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Size matters: relationships between body size, dry weight and ash-free dry weight of common coastal, aquatic invertebrates in the Baltic Sea

Short title: Body size affects weight estimations

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ABSTRACT (410 words)

Background. Organism biomass is one of the most important variables in ecological studies, making estimations of organism weight one of the most common laboratory tasks. Biomass of small macroinvertebrates is usually estimated as dry (DW) or ash-free dry weight (AFDW); a laborious and time consuming process, that often can be speeded up using easily measured and reliable proxy variables like wet/fresh weight and/or body size. Another common way of estimating AFDW - which is the most accurate but also time-consuming estimate of biologically active tissue weight - is the use of AFDW/DW ratios or conversion factors. So far, however, these ratios typically ignore the possibility that the relative weight of biologically active vs. non-active support tissue (e.g. protective exoskeleton or shell) - and therefore, also the AFDW/DW ratio - may change with body size, as previously shown for taxa like spiders, vertebrates and trees.

Methods. We collected samples of aquatic, epibenthic macroinvertebrates (>1 mm) in 32 shallow bays along a 360 km stretch of the Swedish coast along the Baltic Sea; one of the largest brackish water bodies on Earth. We then estimated statistical relationships between the body size (length or height in mm), dry weight and ash-free dry weight for 14 of the most common taxa; five gastropods, three bivalves, three crustaceans and three insect larvae. Finally, we statistically estimated the potential influence of body size on the AFDW/DW ratio per taxon.

Results. For most of the taxa, non-linear regression models describing the power relationship between body size and i) DW and ii) AFDW fit the data well (as indicated by low SE and high R^2). Moreover, for more than half of the taxa studied (including the vast majority of the shelled molluscs), body size had a negative influence on organism AFDW/DW ratios.

Discussion. The good fit of the modelled power relationships suggest that the constants reported here can be used to more quickly estimate organism dry- and ash-free dry weight

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based on body size, thereby freeing up considerable work resources. However, the considerable differences in constants between taxa emphasize the need for taxon-specific relationships, and the potential dangers associated with either ignoring body size or substituting relationships between taxa. The negative influence of body size on AFDW/DW ratio found in a majority of the molluscs could be caused by increasingly thicker shells with organism age, and/or spawning-induced loss of biologically active tissue in adults. Consequently, future studies utilizing AFDW/DW (and presumably also AFDW/wet weight) ratios should carefully assess the potential influence of body size to ensure more reliable estimates of organism biomass.

Keywords: allometry; biometry; estuary; epifauna; infauna; isometric scaling; length:weight relationship; submerged aquatic vegetation; seagrass.

INTRODUCTION

Organism biomass is inarguably one of the more important variables in ecology, playing a central role in studies ranging from ecophysiology and population dynamics, to community interactions, food web regulation and whole-ecosystem metabolism. As a consequence, to accurately estimate organism biomass constitutes one of the most common and important tasks in ecological studies (Rosillo Callé, 2008).

Small invertebrates retained on 0.5-1 mm sieves (hereafter ‘macrofauna’) make up a major part of animal density, diversity and biomass in many ecosystems; e.g. insects and arachnids in terrestrial ecosystems; epibenthic, aquatic crustaceans, echinoderms and molluscs in stands of aquatic vegetation; and infaunal (sediment-dwelling) worms, crustaceans and molluscs in marine sediments. Macrofauna biomass is typically reported as dry- or ash-free dry weight per unit area (e.g. g per m^2), which requires observers to repeatedly identify, sort, dry and weigh individual or pooled organisms; a time-consuming, expensive and tedious process. Many studies have shown that more easily measured proxy variables scale predictably with dry weight and therefore can be used to speed up biomass estimations; e.g. wet (fresh) weight (Brey, Rumohr & Ankar, 1988; Ricciardi & Bourget, 1998) and body size, based on either exact length measurement (Smock, 1980; Frithsen, Rudnick, & Doering, 1986; Sabo, Bastow, & Power, 2002) or retention on sieves of certain mesh sizes (Widbom 1984; Edgar 1990; Casagranda & Boudouresque, 2002). While wet weight can be a very good proxy, we argue that body size (e.g. length) holds several advantages. First, ecological theory supported by empirical data suggest biomass scales predictably with length in the form of power relations (Smock, 1980; Sabo, Bastow, & Power, 2002). Second, while freezing/thawing and fixation in conservation liquids (e.g. EtOH or formalin) can affect both organism wet weight (Howmiller 1972; Mason, Lewis, & Weber, 1983; Leuven, Brock, & van Druten, 1985) and length (Hjörleifsson & Klein-MacPhee, 1992; Kapiris, Miliou, & Moraitou-Apostolopoulou, 1997),

