

NRC FORM 241 (7-1999) U.S. NUCLEAR REGULATORY COMMISSION

REPORT OF PROPOSED ACTIVITIES IN NON-AGREEMENT STATES, AREAS OF EXCLUSIVE FEDERAL JURISDICTION, OR OFFSHORE WATERS

(Please read the instructions before completing this form)

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1. NAME OF LICENSEE (Person or firm proposing to conduct the activities described below) University of South Florida		2. TYPE OF REPORT <input type="checkbox"/> INITIAL <input checked="" type="checkbox"/> REVISION <input type="checkbox"/> CLARIFICATION	
3. ADDRESS OF LICENSEE (Mailing address or other location where licensee may be located) Radiation safety Office 12901 Bruce B. Downs Blvd, MDC35 Tampa, FL 33612-4799		4. LICENSEE CONTACT AND TITLE ADAM WEAVER, RSO	
		5. TELEPHONE NUMBER (include Area Code) 813 974 1194	6. FACSIMILE NUMBER (include Area Code) 813 974 7091

7. ACTIVITIES TO BE CONDUCTED UNDER THE GENERAL LICENSE GIVEN IN 10 CFR 150.20

WELL LOGGING LEAK TESTING AND/OR CALIBRATIONS TELETHERAPY/IRRADIATOR SERVICE

PORTABLE GAUGES OTHER (Specify) ⇒ **Marine research, sampling - in vitro assays on vessel**

RADIOGRAPHY ⇒ REGISTERED AS USER OF PACKAGING (CERTIFICATES OF COMPLIANCE NUMBERS)

8. CLIENT NAME, ADDRESS, CITY/COUNTY, STATE, ZIP CODE University of Miami Rosenstiel School of Marine and Atmospheric Science Miami, FL 33149 R/V F.G. Walton Smith		9. ACTUAL PHYSICAL ADDRESS OF WORK LOCATION (Street and Number or other location. Give as complete an address or directions as possible.) West Florida Shelf (-83.00°W, 27.25°N) ~ 200-300 km west of Tampa Bay	
10. CLIENT TELEPHONE NUMBER (include Area Code) 727 553 1667		11. WORK LOCATION TELEPHONE NUMBER (include Area Code) Vessel - Radio	

12. DATES SCHEDULED	13. NUMBER OF WORK DAYS	14. ADD	15. DELETE	16. LOCATION REFERENCE NUMBER
FROM: October 26, 02 TO: November 1, 02	7			000815

LIST ADDITIONAL WORK SITES ON SEPARATE SHEET(S) TO INCLUDE ALL INFORMATION CONTAINED IN ITEMS 9-16 ABOVE.

17. LIST RADIOACTIVE MATERIAL, WHICH WILL BE POSSESSED, USED, INSTALLED, SERVICED, OR TESTED (include description of type and quantity of radioactive material, sealed sources, or devices to be used.)

5mCi of C-14 bicarbonate, 5mCi of P-33 orthophosphoric acid, 10mCi of H-3; 5mCi H-3 Leucine & 5mCi H-3 Thymidine.

18. AGREEMENT STATE SPECIFIC LICENSE WHICH AUTHORIZES THE UNDERSIGNED TO CONDUCT ACTIVITIES WHICH ARE THE SAME, EXCEPT FOR LOCATION OF USE, AS SPECIFIED IN ITEM 9 ABOVE. (Four copies of the specific license must accompany the initial NRC Form 241.)	LICENSE NUMBER SFRML 806-1	STATE FL	EXPIRATION DATE 12-31-2004
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19. CERTIFICATION (MUST BE COMPLETED BY APPLICANT)

I, THE UNDERSIGNED, HEREBY CERTIFY THAT:

- All information in this report is true and complete.
- I have read and understand the provision of the general license 10 CFR 150.20 reprinted on the instructions of this form; and I understand that I am required to comply with these provisions as to all byproduct, source, or special nuclear material which I possess and use in non-Agreement States or offshore waters under the general license for which this report is filed with the U.S. Nuclear Regulatory Commission.
- I understand that activities, including storage, conducted in non-Agreement States under general license 10 CFR 150.20 are limited to a total of 180 days in calendar year. With the exception of work conducted in off-shore waters, which is authorized for an unlimited period of time in the calendar year.
- I understand that I may be inspected by NRC at the above listed work site locations and at the Licensee home office address for activities performed in non-Agreement States or offshore waters.
- I understand that conduct of any activities not described above, including conduct of activities on dates or locations different from those described above or without NRC authorization, may subject me to enforcement action, including civil or criminal penalties.

