

Size matters: relationships between body size and body mass of common coastal, aquatic invertebrates in the Baltic Sea

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

Methods. We collected 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), body dry mass and ash-free dry mass 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 AFDM/DM ratio per taxon.

Results. For most taxa, non-linear regression models describing the power relationship between body size and i) DM and ii) AFDM 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 AFDM/DM 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 mass 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 ignoring body size. The negative influence of body size on the AFDM/DM 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 AFDM/DM (and presumably also AFDM/wet mass) ratios should carefully assess the potential influence of body size to ensure more reliable estimates of organism body mass.

20 **ABSTRACT (417 words)**

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55 **INTRODUCTION**

56 Organism biomass is inarguably one of the more important variables in ecology, playing a central
57 role in studies ranging from ecophysiology and community and food web regulation, to whole-
58 ecosystem metabolism (e.g. Enquist & Niklas, 2001; Gruner et al., 2008; Perez-Harguindeguy et
59 al., 2013). As a consequence, to accurately estimate organism biomass constitutes one of the most
60 common and important tasks in ecological studies (Rosillo Callé, 2008).

61 Small invertebrates retained on 0.5-1 mm sieves (hereafter ‘macrofauna’) make up a major
62 part of animal density, diversity and biomass in many ecosystems; e.g. insects and arachnids in
63 terrestrial ecosystems; epibenthic, aquatic crustaceans, echinoderms and molluscs in stands of
64 aquatic vegetation; and infaunal (sediment-dwelling) worms, crustaceans and molluscs in marine
65 sediments. Macrofauna biomass is typically reported as dry- or ash-free dry mass per unit area

66 (e.g. g per m²), which requires observers to repeatedly identify, sort, dry and weigh individual or
67 pooled organisms; a time-consuming, expensive and tedious process. Many studies have shown
68 that more easily measured proxy variables scale predictably with dry mass and therefore can be
69 used to speed up biomass estimations; e.g. wet (fresh) mass (Brey, Rumohr & Ankar, 1988;
70 Ricciardi & Bourget, 1998; Brey et al., 2010) and body size, based on either exact length
71 measurement (Smock, 1980; Frithsen, Rudnick & Doering, 1986; Sabo, Bastow & Power, 2002)
72 or retention on sieves of certain mesh sizes (Widbom, 1984; Edgar, 1990; Casagrande &
73 Boudouresque, 2002). While wet mass can be a very good proxy (see e.g. Ricciardi & Bourget,
74 1998), we – as others before – argue that body size (e.g. length) holds several advantages, and
75 lacks several disadvantages associated with wet mass estimations. First, ecological theory
76 supported by empirical data suggest body mass scales predictably with length in the form of power
77 relations (Smock, 1980; Sabo, Bastow & Power, 2002). Second, while freezing/thawing and
78 fixation in conservation liquids (e.g. EtOH or formalin) can affect both organism wet mass
79 (Howmiller, 1972; Mason, Lewis & Weber, 1983; Leuven, Brock & van Druten, 1985) and length
80 (Hjörleifsson & Klein-MacPhee, 1992; Kaporis, Miliou & Moraitou-Apostolopoulou, 1997), wet
81 mass estimations are also very sensitive to exactly how specimens are blotted, centrifuged (to
82 remove excess water), and exposed to air and light before and during weighing (Howmiller, 1972;
83 Mason, Lewis & Weber, 1983; Leuven, Brock & van Druten, 1985). As size estimations do not
84 require blotting, they are less sensitive to observer error, and also faster to perform. Third, body
85 size (e.g. length or height) estimations can more easily be automated, using e.g. image analysis
86 software (Paavo et al., 2008; Mallard, Le Bourlot & Tully, 2013), to rapidly process multiple
87 individuals at a time.

88 In benthic ecology, ash-free dry mass (hereafter ‘AFDM’, in the older literature called ‘ash-

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

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

108

109 **METHODS**

110 **Study area**

111 The Baltic Sea is a 415 000 km² large marginal sea situated in northern Europe (53-66° N; 10-30°

112 E). A main feature is the presence of strong horizontal and vertical gradients in salinity,
113 temperature and oxygen, that also undergo considerable temporal (e.g. seasonal) fluctuations
114 (Voipio, 1981). The Baltic Sea is evolutionary very young (ca 6000 years), and the shallow coastal
115 areas have since the last glaciation been colonized by a mixture of marine, freshwater and brackish
116 organisms, including crustaceans, gastropods, bivalves, polychaetes, hirudineans, nemertean and
117 insect larvae (Hansen, Wikström & Kautsky, 2008). As many marine and freshwater organisms in
118 the Baltic Sea live near their physiological tolerance limits, they grow slower and smaller than in
119 their original environment; e.g. the blue mussel *Mytilus edulis* (Tedengren & Kautsky, 1986). As
120 a consequence, their size ranges - but also size:mass relationships and, potentially, AFDM/DM
121 ratios - could differ from those reported for conspecifics in marine or freshwater areas (Rumohr,
122 Brey & Ankar, 1987). An estimate of the effect of salinity on size:mass or DM:AFDM relationship
123 was beyond the scope of our study, but our results could be compared to relationships in marine
124 populations of the same taxa, if sampled and measured in the same way.

