Copepods use chemical trails to find sinking marine snow aggregates

Lombard, Fabien; Koski, Marja; Kiørboe, Thomas

Link back to DTU Orbit

1 **Copepods use chemical trails to find sinking marine snow aggregates**

2

3 Lombard, F.,[a,b,c,*] M. Koski,[a] and T. Kiørboe[a]

4 [a]Centre for Ocean Life, National Institute for Aquatic Resources, Section for Ocean

5 Ecology and Climate, Technical University of Denmark Charlottenlund, Denmark

6 [b] Laboratoire d'Océanographie Physique et Biogéochimique – Unité Mixte de Recherche

7 6535 (LOPB - UMR 6535) Campus de Luminy, Marseille, France.

8 [c] Université Pierre et Marie Curie-Paris Paris 6, Unité Mixte de Recherche 7093, Laboratoire

9 d'Océanographie de Villefranche, Villefranche sur Mer, France

10 *Corresponding author: lombard@obs-vlfr.fr

11 Running head: Copepods and marine snow chemical trails

18   Abstract

19   Copepods are major consumers of sinking marine particles and hence reduce the efficiency

20   of the biological carbon pump. Their high abundance on marine snow suggests that they can

21   detect sinking particles remotely. By means of laboratory observations we show that the

22   copepod *Temora longicornis* can detect chemical trails originating from sinking marine snow

23   particles (appendicularian houses). The chemical cue was detected by copepods from a

24   distance of > 25 particle radii, with the probability of detection decreasing with distance. The

25   behavior of *T. longicornis* following the trail resembled the behavior of males tracking

26   pheromone trails, although with a lower tracking velocity. Upon finding a house, the copepod

27   would attach for a short period (10-30 s) and feed intensively. Due to short residence times,

28   daily feeding rates were moderate. Our results demonstrate that even *T. longicornis*, a species

29   usually considered a micro-particle feeder, is able to detect and feed on marine snow

30   aggregates. If similar behaviors are displayed by the more dedicated aggregate-feeding

31   copepods, a topic that remains unexplored, the effect of copepods on vertical flux attenuation

32   may be significant.

3

INTRODUCTION

Sinking particles are the main vehicles for the vertical transport of biogenic carbon from the sea surface to the ocean interior and the seafloor (Fowler and Knauer 1986). The downward flux of particles, however, attenuates near exponentially with depth due to the degradation of the particulate material as it sinks (Martin et al. 1987). Bacteria and zooplankton are believed to be approximately equally important for the remineralization of sinking particles (Steinberg et al. 2008). Bacteria rapidly colonize sinking particles, facilitated by their chemotactic behavior (Stocker et al. 2008), and account for a surprisingly constant carbon-specific degradation rate of ca. 0.1 d^{-1} (Ploug and Grossart 2000). This quantitative understanding of the role of bacteria can readily be incorporated in models of flux attenuation (Buesseler and Boyd 2009). The contribution of zooplankton to flux attenuation is much more variable and the underlying processes are poorly known (Steinberg et al. 2008). The lack of knowledge of zooplankton processes has been cited as one of the main factors hampering the advances in modeling biogeochemical fluxes (Boyd and Trull 2007).

Zooplankton may occur in high abundances on marine snow aggregates in the upper ocean (Steinberg et al. 1994; Kiørboe 2000), and their grazing on aggregate constituents may be substantial (Koski et al. 2005). Likewise, large sinking particles may be a significant food source for many zooplankters (Dagg 1993). It is unclear, however, how the zooplankters find these particles. Random encounter is an insufficient process to explain observed abundance of zooplankton on aggregates and remote detection has been invoked to account for the high densities of zooplankton on snow particles (Kiørboe 2000; Kiørboe and Thygesen 2001; Jackson and Kiørboe 2004). Bacterial activity on the surface of sinking particles may cause a substantial leakage of dissolved organic material (Smith et al. 1992), and sinking particles therefore leave an organic solute trail in their wake (Kiørboe 2001). It has been hypothesized

4

58   that cruising zooplankters may encounter such trails, which, in turn, may lead the zooplankter

59   to large, nutritious particles (Kiørboe and Thygesen 2001). Such behavior has been

60   demonstrated in shrimps (*Acetes sibogae*) that follow scent trails left by sinking food particles

61   (Hamner and Hamner 1977). Copepods are also known to be able to follow chemical trails.

62   This has been demonstrated for copepod males in particular that follow pheromone trails left

63   by receptive females (Doall et al. 1998), but copepodites and females may also be able to

64   follow artificial trails of amino acids (Kiørboe 2001) or dextrane (Yen et al. 2004).

65   We here test the hypothesis that copepods may find and follow chemical trails left by

66   sinking marine snow particles. We use discarded appendicularian houses as a source of

67   particles, as it has been demonstrated that the sinking house leaves a trail in its wake

68   (visualized in Lombard and Kiørboe 2010, their fig. 3). Discarded houses are an abundant

69   type of marine snow (Alldredge and Gotschalk 1990) and they may contribute significantly to

70   carbon export from the surface ocean (Robison et al. 2005). Appendicularian houses are

71   loaded with, for instance, algae, bacteria and ciliates that are concentrated on houses up to 2-4

72   orders of magnitude relative to the ambient water (Hansen et al 1996; Lombard et al 2010).

73   The houses are thus concentrated packages of food, likely to be of a high nutritional value

74   (similar to its attached particles). Here, we demonstrate that even a typical suspension feeding

75   copepod (*Temora longicornis*) can feed on sinking marine snow, and that it finds the particles

76   by following the scent trail that marine snow leave in their wake.

