

Appendix A2-2

Protocol for *Artemia* (brine shrimp) preparation

1. Equipment and reagents

Instruments

- Brine shrimp hatchery (A)
 - Air pump with bubbling stone
 - Water heater with thermostat
 - Mesh filter (#150 mesh; 100 µm opening)
- (B) White: mesh-type tea strainer
Green: *Artemia* strainer
(commercial items)

Reagents

- Sea salt
- Brine shrimp eggs (*Artemia* cysts)



← A hatchery for 1-3 L scale
(custom-made item)
Refer to 'Notes' for handmade and
small-scale hatching.

↓ Examples of mesh filters
(commercial items)



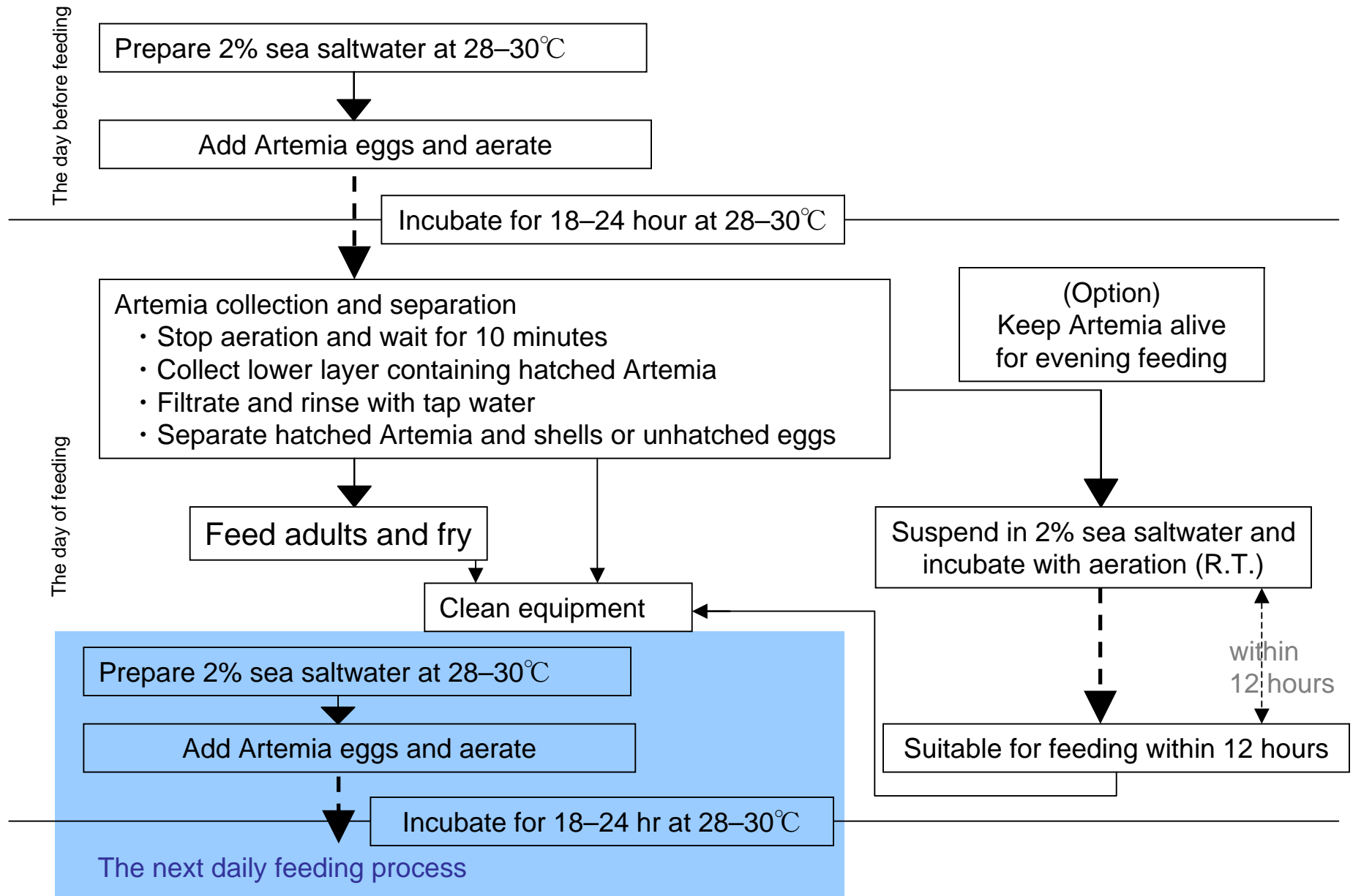
Table A2-2-1 Approximate requirement of *Artemia* eggs per day for number of fish.