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wet weight is also very sensitive to blotting, centrifugation (to remove excess water), and exposure to air and light before and during weighing (Howmiller 1972, Mason, Lewis, & Weber, 1983; Leuven, Brock, & van Druten, 1985). Third, body size (e.g. length or height) estimations can more easily be automated, using e.g. image analysis software (Paavo et al., 2008, Mallard, Bourlot, & Tully, 2013), to rapidly process multiple individuals at a time.

Ash-free dry weight (AFDW) is typically regarded as the most accurate predictor of macrofauna biomass, as it only includes biologically active tissue. Since AFDW estimations require the incineration of dried samples in a furnace at high temperature, adding considerable time and costs to analyses, many studies have reported how AFDW scales with estimations of wet- and dry weight, usually in the form of simple 'conversion factors' (e.g. AFDW/DW, in %) (Rumohr, Brey, & Ankar, 1987; Ricciardi & Bourget, 1998). However, these ratios typically ignore the possibility that the relative weight of biologically active vs. non-active support tissue (e.g. protective exoskeleton or shell) - and therefore the AFDW/DW ratio - may change with macrofauna body size, as previously shown for disparate taxa like spiders (Andersen, 1979), vertebrates (Miller & Birchard, 2005) and trees (Niklas, 1995). This issue is important not only for obtaining accurate biomass conversions and estimations, but also for understanding how organismal investment in one type of structure may limit or constrain investment in other structures across ontogenetic development stages (Lease & Wolf, 2010).

Here we estimate and report relationships between body size, dry-weight and ash-free dry weight for 14 of the most common aquatic, epibenthic invertebrate taxa found in shallow, vegetated habitats of the central Baltic Sea; one of the largest brackish water bodies on Earth. For each taxon we also assess whether the ash-free dry-weight/dry weight ratio changes with body size. Our aim is to provide simple yet reliable size-based relationships that can be used to rapidly estimate organism biomass.

METHODS

Study area

The Baltic Sea is a 415 000 km^2 large marginal sea situated in northern Europe (53-66° N; 10-30° E). A main feature is the presence of strong horizontal and vertical gradients in salinity, temperature and oxygen, that also undergo considerable temporal (e.g. seasonal) fluctuations (Voipio 1981). The Baltic Sea is evolutionary very young (ca 6000 years), and the shallow coastal areas have since the last glaciation been colonized by a mixture of marine, freshwater and brackish organisms, including crustaceans, gastropods, bivalves, polychaetes, hirudineans, nemerteans and insect larvae (Hansen, Wikström, & Kautsky, 2008). As many marine and freshwater organisms in the Baltic Sea live near their physiological tolerance limits, they grow slower and smaller than in their original environment; e.g. the blue mussel *Mytilus edulis* (Tedengren & Kautsky, 1986). As a consequence, their size ranges - but potentially also size:weight relationships and AFDW/DW ratios - could differ from those reported for conspecifics in marine or freshwater areas (Rumohr, Brey, & Ankar, 1987).

Field sampling

During summer (May-Aug) we collected aquatic invertebrate macrofauna (>1mm) in 32 shallow bays situated along a 360 km stretch of the central, Swedish Baltic Sea coastline (Fig. 1). The salinity in the area is generally low (ca. 5-7 psu) but fluctuates strongly with freshwater runoff and upwelling events. In each bay, a snorkeler sampled submerged aquatic vegetation and epibenthic macrofauna in 3-8 randomly selected stations (>30 m apart), by gently placing a 20×20 cm frame (with a 1mm-mesh bag attached) on the sea bed, and collecting all organisms (primarily vegetation and associated invertebrates) found above or on top of the sediment surface. The bag content was immediately transferred to a plastic bag, which was kept cold on ice until frozen (-20° C), in most cases within 1-3 hours.

