, yet further research is required since the factor is not significant when the two species are assessed separately.

The bivalve opening behaviour is characterised by species-dependent inherent rhythms (Liu *et al.*, 2016), daily patterns (Salánki, 1964) and is affected by the physical and chemical environment (Liu *et al.*, 2016; Lummer *et al.*, 2016; Hasler *et al.*, 2017). The opening behaviour and associated filtration rate are also affected by food availability (Risgård *et al.*, 2003; McIvor, 2004; Bontes *et al.*, 2007). In the current study, the highly skewed distribution due to the predominance of open mussels may have resulted in the lack of significant differences. Furthermore, the weak patterns of opening behaviour might be associated with the semi-quantitative nature of the indicator used with the score of 0, 1 and 2. A continuous variable and a continuous record of the behaviour such as through the use of hall sensor technology, which utilises the magnetic field for providing accurate quantitative measures on the distance between the two valves would have been more suitable (Lummer *et al.*, 2016; Hasler *et al.*, 2017).

Further, an interesting behaviour of the positioning of the siphons during opening has been noted in this experiment. While the usual opening behaviour of mussels during feeding was characterised by a wide-open outward-facing inhalant siphon tip as shown in Fig. 1b, during the digestion-period many of the open mussels were often characterised by an inward-facing inhalant siphon as shown in Fig. 1c; no quantitative data are available. Whilst shell opening is usually associated with the filtration rate, the extent to which it is associated with the excretion of bioproducts of digestion is not currently clear. Whether the mussels remain open continuously for excretion purposes or whether periodic opening takes place, requires further investigation. In relation to the oxygen consumption rate, the outward-facing inhalant siphon tip during the digestion-period might be associated with filtration activity. This may explain the significant positive relationship between the opening during the feeding-period by *A. anatina* and the oxygen consumption rate per DM. The inward-facing inhalant siphon tip may be associated with low filtration rates aiming to expel excretions while providing enough oxygen without targeting food uptake. While using hall sensor technology would be adequate for assessing the opening behaviour, for assessing the influence of the inhalant siphon tip on clearance and metabolic activity imaging technology would be necessary.

4.4 Interspecific and mass-specific differences

The oxygen consumption rate per gram of DM is significantly higher for *A. anatina* compared to *U. pictorum*, irrespective of the algal concentration during feeding. The results support our hypothesis and align with previous research by Pusch *et al.* (2001) who recorded a higher oxygen consumption rate by *A. anatina* compared to *U. tumidus* for the populations of the River Spree in Germany. A contrasting pattern has been found by Ravera and Sprocati (1997), who recorded a higher oxygen consumption rate for *Unio mancus* compared to *A. cygnea* for the populations of Lagadone Stream in Italy. However, Ravera and Sprocati (1997) used the wet mass of soft tissue as an indicator of mussel mass. They also demonstrated a higher ratio of wet mass to DM of 5.38 for *U. mancus*, compared to a ratio of 7.58 for *A. cygnea*. Including this information in the analysis would result in a reduced difference between the two species. The results of the current and previous studies show no common trend in the comparisons between the *Unio* and *Anodonta* Genera. The differences may be species- or population-specific and are influenced by the mussel soft tissue mass indicator.

The oxygen consumption per gram of DM recorded in this study (Fig. 5d) are comparable to the temperature-dependent oxygen consumption recorded by Pusch *et al.* (2001), 0.14-0.63 mg O_2 g^{-1} h^{-1} for *A. anatina* (mean DM 3.5 g) and 0.23-0.50 mg O_2 g^{-1} h^{-1} for *U. tumidus* (mean DM 2.5 g). The divergence between the oxygen consumption rate per DM of *A. anatina*, ~0.45 mg O_2 g^{-1} h^{-1}, compared to *U. tumidus*, ~0.35 mg O_2 g^{-1} h^{-1}, at 19 °C may be attributed to the mass-specific differences of the specimens used. Along these lines Ravera and Sprocati (1997) recorded lower oxygen consumption rates per gram of wet weight with the increasing body mass in both *U. mancus* and *A. cygnea*, following the metabolic theory of ecology which states that the metabolic activity per unit decreases with the increase of body mass (Vanni, 2002; Brown *et al.*, 2004).

