

# Simplified Field sampling for Chitobiase Protocol

(K. Young 2022, adapted from K Suchy 2012; Akash Sastri 2008)

## Sampling Equipment

- 40  $\mu\text{m}$  mesh sieves (or 20  $\mu\text{m}$ )
- 500 mL Nalgenes – one for each depth sampled
- carrying container/bucket with handle
- 236  $\mu\text{m}$  (or smaller) zooplankton net

## Incubation Equipment (grey flip-top tote)

- 0.2  $\mu\text{m}$  syringe filters – one for each depth and time subsample
- 10 cc syringes – same # as filters
- tissue grinder (for homogenate; \*at UVic, can use forceps and a scint vial instead)
- 20 mL scintillation vials for subsamples
- cooler to incubate 500 mL Nalgenes (silver cooler on wheels)
- parafilm
- thermometer
- 100-1000 $\mu\text{L}$  pipettor + tips

### 1. Preparation of Crude Homogenate:

Needs to be made before seawater collection – ideally from the net taken at the same station but can be done earlier (within 24 hrs)

1. The crude homogenate is prepared by homogenizing freshly collected 1-3 krill or 5-10 large copepods (depends on size - less is more), in filtered seawater using a tissue grinder (if no grinder is available, use some forceps and squish with 10mL CLEAN filtered seawater in scint vial). Pour mash into 10mL syringe – it shouldn't be too thick, add more filtered seawater if so. Note in plankton log.
2. Pass the homogenate through 0.2  $\mu\text{m}$  (may require 2-3 filters! As the filter clogs, discard the filter and use a new one) into a clean 20mL scint vial and **do not refrigerate**.
3. Steps 4.1. through 4.3. typically produce 8-10 ml of homogenate. This is enough homogenate for ~8 seawater incubations (2 stations). Once 0.2  $\mu\text{m}$  filtered, several homogenates may be pooled into a single vial.
4. Ideally, homogenate should be prepared fresh at each station. Homogenate may be prepared in advance of a station as the activity should remain suitably high for at least 48 hours left at room temperature.
5. The homogenate is passed through a 0.2  $\mu\text{m}$  filter and diluted (1:1000 (v/v)) in seawater samples collected for CBA decay rate estimates. This dilution corresponds to 0.5 ml of 0.2  $\mu\text{m}$  filtered homogenate in 500ml of screened seawater (see step 3.2).

### 2. Screened Seawater Collection:

- 1) Sample seawater from depths: 5, 10, 20, 50, 150, 250 and 350m (or 300m, bottom depth dependant; should be >25m from bottom)
- 2) A net cast corresponding to this range should also be collected
- 3) Upon retrieval of seawater and starting at deepest depth:
  - a) Rinse 40  $\mu\text{m}$  mesh with seawater from Niskin
  - b) Conventional 3X rinse of Nalgene (500 mL) with water passed through mesh
  - c) Fill Nalgene with screened seawater (note time on chito logsheet)

Station Info: LB08 Event: 27  
 Date: 25 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note
CBAnat	32	32 A	100	temp from CTD cast for Time 0
	36	↓	50	
	39		20	
	41		10	
	44		5	
				Time Start: 23:00
				Time End: 23:10

0 Time Sampled	Sample #	Rep # B	Incubator Temperature:	Notes
Spiked			13.2	→ From surface seawater
	Time Start:	23:27		
	Time End:	23:31		

1 Time Sampled	Sample #	Rep # C	Incubator Temperature:	Notes
3 hrs after (-ish)			13.5	
~ 02:30	Time Start:	02:30		
	Time End:	02:35		

2 Time Sampled	Sample #	Rep # D	Incubator Temperature:	Notes
6 hr sample			14.7	
~ 05:30	Time Start:	05:31		
	Time End:	05:37		

3 Time Sampled	Sample #	Rep # E	Incubator Temperature:	Notes
12 hr sample			16.5	
~ 11:30	Time Start:	13:00		
	Time End:	13:10		added ice pack.

