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Resolving the paradox of the ambush feeding cyclopoid copepod *Apocyclops royi* being microphageous

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Running head: Feeding behavior of *Apocyclops royi*

Author contributions: AZ, BWH, TK, and FR conceived the study designed the experiments. AZ and FR performed the experiments. All authors contributed to data analysis and interpretation. AZ and FR wrote the manuscript with significant input from BWH and TK.

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Keywords: *Apocyclops royi*, prey-capture, ambush feeding, escaping capacity

Abstract

The cyclopoid copepod *Apocyclops royi* is assumed to be an ambush feeder that passively waits for its prey and captures it by a fast surprise attack. This feeding strategy requires an acute sensibility to hydromechanical signals generated by moving prey. However, *A. royi* in long-term cultures is able to survive microphageously fed solely on Baker's yeast (*Saccharomyces cerevisae*), a small, non-motile prey. In this study, we investigate the feeding behavior of *A. royi* and how it senses *S. cerevisae* cells. Using high-speed video, we find that *A. royi* still exhibits an ambush feeding behaviour when fed *S. cerevisae*. Moreover, we characterise the distance and the duration of attack jumps and evaluate the sensitivity of *A. royi* to fluid disturbances by filming its escaping behaviour when caught in a suction flow simulating a predator e.g., a fish larva. We demonstrate that its sensitivity to fluid disturbances is very similar to that of other copepod species. Thus, we find that remote detection of *S. cerevisae* cells due to hydromechanical signals is unlikeky as the particles are small (3.8 ± 1.3 µm) and non-motile, and that *A. royi* likely senses *S. cerevisae* cells by randomly touching them with setae on their first antennae.

Introduction

The cyclopoid copepod *Apocyclops royi* is one of two dominant zooplankton species in brackish Taiwanese aquaculture ponds (Jepsen *et al.*, 2021). It is well adapted to these waters that are characterized by extreme fluctuations in salinity, oxygen, and food availability (Blanda *et al.*, 2017).

A. royi is able to survive and reproduce on very poor monospecific diets, such as Baker's yeast (*Saccharomyces cerevisae*) (Nielsen *et al.*, 2019, 2020). This is because *A. royi* can synthesise n-3 polyunsaturated fatty acids (PUFA) and produce docosahexanoic acid (DHA) even when fed low-PUFA diets. These discoveries provide the metabolic reason why *A. royi* can survive on Baker's yeast that is practically deprived of long-chained fatty acids, but do not provide any information on how these copepods sense and catch these small (3–4 μm) non-motile particles acting microphageously, i.e., feeding upon tiny particles. *A. royi* nauplii exhibit ambush feeding when fed the motile microalga *Isochrysis galbana* (~6 μm), one of its natural prey (Wu *et al.*, 2011). However, there are no observations on the feeding behavior of later life-cycle stages of this presumably ambush feeding copepod. Ambush-feeding copepods generally passively wait for motile prey to swim within their sensory sphere, and then jump towards the prey and catch it by creating a vacuum (Svensen and Kiørboe, 2000). *Oithona davisae*, an obligatory cyclopoid ambush feeder, and *Acartia tonsa*, a calanoid partial ambush feeder, both elicit an attack when the prey comes within ~0.2 mm of their first antennae (Kiørboe *et al.*, 2009). This suggests that the prey is percieved by setae on their antennae acting as mechanoreceptors that perceives the fluid disturbance generated by the motile prey (Strickler and Bal, 1973). However, as opposed to the microalgae usually fed to ambush feeding copepods in cultures, *S. cerevisae* cells are not motile and do not generate a fluid signal. How, then, does *A. royi* manage to feed on Baker's yeast?

Here, we study the foraging and escape behavior of *A. royi* by direct observations using high-speed videography. We find that *A. royi* elicits ambush feeding behavior even when fed the small non-

motile *S. cerevisae*. We evaluate the capability of *A royi* to perceive fluid disturbances by quantifying their escape response to artificial fluid signals and find that their sensitivity is similar to that found in other copepods. We therefore propose that direct encounter, rather than a fluid signal, triggers a feeding jump towards these small non-motile prey.

Materials and methods

Experimental organisms

We used *Apocyclops royi* from Donggang in Taiwan that were established in culture at LOG-Marine Station of Wimereux in France (Pan *et al.*, 2016). The culture was initiated at Roskilde University, Denmark and kept for 100+ generations in a 25 °C walk-in temperature controlled room, with a 12:12 light:dark cycle (Jepsen *et al.*, 2021). Copepods were kept in 0.2 µm filtered 20 psu seawater and cultures were fed either the microalga *Rhodomonas salina* or Baker's yeast (*Saccharomyces cerevisiae*) in excess.