Based on the findings of the current study, the oxygen consumption rate per DM decreases with DM for *A. anatina* (Fig. 6b), following the metabolic theory of ecology. The prominent slope of -0.546 for *A. anatina* clearly supports our hypothesis for this species while a contradicting pattern is recorded for *U. pictorum*. Considering that the experiments of this study were conducted at 19 ± 1 °C, a prominent pattern could be expected since Ravera and Sprocati, (1997) found that the body mass dependency of oxygen consumption rate per wet weight in *A. cygnea* and *U. mancus* was more evident at temperatures higher than 12 °C and in specimens of less than 8 g of wet soft tissue weight. In the current study, the wet mass of the specimens used was for *A. anatina* between 3.2 and 9.9 g and for *U. pictorum* between 11.7 and 27.9 g. This is the potential reason for not recording a reduced oxygen consumption per DM with the increasing body mass in *U. pictorum*.

Pusch *et al.* (2001) also recorded a diverging pattern between *Unio* and *Anodonta* Genera in relation to their metabolic rate and filtration rates. A negative correlation was shown between the clearance rate and the respiration rate per DM unit *U. tumidus*, with a slope of -0.21, and a positive one by *A.*

anatina, with a slope of 0.78. This confirms the complexity of unionid metabolism and indicates that filtration does not determine, and cannot be used as an indicator of, the overall metabolic activity.

As discussed above, the unionid metabolic activity is dependent on body mass, which, according to Agrell (1948) is further influenced by the trophic state. Agrell (1948) found an increased body mass for *A. anatina* and *U. pictorum*, and a decreased for *U. tumidus*, collected from natural habitats with increasing trophic state. Consequently, changes in the body mass in response to changes in the trophic level may be observed within an animals lifespan or a population following successive reproduction cycles. For increasing the effectiveness of bioremediation models and reducing the chances of anoxia, changes in unionid body mass and their association with oxygen consumption need to be taken into consideration. Furthermore, oxygen consumption is the result of multiple metabolic and behavioural processes. As Lurman *et al.* (2014a, 2014b) showed, the behavioural responses associated with locomotion, opening and burrowing recorded for unionids, such as *U. tumidus* and *A. anatina*, are influenced by temperature fluctuations. Hence, assessing the influence of eutrophication and its seasonal effects is necessary for both the overall metabolic activity and the individual processes.

4.5 Further considerations

Temperature and pH

Despite the significant differences between the treatments, temperature did not significantly affect oxygen consumption or oxygen consumption per DM dependent on body mass. Hence, the temperature differences observed are not expected to have significantly affected the results.

From the results, the minimum oxygen concentration and saturation recorded at the end of the digestion-period, 7.59 mg l^{-1} and 82.9 %, respectively, are comparable to those found in the specimen collection sites and are not expected to have interfered with the unionid metabolism and data collection. However, a potential limitation of the experimental design may be the animals' responses to metabolic products, such as ammonium excretions, that are toxic to unionids (Augspurger *et al.*, 2003; Newton and Bartsch, 2007; Wang *et al.*, 2011). Interestingly, *U. pictorum* is not particularly sensitive to ammonium excretions (Beggel *et al.*, 2017). In further work taking readings of phosphate, nitrate and nitrite concentrations generated during digestion could provide further insights on the chemical products of unionid metabolism and digestion and the role of unionids on nutrient recycling in benthic ecosystems.

