# Behavioural responses of *Anodonta anatina* and *Unio pictorum* to temperature and algal concentration

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**ABSTRACT**

Using, time-lapse photography in a laboratory setting, we exposed *Anodonta anatina* and *Unio pictorum* for 4 h to algal (*Chlorella vulgaris*) concentrations ranging from 0.5 to 20.0 mg ash free dry mass (AFDM) l-1 and to three different temperatures (11, 15 and 19 ± 1 °C). We analysed the proportion of mussels in locomotion, duration of locomotory activities, posterior tip movement and valve opening behaviour. The proportion of mussels in locomotion was significantly higher for *A. anatina* and for *A. anatina* was significantly lower at 11 °C. For both species, the proportion of mussels in locomotion, locomotory duration and posterior tip movement decreased with increasing algal concentration, and locomotory duration was significantly shorter in *U. pictorum*. In both species, valve opening peaked at intermediate algal concentrations, with the deviation from the peak being more prominent in *A. anatina*. Finally, we recorded a contrasting locomotory strategy for the two species (*A. anatina* crawled on the sediment surface, whereas *U. pictorum* moved through the sediment) and identified potential density-dependence in behavioural adaptation.

**INTRODUCTION**

Research on the effects of food concentration in unionids has focused primarily on filtration rates and intermediate microbial and/or organic matter concentrations in the seston have been shown to be associated with a peak of unionid clearance (Vanderploeg *et al.*, 1995; Pusch *et al.*, 2001; Bontes *et al.*, 2007; Tuttle-Raycraft *et al.*, 2017), paralleling trends recorded for marine bivalves (Riisgård & Randløv, 1981; Riisgård, 2001; Riisgård *et al.*, 2003; Pascoe *et al.*, 2009). In addition, valve opening behaviour in unionids is temperature-dependent (Rodland *et al.*, 2009) and species increase filtration with temperature depending on their thermal optima (Vanderploeg *et al.*, 1995; Kim *et al.*, 2011). At the same time, there is a gap in our knowledge of the effects of food concentration and temperature on other aspects of unionid behaviour, such as movement. These effects are ecologically important as the movement of benthic invertebrates influences fluxes in nutrients, oxygen and sediment at the water–sediment interface (Mermillod-Blondin & Rosenberg, 2006).

Mussel movements, both horizontal and vertical (e.g. burrowing), affect the spatial distribution of mussels and their distribution gradients and patchiness (Amyot & Downing, 1997; Schwalb & Pusch, 2007; Zieritz, Geist, & Gum, 2014; Zając, Zając & Ćmiel, 2016; Ożgo *et al.*, 2021). Burrowing behaviour can provide shelter and there are species-specific and stage-specific differences, with juveniles being more likely to burrow in the sediments than adult specimens (Saarinen & Taskinen, 2003; Zieritz *et al.*, 2014; Ożgo *et al.*, 2021). Horizontal movements are beneficial for location selection during fertilization and larval dispersal (Amyot & Downing, 1997; Vicentini, 2005). Distribution may also be associated with food availability, and migration is a behaviour associated with trophic cues and phytoplankton availability in multiple taxa of marine bivalves (Forêt *et al.*, 2018).

For the purposes of this study, we define ‘locomotion’ as solely horizontal unionid movements, excluding all vertical movements within the sediments and all movements of the posterior tip. Unionid burrowing and locomotion are affected by seasonality (Amyot & Downing, 1997; Watters *et al.*, 2001; Saarinen & Taskinen, 2003; Schwalb & Pusch 2007; Allen & Vaughn, 2009; Block *et al.*, 2013; Lurman *et al.*, 2014a), and burrowing speed, locomotory speed and locomotory distance are known to increase with temperature (Schwalb & Pusch, 2007; Block *et al.*. 2013; Lurman *et al.*, 2014b, 2014a). Saarinen & Taskinen (2003) recorded crawling tracks on lake bottoms for several European unionid species and found that the distances covered were up to 1.9 ± 0.5 m for *Anodonta anatina* (Lopes-Lima *et al.*, 2017; as *A. piscinalis*), 0.9 ± 0.3 m for *Unio pictorum* and 0.1 m for *Pseudanodonta complanata*. Schwalb & Pusch (2007) found that locomotory distances for *A. anatina*, *U. pictorum* and *Unio tumidus* were considerably shorter in lotic conditions, with *c.* 90% of specimens moving up to 0.25 m week-1 and maximum rates being up to 2.26 m week-1. Despite these data quantifying movement and the influence of seasonality, the factors influencing unionid locomotion remain understudied. While Bovbjerg (1957) showed that there was increased locomotory activity in the unionid *Lampsilis siliquoidea* under food limitation, it remains unknown if food availability affects locomotion in the unionid tribes Unionini and Anodontini. In addition, information is scarce on the concentration range of microbial organisms and/or organic matter in the seston, that may trigger or hinder locomotion.

In this study, we assess the behaviour of the European unionids *Anodonta anatina* and *Unio pictorum* in response to a range of temperatures and food concentrations under lentic conditions. The behavioural responses studied were probability of locomotion, duration of locomotion, posterior tip movement and valve opening behaviour. We made four predictions and evaluated them in turn. (1) The proportion of mussels in locomotion increases with water temperature between 10 to 20 °C (Block *et al.*, 2013) and is not affected by the species and by shell length in adult mussels (Saarinen & Taskinen, 2003). (2) The proportion of mussels in locomotion, locomotory duration and the posterior tip movement rate decrease with increasing algal concentrations (Bovbjerg, 1957). (3) The locomotory duration of *A. anatina* is longer than by *U. pictorum* (Saarinen & Taskinen, 2003). (4) Valve opening peaks at intermediate food concentration levels (Bontes *et al.*, 2007; Pascoe *et al.*, 2009).