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Length estimations

Following thawing in room temperature, we identified intact invertebrate organisms to the highest taxonomic resolution feasible using standard literature. For the 14 most common taxa we then selected and measured the body size of 12-459 individuals per taxa (3220 individuals in total), chosen to capture the full range of body sizes found across the 32 bays. The taxa included five gastropods (*Theodoxus fluviatilis*, *Hydrobia* spp., *Radix balthica, Potamopyrgus antipodarum, Bithynia tentaculata*), three bivalves (*Mytilus edulis*, *Limecola* (*Macoma*) *balthica* and *Cardidae* spp. [numerically dominated by *Parvicardium hauniense*], three crustaceans (*Amphibalanus improvisus, Idotea* spp., *Gammarus* spp.) and three insects (larval stages of *Chironomidae* spp., *Agraylea* spp. and *Limnephilidae* spp.) (see also Table 1). Body size (to the nearest 1 mm) was measured (based on standard procedures) as; i) gastropod height along the central shell axis, ii) bivalve length from anterior to posterior side, iii) total length of *Gammarus* and *Idotea* spp. from tip of rostrum to last urosome, iv) body width for *Amphibalanus improvisus*, and v) total length of insect larvae from end of head to last segment. A higher size accuracy is definitely possible (e.g. to 0.1 or 0.01 mm using calipers or stereo lenses), but as most studies utilizing this type of data (including ours) will depend on 1000s of length measurements, the accuracy chosen was a realistic trade-off between time and precision.

Estimations of dry- and ash-free dry-weight

Following size estimations, the measured individuals were transferred to pre-dried and -weighed (nearest 0.0001 g) porcelain crucibles. For most size classes (except for very large and rare individuals), multiple individuals were typically pooled into the same crucible. This step underestimates actual biomass variability between individuals, but was necessary as the low individual weights (particularly AFDW) were near or below the reliable detection limit of

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the scale. We included multiple estimations of the same sizes, so that the number of weight estimations (N) ranged from 10 to 42 per taxa. Samples were then dried at 60 °C for >48h (until constant weight), and cooled to room temperature in a desiccator before weighing. To estimate ash-free dry weight, the crucibles were then transferred to a muffle furnace, incinerated (550 °C for 3 hours), cooled and weighed again. Ash-free dry weight was calculated as dry weight minus ash weight.

Statistical analyses

We estimated taxon-specific body size:biomass relationship using non-linear regression in the form of the power equation:

$$biomass = \alpha \times size^{\beta}$$

where *biomass* is the individual weight (mg DW or AFDW), *size* is the body size (length/height, in mm), α is a normalization constant, and β is the scaling constant. Individual biomass typically scales with size in a power relationship, and initial data exploration showed that power equations provided a superior fit compared to linear, log or exponential relationships. As regression coefficients (R^2) are an inadequate measure of fit for non-linear regression models (Spiess & Neumeyer, 2010), we report SE for α and β. However, for the sake of simplicity we also estimated the linear log-log relationship between body size and biomass, and report the R^2 for those models (see e.g. Lease & Wolf, 2010).

For each taxon we also calculated the mean (± 1 SE) AFDW/DW ratio (in %); a commonly used conversion factor in macroinvertebrate studies (see e.g. Ricciardi & Bourget, 1998). We then used linear regression to test whether body size (in mm) affected the AFDW/DW ratio. Prior to analyses we checked assumptions of normality (by plotting predicted vs. observed quantiles) and homoscedasticity (by plotting predicted vs. observed residuals). All analyses were conducted in R v. 3.2.3 (R Core Team, 2016).