77

78   **METHODS**

79   ***Origin and culture of animals***

80   Experiments were conducted using both cultivated and freshly collected copepods and

81   appendicularians ('culture' and 'wild' conditions). Cultured appendicularians (*Oikopleura*

82   *dioica*) and copepods (*Temora longicornis*), originally collected in the Øresund (56°03'N;

5

83   12°39'E), western Baltic Sea, were grown in the laboratory (18°C; salinity 35) for > 10

84   generations following standard protocols (Lombard and Kiørboe 2010). The appendicularians

85   were fed a mixture of diatoms (*Thalassosira pseudonana*) and phytoflagellates (*Isochrysis*

86   *galbana* and *Rhodomonas salina*). Under these conditions *O. dioica* produces 7-8 houses d^{-1}

87   (Fenaux 1985). *Temora longicornis* was fed a mixture of *R. salina*, *Thalassiosira weissflogii*

88   and dinoflagellates (*Prorocentrum minimum* and *Heterocapsa* sp.) in excess, which allowed

89   for relatively high ingestion and egg production rates.

90   Wild copepods and appendicularians were collected from a pier in the Øresund (55°45' N;

91   12°35.5' E) 24 h prior to the experiment. Animals were collected with a bucket, which

92   ensured their good condition. Wild animals were only used for behavioral observations.

93   Salinity at the time of collection was 17.

94   All experiments were conducted at 18°C.

95

96   ***Behavioral observations***

97   We recorded the behavior of *Temora longicornis* in the presence of sinking houses with a

98   black and white Charge-Coupled Device camera (Watex, WAT-535EX, 25 Hz frame rate)

99   equipped with a 35 mm lens to yield a field of view of ca. 5 x 4 cm. The experimental arena

100   consisted of a 500 mL transparent plexiglas chamber (dimensions 5 x 5 x 20 cm). The

101   chamber was placed in a dark room and illuminated with collimated infrared light from a

102   light-emitting diode that was shined through the chamber towards the camera. This way

103   shadow images of copepods and sinking houses were obtained. Both the camera and the light

104   were mounted on a motorized support which allowed moving the whole system in two

105   dimensions. Individual particles could thus be followed as they were sinking through the

106   water column without modifying the settings (size calibration) of the camera.

107   For behavioral observations we used either newly discarded houses (5-10 min), or houses

108   that had aged for 1-2 h. Fresh and aged houses differ in their properties, because houses

109   deflate rapidly once discarded. Thus, fresh houses leak internal water and food particles

110   (Lombard and Kiørboe 2010). Prior to an experiment, the chamber was filled with filtered

111   water from the appendicularian culture (or freshly collected sea water for wild conditions).

112   100-200 copepods were then added and allowed 24 h to acclimate. The high density of

113   copepods was chosen to increase the number of encounter events during the video recordings

114   and was assumed not to affect the swimming behavior (Dur et al. 2011).

115   Observations were made by gently introducing appendicularian houses (one at a time, up to

116   15 times) in the surface of the chamber, and then following the sinking house and nearby

117   copepods with the camera until the house reached the bottom of the chamber (~5 min). Video

118   sequences were later analyzed using a MatLab script that follows frame by frame the position

119   of targeted objects. The following parameters were retrieved: sizes, sinking trajectories, and

120   speeds of appendicularian houses; swimming trajectories and speeds of copepods that reacted

121   to a house (before and after encountering the chemical plume of the house); and copepod

122   residence time on the houses. A total of 232 copepod reactions were recorded and analyzed.

123

124   *Feeding experiment*

125   We estimated the feeding of copepods on discarded appendicularian houses by an indirect

126   approach, by quantifying the number of fecal pellets that copepods produce when offered

127   houses. Although dependent on food type, pellet production can be considered to be linearly

128   related to feeding rate (Besiktepe and Dam 2002). Ten adult *T. longicornis* were incubated for

129   48 h in 600 mL screw-cap bottles in 0.2-μm filtered sea water, containing a variable number

130   of aged 2-3 mm diameter houses (0; 5; 10; 20; 30; 40; 50 houses per bottle). The highest

131   tested densities of houses are in the upper end of that observed in nature, representing bloom

7

132 conditions, where densities of appendicularians (each producing 13-26 houses d^{-1};Sato et al.

133 2003) may exceed 50, 000 m^{-3} (Uye and Ishino 1995). All incubations were done in

134 triplicates. The bottles were mounted on a slowly rotating wheel (1 round per minute) and

135 incubated in darkness. Water and houses were changed after 24 h and the pellets were counted

136 at 24 and 48 h. For each counting, the content of the bottles was gently filtered onto a

137 submerged 10 μm filter and the copepods were immediately transferred to the new water.

138   Pellet production was used to qualitatively verify feeding on houses. Pellet production was

139 also converted to a crude quantitative estimate of feeding rate, by assuming a pellet volume of

140 3.75 10^5 μm^3 (Dutz et al. 2008), a carbon content of 0.052 pg μm^{-3} (González and Smetacek

141 1994), and a carbon assimilation efficiency of 0.75 (Besiktepe and Dam 20020). The obtained

142 feeding rates should be taken as conservative, since pellets volume and in particular carbon

143 content are strongly dependent on copepod diet and could be substantially higher than the

144 values used here (*e.g.* Ploug et al. 2008). Carbon content of female copepods from our culture

145 was 7.5 μg C individual (ind.)$^{-1}$ (Dutz et al. 2008).

146

147 **RESULTS**

148 ***Behavioral observations***

149   We observed two different reactions of copepods to sinking houses (*see* also video A1 in

150 the Web Appendix www.aslo.org/lo/toc/vol_xx/issue_x/xxxxa.xxx: 1) Direct encounter of a

151 sinking house by a cruising copepod and subsequent attachment of the copepod to the house

152 (as shown in video A1). This reaction was observed 86 times. 2) Remote detection and

153 response to the house: When passing directly above the house the copepod abruptly changes

154 its swimming direction and accelerates downward towards the house, following the presumed

155 scent trail (as shown in video A1 and A2). The position where this happens is considered the

156 detection point. While following the trail, the copepod doubles its swimming speed to 6-8 mm

8

157   s^{-1} (Table 1, Fig. 1) and swims in a zigzag course towards the particle. Of the 146 cases of

158   remote detection, the copepods were successful in actually finding and attaching to the

159   particle in only half of these (72).

