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# Larval growth of the polychaete *Arenicola marina* under different temperature and food conditions: consequences on bioenergetic models

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1 **Larval growth of the polychaete *Arenicola marina* under different**  
2 **temperature and food conditions: consequences on bioenergetic models**

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15 **Lay summary**

16 Biphasic growth models of *Arenicola marina* larvae highlight an optimal temperature at 15°C  
17 with a lower temperature tolerance range compared to juveniles and adults. We claim that two  
18 sets of Arrhenius temperatures depending on the life-history stages should be implemented  
19 when using an abj-DEB model in this species.

20

21 **Total word counted 6698**

22 **Abstract**

23 *Arenicola marina*, a marine benthic polychaete, is widespread on sandy beaches in Europe  
24 and considered as an ecosystem engineer despite commonly used as bait by fishermen. Data  
25 regarding the bioenergetics of the lugworm larval stages are still incomplete. Trochophore is  
26 initially lecithotroph and then becomes planktotroph while growing as metatrochophore on  
27 subtidal area, a quite stable daily temperature environment compared to the foreshore, where  
28 juveniles and adult live, with daily temperature fluctuating up to 15°C. These discrepancies in  
29 temperature ranges may influence the temperature corrections that control metabolic rates  
30 during the life cycle of *A. marina*. We carried out laboratory experiments in microcosms by  
31 inducing artificial spawning of lugworms, and then undertaken *in vitro* fertilization to obtain  
32 embryos and finally to follow, the larval development up to 10 segments with chaetae for 50  
33 days under three temperature conditions (13°C, 15°C and 17°C) and two food conditions  
34 ('fed' and 'non-fed'). The first feeding ('birth') of *A. marina* larvae was deciphered  
35 anatomically for a size between ~ 450–500 µm and described at 17 days post-fertilization for  
36 larvae reared at 15°C and 17°C. Using a biphasic model with a von Bertalanffy growth before  
37 'birth' and, an exponential growth after 'birth', among the three temperature treatments, the  
38 15°C condition exhibited the best larval performance. Temperature corrections (TC) based on  
39 embryonic and larval metabolic rates gave an Arrhenius temperature of ~ 6661 K and a higher  
40 boundary temperature tolerance range of ~ 294.5 K. Both temperature values differ from  
41 those calculated from TC based mostly on juvenile and adult metabolic rates. We claim to use  
42 two sets of Arrhenius temperatures according to the life-history stages of *A. marina* while  
43 using Dynamic Energy Budget model. This model was developed initially in order to manage  
44 the conservation of the lugworm species.

45 Key words: Growth rate; lugworm; polychaetes; biphasic model; DEB model; larval stages;  
46 Arrhenius temperatures.

## 47 **Introduction**

48 Polychaeta are mainly marine metazoans and represent significant part of the benthic biomass  
49 (Grémare *et al.*, 1998). They play a major role in the functioning of benthic ecosystems and  
50 serve as bio-indicators of the marine environment health status (Giangrande *et al.*, 2005;  
51 Sivadas *et al.*, 2010). Polychaetes have a market value in fisheries where they are used as bait  
52 by fishermen (Watson *et al.*, 2017). In aquaculture, polychaetes may be used either as food  
53 supplements due to their high nutritional value for cultured aquatic species (Pairohakul *et al.*,  
54 2021) or by their abilities in waste depollution in integrated aquaculture (Jansen *et al.*, 2019;  
55 Jerónimo *et al.*, 2020). Finally, studies have shown the therapeutic interest which certain  
56 species of polychaetes may have for applications in human health (Kuijk & van Die, 2010;  
57 Singh *et al.*, 2014). For all these reasons, the breeding and marketing of polychaetes are of  
58 growing interest and are current issues (Olive, 1993; Olive, 1994; Micael *et al.*, 2016). This  
59 attractiveness causes intensive harvesting of these species, mainly on foreshore, which results  
60 in an alteration of the environment and therefore has a deleterious effect on benthic  
61 ecosystems (Beukema, 1989; Clarke *et al.*, 2017). Moreover, this overexploitation of the  
62 resource endangers the survival of some species of polychaetes (Cole *et al.*, 2018; De Cubber  
63 *et al.*, 2018). To overcome these issues, some countries have implemented regulations that  
64 aimed at regulating the rate of withdrawals over the years in sensitive areas e.g. in Portugal  
65 (Xenarios *et al.*, 2018), in USA (Sypitowski *et al.*, 2009), in Australia (Cole *et al.*, 2018) and  
66 in UK (Watson *et al.*, 2015). Another way to avoiding the depopulation of polychaetes is to  
67 develop the domestication of species of high economic interest; thus, farms of *Atilia virens*  
68 (Olive, 1999; Sustainable Feeds Ltd™), *Arenicola marina* (Olive *et al.*, 2001; Hemarina  
69 Ltd™), *Arenicola defodiens* (Olive *et al.*, 2001), *Hediste diversicolor* (Bischoff *et al.*, 2009),  
70 *Diopatra aciculata* (Safarik *et al.*, 2006), *Perinereis cf. nuntia* (Poltana *et al.*, 2007) and  
71 *Perinereis helleri* (Palmer *et al.*, 2016) have emerged. However, a complete knowledge of the

72 physiology of these polychaetes and in particular of the early stages of their development, is  
73 necessary to carry out these conservation and cultivation projects.

74         The lugworm *Arenicola marina* (Linnaeus, 1758) is one of the most used bait for  
75 professional and recreational fishing in Western Europe, where it is intensively harvested  
76 from the Arctic to the Mediterranean (Watson *et al.*, 2017; De Cubber *et al.*, 2018). Moreover,  
77 the strong affinity of its hemoglobin for oxygen has led to the production of this worm for  
78 therapeutic uses in human health, whether as an organ preservative during transplants but also  
79 as a possible blood substitute (Rousselot *et al.*, 2006; Batool *et al.*, 2021). *A. marina* lives in 5  
80 to 40 cm deep U-shaped burrows in soft foreshore sediments in the intertidal area, from  
81 mediolittoral to infralittoral (De Cubber *et al.*, 2020). The life cycle of *A. marina* has been  
82 described in details (Newell, 1948; Newell, 1949; Farke & Berghuis, 1979a,b; Reise *et al.*,  
83 2001; De Cubber *et al.*, 2019). Juveniles and adults live in burrows, where they swallow the  
84 sediment at the surface being psammivorous. Lugworms may move backwards in the burrow,  
85 where they expulse their faeces by their pygidium that forms a characteristic sand-pile called  
86 castings. Breeding season occurs in autumn where lugworm's population have annual  
87 epidemic spawning of few days (Watson *et al.*, 2000). Females spawn their oocytes within the  
88 gallery, while males release sperm puddles on to the sediment surface that will be diluted by  
89 the incoming tide and then drawn into female's gallery by pumping. Fertilization takes place  
90 inside the gallery (Williams *et al.*, 1997) where embryos remain until hatching at the  
91 trochophore larval stage. Trochophores and then metatrochophores are lecithotrophic larvae  
92 dispersing several days (depending on temperature) in the water column until temporally (few  
93 months) settling on subtidal marine habitats such as macroalgae or mussel beds (Farke &  
94 Berghuis, 1979a,b; De Cubber *et al.*, 2019). During that first settling period, the first food  
95 intake ('birth'; Dynamic Energy Budget (DEB) theory see after; Kooijman, 2010) occurs,  
96 where larvae will live in a mucus tube but going out of their tube to collect organic matter or

97 phytoplankton. Larvae will develop segments with chaetae called setigers (up to 19 setigers)  
98 until the completion of metamorphosis that could last up to 7 months (Farke & Berghuis,  
99 1979a,b; De Cubber *et al.*, 2019). When metamorphosis will be completed, a second phase of  
100 dispersal will occur into the water column allowing post-larval stages to reach the foreshore.  
101 These post-larvae will then settle on high part of the shore, burrowing themselves and  
102 becoming a psammivorous juvenile as the adults. While growing and acquiring maturity to  
103 become an adult, lugworms will migrate lower on the shore (De Cubber *et al.*, 2020).  
104 Although the overall functioning of the life cycle is known, knowledge was still poor  
105 regarding the fine tune of the larval stage development of *A. marina* on the subtidal area  
106 (Newell, 1948; Farke & Berghuis, 1979a,b; De Cubber *et al.*, 2019).