RESULTS

Relationships between body size and individual biomass

The relationships between body size (mm), individual dry weight (mg DW) and ash-free dry weight (mg AFDW) for all 14 taxa are displayed in Figure 2a-h, and the parameters (and their fit) are presented in Table 1. For most of the taxa, body size was a very good predictor of individual DW, as demonstrated by low SE and R^2 near 1. The model fits were slightly poorer for the three insect taxa (R^2 = 0.60-0.82) and the gastropod *Bithynia tentaculata* (R^2 = 0.85) than for the other ten taxa. For a majority (12 out of 14) of the taxa, the scaling constants (β) were well above 2 (2.110-3.590). The exceptions were the small gastropod *Potamopyrgus antipodarum* and chironomid larvae, that had constants closer to 1 (β = 1.368 and 1.383, respectively).

Body size was also a very good predictor of AFDW, even though model fits (based on SE and R^2) were slightly poorer than for DW (Table 1). Just as for DW relationships, the model fits (based on SE and R^2) were best for gastropods, molluscs and crustaceans. The scaling constants (β) were for most taxa quite similar to those reported for the DW relationships, with the exception of a higher constant for *P. antipodarum* (β = 2.447) and a lower constant for *Bithynia tentaculata* (β = 1.360).

Influence of organism body size on AFDW/DW ratios

The AFDW/DW ratios (mean %±SE) per taxa are also presented in Table 1. As expected, there were consistent differences between the four major taxonomic groups studied, with low AFDW content in bivalves and gastropods (12-27%), who's calcium carbonate shell makes up the major part of whole-body biomass, to higher AFDW content in chitin-shelled crustaceans (ca 60%), and the highest content in insect larvae (86-92%).

Results of simple linear regression showed that for more than half (8 out of 14) of the taxa surveyed, body size clearly affected the AFDW/DW ratio (Table 1, Fig. 2i-l). For four out of five gastropods, two out of three bivalves, as well as the sessile, calcite-shelled crustacean *Amphibalanus improvisus*, the AFDW/DW ratio decreased linearly with body size. For the small gastropod *Potamopyrgus antipodarum* body size instead had a positive influence on AFDW/DW. However, the *P. antipodarum* size range was very narrow (2-4mm) and the intercept was not different from 0 (Table 1), suggesting a relatively poor model. Moreover, there was no size effect found in the blue mussel *Mytilus edulis* (Table 1). Finally, in contrast to the size effects found for most of the hard-shelled molluscs, there was no influence of body size on AFDW/DW in any of the chitin-shelled crustaceans or insect larvae (Table 1, Fig 2i-l).

DISCUSSION

Estimating organism biomass is one of the most common, important but also resource-consuming tasks in ecological work, particularly when it comes to small-bodied, highly abundant and diverse macroscopic invertebrates. Many previous studies have successfully shown that more easily measured variables like invertebrate wet (fresh) weight (e.g. Ricciardi & Bourget, 1998) or body size (e.g. Smock, 1980) can be used as proxies to reliably predict both the dry- and ash-free dry weight, thereby simplifying and speeding up biomass estimations. Here, we first complement this literature by reporting how individual biomass scales with body size for 14 of the most common epibenthic invertebrate taxa found in shallow coastal areas of the Baltic Sea. Moreover, we demonstrate that for a majority of the studied molluscs, the ratio between organism dry- and ash-free dry weight – an often-used conversion factor (Rumohr, Brey, & Ankar, 1987; Ricciardi & Bourget, 1998) – decreases predictably with body size. Thus, our results can be used to quickly estimate the biologically active biomass of individual organisms based on their size, and when combined with density data, accurately estimate biomass per unit area.

Body size as a proxy for dry- and ash-free dry weight

For a majority of the studied taxa, body size was a good predictor of both dry weight and ash-free dry weight. The model fits were slightly poorer for ash-free dry weight (AFDW); most likely a consequence of the fact that even though multiple individuals of the same size were pooled, the low individual AFDW of many organisms (in the vicinity of 1 mg) challenged the accuracy of the scale. Comparisons between the 14 taxa studied (Table 1) show that particularly within the gastropods and crustaceans, the scaling (β) constants differ quite substantially between taxa (see the different slopes in Fig. 2 and β coefficients in Table 1). These differences emphasize the need for taxon-specific relationships to accurately predict biomass, and the potential dangers in either ignoring body size or substituting relationships between taxa. Consequently, our power equations (Table 1) can be used in a simple yet reliable way to estimate organism dry- or ash-free dry weight based on standard body size measurements. Size-based biomass estimations are likely to speed up laboratory work considerably; for example, Casagranda and Boudouresque (2002) showed that sieve-based size estimations speeded up estimations of body biomass of the gastropod *Hydrobia ventricosa* by 20-30 times. Consequently, our size-based estimations of invertebrate biomass are likely to free up considerable work resources (time, man-power, money) that can be used to e.g. collect and process more samples.