4 Time Sampled	Sample #	Rep # F	Incubator Temperature:	Notes
18 hr sample			15.7	
~ 17:30	Time Start:	17:48		
	Time End:	17:57		added ice pack

5 Time Sampled	Sample #	Rep # G	Incubator Temperature:	Notes
24 hr sample			15.6	
~ 23:30	Time Start:	00:05		
	Time End:	00:10		

Station Info: LC04 Event: 51  
 Date: 27 AUG 2023

0 Time Sampled	Sample #	Label	Depth	Note
CBAnat	121		150	temp from CTD cast for Time 0
	124		100	
	127		50	
	130		20	
	132		10	Time Start: 01:28
	135		5	Time End: 01:32

0 Time Sampled	Sample #	Rep A	Incubator Temperature:	Notes
Spiked	Time Start:	01:35	15.7	
	Time End:	01:45		

1 Time Sampled	Sample #	Rep B	Incubator Temperature:	Notes
3 hrs after (-ish)	Time Start:	04:50	16.0	
~ 04:30	Time End:	05:00		ice pack added.

2 Time Sampled	Sample #	Rep C	Incubator Temperature:	Notes
6 hr sample	Time Start:	7:24	14.7	
~ 07:30	Time End:	7:35		

3 Time Sampled	Sample #	Rep D	Incubator Temperature:	Notes
12 hr sample	Time Start:	1338	16.0°C	ice pack added.
* ~ 13:30	Time End:	1339.		

4 Time Sampled	Sample #	Rep E	Incubator Temperature:	Notes
18 hr sample	Time Start:	1934	15.5	added ice pack.
* ~ 19:30	Time End:	1939		

5 Time Sampled	Sample #	Rep F	Incubator Temperature:	Notes
24 hr sample	Time Start:	00:55	15.7	
~ 01:30	Time End:	01:00		

Station Info: LC09 Event: 61  
 Date: 27 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note
CBAnat	159		250	temp from CTD cast for Time 0
	163		150	
	168		50	
	171		20	
	173		10	Time Start: 11:35
	176		5	Time End: 11:43

0 Time Sampled	Sample #	Rep A
Spiked	Time Start: 11:48	Incubator Temperature: 15.7
	Time End: 11:52	Notes

★

1 Time Sampled	Sample #	Rep B
3 hrs after (-ish) ~ 15:00	Time Start: 15:55	Incubator Temperature: 15.0 added new ice pack
	Time End: 16:00	Notes

★

2 Time Sampled	Sample #	Rep C
6 hr sample ~ 18:00	Time Start: 19:29	Incubator Temperature: 15.5 added 1 ice pk.
	Time End: 19:29	Notes

3 Time Sampled	Sample #	Rep D
12 hr sample ~ 00:00	Time Start: 00:00	Incubator Temperature: 15.7
	Time End: 00:08	Notes: added ice pack

4 Time Sampled	Sample #	Rep E
18 hr sample ~ 06:00	Time Start: 5:32	Incubator Temperature: 15.0
	Time End: 5:39	Notes

5 Time Sampled	Sample #	Rep F
24 hr sample ~ 12:00	Time Start: 12:30	Incubator Temperature: 15.5
	Time End: 12:35	Notes

Station Info: LG02 Event: 90  
 Date: 28 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note
→ CBAnt	266		Bot-10	temp from CTD cast for Time 0
	267		75	
	269		50	
	271		20	
	274		10	Time Start: 23:53
	<del>277</del>	<del>267</del>	5	Time End: 0:03

0 Time Sampled	Sample #	Rep A	Incubator Temperature:	Notes
Spiked		008	18.0	
	Time Start:	0:08		
	Time End:	0:10		ice packs added in w bottles

1 Time Sampled	Sample #	Rep B	Incubator Temperature:	Notes
→ 3 hrs after (-ish)		2:59	16.4	
~03:00	Time Start:	2:59		
	Time End:	3:05		ice pack added

2 Time Sampled	Sample #	Rep C	Incubator Temperature:	Notes
6 hr sample		05:57	15.2	
~06:00	Time Start:	05:57		
	Time End:	06:05		