160   The positions of detection points (Fig. 1) describe the extension of the chemical trail within

161   which the concentration of attractant exceeds the threshold for detection and response. The

162   length of the trail is about 35 mm, corresponding to more than 25 particle radii, its maximum

163   width about 4 mm, and it is tapered toward its distal part. If the copepod detects the chemical

164   signal with sensors at the tip of the antennules, then the 'real' width of the plume is 2 x

165   antennules length (~ 2 x 0.6 mm) less that suggested by the detection points, i.e., 2-3 mm. The

166   width of the plume can alternatively be estimated from the amplitude of the zigzag behavior

167   (~0.7 mm; Table 1) plus 2 x antennules length; again ca. 2 mm.

168   The probability of detection declines hyperbolically with the distance to the particle (Fig.

169   2). There was no difference in average detection distance between fresh and aged houses,

170   whereas there was a difference between the reactions in wild vs. culture conditions. Houses

171   from wild appendicularians, were detected at a shorter distance by wild copepods than

172   cultured copepods detected cultured houses (Table 1).

173   Copepods that managed to find a house attached to it and fed intensely from its surface as

174   evidenced by constant activity of the feeding appendages. Sometimes the copepod would grab

175   the house with its mandibles and apparently attempt to remove part of the house as evidenced

176   by vigorous kicking of the swimming legs (*see* the Web Appendix). The copepods resided on

177   the house for a relatively short (< 1 min) but intense period, which was more than twice as

178   long for wild than for cultured conditions (Table 1).

179

180   ***Grazing and fecal pellets production***

9

181   This feeding activity resulted in the production of fecal pellets, and the production of

182   pellets increased with the concentration of houses. Fitting a Holling Type II response to the

183   observations yields a saturated pellet production rate of 34 ±13 pellets ind.$^{-1}$ d^{-1} (Fig. 3). This

184   rate was however not reached, even at the highest concentration of houses (83 L^{-1}). The

185   maximum observed pellet production, ca. 15 pellets ind.$^{-1}$ d^{-1}, corresponds to an ingestion of

186   ~0.45 μg C ind.$^{-1}$ d^{-1} (6% body weight d^{-1}). The saturated consumption rate of appendicularian

187   houses would then be about 0.9 μg C ind.$^{-1}$ d^{-1} (12% body weight d^{-1}), a sufficiently high rate

188   to support copepod metabolism, while not allowing for a high growth or reproduction rate

189   (Hernandez-León and Ikeda 2005). Thus even in saturating concentration of houses those only

190   partly fulfill this copepod needs.

191

192   **DISCUSSION**

193   ***Chemical or hydromechanical cues?***

194   Our results demonstrate that the copepod *Temora longicornis* is able to remotely detect

195   sinking appendicularian houses, to subsequently follow the sinking track to the particle, and

196   eventually to grab the particle and feed on it. To our knowledge, our results constitute the first

197   demonstration of such particle tracking behavior in a copepod using realistic particles

198   produced both under laboratory and wild conditions. Similar observations have previously

199   been reported for planktonic shrimps tracking sinking detritus (Hamner and Hamner 1977),

200   while the general assumption of the ability of copepods to follow chemical particle trails has

201   until present been mainly based on theoretical considerations (Jackson and Kiørboe 2004).

202   The detection distance to a sinking 0.25 cm radius house was up to 3.5 cm at a position that

203   was left by the particle over 25 seconds earlier. This strongly suggests that the cue utilized by

204   the copepod is chemical rather than fluid mechanical. All our observations correspond to

205   Reynolds numbers (Re) between 1 to 6. Fluid disturbances at low Re dissipate rapidly due to

10

206 viscosity; thus, the viscous time scale (L^2/η, where L is the linear dimension of the disturbance

207 and η the kinematic viscosity of the water) for a fluid signal of a spatial extension similar to

208 the size of the sinking particles is ~6 s. The fluid disturbance also attenuates spatially, and the

209 signal generated by the sinking particle at a distance of 3.5 cm can be estimated assuming

210 Stokes flow for a translating sphere. Using the model developed by (Visser 2001), and

211 assuming that the fluid velocity signal has to be ≥ 100 μm s^{-1} to be detected (Kiørboe 2011),

212 the hydromechanical detection distance can be estimated as 0.4 cm; if a 10x smaller detection

213 signal is sufficient, this estimate increases to 1.2 cm.

214   The behavior of the tracking copepods is consistent with them following a chemical trail. It

215 resembles the behavior described for males tracking pheromone trails deposited by swimming

216 females, although tracking velocities observed here (< 10 mm s^{-1}; Fig. 1, Table 1) are

217 substantially lower than the swimming speed of female-tracking males of the same species

218 (15-40 mm s^{-1}; Doall et al. 1998) and thus cannot be confused. The constant zigzagging along

219 the invisible trail has been termed 'casting behavior' (Weissburg et al. 1998). It serves the

220 purpose of constantly checking the radial extension of the trail, and is a prerequisite for being

221 able to follow the trail. The tracking behavior of the copepods and the weakness of fluid

222 mechanical disturbances generated by the sinking particles lead us to conclude that the cue

223 used by the copepod to detect the sinking particle is chemical.

224   The spatial extension of the chemical plume, as evidenced by the distribution of detection

225 points, is only partly consistent with that predicted from a model that describes the flow and

226 concentration fields around a sinking sphere that leaks solutes (Kiørboe et al. 2001; *see* also

227 Fig. 1A). The predicted solute distribution depends mainly on the Péclet number (Pe = aU/D,

228 where a is the sphere radius, U the sinking velocity, and D the diffusivity of the leaking

229 substance). The slower the particle sinks, and the higher the diffusivity of the leaked

230 molecules, the broader the deposited trail. Assuming a diffusivity of $D = 10^{-5}$ cm^2 s^{-1}, which is

11

231   typical for small biological molecules, and the sinking velocities recorded for the discarded

232   houses (Table 1), we get a Péclet number of about 10^3, and a predicted solute distribution as

233   shown in Fig. 1A. Obviously, the observed plume is much broader than that predicted, even

234   when accounting for the length of the antennules of the copepods. The distribution rather

235   resembles a situation for a Péclet number of order 100 or less (Fig. 1A). We argue that this is

236   due to a much higher effective diffusivity of leaked substances in our experiments due to the

237   mixing effect of both the swimming copepods and of the unavoidable convection currents in

238   the experimental chamber. Observations and theory become consistent with one another if we

239   assume a 10-fold higher effective diffusivity due to our experimental setup.