The influence of body size on AFDW/DW ratios

For most of the taxa with a calcium-carbonate (molluscs) or calcite shell (the barnacle *Amphibalanus improvisus*), we found a significant negative influence of body size on the

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AFDW/DW ratio; a commonly reported and often-used conversion factor in invertebrate studies (Rumohr, Brey, & Ankar, 1987; Ricciardi & Bourget, 1998). In other words, the proportional weight of biologically active vs. non-active tissue (shell, hard mouth parts, etc.) decreased with body size. There are at least two possible and complementary explanations for this relationship. First, while mollusc shell length increase per unit of time typically decreases with age, new shell layers are consistently added on a yearly basis (Negus, 1966). This results in increasingly thicker, and therefore disproportionally heavier, shells with mussel length, and a higher shell:tissue weight ratio. Second, our sampling was conducted during summer; a season when a majority of adult molluscs (here represented by the larger individuals per taxa) most likely had spawned and temporarily lost a considerable proportion of their biologically active tissue (Kautsky, 1982). The slopes of the significant regressions (Table 1, median = -1.26) suggest that failing to incorporate the potential influence of body size can strongly reduce the accuracy of AFDW estimations based on dry weight (and presumably also wet weight) - particularly if there is considerable variability in body size in the samples. The somewhat surprising lack of size influence in the common blue mussel *Mytilus edulis* was not investigated in detail, but could be caused by i) the lack of small shell-crushing mussel predators in the area (e.g. crabs), who otherwise are known to trigger thicker mussel shells (Freeman, 2007), and/or ii) the relatively low salinity, which results in that the small, osmotically stressed *M. edulis* invests considerably more energy into osmosis and soft tissue production, than in thicker shells (Kautsky, Johannesson, & Tedengren, 1990).

In contrast to the results for molluscs, there was no size effect on AFDW/DW ratios for the chitin-shelled insects and crustaceans. These results fit well with those reported in previous studies, for example of terrestrial insects, for which exoskeletal chitin scales isometrically (1:1) with body size (Lease & Wolf, 2010). In summary, our results suggest that body size can play

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an important but hitherto underestimated role when estimating organism AFDW based on dry (and possibly, wet) weight, particularly for shelled molluscs.

Conclusions

Using samples of epibenthic macroinvertebrates collected in 32 shallow bays along a 360 km stretch of the Swedish Baltic Sea coastline, we show that for 14 of the most common macrofauna taxa, organism body size scales predictably with individual dry weight and ash-free dry weight in the form of power relations. The good model fits suggest the taxon-specific equations reported here can be used to predict individual biomass based on organism size, thereby speeding up estimations of macrofauna biomass. Moreover, for the vast majority of the molluscs studied, we find a negative relationship between body size and AFDW/DW ratio; a commonly used conversion factor in macrofauna studies. Consequently, future studies utilizing AFDW/DW ratios should carefully assess the potential influence of body size to ensure reliable biomass estimations.

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Table 1. Results of regression analyses estimating i) the non-linear power relationship between body size and dry weight (DW) and ii) ash-free dry weight (AFDW), iii) the mean ± 1SE AFDW/DW ratio (in %), and iv) the linear relationship between body size and AFDW/DW ratio (in %). α and *β*: normalization and scaling constant for power equations, respectively. ns: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001. Values in bold mark those significant (at α = 0.05). Note: R^2 were derived from linear log-log models.