107         To overcome this, and thus have a better knowledge of the different life stages of this  
108 species, an abj-DEB model was developed by De Cubber *et al.* (2019). Indeed, DEB models  
109 allow to predict the physiological processes (such as growth, assimilation, respiration,  
110 reproduction) of a species across its whole life cycle according to environmental conditions  
111 (such as food availability and temperature) (Kooijman, 2010). When applying DEB theory  
112 (Kooijman, 2010), abj-DEB model (Marques *et al.*, 2018) differs from a standard DEB model  
113 by adding an extra juvenile life stage that takes place between the first feeding of the larval  
114 stage (birth, ‘*b*’) to the end of the metamorphosis (‘*j*’) at the post-larval stage, where  
115 metabolic acceleration ( $s_M$ ) occurs leading to an exponential growth of the individual  
116 (Kooijman, 2014), compared to a classical von Bertalanffy growth before ‘birth’ and from the  
117 juvenile to adult stages (Kooijman, 2010). However, data used for the abj-DEB model  
118 developed by De Cubber *et al.* (2019) were not supported by data for the early-life stages  
119 between the trochophore and the post-larval stages despite some predictions of age and length  
120 were obtained by simulation. No experimental studies have described so far, the early larval  
121 stages of *A. marina* into details regarding the age versus length according to temperature and

122 food level. Most studies were focused on fertilization success and temperature effect during  
123 embryogenesis (prior the trochophore stage) at a stage that embryos still live into the female  
124 gallery on the foreshore (Lewis *et al.*, 2002; Watson *et al.*, 1998). In addition, abiotic factors  
125 such as temperature and food availability have not been tested in the laboratory to determine  
126 their effect on larval growth and development.

127 Thus, we carried out an experimental study in laboratory in order to deepen our  
128 knowledge on the influence of temperature and food on the physiology of the larval stages of  
129 *A. marina*. The study aimed to determine precisely when the first feeding ('the birth': in DEB  
130 theory) occurs (age at 'birth' and length at 'birth') in order to describe the biphasic growth  
131 before and after 'birth' according to different temperature and food conditions. The second  
132 goal of this paper was to decipher if there was a difference into the thermal tolerance during  
133 the life cycle of *A. marina* between different life stages as larval stages live in the subtidal  
134 areas, a quite stable daily temperature, whereas juveniles and adults live in the intertidal areas,  
135 where daily temperature can fluctuate up to 15 °C. These discrepancies in temperature ranges  
136 in these two marine habitats may result in different sets of Arrhenius temperatures (Kooijman,  
137 2010) that control metabolic rates of the lugworms according to its life stage. Overall data  
138 could be used to improve the existing abj-DEB model that has been developed initially in  
139 order to help stakeholders to make decision for preserving *A. marina* in areas with high  
140 anthropogenic pressure or to improve the farming of this species in aquaculture.

141

## 142 **Materials and methods**

### 143 Study area and sampling

144 For the need of our experiment, 180 adult lugworms were collected at Wimereux (50°46'N,  
145 1°36'E), located on the Eastern English Channel, part of a marine protected area (MPA)  
146 created in 2012. The coastline is principally composed of sandy beaches as well as rocky

147 shores mainly colonized by algae and mussels on the intertidal and subtidal areas (Rolet *et al.*,  
148 2015). In this MPA, adult population of *Arenicola marina* are found on the high and mid-  
149 shore (De Cubber *et al.*, 2018). From September 2<sup>nd</sup> to 16<sup>th</sup> 2019, 180 adults of *A. marina*  
150 were sampled in total, using a shovel and a bait pump (Decathlon Ltd.) on the mid-shore at low  
151 tide.

152

### 153 Broodstock selection and maintenance

154 At the Wimereux Marine Station, collected lugworms ( $n = 180$ ) were maintained in a 300 L  
155 tank with a continuous seawater flow ( $300 \text{ L.h}^{-1}$ ), placed on a thermostatically controlled  
156 room ( $15^\circ\text{C}$ ). A continuous flow of water mixed the tank for 24 hours in order to clean the  
157 worms by removing sand and micro-algae residues. Then, to assess the reproductive status of  
158 each worm, biopsies of the coelomic fluid were performed using a sterile hypodermic syringe  
159 on anaesthetized individuals *Arenicola marina* in three successive ethanol solution (1%, 2.5%  
160 and 5%) in twice-filtered seawater solutions (TFSW,  $0.45 \mu\text{m}$  and  $0.2 \mu\text{m}$ ) (Gaudron &  
161 Bentley, 2002). Observations using an optic microscope (Motic® BA210) allowed to  
162 establish the state of maturity of the gametes and to differentiate the sexes. After sex  
163 determination, males and females were separated and kept in two different tanks with  
164 continuous seawater flow. While maintaining the lugworms, regular gametes observations  
165 using the optic microscope, were carried out randomly on biopsies of five males and five  
166 females in order to estimate the reproductive status of each individual. For females, 30  
167 random oocytes were measured using the optic microscope equipped with Motic Image Plus©  
168 3.0 software. Female gametes were estimated to be ready for fertilization when mean oocytes  
169 diameter was at  $180 \mu\text{m}$  (Watson *et al.*, 1998). For male gametes, maturity was fixed when 80  
170 % rate of spermatocytes were in the morula stage (Dillon & Howie, 1997).

171



172 Spawning induction, artificial fertilization

173 Five females and five males with the most mature gametes were selected as broodstock for  
174 artificial fertilization. Lugworms were washed with autoclaved TFSW and then placed in  
175 individual tanks (15.0 x 8.0 x 10.0 cm) filled with 1 L of TFSW. Each selected female was  
176 injected with two prostomial homogenates (Howie, 1961) and kept for 24 to 48 hours in an  
177 individual tank at 15°C until the release of the oocytes. After spawning, females were  
178 removed from their tanks and oocytes were collected with a 63 µm-mesh. Then, female  
179 gametes were washed twice with TFSW and stored in 5 mL microtubes at 4°C.

180 Just after the release of oocytes, each male was injected with two prostomial homogenate  
181 (Pacey & Bentley, 1992) and monitored until gametes release. After ejection by male's  
182 nephridiopores, 'dry' sperm was collected immediately with a micropipette and placed in 1  
183 mL microtubes on ice (Williams *et al.*, 1997). Male gametes were counted using a Neubauer  
184 counting chamber (Sigma Ltd.) under the optic microscope. Before the artificial fertilization,  
185 females ( $n = 5$ ) and males ( $n = 5$ ) gametes were pooled together to increase fertilization  
186 success. Approximately  $10^6$  oocytes were mixed with a concentration of  $10^4$  sperm per egg in  
187 a 2 L autoclaved glass container filled with 1 L of TFSW for a 10 minutes sperm-egg contact  
188 time to avoid polyspermy (Williams *et al.*, 1997). Then, fertilized oocytes were removed and  
189 washed twice with TFSW before being distributed ( $\sim 10^5$  oocytes per container) in ten  
190 different 1 L autoclaved glass containers filled with 500 mL of TFSW and placed in the dark  
191 at 15°C.

192

193 Experimental design for larval rearing

194 After 48h post fertilization, TFSW was changed every two days with embryos retained and  
195 washed in a 63-µm mesh. Some subsamples were fixed in 4% formaldehyde for further

196 observations. From day 4, the larvae began to secrete a lot of mucus, and to avoid clogging,  
197 they were gently resuspended with Pasteur pipette every day until day 12.

198 On day 6, the ten glass containers (1 L filled with 500 mL of TFSW) were placed in three  
199 different thermostatically controlled rooms with respectively 3 glass containers at 13°C and  
200 17°C, and 4 glass containers at 15°C.

201 After 24h of acclimation of these new temperature conditions at day 7, one glass container per  
202 room at 13°C and 17°C and two glass containers at 15°C were supplemented with a solution  
203 of microalgae ( $4 \cdot 10^4$  cell/mL concentration of RGcomplete APBreed™, Planktovie ltd.) every  
204 two days and called the ‘fed’ conditions, while the remained containers at 13°C, 15°C and  
205 17°C were called the ‘non-fed’ conditions. TFSW was changed initially every two days but  
206 after day 22 it was extended to three to five days. The experiments lasted for 50 days.

207

#### 208 Monitoring of larval morphology and biometry

209 The larval development from artificially fertilized oocytes was monitored daily for the first  
210 three weeks, then twice a week thereafter, using the optic microscope equipped with Motic  
211 Image Plus© 3.0 software. Times required reaching the following stages of trochophore and  
212 metatrochophore were recorded for each temperature (13°C, 15°C and 17°C) and food  
213 conditions (‘fed’ and ‘non-fed’). For each temperature condition, fifteen to thirty larvae per  
214 glass container were collected at random and sacrificed for morphological observations and  
215 biometry. The selected larvae were anesthetized (Gaudron & Bentley, 2002). The observation  
216 of the number of setigers (segments bearing setae), as well as the opening of the mouth, the  
217 anus and the appearance of the digestive tract were carried out using the optic microscope. In  
218 addition, taking photographs allowed to measure the total length of each larva (Motic Image  
219 Plus© 3.0 software).