125

126 **Field sampling**

127 During summer (May-Aug) 2014 we collected aquatic invertebrate macrofauna (>1mm) in 32
128 shallow bays situated along a 360 km stretch of the central, Swedish Baltic Sea coastline (Fig. 1).
129 The salinity in the area is generally low (ca. 5-7 psu) but fluctuates strongly with freshwater runoff
130 and upwelling events. In each bay, a snorkeler sampled submerged aquatic vegetation and
131 epibenthic macrofauna in 3-8 randomly selected stations (>30 m apart), by gently placing a 20×20
132 cm frame (with a 1mm-mesh bag attached) on the sea bed, and collecting all organisms (aquatic
133 vegetation and associated invertebrates) found above or on top of the sediment surface. The bag

134 content was immediately transferred to a plastic bag, which was kept cold on ice until frozen (-20°
135 C), in most cases within 1-3 hours.

136

137 **Body size estimations**

138 Following thawing in room temperature, we identified intact invertebrate organisms to the highest
139 taxonomic resolution feasible using standard literature. For the 14 most common taxa we then
140 selected and measured the body size of 12-459 individuals per taxa (3220 individuals in total),
141 chosen to capture the full range of body sizes found across the 32 bays. The taxa included five
142 gastropods (*Theodoxus fluviatilis*, *Hydrobia* spp., *Radix balthica*, *Potamopyrgus antipodarum*,
143 *Bithynia tentaculata*), three bivalves (*Mytilus edulis*, *Limecola (Macoma) balthica* and *Cardidae*
144 spp. [numerically dominated by *Parvicardium hauniense*], three crustaceans (*Amphibalanus*
145 *improvisus*, *Idotea* spp., *Gammarus* spp.) and three insects (larval stages of Chironomidae spp.,
146 *Agraylea* spp. and Phryganeidae spp.) (see also Table 1). Body size (to the nearest 1 mm) was
147 measured (based on standard procedures; Hayward & Ryland, 1995) as; i) gastropod height along
148 the central shell axis, ii) bivalve length from anterior to posterior side, iii) total length of *Gammarus*
149 and *Idotea* spp. from tip of rostrum to last urosome, iv) body width for *Amphibalanus improvisus*,
150 and v) total length of insect larvae from end of head to last segment. A higher size accuracy is
151 definitely possible (e.g. to 0.1 or 0.01 mm using calipers or stereo lenses), but as most studies
152 utilizing this type of data (including ours) will depend on 1000s of length measurements, the
153 accuracy chosen was a realistic trade-off between time and precision.

154

155 **Estimations of dry- and ash-free dry mass**

156 Following size estimations, the measured individuals were transferred to pre-dried and -weighed
157 (nearest 0.0001 g) porcelain crucibles. For most size classes (except for very large and rare
158 individuals), multiple individuals were typically pooled into the same crucible. This step
159 underestimates actual variability in body mass between individuals, but was necessary as the low
160 individual body masses (particularly AFDM) were near or below the reliable detection limit of the
161 scale. We included multiple estimations of the same sizes, so that the number of biomass
162 estimations (N) ranged from 10 to 42 per taxa. Samples were then dried at 60 °C for >48h (until
163 constant mass), and cooled to room temperature in a desiccator before weighing. To estimate ash-
164 free dry mass, the crucibles were then transferred to a muffle furnace, incinerated (550 °C for 3
165 hours), cooled and weighed again. Ash-free dry mass was calculated as dry mass minus ash mass.

166

167 **Statistical analyses**

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

170

$$body\ mass = \alpha \times size^{\beta}$$

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

179 For each taxon we also calculated the mean (± 1 SE) AFDM/DM ratio (in %); a
180 commonly used conversion factor in macroinvertebrate studies (see e.g. Ricciardi & Bourget,
181 1998). We then used linear regression to test whether body size (in mm) affected the AFDM/DM
182 ratio. Prior to analyses we checked assumptions of normality (by plotting predicted vs. observed
183 quantiles) and homoscedasticity (by plotting predicted vs. observed residuals). All analyses were
184 conducted in R v. 3.2.3 (R Core Team, 2016).