			Body size vs. DW			**Body size vs. AFDW**			**AFDW/DW**	**Body size vs. AFDW/DW**		
Taxon	**Class**	**N**	***α*±SE**	***β*±SE**	**R^2**	***α*±SE**	***β*±SE**	**R^2**	**Mean %±1SE**	**Intercept±SE**	**slope±SE**	**R^2**
Bithynia tentaculata L.	Gastropoda	25	0.598±0.484^{ns}	**2.117±0.351*** **	0.847	0.479±0.511^{ns}	**1.36±0.472** **	0.668	19.133±2.207	**33.162±3.878*** **	**-1.91±0.452* **	0.424
Hydrobia spp.	Gastropoda	24	**0.239±0.041*** **	**2.134±0.095*** **	0.952	**0.079±0.029* **	**1.441±0.22*** **	0.758	13.737±1.155	**19.791±2.855*** **	**-0.633±0.715* **	0.155
Potamopyrgus antipodarum Gray	Gastropoda	17	0.479±0.511 ^{ns}	**1.360± (0.472** **	0.919	0.021±0.012 ^{ns}	**2.447±0.395*** **	0.898	16.051±1.399	6.063±4.616^{ns}	**2.653±1.180* **	0.202
Radix balthica L.	Gastropoda	20	**0.137±0.035** **	**2.355±0.115*** **	0.956	**0.046±0.018* **	**2.119±0.177*** **	0.906	27.087±2.233	**35.338±3.558*** **	**-1.794±0.650* **	0.258
Theodoxus fluviatilis L.	Gastropoda	29	**0.221±0.065** **	**2.683±0.148*** **	0.9492	**0.015±0.006* **	**2.915±0.194*** **	0.912	13.044±1.083	**18.52±2.396*** **	**-0.242±0.494* **	0.159
Cardidae spp.	Bivalvia	33	0.134±0.094 ^{ns}	**2.848±0.347*** **	0.924	0.014±0.013 ^{ns}	**2.806±0.486*** **	0.879	12.358±0.852	**18.075±1.468*** **	**-0.429±0.325* **	0.364
Limecola balthica L.	Bivalvia	18	**0.069±0.024* **	**2.820±0.134*** **	0.991	0.001±0.002 ^{ns}	**3.479±0.673*** **	0.92	12.717±1.934	**21.429±2.98*** **	**-0.264±0.372* **	0.383
Mytilus edulis L.	Bivalvia	24	**0.030±0.015* **	**2.933±0.153*** **	0.991	**0.006±0.003* **	**2.844±0.147*** **	0.978	14.189±0.504	**13.162±1.044*** **	0.078±0.069 ^{ns}	0.011
Amphibalanus improvisus Darwin	Crustacea	13	0.314±0.205 ^{ns}	**2.515±0.289*** **	0.976	0.036±0.022 ^{ns}	**2.289±0.276*** **	0.961	8.939±0.550	**11.044±1.064*** **	**-0.397±0.179* **	0.246
Gammarus spp.	Crustacea	37	0.047±0.032 ^{ns}	**2.111±0.265*** **	0.926	0.033±0.028 ^{ns}	**2.05±0.32*** **	0.863	58.966±1.519	**63.062±2.616*** **	-0.389±0.307 ^{ns}	0.017
Idothea spp.	Crustacea	42	0.001±0.001 ^{ns}	**3.592±0.200*** **	0.949	0.001±0.001 ^{ns}	**3.850±0.249*** **	0.919	61.505±1.659	**66.183±3.457*** **	-0.550±0.358 ^{ns}	0.032
Agraylea spp. (larvae)	Insecta	13	0.001±0.002 ^{ns}	**3.410±0.721** **	0.820	0.001±0.002 ^{ns}	**3.432±0.769*** **	0.833	85.967±3.769	**88.893±7.725*** **	0.570±1.277 ^{ns}	-0.097
Chironomidae spp. (larvae)	Insecta	38	0.014±0.016 ^{ns}	**1.383±0.290*** **	0.600	0.008±0.006 ^{ns}	**1.544±0.321*** **	0.533	79.307±2.643	**78.633±6.947*** **	0.070±0.688 ^{ns}	-0.027
Limnephilidae spp. (larvae)	Insecta	10	0.001±0.001 ^{ns}	**3.176±0.649*** **	0.746	0.001±0.001 ^{ns}	**3.207±0.611*** **	0.789	91.851±2.137	**86.64±3.558*** **	0.382±0.185 ^{ns}	0.290

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Fig. 1. Maps of Sweden (small image) and the sampling area, marking the position of the 32 bays with black circles.