CERTIFYING OFFICER - RSO or Management Representative (Name and Title) ADAM S. WEAVER, RSO	SIGNATURE <i>Adam S. Weaver</i>	DATE 10-8-2002
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WARNING: False statements in this certificate may be subject to civil and/or criminal penalties. NRC regulations require that submissions to the NRC be complete and accurate in all material respects. 18 U.S.C. Section 1001 makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

FOR NRC USE ONLY	RE Janice H. Kirby Licensing Assistant	TITLE Janice Kirby	SIGNATURE <i>Janice Kirby</i>	DATE 10/9/02	TOTAL USAGE - DAYS TO DATE 26
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Radiation Cruise Plan
R/V F.G. Walton Smith
Karenia Research Cruise
October 26 – November 1, 2002

Principal Investigator: Dr. Cynthia Heil
 College of Marine Science, University of South Florida,
 140 7th Ave S., St. Petersburg, Fl 33701
 (tel) 727-553-1667, (fax) 727-553-1189
 e-mail: cheil@seas.marine.usf.edu)

Departure/Return Port: USF Bay Campus, St. Petersburg, Fl.

Destination: West Florida Shelf (-83.00°W, 27.25°N). ~200-300 km west of Tampa Bay.

Vessel: R/V Walton Smith, University of Miami, Miami, Florida

Miami Radiation Contact: Edward Pombier (phone) 305-373-3830, (fax) 305-547-1658

USF Radiation Contact: Adam Weaver (phone) 813-974-1194

Project: (*Collaborative Research*): Fate of recently fixed N_2 in the eastern Gulf of Mexico: Does the regeneration of N by *Karenia* support the development of *Gymnodinium breve* blooms?

Funding Agency: National Science Foundation, Biological Oceanography Division

Radiation Users:

<u>User</u>	<u>Isotope</u>	<u>Institution</u>
Dr. Cynthia Heil	^{14}C , ^{33}P	Univ. of South Florida
Dr. Judith O'Neil	^{14}C , ^{33}P	Univ. of Queensland, Univ. of Maryland
George Boneillo	3H	Old Dominion University
Pete Bernhardt	3H	Old Dominion University
Michelle Watson	3H	Old Dominion University

Project Summary:

The overall goal of this cruise is to examine the nitrogen dynamics of the N_2 fixing cyanophyte *Karenia*. It's contribution to bacterial and primary production and effects upon phytoplankton and zooplankton community structure on the west Florida shelf. This will be accomplished by locating a *Karenia* population on the shelf, deploying a radio-drogue within this *Karenia* population, following the drogue for 6 days during which various biological, chemical and physical measurements in the different areas of the bloom.

Summary of Radioisotope Use:

During this cruise, phytoplankton primary production will be measured by $H^{14}CO_3^-$ uptake on water samples collected with Niskin bottles mounted on a rosette and on *Karenia* picked from net tows. It is expected that ~6 experiments (=measurements of primary

production) will be conducted which will require use of ^{14}C -bicarbonate. Additional experiments (~6 total) will also be conducted in which populations of *Karenia* are labeled with ^{14}C in incubations, then fed to zooplankton isolated from tows to determine grazing rates. ^{14}C emits beta particles and has a $T_{1/2}$ of 5,739 yrs. It is anticipated that the total activity of ^{14}C brought on board will not exceed 5 mCi.

Measurements will also be made of bacterial productivity on water samples using both ^3H -leucine and ^3H -thymidine. It is expected that ~6 experiments (3 measurements of bacterial production x 2 compounds) will be conducted which will require use of ^3H -labelled compounds. ^3H emits beta particles and has a $T_{1/2}$ of 12.3 yrs. It is anticipated that the total activity of ^3H brought on board will be 1 mCi of ^3H -leucine and 1 mCi of ^3H -thymidine for a total of 2 mCi of ^3H .