**MATERIAL AND METHODS**

*Specimen collection and husbandry*

We collected 30 *Unio pictorum* specimens from Mapperley Reservoir, Ilkeston, UK (Grid reference SK 43478 43600) on 11 January 2018 and 41 specimens of *Anodonta anatina* from Markeaton Brook, Derby, UK (SK 33973 37293) on 14 January 2018. We cleaned the shells with a brush and removed the attached flora and fauna. Despite collecting *U. pictorum* in January, we rinsed the shells of this species with 5% acetic acid so as to kill any *Dreissena polymorpha* larvae that may have become attached to these mussels ( DiVittorio *et al.*, 2012; Davis et al., 2015).

*Unio pictorum* and *A. anatina* were each maintained in separate 120-l recirculating aquaculture systems with a water pump running at 1800 lh-1; the water pump was connected to an external filter and a powerhead of 1200 lh-1. The temperature was set at 10.5 ± 0.5 °C and increased by 2 °C day-1 until reaching 19.5 ± 0.5 °C to meet the desired experimental temperature. Light was provided from 08.00 until 20:.00 h by a 48" Arcadia Original Tropical fluorescence T8 lamp (Arcadia aquatic, Ely, UK). Dechlorinated tap water of hardness between 5 and 7 °KH was used. Phosphate (‎PO3−) was removed by a RowaPhos phosphate reactor (Weil Industrieanlagen GmbH, Osnabrück, Germany) and ammonium (NH4+), nitrate (NO3-) and nitrite (NO2-) ions were removed by an external filter containing Sera siporax enhanced by the addition of nitrifying bacteria with Sera bio-nitrivec (Sera GmbH, Heinsberg, Germany). Water quality was monitored using an API Fishcare Kit (Mars Incorporated, McLean, Virginia, USA). Water changes comprising 20–30 % of the volume were made on a weekly basis and more often when nutrient concentrations exceeded at least one of four thresholds (NH4+ > 0.2 mg l-1, NO3- > 10 mg l-1, NO2- > 0.0 mg l-1 or PO3− > 1.0 mg l-1).

*Experimental design*

Our study consisted of two sets of experiments: study 1 and study 2. In study 1, we assessed the influence of temperature, algal concentration and mussel size on the proportion of mussels that engaged in locomotion (proportion in locomotion). In study 2, we assessed the influence of algal concentration on unionid locomotory duration, posterior tip movement and valve opening behaviour, and we used the results of study 1 to inform study 2.

All experiments were conducted between 2 February and 2 March 2018 in transparent acrylic tanks with base dimensions of 37 x 21 cm. The tanks were filled with sediment (3–5mm grain size) to a depth of 5 cm and 9 l of dechlorinated tap water was added; the tanks were then placed in a water bath (Fig 1A). In each tank, we placed six randomly selected mussels of the same species that had not been fed for 44 h. The mussels were placed in the tanks 1 h before each experiment to acclimate and attain their preferred position in the sediment. All experiments started between 10.40 and 11.10 h and lasted for 4 h. All unionid behaviour that occurred during the 4-h experimental duration were recorded using vertical time-lapse photography (see details indata collection section).

*Study 1: effect of temperature and food availability on the proportion of mussels in locomotion*

We conducted 24 experiments. Each of these involved a different treatment and consisted of six randomly selected mussels of each species in a transparent tank (density of 77 mussels m-2; Fig 1B). We exposed the mussels to the alga *Chlorella vulgaris* (strain CCAP 211/74) at concentrations of 3.0, 10.0 and 20.0 mg ash free dry mass (AFDM) l-1 and at three temperature levels, 11.0 ± 1.0, 15.0 ± 1.0 and 19.0 ± 1.0 °C. This two-way orthogonal fixed factor experimental design gave 9 combinations for each species (two unionid species x three algal concentrations x three temperature levels = 18 treatments). The shell length (maximum distance from the posterior tip to the anterior tip) of the specimens used in these treatments was 40–60 mmfor *A. anatina* and 80–100 mm for *U. pictorum*. For a direct comparison, we conducted an additional treatment for both species at each temperature level and with the algal concentration held constant at 3.0 mg AFDM l-1 (for details of how AFDM was measured, see Supplementary Material Appendix S1); in these treatments all exposed specimens were 60–80 mm in shell length (two unionid species x one algal concentration x three temperature levels = six treatments). We recorded the locomotory activity of mussels for each treatment using time-lapse photography.

*Study 2: effect of food availability on locomotory proportion and duration, posterior tip movement and valve opening*

We conducted 32 experiments, with three 11 x 17 cm trays in each transparent tank and two randomly selected conspecific specimens in each tray (density = 106 mussels m-2; Fig 1C). The trays contained 5 cm of the same gravel used in study 1. Each specimen was placed on a tercile point of the longest middle axis of a tray, giving an equal distance between them and the end of the tray. For each species, we conducted four experiments at a temperature of 19.0 ± 1.0 °C and algal concentrations of 0.05, 1.0, 3.0 and 6.0 mg AFDM l-1; the range of algal concentration used was selected to facilitate clear imaging and to examine whether the range AFDM l-1 (between 1 and 2 mg) AFDM l-1 that affects unionid filtration rates (Bontes *et al.*, 2007) also influences locomotory behaviour. Due to the shell-size range of the mussels available to us, we used specimens with shell lengths of 50–70 mm for *A. anatina* and 80–100 mm for *U. pictorum*. We recorded the locomotory duration (in min), rate of movement at the posterior tip during periods of no locomotion in a two-dimensional horizontal plane (mm h-1) and the semi-quantitative valve opening behaviour in a two-dimensional plane (see detailsin the section on statistical analysis). We used the mean values for each tray as replicates (four experiments x three trays = 12 treatments in total for each species at each algal concentration).