<i>Artemia</i> eggs (g)	2% salt solution (L)	Salt (g)	Water (L)	Adult fish
1	0.5	10	0.5	100
5	2	40	2	500
10	4	80	4	1,000
25	10	200	10	2,500
50	20	400	20	5,000

This protocol's scale →

Artemia cysts have a specific gravity about 0.62 g/cc and contain about 250,000 eggs per gram.

2. Flowchart of *Artemia* (brine shrimp) preparation



3. Procedure for preparing *Artemia* as food for medaka

-- 24 hours before feeding (Steps 1–5) --

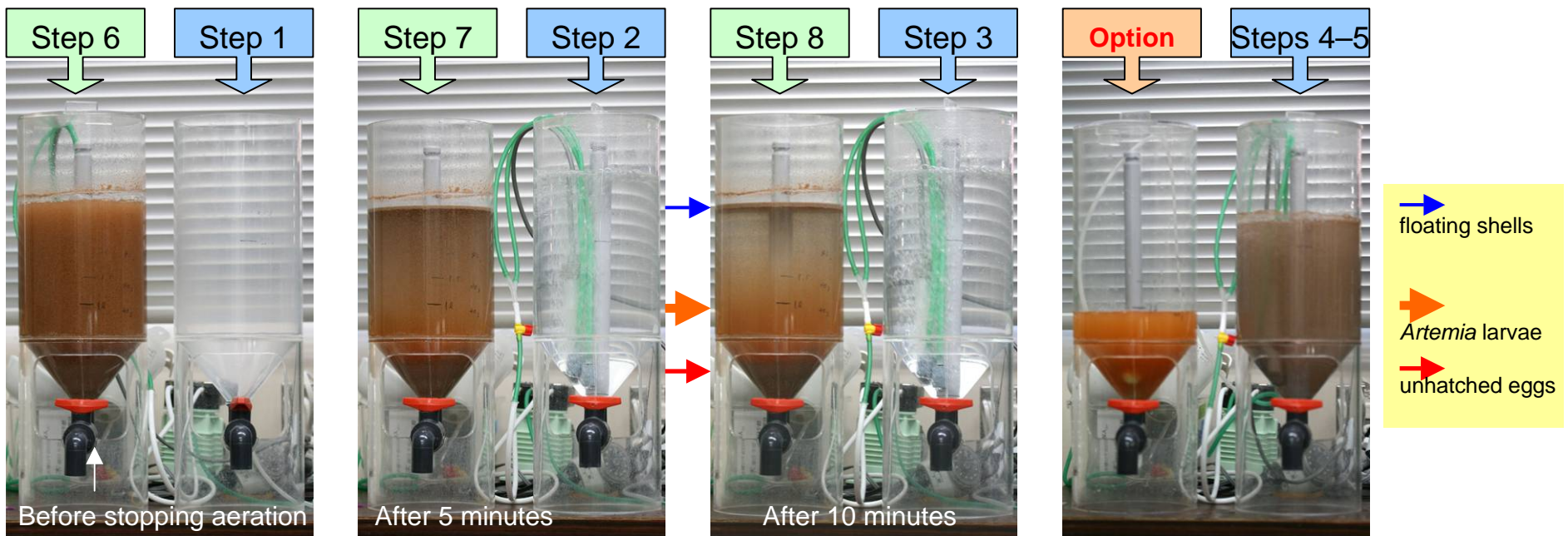
1. Set up equipment (hatchery, air pump, bubbling stone, and heater).
In daily work, this step can be done within Steps 7–8.
2. Fill with 2% saltwater and aerate (if necessary, use heater).
3. Wait for dissolution of salt and warming solution.
4. Add *Artemia* eggs into saltwater. (Do not add eggs before dissolution of salt, or the hatching rate will decrease notably.)
5. Incubate and aerate for 24 hours.

-- Steps on the feeding day (Steps 6–14) --

6. Wait for 24 hours after incubation.
7. Stop aeration (photo is 5 minutes after stopping).
8. Wait for hatched *Artemia* to sink (10 minutes).

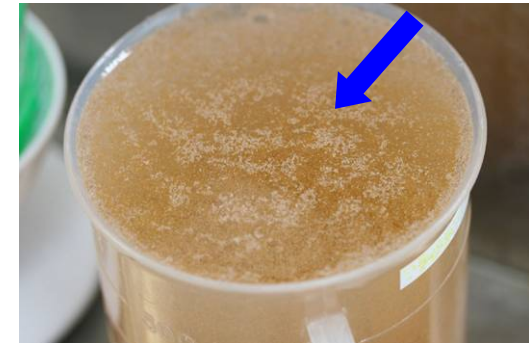
-- Photos for Steps 9–14 are on the next page --

9. Transfer larval sediment to another vessel.
10. Filter using the mesh filter to remove salt.
11. Suspend larvae in tap water.
12. Wait to allow larvae to sink and shells to float.
13. Collect only larvae by decantation, and filtrate. (Remove shells and unhatched eggs.)
14. Suspend in tap water and feed to fish.
-- Option for evening feeding (Option) –
Suspend larvae in 2% saltwater and aerate.





