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NOTE

High mucous-mesh production by the ascidian *Herdmania momus*

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ABSTRACT: Suspension feeders, including ascidians (Phylum Chordata, Class Ascidiacea), experience a dilute prey field composed of extremely small particles. The filtration apparatus of ascidians is based on a mucous-mesh that is continuously secreted and ingested. The rate and metabolic cost of this mesh secretion has not been quantified to date. We used video boroscopy to quantify the mucous-mesh production rate of the solitary ascidian *Herdmania momus* under different food and temperature treatments. *H. momus* individuals with an average (±95% CI) biomass of 30.7 ± 1.1 mg and a branchial sac area of 10.3 ± 1.2 cm^2 produced an average of 276 ± 33.5 cm^2 of mucous-mesh h^{-1}, corresponding to a median turnover rate of 625 ± 82 mesh d^{-1}. Since the mean mesh mass was 2.44 ± 0.58 mg, this production rate corresponds to roughly 50 ± 8 times the individual's biomass per day. Food concentration had no detectable effect on mesh production rate, whereas a temperature difference of ~9°C (20 vs. 29°C) moderately increased mesh production by 30–50%. The current study reveals that the feeding process of *H. momus* involves a high expenditure on mucous-mesh synthesis that, combined with low food availability, may limit its growth in oligotrophic waters and under changing climate regimes.

KEY WORDS: Filter-feeding invertebrates · Invasive species · Metabolism · Oligotrophic conditions · Suspension feeding · Tunicate biology

1. INTRODUCTION

Benthic suspension feeders remove suspended food particles from the water column. In oligotrophic waters such as the East Mediterranean, suspension feeders face a dual challenge: the particulate organic content is 1–2 orders of magnitude lower (<0.3 ppm) than in temperate waters, and the dominant phytoplanktonic cells are much smaller (<2 µm) (Jørgensen 1975, Jacobi et al. 2018). Suspension feeders residing in these waters may possess specialized filtration organs and associated mechanisms (Riisgård & Larsen 1995, reviewed by Gili & Coma 1998) and/or process much more water to gain the same amount of food as ascidians residing in more productive waters. To date, the energetic cost of pumping water is considered negligible (normally <1% of the metabolic rate; Riisgård 1988). Therefore, it is unclear if, and to what extent, food availability limits benthic suspension feeders' metabolism and growth, especially in regions with low productivity.

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Ascidians are sessile invertebrates that inhabit shallow waters of all oceans (Monniot et al. 1991). Like many suspension feeders, ascidians use mucus as a central part of their feeding mechanism (Jørgensen et al. 1984). The process of ascidian feeding has been thoroughly described by Millar (1971) and Conley et al. (2018). Briefly, cilia draw water into the inhalant siphon and pharyngeal cavity, where it is forced along and through the branchial basket. The mucous-mesh suspended across the branchial basket efficiently captures suspended particles. The mucous-mesh and retained food particles are transported across the basket, rolled into a strand, and ultimately digested. Filtered water then flows through small slits in the basket (stigmata), into the atrial cavity, and then leaves as a jet through the exhalant siphon. Through this process, ascidians transfer substantial amounts of material from the pelagic zone to the benthic habitat (Petersen & Riisgård 1992, Riisgård et al. 1998), and thus play an important role in benthic–pelagic linkage (Marcus 1998).

In this study, we sought to advance our understanding of ascidian filtration mechanisms in order to resolve the question of how benthic suspension feeders survive in the highly dilute prey field and the wide range of temperatures (17–31°C; Coll et al. 2010) of the Eastern Mediterranean. Previous observations of ascidian mucous-mesh speeds suggest that this feeding strategy may be metabolically demanding (Flood 1982, Armsworthy et al. 2001), particularly if mesh secretion and transport occurs continuously (MacGinitie 1939, Armsworthy et al. 2001). However, the rate and metabolic cost of ascidian mucus secretion have not been previously analyzed or quantified.