220 Scanning electron microscope (SEM) was used for better visualization of ontogeny.  
221 For this, some larvae fixed in 4% formaldehyde were washed in MilliQ water (Millipore) in  
222 40- $\mu$ m mesh, and were gradually dehydrated by placing them successively for 1 hour in  
223 ethanol (Merck, Normapur) baths ranging from 30% to 100% with a step of 10%. Following  
224 this dehydration, and in order to fix and dry the larvae, they were put twice in a row, for one  
225 hour, in a bath of hexamethyldisilane (HMDS, Molekula). The larvae were collected  
226 individually using micro forceps and stuck on aluminium stubs (Agar Scientific) with double  
227 sticky carbon tabs (Agar Scientific), which was finally sputter coated under Argon flow with  
228 Au/Pd (Polaron SC 7620) for 90 seconds. SEM observations were carried out under the SEM  
229 LEO 438 VP using a secondary electron detector for topography at 20 keV.

230

231 Data analyses

232 Definition of ‘birth’

233 The date of the first exogenous food intake, called ‘birth’ (DEB theory; Kooijman, 2010) in  
234 our study, corresponds to the concomitant appearance of the opening of the mouth, of the anus  
235 and the appearance of the gut. Initially *Arenicola marina* larvae are lecithotroph living on  
236 maternal reserve and this is called the ‘embryo’ stage in the DEB theory (Kooijman, 2010)  
237 having a von Bertalanffy growth curve. Then when the larva starts to feed on exogenous food  
238 (planktotroph) by developing a functional gut, the growth is exponential until the end of the  
239 metamorphosis. The transition between a lecithotrophic larva and the feeding larval stage has  
240 been described for each temperature conditions through microscopic observation.

241

242 Biphasic bioenergetic modelling

243 Larval growth was modelled using a biphasic time-dependent model described by a set of two  
244 equations. The change depends on the time of ‘birth’ ( $tb$ ), where growth before ‘birth’ follows

245 the laws of von Bertalanffy (von Bertalanffy, 1957) (Equation 1) and, after ‘birth’ it is  
246 exponential (Equation 2).

247 For length data, the growth equation is written as follows:

$$248 \quad L_1(t) = L_{inf} - (L_{inf} - L_0) * \exp^{-bt} \text{ with } L_{inf} = \frac{a}{b} \text{ for } t \leq tb \text{ i.e. before ‘birth’} \quad (1)$$

$$249 \quad L_2(t) = L_1(tb) * \exp^{ct} \quad \text{for } t > tb \text{ i.e. after ‘birth’} \quad (2)$$

250  $L_1(t)$  and  $L_2(t)$  are lengths as a function of time ( $t$ ) with  $L_0$  and  $L_1(tb)$  are length at time 0 and  
251 at  $tb$  respectively.  $L_{inf}$  is the asymptotic length,  $a$  and  $b$  are the size-specific rates of energy  
252 acquisition and energy use for body maintenance (the von Bertalanffy growth rate between  
253 fertilization and  $tb$ ), respectively, and  $c$  the exponential growth rate after  $tb$ .

254

255 Temperature range for metabolic responses in larval stages

256 All metabolic rates depend on body temperature (Kooijman, 2010), and in ectotherms it  
257 corresponds to the external temperature such as in polychaetes. Thus, a temperature correction  
258 (TC) is usually applied on metabolic rates using the Equation (3), where  $T_A$  is the Arrhenius  
259 temperature (in K),  $T_{ref}$ , the reference temperature (293.15 K), and  $T$  is the experimental  
260 temperature (in K):

$$261 \quad TC = \exp\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right) \quad (3)$$

262 Outside the lower and higher boundaries of the species-specific temperature tolerance range  
263 (respectively  $T_L$  and  $T_H$ ), the TC shape differs and is calculated adding an extra term to the  
264 Equation (3) as presented in Equation (4), with  $T_{AL}$  the Arrhenius temperature below the lower  
265 boundary of the species-specific temperature tolerance range (in K) and  $T_{AH}$  the Arrhenius  
266 temperature above the higher boundary of the species-specific temperature tolerance range (in  
267 K) (Kooijman, 2010).

268 TC =

269  $\exp\left(\frac{T_A}{T_{ref}} -$

270  $\frac{T_A}{T}\right) \left[ \frac{1 + \exp\left(\frac{T_{AL}}{T_{ref}} - \frac{T_{AL}}{T_L}\right) + \exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T_{ref}}\right)}{1 + \exp\left(\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right) + \exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T_{ref}}\right)} \right] \quad (4)$

271 A simpler version of this equation for the higher boundary of the temperature tolerance range

272 only is as follows:

273  $TC = \exp\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right) * \left[ \frac{1 + \exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T_{ref}}\right)}{1 + \exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T_{ref}}\right)} \right] \quad (5)$

274 The Arrhenius temperature of *A. marina* has been previously estimated, using Equation 3,

275 together with other DEB parameters using the DEBtool package (De Cubber *et al.*, 2019;

276 Marques *et al.*, 2018). In addition, the temperature tolerance range and the Arrhenius

277 temperatures of Equation 4 have been estimated for the species from data collected mainly in

278 juveniles and adults (De Cubber *et al.*, 2020). Hence, new data on larval stages of *A. marina*

279 were used to re-estimate the Arrhenius temperatures i.e.  $T_A$ ,  $T_{AH}$  and to estimate the higher

280 boundary of the temperature tolerance range,  $T_H$ , using Equation 5. As no data were available

281 below 5°C, it was not possible to estimate the lower boundary of Equation 4. The new data set

282 consisted in the parameters ( $a$ ,  $b$  and  $c$ ; Equations 1 and 2) of the biphasic growth model at

283 13°C, 15°C and 17°C as well as the data from several fertilization success experiments carried

284 out at 5°C, 10°C, 13°C, 15°C, 18°C, 20°C and 22°C by Lewis *et al.* (2002). Each data set was

285 standardized by its maximum value to get values between 0 and 1 in line with the temperature

286 correction (Equation 5).

287

288 Statistics and fittings

289 All growth curve fitting processes and associated statistics were coded in R version 4.0.3  
290 (2020). A nonlinear least squares method (package ‘nls2’; Grothendieck, 2013) was used to fit  
291 Equations 2 and 5 as it allows multiple starting values to avoid local minima problems in  
292 parameter estimation. This package provides parameter best estimates and standard errors,  
293 and parameter significances by *t*-test. Further, bioenergetics models were tested for either  
294 differences in the temperature effect (3 modalities) or differences in the food condition (‘fed’  
295 and ‘non-fed’) within each temperature (2 modalities) following the method of Ritz &  
296 Streibig (2008) and using analysis of variance (ANOVA). For the temperature factor, the sum  
297 of the residual sum of squares ( $RSS_{ind}$ ) of the three fitted models for each temperature (3  
298 parameters per model, 9 in total, ‘ $n_{par_{ind}}$ ’) were compared to the  $RSS_{all}$  of a model grouping  
299 all data and fitted with only 3 parameters (‘ $n_{par_{all}}$ ’). For the food condition factor, we  
300 assumed there was no effect of food condition before *tb* and then, the von Bertalanffy phase  
301 of the biphasic model has  $L_{inf}$  and  $b$  as common parameters for a given temperature. Hence,  
302 the sum of  $RSS_{ind}$  of the two fitted models for each food condition (2 common parameters  
303 plus one  $c$  parameter per model, ‘ $n_{par_{ind}} = 4$ ’) were compared to the  $RSS_{all}$  of a model  
304 grouping all data for a given temperature and fitted with only 3 parameters (‘ $n_{par_{all}} = 3$ ’).  
305 The  $F$  statistic was calculated as follows:

$$F = \frac{\frac{RSS_{all} - RSS_{ind}}{(N - n_{par_{all}}) - (N - n_{par_{ind}})}}{\frac{RSS_{ind}}{N - n_{par_{ind}}}}$$

306 With  $N$  the total number of individuals. The  $P$  value was then determined by searching for the  
307  $F$  value in the  $F$  distribution with degrees of freedom ( $n_{par_{ind}} - n_{par_{all}}, N - n_{par_{ind}}$ )  
308 using the function ‘*pf*’ of the R statistical package.