R. salina was from a culture at Roskilde University and *S. cerevisiae* was from the yeast production company De Danske Gærfabrikker A/S and available at most supermarkets. The equivalent spherical diameter was 7.6 ± 1.2 µm and 3.8 ± 1.3 µm for *R. salina* and *S. cerevisiae*, respectively (Fig. 1).

Foraging experiment

Between five and eights copepods (0.38 ± 0.06 mm prosome length, stages CII-CIII) were pipetted into a 4.5 mL squared glass cuvette containing either *R. salina* or *S. cerevisiae* in excess. Filming was conducted at 18–20 °C in a temperature controlled room in darkness. Foraging behavior was filmed by a Phantom V210 high-speed camera (Vision Research, New Jersey, USA) equipped with lenses to provide a 2.1×1.3 mm^2 field of view. Collimated infrared- (*R. salina*) or white light (*S. cerevisiae*) shining through the experimental cuvette towards the camera was the only source of illumination. The focal plane of the camera was in the middle of the experimental cuvette to

minimize wall effects. Whenever a feeding copepod was in focus, we recorded a 2.7 s video at 2000 frames per second (fps). Videos were analyzed using the software ImageJ version 1.53n (National Institutes of Health, USA).

Escape response experiment

The setup and the protocol for the escape experiment were taken up from Kiørboe *et al*. (1999) with some adaptations. A pipette sucked water from an aquarium, and the response of the copepods to the flow generated was quantified. Our experimental container was a 15×15×20 cm^3 aquarium with a mirror dividing it into two halfs by the diagonal. We used the same camera as above, but with an objective providing a 66×50 mm^2 field of view. The infrared light was placed perpendicular to the camera and shined towards a mirror that reflected the light into the camera. The pipette was placed on the bottom of the aquarium and was attached to a tube. The water flow was regulated by a faucet and a clamp. The gravity-driven flow was fixed at Q = 1.42 mL s^{-1} (Kiørboe *et al.*, 1999). The tip of the pipette was 3 mm wide and the pipette extended 1.5 cm into the water. We recorded 143 second videos at 50 fps. During recordings we did not return water into the tank as to not generate turbulence. We recorded escape behaviors only about 2 cm around the tip of the pipette. Some of the copepods jumped multiple times to escape from the flow field but we only considered the first jump. The experiment was repeated three times with different copepod size fractions. The videos were analyzed using ImageJ. Due to the mirror in our experimental tank, we had both the *(x, y)* and the *(z, y)* coordinate planes on the same image. Therefore, we could estimate the escape distances from the *x*-*y*-*z* coordinates. We also determined the coordinates of the copepod at the end of its first jump in order to estimate the average jump speed.

Data analysis

Using the escape distances measured in the pipette experiment we calculated the threshold maximum deformation rate that elicits an escape. The maximum deformation rate (△) is the

deformation rate along the length axis that yields the highest absolute value (Kiørboe and Visser, 1999):

$$\triangle = \frac{Q}{2\pi r^3}, \quad (1)$$

where Q the volume flow (mL s^{-2}) and r the escape distance (cm). The maximum velocity difference (signal strength, S) between the copepod and the ambient fluid due to fluid deformation is:

$$S = \triangle \times L, \quad (2)$$

where L is the radius of the copepod. A potential limit to this experiment is that in the boundary layer around the pipette, friction may be important and decelerate the flow, and thus we disregarded responses occuring within that distance from the side of the pipette. To calculate its thickness (δ), we used the same definition as (Kiørboe *et al.*, 1999) who considered it as the layer within which the flow deviates more than l % of the free-stream fluid velocity. In our experiment, the thickness of the boundary layer was estimated as $\delta \approx 0.25$ cm. The average speed of the first jump was calculated with the coordinates at the beginning and the end of the jump. To test for statistical differences between threshold deformation rates and jump speeds, bilateral and unilateral t-tests were used ($\alpha = 0.05$).

Results

Foraging experiment

Apocyclops royi exhibits ambush feeding when offered both the motile microalga *Rhodomonas salina* and the non-motile yeast cells *Saccharomyces cerevisiae*. The copepod sinks in the water column, occasionally adjusting its position with short jumps. When prey is close to the first antennae, *A. royi* jumps towards the prey and creates a vacuum using its feeding appendages to ingest the prey (Fig. 2–3; Videos S1–S2). We were unable to determine whether or not prey cells touch the setae of the antennules.