240

241   ***Efficiency of particle detection***

242   From our observations, *T. longicornis* has almost twice the chance of remotely detecting a

243   sinking house rather than directly encountering it (146 vs. 86 observations). However, judged

244   from the observed ratio of direct collision-encounters to particle encounters facilitated by

245   remote detection of sinking particles (86 vs. 71), remote detection only has a rather modest

246   effect on the efficiency by which copepods can find sinking particles. However, we argue that

247   our experimental design leads to an underestimation of this efficiency due to the mixing

248   effects of the swimming copepods and small convection currents, but also that we can roughly

249   quantify the error and estimate the real efficiency from our observations.

250   We use the 'moving point source model' of Jackson and Kiørboe (2004) to evaluate our

251   results. According to this model, the length (L), maximum width (d; derived from Jackson and

252   Kiørboe 2004), and cross-sectional are (A_0) of the plume can be estimated as

253    $$L = \frac{Q}{4\pi D C^*}$$ (1)

12

254 $$d = 2.43\left(\frac{DL}{v}\right)^{0.5} \qquad (2)$$

255 $$A_0 = \frac{0.24}{Dv^{0.5}}\left(\frac{Q}{\pi C*}\right)^{1.5} \qquad (3)$$

256 where Q (mol s^{-1}) is the rate at which solutes leak from the particle, $C*$ (mol cm^{-3}) the

257 threshold concentration for detection, and v (cm s^{-1}) the sinking velocity of the particle.

258 Equation 2 allows us to estimate the effective diffusivity of the chemical substance from the

259 observed length (3.5 cm) and width (0.2 cm) of the plume and sinking velocity of the particles

260 (0.05-0.15 cm s^{-1}). The effective diffusivity thus estimated is about 1-3 10^{-4} cm^2 s^{-1}, one order

261 of magnitude larger than the molecular diffusivity of small biological molecules, as also

262 proposed above.

263 Kiørboe and Thygesen (2001) and Jackson and Kiørboe (2004) compiled empirical

264 evidence to suggest that if the substance to which copepods respond is amino acids, then Q

265 (mol s^{-1}) $\approx 10^{-12}a^{1.5}$ and $C* \approx 10^{-11}$ mol cm^{-3}, where a is the radius of the sinking particle (0.15

266 cm). If we accept these estimates and assume an effective diffusivity of 1-3 10^{-4} cm^{2}s^{-1} then

267 the model predicts a plume length in our experiments of 1.67-5 cm, in the range of our

268 observations (<3.5 cm). Thus, our experimental observations are internally consistent and

269 moreover consistent with independent estimates of particle leakage rates and copepod

270 chemical sensitivity.

271 The encounter cross section of the chemical plume can be estimated as

272 $$A = A_0 + 2SL \qquad (4)$$

273 where S is the length of the antennules (0.6 mm). This may be compared to the encounter

274 cross section of the sinking particle itself ($\pi(a+S)^2$) and used to estimate the enhancement in

275 potential encounter rate due to chemical signals which are comparable to observed

276 enhancement of encounter irrespectively to copepods or houses concentrations. Using the

13

277 numbers above and a diffusivity not affected by experimental mixing effect, the enhancement

278 for 2.5 mm diameter sinking houses is about two orders of magnitude. This estimate does not

279 take into account the decreasing detection probability with increasing distance to the particle

280 (Fig. 2), and it does not consider the effect of turbulent mixing (Visser and Jackson 2004).

281 One may argue that the starved copepods used in our experiments were particularly

282 responsive to food signals. As food in the ocean tends to be patchily distributed (Mitchell and

283 Fuhrman 1989) and as starvation tolerance of neritic copepods such as *Temora longicornis* is

284 limited (Koski and Klein Breteler 2003), an increased ability of starved copepods to detect

285 food signals would be a relevant trait. However, our study was not designed to investigate the

286 effect of starvation on behavior, but simply to demonstrate that chemical plumes behind

287 sinking particles may significantly increase the possibility for zooplankters to find these

288 nutritious particles.

289

290 ***Feeding on sinking particles***

291 Jackson and Kiørboe (2004) explored the potential for zooplankters to find and feed on

292 large sinking particles using the above model and parameter values consistent with those

293 estimated here. While copepods are generally thought of as microphageous that mainly feed

294 on microscopic phytoplankton and protozoans, the analysis of Jackson and Kiørboe (2004)

295 suggested that large sinking particles may also be an important source of nutrition. The

296 importance of sinking particles in the nutrition of zooplankton has also been suggested based

297 on the metabolic demands of the zooplankton communities in the mesopelagic zone

298 (Steinberg et al. 2008) and analysis of their gut fatty acid and pigment compositions (Wilson

299 and Steinberg 2010).

300 The residence times of the copepods on the houses are short, consistent with earlier

301 laboratory observations (Alldredge 1972). Reported residence times vary from minutes for

302   pelagic organisms (Shanks and Walters 1997) to hours for semi-benthic species (Koski et al.

303   2005). It has been suggested that the short residence times are the result of a trade-off between

304   the good feeding conditions and the elevated predation risk when attached to an aggregate,

305   and that a copepod should remain on the aggregate only long enough to fill its gut (Kiørboe

306   and Thygesen 2001). From estimated house encounter rates (calculated from observed

307   copepods swimming speed, and sizes of particles and chemical trail) and consumption for *T.*

308   *longicornis*, one can estimate that each house encounter leads to the ingestion of 3-5 10^{-4} μg

309   C, and an ingestion rate during attachment of 0.1-0.2 μg C h^{-1}. This is a very high

310   instantaneous ingestion rate, but it would still take a copepod about 7-15 min to fill its gut

311   (assumed to be the equivalent of one fecal pellet).