3 Time Sampled	Sample #	Rep D	Incubator Temperature:	Notes
12 hr sample		14:36	16.4°C	
~12:00	Time Start:	14:36		
	Time End:	14:45		

4 Time Sampled	Sample #	Rep E	Incubator Temperature:	Notes
18 hr sample		19:30	16.8°C	
~18:00	Time Start:	19:30		
~19:00	Time End:	19:41		

5 Time Sampled	Sample #	Rep F	Incubator Temperature:	Notes
24 hr sample		11:50	17.6	
~00:00	Time Start:	11:50		
	Time End:	00:00		ice pack added for other bottles

Put  
in  
other  
bottles  
fudge

Station Info: LG09  
 Date: 29 AUG 2023

Event: 105

(Green tope)

0 Time Sampled	Sample #	Label	Depth	Note
	306		250	temp from CTD cast for Time 0
	307		150	
	308		50	
	309		20	
	310		10	Time Start: 14:49
	311		5	Time End: 14:56

0 Time Sampled	Sample #	Rep A	Incubator Temperature:	Notes
Spiked			16.0°C	Both placed in drawer @ 25°C

1 Time Sampled	Sample #	Rep B	Incubator Temperature:	Notes
3 hrs after (-ish)			16.4°C	accidentally left Nalgene's out until 19:30 :- (@ 25°C for ~90min)
~18:00				

2 Time Sampled	Sample #	Rep C	Incubator Temperature:	Notes
6 hr sample			17.2°C	ice pack added.
~21:00				

3 Time Sampled	Sample #	Rep D	Incubator Temperature:	Notes
12 hr sample			15.9°C	
~03:00				

4 Time Sampled	Sample #	Rep E	Incubator Temperature:	Notes
18 hr sample			17.3	ice pack added.
~09:00				

5 Time Sampled	Sample #	Rep F	Incubator Temperature:	Notes
24 hr sample			16.7	
~15:00				

noon 5h-F4

Station Info: LBP8 ~~LBP8~~ Event: 116  
 Date: 30 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note
CBAnat	313		250 150	temp from CTD cast for Time 0
	314		150 100	
	315		50	
	317		20	
	318		10	Time Start: 09:15
	319		5	Time End: 09:20

0 Time Sampled	Sample #	Rep A
Spiked	Time Start:	09:22 Incubator Temperature: 17.3
	Time End:	09:26 Notes

1 Time Sampled	Sample #	Rep B
3 hrs after (-ish)	Time Start:	12:47 Incubator Temperature: 16.2
~12:30	Time End:	12:57 Notes

2 Time Sampled	Sample #	Rep C
6 hr sample	Time Start:	15:55 Incubator Temperature: 16.7
~15:30	Time End:	16:03 Notes

3 Time Sampled	Sample #	Rep D
12 hr sample	Time Start:	22:10 Incubator Temperature: 16.9
~21:30	Time End:	22:21 Notes ice added

4 Time Sampled	Sample #	Rep E
18 hr sample	Time Start:	03:10 Incubator Temperature: 16.0
~03:30	Time End:	03:15 Notes ice added.

5 Time Sampled	Sample #	Rep F
24 hr sample	Time Start:	09:37 Incubator Temperature: 16°C
~09:30	Time End:	09:43 Notes changed ice pack

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Station Info: LBP3 ~~LBP8~~ Event: 123  
 Date: 30 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note	
CBA <sub>nat</sub>	356		<del>150</del> 250		temp from CTD cast for Time 0
	358		100 <del>150</del>		
	361		50		
	364		20		
	366		10	Time Start:	18:14
	369		5	Time End:	18:24

0 Time Sampled	Sample #	Rep A		
Spiked 18:26	Time Start:	18:28	Incubator Temperature:	16.4
	Time End:	18:36	Notes	

13  
FL →

1 Time Sampled	Sample #	Rep B		
3 hrs after (-ish)	Time Start:	21:27	Incubator Temperature:	16.7
~ 21:30	Time End:	21:36	Notes	ice added