309

310 Results

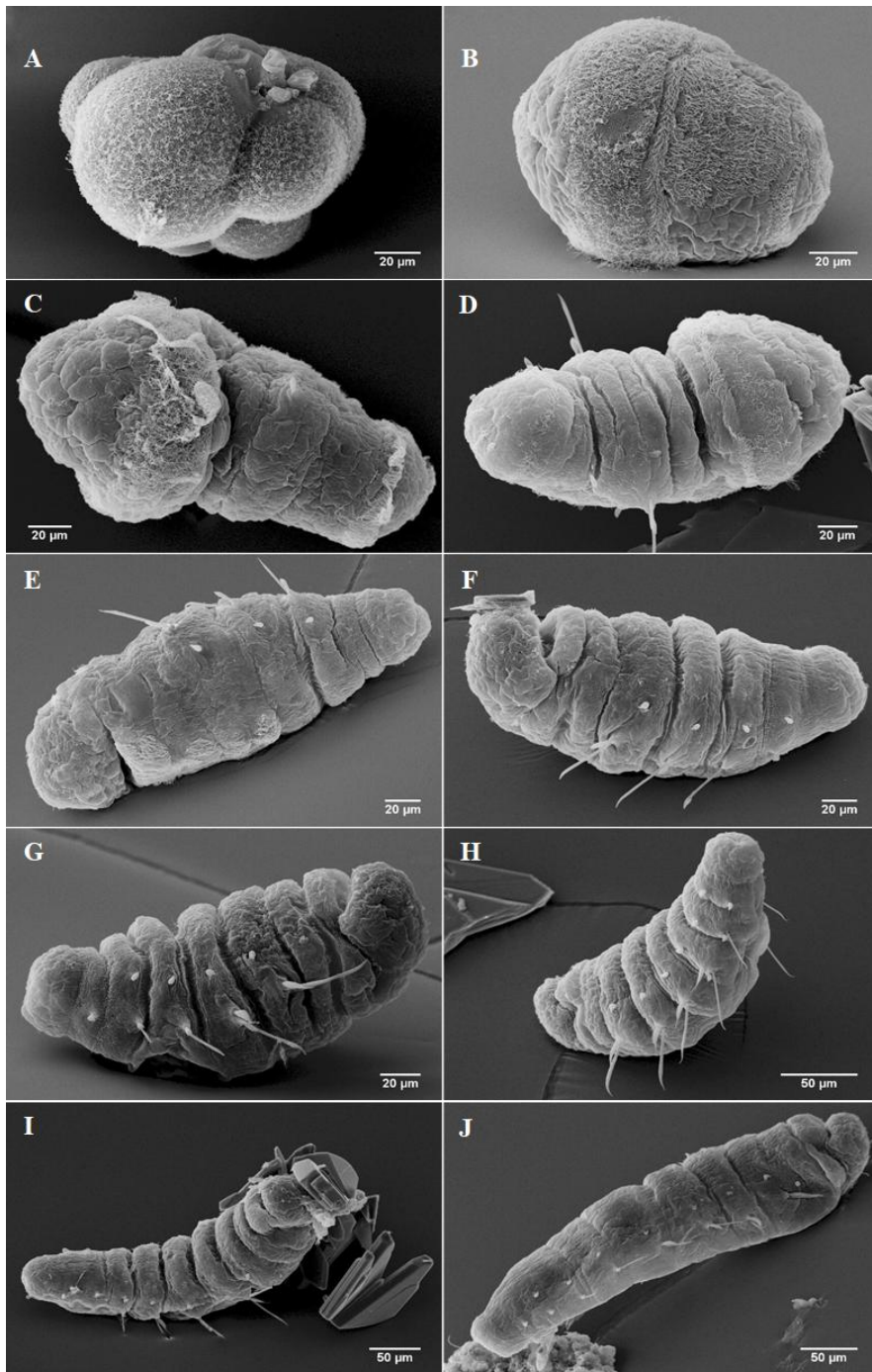
311 Effect of temperature on larval development of *Arenicola marina*

312 According to the 13°C, 15°C and 17°C exposed temperature respectively, the chronology of  
313 *A. marina* larval development (Fig. 1) and their biometry were recorded (Table 1). The  
314 fertilized oocytes had an average diameter of  $176 \pm 6 \mu\text{m}$  and develops to embryo by cell  
315 division during embryogenesis (Fig. 1A). Larvae hatch at the trochophore stage (Fig. 1B) at  
316 the end of the gastrulation at 3 days post fertilization (dpf) with a mean total length of  $169 \pm$   
317  $14 \mu\text{m}$  (Table 1). The larvae developed their first setiger at 6 dpf (Fig. 1C) with a mean total  
318 length of  $255 \pm 28 \mu\text{m}$ , becoming a metatrochophore. All larvae were still conditioned at  
319 15°C at that time (Table 1). After 6 dpf to 50 dpf, the larvae were raised to three different  
320 temperature conditions (13°C, 15°C and 17°C), and the appearance of new setigers (up to 10  
321 setigers) were not tuned between the three treatments. Indeed, at 15°C the larvae have  
322 developed 4 setigers (S) at 14 dpf and 6 S at 24 dpf (Table 1; Figs. 1F&H). While the larvae  
323 placed at 13°C needed 16 and 24 dpf to reach 4 S and 6 S respectively (Table 1), those placed  
324 at 17°C reached 4 S and 6 S at 14 and 21 dpf respectively (Table 1). At 50 dpf, larvae had  
325 reached 7 S (Fig. 1I) with a mean total length of  $780 \pm 130 \mu\text{m}$  (Table 1) at 13°C, 8 S (Fig.  
326 1J) with a mean total length of  $746 \pm 258 \mu\text{m}$  (Table 1) at 15°C and 10 S with a mean total  
327 length of  $544 \pm 186 \mu\text{m}$  (Table 1) at 17°C. Although, there was a time lag of larval  
328 development as a function of temperature, larvae had equivalent size for each stage. Indeed,  
329 for example for the 4 S stage, larvae measured  $471 \pm 69 \mu\text{m}$ ,  $476 \pm 33 \mu\text{m}$  and  $454 \mu\text{m} \pm 31$   
330  $\mu\text{m}$  at 13°C, 15°C and 17°C respectively (Table 1).

331

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334

335 **Figure 1.** Scanning electron microscopy photographs of ten larval stages of *Arenicola marina*. A)  
 336 Embryo at early stage of cell division before hatching; B) Trochophore stage after hatching;  
 337 Metatrochophore with C) 1 setiger (segment with chaetae); D) 2 setigers; E) 3 setigers; F) 4 setigers;  
 338 G) 5 setigers; H) 6 setigers; I) 7 setigers; J) 8 setigers.

339

340 Effect of diet on larval development of *Arenicola marina*

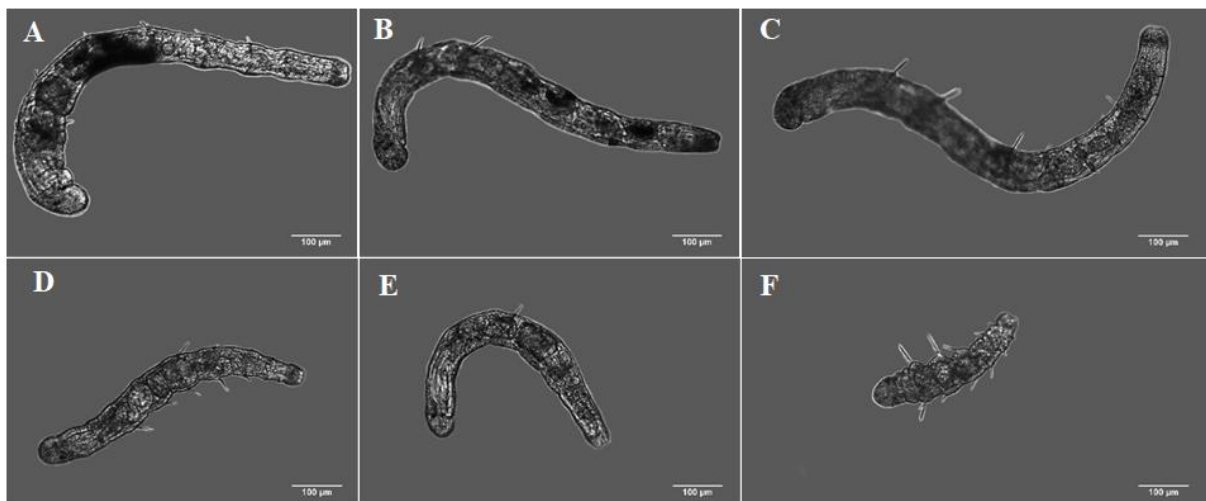
341 The dietary transition between lecithotrophic larvae to planktotrophic larvae ('birth') occurred

342 at a size between ~ 450–500  $\mu\text{m}$ , regardless of temperature (Fig. 1F; Table 1; 1<sup>st</sup> time that a



343 row has 'yes' in the last two columns). In terms of duration, the age at 'birth' has taken place  
344 at 17 dpf for the larvae reared at both 15°C (4 S stage) and 17°C (5 S stage) and, at 21 dpf for  
345 those placed at 13°C (5 S stage) (Table 1).

