NAC SETAC Workshop
Microplastics & Marine Shellfish
Evan Ward, Sandy Shumway
May 11, 2021

Part A. – Husbandry of experimental animals
Keeping them happy and in top shape

1. What do bivalves eat?
2. Maintaining bivalves in the laboratory
3. How much food to provide

Part B. – Measuring Suspension Feeding
The good, the bad and the ugly

In this part of the workshop we will define the most common feeding processes that are often used in studies examining impacts of microplastics on bivalves, describe the two most common methods to measure feeding rates, and perform mock experiments that demonstrated some of the pitfalls that can occur with these methods. We will end with some thoughts on best practices for conducting feeding rate assays.

A. Setting the Stage

1. Definitions of feeding processes (also, see Ward & Shumway 2004, Cranford et al. 2011, Rosa et al. 2018)
    a. Pumping rate (PR): volume of water pumped from the animal per unit time (vol/time; difficult to measure)
    b. Clearance rate (CR): volume of water cleared of particles per unit time (vol/time; CR=PR only if all quantified particles are captured at 100% efficiency)
    c. Filtration rate (FR): number or mass of particles removed from suspension per unit time (# or mass/time)
    d. Pseudofeces production rate (PsR): number or mass of particles rejected per unit time (# or mass/time). See note on pseudofeces.
    e. Ingestion rate (IR): number or mass of particles removed from suspension minus number or mass of particles rejected per unit time (FR-PsR=IR; # or mass/time)

2. A note on pseudofeces (particles captured but rejected prior to ingestion)
    a. All bivalves produce pseudofeces as a byproduct of particle selection
    b. The anatomical location of pseudofeces rejection is species specific
    c. So called 'pseudofeces threshold' refers to a low concentration of suspended particles that elicits so little pseudofeces that it can no longer be seen by the unaided eye (video).
    d. With microplastics, which occur at a low concentration, even small amounts of pseudofeces can skew the estimates of ingestion.
    e. Therefore, it is critical to use microscope when collecting pseudofeces in MP studies (see Ward et al. 2019).

3. Two main methods for measuring clearance rates

a. Static method (see Coughlan 1969)
b. Flow through method (see Hildreth & Crisp 1976, Riisgård 1977)

B. Tackling the Methods

1. Static method to measure CR
   a. Equation of Coughlan (1969) should be used to determine clearance rate. Appropriate controls for settling and the division of phytoplankton need to be included in the experimental design. Settling and division rates need to be accounted for in the final estimates of rates (see equation).

$$CR = \frac{M}{N \cdot T}\left[\text{Log}_e\left(\frac{C_0}{C_t}\right) - \text{Log}_e\left(\frac{C_0\,\text{blank}}{C_t\,\text{blank}}\right)\right]$$

   Where: M = volume of water at beginning of each time ‘t’, n = number of animals in beaker, T = time elapsed, C_o = concentration of particles at start of t, C_t = concentration of particles after time t.

   b. Animal size vs chamber volume (rule of thumb: 3-5 L/hr/g dry mass)

   c. Appropriate sampling intervals – The intervals between sampling times need to be considered in reference to ‘b.’ above.
      i. The relationship between the decrease in particle concentration and time is not linear over its entire range. Rather, the relationship approximates an exponential decay function with the concentration approaching a value of zero (Williams 1982).
      ii. To measure clearance rate, only the linear portion of that relationship (curve) should be used, which gives a value that is comparable to what one would obtain in a volume of water with a constant number of particles (e.g., natural environment).
      iii. Considering that clearance rates of many bivalves (above), the depletion of particles that are retained with ca. 100% efficiency can occur over a period of minutes depending on experimental conditions and size of the animal under study. Therefore, water samples for clearance rate calculations need to be taken within this timeframe to capture the linear portion of the relationship between decrease in particle concentration and time.
      [**Mock experiment 1**]

   d. Importance of capture efficiency - In addition, sampling on a timescale that exceeds the turnover time of water in the chamber can result in calculation of erroneously high capture efficiencies for inefficiently retained particles (e.g., < 2 µm).
      i. This outcome is a result of particles and water passing through the gills multiple times, inflating CE with each pass.
      [**Mock experiment 2**]

   e. Importance of blanks (no animal) – Chambers with no animals should be used to control for settling and the division of phytoplankton.

i. Plastic particles can be hydrophobic and form agglomerations with other particles (e.g., microalgal cells) in suspension. These agglomerates can sink or stick to the sides of experimental containers. Such loss can inflate CR calculations.

ii. Conversely, microalgae can divide thus increasing particle concentration in the beakers and produce underestimates of CR.