We used video boroscopy to quantify the mesh production rate of the solitary ascidian *Herdmania momus* (Order: Stolidobranchia). This non-indigenous species originated from the Indo-west Pacific, where water temperatures range from 20–27°C (Shenkar & Loya 2008). Ascidians were examined under 4 combinations of temperature and food concentrations relevant to the Eastern Mediterranean to simulate food-deprived and food-replete conditions. We sought to estimate the rate of mesh production and to test if, and to what extent, ascidians control and adjust the mesh production rate in response to varying environmental conditions. Our working hypothesis was based on Armsworthy et al. (2001), who studied the ascidian *Halocynthia pyriformis*. As expected of poikilotherms (Bullock 1955), we hypothesized that the mesh production rate would increase when exposed to a higher temperature. We further anticipated an increase in mesh production under higher food concentrations (to reduce mesh load and clogging) and a reduction in mesh production under low food concentrations, which should minimize the investment in new mesh production.

2. METHODS IN BRIEF

An extended version of this section is provided in Text S1 in the Supplement at www.int-res.com/articles/suppl/m663p223_supp/.

Ascidians were collected by SCUBA diving from artificial structures in the central Mediterranean coast of Israel (Eastern Mediterranean). Following a 1 wk acclimation, each animal was transferred to a 1 l beaker equipped with temperature-controlled water supply at either 20 ± 1 or 29 ± 1°C (hereafter 'cold' and 'warm' treatments, respectively; see Table 1). Ascidians were fed a slurry of previously frozen *Nannochloropsis* sp. cells (~2 µm diameter) at either 10^2 or 5×10^4 cells ml^{-1} (hereafter 'low' and 'high' treatments, respectively). Animals were allowed to acclimate for at least 48 h to the new temperature before the boroscopic measurements commenced. These measurements lasted from 1–4 d treatment^{-1}.

Our experiment was originally planned as a repeated measures design in which each individual would have been examined under each of the treatments. Due to logistic constraints, not all animals were exposed to all treatments and therefore, we had to revert to a partial factorial design with 12 animals that were exposed, in a random order, to 1 or 2 temperatures (cold and/or warm) and 1 or 2 food concentrations (low and/or high). For further details, see Text S3 in the Supplement at www.int-res.com/articles/suppl/m663p223_supp/.

Boroscope observations were conducted as described in Conley et al. (2018) (2160 × 3840 pixel resolution, 29.98 frames s^{-1}; Fig. 1b,c). Briefly, the optical insertion tube was gently inserted into the inhalant siphon of the animal using a micromanipulator. While the animal was actively pumping, 10 µm spherical fluorescent microspheres (Polysciences Fluoresbrite® carboxylate microspheres, hereafter referred to as 'beads') were gently injected approximately 1 cm above the inhalant siphon using a plastic Pasteur pipette. The water current produced by the animal drew the beads into the pharyngeal basket. Video recording started whenever the beads were observed to adhere to and be transported by the mucous-mesh.

At the end of the experiment, animals were dissected, and the branchial basket area and ash free

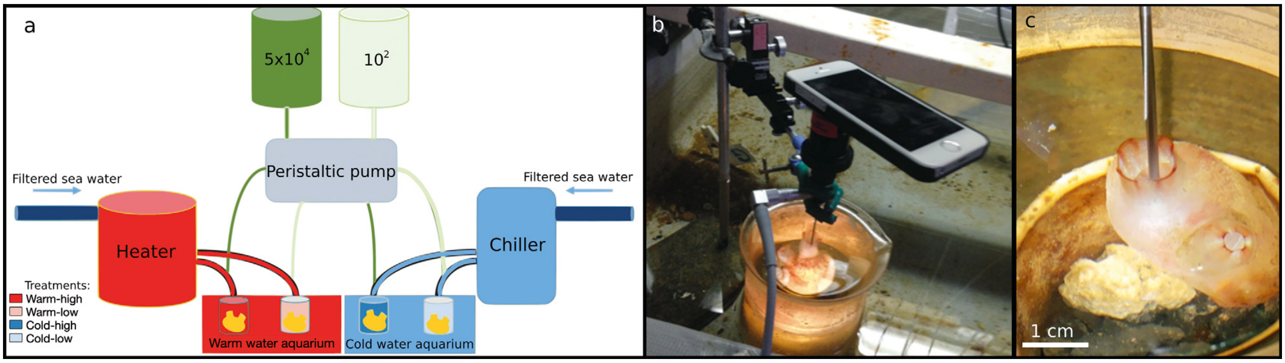

Fig. 1. Experiment setup. (a) Temperature control and food dosing system. (b,c) The borescope inserted into the inhalant siphon of *Herdmania momus* to visualize the mesh movement inside the branchial basket

dry weight (AFDW) of the body and tunic of each animal were measured using standard methods.