*Feeding*

For feeding, we added 1l of dechlorinated tap water containing an algal concentration necessary for reaching the targeted algal concentration of each treatment. *Chlorella vulgaris* was reared in Jaworski's medium in shaking batch cultures with light provided by an Arcadia T5 Tropical Pro 54W 46" lamp (model FO54T5). We homogenised the algal distribution by a 5-min aeration before starting each experiment. We previously developed standard curves of *C. vulgaris* absorbance and measured the algal concentration with a Cecil CE 1011 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK) at 750 nm to minimize the potential effects of the culturing conditions (Griffiths *et al.*, 2011). The samples of *C. vulgaris* used in our experiments were always collected during the growth phase.

*Data collection*

In both studies 1 and 2, we measured water temperature, pH, oxygen concentration and oxygen saturation at the start and the end of the 4-h experiments. This was done using an HQ40d multimeter with a luminescent optical dissolved oxygen probe (IntelliCAL™ LDO101) and a pH liquid probe (PHC30101; Hach company, Colorado, USA). For time-lapse photography, we took vertical images with Go-pro cameras (model: Hero 5 black) for 4 h at 60-s intervals. We created time-lapse videos of 3904 x 2304 resolution at the rate of 2 frames sec-1 on Adobe Photoshop CC 2018 (Adobe Inc., 2017). We analysed the videos manually using the software Kinovea 0.8.15 (Charmant, J. & Contrib., 2006–2011).

*Statistical analyses*

All analyses were conducted using R v. 3.5.1 (R Core Team, 2015); we checked model assumptions using standard residual diagnostics (Zuur *et al.*, 2009). In study 1, we assessed the influence of the mussel species, shell length, temperature and food availability on the locomotory proportion. We conducted two-sided probability theory analyses with 95% confidence intervals between the different treatments.

For study 2, we conducted a logistic regression on the proportion of mussels in locomotion based on algal concentration. We applied a generalised linear model (GLM), with a binomial error structure and logit link structure, and gave a score of 1 to any mussel in locomotion and 0 to specimens not in locomotion. Due to heteroscedasticity, we ran a separate model for each species.

We used a GLM to analyse the dependency of locomotory duration on mussel species, algal concentration, tank and tray, and applied stepwise backwards selection using the Akaike information criterion. To assess the extent of valve opening, we used a semiquantitative metric and gave the opening score of 0 for closed valves, a score of 1 for partly-open valves with no visible siphons and a score of 2 for widely open valves (Fig 1D–F). We summed the scores of all images throughout each experiment for each specimen, giving the range between 0 and 480. The mean values of locomotory duration and valve opening score per tray (*n* = 12 per algal concentration) were analysed by GLMs dependent on algal concentration for each species. Models used a log link function and Poisson error distribution. To compare the locomotory duration between the two species, we applied a square root transformation and improved the normality of the residuals. The GLM for valve opening score utilized a quadratic power form to reflect the peak of valve opening at mid-algal concentrations (observed during data exploration). To deal with overdispersion, we used quasipoisson models (*A. anatina* and *U. pictorum* locomotory duration, *A. anatina* and *U. pictorum* valve opening score).

To analyse posterior tip movement, we included the data for specimens that remained open for at least 3 of the 4 h. We standardized the values to 75 mm shell length (i.e. we divided the 2-dimensional movement rate by the shell length and multiplied by 75). Due to heteroscedasticity, we applied a generalised least squares (GLS) extension to the general linear regression modelling framework and used backwards stepwise selection to obtain the minimum adequate model; the independent variables of algal concentration and unionid species were included in the final model.

During the time-lapse video analysis, we observed contrasting locomotory strategies between the two species. *Anodonta anatina* tended to mainly crawl on the sediment, while *U. pictorum* moved within the sediment with the umbo covered. From the videos obtained in studies 1 and 2, we obtained the total duration (in min) that each species engaged in each locomotion and conducted a Pearson's χ2 test.

**RESULTS**

*Study 1*

We recorded 13 locomotory activities, 11 by *Anodonta anatina* and 2 by *Unio pictorum* (Table 1). The locomotory proportion ranged between 0 and 0.5 in all treatments. The locomotory proportion was significantly higher in *A. anatina* (*P* = 0.001; Table 1) and in both species was unaffected by shell length (*P* > 0.5; Table 1). The locomotory proportion of *A. anatina* was significantly higher at 15 and 19 °C as compared to that at 11 °C (*P* = 0.044; Table 1). Similar results were obtained in response to algal concentration: we found that locomotory proportion was significantly higher at an algal concentration of 3 mg AFDM as compared to concentrations of 10 and 20 mg AFDM l-1 (*P* = 0.003; Table 1). The locomotory proportion of *U. pictorum* was influenced neither by temperature (*P* = 0.259; Table 1) nor algal concentration (*P* > 0.5; Table 1).

In all treatments, the temperature kept within the targeted range and, in most treatments, the algal concentration slightly exceeded the targeted concentration (Table 2). The pH ranged from 7.60–8.59, oxygen concentration ranged from 6.26–10.81 mg l-1 and oxygen saturation ranged from 69.90–105.40 % (Table 2).

*Study 2*

Locomotion counts decreased significantly with respect to increasing algal concentration in both *A. anatina* (algal conc. coefficient = -0.23, df = 94, z = -2.15, *P* = 0.032) and *U. pictorum* (algal conc. coefficient = -0.67, df = 94, z = -2.06, *P* = 0.039) (Fig. 2A, B). We also found that locomotion counts were significantly lower for *U. pictorum* (algal conc. coefficient = -0.31, df = 189, z = -3.00 *P* = 0.003, species coefficient = -1.26, z = -2.78 *P* = 0.005; Supplementary Material Table S1). In contrast to *A. anatina*, *U. pictorum* showed no locomotory activityat algal concentrations higher than 2.0 mg AFDM l-1. The locomotory duration decreased significantly with increasing algal concentrations in both *A. anatina* (GLM: df = 46, residual deviance = 2223.5, t = -2.11, *P* = 0.040) and *U. pictorum* (GLM: df = 46, residual deviance = 1561.6, t = -2.04, *P* < 0.047) (Fig. 2C). For *U. pictorum*, the locomotory duration was significantly lower(df = 93, residual deviance = 424.8, t = -2.72, *P* = 0.007; Supplementary Material Table S2).