Measurements of phosphorus uptake and regeneration by both *Karenia* and natural phytoplankton populations will be made with $\text{H}_3^{33}\text{PO}_4$. It is expected that ~6-8 experiments will be conducted to determine the uptake kinetics of *Karenia* and natural phytoplankton populations. 2 additional experiments will examine the role of zooplankton grazing in P regeneration by prelabeling *Karenia* or ambient phytoplankton communities with ^{33}P , then feeding these labeled cells to zooplankton isolated from tows. Three additional experiments will examine P regeneration via a 2nd method, in which a 2 L sample is labeled with ^{33}P , and then subdivided into different size fractions. High concentrations of inorganic P are added to each fraction to inhibit uptake and the increase of ^{33}P activity in the dissolved fraction is sampled over a 7 hr period. The total activity of P-33 Orthophosphoric Acid brought on board will not exceed 5 mCi.

Radioisotope Protocol for ^{14}C and ^3H

1. All isotope usage will be confined to the wet lab of the R/V Walton Smith (which will be dedicated solely for radiation use during this cruise) and the on-deck incubation container. All ^{14}C , ^{33}P and ^3H isotope stocks will be stored in a small refrigerator in the wet lab in a lock box when not in use. ^3H and ^{33}P liquid waste will be combined, but kept separate from the ^{14}C liquid waster (due to the addition of TCA (trichloroacetic acid) as a rinse to ^3H incubations). All liquid waste will be stored in 20 L Nalgene carboys double wrapped within plastic bags within drums lashed to the boat out of the way of daily operations.
2. A survey of the proposed usage area (wet lab) on the R/V Walton Smith will be conducted using a Geiger counter survey meter and swipes (at least 6 wipe samples per survey) prior to loading the isotope aboard the ship, after each experiment, and after lab clean up immediately prior to return to the dock. Wipe activity will be determined immediately after wipes using a scintillation counter provided by University of Miami. Upon return to the dock the radiation use area on board will be sealed until activity of final swipes by USF radiation safety staff are read. If no activity above 200 cpm open window LSC counting is found, then the radiation use area will be reopened.
3. Only personnel authorized for the use of ^{14}C , ^3H and/or ^{33}P by USF Radiation Safety will conduct the experiments and handle radioactive samples and waste. All authorized users will have provided USF Radiation Safety Office Proof of Radioisotopic training prior to the cruise.
4. Double gloves and lab coats will be used during all experiments.

5. All areas for radioisotope use will be clearly labeled with tape and covered with benchcote. All equipment used for radioisotopic work will be labeled with "Caution Radioactive Material" tape and dedicated solely for use with ^{14}C , ^{33}P or ^3H .
6. All stock solutions of $\text{H}^{14}\text{CO}_3^-$, ^3H -leucine, ^3H -thymidine and $\text{H}_3^{33}\text{PO}_4$ will be stored in separate containers in a locked Plexiglass box inside a refrigerator located within the wet lab. All ^{14}C and ^3H stocks, solid waste and vial samples will be kept separated at all times. Solid ^{14}C waste will be separated into solid waste and vials, each of which will be double-bagged and stored separately inside the wet lab. Solid ^3H and ^{33}P waste will be treated in a similar manner. All waste solutions will be stored in 20 L carboys provided by Dr G. Vargo, which will be double bagged, placed with Solid-A-Sorb inside a large plastic waste container and returned to USF for proper disposal. All waste will be tagged with the appropriate USF waste tag.
7. All additions of ^{14}C , ^{33}P and ^3H to sample bottles and filtering of samples will be conducted inside the wet lab.
8. All incubations will be conducted in Coleman coolers to contain any spills and drips. These coolers will never be used for storage of food or ice for human consumption and will be appropriately labeled with "Caution Radioactive Material" tape.

Summary of Radiation Use and Storage

All isotope use will be restricted to the wet lab on the R/V F.G. Walton Smith, except when samples need to be incubated under natural light conditions, when samples will be placed in a radiation use only cooler plumbed with flowing seawater.

Solid Waste: All ^3H , ^{33}P and ^{14}C solid waste will be stored under bench in the wet lab in large plastic bags provided by USF Radiation Safety. H-3 and C-14 can be combined. P-33 waste will be kept in a separate container.

Liquid Waste: All ^3H , ^{14}C and ^{33}P liquid waste will be stored in 20 L Nalgene plastic carboys wrapped in plastic bags within larger plastic barrels with solid absorbant, with barrels stored lashed on deck.

Vials: All ^3H , ^{33}P and ^{14}C in vials will be stored within radiation van

Isotope Storage: all isotopes will be stored in locked box within refrigerator in wet lab.