185

186 RESULTS

187 *Relationships between body size and individual biomass*

188 The relationships between body size (mm), individual dry mass (mg DM) and ash-free dry mass
189 (mg AFDM) for all 14 taxa are displayed in Figure 2a-h, and the parameters (and their fit) are
190 presented in Table 1. For most of the taxa, body size was a very good predictor of individual DM,
191 as demonstrated by low SE and R^2 near 1. The model fits were slightly poorer for the three insect
192 taxa ($R^2 = 0.60$ - 0.82) and the gastropod *Bithynia tentaculata* ($R^2 = 0.85$) than for the other ten
193 taxa. For a majority (12 out of 14) of the taxa, the scaling constants (β) were well above 2 (2.110-
194 3.590). The exceptions were the small gastropod *Potamopyrgus antipodarum* and chironomid
195 larvae, which had constants closer to 1 ($\beta = 1.368$ and 1.383 , respectively).

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

202

203 *Influence of organism body size on AFDM/DM ratios*

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

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

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220

221 **DISCUSSION**

222 Estimating organism biomass is one of the most common, important but also resource-consuming
223 tasks in ecological work, particularly when it comes to small-bodied, highly abundant and diverse
224 macroscopic invertebrates. Many previous studies have shown that more easily measured variables

225 like invertebrate wet (fresh) mass (e.g. Ricciardi & Bourget, 1998) or body size (e.g. Smock, 1980)
226 can be used as proxies to reliably predict both the dry- and ash-free dry body mass, thereby
227 simplifying and speeding up biomass estimations. Here, we first complement this literature by
228 reporting how body mass scales with body size for 14 of the most common epibenthic invertebrate
229 taxa found in shallow coastal areas of the Baltic Sea. Moreover, we demonstrate that for a majority
230 of the studied molluscs, the ratio between organism dry- and ash-free dry mass – an often-used
231 conversion factor (e.g. Rumohr, Brey & Ankar, 1987; Ricciardi & Bourget, 1998) – decreases
232 predictably with body size. Thus, our results can be used to quickly estimate the biologically active
233 biomass of individual organisms based on their size, and when combined with density data,
234 accurately estimate biomass per unit area.

235

236 *Body size as a proxy for dry- and ash-free dry mass*

237 For a majority of the studied taxa, body size was a good predictor of both dry mass and ash-free
238 dry mass. The model fits were slightly poorer for ash-free dry mass (AFDM); most likely a
239 consequence of the fact that even though multiple individuals of the same size were pooled, the
240 low individual AFDM of many organisms (in the vicinity of 1 mg) challenged the accuracy of the
241 scale. Comparisons between the 14 taxa studied (Table 1) show that particularly within the
242 gastropods and crustaceans, the scaling (β) constants differ quite substantially between taxa (see
243 the different slopes in Fig. 2 and β coefficients in Table 1). These differences emphasize the need
244 for taxon-specific relationships to accurately predict biomass, and the potential dangers in either
245 ignoring body size or substituting relationships between taxa. Consequently, our power equations
246 (Table 1) can be used in a simple yet reliable way to estimate organism dry- or ash-free dry mass
247 based on standard body size measurements. Future studies should ideally also assess how these

248 relationships vary in time and space (e.g. over seasons), for even more accurate biomass
249 estimations. Size-based biomass estimations are likely to speed up laboratory work considerably;
250 for example, Casagrande and Boudouresque (2002) showed that sieve-based size estimations
251 speeded up estimations of body biomass of the gastropod *Hydrobia ventricosa* by 20-30 times.
252 Consequently, our size-based estimations of invertebrate biomass are likely to free up considerable
253 work resources (time, man-power, money) that can be used to e.g. collect and process more
254 samples.

255

256 *The influence of body size on AFDM/DM ratios*

257 For most of the taxa with a calcium-carbonate (molluscs) or calcite shell (the barnacle
258 *Amphibalanus improvisus*), we found a significant negative influence of body size on the
259 AFDM/DM ratio; a commonly reported and often-used conversion factor in macrofauna studies
260 (e.g. Rumohr, Brey & Ankar, 1987; Ricciardi & Bourget, 1998). In other words, the proportional
261 mass of biologically active vs. non-active tissue (shell, hard mouth parts, etc.) decreased with body
262 size. There are at least two possible and complementary explanations for this relationship. First,
263 while the rate of growth in length of mollusc shells typically decreases with age, new shell layers
264 are consistently added on a yearly basis (Negus, 1966). This results in increasingly thicker, and
265 therefore disproportionally heavier, shells with mussel length, and a higher shell:tissue mass ratio.
266 Second, our sampling was conducted during summer; a season when a majority of adult molluscs
267 (here represented by the larger individuals per taxa) most likely had spawned and temporarily lost
268 a considerable proportion of their biologically active tissue (Kautsky, 1982). The slopes of the
269 significant regressions (Table 1, median = -1.26) suggest that failing to incorporate the potential
270 influence of body size can strongly reduce the accuracy of AFDM estimations based on dry mass

271 (and presumably also wet mass), particularly if there is considerable variability in body size in the
272 samples. The somewhat surprising lack of size influence in the common blue mussel *Mytilus edulis*
273 was not investigated in detail, but could be caused by i) the lack of small shell-crushing mussel
274 predators in the area (e.g. crabs), who otherwise are known to trigger thicker mussel shells
275 (Freeman, 2007), and/or ii) the relatively low salinity, which causes the small, osmotically stressed
276 *M. edulis* to invest considerably more energy into osmosis and soft tissue production, than in
277 thicker shells (Kautsky, Johannesson & Tedengren, 1990).