Step 12 Floating shells



Step 13 After discarding the floating shells, decant larvae into mesh filter.



Discard unhatched egg sediment.



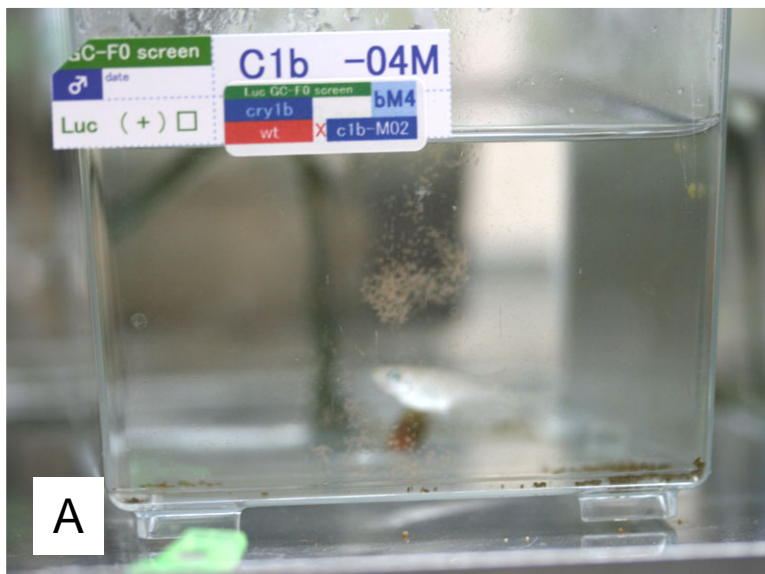
Step 14 Feeding to fish

← with a transfer pipette
with a squeeze bottle →

Refer to the next slide for moderate amount of *Artemia* feed in each case.



Informative photos for moderate amount of *Artemia* feed



This is a guide for the amount of feed according to the number of adult fish.

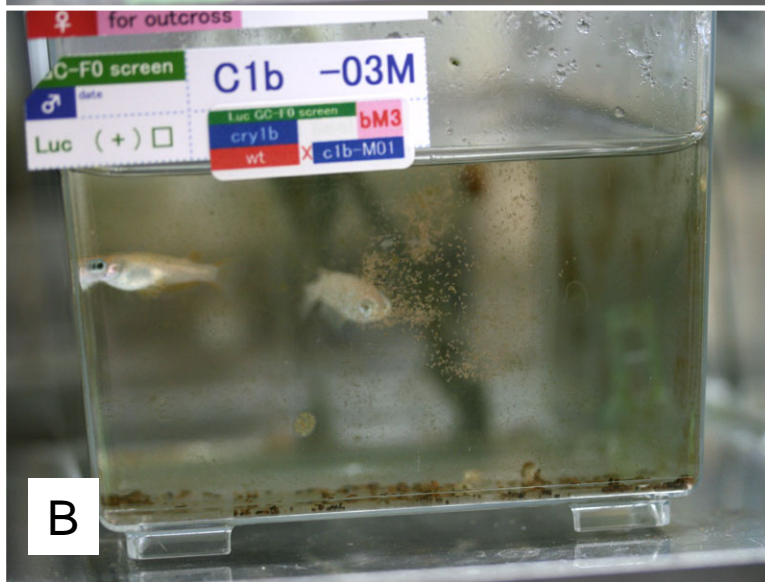
A: per 1 adult

B: per 2 adults (per pair)

C: per 10 adults

Give these amounts of *Artemia* at scheduled feedings (twice a day).

Young fish (from 1-month-old fry to adults) have a hearty appetite, so give nearly as much *Artemia* as for adult fish.



4. Notes

The best timing for feeding Artemia is just after they've hatched, as they expend nutrition for their growth. Feed as soon as possible!

About the equipment

- The hatchery can be made from plastic bottles, e.g., PET bottles. When using a PET bottle, cut away the bottom and create a hole on the cap, then connect a long tube to the hole for aeration. The important point is to avoid the disruption of the convective flow by aeration. When using a 'flat-bottom' hatchery, the eggs pile up at the corners and the hatching ratio of Artemia will decrease.

About the protocol

- Optimal incubation temperature is 28-30°C. But you can incubate Artemia eggs at room temperature unless the room temperature is below 20°C. A longer hatching time is required for low-temperature incubation relative to that at the optimal temperature.
- Add Artemia eggs after dissolving the salt. Deficient dissolution of salt causes a low hatching ratio.
- Do not allow larvae to sink for more than 20 min. Larvae cannot live without aeration (for steps 8 and 12).



A handmade hatchery.
(2 L PET bottle for 0.5-1 L hatchery)



A small-scale hatchery
(500 ml scale)
(commercial item)