

Simplified Field sampling for Chitobiase Protocol

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

Sampling Equipment

- 40 μm mesh sieves (or 20 μm)
- 500 mL Nalgenes – one for each depth sampled
- carrying container/bucket with handle
- 236 μm (or smaller) zooplankton net

Incubation Equipment (grey flip-top tote)

- 0.2 μm 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 μL 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 μm (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 μm 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 μm 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 μm 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 μm 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

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0 Time Sampled	Sample #	Label	Depth	Note
CBA nat	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
Spiked	Time Start: 23:27	Incubator Temperature: 13.2 → From surface seawater
	Time End: 23:31	Notes

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

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

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

4 Time Sampled	Sample #	Rep # F
18 hr sample	Time Start: 17:48	Incubator Temperature: 15.7
~ 17:30	Time End: 17:51	Notes: added ice pack

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

Station Info: LC04
Date: 27 AUG 2023

Event: 51

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0 Time Sampled	Sample #	Label	Depth	Note
CBA nat	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
Spiked	Time Start: 01:35	Incubator Temperature: 15.7
	Time End: 01:45	Notes

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

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

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

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

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

Station Info: LC09

Event: 61

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Date: 27 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note	
CBAnt	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)	Time Start:	15:55	Incubator Temperature:	15.0	added new ice pack.
~ 15:00	Time End:	16:00	Notes		

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

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

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

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

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

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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
	277		5	Time End: 0:03

0 Time Sampled	Sample #	Rep A
Spiked	Time Start: 00:08	Incubator Temperature: 18.0
	Time End: 0:10	Notes: ice packs added in w/ bottles

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

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

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

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

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

Put
in
clean
lids

Station Info: LG09
Date: 29 AUG 2023

Event: 105

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(Green
toys)

0 Time Sampled	Sample #	Label	Depth	Note
CBAnt	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
Spiked	Time Start: 15:02	Incubator Temperature: 16.0°C
	Time End: 15:05	Notes

1 Time Sampled	Sample #	Rep B
3 hrs after (-ish)	Time Start: 18:07	Incubator Temperature: 16.4°C
~18:00	Time End: 18:13	Notes

2 Time Sampled	Sample #	Rep C
6 hr sample	Time Start: 21:09	Incubator Temperature: 17.2°C
~21:00	Time End: 21:16	Notes

3 Time Sampled	Sample #	Rep D
12 hr sample	Time Start: 3:35	Incubator Temperature: 15.9°C
~03:00	Time End: 3:42	Notes

4 Time Sampled	Sample #	Rep E
18 hr sample	Time Start: 09:15	Incubator Temperature: 17.3
~09:00	Time End: 09:20	Notes

5 Time Sampled	Sample #	Rep F
24 hr sample	Time Start: 15:43	Incubator Temperature: 16.7
~15:00	Time End: 15:52	Notes

Both placed
in drawer
@ 25°C

accidentally left Nalgene's out until 19:30 :- (@ 25°C for ~90m)

ice pack added.

ice pack added.

noon
5h-F

Station Info: LBP8
 Date: 30 Aug 2023

Event: 116

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0 Time Sampled	Sample #	Label	Depth	Note
CBAnt	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

FL
→
200g
Shift

Station Info: LBP 3 ~~LBP 8~~

Event: 123

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Date: 30 Aug 2023

0 Time Sampled	Sample #	Label	Depth	Note	
CBA nat	356		150 250		temp from CTD cast for Time 0
	358		100 150		
	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		

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	

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

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

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	

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

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0 Time Sampled	Sample #	Label	Depth	Note	
CBAnt	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 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
~ 04:00	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
~ 12:00	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
 Date: 1 SEPT 2023

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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
	438 451		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		

Station Info: 12 Event: 183
 Date: 2 Sep 2023

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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	

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	

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	

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

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

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		
CBAnat	494		350	Note	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			
Spiked	Time Start:	10:08	Incubator Temperature:	15.8°	
	Time End:	10:16	Notes	changed ice pack	

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

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

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

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

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

Station Info: 42 Event: _____
 Date: 3 Sep 23

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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	

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	

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

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

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

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