Jump distance, duration, and average speed are presented in Table 1. A jump is considered from the detection of the prey to the opening of the feeding appendages (Kiørboe *et al.*, 2009).

Escape response experiment

Copepods drawn with the flow towards the tip of the pipette respond to the flow at a characteristic distance to the pipette. These reponse distances increase with the length (L) of the copepods. Consequently, the threshold deformation rate required to elicit an escape varied inversely with body length (Table 2). The smallest size fraction reacted to a significantly higher deformation rate compared to the two larger fractions (T-test, $p < 0.01$). Jump speed generally increased with body length, and was significantly higher for the two larger size fractions compared to the smallest one (T-test, $p < 0.001$). We also estimated the threshold strength of the signal (S) due to fluid deformation (Eq. 3). We consider the copepod as a sphere, therefore its body length is twice its radius. Our estimation of the average threshold strength of the signal is $S = 0.015 \pm 0.003$ cm s $^{-1}$.

Discussion

Feeding and prey perception

Apocyclops royi displayed an ambush feeding behaviour when fed both *R. salina* and *S. cerevisae*. The prey attack mechanism in *A. royi* fed Baker's yeast is very similar to the one described for the ambush feeding copepods *Oithona davisae* and *Acartia tonsa* by (Kiørboe *et al.*, 2009). Jump distance, duration and speed are in the same range as for *O. davisae*, a obligatory ambush feeder about the same size as *A. royi*. For both preys, *A. royi* elicited an attack when the prey was close to or touching the first antennae (Video S1–S2). Thus, *A. royi* is capable of sensing smaller, non-motile particles (*S. cerevisae*) the same way it senses the motile *R. salina* cells. Remote prey detection can be elicited by a chemical or a hydromechanical signal, however chemical detection of individual prey is unlikely in ambush-feeding zooplankters (Svensen and Kiørboe, 2000; Kiørboe, 2011). While we cannot entirely exclude the possibility of chemical detection, we will provide

arguments to why we find it unlikely also for *A. royi* feeding on *S. cerevisae*. Several previous studies have addressed the issue of chemosensory prey detection in copepods, and has found it improbable in both ambush- and feeding-current feeding copepods feeding on both motile and non-motile prey (Légier-Visser *et al.*, 1986; Svensen and Kiørboe, 2000; Tiselius *et al.*, 2013; Gonçalves and Kiørboe, 2015). This is because the 'diffusion speed' of the substances leaking from the cell must be greater than the velocity of the cell travelling towards the predator, whether it does so by swimming or sinking. Chemical signals leaking from smaller cells disappear almost immediately due to molecular diffusion, and the estimated phycosphere, i.e., where the concentration of solutes around the cell is 50% greater than the background, for a similarly sized phytoplankton to *S. cerevisae* (~5 µm) is estimated to protrude just a few microns from the cell surface (Seymour *et al.*, 2017). This distance is similar to the sinking distance covered by phytoplankton cells this size (Kiørboe, 2008). Thus, the cell would encounter the mechanoreceptors on the setae extending from the first antennae before the first antennae itself. Even when entrained in a feeding current, where the phycosphere is stretched out towards the grazer, the estimated lower size limit for a cell to trigger chemical detection is in the range of 50–70 µm (Légier-Visser *et al.*, 1986; Tiselius *et al.*, 2013).

Rather, we suggest that *A. royi* perceives particles by the mechanoreceptory setae on the antennules, similar to other ambush feeding copepods. Assuming that *A. royi* has a similar sensitivity to fluid disturbances than the ambush feeding copepod *Oithona similis, R. salina* cells are clearly in the size- and speed range of remote detectability (Kiørboe and Visser, 1999). However, it is not clear how *S. cerevisae* cells are perceived. In fact, *O. similis* can only perceive the fluid signal from non-motile prey larger then 80 µm that create a sufficiently strong fluid disturbance by sinking (Kiørboe and Visser, 1999). The escape response experiment suggests that *A. royi* does not have a sensitivity to fluid disturbances different from that of other copepod species (Burdick *et al.*, 2007; Kiørboe *et al.*, 2009). Therefore, remote detection of *S. cerevisae* cells seems unlikely. Instead, *A. royi* most likely senses single *S. cerevisae* cells by randomly touching them with the first antenna. In a dense