In both species, the posterior tip movement rate decreased significantly with increasing algal concentrations (GLS: df = 85, residual standard error = 19.4, t = -11.99, *P* < 0.001; Fig. 2D; Supplementary Material Table S3) and no interspecific difference was observed (*P* = 0.355). The highest posterior tip rate predicted by the model in the absence of algae was 19.5 mm h-1.

The valve opening scores peaked at algal concentrations of 2.8–3.8 mg AFDM l-1 for both *A. anatina* (GLM: df = 45, residual deviance = 1391.9, t = -2.46, *P* = 0.018) and *U. pictorum* (GLM: df = 44, residual deviance = 105.9, t = -2.36, *P* = 0.026) (Fig. 2E; Supplementary Material Table S4). However, we found that *A. anatina* was more likely to be fully open when algal concentrations deviated from the optimum as compared to *U. pictorum*. We did not assess this statistically as the model combining both species violated the assumptions of both normality and homoscedasticity.

The recorded temperature was primarily at the lower limits of the targeted range and algal concentrations slightly exceeded the targeted concentration (Table 3). The pH ranged from 7.80–8.43, oxygen concentration ranged from 6.86–9.44 mg l-1 and oxygen saturation ranged from 72.40–101.50 % (Table 3).

*Locomotory pattern*

The locomotion of *A. anatina* largely involved crawling on the sediment, whereas that of *U. pictorum* mainly involved movement through the sediment, with the umbo covered (χ2 value = 1702.2, df = 1, *P* < 0.001; Figs 1G–J, 2F). *Unio pictorum* was observed crawling on the sediment on only three occasions: during two locomotory activities in study 1 and for a short period during one activity in study 2.

**DISCUSSION**

The aim of our investigation was to assess the locomotory probability of *Anodonta anatina* and *Unio pictorum* at different temperatures and over a range of algal concentrations. For the optimal thermal range, we recorded a decrease in the proportion of mussels in locomotion in response to decreasing temperature and increasing algal concentrations. The locomotory duration and posterior tip movement decreased with increasing algal concentrations and valve opening peaked at algal concentration of 2.8–3.8 mg AFDM l‑1. Our findings suggest that, similarly to marine bivalve taxa, trophic cues and the concentration of microalgae in the seston influence locomotory activity in unionids and may affect their distribution (Forêt *et al.*, 2018).

*Locomotion and posterior tip movement*

In agreement with prediction 1, the locomotory proportion increased with temperature for *A. anatina* but there was no difference between 15 and 19 °C (Table 1), suggesting that there is a temperature threshold between 11 and 15 °C, which triggers or allows locomotion. These results are consistent with other published studies. Lurman *et al.* (2014) recorded that the locomotory and burrowing speed of *A. anatina* increases with temperature. Similarly, the burrowing activity of *Potamilus alatus* increases with temperature (Block *et al.*, 2013). Identifying the temperature threshold in *A. anatina* is necessary to understand any potential impacts of thermal pollution (Råman Vinnå *et al.*, 2017; Ferreira-Rodríguez *et al.*, 2019). Due to the low number of locomotory activities by *U. pictorum* in study 1, we could not assess temperature influences.

Consistent with prediction 3, we recorded a higher locomotory proportion and a longer locomotory duration for *A. anatina* as compared with *U. pictorum.* The recorded locomotory proportions (0–0.5) agree with those recorded by Saarinen & Taskinen (2003) for lakes during the summer (0–0.57). These authors also recorded population-dependent seasonality in locomotory behaviour for *A. anatina*, *U. pictorum* and *P. complanata*, with *A. anatina* alone showing higher locomotory activity in August compared to June. While Saarinen & Taskinen (2003) recorded no significant difference in the crawling path length of *U. pictorum* and *A. anatina*, we recorded a difference in locomotory duration, suggesting that the locomotory speed of *U. pictorum* and *A. anatina* may be species- or population-dependent.

The higher locomotory proportion and longer duration we recorded for *A. anatina* and *U. pictorum* at lower algal concentrations support prediction 2. The findings also support the hypothesis of Bovjerg (1957), who recorded an increase in the locomotion of *Lampsilis siliquoidea* during periods of low food availability and explained this as an attempt by this species to move to areas richer in food resources. We recorded no activity by *U. pictorum* at algal concentrations higher than 2.0 mg AFDM l-1, potentially suggesting a lower algal concentration threshold for *U. pictorum* as compared with *A. anatina*. If under food limitation, locomotion functions as a foraging behaviour associated with pedal feeding (Raikow & Hamilton, 2001; Klauke, 2007, as cited in Brendelberger & Klauke, 2009), the lower threshold for *U. pictorum* may be associated with a lower metabolic activity as compared with *A. anatina* (Zapitis, Huck & Ramsey, 2021). It is worth noting that the locomotory distance of *A. anatina* decreases at soluble reactive phosphorous concentrations of 1000 μg l-1 or higher (Reynolds & Guillaume, 1998). The trend aligns with the decreasing locomotory duration we recorded in response to increasing algal concentration. Further investigations are needed to identify if this trend is valid for other nutrients associated with primary production.

The posterior tip movement rate significantly decreased with increasing algal concentrations. It is unclear if these movements actively contribute to increasing food accessibility by repositioning the inhalant siphon in the water column or indirectly result from mussel movement and, possibly also, pedal feeding under conditions of food scarcity.