13

2 Time Sampled	Sample #	Rep C		
6 hr sample	Time Start:	00:46	Incubator Temperature:	15.2
~ 00:30	Time End:	00:49	Notes	

16

3 Time Sampled	Sample #	Rep D		
12 hr sample	Time Start:	06:52	Incubator Temperature:	15.4
~ 06:30	Time End:	06:55	Notes	

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4 Time Sampled	Sample #	Rep E		
18 hr sample	Time Start:	11:40	Incubator Temperature:	14.6°C
~ 12:30	Time End:	11:43	Notes	changed ice pack

FL →

5 Time Sampled	Sample #	Rep F		
24 hr sample	Time Start:	18:17	Incubator Temperature:	15.7
~ 18:30	Time End:	18:24	Notes	

Station Info: CS02 Event: 142  
 Date: 31 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note
CBAnat	396		250	temp from CTD cast for Time 0
	399		150	
	404		50	
	407		20	
	409		10	Time Start: 16:53 → collected
	411		5	Time End: 17:08 - 17:20 CBA Nat + SARK @ 17:08

0 Time Sampled	Sample #	Rep A
Spiked	Time Start: 17:08	Incubator Temperature: 15.5
	Time End: 17:20	Notes

1 Time Sampled	Sample #	Rep B
3 hrs after (-ish)	Time Start: 20:00	Incubator Temperature: 16.1
~ 20:00	Time End: 20:05	Notes: changed ice pack

2 Time Sampled	Sample #	Rep C
6 hr sample	Time Start: 23:00	Incubator Temperature: 15.2
~ 23:00	Time End: 23:07	Notes

3 Time Sampled	Sample #	Rep D
12 hr sample	Time Start: 04:45	Incubator Temperature: 16.4
<del>04:00</del> ~ 05:00	Time End: 04:51	Notes: added ice pack.

4 Time Sampled	Sample #	Rep E
18 hr sample	Time Start: 11:32	Incubator Temperature: 16.0
<del>10:00</del> ~ 11:00	Time End: 11:35	Notes: changed ice pack

5 Time Sampled	Sample #	Rep F
24 hr sample	Time Start: 17:38	Incubator Temperature: 15.6
~ 17:00	Time End: 17:44	Notes

Station Info: CS09

Event: 173

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Date: 1 SEPT 2023

0 Time Sampled	Sample #	Label	Depth	Note
CBAnat	438		150	temp from CTD cast for Time 0
	441		100	
	444		50	
	447		20	
	449		10	Time Start: 20:52
	<del>438</del> 451		5	Time End: 20:57

0 Time Sampled	Sample #	Rep A
Spiked 21:07	Time Start: 21:10	Incubator Temperature: 16.4
	Time End: 21:16	Notes

1 Time Sampled	Sample #	Rep B
3 hrs after (-ish)	Time Start: 00:20	Incubator Temperature: 16.7
00:16	Time End:	Notes: ice pack changed

2 Time Sampled	Sample #	Rep C
6 hr sample	Time Start: 03:30	Incubator Temperature: 15.1
03:16	Time End: 03:36	Notes

3 Time Sampled	Sample #	Rep D
12 hr sample	Time Start: 09:15	Incubator Temperature: 16.1
09:16	Time End: 09:20	Notes: added ice.

4 Time Sampled	Sample #	Rep E
18 hr sample	Time Start: 15:18	Incubator Temperature: 15.2
15:16	Time End: 15:24	Notes

5 Time Sampled	Sample #	Rep F
24 hr sample	Time Start: 21:15	Incubator Temperature: 16.4
21:16	Time End: 21:21	Notes

+3

+3

+b

+b

+b

Station Info: 12

Event: 183

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Date: 2 Sep 2023

0 Time Sampled	Sample #	Label	Depth		
CBAnt	472		300	Note	temp from CTD cast for Time 0
	473		250		
	476		150		
	480		50		
	483		20	Time Start:	19:20
	484		10	Time End:	19:26
	485		5		