178 culture, this presumably happens frequently enough to accomodate food intake needs. Once it has
179 located the prey, the copepod adopts the previously described ambush feeder behaviour. We were
180 from our videos unable to determine the fraction of attack-jumps that ended in a successful prey
181 ingestion, however, we believe the fraction to be high, given the unability of non-motile cells to
182 utilize escape behaviors commonly found in motile protists (Jakobsen, 2001). **The energetic cost of**
183 **jumping *vs*. the nutritional value of prey**

184 The energetic cost of swimming and jumping in copepods is considered relatively low (Titelman
185 and Kiørboe, 2003). However, in order to sustain growth on a diet of Baker's yeast, the energy
186 expenditure of the jump must be less than the gain from ingesting a cell. The energetic content of
187 the *S. cerevisae* used here is roughly 4770 J g^{-1} (De Danske Gærfabrikker,
188 https://oekologiskgaer.dk/). Using a density of 1.0952 g mL^{-1} (Reuss *et al.*, 1979) one cell (~4 µm
189 ESD) contains roughly 1.8×10^{-7} J. This is an order-of-magnitude lower than the estimate for
190 *Rhodomonas* sp. using 47 pg C $cell^{-1}$ (Berggreen *et al.*, 1988) and a carbon to calorie ratio of 11.4
191 cal g C^{-1} (Platt and Irwin, 1973).

192 The energy expenditure (J, kg m^2 s^{-2}) from a jump can be estimated from:

$$E = F \times d, \tag{3}$$

193 where F is the force (N, kg m s^{-2}) and d the jump distance (m). F is in turn estimated as:

$$F = 0.5\rho C_D U^2 A, \tag{4}$$

194 where ρ is the density of the water (~1 kg m^3), C_D is the drag coefficient, U is the jump speed (m
195 s^{-1}), and A is the cross-section area of the copepod (m^2). For simplicity, the copepod is assumed to
196 be spherical. The drag coefficient is estimated as (Massey, 1968):

$$C_D = \frac{24}{Re}\left(1 + \frac{3}{16}Re\right)^{\frac{1}{2}}, \tag{5}$$

197 where Re is the Reynolds number (~30 in our case). Using values from Table 1 gives $E = 5 \times 10^{-13}$
198 J if we assume an efficiency of 1%. If we assume that the attack-jump velocity scales to the same

power 0.6 as escape jumps (Kiørboe *et al.*, 2009), a large adult female (~0.95 mm length) still only spends 1.9×10^{-10} J on an attack jump, three orders-of-magnitude less than the energetic content of a yeast cell. Thus, the lower nutritional value of yeast compared to that of phytoplankton should not be an issue for *A. royi*.

Conclusions

Our observations experiments demonstrates that *Apocyclops royi* use an ambush feeding strategy even when microphageously fed small, non-motile Baker's yeast cells (*Saccharomyces cerevisae*). We also suggest that its foraging and escape behaviour are both elicited by fluid disturbances or direct contact with the prey, thus, sensed by mechanoreceptors. Finally, we can confirm that *A. royi* is not more sensitive to the presence of a predator than *Acartia tonsa,* a copepod suggested used as live feed in aquaculture. Due to its high adaptability, self enriching fatty acid biosynthesis and the fact that copepods are generally considered as a better live feed for fish larvae than the widely used rotifers and *Artemia* sp., *A. royi* is indeed an interesting candidate for intensive aquaculture uses (Abate *et al.*, 2016; Jepsen *et al.*, 2021). Evidently, the escape behaviour of *A. royi* would not be a barrier for its aquaculture uses.

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283 Table legends

284 Table 1. Overview of prey attacks in *Apocyclops royi* fed Baker's yeast (*S. cerevisae*). Copepod
285 length refers to the prosome. *n* = number of observations.

286 Table 2. Escape response of *Apocyclops royi*. Reaction distance (mean ± SD) to mouth of the
287 pipette for different developmental stages in a siphon flow (*R*), calculated threshold deformation
288 rate (mean and 95 % confidence interval) at the point of escape, and speed of the first escape jump
289 (mean ± SD). *n* = number of observations.

290

291

292 Figure legends

293 Figure 1. Size spectra of Baker's yeast (*Saccharomyces cerevisae*) and *Rhodomonas salina* as
294 measured by a Beckman Coulter Multisizer 4 (Brea, California, USA).

295 Figure 2. Prey capture in *A*pocyclops *royi* fed *Rhodomonas salina*. The position of the
296 phytoplankton cell is indicated by the red circle. Duration of the jump is indicated.

297 Figure 3. Prey capture in *A*pocyclops *royi* fed *Saccharomyces cerevisae*. The position of the yeast
298 cell is indicated by the red circle. Duration of the jump is indicated.