*Valve opening behaviour*

In agreement with published research on marine bivalves (Riisgård, 2001; Pascoe *et al.*, 2009) and in support of prediction 4, we recorded a peak in valve opening at intermediate algal concentrations. This was primarily due to the high valve opening rates recorded and the decreased score resulting primarily from partly-open instead of closed mussels. Valve opening behaviour did not appear to affect the locomotory proportion and duration (see Supplementary Material Fig. S1 for additional models that exclude the partly-open category).

While the degree of valve opening in unionids is not a direct indicator of the filtration rate (McIvor, 2004) and valve opening is affected by the composition of seston and chemical stressors (Hartmann *et al.*, 2016; Lummer, Auerswald & Geist, 2016), our results for higher closures at concentrations lower than 3 mg AFDM l-1 correspond to findings on decreased clearance rates for *A. anatina* at algal concentrations between 1 and 2 mg AFDM l-1 (Bontes *et al.*, 2007). In addition to affecting clearance rates, the effect of algal concentration on valve opening behaviour in unionids may affect interspecific interactions. For example, the European bitterling *Rhodeus sericeus* shows host preference for egg deposition on the ctenidia of unionid species and the number of eggs deposited vary between different host species (Mills & Reynolds, 2002). Thus, algal concentration may affect the preferences shown by *R. sericeus* for different host species.

*Locomotory strategy*

The locomotion of *A. anatina* was found to consist predominantly of crawling on the sediment surface, whereas *U. pictorum* moved through sediments. Our findings agree with the burrowing behaviour of the two species and the differences in their vertical distribution in the sediment, with a higher percentage of adult *A. anatina* (83.3%) inhabiting the surface layer as compared to *U. pictorum* (74.5%; Ożgo *et al.*, 2021). These contrasting locomotory strategies may influence sediment mixing, surface area and depth, and affect fluxes in nutrients, oxygen and sediment particles at the sediment–water interface (Mermillod-Blondin & Rosenberg, 2006). Crawling by *U. pictorum* took place in study 1 at densities of 77 mussels m-2 and breaking through the sediment took place in study 2 at 106 mussels m-2. This suggests potential density dependence, so we analysed two trial time-lapse videos that were recorded before the experiments with *U. pictorum* at 19 °C, with no *C. vulgaris* andat the density of 77 mussels m-2. In both trials, just four out of six mussels moved and exhibited crawling behaviour—this supports the density-dependence hypothesis. In high-density mussel beds, moving through the sediments may enhance anchoring and reduce the risk of being washed away by currents. Based on this hypothesis, we would expect A. anatina from the brook to break through the sediment and U. pictorum from the reservoir to crawl, but we observed the opposite trend. One explanation for this is that the mussels adapt their behaviour to the lentic conditions of the experimental setup. Alternatively, it may be that as the *A. anatina* specimens used in our study were small, higher densities were necessary for inducing the behavioural response shown by *U. pictorum*.

*Further considerations*

In natural ecosystems inhabited by unionids, the sediments present consist of a range of grain sizes and contain organic matter, while the surrounding water contains a range of phytoplankton species (Lopes-Lima *et al.*, 2014; Marroni *et al.*, 2017). We used sediment particles of relatively uniform size and lacking organic content, and provided only one algal species (*C. vulgaris*)to standardize the conditions and exclude the influence of natural heterogeneity. This may have affected mussel behaviour and so our findings require *in situ* validation. Additionally, the long-term influence of seston concentration (including microbes, suspended sediments and organic matter) and the potential effects of circadian rhythms require further investigation.

As we have conducted the experiments in contained areas, there was a risk that the limited space available would affect the locomotory duration of the mussels. However, the recordings showed that the mussels did not stop moving when they reached the sides of the tanks or trays and kept moving along the perimeter. Hence, we consider our results to be reliable. Due to the assessment method of vertical time-lapse photography, we only recorded horizontal movements and excluded vertical movements. Our results may thus not reflect the locomotory activity of mussels in natural ecosystems and this requires further in situ investigations.

The clearance of unionids shows ecotypic responses and intraspecific specialization to their hydrodynamic habitats (Vanden Byllaardt & Ackerman, 2014). Our experimental setup was broadly speaking lentic in nature. However, although the specimens of *U. pictorum* used in our study were collected from a lentic ecosystem, the individuals of *A. anatina* were from a lotic ecosystem. Specimens of both species from both lentic and lotic ecosystems need to be studied to assess ecotypic locomotory responses. Finally, while we used only adult specimens, intraspecific differences in locomotory behaviour may be more prominent in juvenile stages when mussels rely on pedal feeding (Schartum *et al.*, 2017; Lavictoire *et al.*, 2018). Therefore, future studies should investigate locomotion in both adults and juveniles.

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Table 1. Counts of locomotion grouped by mussel size, temperature and algal concentration and the *P* values from the two-sided probability theory analyses conducted on the pooled number of locomotory activities of study 1.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Factor assessed** | **Group 1** | | **Group 2** | | ***P*** |
| **Treatment** | **Mussels moved / total number used** | **Treatment** | **Mussels moved / total number used** |
| **Species** | A | 11 / 72 | U | 2 / 72 | 0.001 |
| **Mussel size class (mm)** | A: 60–80 | 6 / 18 | A: 40–60 | 5 / 18 | 0.804 |
| U: 80–100 | 6 / 18 | U: 60–80 | 0 / 18 | 0.622 |
| **Temperature (**°C**)** | A: 15 | 5 / 24 | A: 11 °C | 1 / 24 | 0.044 |
| A: 19 | 5 / 24 | A: 11 °C | 1 / 24 | 0.044 |
| A: 19 | 5 / 24 | A: 15 °C | 5 / 24 | 1 |
| U: 11 | 2 / 24 | U: 15 °C | 0 / 24 | 0.259 |
| U: 11 | 2 / 24 | U: 19 °C | 0 / 24 | 0.259 |
| U: 15 | 0 / 24 | U: 19 °C | 0 / 24 | \* |
| **Algal concentration**  **(mg AFDM** l-1) | A: 3 | 11 / 36 | A: 10 | 0 / 18 | 0.003 |
| A: 3 | 11 / 36 | A: 20 | 0 / 18 | 0.003 |
| A: 10 | 0 / 18 | A: 20 | 0 / 18 | \* |
| U: 3 | 1 / 36 | U: 10 | 0 / 18 | 1 |
| U: 20 | 1 / 18 | U: 3 | 1 / 36 | 0.722 |
| U: 20 | 1 / 18 | U: 10 | 0 / 18 | 0.622 |