346 Growth retardation was observed visually at 50 dpf between larvae fed with  
347 microalgae ('fed') and those non-feds whatever the temperature treatments (Fig.2). For the  
348 three-temperature conditions, the mean total lengths of larvae at 50 dpf of the 'non-fed'  
349 conditions were lower than those of the 'fed' conditions (Table 2), but at 13°C mean total  
350 length ( $659 \pm 96 \mu\text{m}$ ) of the 'non-fed' condition, was not significantly different than that of  
351 the 'fed' condition ( $800 \pm 129 \mu\text{m}$ ) ( $t$ -test;  $P = 0.08$ ); at 15°C, the mean total length of larvae  
352 from 'non-fed' condition ( $487 \pm 110 \mu\text{m}$ ) was highly significantly different than that of the  
353 'fed' condition ( $746 \pm 258 \mu\text{m}$ ) ( $t$ -test;  $P < 0.001$ ); at 17°C, the mean total length of larvae  
354 from 'non-fed' condition ( $506 \pm 212 \mu\text{m}$ ) was not significantly different than that of the 'fed'  
355 condition ( $544 \pm 186 \mu\text{m}$ ) ( $t$ -test;  $P = 0.56$ ). It seems that some shrinkage had occurred in  
356 larvae from the 'non-fed' treatments between 43 dpf to 50 dpf both at 15°C and 17°C (Table  
357 2).



358

359 **Figure 2.** Images obtained with an optic microscope of *Arenicola marina* larvae at different food  
360 levels and temperature conditions at 50 days post-fertilization. A) 13°C and 'fed' conditions; B) 15°C  
361 and 'fed' conditions; C) 17°C and 'fed' conditions; D) 13°C and 'non-fed' conditions; E) 15°C and 'non-  
362 fed' conditions; F) 17°C and 'non-fed' conditions.

363 Effect of temperature and diet conditions on bioenergetic of *Arenicola marina* larvae  
364 Temperature had a significant effect on the biphasic growth models ( $F_{(6, 1338)} = 9.72$ ;  $P <$   
365  $0.001$ ). In the first phase of the model (von Bertalanffy), growth rate  $b$  gave better  
366 performance at  $15^{\circ}\text{C}$  ( $0.263\text{ d}^{-1}$ ) and  $17^{\circ}\text{C}$  ( $0.216\text{ d}^{-1}$ ) compared to  $13^{\circ}\text{C}$  ( $0.107\text{ d}^{-1}$ ), whereas  
367 in the second phase of the model, exponential growth rates  $c$  were greater at  $13^{\circ}\text{C}$  ( $0.012\text{ d}^{-1}$ )  
368 and  $15^{\circ}\text{C}$  ( $0.013\text{ d}^{-1}$ ) compared to  $17^{\circ}\text{C}$  ( $0.006\text{ d}^{-1}$ ) (Table 3; Fig. 3). The effect of food on the  
369 biphasic growth model is highly significant at  $15^{\circ}\text{C}$  ( $F_{(1, 556)} = 59.44$ ;  $P < 0.001$ ), where the  
370 growth model gave better results in ‘fed’ conditions compared to the ‘non-fed’ condition  
371 (Fig.3B). The effect of food is marginally significant at  $13^{\circ}\text{C}$  ( $F_{(1, 285)} = 2.77$ ;  $P = 0.097$ ), but  
372 still the biphasic growth model gave better performance in ‘fed’ condition compared to the  
373 ‘non-fed’ condition (Fig.3A). At  $17^{\circ}\text{C}$ , there is no effect of the food conditions on the  
374 biphasic growth model ( $F_{(1, 494)} = 0.0$ ;  $P = 0.98$ ), where both biphasic models were similar  
375 given bad performance regarding larval growth (Table 3; Fig.3C).

376

377 Temperature correction on metabolic rates of *Arenicola marina* across different life-history  
378 stages

379 The estimates of the biphasic larval growth models (Table 3) along with data from Lewis et  
380 al. (2002) after being standardized by their maximum values, helped to re-estimate the  
381 temperature corrections using Equation 5. As  $T_{AH}$  was non-significant in the first regression  
382 fit ( $P = 0.14$ ),  $T_{AH}$  (82380 K) from De Cubber et al. (2020) was set in the Equation 5. New  
383 temperature corrections were calculated with a new  $T_A$  equaled to  $6661.79\text{ K} (\pm 1241.5)$ ;  $P <$   
384  $0.001$ ) and a new  $T_H$  equaled to  $294.44\text{ K} (\pm 0.42)$ ;  $P < 0.001$ ). Overall, these new Arrhenius  
385 temperature datasets were different from those of De Cubber et al. (2020), where the  $T_H$  from  
386 this study (blue line; Fig.4) issued from larval metabolic rates, was lower than that of De  
387 Cubber et al. (2020) issued from juvenile/adult metabolic rates (black line; Fig.4), and the  $T_A$

388 from our datasets (slope of the blue line on the left part of the curve; Fig.4) was higher than  
389 that of De Cubber et al. (2020) (slope of the dark line on the left part of the curve; Fig.4).

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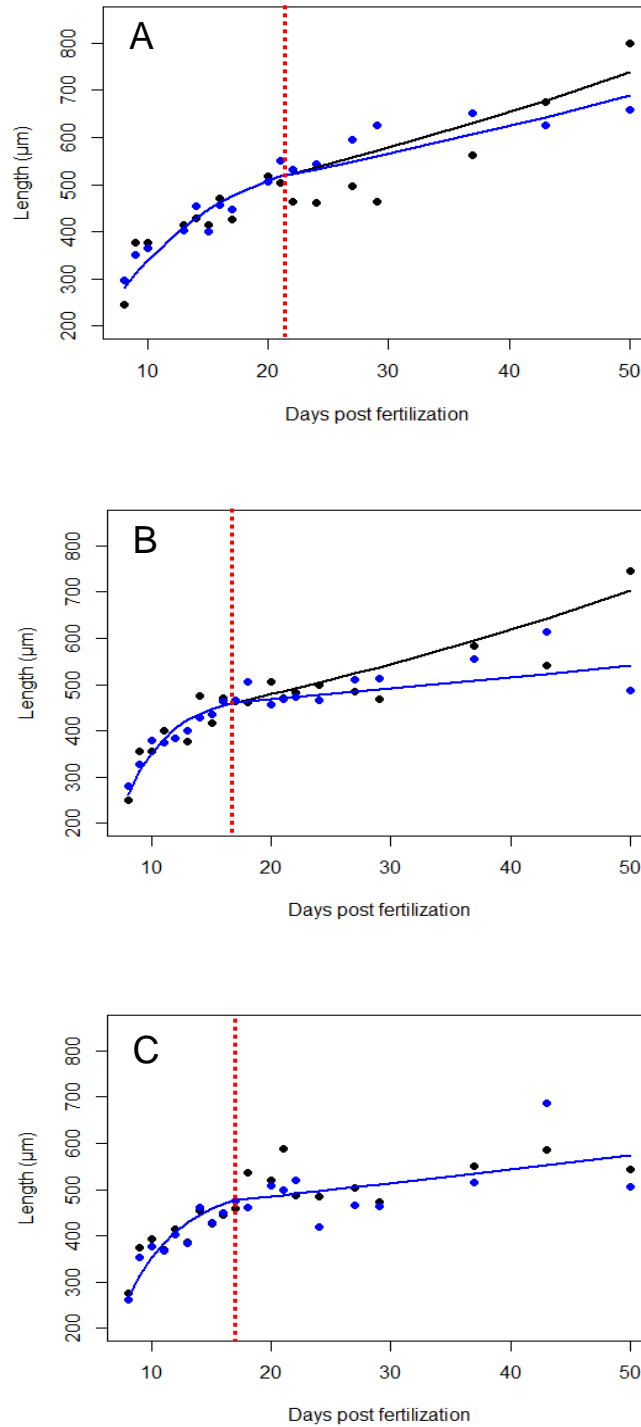
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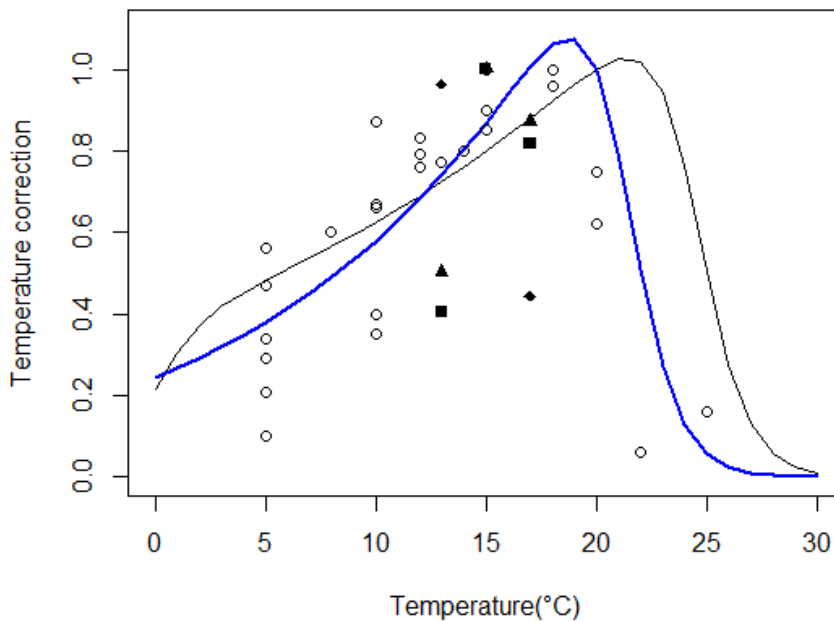
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409 **Figure 3.** Evolution of the total length of *Arenicola marina* larvae at three different temperatures  
410 following days post-fertilization. A) At 13°C, where the age at first food intake ('birth') is indicated by  
411 the red dotted vertical line (21 days); B) At 15°C, where the age at first food intake ('birth'), is



412 indicated by the red dotted vertical line (17 days); C) At 17°C, where the age at first food intake  
 413 ('birth') is indicated by the dotted red vertical line (17 days). Lines are simulations of the models:  
 414 classic von Bertalanffy (first phase of the biphasic growth model) and exponential (second phase of  
 415 the biphasic growth model). Larval growth for the 'fed' condition is in black, and for the 'non-fed'  
 416 condition is in blue.