[**Mock experiment 3**]

2. Flow-through method to measure CR (much more difficult)
   a. For experimental designs using a flow-through method, the equation of Hildreth and Crisp (1976) or Riisgård (1977) should be used. For example:

$$CR = F\left(\frac{[C_{o1} - C_{o2}]}{C_{o2}}\right)$$

Where: F = flow rate of water through the chamber, C_{o1} = particle concentration exiting each individual animal chamber (assumes 1 animal in each), and C_{o2} = particle concentration exiting the control chamber (no animal).

   b. Importantly, researches should fully investigate the many papers that describe specific conditions for such a design to avoid artifacts, including flow rate, chamber geometry, and sampling protocols (e.g., Filgueira et al. 2006, Larsen & Riisgård 2011).

3. Other common pitfalls
   a. Problem with measuring only depletion of particles in bioassays. Doing so can lead to erroneous conclusions. For example, at a constant CR, increasing particle concentration will lead to increased particle removal, which has no behavioral or physiological basis.

   b. Resuspension of pseudofeces and feces. Agitation that is too vigorous can cause release of particles from these biodeposits.

C. Final Thoughts

D. References

Coughlan, J. 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2:356–358.

Cranford, P.J., J.E. Ward and S. Shumway, 2011. Bivalve filter feeding: variability and limits of the aquaculture biofilter. In: S.E. Shumway (ed.), Shellfish Aquaculture and the Environment, John Wiley & Sons Publ., 81-124.

Filgueira, R., U. Labarta and M.J. Fernandez-Reiriz, 2006. Flow-through chamber method for clearance rate measurements in bivalves: design and validation of individual chambers and mesocosm. Limnol. Oceanogr. Methods 4, 284–292.

Hildreth, D. J. & D. J. Crisp, 1976. A corrected formula for calculation of filtration rate of bivalve molluscs in an experimental flowing system. J. Mar. Biol. Assoc. U.K. 56:111–120.

Larsen, P.S. and H.U. Riisgård, 2011. Validation of the flow-through chamber (FTC) and steady-state (SS) methods for clearance rate measurements in bivalves. Biology Open doi: 10.1242/bio.2011011, 1–6.

Riisgård, H.U. 1977. On measurements of the filtration rates of suspension feeding bivalves in a flow system. Ophelia 16: 167 – 173.

Rosa, M., J.E. Ward & S.E. Shumway, 2018. Selective capture and ingestion of particles by suspension-feeding bivalve molluscs: A review. J. Shellfish Res. 37: 727-746.

Ward, J.E. and S.E. Shumway, 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300:83–130.

Williams, L.G., 1982. Mathematical analysis of the effects of particle retention efficiency on determination of filtration rate. Mar. Biol. 66, 171–177.

Exercises

Mock Experiment 1 – CR and time

1. Using the data obtained by Dr. Gallant and Dr. Goofus for particles 5-10 µm, calculate the clearance rates of the bivalves used in the experiment. Use the equation by Coughlan (below) and assume that settling and division of particles is zero. The volume of water (mixed with algae and microplasics) in each container was initially 1000 ml. Each sample removes 20 ml from the container.

$$CR = \frac{M}{N \cdot T}\left[\mathrm{Log_e}\left(\frac{C_0}{C_t}\right) - \mathrm{Log_e}\left(\frac{C_0\,\mathrm{blank}}{C_t\,\mathrm{blank}}\right)\right]$$

where: M = vol of water at beginning of each time 't', n = number of animals in beaker, T = time elapsed, Co = concentration of particles at start of t, Ct = concentration of particles after time t.

2. Compare and contrast the results obtained from Dr. Gallant and Dr. Goofus. Are they different? If so, why?

Mock Experiment 2 – CR of different sized particles (capture efficiency)

1. Using the data obtained by Dr. Gallant and Dr. Goofus, calculate the clearance rates of the bivalves for the two size classes of particles (1-3 µm, 5-10 µm). Again, use the equation by Coughlan (above) and assume that settling and division of particles is zero. The volume of water (mixed with algae and microplasics) in each container was initially 1000 ml. Each sample removes 20 ml from the container.

2. Compare and contrast the results obtained from Dr. Gallant and Dr. Goofus. Are they different? What erroneous conclusion might Dr. Goofus draw?

Mock Experiment 3 – CR obtained with and without blanks

1. Using the data obtained by Dr. Gallant and Dr. Goofus for particles 5-10 µm, calculate the clearance rates of the bivalves used in the experiment. Again, use the equation by Coughlan (above) but this time include the data from the blank chambers. The volume of water (mixed with algae and microplasics) in each container was initially 1000 ml. Each sample removes 20 ml from the container.

2. Compare and contrast the results obtained from Dr. Gallant and Dr. Goofus. Are they different? What erroneous conclusion might Dr. Goofus draw?