312   Our observations show that *T. longicornis* feed on discarded houses but even saturated

313   feeding rates are only 10% of those reported for grazing on phytoplankton (Besiktepe and

314   Dam 2002). The feeding rates (~4 x 10^{-4} μg C encounter^{-1}) extrapolated to natural

315   concentrations of discarded appendicularian houses (e.g., max. 6 L^{-1} in the North Sea; Koski

316   et al. 2007; encounter rate calculated with a normal case of effective diffusivity) would be

317   low, around 0.5 μg C ind. d^{-1}. However, appendicularian houses are only one example of

318   sinking particles, and copepods may also feed on other types of marine snow, such as diatom

319   flocks or fecal pellets. Further, the feeding rates observed in the laboratory may underestimate

320   those in the field. As suggested by the difference between cultured and wild copepods in our

321   experiments, the wild copepods are more efficiently feeding on appendicularian houses. This

322   may be due to the immediate history of the animals, e.g., hunger level or age, body size, or the

323   different behaviors of the cultured and wild copepods (Tiselius et al. 1995).

324   *T. longicornis* is probably not a typical aggregate feeder and other zooplankters have

325   morphologies and behaviors that make them particularly adapted to feed on marine snow

326   aggregates. Copepods that are typically found on marine snow aggregates are harpacticoids,

15

327 such as *Microsetella norvegica*, and copepods of the genus *Oncaea* (Shanks and Walters

328 1997). These species are not able to feed efficiently on suspended food, but are dependent on

329 aggregates for feeding (Koski et al. 2005), and behavioral observations suggest that they

330 readily feed on appendicularian houses (Ohtsuka and Kubo 1991). *M. norvegica* and semi-

331 benthic harpacticoids can have feeding rates ranging from 40% to 200% body weight^{-1} d^{-1} on

332 appendicularian houses and settled diatoms (Koski et al. 2005; Koski et al. 2007), thus being

333 considerably more efficient in aggregate feeding than *T. longicornis*.

334 Published estimates of the effect of copepods on the attenuation of sinking flux suggest

335 potentially high degradation rates both in and below the euphotic zone, mainly depending on

336 the abundance of potential grazers (Kiørboe 2000; Koski et al. 2005; Steinberg et al. 2008).

337 Calanoid copepods, like *Temora longicornis,* are not normally considered feeders on large,

338 sinking particles, and the feeding rates found here were lower than typically observed for *T.*

339 *longicornis* feeding on suspended food (e.g., diverse phytoplankton). However, the

340 degradation of sinking aggregates due to zooplankton grazing may be intense if typical

341 detritivorous zooplankton exhibit similar aggregate-finding behavior as that reported here for

342 *T. longicornis*. As calculations based on the abundance of some copepod species (typically

343 *Microsetella norvegica* and *Oncaea* spp.) on marine snow suggest that random encounter is

344 not sufficient to explain their high concentrations on aggregates (Kiørboe 2000), this seems

345 likely. An efficient mechanism to find and colonize particles, together with their at times high

346 abundances (up to 100 ind. L^{-1}; Uye et al. 2002), suggest a potentially high effect of particle-

347 colonizing copepods on the attenuation of vertical flux.

348 We have here demonstrated a mechanism by which copepods may locate sinking marine

349 snow aggregates. This mechanism may account for the high abundances of copepods

350 observed on aggregates, and is consistent with copepods contributing significantly to the

351 vertical attenuation of particle flux in the ocean. Our observation is one step further in

16

352   understanding the controls of flux attenuation and suggests that remote detection of chemical

353   cues can enhance the encounter and degradation rates by orders of magnitude from what

354   would be predicted based on the particle sizes and concentrations alone.

355   *References*

356   Alldredge, A. L., and C. C. Gotschalk. 1990. The relative contribution of marine snow of

357   different origins to biological processes in coastal waters. Cont. Shelf Res. **10:** 41-58.

358   Besiktepe, S., and H. G. Dam. 2002. Coupling of ingestion and defecation as a function of

359   diet in the calanoid copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. **229:** 151-164.

360   Boyd, P. W., and T. W. Trull. 2007. Understanding the export of biogenic particles in oceanic

361   waters: Is there consensus? Prog. Oceanogr. **72:** 276-312.

362   Buesseler, K. O., and P. W. Boyd. 2009. Shedding light on processes that control particle

363   export and flux attenuation in the twilight zone of the open ocean. Limnol. Oceanogr.

364   **54:** 1210-1232.

365   Dagg, M. 1993. Sinking particles as a possible source of nutrition for the large calanoid

366   copepod *Neocalanus cristatus* in the Sub-Arctic Pacific-Ocean Deep-Sea Res. I **40:**

367   1431-1445.

368   Doall, M. H., S. P. Colin, J. R. Strickler, and J. Yen. 1998. Locating a mate in 3D: The case of

369   *Temora longicornis*. Phil. Trans. R. Soc. B **353:** 681-689.

370   Dur, G., S. Souissi, F. Schmitt, F.-G. Michalec, M.-S. Mahjoub, and J.-S. Hwang. 2011.

371   Effect of animal density, volume, and the use of 2D/3D recording on behavioral

372   studies of copepods. Hydrobiol. **666**: 197-214.

373   Dutz, J., M. Koski, and S. H. Jonasdottir. 2008. Copepod reproduction is unaffected by

374   diatom aldehydes or lipid composition. Limnol. Oceanogr. **53:** 225-235.