\* Analysis was not conduced since the expected proportion had no recorded locomotion activity.

Comparison of the locomotory proportion by species across treatments, mussel size class at 3.0 mg AFDM l-1 across all temperatures, temperature across all algal concentrations and algal concentration across all temperatures. ‘A’ and ‘U’ indicate *Anodonta anatina* and *Unio pictorum*, respectively, followed by the value of the relevant parameter and the number of mussels that moved divided by the total number of mussels tested. Comparisons of frequencies between groups were done with either group acting as the reference frequency. Taking a conservative approach, both tests were conducted, and the one resulting in the highest *P* value is presented.

Table 2. Parameters measured at the start and at the end of study 1.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Time** | **Parameter** | **A at 11 °C** | **A at 15 °C** | **A at 19 °C** | **U at 11 °C** | **U at 15 °C** | **U at 19 °C** |
| **Start** | **Algal conc. at 3 mg AFDW l-1; size class 1 (mg AFDW l-1)** | 3.31 | 2.98 | 3.14 | 3.14 | 3.31 | 4.30 |
| **Algal conc. at 3 mg AFDW l-1; size class 2 (mg AFDW l-1)** | 3.31 | 2.98 | 3.31 | 3.47 | 3.31 | 3.64 |
| **Algal conc. at 10 mg AFDW l-1; size class 1 (mg AFDW l-1)** | 9.27 | 9.60 | 10.26 | 9.93 | 10.92 | 11.58 |
| **Algal conc. at 20 mg AFDW l-1; size class 1 (mg AFDW l-1)** | 21.18 | 21.18 | 21.51 | 21.84 | 22.84 | 21.84 |
| **Temp. (°C)** | 11.10 (0.14) | 15.05 (0.06) | 18.50 (0.08) | 11.10 (0.20) | 14.93 (0.05) | 19.70 (0.08) |
| **pH** | 8.2 (0.05) | 8.4 (0.03) | 8.4 (0.13) | 8.1 (0.06) | 8.5 (0.02) | 8.2 (0.04) |
| **O2 conc. (mg l-1)** | 10.8 (0.05) | 10.3 (0.01) | 9.6 (0.08) | 10.3 (0.04) | 10.4 (0.02) | 9.0 (0.01) |
| **O2 sat. (%)** | 98.6 (0.19) | 102.1 (0.06) | 104.2 (0.88) | 94.0 (0.19) | 102.5 (0.13) | 101.2 (0.17) |
| **End** | **Temp. (°C)** | 11.13 (0.25) | 15.20 (0.08) | 19.05 (0.10) | ND | 15.23 (0.05) | 19.75 (0.06) |
| **pH** | 8.1 (0.01) | 8.3 (0.08) | 8.2 (0.13) | ND | 8.1 (0.06) | 7.7 (0.06) |
| **O2 conc. (mg l-1)** | 10.1 (0.17) | 9.4 (0.36) | 8.3 (0.39) | ND | 8.6 (0.23) | 6.5 (0.34) |
| **O2 sat. (%)** | 92.6 (2.01) | 94.2 (3.68) | 90.4 (4.18) | ND | 85.8 (2.31) | 72.9 (3.85) |

ND indicates that no data were collected for the parameter for the treatment.

For each species, four experiments were conducted at each temperature (*n* = 24). The algal concentration measured at the start is provided for each experiment. The mean temperature, pH, oxygen concentration and saturation of the four treatments are provided with SDs shown within parentheses. ‘A’ and ‘U’ denote *Anodonta anatina* and *Unio pictorum*; the number next to the species label indicates the targeted temperature For *A. anatina*, size class 1 refers to 40–60 mm long shells and size class 2 to 60–80-mm long shells. For *U. pictorum*, size class 1 refers to 80–100-mm long shells and size class 2 refers to 60–80-mm long shells.