417

418 **Figure 4.** Temperature corrections in different life stages of *Arenicola marina*. The black line  
 419 represents the temperature correction used in the abj-DEB model mostly on juvenile/adult stages  
 420 from De Cubber *et al.* (2020) and the blue line, the temperature correction using datasets from  
 421 fertilization success rate (circle, Lewis *et al.*, 2002) and from this study on larval growth with  
 422 parameters from the first phase of the biphasic growth model ( $a$  = black triangle;  $b$  = black square;  
 423 equation (1) ) and from the second phase of the biphasic growth model ( $c$  = black diamond; equation  
 424 (2)) at several temperatures.

425

## 426 Discussion

427 The present work shows that a cohort of *Arenicola marina* was successfully fertilized *in vitro*  
 428 getting the embryo stage and then reared for fifty days under favorable experimental growth  
 429 conditions allowing the cohort to hatch to different larval stages including both a  
 430 lecithotrophic stage using maternal reserves and then, using the exogenous food provided by  
 431 the algal culture being then planktotrophic. This experiment enabled to strengthen knowledge  
 432 on the first life-history stages of the lugworm species *A. marina*, and in particular the precise

433 age and length of the occurrence of the ‘birth’ stage with the biphasic growth before and after  
434 ‘birth’ under temperature and food control conditions. These data will be useful to consolidate  
435 the abj-DEB model developed by De Cubber *et al.* (2019; 2020).

436

437 Early larval stages of *Arenicola marina* and ‘birth’ stage

438 Most of earlier work on developmental larval stages of *A. marina* were reported (Newel,  
439 1948, 1949; Farke & Berghuis, 1979a,b) at a time that the species delimitation between *A.*  
440 *marina* and *A. defodiens*, a sympatric species that might occur at some beach in European  
441 marine habitats, was not yet known (Cadman & Nelson-Smith, 1993; De Cubber *et al.*, 2018)  
442 mixing the different ontogeny larval stages between the two species. The best study on larval  
443 development was carried out by Farke & Berghuis (1979b) in laboratory where authors  
444 develop a genius microsystem enabling mature adult lugworms (supposedly *A. marina*) to  
445 spawn and larvae to develop in the laboratory. However, timing of spawning events, larvae  
446 occurrence and control of temperature conditions could not be recorded precisely. Despite  
447 this, previous authors (Farke & Berghuis, 1979b) described nicely the behavior, habitats and  
448 biometry of three larval stages of *A. marina*. Newly hatched trochophore larvae were in  
449 female gallery and had a size around 0.25 mm in length. In our study the trochophore larval  
450 stage was lesser in length and it was the larvae of one setiger that reached 0.25 mm.

451 Metatrochophores with 3 setigers were seen swimming by ciliary movements and measured  
452 around 0.5 mm (Farke & Berghuis, 1979b). In our study the 3S larval stage was ~ 0.4 mm  
453 closed to what was measured by Newel (1948, 1949) from its *in situ* sampling larvae of *A.*  
454 *marina*. After this 3S stage, larvae started to secrete a mucus tube in order to adhere to hard  
455 substrate and they changed their behavior (Farke & Berghuis, 1979b). Larvae could leave  
456 their mucus tube in order to crawl and feed on particles deposited around the tube being  
457 deposit-feeder (Farke & Berghuis, 1979b). Only larvae with 6 setigers were shown to contain

458 food particles in their gut with a size of 0.8 mm (Farke & Berghuis, 1979b). In our study the  
459 first food intake ('the birth stage') was observed earlier at the 4S/5S larval stage at a size  
460 between 450–500  $\mu\text{m}$ . Marty et al. (1997) had followed the appearance of setigers following  
461 time in the larvae of the polychaete *Hediste diversicolor*. Larvae of 3S ( $425 \pm 30 \mu\text{m}$ ) were  
462 starting to feed ('birth') on non-fertilized oocytes in females gallery being cannibalistic and  
463 adelphophagic. This length at first feeding is very close to that observed in the larvae of *A.*  
464 *marina*.

465         Within an abj-DEB model, two primary parameters depends on the metabolic  
466 acceleration ( $s_M$ ) that occurs between the 'birth' stage to the end of the metamorphosis  
467 (Kooijman, 2010; 2014): 1) The maximum assimilation rate after metamorphosis  $\{\dot{p}_{Am}\}_j =$   
468  $\{\dot{p}_{Am}\}_b s_M$  and; 2) The energy conductance values ( $\dot{v}$ ) after metamorphosis  $\dot{v}_j = \dot{v}_b s_M$ . The  
469 metabolic acceleration is calculated as the ratio of the structural length at metamorphosis to  
470 the structural length at 'birth':  $s_M = L_j/L_b$ . Within the abj-DEB model developed on *A.*  
471 *marina* (De Cubber et al., 2019), the physical length at 'birth' ( $L_{wb}$ ) was set at 230  $\mu\text{m}$  (twice  
472 lower to what is observed in this study) and this might have changed the estimation of the  
473 metabolic acceleration  $s_M$ . In this study, we managed to describe precisely the length at  
474 'birth' ( $\sim 450 \mu\text{m}$ ) and this will complete the dataset of the abj-DEB model of *A. marina*  
475 developed by De Cubber et al. (2019).

476

477 Effect of abiotic factors on the first food intake ('birth') in *Arenicola marina*

478 When the larvae hatch at the trochophore stage, and until the development of the complete  
479 digestive tract occurring at the 4S/5S metatrochophore stage, the larvae draw their energy  
480 from the yolk reserves (lecithotrophy) for growth, maintenance and the complexity of its  
481 maturity in DEB theory (Kooijman, 2010). Thus, the availability of food in the environment  
482 has no influence on the transition from the lecithotrophic stage to the planktotrophic stage

483 ('birth'), but temperature does. According to our results, 'birth' appeared earlier in  
484 metatrochophores subjected to warmer temperatures (17 days at both 15°C and 17°C)  
485 compared to lower temperature (21 days at 13°C). This is not in line with the age at 'birth'  
486 estimated by the abj-DEB model proposed by De Cubber *et al.* (2019), where simulation  
487 carried at 10°C gave a 'first feeding' at 10.52 days post fertilization, twice much lower than  
488 that observed at 13°C. However, when De Cubber *et al.* (2019) simulated the temperature  
489 conditions for a whole year at Wimereux (Eastern English Channel) using real *in situ* data  
490 from 5.5°C to 20°C, a closer simulated value of the age at 'birth' was estimated (15.5 days  
491 closed to the 17 days observed for 15°C in our experimental set up). In the field, Newell  
492 (1948, 1949) observed metatrochophores of *Arenicola* sp. ready to become planktotrophic at  
493 2-3 weeks post spawning at Whistable (UK). In this study we managed to describe precisely  
494 the age at 'birth' for three different temperatures and this will complete again the dataset of  
495 the abj-DEB model of *A. marina* developed by De Cubber *et al.* (2019).

496

497 Abiotic factors on growth rates of *Arenicola marina* larvae

498 The increase in seawater temperature has induced an acceleration of larval  
499 development giving at 50 days post fertilization, metatrochophores with more developed  
500 setigers (10 S) in higher temperature conditions (17°C) compared to lower temperature; e.g. at  
501 13°C only metatrochophores with 7 segments with chaetae were recovered. Thus, larvae  
502 reared at 17°C changed larval stages faster than those exposed at 13°C meaning the energy  
503 allocated to the complexity of the larvae was greater ( $E_H$  in DEB theory; Kooijman, 2010).  
504 However, the mean total length of the larvae reared at 17°C (~ 544 µm) was lower compared  
505 to those reared at 13°C (~ 800 µm) at 50 dpf meaning in DEB interpretation that less energy  
506 was allocated to somatic growth while more energy was allocated to the complexity of the  
507 larvae reared in higher temperature. The discrepancy in mean length was enhanced by the

508 poor food conditions treatment ('non-fed') that induced a kind of starvation at 50 dpf for both  
509 15°C and 17°C treatments. In DEB theory (Kooijman, 2010), energy is needed in priority for  
510 maintenance of maturity and growth when less energy is available from mobilization; what is  
511 seen here is the larvae seem to shrink and some lysis of cells might have occurred.