375   Fenaux, R. 1985. Rhythm of secretion of Oikopleurid's houses. Bull. Mar. Sci. **37:** 498-503.

376   Fowler, S. W., and G. A. Knauer. 1986. Role of large particles in the transport of elements

377   and organic compounds through the ocean water column. Prog. Oceanogr. **16:** 147-

378   194.

379 Fransz, H. G., J. M. Colebrook, J. C. Gamble, and M. Krause. 1991. The zooplankton of the

380   North Sea. Neth. J. Sea Res. **28:** 1-52.

381 González, H. E., and V. Smetacek. 1994. The possible role of the cyclopoid copepod *Oithona*

382   in retarding vertical flux of zooplankton faecal material. Mar. Ecol. Prog. Ser. **113:**

383   233-246.

384 Hamner, P., and W. M. Hamner. 1977. Chemosensory tracking of scent trails by planktonic

385   shrimp *Acetes Sibogae Australis*. Science **195:** 886-888.

386 Hansen, J.L.S., T. Kiørboe, and A. L. Alldredge. 1996. Marine snow derived from abandoned

387   larvacean houses: Sinking rates, particle content and mechanisms of aggregate

388   formation. Mar. Ecol. Prog. Ser. **141**: 205-215.

389 Hernandez-León, S., and T. Ikeda. 2005. A global assessment of mesozooplankton respiration

390   in the ocean. J. Plankton Res. **27:** 153-158.

391 Jackson, G. A., and T. Kiørboe. 2004. Zooplankton use of chemodetection to find and eat

392   particles. Mar. Ecol. Prog. Ser. **269:** 153-162.

393 Kiørboe, T. 2000. Colonization of marine snow aggregates by invertebrate zooplankton:

394   Abundance, scaling, and possible role. Limnol. Oceanogr. **45:** 479-484.

395 Kiørboe, T. 2001. Formation and fate of marine snow: Small-scale processes with large-scale

396   implications. Sci. Mar. **65:** 57-71.

397 Kiørboe, T. 2011. How zooplankton feed: Mechanisms, traits and trade-offs. Biol. Rev. **86:**

398   311-339.

399 Kiørboe, T., H. Ploug, and U. H. Thygesen. 2001. Fluid motion and solute distribution around

400   sinking aggregates. I. Small-scale fluxes and heterogeneity of nutrients in the pelagic

401   environment. Mar. Ecol. Prog. Ser. **211:** 1-13.

402 Kiørboe, T., and U. H. Thygesen. 2001. Fluid motion and solute distribution around sinking

403   aggregates. II. Implication for remote detection by colonizing zooplankters. Mar. Ecol.

404   Prog. Ser. **211:** 15-25.

405 Koski, M., and W. C. M. Klein Breteler. 2003. Influence of diet on copepod survival in the

406   laboratory. Mar. Ecol. Prog. Ser. **264**: 73-82.

407 Koski, M., T. Kiorboe, and K. Takahashi. 2005. Benthic life in the pelagic: Aggregate

408   encounter and degradation rates by pelagic harpacticoid copepods. Limnol. Oceanogr.

409   **50:** 1254-1263.

410 Koski, M., E. F. Moller, M. Maar, and A. W. Visser. 2007. The fate of discarded

411   appendicularian houses: Degradation by the copepod, *Microsetella norvegica*, and

412   other agents. J. Plankton Res. **29:** 641-654.

413 Lombard, F., and T. Kiørboe. 2010. Marine snow originating from appendicularian houses:

414   Age-dependent settling characteristics. Deep-Sea Res. I **57:** 1304-1313.

415 Lombard, F., D. Eloire, A. Gobet, L. Stemmann, J. Dolan, and G. Gorsky. 2010. Experimental

416   and modeling evidence of ciliates-appendicularians interactions. Limnol. Oceanogr.

417   **55**: 77-90.

418 Martin, J. H., G. A. Knauer, D. M. Karl, and W. W. Broenkow. 1987. VERTEX - Carbon

419   cycling in the Northeast Pacific. Deep-Sea Res. A **34:** 267-285.

420 Mitchell, J. G., and J. A. Fuhrman. 1989. Centimeter scale vertical heterogeneity in bacteria

421   and chlorophyll *a*. Mar. Ecol. Prog. Ser. **54**: 141-148.

422 Ohtsuka, S., and N. Kubo. 1991. Larvaceans and their houses as important food for some

423   pelagic copepods. Bull. Plankton Soc. Japan **Spec. Vol.:** 535-551.

424 Ploug, H., and H. P. Grossart. 2000. Bacterial growth and grazing on diatom aggregates:

425   Respiratory carbon turnover as a function of aggregate size and sinking velocity.

426   Limnol. Oceanogr. **45:** 1467-1475.

20

427 Ploug, H., M. H. Iversen, M. Koski, and E. T. Buitenhuis. 2008. Production, oxygen

428   respiration rates, and sinking velocity of copepod fecal pellets: Direct measurements

429   of ballasting by opal and calcite. Limnol. Oceanogr. **53:** 469-476.

430 Robison, B. H., K. R. Reisenbichler, and R. E. Sherlock. 2005. Giant larvacean houses: Rapid

431   carbon transport to the deep sea floor. Science **308:** 1609-1611.

432 Sato, R., Y. Tanaka, and T. Ishimaru. 2003. Species-specific house productivity of

433   appendicularians. Mar. Ecol. Prog. Ser. **259**: 163-172.

434 Shanks, A. L., and K. Walters. 1997. Holoplankton, meroplankton, and meiofauna associated

435   with marine snow. Mar. Ecol. Prog. Ser. **156:** 75-86.

436 Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intensive hydrolytic activity on

437   marine aggregates and implications for rapid particle dissolution. Nature **359:** 139-

438   142.

439 Steinberg, D. K., M. W. Silver, C. H. Pilskaln, S. L. Coale, and J. B. Paduan. 1994. Midwater

440   zooplankton communities on pelagic detritus (giant larvacean houses) in Monterey

441   Bay, California. Limnol. Oceanogr. **39:** 1606-1620.

442 Steinberg, D. K., B. A. S. Van Mooy, K. O. Buesseler, P. W. Boyd, T. Kobari, and D. M.

443   Karl. 2008. Bacterial vs. zooplankton control of sinking particle flux in the ocean's

444   twilight zone. Limnol. Oceanogr. **53:** 1327-1338.

