Food pellets for diet experiments

1. Purpose

The purpose of this manual is the standardized creation of agarose-based food pellets that can be fed to isopods. The substrate is first boiled/autoclaved, then pipetted and, after drying/solidifying, stored in the fridge or freezer. When added to water, the pellet will become soft and palatable to isopods. Depending on the size of an isopod, a single pellet will last between 5 and 14 days before consumed, but the shorter the better, as the pellet may be colonized by unwanted biofilm.

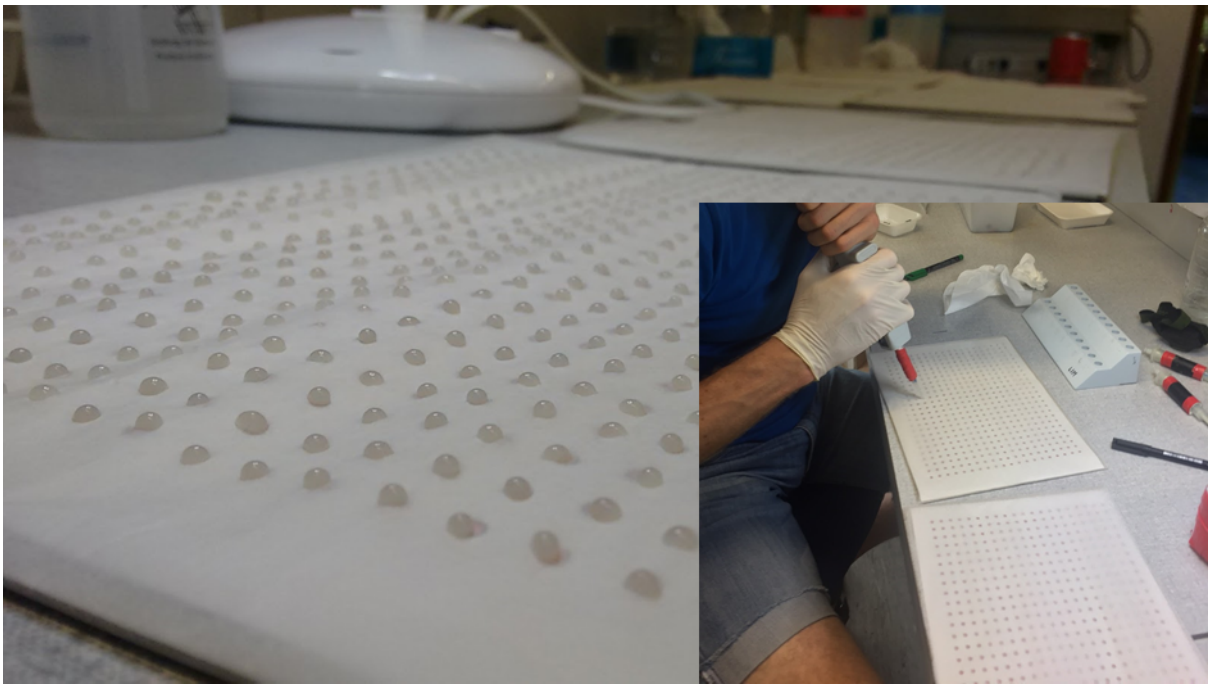

Fig. 1 - The pellet substrate is boiled or autoclaved, and then, while still warm and liquid, pipetted onto a template (can be downloaded - see link below) that is coated with baking paper. After drying, the pellets can be stored in the fridge or freezer (ideally kept dry and in darkness).

2. Requirements

- A stove or mini-autoclave, ideally, something that can be temperature regulated
- A repeater pipette and a 10-20 ml reservoir tip
- Baking paper and cardboard (roughly A4 sized), and a pipetting-template (download from asellus.org/wiki)
- Agarose, forms the basis of the pellet (from the baking section in the supermarket will do, but high-grade chemical supply agarose works too)
- Corn or potato starch and dried yeast, to make the pellet nutritious (this is to balance the stoichiometry of C, N and P - see Lürig and Matthews (2021) for details. Again, either consumer grade or high-grade substrates will work)
- *Any other agent you wish to add to the pellet*

3. Procedure

Composition - general remarks

Mix the agar and differing combinations of yeast (commercial grade yeast), starch (commercial grade cornstarch), and any other substrate you want to add (for the ratios see below). Depending on the substrate it might make sense to either mix it in directly with agarose, yeast and starch, or with water instead. The ratio of yeast and starch will determine diet stoichiometry, and thus its nutritional "quality" for the isopods. For instance, a low C:P/C:N ratio or one that is as close to the composition of the organism could be considered "high quality" (Elser et al., 2000). Such high- and low-quality diet ratios or described and Lürig and Matthews (2021), where yeast and starch have been mixed in 1:4 and 4:1 ratios, to create contrasting diets (Fig. 2).