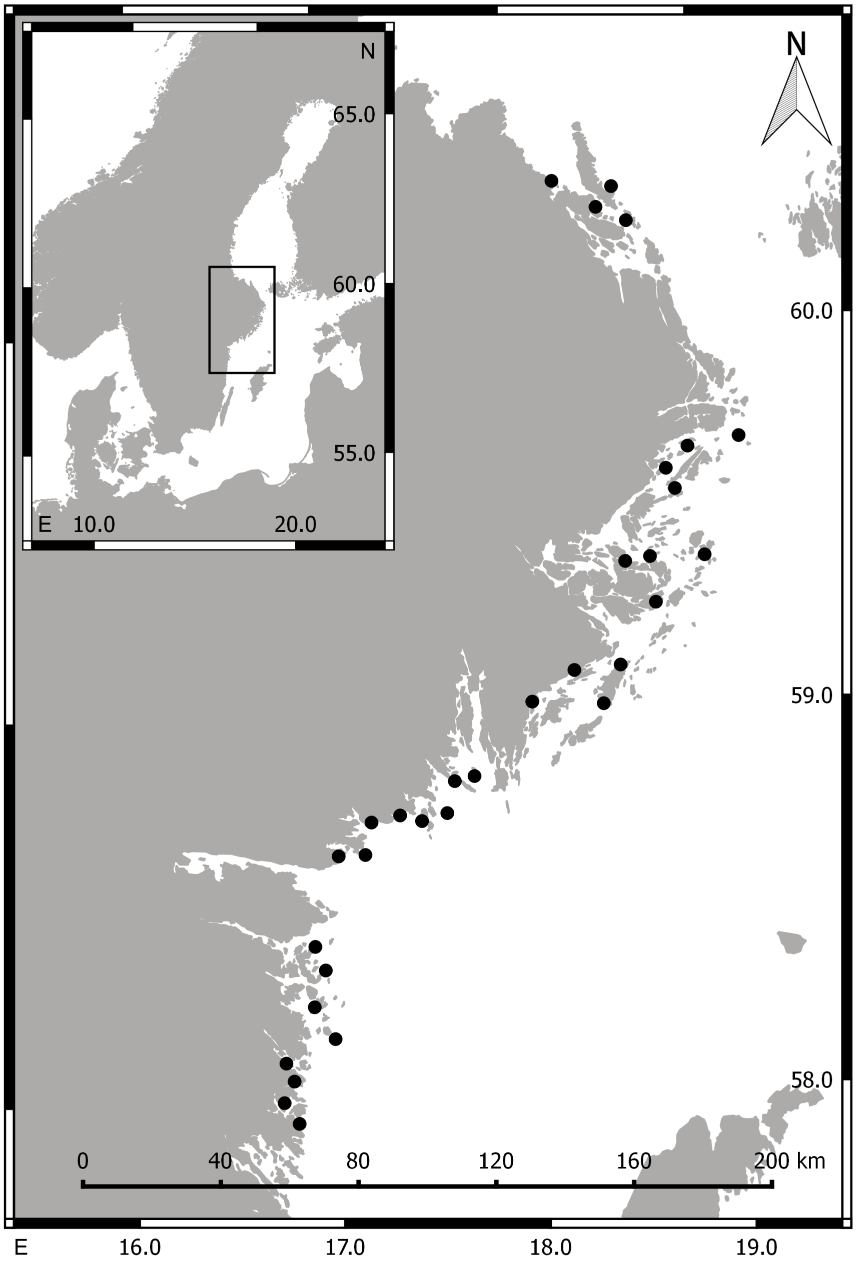

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Fig. 2. Best-fitting relationships between body size (length or height, see methods) and a-d) dry weight (mg. DW), e-h) ash-free dry weight (mg. AFDW) and i-l) AFDW/DW ratio (% AFDW), for 14 taxa - five gastropods, three bivalves, three crustaceans and three insect larvae - sampled in coastal areas of the central Baltic Sea. For model parameters and estimates of fit, see Table 1.