Reproductive stage

Specifically, in bivalves such as unionids, oxygen consumption is affected by larval brooding with gravid females showing a lower oxygen consumption and ammonia production than non-gravid specimens (Baker and Hornbach, 2001). Particularly in freshwater mussels, the production of

glochidia grown on their ctenidia results in a reduced particle retention efficiency (Tankersley and Dimock, 1993; Tankersley, 1996). Brooding reduces the surface area of gas exchange and the net water transport through ctenidia with brooding females potentially requiring a higher expense of metabolic energy through an increased filtration rate (Tankersley and Dimock, 1992). The increased cirri activity recorded for the pre-brooding period females, compared to males, of the American unionid *Anodonta cataracta* (Tankersley and Dimock, 1993) supports this assumption. Nonetheless, the energetic cost of pumping water in bivalves, calculated based on *M. edulis*, is lower than 2 % of the total cost (Jørgensen *et al.*, 1986; Riisgård *et al.*, 2014). Therefore, an increase in the pumping rate might not significantly influence the overall metabolism. Furthermore, a counteraction to this potentially increased activity may arise by a reduced expense of metabolic energy in digestion associated with a lower food uptake. This could explain the reduced ammonia production and oxygen consumption of gravid females recorded by Baker and Hornbach (2001).

In this study, the specimens' sex and their reproductive stage were not recorded. The reproductive period of the two species examined in this study has been documented by Aldridge (1999); for *U. pictorum* the embryonic development and the mature glochidia were found between April and July. These findings align with the *U. pictorum* sperm release recorded in our facilities in February 2018, during a period that specimens were exposed to gradually increasing temperatures. Based on the timing, it is unlikely that mature glochidia were still found in August when the experiments were conducted. Nonetheless, it cannot be ruled out that gravid females were used for the experiments. For *A. anatina*, the embryonic development has been recorded for the period August to December (Aldridge, 1999) and is therefore possible that gravid females might have been used, affected thus the results.

Starvation- and feeding-period

What is described here as a 24-hour "starvation" prior to each experiment refers to the conscious decision of not feeding algae to the mussels the day before the experiments. Nonetheless, the water contains bacteria and other picoplankton, which also constitute part of unionid diet (Baker and Levinton, 2003; Atkinson *et al.*, 2011; Ismail *et al.*, 2015). Therefore, the mussels cannot be considered fully starved at the beginning of the experiments. Similarly, during the experiments, the algal concentration was monitored spectrophotometrically while the bacterial load, which was also available during the digestion-period, was not.

Patterson *et al.* (1999) showed that starvation and food availability affect the unionids' glycogen content, a primary energy reserve. Furthermore, Bontes *et al.* (2007) have shown that the ingestion rate of *A. anatina* is dependent on food availability and food concentration while the pseudofaeces production varies significantly based on the algal species fed. Consequently, to compensate for the

glycogen loss associated with starvation, it is possible that mussels adjust their digestion time based on food availability and further research is required. If this assumption is valid and the mussels digest the food quicker at lower quantities, the readings taken after the 2-hour digestion-period for the 0.05 mg AFDM *C. vulgaris* l^{-1} treatment depict the animals' standard metabolic rate. In contrast, at lower food availabilities mussels may delay the initiation of the digestion process until the food quantity in their stomach reaches a certain threshold instead of continually digesting smaller quantities. To enhance our understanding of how unionids affect their environments and how the increased trophic state may affect these ecological interactions, these assumptions require further investigation. In further research, the oxygen consumption rate should be addressed as a factor of feeding- and starvation-periods and their synergistic impacts with food availability.