Table 1 Details of Isotopes to be used in experiment

Isotope	Type emitted	Range in Air	Decays to	Max Energy (MeV)	Shielding
^{14}C	Beta	0.75 ft	^{14}N	0.16	None
^3H	Beta	0.02 ft	^3He	0.018	None
^{33}P	Beta	2 ft	^{33}S	0.25	None

Table 2. Summary of isotope use, expected waste activities during cruise

	Isotope	Total Amt Brought	Amt Used	Liquid		Solid	Vials
				Activity	Vol.		
Primary Production	H ¹⁴ CO ₃	5000 μCi	2880 μCi	2736 μCi	14. L	115.2 μCi	29.952 μCi
Zooplankton Grazing	H ¹⁴ CO ₃		150 μCi	142.5 μCi	2.46 L	6.0 μCi	1.5 μCi
Zooplankton Grazing	H ₃ ³³ PO ₄		300 μCi	142.5 μCi	2.46 L	6.0 μCi	1.5 μCi
Bacterial Production	³ H-Thymidine	5000 μCi	432 μCi	410 μCi	0.43 L	17.28 μCi	4.32 μCi
Bacterial Production	³ H-Leucine	5000 μCi	432 μCi	410 μCi	0.43 L	17.28 μCi	4.32 μCi
P Regeneration	H ₃ ³³ PO ₄	5000 μCi	900 μCi	855 μCi	18 L	36 μCi	9 μCi
P Uptake	H ₃ ³³ PO ₄		1000 μCi	950 μCi	20 L	40 μCi	10 μCi

Experimental Protocol

A.) H¹⁴CO₃ Uptake (Primary Production)

- 1.) All manipulations of ¹⁴C stock solutions and filtering of samples will be conducted in the wet lab.
- 2.) 3 treatments will be used: 1) unfiltered station seawater, 2) filtered (0.2 μm) station seawater and 3) filtered (0.2 μm) station seawater to which *Karenia* populations have been added. 4x1200 ml samples for each treatment will be added to 125 ml Nalgene bottles, with 2 of the 4 bottles incubated in 100% light and 2 in 0% light. Each bottle will be inoculated with ~20 μCi of ¹⁴C labeled bicarbonate from a secondary stock solution of ¹⁴C (made by placing a known amount of ¹⁴C into ~25 ml sterile filtered seawater). Note: One secondary ¹⁴C stock solution will be made up at the start of the cruise which will be used for the entire cruise.
 - a. Duplicate light and dark bottles for each of 3 treatments/station= 12 bottles
 - b. 2 depths = 24 bottles total/experiment
 - c. 1 experiment/day*6 days= 144 bottles*100 ml/bottle=14.4 L ¹⁴C liquid waste
 - d. Total ¹⁴C activity used = 144 bottles * 20 μCi/bottle = 2.88 mCi ¹⁴C
- 3.) Remove triplicate initial samples (100 μl each) from the secondary stock ¹⁴C solution with pipette and place in scintillation vial with scintillation fluid
- 4.) Incubate sample bottles in 30 L Coleman coolers under *in situ* light conditions with flowing seawater.
- 5.) After 2-4 hr incubation, the contents of each bottle will be filtered onto 0.45 μm nucleopore filters. Place filters immediately into scintillation vials with scintillation fluid. For each experiment it is expected that the ¹⁴C activity on filters will be ~4.8 μCi ¹⁴C, activity in solid waste will be 19.2 μCi and the activity in liquid waste will

be 456 μCi . Total activity used in 6 experiments will be 2880 μCi ^{14}C , with final activities of 29.952 μCi on filters, 115.2 μCi in solid waste and 2736 μCi in waste liquids (~14.4 L).

- 6.) All liquid waste from filtration and sample bottles will be stored in plastic containers double bagged with plastic, placed in larger plastic waste barrels with solid-A-sorb.
- 7.) It is anticipated that 6 experiments examining uptake will be conducted during the cruise
- 8.) Surveys of the radiation van will be conducted immediately at the start of the cruise, after each experiment and after clean up prior to return to dock.

B.) Zooplankton grazing on ^{14}C labeled *Karenia*.