Mucous-mesh speed measurements were conducted by tracking the speed of particles trapped on the mesh. Only beads that were in focus were tracked and analyzed (Video clip S1 at www.int-res.com/articles/suppl/m663p223_supp/). Bead size was used as an internal scale for the analysis. Particle tracking and distance measurements were made with 'DLTvd', a MATLAB tool developed by the Hedrick lab (http://biomech.web.unc.edu/dltdv/, Fig. S1 in the Supplement; see Ben Tal 2019 for details). We tracked 30–150 particles treatment^{-1} and calculated the mesh speed as the distance travelled by a particle between each frame.

For the determination of the mucous-mesh dry weight, we anesthetized ascidians with a menthol solution and gently collected the mesh with a Teflon toothpick. Each mucus sample was then placed on a pre-weighted 13 mm glass fiber filter and its biomass determined using standard methods.

The speed of the mucous-mesh (cm h^{-1}) was determined by averaging the distance that 30 trapped beads travelled between subsequent frames (29.9 frames s^{-1}). Mesh production (cm^2 h^{-1}) was calculated for each individual and at each treatment as the product of the mesh speed and the endostyle length, assuming that the mucous-mesh completely covered the branchial basket and was continuously secreted at the same rate along the entire endostyle length. Since, under normal conditions, the mucous-mesh covers the branchial basket (but see Armsworthy et al. 2001), the basket area should equal that of the mesh. To calculate the mesh turnover rate (mesh h^{-1}), we divided the mesh production rate by the branchial basket area (cm^2). To determine the body mass equivalent for mucous-mesh replacements per hour, we divided the product of the average mesh biomass (mg), and the mesh turnover rate (mesh h^{-1}) by the average biomass (i.e. AFDW) of the zooids (mg). Throughout the text, data are reported as mean ± 95% confidence intervals for the mean.

3. RESULTS

Under the experimental conditions (Table 1), the mucous-mesh speed of *Herdmania momus* reached a maximum of 252 µm s^{-1} with an average speed of 146 ± 16.2 µm s^{-1} (Fig. 2), corresponding to 53 ± 6 cm h^{-1}. Mean mesh production was significantly elevated (30–50%) in the 29°C (warm) treatments in comparison to the 20°C (low) treatment (2-way ANOVA, $F_{1,30}$ = 9.6, $p < 0.001$). In contrast, food concentration (low, 10^2 cells ml^{-1} vs. high, 5×10^4 cells ml^{-1}) had no effect on the rate of the mucous-mesh production (2-way ANOVA, $F_{1,30}$ = 0.26, $p = 0.6$), and there was no significant interaction between the food concentration and temperature treatments ($p = 0.57$; Table 2). The statistical power of our experimental design to detect medium size effects, >20%, was sufficiently high (>90%) to rule out these effects. Post hoc pairwise analysis indicated that the only significant difference was between the cold–high-food, and warm–low-food treatments (Student-Newman-Keuls test, $p = 0.04$) but this effect was minor (Q_{10} = 0.62; Ben Tal 2019).

The experimental animals used for the mesh speed experiments were relatively large individuals with an average branchial basket area of 10.3 ± 1.2 cm^2 and an average endostyle length of 5.2 ± 0.3 cm. Zooid biomass (30.7 ± 1.1 mg AFDW) was linearly correlated to the square root of the branchial basket area (R^2 = 0.94; Fig. S2c in the Supplement).