Further to food availability, food accessibility, which as demonstrated in other species is affected by the presence of predators (Griffiths and Richardson, 2006; Maire *et al.*, 2010; Antoł *et al.*, 2018), is an essential factor to be taken into consideration. Chemical detection of predatory crabs has been demonstrated to increase the burrowing depth of the marine bivalve *Limecola balthica*, a synonym for *Macoma balthica* (Griffiths and Richardson, 2006). Additionally, the exposure of *L. balthica* to sublethal predation by shrimps, detected by tactile stimuli, is associated with the retraction of the feeding siphon and a reduction in feeding activity. Similarly, the chemical detection of invertebrate fed predatory fish by the freshwater bivalve *D. polymorpha* was shown to initiate a reduced metabolic activity as an antipredator response (Antoł et al., 2018). The predator avoidance behaviour mentioned above directly influence food accessibility. The predator avoidance behaviour mentioned above directly influence food accessibility. Considering the high pressure of lethal predation by medium-sized predators, such as otters (*Lutra lutra*) and muskrats (*Ondatra zibethicus*) (Diggins and Stewart, 2000; Zając, 2014), and effects of invertebrate predators, such as the crayfishes *Procambarus clarkii* and *Pacifastacus leniusculus* (Meira *et al.*, 2019), on unionid survival and fitness, predation may significantly influence the feeding period. Hence, unionid metabolic activity needs further examination in relation to food accessibility.

5. Conclusion

In the present study, the importance of digestion in metabolic activity has been demonstrated along with the importance of food availability for the unionids *A. anatina* and *U. pictorum*. The findings indicate the necessity of understanding the role of trophic state and cultural eutrophication on oxygen fluxes in unionid dominated benthic systems. Furthermore, similarly to many organisms (Vanni, 2002), the recorded mass-specific oxygen consumption is inversely related to body size and affects nutrient recycling rates. Further to the effects of food availability on oxygen uptake, filtration and digestion, its influence on nutrient recycling associated with locomotion behaviours need to be

assessed. Enhancing the importance of the locomotion-dependent metabolic activity, nutrient cycling and oxygen fluxes are further affected by sediment reworking, bioturbation and nutrient release, topics requiring further research attention.

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Appendix

Table 1 Details of the quadratic linear models (LM) describing the relationship between the dry soft-tissue mass (DM)(g) and the Ash Free Dry Mass (AFDM)(g) dependent on the shell length (mm) and of the LM describing percentage organic matter content soft-tissue content (%) dependent on specimens' DM (Fig. 2). The details of each model and each coefficient are provided on the left and the right of the vertical line, respectively. DF – degrees of freedom, Res. SD – residual standard error, Adj. R^2 – adjusted R squared, Intercept. – Y-axis intercept, SE – standard error.

Fig. 2	Model	DF	Res. SE	Adj. R^2	F-statistic	p-value	Coefficient	Value	SE	t-value	p-value
a	DM *A. anatina*	48	0.157	0.886	195.3	< 0.001	Intercept	2.311	0.601	3.846	< 0.001
							Length	-0.089	0.019	-4.72	< 0.001
							$Length^2$	$9.66*10^{-4}$	$1.45*10^{-4}$	6.629	< 0.001
	DM *U. pictorum*	24	0.238	0.95	246.2	< 0.001	Intercept	-0.021	0.784	-0.027	0.979
							Length	-0.02	0.021	-0.944	0.355
							$Length^2$	$5.06*10^{-4}$	$1.33*10^{-4}$	3.814	< 0.001
b	AFDM *A. anatina*	26	0.058	0.858	85.71	< 0.001	Intercept	0.986	0.281	3.504	< 0.01
							Length	-0.041	0.009	-4.373	< 0.001
							$Length^2$	$4.36*10^{-4}$	$7.54*10^{-5}$	5.779	< 0.001
	AFDM *U. pictorum*	24	0.096	0.644	24.5	< 0.001	Intercept	0.061	0.317	0.193	0.849
							Length	-0.004	0.008	-0.488	0.630
							$Length^2$	$7.46*10^{-5}$	$5.36*10^{-5}$	1.39	0.177
c	Organic content *A. anatina*	23	5.47	0.435	19.51	< 0.001	Intercept	84.825	2.511	33.775	< 0.001
							DM	-21.731	4.92	-4.417	< 0.001

Table 2 Details of the LMs describing the relationships between shell length (mm), mass (g) and volume (cm^3) of the living specimens (Fig. 3).