0 Time Sampled	Sample #	Rep A			
Spiked 19:30	Time Start:	19:32	Incubator Temperature:	16.3	
	Time End:	19:39	Notes	added ice pack	

+3	1 Time Sampled	Sample #	Rep B		
	3 hrs after (-ish)	Time Start:	22:36	Incubator Temperature:	16.4
	~22:30	Time End:	22:43	Notes	added ice pack

+3	2 Time Sampled	Sample #	Rep C		
	6 hr sample	Time Start:	01:17	Incubator Temperature:	15.4
	~01:30	Time End:	01:23	Notes	ice pack changed

+b	3 Time Sampled	Sample #	Rep D		
	12 hr sample	Time Start:	08:03	Incubator Temperature:	15.4
	~07:30	Time End:	08:08	Notes	

+b	4 Time Sampled	Sample #	Rep E		
	18 hr sample	Time Start:	13:16	Incubator Temperature:	14.6
	~13:30	Time End:	13:23	Notes	

+b	5 Time Sampled	Sample #	Rep F		
	24 hr sample	Time Start:	20:15	Incubator Temperature:	16.1
	~19:30	Time End:	20:20	Notes	added ice

Station Info: GEO1 Event #: 98

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Date: 3 Sep 2023

0 Time Sampled	Sample #	Label	Depth	Note
	494		350	temp from CTD cast for Time 0
	495		250	
	496		150	
	497		50	
	498		20	Time Start: 10:00
	499		10	Time End: 10:08
	500		5	

0 Time Sampled	Sample #	Rep A	Incubator Temperature:	Notes
Spiked	Time Start:	10:08	15.8°	
	Time End:	10:16		changed ice pack

1 Time Sampled	Sample #	Rep B	Incubator Temperature:	Notes
3 hrs after (-ish)	Time Start:	13:00	14.1	
~13:00	Time End:	13:07		

2 Time Sampled	Sample #	Rep C	Incubator Temperature:	Notes
6 hr sample	Time Start:	16:10	15.1	
~16:00	Time End:	16:13		added ice

3 Time Sampled	Sample #	Rep D	Incubator Temperature:	Notes
12 hr sample	Time Start:	21:55	12.7	
~22:00	Time End:	21:58		

4 Time Sampled	Sample #	Rep E	Incubator Temperature:	Notes
18 hr sample	Time Start:	05:30	12.0	
~04:00	Time End:			

5 Time Sampled	Sample #	Rep F	Incubator Temperature:	Notes
24 hr sample	Time Start:	10:25	13.6.	
~10:00	Time End:	10:30		

Station Info: 42 Event: \_\_\_\_\_  
 Date: 3 Sep 23

0 Time Sampled	Sample #	Label	Depth		
CBAnat	503		250	Note	temp from CTD cast for Time 0
	504		150		
	505		50		
	506		20		
	507		10	Time Start:	12:35
	508		5	Time End:	12:40

0 Time Sampled	Sample #	Rep A			
Spiked 12:42			Time Start:	12:46	Incubator Temperature: 14.1
			Time End:	12:52	Notes

+3

1 Time Sampled	Sample #	Rep B			
3 hrs after (-ish)			Time Start:	15:58	Incubator Temperature: 15.1
15:45			Time End:	16:04	Notes

+3

2 Time Sampled	Sample #	Rep C			
6 hr sample			Time Start:	18:25	Incubator Temperature: 16.1
18:45			Time End:	18:32	Notes

+6

3 Time Sampled	Sample #	Rep D			
12 hr sample			Time Start:	23:00	Incubator Temperature: 12.8
00:45			Time End:	23:05	Notes

+6

4 Time Sampled	Sample #	Rep E			
18 hr sample			Time Start:	05:50	Incubator Temperature: 12.0
06:45			Time End:	05:57	Notes

+6

5 Time Sampled	Sample #	Rep F			
24 hr sample			Time Start:	10:25	Incubator Temperature: 13.6
12:45			Time End:	10:30	Notes