H. momus individuals produced between 114 and 474 cm^2 of mucus per hour (average: 275 ± 33 cm^2 h^{-1}).

Table 1. Experimental design to test the effect of temperature and food concentration on mesh production rate of the solidary ascidian *Herdmania momus*. Table values are the number of animals subjected to each treatment

Water temperature (°C)	— Food levels (cells ml^{-1}) — Low (10^2)	High (5 × 10^4)
Cold: 20	4	6
Warm: 29	11	5

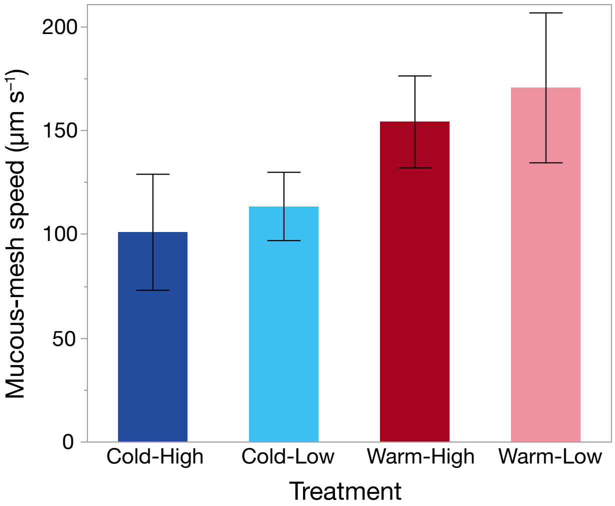

Fig. 2. Mean (±95% CI) mucous-mesh speeds of the ascidian *Herdmania momus* under different food conditions (high or low concentration) and temperature conditions (high or low temperature). See Table 1 for experimental conditions and number of replicates in each treatment

Under the cold treatments, mesh production was 35% lower than production in the warm treatments (224 ± 53 and 303 ± 39 cm^2 h^{-1}, respectively). This mucus production for cold and warm treatments corresponds to 6720 ± 722 cm^2 d^{-1} with maximal values of >1 m^2 mesh d^{-1}, suggesting a mean turnover rate of 625 ± 82 mesh d^{-1}. In cold treatments, mesh turnover rate ranged between 488 and 501 d^{-1}, whereas in warm treatments mesh turnover rates were between 646 and 715 d^{-1}.

Based on the analysis of 20 meshes harvested from 20 individual ascidians, the mean dry weight of a single mucous-mesh was 2.44 ± 0.58 mg. This suggests that an average ascidian zooid with an AFDW of 30.7 ± 1.1 mg and mesh turnover rate of 625 ± 82 mesh d^{-1} produced 1.5 ± 0.2 g mucous-mesh d^{-1}, potentially recycling 50 ± 8 times its body weight each day. This figure should be regarded as a lower bound estimate, since it is unlikely that 100% of the mesh was obtained from each animal through our sampling techniques.

Table 2. Mucous-mesh production parameters for the ascidian *Herdmania momus* (mean ± 95% CI). See Table 1 for experimental conditions and number of animals in each treatment

Treatment	Average mucous-mesh speed (µm s^{-1})	Average mucous-mesh production rate (cm^2 h^{-1})
Warm–Low	171 ± 34	303 ± 38
Warm–High	154 ± 21	301 ± 92
Cold–Low	113 ± 15	247 ± 106
Cold–High	101 ± 25	213 ± 69
Total	**146 ± 16**	**275 ± 33**

4. DISCUSSION

Ascidians feed mostly on phytoplankton, even in oligotrophic waters where phytoplankton account for less than 50% of the nano- and pico-planktonic biomass (Dadon-Pilosof et al. 2017, Jacobi et al. 2018). This necessitates that ascidians process large volumes of water in order to sustain maintenance, growth, and reproduction. Ascidian water processing is considered energetically negligible (Riisgård 1988, Petersen 2007), but our results suggest that ascidian mucous-mesh production may require high investment, particularly under food-replete conditions. Counter to our expectations that mucus production would increase in response to increased food availability, we observed no such response with experimental food exposure of up to 5 × 10^4 algae cells ml^{-1}. This suggests that *Herdmania momus* is capable of processing food concentrations much higher than the maximum levels this species typically experiences in the East Mediterranean (Dadon-Pilosof et al. 2017, Jacobi et al. 2018).