512 In this study, the first phase (von Bertalanffy growth) of the biphasic growth model of  
513 *A. marina* larvae that encompasses trochophores and metatrochophores up to 3S (before  
514 'birth') was better at 15°C and 17°C. These larval stages occur first within the female gallery  
515 on the intertidal foreshore and then disperse in the water column. Then, after 'birth', at the  
516 larval stage of 4S, the second phase (exponential growth) of the biphasic growth model was  
517 greater at 13°C and 15°C, where at these larval stages, *A. marina* larvae are living on the  
518 subtidal areas. For both biphasic growth phases, the optimal temperature was shown to be at  
519 15°C before and after 'birth'. Lewis *et al.* (2002) found for different populations of lugworms  
520 in the UK that the optimal temperature for fertilization success (embryos stages) was between  
521 15–18°C. Lewis *et al.* (2002) were quite astonished by their results as spawning periods of *A.*  
522 *marina* occurred at lower temperature in the UK (10–12°C), where embryos develop in  
523 female gallery on the intertidal habitat. Lewis *et al.* (2002) concluded that lugworms were not  
524 breeding at their optimal temperature and other selective pressures were certainly be acting. In  
525 our study, the optimal temperature was found at 15°C and this, for others life-history stages  
526 (trochophores and metatrochophores) of *A. marina* that live not anymore on the intertidal area  
527 but on the subtidal area (Farke & Berghuis 1979a; Newell 1948, 1949). At Wimereux  
528 (Eastern English Channel), *A. marina* population spawns from the end of September to early  
529 October (De Cubber *et al.*, 2018) where temperature drop from 15°C to 14°C but larvae  
530 seems to be in their optimal temperature at least during the onset of larval development as  
531 temperature fall in winter to temperatures up to 5.5°C (De Cubber *et al.*, 2019). At a regional  
532 scale, other populations of *A. marina* breed later until mid-November on the Eastern English



533 Channel (De Cubber *et al.*, 2018). In mid-November, temperature is around 10°C as seen in  
534 the UK in Lewis *et al.* (2002). *A. marina* populations are widespread in Europe and some  
535 population live in South of Europe such as in Portugal (Pires *et al.*, 2015) where the mean  
536 seawater temperature is much higher in winter but in the range of the optimal temperature for  
537 larvae and in spring and summer in the range of juvenile/adult optimal temperature. This may  
538 explain the well-establishment of this species in South of Europe, where in Portugal the  
539 lugworm is seen as an invasive species (Pires *et al.*, 2015).

540

541 Applications in DEB theory and in aquaculture

542 Intertidal species (mostly ectotherms) such as polychaetes, bivalves and gastropods can  
543 experience during low tide a great variation (up to 20°C) of daily temperature either in winter  
544 or in summer (Seuront *et al.*, 2019; Moisez *et al.*, 2020; De Cubber *et al.*, 2020) compared to  
545 species living in a more stable daily temperature environment such as in the subtidal area. As  
546 reported by Kooijman (2010), these species have enzymes involved in metabolic reaction that  
547 function in this broad temperature range with the consequence to have a relatively low  
548 Arrhenius temperature ( $T_A$ ) (around 6000 K), compared to species that live in more constant  
549 daily temperature having a higher Arrhenius temperature (around 12 000 K).  $T_A$  calculated  
550 using DEB tool (Add-my-pet-database) of the polychaete *Hediste diversicolor* and the cockle  
551 bivalve *Cerastoderma edule*, living both on intertidal mud flat, were found respectively to be  
552 4877 K and 5290 K respectively. De Cubber *et al.* (2020) have estimated a  $T_A$  of 4014 K for  
553 *Arenicola marina*, a correct value for an intertidal species. The calculation was based on  
554 metabolic rates of life-history stages of the lugworms (embryos, juveniles and adults) that live  
555 on the foreshore. In our study, a new set of Arrhenius temperatures ( $T_A$  and  $T_H$ ) was calculated  
556 based on temperature corrections of metabolic rates of only early-life stages of *A. marina*  
557 (embryos and larvae).  $T_A$  of early-life stages of *A. marina*, that spend most of their time in the

558 subtidal area (a more stable environment), as expected, was found higher (~ 6661 K)  
559 compared to the  $T_A$  (~ 4014 K) (De Cubber et al. 2020) of life-stages of *A. marina* that live on  
560 the foreshore (a highly variable environment). Likewise, the higher boundary temperature  
561 value ( $T_H = 294.4$  k; ~ 21.25°C) of the early-life stages was lower than that of the  
562 juvenile/adult stages ( $T_H = 297.7$  k; ~ 24.55°C; De Cubber *et al.*, 2020). As already reported  
563 by Kooijman (2010), larvae of intertidal species that live in pelagic environment, have a  
564 higher Arrhenius temperature as this  $T_A$  can change with the life stage of a species. We  
565 therefore support the idea that two sets of Arrhenius temperatures should be used in all  
566 intertidal Lophotrochozoan species that have a larval life in pelagic area when using an abj-  
567 DEB model. As the authors are aware only one Arrhenius temperature is usually including  
568 into any DEB model even if, a species may experience different temperature ranges during  
569 their life cycle. For instance, in the mollusc bivalve *Magdallena gigas*, that is a commercial  
570 species and intertidal, in the AMP database, the value of  $T_A$  is set at 8000 K despite that Rico-  
571 Villa et al. (2010) calculated a higher value of  $T_A$  (11 000 K) for the larvae after rearing them  
572 at 5 different temperatures from 17°C to 32°C.

573 *A. marina* has been cultured since the late 90' in Northeast England (Northumbland  
574 Seabait Ltd.) with a number of patents issued from this bait farming (e.g. Olive et al., 2001  
575 (WO2003007701A2); Craig & Olive, 2005 (WO2005043994A1)). The initial purpose of the  
576 culture of lugworms in the UK was to support the demands of fishermen that were digging  
577 intensively the worms used for bait (Olive, 1993; 1994; Olive & Cowin, 1994). Recently, *A.*  
578 *marina* is reared in a farm in Noirmoutiers Island in West of France (Hemarina Ltd<sup>TM</sup>) for  
579 medical purposes, where a numerous of exiting research is carried out on the medical  
580 potential and application of the lugworm haemoglobin (Asong-Fontem *et al.*, 2021; Batool *et*  
581 *al.*, 2021; Le Daré *et al.*, 2021; Le Meur *et al.*, 2021). Our study on larval physiology

582 highlight that the optimal temperature for growth is around 15°C with a maximal tolerance of  
583 21°C and this could have interesting application in aquaculture.

584

## 585 **Conclusion**

586 Overall, our data on the early larval stages of *A. marina* will be valuable in improving the  
587 existing abj-DEB model for this engineer species. These include life traits such as age at birth,  
588 size at birth but also Arrhenius temperatures and length over time for two food levels. DEB  
589 modeling allows to predict functional traits of the species such as size at first maturity, life  
590 span, number of oocytes during the whole life cycle (total reproductive output), growth rate,  
591 maximum length (Lmax), etc. This model outputs can help marine conservation managers  
592 make decisions to preserve the *A. marina* population exploited by bait fishing. In particular, it  
593 helps stakeholders to establish regulatory measures such as catch size limits or the number of  
594 individuals that can be harvested. One of the solutions to overexploitation of lugworms is  
595 aquaculture farming. Our data underline that the optimal temperature for rearing lugworm  
596 larvae is 15°C and that it is necessary to feed them with microalgae after the 'birth' period  
597 which occurs 17 days after fertilization.

598

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603

## 604 **Conflicts of interest**

605 The authors declare no financial and personal conflict of interest.

606

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612 whom we had our first concerns during the DEB telecourse in 2017 regarding the use of two  
613 sets of Arrhenius temperatures for species living in different marine habitats during their life  
614 cycle.

615

616 Data availability statements

617 The data underlying this article are available in the article.

618

## 619 **References**

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**Table 1.** Larval development of *Arenicola marina* at 13°C, 15 °C and 17°C. At 7 days post-fertilization (Time), larvae were fed with microalgae. Larval stages correspond to the number of setigers (S). Total length is the mean of the n replicates with its standard deviation ( $\pm$ ).