445 Stocker, R., J. R. Seymour, A. Samadani, D. E. Hunt, and M. F. Polz. 2008. Rapid

446   chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient

447   patches. Proc. Natl. Acad. Sci. USA **105:** 4209-4214.

448 Tiselius, P., B. Hansen, P. Jonsson, T. Kiørboe, T. G. Nielsen, S. Piontkovski, and E. Saiz.

449   1995. Can we use laboratory reared copepods for experiments? A comparison of

450   feeding behavior and reproduction between a field and a laboratory population of

451   *Acartia tonsa*. ICES J. Mar. Sci. **52:** 369-376.

452 Uye, S., and S. Ichino, 1995. Seasonal variations in abundance, size composition, biomass and

453   production rate of *Oikopleura dioica* (Fol) (Tunicata: Appendicularia) in a temperate

454   eutrophic inlet. J. Exp. Mar. Biol. Ecol. **189**: 1-11.

455 Uye, S., I. Aoto, and T. Onbé. 1992. Seasonal population dynamics and production of

456   *Microsetella norvegica*, a widely distributed but little-studied marine planktonic

457   harpacticoid copepod. J. Plankton res. **24**: 143-153.

458 Visser, A. W. 2001. Hydromechanical signals in the plankton. Mar. Ecol. Prog. Ser. **222:** 1-

459   24.

460 Visser, A. W., and G. A. Jackson. 2004. Characteristics of the chemical plume behind a

461   sinking particle in af turbulent water column. Mar. Ecol. Prog. Ser. **283:** 55-71.

462 Weissburg, M. J., M. H. Doall, and J. Yen. 1998. Following the invisible trail: Kinematic

463   analysis of mate-tracking in the copepod *Temora longicornis*. Phil. Trans. R. Soc. B

464   **353:** 701-712.

465 Wilson, S. E., and D. K. Steinberg. 2010. Autotrophic picoplankton in mesozooplankton guts:

466   evidence of aggregate feeding in the mesopelagic zone and export of small

467   phytoplankton. Mar. Ecol. Prog. Ser. **412:** 11-27.

468 Yen, J., A. Prusak, M. Caun, M. H. Doall, J. Brown, and J. R. Strickler. 2004. Signaling

469   during mating in the pelagic copepod, *Temora longicornis*, p. 149-159. *In* L. Seuront

470   and P. Strutton [eds.], Scales in aquatic ecology: measurements, analysis, modelling.

471   CRC Press.

22

Tables

Table 1 : Summary of copepod reactions to sinking aggregates at the different experimental conditions: Total number of observations (*n*), number of direct encounters, number of chemical tracking events (including copepods that lose the trail), mean house size and sinking speed, reaction distance of the copepods to the sinking house, mean zigzag amplitude, residence time, and swimming velocity before and after encountering the chemical trail (± SD). Differences between new and old houses have been tested as no significantly different for all observations (*t*-test, *p* > 0.05) except houses size (*t*-test, df = 230, *p* < 0.001). Differences between 'wild' and 'cultured' conditions are significant with respect to detection distance (*t*-test, df = 148, *p* < 0.001), residence time (*t*-test, df = 110, *p* = 0.012) and swimming and tracking velocities (*t*-tests, df = 109, *p* = 0.048 and df = 108, *p* < 0.001, respectively).

Observation conditions		*n*	Encounter		House size (mm)	House sinking speed (m d⁻¹)	Mean reaction distance (mm)*	Mean zigzags amplitude (mm)	Time on particle (s)	Copepods speed (mm s⁻¹)	
			direct	chemical (lose)						before encoutering the trail	following the trail*
culture	new	127	50	77 (37)	3.5 (±0.8)	129	12.7 (±8.3)	0.78	11.0 (±22.3)	4.1 (±1.8)	7.5 (±1.4)
	old	35	13	22 (9)	2.4 (±0.3)	132	12.3 (±9.8)	0.62	12.9 (±17.4)	4.4 (±1.9)	8.0 (±2.3)
	total	162	63	99 (46)	3.3 (±0.9)	130	12.6 (±8.7)	0.75	11.3 (±21.6)	4.2 (±1.8)	7.6 (±1.6)
wild	new	33	13	20 (12)	3.5 (±0.4)	48	7.9 (±5.6)	0.56	23.2 (±16.6)	3.6 (±1.4)	6.3 (±1.2)
	old	37	10	27 (16)	2.8 (±0.5)	57	8.6 (±6.6)	0.62	27.7 (±26.7)	3.5 (±1.5)	6.5 (±1.6)
	total	70	23	47 (28)	3.1 (±0.6)	54	8.3 (±6.2)	0.60	26.1 (±23.7)	3.5 (±1.5)	6.5 (±1.5)

* only consider chemical reactions

Figure legends

473 Figure 1 : (A) Relative position of *Temora longicornis* when it reacts to the chemical trail left

474 by sinking discarded house (represented here as a large dot). The two inserted images

475 represent the theoretical chemical plume assuming two different Péclet numbers (adapted

476 from Kiørboe et al. 2001). (B) Example of *T. longicornis* swimming pattern and speed (mm s^{-}

477 ^{1}) relative to position of sinking discarded houses. The detection point is marked with a black

478 arrow. Note the increased speed and the zigzagging behavior when following the chemical

479 trail.

480

481 Figure 2 : Frequency distribution of detection distance of *T. longicornis* to the sinking

482 aggregates. Wild and culture conditions were plotted separately since they were tested as

483 significantly different (χ^2-test *p*>0.05).