- 1.) ~40-50 ml of concentrated *Karenia* sample will be transferred to filtered seawater in a 100 ml polycarbonate bottle.
- 2.) 25 μCi of H^{14}CO_3 will be added to the bottle.
 - a. 25 μCi /bottle * 1 bottle/expt * 1 expt/day * 6 days = 150 μCi ^{14}C used
 - b. 150 μCi ^{14}C stock used, with 142.5 μCi in liquid waste, 6 μCi in solids waste and 1.5 μCi in vials
- 3.) Sample bottle will be wrapped in neutral density screening and incubated in cooler for 2-4 hr.
- 4.) Incubate sample bottles in coolers to contain spills.
- 5.) Incubations will be terminated by transferring and concentrating (2 μm plexiglass sieve) 'hot' ^{14}C labeled *Karenia* to a beaker of 'cold' filtered seawater to wash off any unincorporated ^{14}C , then the concentrated sample will be transferred to 40 ml polycarbonate test tubes containing 1-3 copepods each.
- 6.) Uptake of ^{14}C label into the copepods will be determined over time course incubations of 0, 30, 60 min with 3 replicates for each time point.
 - a. 3 reps/time * 3 times * 40 ml/rep = 0.36 L/experiment + 50 ml from original incubation = 0.41 L
 - b. 0.41 L waste/experiment * 1 experiment/day * 6 days = 2.46 L ^{14}C liquid waste
- 7.) All liquid waste from filtration and sample bottles will be stored in plastic containers double bagged with plastic, placed in larger plastic waste barrels with solid-A-sorb.
- 8.) Experiments will be terminated by filtering samples onto pre-weighed Nucleopore filters, which will then be rinsed with 6% ammonium formate.
- 9.) Filters will be placed in scintillation fluid in vials and counted.
- 10.) Surveys of the radiation van will be conducted immediately at the start of the cruise, after each experiment and after clean up prior to return to dock.

C.) ^3H Leucine Bacterial Production

- 1.) Bacterial activity of natural bacterial populations will be determined by measuring the uptake of L-[4,5- ^3H] Leucine.
- 2.) A *Karenia* sample will be concentrated (2 μm plexiglass sieve) and transferred to filtered seawater.
- 3.) Treatments will consist of 12 mls of seawater with and without added *Karenia* sample in 50 ml centrifuge tubes
- 4.) 12 μCi of ^3H Leucine will be added to each treatment

- a. 6 expts * 3 reps/treatment * 2 treatments/expt * 12 uCi/rep = 432 uCi leucine stock used
 - b. 432 uCi used, with 410.4 uCi in ³H liquid waste, 17.28 uCi in solid ³Hwaste and 4.32 uCi in vials
 - c. Volume generated: 6 expts * 2 treatments/expt * 3 reps/treatment * 12 ml/rep * 1L/10³ ml = 0.432 ml/cruise
- 5.) All samples will be incubated in the cooler with flowing seawater for 30 min.
 - 6.) Incubations will be terminated by adding TCA and heating for 30 min to 80°C. Samples will then be filtered onto nitrocellulose filters and rinsed with 5% TCA and ethanol. Filters will then be placed in scint vials, dried overnight, then dissolved in ethyl acetate.
 - 7.) Scintillation fluid will be added to vials and activity counted.

D.) ³H Thymidine Bacterial Production

- 1.) Bacterial activity of natural bacterial populations will be determined by measuring the uptake of [methyl-³H] Thymidine.
- 2.) All procedures and activities for ³H-thymidine uptake are the same as for ³H-leucine uptake except after incubation, samples are filtered using a Hoffer unit with cold rinses of 5% TCA and ethanol.
- 3.) Filters will then be placed in scint vials, dried overnight, then dissolved in ethyl acetate.
- 4.) Scintillation fluid will be added to vials and activity counted.
- 5.) Summary of ³H Thymidine Use:
 - a. 12 μCi of ³H Thymidine will be added to each treatment
 - b. 6 expts * 3 reps/treatment * 2 treatments/expt * 12 uCi/rep = 432 uCi thymidine stock used
 - c. 432 uCi used, with 410.4 uCi in ³H liquid waste, 17.28 uCi in solid ³Hwaste and 4.32 uCi in vials
 - d. Volume generated: 6 expts * 2 treatments/expt * 3 reps/treatment * 12 ml/rep * 1L/10³ ml = 0.432 ml/cruise