Table 3 Mean (and SD) algal concentration, temperature, pH, oxygen concentration and saturation at the start and at the end of study 2.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Time** | **Parameter** | **A at 00.05** | **A at 01.00** | **A at 03.00** | **A at 06.00** | **U at 00.05** | **U at 01.00** | **U at 03.00** | **U at 06.00** |
| **Start** | **Algal conc. (mg AFDM l-1)** | 0.05\* | 1.27 (0.20) | 3.01 (0.47) | 7.78 (1.21) | 0.05\* | 1.44 (0.22) | 3.65 (0.57) | 7.57 (1.18) |
| **Temp. (°C)** | 17.93 (0.22) | 18.35 (0.06) | 18.28 (0.10) | 18.43 (0.05) | 18.23 (0.19) | 17.98 (0.05) | 18.18 (0.05) | 18.73 (0.10) |
| **pH** | 8.34 (0.03) | 8.40 (0.02) | 8.28 (0.03) | 8.27 (0.03) | 8.30 (0.02) | 8.40 (0.01) | 8.33 (0.03) | 8.24 (0.02) |
| **O2 conc. (mg l-1)** | 9.38 (0.06) | 9.28 (0.02) | 9.18 (0.06) | 9.10 (0.02) | 9.33 (0.07) | 9.38 (0.01) | 9.38 (0.05) | 9.07 (0.14) |
| **O2 sat. (%)** | 101.28 (0.26) | 98.25 (0.17) | 98.03 (0.60) | 96.20 (0.28) | 100.35 (0.52) | 98.73 (0.15) | 98.35 (0.51) | 97.18 (1.27) |
| **End** | **Temp. (°C)** | 18.80 (0.08) | 18.40 (0.14) | 18.3 (0.17) | 18.7 (0.06) | 18.1 (0.17) | 18.0 (0.08) | 18.4 (0.14) | 18.6 (0.08) |
| **pH** | 8.1 (0.03) | 8.2 (0.06) | 8.1 (0.02) | 8.1 (0.06) | 7.9 (0.01) | 8.0 (0.07) | 8.0 (0.05) | 7.8 (0.03) |
| **O2 conc. (mg l-1)** | 8.4 (0.20) | 8.1 (0.14) | 8.4 (0.07) | 8.5 (0.10) | 7.3 (0.19) | 6.9 (0.06) | 7.2 (0.14) | 7.2 (0.21) |
| **O2 sat. (%)** | 92.6 (2.25) | 86.3 (1.20) | 89.6 (0.72) | 89.9 (0.73) | 78.2 (1.96) | 73.1 (0.53) | 75.5 (1.62) | 76.6 (2.31) |

\* Concentration below the threshold level; the value was estimated based on the algal volume before dilution.

For each algal concentration treatment, 4 tanks (each containing 3 trays with 2 specimens) were used per mussel species (32 experiments in total). ‘A’ and ‘U’ denote *Anodonta anatina* and *Unio pictorum*. The number next to the species label shows the targeted algal concentration in mg AFDM l-1.

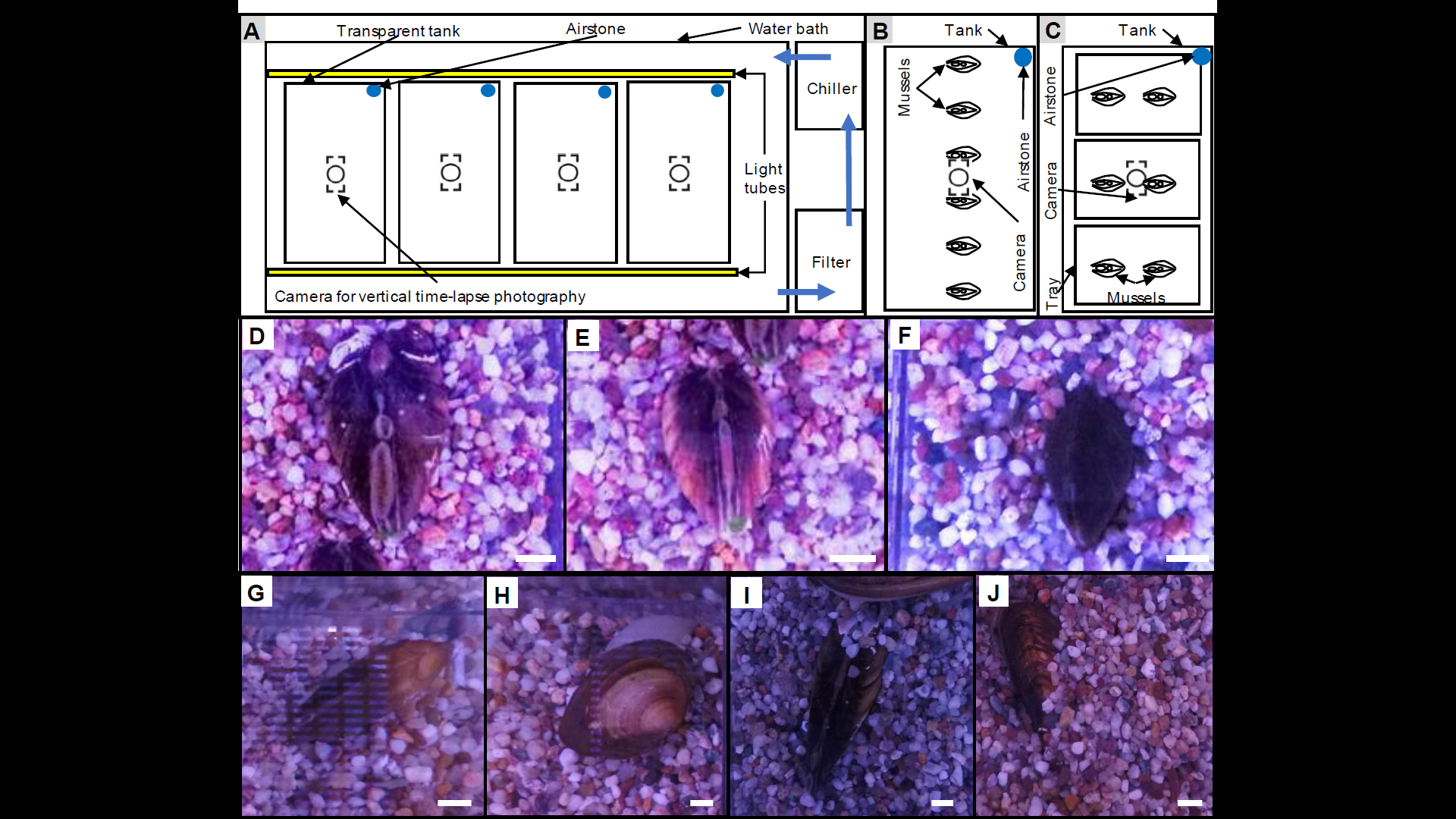


Figure 1. Experimental design and photographs of recorded mussel behaviours. A. Water bath with transparent tanks used for studies 1 and 2. B. Mussel location in each tank at the start of the experiments in study 1. C. Mussel location in each tray at the start of the experiments in study 2. D–F. Examples of valve opening behaviour. D. *Unio pictorum* with open valves and siphons extended (score 2). E. *Unio pictorum* with open valves and siphons not extended (score 1). F. *Anodonta anatina* with closed valves (score 0). G–J. Commonly recorded locomotory strategy of *A. anatina* (G, H) and *U. pictorum* (I, J). Scale bars for D–J are 1 cm.