278 In contrast to the results for molluscs, there was no size effect on AFDM/DM ratios for the
279 chitin-shelled insects and crustaceans. These results fit well with those reported in previous studies,
280 for example of terrestrial insects, for which exoskeletal chitin scales isometrically (1:1) with body
281 size (Lease & Wolf, 2010). In summary, our results suggest that body size can play an important
282 but hitherto underestimated role when estimating organism AFDM based on dry (and possibly,
283 wet) mass, particularly for shelled molluscs.

284

285 **Conclusions**

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

294 assess the potential influence of body size and spatial-temporal variability, to ensure reliable
295 biomass estimations.

296

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303

304 **References (count = 36)**

- 305 Andersen SO. 1979. Biochemistry of Insect Cuticle. *Annual Review of Entomology* 24:29-59. DOI:
306 10.1146/annurev.en.24.010179.000333
- 307 Brey T, Muller-Wiegmann C, Zittier ZMC, and Hagen W. 2010. Body composition in aquatic
308 organisms - A global data bank of relationships between mass, elemental composition and
309 energy content. *Journal of Sea Research* 64:334-340. DOI: 10.1016/j.seares.2010.05.002
- 310 Brey T, Rumohr H, and Ankar S. 1988. Energy content of macrobenthic invertebrates: general
311 conversion factors from weight to energy. *Journal of Experimental Marine Biology and*
312 *Ecology* 117:271-278. DOI: 10.1016/0022-0981(88)90062-7
- 313 Casagrande C, and Boudouresque CF. 2002. A sieving method for rapid determination of size-
314 frequency distribution of small gastropods. Example of the mud snail *Hydrobia ventrosa*.
315 *Hydrobiologia* 485:143-152. DOI: 10.1023/A:1021371308753

- 316 Edgar GJ. 1990. The use of the size structure of benthic macrofaunal communities to estimate
317 faunal biomass and secondary production. *Journal of Experimental Marine Biology and*
318 *Ecology* 137:195-214. DOI: 10.1016/0022-0981(90)90185-F
- 319 Enquist BJ, and Niklas KJ. 2001. Invariant scaling relations across tree-dominated communities.
320 *Nature* 410:655-660. DOI: 10.1038/35070500
- 321 Freeman AS. 2007. Specificity of induced defenses in *Mytilus edulis* and asymmetrical predator
322 deterrence. *Marine Ecology Progress Series* 334. DOI: 10.3354/meps334145
- 323 Frithsen JB, Rudnick DT, and Doering PH. 1986. The determination of fresh organic carbon
324 weight from formaldehyde preserved macrofaunal samples. *Hydrobiologia* 133:203-208.
325 DOI: 10.1007/BF00005591
- 326 Gruner DS, Smith JE, Seabloom EW, Sandin SA, Ngai JT, Hillebrand H, Harpole WS, Elser JJ,
327 Cleland EE, Bracken MES, Borer ET, and Bolker BM. 2008. A cross-system synthesis of
328 consumer and nutrient resource control on producer biomass. *Ecology Letters* 11:740-755.
329 DOI: 10.1111/j.1461-0248.2008.01192.x
- 330 Hansen JP, Wikström SA, and Kautsky L. 2008. Effects of water exchange and vegetation on the
331 macroinvertebrate fauna composition of shallow land-uplift bays in the Baltic Sea.
332 *Estuarine, Coastal and Shelf Science* 77:535-547. DOI: 10.1016/j.ecss.2007.10.013
- 333 Hayward PJ, and Ryland JS. 1995. Handbook of the Marine Fauna of North-West Europe. New
334 York, USA: Oxford University Press. p 800.
- 335 Hjörleifsson E, and Klein-MacPhee G. 1992. Estimation of live standard length of winter flounder
336 *Pleuronectes americanus* larvae from formalin-preserved, ethanol-preserved and frozen
337 specimens. *Marine Ecology Progress Series* 82:13-19. DOI: 10.3354/meps082013