E.) ³³P uptake experiments

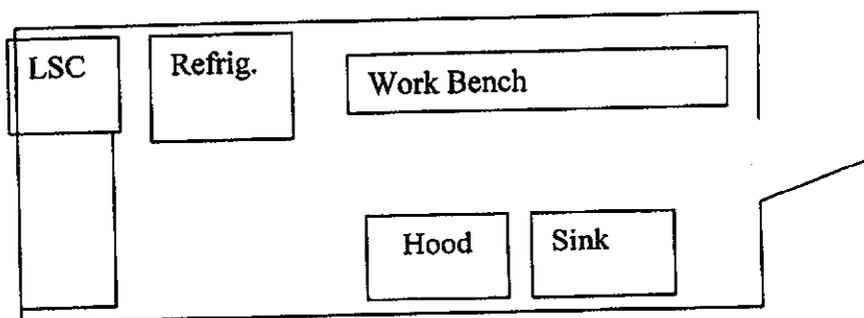
- 1.) All manipulations of ³³P stock solutions and filtering of samples will be conducted in the wet lab.
- 2.) 2 samples will be used: 1) unfiltered station seawater from the deep chl maximum and 2) filtered (0.2 μm) station seawater to which *Karenia* populations have been added. Duplicate 100 ml samples for of each sample will be added to 125 ml plastic bottles. For each sample, a series of 8 treatments will be made with P (as unlabeled PO₄) additions ranging from 0 to 5 μM PO₄. (2 samples * 8 P treatments/sample * 2 reps/treatment = 32 bottles total). 4 controls will consist of 1) filtered surface seawater, 2) filtered DCM seawater, 3) killed *Karenia* and 4) killed DCM sample. Each control will have 2 reps with 100 ml/bottle. Each bottle will be inoculated with 5 μCi of ³³P from a secondary stock solution of ³³P (made by placing a known amount of ³³P into ~25 ml sterile filtered seawater). Note: One secondary ³³P stock solution will be made up at the start of the cruise which will be used for the entire cruise. Uptake measurements made each day for 5 days will consist of:

- a. *Karenia*:
 1. 8 P treatments * 2 reps/treatment * 100 ml/rep = 1600 ml
 2. 8 treatments * 2 reps/treatment * 5 uCi ³³P/rep = 80 uCi ³³P
 - b. Water from Deep Chl Maximum (DCM) =
 1. 8 P treatments * 2 reps/treatment * 100 ml/rep = 1600 ml
 2. 8 treatments * 2 reps/treatment * 5 uCi ³³P/rep = 80 uCi ³³P
 - c. Controls:
 1. Killed *Karenia*:
 - A.) 2 reps * 100 ml/rep = 200 ml
 - B.) 2 reps * 5 uCi/rep = 10 uCi ³³P
 2. Killed DCM:
 - A.) 2 reps * 100 ml/rep = 200 ml
 - B.) 2 reps * 5 uCi/rep = 10 uCi ³³P
 3. Surface Filtered Seawater:
 - A.) 2 reps * 100 ml/rep = 200 ml
 - B.) 2 reps * 5 uCi/rep = 10 uCi ³³P
 4. DCM filtered Seawater:
 - A.) 2 reps * 100 ml/rep = 200 ml
 - B.) 2 reps * 5 uCi/rep = 10 uCi ³³P
 - d. Totals/day:
 1. ³³P used: 80 uCi+80 uCi+10 uCi+10 uCi+10 uCi+10 uCi = 200 uCi
 2. Liquid waste generated =1600ml + 1600ml + 200ml + 200ml + 200ml +200ml= 4000 ml
 - e. Totals/cruise:
 1. ³³P used: 200 uCi/day * 5 days = 1000 uCi
 2. Liquid waste generated =4000 ml/day * 5 days = 20,000 ml=20L
 - 3.) Remove triplicate initial samples (100 µl each) from the secondary stock ¹⁴C solution with pipette and place in scintillation vial with scintillation fluid
 - 4.) Incubate sample bottles in 30 L Coleman coolers under *in situ* light conditions with flowing seawater.
 - 5.) After 2 hr incubation, the contents of each bottle will be filtered onto 0.45 µm nucleopore filters and a subsample of the filtrate taken for determination of dissolved ³³P activity. Place filters immediately into scintillation vials with scintillation fluid. For each experiment it is expected that the ³³P activity on filters will be ~2 µCi ¹⁴C, activity in solid waste will be 8 µCi and the activity in liquid waste will be 190 µCi. Total activity used in 5 experiments will be 1000 µCi ³³P, with final activities of 10 µCi on filters, 40 µCi in solid waste and 950 µCi in waste liquids (~29 L).
 - 6.) All liquid waste from filtration and sample bottles will be stored in plastic containers double bagged with plastic, placed in larger plastic waste barrels with solid-A-sorb.
 - 7.) It is anticipated that 5 experiments examining uptake will be conducted during the cruise
 - 8.) Surveys of the radiation van will be conducted immediately at the start of the cruise, after each experiment and after clean up prior to return to dock.
- F.) ³³P regeneration experiments