Fig. 3	Model	DF	Res. SE	Adj. R^2	F-statistic	p-value	Coefficient	Value	SE	t-value	p-value
a	Length-Mass *A. anatina*	41	3.20	0.92	234.4	< 0.001	Intercept	14.5	31.7	0.46	0.651
							Length	-0.87	1.00	-0.87	0.391
							$Length^2$	0.018	0.008	2.28	0.028
	Length-Mass *U. pictorum*	23	3.87	0.95	255.1	< 0.001	Intercept	109.5	71.9	1.52	0.142
							Length	-3.28	1.67	-1.97	0.061
							$Length^2$	0.031	0.010	3.18	< 0.01
b	Length-Volume *A. anatina*	41	3.26	0.89	175.4	< 0.001	Intercept	52.8	32.4	1.63	0.110
							Length	-2.35	1.02	-2.29	0.027
							$Length^2$	0.028	0.008	3.51	0.001
	Length-Volume *U. pictorum*	24	5.78	0.83	64.1	< 0.001	Intercept	117.6	69.6	1.69	0.104
							Length	-3.05	1.57	-1.96	0.062
							$Length^2$	0.024	0.008	2.79	0.010
c	Volume-Mass *A. anatina*	42	3.23	0.91	457.7	< 0.001	Intercept	12.4	1.02	12.2	< 0.001
							Volume	1.07	0.05	21.4	< 0.001
	Volume-Mass *U. pictorum*	25	7.04	0.89	202.1	< 0.001	Intercept	5.79	4.24	1.36	0.185
							Volume	1.40	0.099	14.2	< 0.001

Table 3 Model details for the LM of (Fig. 5a) temperature (°C) at the end of the 2-hour digestion-period based on mussel species and algal concentrations (mg AFDM *C. vulgaris* l^{-1}), (Fig. 5b) oxygen consumption rate per DM (mg O_2 g^{-1} h^{-1}) unit during the 2-hour period in the glass jars dependent on temperature, (Fig. 5c) pH dependent on oxygen consumption (mg O_2 h^{-1}) after the 2-hour digestion-period in the sealed jars, and (Fig. 5d) oxygen consumption rate per DM unit of *A. anatina* and *U. pictorum* during the 2-hour digestion-period.

Fig. 5	Model	DF	Res.SE	Adj. R^2	F-statistic	p-value	Coefficient	Value	SE	t-value	p-value
a	Temperature - Algal concentration in both species	140	0.15	0.90	454.1	< 0.001	Intercept	18.5-19.5	0.026	717.2-756.3	< 0.001
							Species: Aa-Up	-0.143	0.026	-5.55	< 0.001
							0.05 - 6	0.017	0.032	0.53	0.599
							0.05 - 12	1.01	0.032	31.86	< 0.001
							6 - 12	0.99	0.032	31.33	< 0.001
b	Oxygen consumption and temperature in both species	141	0.13	0.75	215.1	< 0.001	Intercept	-0.833	0.430	-1.94	0.055
							Temp.	0.077	0.023	3.43	< 0.001
							Species: Aa-Up	-0.447	0.023	-19.75	<0.001
c	pH-Oxygen consumption	142	0.041	0.35	79.55	< 0.001	Intercept	8.25	0.012	670.96	< 0.001
							O_2 cons.	-0.25	0.028	-8.92	< 0.001
d	Oxygen consumption - Algal concentration in both species	140	0.13	0.75	144.7	< 0.001	Intercept	0.59-0.69	0.022	26.5-31.0	< 0.001
							Species: Aa-Up	-0.458	0.022	-20.51	< 0.001
							0.05 - 6	0.046	0.027	1.698	0.092
							0.05 - 12	0.101	0.027	3.683	< 0.001
							6 - 12	0.054	0.027	-20.51	< 0.001
	Oxygen consumption - Algal concentration *A. anatina*	69	0.18	0.065	3.47	0.036	Intercept	0.57-0.71	0.037	15.3-19.0	< 0.001
							0.05 - 6	0.066	0.053	1.25	0.217
							0.05 - 12	0.140	0.053	2.63	0.010
							6 - 12	0.639	0.037	17.05	0.170
	Oxygen consumption - Algal concentration *U. pictorum*	66	0.034	0.364	20.46	< 0.001	Intercept	0.16-0.22	0.007	22.4-31.4	< 0.001
							0.05 - 6	0.026	0.010	2.60	0.012
							0.05 - 12	0.064	0.010	6.36	< 0.001
							6 - 12	0.038	0.010	3.76	< 0.001