- 338 Howmiller RP. 1972. Effects of Preservatives on Weights of Some Common Macrobenthic
339 Invertebrates. *Transactions of the American Fisheries Society* 101:743-746. DOI:
340 10.1577/1548-8659(1972)101<743:EOPOWO>2.0.CO;2
- 341 Kapiris K, Miliou H, and Moraitou-Apostolopoulou M. 1997. Effects of formaldehyde
342 preservation on biometrical characters, biomass and biochemical composition of *Acartia*
343 *clausi* (Copepoda, Calanoida). *Helgolander Meeresuntersuchungen* 51:95-106. DOI:
344 10.1007/BF02908757
- 345 Kautsky N. 1982. Quantitative studies on gonad cycle, fecundity, reproductive output and
346 recruitment in a baltic *Mytilus edulis* population. *Marine Biology* 68:143-160. DOI:
347 10.1007/BF00397601
- 348 Kautsky N, Johannesson K, and Tedengren M. 1990. Genotypic and phenotypic differences
349 between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal
350 transplantations. I. Growth and morphology. *Marine Ecology Progress Series* 59:203-210.
351 DOI: 10.3354/meps059203
- 352 Lease HM, and Wolf BO. 2010. Exoskeletal chitin scales isometrically with body size in terrestrial
353 insects. *Journal of Morphology* 271:759-768. DOI: 10.1002/jmor.10835
- 354 Leuven RSEW, Brock TCM, and van Druten HAM. 1985. Effects of preservation on dry- and ash-
355 free dry weight biomass of some common aquatic macro-invertebrates. *Hydrobiologia*
356 127:151-159. DOI: 10.1007/BF00004193
- 357 Mallard F, Le Bourlot V, and Tully T. 2013. An automated image analysis system to measure and
358 count organisms in laboratory microcosms. *Plos ONE* 8:e64387. DOI:
359 10.1371/journal.pone.0064387

- 360 Mason WT, Lewis PA, and Weber CI. 1983. An evaluation of benthic macroinvertebrate biomass
361 methodology. *Environmental Monitoring and Assessment* 3:29-44. DOI:
362 10.1007/BF00394030
- 363 Miller K, and Birchard GF. 2005. Influence of Body Size on Shell Mass in the Ornate Box Turtle,
364 *Terrapene ornata*. *Journal of Herpetology* 39:158-161. DOI: 10.1670/0022-
365 1511(2005)039[0158:IOBSOS]2.0.CO;2
- 366 Negus C. 1966. A Quantitative Study of Growth and Production of Unionid Mussels in the River
367 Thames at Reading. *Journal of Animal Ecology* 35:513-532. DOI: 10.2307/2489
- 368 Niklas KJ. 1995. Size-dependent allometry of tree height, diameter and trunk-taper. *Annals of*
369 *Botany*. p 217-227. DOI: 10.1006/anbo.1995.1015
- 370 Paavo B, Ziegelmeyer A, Lavric E, and Probert PK. 2008. Morphometric correlations and body
371 mass regressions for *Armandia maculata*, *Aglaophamus macroura* (Polychaeta), and
372 *Zethalia zelandica* (Gastropoda). *New Zealand Journal of Marine and Freshwater*
373 *Research* 42:85-91. DOI: 10.1080/00288330809509938
- 374 Perez-Harguindeguy N, Diaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS,
375 Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L,
376 Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quetier F,
377 Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L,
378 Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, and Cornelissen
379 JHC. 2013. New handbook for standardised measurement of plant functional traits
380 worldwide. *Australian Journal of Botany* 61:167-234. DOI: 10.1071/bt12225
- 381 R Core Team. 2016. R: A Language and Environment for Statistical Computing. R Foundation for
382 Statistical Computing. Vienna Austria.