484

485 Figure 3 : *T. longicornis* fecal pellet production when offered discarded house at different

486 concentrations (number L^{-1}). Error bars represent the standard error originating from

487 triplicates. Relationship is: Y= 33.8 (±13.0)X/(X+133 (±73)) (R^2 = 0.84; *n* = 27) dotted lines

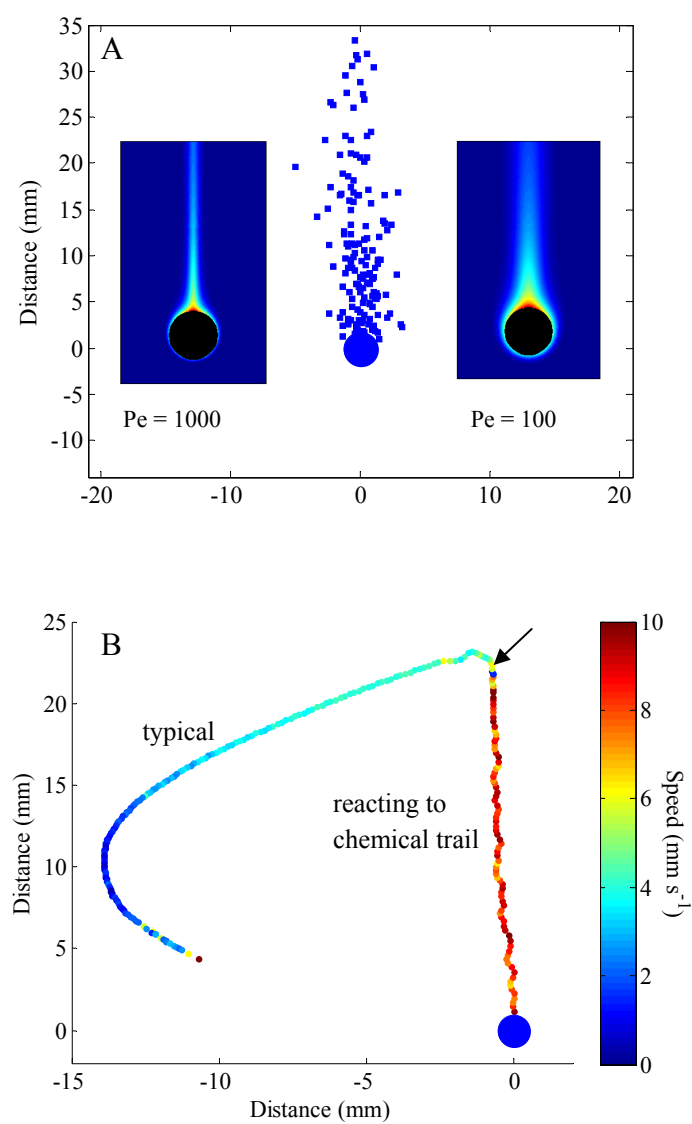488 represent the 95% confidence limits intervals of this relationship.
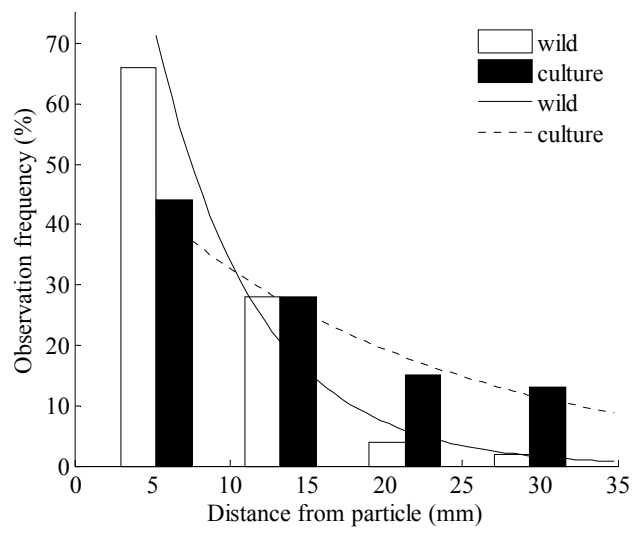
489

24

Figure 1

Figure 2

Figure 3

27

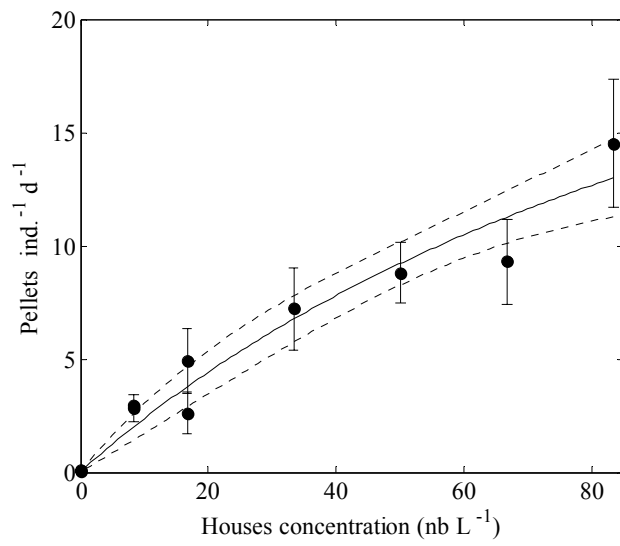**Web appendix**

These three video sequences illustrate the different behavioral observations described in the accompanying manuscript.

Video A1: Example of *Temora longicornis* interacting with sinking marine snow (discarded appendicularian house) using infra red light. All the different behavior (chemical trail detection, direct encounter) are illustrated.

Available online at:

http://fabien.lombard1.free.fr/temoratrackIR2.wmv

Video A2: Example of *T. longicornis* following a chemical trail left by a sinking marine snow (discarded appendicularian house) using white light illumination inside a rotating tank:

http://fabien.lombard1.free.fr/temoratrackwhiteligth.wmv

Video A3: Close-up view of the feeding behavior of *T. longicornis* on marine snow (discarded appendicularian houses) using a binocular lens (inside a petri dish).

Available online at

http://fabien.lombard1.free.fr/closeupfeeding.wmv