Temperature	Time (days)	Larval stage	Total length ( $\mu\text{m}$ )	n	Mouth & anus opening	Presence of a digestive tract
15°C	0 – 2	Embryo	159 $\pm$ 8	73	No	No
	3	Trochophore	169 $\pm$ 14	91	No	No
	6	1S	255 $\pm$ 28	48	No	No
	9	2S	356 $\pm$ 15	8	No	No
	12	3S	383 $\pm$ 34	12	No	No
	14	4S	476 $\pm$ 33	23	Yes	No
	17	4S	465 $\pm$ 52	22	Yes	Yes
	20	5S	506 $\pm$ 66	16	Yes	Yes
	24	6S	499 $\pm$ 86	16	Yes	Yes
	43	7S	541 $\pm$ 157	6	Yes	Yes
	50	8S	746 $\pm$ 258	22	Yes	Yes
13°C	8	2S	246 $\pm$ 11	5	No	No
	13	3S	415 $\pm$ 30	10	No	No
	16	4S	471 $\pm$ 69	8	Yes	No
	21	5S	510 $\pm$ 46	5	Yes	Yes
	24	6S	461	1	Yes	Yes
	43	7S	674 $\pm$ 76	4	Yes	Yes
	50	7S	800 $\pm$ 129	5	Yes	Yes
17°C	8	2S	276 $\pm$ 19	11	No	No
	11	3S	371 $\pm$ 21	9	No	No
	14	4S	454 $\pm$ 30	19	Yes	No
	16	5S	445 $\pm$ 40	8	Yes	No
	17	5S	459 $\pm$ 45	10	Yes	Yes
	21	6S	588 $\pm$ 95	6	Yes	Yes
	37	7S	551 $\pm$ 155	18	Yes	Yes
	43	8S	586 $\pm$ 204	20	Yes	Yes
	50	10S	544 $\pm$ 186	21	Yes	Yes

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818 **Table 2.** Larval development after 'birth' according to diet condition for each temperature treatment.

819 Times correspond to days post-fertilization. Total length is the mean of the n replicates with its

820 standard deviation ( $\pm$ ). na for not available.

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Temperature	Time (days post-fertilization)	Total length ( $\mu\text{m}$ ) for 'non-fed' condition	Total length ( $\mu\text{m}$ ) for 'fed' condition
13°C	21	551 $\pm$ 74 ( $n = 12$ )	505 $\pm$ 51 ( $n = 4$ )
	24	543 $\pm$ 79 ( $n = 13$ )	461 $\pm$ na
	27	594 $\pm$ 65 ( $n = 8$ )	497 $\pm$ 64 ( $n = 9$ )
	29	626 $\pm$ 132 ( $n = 7$ )	463 $\pm$ 25 ( $n = 2$ )
	37	651 $\pm$ 120 ( $n = 14$ )	561 $\pm$ 64 ( $n = 5$ )
	43	626 $\pm$ 102 ( $n = 11$ )	674 $\pm$ 76 ( $n = 4$ )
	50	659 $\pm$ 96 ( $n = 12$ )	800 $\pm$ 129 ( $n = 5$ )
15°C	17	465 $\pm$ 84 ( $n = 12$ )	465 $\pm$ 52 ( $n = 22$ )
	21	468 $\pm$ 80 ( $n = 7$ )	472 $\pm$ 34 ( $n = 7$ )
	24	465 $\pm$ 71 ( $n = 12$ )	499 $\pm$ 86 ( $n = 16$ )
	27	511 $\pm$ 95 ( $n = 19$ )	485 $\pm$ 78 ( $n = 13$ )
	29	514 $\pm$ 113 ( $n = 15$ )	467 $\pm$ 68 ( $n = 15$ )
	37	556 $\pm$ 77 ( $n = 13$ )	583 $\pm$ 85 ( $n = 14$ )
	43	613 $\pm$ 154 ( $n = 9$ )	541 $\pm$ 157 ( $n = 6$ )
	50	487 $\pm$ 110 ( $n = 23$ )	746 $\pm$ 258 ( $n = 22$ )
17°C	17	476 $\pm$ 57 ( $n = 11$ )	459 $\pm$ 45 ( $n = 10$ )
	21	500 $\pm$ 70 ( $n = 11$ )	588 $\pm$ 95 ( $n = 6$ )
	24	420 $\pm$ 70 ( $n = 10$ )	485 $\pm$ 66 ( $n = 8$ )
	27	466 $\pm$ 133 ( $n = 10$ )	505 $\pm$ 107 ( $n = 12$ )
	29	463 $\pm$ 72 ( $n = 11$ )	473 $\pm$ 57 ( $n = 8$ )
	37	514 $\pm$ 125 ( $n = 13$ )	551 $\pm$ 155 ( $n = 18$ )
	43	687 $\pm$ 265 ( $n = 16$ )	586 $\pm$ 204 ( $n = 20$ )
	50	506 $\pm$ 212 ( $n = 17$ )	544 $\pm$ 186 ( $n = 21$ )

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823 **Table 3.** Biphasic growth modelling parameters where  $a$  and  $b$  are from Von Bertalanffy's phase and  
 824  $c$  results from the exponential phase. The parameter  $a$  was estimated from  $L_{inf}$  and  $b$  using Equation  
 825 2.

Treatment	Parameters	Unit	Value	Standard error	<i>P</i> -value
13°C	$L_{inf}$	µm	595.83	43.77	< 0.001
	$a$	µm/d	63.71	-	-
	$b$	/d	0.107	0.026	< 0.001
Fed	$c$	/d	0.012	0.001	< 0.001
Non-fed	$c$	/d	0.010	0.001	< 0.001
15°C	$L_{inf}$	µm	481.59	15.02	< 0.001
	$a$	µm/d	126.92	-	-
	$b$	/d	0.263	0.051	< 0.001
Fed	$c$	/d	0.013	0.001	< 0.001
Non-fed	$c$	/d	0.005	0.001	< 0.001
17°C	$L_{inf}$	µm	512.61	29.15	< 0.001
	$a$	µm/d	110.61	-	-
	$b$	/d	0.216	0.060	< 0.001
Fed	$c$	/d	0.006	0.001	< 0.001
Non-fed	$c$	/d	0.006	0.001	< 0.001

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841 Figures caption

842 **Figure 1.** Scanning electron microscopy photographs of ten larval stages of *Arenicola*  
843 *marina*. A) Embryo at early stage of cell division before hatching; B) Trochophore stage after  
844 hatching; Metatrochophore with C) 1 setiger (segment with chaetae); D) 2 setigers; E) 3  
845 setigers; F) 4 setigers; G) 5 setigers; H) 6 setigers; I) 7 setigers; J) 8 setigers.

846 **Figure 2.** Images obtained with an optic microscope of *Arenicola marina* larvae at different  
847 food levels and temperature conditions at 50 days post-fertilization. A) 13°C and 'fed'  
848 conditions; B) 15°C and 'fed' conditions; C) 17°C and 'fed' conditions; D) 13°C and 'non-  
849 fed' conditions; E) 15°C and 'non-fed' conditions; F) 17°C and 'non-fed' conditions.

850 **Figure 3.** Evolution of the total length of *Arenicola marina* larvae at three different  
851 temperatures following days post-fertilization. A) At 13°C, where the age at first food intake  
852 ('birth') is indicated by the red dotted vertical line (21 days); B) At 15°C, where the age at  
853 first food intake ('birth'), is indicated by the red dotted vertical line (17 days); C) At 17°C,  
854 where the age at first food intake ('birth') is indicated by the dotted red vertical line (17 days).  
855 Lines are simulations of the models: classic von Bertalanffy (first phase of the biphasic  
856 growth model) and exponential (second phase of the biphasic growth model). Larval growth  
857 for the 'fed' condition is in black, and for the 'non-fed' condition is in blue.

858 **Figure 4.** Temperature corrections in different life stages of *Arenicola marina*. The black line  
859 represents the temperature correction used in the abj-DEB model mostly on juvenile/adult  
860 stages from De Cubber *et al.* (2020) and the blue line, the temperature correction using  
861 datasets from fertilization success rate (circle, Lewis *et al.*, 2002) and from this study on  
862 larval growth with parameters from the first phase of the biphasic growth model ( $a$  = black  
863 triangle;  $b$  = black square; equation (1)) and from the second phase of the biphasic growth  
864 model ( $c$  = black diamond; equation (2)) at several temperatures.

865 Tables caption

866 **Table 1.** Larval development of *Arenicola marina* at 13°C, 15 °C and 17°C. At 7 days post-  
867 fertilization (Time), larvae were fed with microalgae. Larval stages correspond to the number  
868 of setigers (S). Total length is the mean of the n replicates with its standard deviation ( $\pm$ ).

869

870 **Table 2.** Larval development after ‘birth’ according to diet condition for each temperature  
871 treatment. Times correspond to days post-fertilization. Total length is the mean of the n  
872 replicates with its standard deviation ( $\pm$ ). na for not available.

873

874 **Table 3.** Biphasic growth modelling parameters where  $a$  and  $b$  are from Von Bertalanffy’s  
875 phase and  $c$  results from the exponential phase. The parameter  $a$  was estimated from  $L_{inf}$  and  $b$   
876 using Equation 2.

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