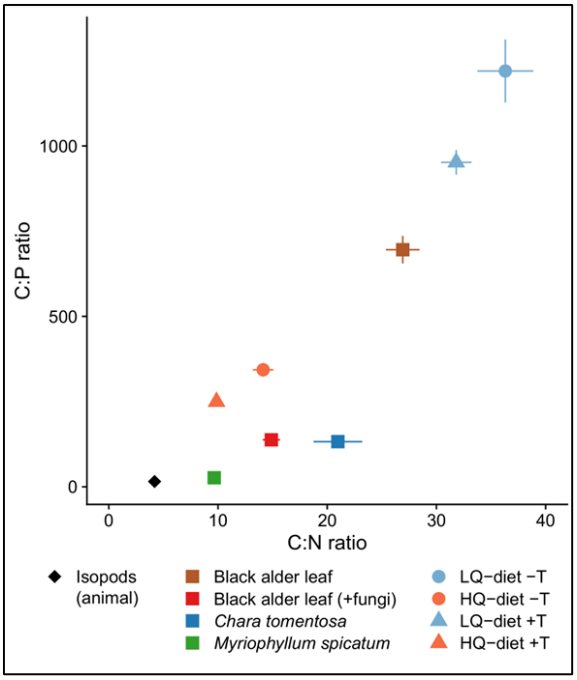

Fig. 2 - Elemental composition of various natural food items that isopods encounter in nature, as well as artificial diets used in Lürig and Matthews (2021): LQ, low quality/high elemental ratio; HQ, high quality/low elemental ratio;−T, without tryptophan supplement; +T, with tryptophan supplement). This panel also shows the elemental composition of isopods collected from Lake Lucerne (black diamond). Elemental ratios are scaled by the molar mass of the respective elements (reproduced from Lürig and Matthews (2021)).

Composition - an example case

Exemplary mixtures could be composed as follows: in Lürig et. al (2019) and Lürig and Matthews (2021), a "low quality" diet was composed of 2 g of yeast and 8 g of starch, whereas the high quality diet contained 8 g of yeast and 2 g of starch. Together with 2g of agarose, the ingredients of the diets were added to 50 mL ultrapure water and then autoclaved for 10 min at 121 °C. Dietary pellets were made by pipetting 50 µL of the mixture onto wax paper and letting it cool at 4 °C for 2 days. It was then stored in -20 °C until used.

4. Comments

- It is important to create a sterile work environment! Wear gloves, and keep surfaces clean (e.g. wipe down the baking paper before pipetting, and dry the pellets in a clean environment without dust. This will reduce the risk of contaminations when rehydrating the pellets in water.
- It is important to inactivate the yeast (or any additional ingredients with biological agents) before using it, otherwise it will metabolize the starch or agarose and this may alter the chemical composition. Boiling water, an ultrasonic bath, or both may do the job.
- Once the agarose is boiling, work fast: the “dough” will harden quickly once it’s cooling down. It is a good idea to keep the stove on medium heat to keep everything liquid (and, given the low volumes, mind evaporation). It might help to work with multi pipettes that have a large reservoir, as this will stay warm longer. Insulating those reservoir syringes also might help.
- Mixing the ingredients with more water (e.g., for the example case, 100 ml instead of 50) will make the resulting pellets less “dense”, i.e., isopods will have to consume more to ingest the same amount of calories or nutrients. This may require to adjust the amount of agarose that is used so that the pellet can solidify.

5. References

Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S. S., McCauley, E., Schulz, K. L., Siemann, E. H., & Sterner, R. W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, *408*(6812), 578–580. https://doi.org/10.1038/35046058

Lürig, M. D., Best, R. J., Svitok, M., Jokela, J., & Matthews, B. (2019). The role of plasticity in the evolution of cryptic pigmentation in a freshwater isopod. *The Journal of Animal Ecology*, *88*(4), 612–623. https://doi.org/10.1111/1365-2656.12950

Lürig, M. D., & Matthews, B. (2021). Dietary-based developmental plasticity affects juvenile survival in an aquatic detritivore. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1945), 20203136. https://doi.org/10.1098/rspb.2020.3136