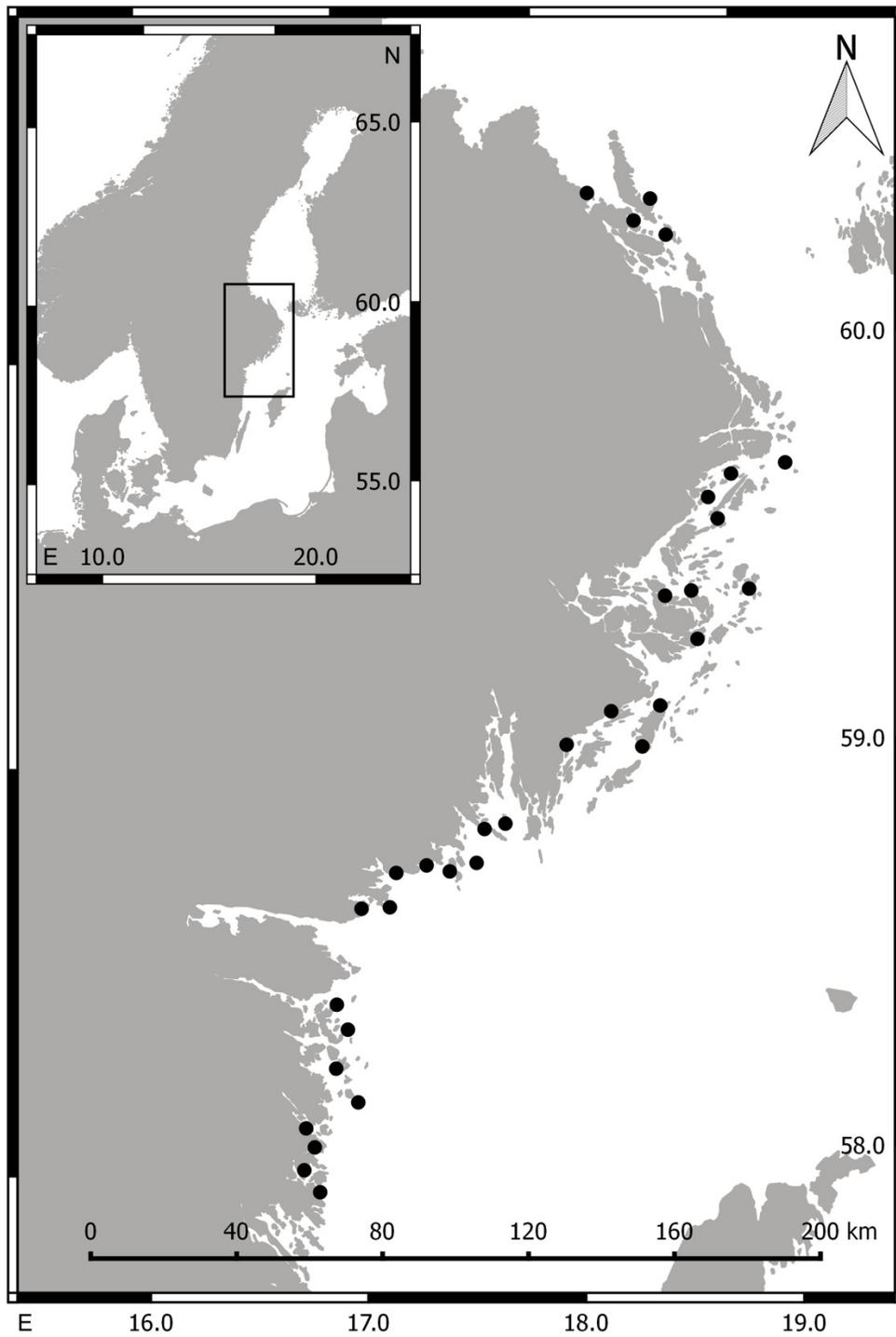
- 383 Ricciardi A, and Bourget E. 1998. Weight-to-weight conversion factors for marine benthic
384 macroinvertebrates. *Marine Ecology-Progress Series* 163:245-251. DOI:
385 10.3354/meps171245
- 386 Rosillo Callé F. 2008. *The biomass assessment handbook: bioenergy for a sustainable*
387 *environment*. Routledge.
- 388 Rumohr H, Brey T, and Ankar S. 1987. A compilation of biometric conversion factors for benthic
389 invertebrates of the Baltic Sea. *The Baltic Marine Biologists* 9:1-56. DOI:
390 10.3354/meps171245
- 391 Sabo JL, Bastow JL, and Power ME. 2002. Length-Mass relationships for adult aquatic and
392 terrestrial invertebrates in a California watershed. *Journal of the North American*
393 *Benthological Society* 21:336-343. DOI: 10.2307/1468420
- 394 Smock LA. 1980. Relationships between body size and biomass of aquatic insects. *Freshwater*
395 *Biology* 10:375-383. DOI: 10.1111/j.1365-2427.1980.tb01211.x
- 396 Spiess A-N, and Neumeier N. 2010. An evaluation of R² as an inadequate measure for nonlinear
397 models in pharmacological and biochemical research: a Monte Carlo approach. *BMC*
398 *pharmacology* 10:6. DOI: 10.1186/1471-2210-10-6
- 399 Tedengren M, and Kautsky N. 1986. Comparative study of the physiology and its probable effect
400 on size in Blue Mussels (*Mytilus Edulis* L.) from the North Sea and the Northern Baltic
401 Proper. *Ophelia* 25:147-155. DOI: 10.1080/00785326.1986.10429746
- 402 Voipio A. 1981. *The Baltic Sea*. Elsevier Science.
- 403 Widbom B. 1984. Determination of average individual dry weights and ash-free dry weights in
404 different sieve fractions of marine meiofauna. *Marine Biology* 84:101-108. DOI:
405 10.1007/BF00394532

407 Table 1. Results of regression analyses estimating i) the non-linear power relationship between body size and dry mass (DM) and ii)
 408 ash-free dry mass (AFDM), iii) the mean \pm 1 SE AFDM/DM ratio (in %), and iv) the linear relationship between body size and
 409 AFDM/DM ratio (in %), for 14 macroinvertebrate taxa in shallow coastal areas of the Baltic Sea. Letters within parentheses after taxa
 410 names denote classes, where G: Gastropoda, B: Bivalvia, C: Crustacea, and I: Insecta (larvae). α and β normalization and scaling
 411 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
 412 = 0.05). Note: R^2 were derived from linear log-log models.