Consistent with expectations for poikilotherms (Bullock 1955), *H. momus* significantly increased its rate of mucous-mesh production (30–50%) when exposed to a higher temperature. We therefore suggest that the low ascidian abundance in the east Mediterranean (2–4 m^2; R. Diga & G. Yahel unpubl. data) may be due to low food availability that necessitates the processing of a higher volume of water and hence the production of more mesh that later needs to be digested and recycled. Reported densities of ascidians in oligotrophic environments such as the Red Sea and Eastern Mediterranean, where phytoplankton biomass averages <7 µg C l^{-1} (Jacobi et al. 2018), range from 2–5 ind. m^{-2} (Genin et al. 2009, Gewing et al. 2019). In contrast, densities higher than 75 ind. m^{-2} have been reported for eutrophic waters such as Shark Bay (Western Australia; Wells et al. 1985) and Odense Fjord (Denmark; Riisgård et al. 2004), where phytoplankton biomass averages ~200 µg C l^{-1} (Riisgård et al. 2016). Ascidians in these different prey regimes must process strikingly different volumes of water. For example, at Odense Fjord, *Ciona intestinalis* gains ~20 µmol of carbon from each 1 l it pumps, assuming 100 % capture efficiency of phytoplankton (Jørgensen 1955, Petersen et al. 1995). In contrast, *H. momus* residing in the oligotrophic waters of the eastern Mediterranean should process ~30 times as much water to gain an equivalent amount of phytoplanktonic carbon (Jacobi et al. 2018).

Our estimates of mesh secretion by *H. momus* are consistent with previously published rates for other solitary and colonial ascidian species. The mean speed of mucus secretion by *H. momus* was 146 ± 16.2 µm s^{-1}, similar to rates reported for the transparent colonial ascidian *Clavelina lepadiformis* (Aplousobranchiata; Flood 1982). Flood (1982) showed that mucous-mesh secretion speed may range from 20–220 µm s^{-1}, with larger individuals having faster secretion rates. A similar order of magnitude was also reported by Armsworthy et al. (2001), who measured a mean mesh production speed of 74 µm s^{-1} for the temperate ascidian *Halocynthia pyriformis*.

H. momus belongs to the order Stolidobranchia, which is characterized by a folded branchial basket that enlarges the basket surface area (Monniot et al. 1991). The measurements of the branchial basket area we report here were derived from measurements of dissected animals (see Text S2 in the Supplement) and are not corrected to incorporate the additional area of all the folds in the basket visualized by an MRI scan (Fig. S2b); hence, these measurements should be regarded as a minimum estimate. This putative underestimation of the mesh area has a direct bearing on our estimate of the mucous-mesh turnover rate, which was calculated as the ratio of mesh production rate (cm^2 h^{-1}) to mesh area (Table 2). If the projected branchial basket area is underestimated due to the exclusion of some of the folds, then the mesh turnover rate will be overestimated. Conversely, our estimates of mesh mass are likely to be an underestimation, as our mesh harvesting method from live animals is not guaranteed to extract the entire mesh from each of the experimental animals.

The mucus production rates reported here, amounting to >50 times (5000 %) the body mass per day, is high. For comparison, the amount of mucus discharge by land snails may reach up to 3 % of their body mass per day, slugs discharge up to 20 % of their body mass per day as mucus (Bába 2000), and appendicularians produce 1–3 times (120–300 %) their biomass per day as mucus (Hopcroft & Roff 1998, Sato et al. 2001). These rates are considerably lower than that of ascidians, but unlike ascidians that ingest the produced mucous and probably recycle most of it (Petersen 2007), slugs and snails leave their mucus trails behind, whereas appendicularians discard the exterior mucus filter (Hopcroft & Roff 1998, Bába 2000, Sato et al. 2001). Although the energetic cost of mucous-mesh production could not be directly quantified in the current study, our findings suggest that particle capture involves a high investment in the mucous-mesh production, which may impose a major toll on the ascidian feeding process. We therefore suggest that the production of the mucous-mesh and its subsequent processing, rather than the water pumping, may limit the ascidian filtration rate. Future studies on ascidian physiology should couple the energetic budget (e.g. by measuring respiration rate) with mesh production rate to further advance our understanding of the limitations of ascidian growth and metabolic constraints.

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