299

300 Table 1

	Mean ± SD	Range	*n*
Copepod length (mm)	0.38 ± 0.06	(0.35 – 0.40)	18
Jump distance (mm)	0.42 ± 0.20	(0.30 – 0.50)	10
Jump duration (ms)	26.3 ± 5.91	(22.20 – 29.70)	10
Average jump speed (mm s^{-1})	16.0 ± 5.23	(12.10 – 18.90)	10

301

302

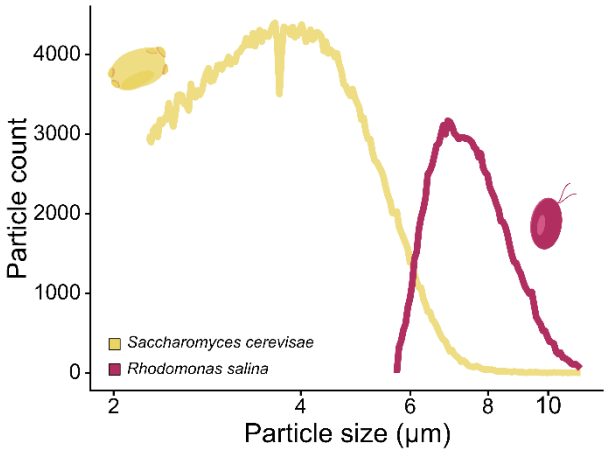
303 Table 2

Average length (µm)	Stage	*R* (cm)	Deformation rate (Δ) (s^{-1})	Jump speed (mm s^{-1})	*n*
117 ± 9	NI - NII	0.48 ± 0.08	2.16 (1.86 – 2.50)	12.6 ± 7.9	38
328 ± 78	CI - CIII	0.67 ± 0.21	0.88 (0.85 – 0.91)	28.0 ± 27.0	52
483 ± 25	CIII - CIV	0.69 ± 0.19	0.79 (0.61 – 1.01)	22.3 ± 9.9	45

304

305

306 Figure 1



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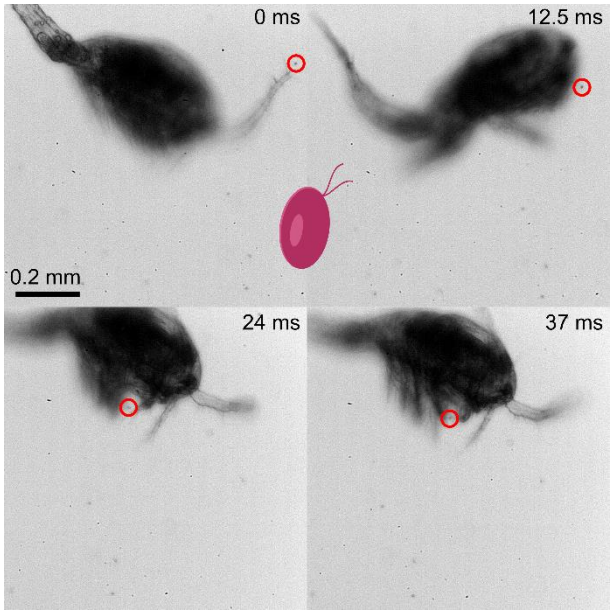
308

309 Figure 2



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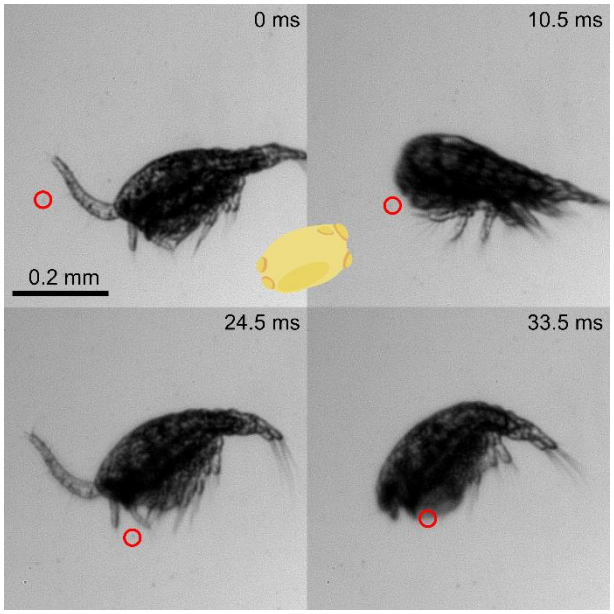
312 Figure 3



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