- 1.) ^{33}P regeneration of 3 samples (water from surface and deep chl maximum and filtered surface seawater with *Karenia* added) will be determined 3X during the cruise.
 - 2.) 2 L from each of the 3 water samples will be placed in a 2.3 L clear Nalgene Bottles and subsampled for total phosphorus.
 - 3.) Each bottle will be spiked with ^{33}P to a final concentration of 50 uCi/L
 - a.) $3 \text{ expts/cruise} * 3 \text{ samples/expt} * 2 \text{ L/sample} * 50 \text{ uCi/L} = 900 \text{ uCi/cruise}$
 - b.) Volume generated: $3 \text{ expts/cruise} * 3 \text{ samples/expt} * 2 \text{ L/sample} = 18 \text{ L/cruise}$
 - 4.) All 3 bottles will be incubated in flowing seawater for 17 hr.
 - 5.) At the end of the incubation period, 100 ml subsamples from each bottle will be subsampled into a $<0.8 \mu\text{m}$ (Poetics filter), $<40 \mu\text{m}$ (Nitex screening) and bulk (no fractionation) fractions in 1.25 L bottles, each of which will be sampled for ^{33}P activity in the filtrate.
 - 6.) PO_4 (unlabelled) will be added to each bottle to a final concentration of 500 ug/L P.
 - 7.) Each bottle will be subsampled for ^{33}P activity in the filtrate at 30 minute intervals for 7-8 hrs.
- G.) Zooplankton grazing on ^{33}P labeled *Karenia*
- 1.) ~40-50 ml of concentrated *Karenia* sample will be transferred to filtered seawater in a 100 ml polycarbonate bottle.
 - 2.) 25 μCi of $\text{H}_3^{33}\text{PO}_4$ will be added to the bottle.
 - a.) $25 \text{ uCi/bottle} * 1 \text{ bottle/expt} * 1 \text{ expt/day} * 6 \text{ days} = 150 \text{ uCi } ^{33}\text{P}$
 - b.) 150 uCi ^{14}C stock used, with 142.5 uCi in liquid waste, 6 uCi in solids waste and 1.5 uCi in vials
 - 3.) Sample bottles will be wrapped in neutral density screening and incubated in cooler for 2-4 hr. Coolers will be used to contain spills
 - 4.) Incubations will be terminated by transferring and concentrating (2 μm plexiglass sieve) 'hot' ^{33}P labeled *Karenia* to a beaker of 'cold' filtered seawater to wash off any unincorporated ^{33}P , then the concentrated sample will be transferred to 40 ml polycarbonate test tubes containing 1-3 copepods each.
 - 5.) Uptake of ^{33}P label into the copepods will be determined over time course incubations of 0, 30, 60 min with 3 replicates for each time point.
 - a.) $3 \text{ reps/time} * 3 \text{ times/expt} * 40 \text{ ml/rep} = 0.36 \text{ L/experiment} + 50 \text{ ml in original incubation} = 0.41 \text{ L water}$
 - b.) $0.41 \text{ L waste/experiment} * 1 \text{ experiment/day} * 6 \text{ days} = 2.46 \text{ L } ^{33}\text{P liquid waste}$
 - 6.) All liquid waste from filtration and sample bottles will be stored in plastic containers double bagged with plastic, placed in larger plastic waste barrels with solid-A-sorb.
 - 7.) Experiments will be terminated by filtering samples onto pre-weighed Nucleopore filters, which will then be rinsed with 6% ammonium formate.
 - 8.) Filters will be placed in scintillation fluid in vials and counted.
 - 9.) Surveys of the radiation van will be conducted immediately at the start of the cruise, after each experiment and after clean up prior to return to dock.

References for methods

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**Fig. 1 Schematic of Wet lab
R/V F.G. Walton Smith**