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Taxon	N	Body size vs. DM			Body size vs. AFDM			AFDM/DM	Body size vs. AFDM/DM		
		$\alpha \pm SE$	$\beta \pm SE$	R^2	$\alpha \pm SE$	$\beta \pm SE$	R^2	Mean $\% \pm 1SE$	Intercept $\pm SE$	slope $\pm SE$	R^2
<i>Bithynia tentaculata</i> L. (G)	25	0.598 \pm 0.484 ^{ns}	2.117\pm0.351***	0.847	0.479 \pm 0.511 ^{ns}	1.36\pm0.472**	0.668	19.133 \pm 2.207	33.162\pm3.878***	-1.91\pm0.452*	0.424
<i>Hydrobia</i> spp. (G)	24	0.239\pm0.041***	2.134\pm0.095***	0.952	0.079\pm0.029*	1.441\pm0.22***	0.758	13.737 \pm 1.155	19.791\pm2.855***	-0.633\pm0.715*	0.155
<i>Potamopyrgus antipodarum</i> Gray (G)	17	0.479 \pm 0.511 ^{ns}	1.360\pm(0.472**)	0.919	0.021 \pm 0.012 ^{ns}	2.447\pm0.395***	0.898	16.051 \pm 1.399	6.063 \pm 4.616 ^{ns}	2.653\pm1.180*	0.202
<i>Radix balthica</i> L. (G)	20	0.137\pm0.035**	2.355\pm0.115***	0.956	0.046\pm0.018*	2.119\pm0.177***	0.906	27.087 \pm 2.233	35.338\pm3.558***	-1.794\pm0.650*	0.258
<i>Theodoxus fluviatilis</i> L. (G)	29	0.221\pm0.065**	2.683\pm0.148***	0.9492	0.015\pm0.006*	2.915\pm0.194***	0.912	13.044 \pm 1.083	18.52\pm2.396***	-0.242\pm0.494*	0.159
Cardidae spp. (B)	33	0.134 \pm 0.094 ^{ns}	2.848\pm0.347***	0.924	0.014 \pm 0.013 ^{ns}	2.806\pm0.486***	0.879	12.358 \pm 0.852	18.075\pm1.468***	-0.429\pm0.325*	0.364
<i>Limecola balthica</i> L. (B)	18	0.069\pm0.024*	2.820\pm0.134***	0.991	0.001 \pm 0.002 ^{ns}	3.479\pm0.673***	0.92	12.717 \pm 1.934	21.429\pm2.98***	-0.264\pm0.372*	0.383
<i>Mytilus edulis</i> L. (B)	24	0.030\pm0.015*	2.933\pm0.153***	0.991	0.006\pm0.003*	2.844\pm0.147***	0.978	14.189 \pm 0.504	13.162\pm1.044***	0.078 \pm 0.069 ^{ns}	0.011
<i>Amphibalanus improvisus</i> Darwin (C)	13	0.314 \pm 0.205 ^{ns}	2.515\pm0.289***	0.976	0.036 \pm 0.022 ^{ns}	2.289\pm0.276***	0.961	8.939 \pm 0.550	11.044\pm1.064***	-0.397\pm0.179*	0.246
<i>Gammarus</i> spp. (C)	37	0.047 \pm 0.032 ^{ns}	2.111\pm0.265***	0.926	0.033 \pm 0.028 ^{ns}	2.05\pm0.32***	0.863	58.966 \pm 1.519	63.062\pm2.616***	-0.389 \pm 0.307 ^{ns}	0.017
<i>Idothea</i> spp. (C)	42	0.001 \pm 0.001 ^{ns}	3.592\pm0.200***	0.949	0.001 \pm 0.001 ^{ns}	3.850\pm0.249***	0.919	61.505 \pm 1.659	66.183\pm3.457***	-0.550 \pm 0.358 ^{ns}	0.032
<i>Agraylea</i> spp. (I)	13	0.001 \pm 0.002 ^{ns}	3.410\pm0.721**	0.820	0.001 \pm 0.002 ^{ns}	3.432\pm0.769***	0.833	85.967 \pm 3.769	88.893\pm7.725***	0.570 \pm 1.277 ^{ns}	-0.097
Chironomidae spp. (I)	38	0.014 \pm 0.016 ^{ns}	1.383\pm0.290***	0.600	0.008 \pm 0.006 ^{ns}	1.544\pm0.321***	0.533	79.307 \pm 2.643	78.633\pm6.947***	0.070 \pm 0.688 ^{ns}	-0.027
Phryganeidae spp. (I)	10	0.001 \pm 0.001 ^{ns}	3.176\pm0.649***	0.746	0.001 \pm 0.001 ^{ns}	3.207\pm0.611***	0.789	91.851 \pm 2.137	86.64\pm3.558***	0.382 \pm 0.185 ^{ns}	0.290