* The range in the Intercept coefficient details results includes all the values for the models run with each of the nominal variables for algal concentration—0.05, 6.0 and 12.0 mg AFDM *C. vulgaris* l^{-1}—as baseline

Table 4 Model details of oxygen consumption rate (mg O_2 h^{-1}) dependent on DM (g) for *A. anatina* and *U. pictorum* and oxygen consumption rate per gram of DM (mg O_2 g^{-1} h^{-1}) dependent on DM for *U. pictorum*. (Fig. 6).

Fig. 6	Model	DF	Res.SE	Adj. R^2	F-statistic	p-value	Coefficient	Value	SE	t-value	p-value
a	OC *A. anatina*	70	0.10	0.149	13.44	< 0.001	Intercept	0.296	0.036	8.168	< 0.001
							DM	0.178	0.049	3.66	< 0.001
	OC *U. pictorum*	67	0.104	0.341	36.12	< 0.001	Intercept	0.106	0.058	1.839	< 0.001
							DM	0.143	0.024	6.01	0.061
b	OC DM^{-1} *A. anatina*	70	0.139	0.468	63.4	< 0.001	Intercept	1.03	0.051	20.08	< 0.001
							DM	-0.546	0.069	-7.96	< 0.001

Table 5 Model details of the multidimensional LM and the factors significantly affecting the oxygen consumption rate (mg O_2 h^{-1}) in both species and the oxygen consumption rate per DM (mg O_2 g^{-1} h^{-1}) in *A. anatina*. The dependent variables are depicted in Fig. 6. For the LM of oxygen consumption rate in both species the minimum adequate model was selected by including the recorded independent variables—unionid species, DM (g), algal concentration (mg AFDM *C. vulgaris* l^{-1}), mean opening score during feeding, mean opening score during digestion and temperature (°C)—as well as the two-way interaction effects between species, DM and algal concentration were tested. For the LM of oxygen consumption rate per DM of *A. anatina*, no interaction effects were included. * indicates an interaction term

Model	DF	Res.SE	Adj. R^2	F-statistic	p-value	Coefficient	Value	SE	t-value	p-value
OC both species	137	0.11	0.223	7.86	< 0.001	Intercept	0.082-0.20	0.071-0.062	1.16-2.76	< 0.01-0.249
						Species: Aa -Up	0.07	0.066	1.07	0.287
						DM	0.18	0.055	3.37	< 0.001
						0.05 - 6	0.089	0.025	3.56	< 0.001
						0.05 - 12	0.11	0.022	5.07	< 0.001
						6 - 12	0.023	0.025	0.95	0.345
						Digestion opening	0.076	0.028	2.76	0.006
						Species*DW	-0.14	0.06	-2.38	0.018
OC DM^{-1} *A. anatina*	67	0.12	0.572	24.72	< 0.001	Intercept	0.83-0.96	0.074-0.76	11.11-12.71	< 0.001
						DM	-0.562	0.062	-9.07	< 0.001
						0.05 - 6	0.074	0.036	2.06	0.043
						0.05 - 12	0.135	0.036	3.75	< 0.001
						6 - 12	0.061	0.036	1.69	0.096
						Feeding opening	0.077	0.034	2.27	0.027

* The range in the Intercept coefficient details results includes all the values for the models run with each of the nominal variables for algal concentration—0.05, 6.0 and 12.0 mg AFDM *C. vulgaris* l^{-1}—as baseline