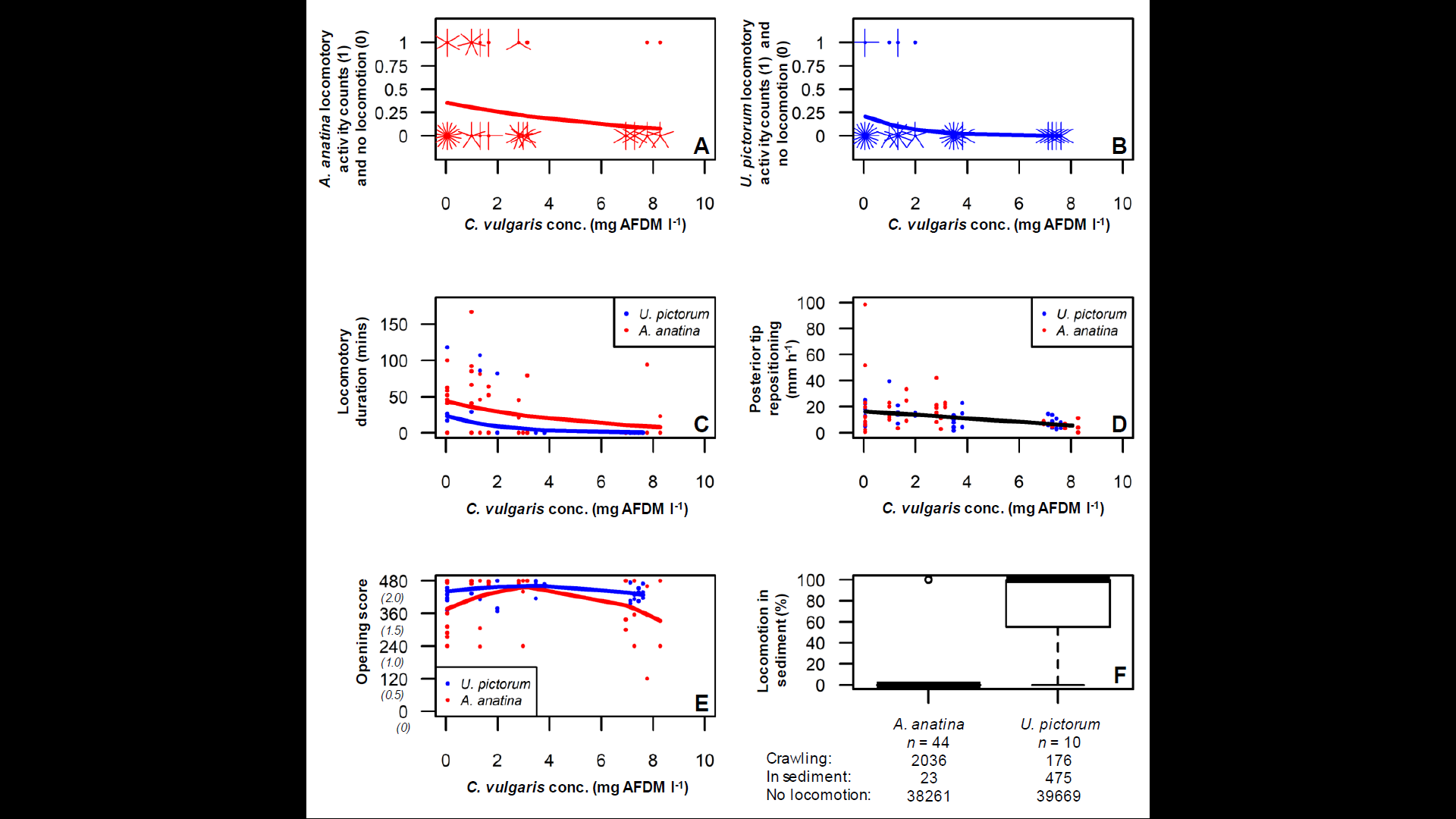


Figure 2. Parameters studied (counts of locomotion, locomotory duration, posterior tip movement rate and valve opening score) at algal concentrations between 0.05 and 8.27 mg AFDM l-1 in study 2 (A–E) and the percentage contribution of each locomotory strategy in studies 1 and 2 (F). A, B. Counts of locomotory activities at each algal concentration for *Anodonta anatina* (A) and *Unio pictorum* (B). Each point indicates a single count and for multiple counts the number of lines radiating from a single point indicates the count number. C. Mean locomotory duration per tray containing two specimens of *A. anatina* or *U. pictorum*. D. Mean posterior tip movement rate per h standardized to 75-mm shell length. E. Mean valve opening score per tray containing two specimens of *A. anatina* or *U. pictorum*. Mean valve opening scores out of 2, a measure intuitively easier to understand, are the numbers shown in italics within parentheses. Red points represent *A. anatina* and blue points *U. pictorum*. Model details for A–F are provided in Supplementary Material Tables S1–4. F. The percentage contribution of each locomotory strategy for each species in studies 1 and 2. The total duration of recorded locomotory activity by strategy (crawling on sediment *vs* moving through sediment) and duration of no locomotory activity (in min) are shown for each species. Rectangle indicates the first and third quartiles, and circles denote outliers; bold and dotted lines indicate the median and the range. Model details for A–F are provided in Supplementary Material Tables S1–4.