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



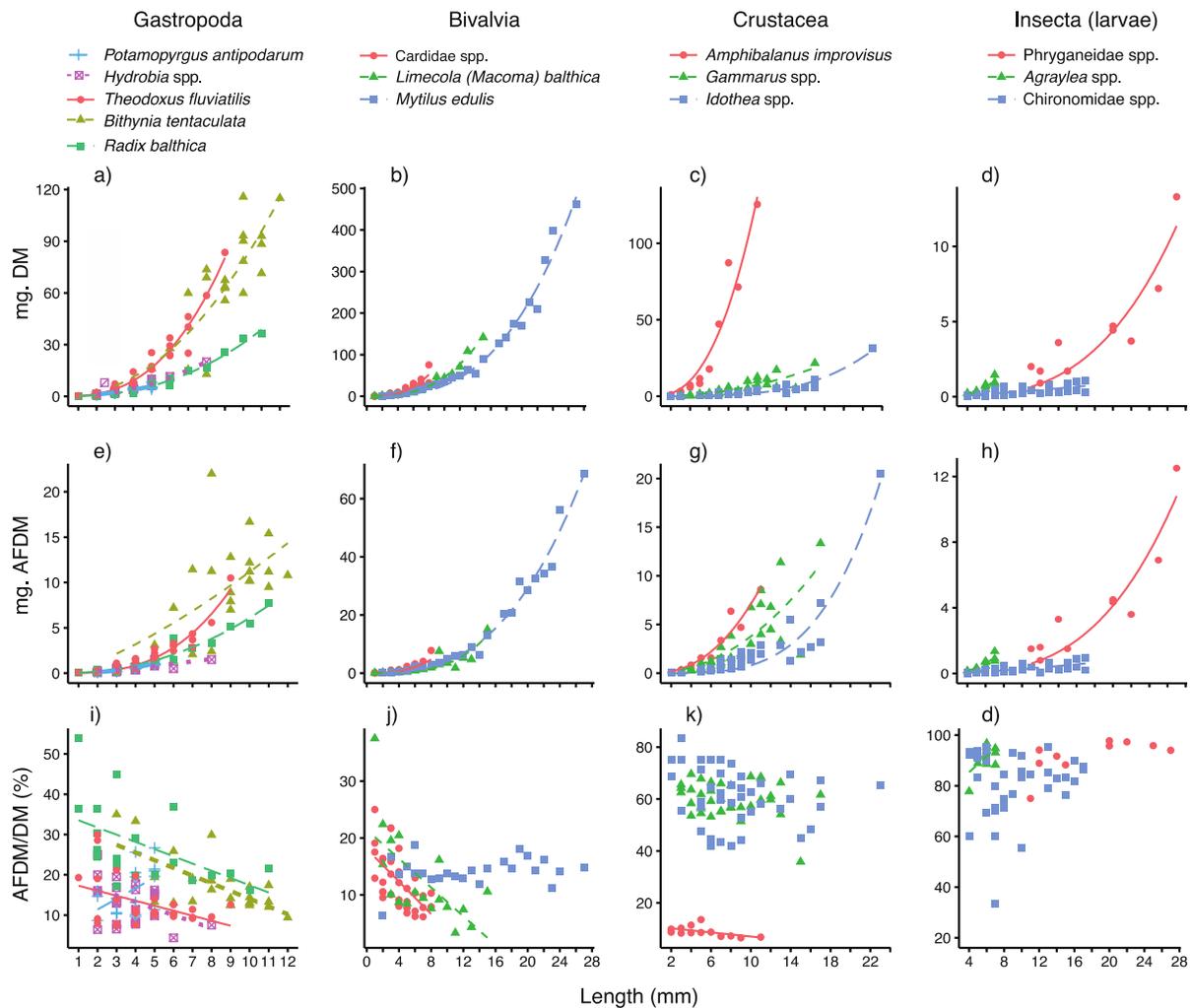
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420 Fig. 2. Best-fitting relationships between body size (length or height, see Methods) and a-d) dry
 421 mass (mg. DM), e-h) ash-free dry mass (mg. AFDM) and i-l) AFDM/DM ratio (% AFDM), for 14
 422 taxa - five gastropods, three bivalves, three crustaceans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

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