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

APPROVED BY OMB: NO. 3150-0013 EXPIRES: 07/31/2002

Estimated burden per response to comply with this mandatory collection request: 15 minutes. This notification is required so that NRC may schedule inspection of the activities to ensure that they are conducted in accordance with requirements for protection of the public health and safety. Send comments regarding burden estimate to the Records Management Branch (T-6 E6), U.S. Nuclear Regulatory Commission, Washington, DC 20555-0001, or by internet e-mail to bjs1@nrc.gov, and to the Desk Officer, Office of Information and Regulatory Affairs, NEOB-10202, (3150-0013), Office of Management and Budget, Washington, DC 20503. If a means used to impose an information collection does not display a currently valid OMB control number, the NRC may not conduct or sponsor, and a person is not required to respond to, the information collection.

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

(Please read the instructions before completing this form)

1. NAME OF LICENSEE *(Person or firm proposing to conduct the activities described below)*
University of South Florida

2. TYPE OF REPORT
☒ INITIAL ☐ REVISION ☐ 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 board 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	TO				NUMBER TO BE ASSIGNED BY NRC
7/9/2002	7/16/2002	8			00815

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.)*
5 millicuries of Carbon 14 - bicarbonate, 5mCi of Phosphorus 33 - ortho-phosphoric acid, and 2mCi of Hydrogen 3 - 1mCi H-3 leucine & 1mCi H-3 Tymidine.

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	STATE	EXPIRATION DATE
SFRML 806-1	FL	12-31-2004

19. CERTIFICATION *(MUST BE COMPLETED BY APPLICANT)*

I, THE UNDERSIGNED, HEREBY CERTIFY THAT:

a. All information in this report is true and complete.

b. 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.

c. 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.

d. 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.

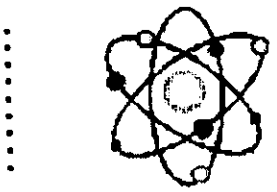
e. I understand that conduct of any activities not described above, including conduct of activities on dates or locations different from those described above or without NRC authorization, may subject me to enforcement action, including civil or criminal penalties.

CERTIFYING OFFICER - RSO or Management Representative *(Name and Title)*	SIGNATURE	DATE
[illegible]	ADAM S. WEAVER, RSO	6/13/2002

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 represent[illegible] department or agency of the United States as to any matter within its jurisdiction.

FOR NRC USE ONLY	REVIEW	Janice H. Kirby Licensing Assistant	SIGNATURE	DATE	TOTAL USAGE - DAYS TO DATE
				6/14/02	8

NRC FORM 241 (7-1999) PRINTED ON RECYCLED PAPER



USF University of South Florida

Facsimile Transmittal

To:	**Ms. Janice H. Kirby Licensing Assistant NRC Region II**	**Fax:**	**404-562-4955**
From:	Adam S. Weaver, RSO	**Date:**	**June 13, 2002**
Re:	Research cruise Initial report CY 2002	**Pages:**	**20**
For Review	***Please Reply***		

The University of South Florida (USF), a State of Florida Agreement State licensee (SFRML 806-1), submits a NRC form 241 for offshore water research cruises with radioactive materials, initial report for calendar year 2002.

We request reciprocity for using 5 millicuries of carbon-14, 2 millicuries of hydrogen-3 and 5 millicuries of phophorus-33 on board a research vessel in offshore waters (west Florida shelf). July 9 – July 16, 2002 (8 days)

The University of South Florida is a non-profit educational institution and is exempted by 10 CFR 170.11(a)(4) from payment of Reciprocity Recognition Fee required by CFR 170.31(16).

Attached:	
NRC form 241	1 page
Research cruise plan for R/V F.G. Walton Smith.	10 pages
USF State of Florida radioactive materials license	8 pages

If you have any questions about this request, please call me at 813-974-1194

Thanks for the assistance

Adam Weaver, CHP University of South Florida 12901 Bruce B. Downs Blvd., MDC 35
Tampa, FL 33612-4799 Phone 813-974-1194 Fax 813-974-7091

Radiation Cruise Plan
R/V F.G. Walton Smith
Trichodesmium Research Cruise
July 9-16, 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). *Trichodesmium* ~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 Trichodesmium support the development of Gymnodinium breve blooms?*

Funding Agency: National Science Foundation, Biological Oceanography Division

Radiation Users:

User	Isotope	Institution
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
Pete Bernhardt	^{3}H	Old Dominion University
Ester Cornfield	^{3}H	Old Dominion University
George Boneilo	^{3}H	Old Dominion University

Project Summary:

The overall goal of this cruise is to examine the nitrogen dynamics of the N_2 fixing cyanophyte *Trichodesmium*. 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 *Trichodesmium* populations on the shelf, deploying a radio-drogue within this Trichodesmium 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

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

Measurements of phosphorus uptake and regeneration by both *Trichodesmium* and natural phytoplankton populations will be made with $H_3{}^{33}PO_4$. It is expected that ~6-8 experiments will be conducted to determine the uptake kinetics of *Trichodesmium* and natural phytoplankton populations. 2 additional experiments will examine the role of zooplankton grazing in P regeneration by prelabeling *Trichodesmium* 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 ^{3}H

1. All isotope usage will be confined to the wet lab of the R/V Walton Smith (which will dedicated solely for radiation use during this cruise) and the on-deck incubation container. All ^{14}C, ^{33}P and ^{3}H isotope stocks will be stored in a small refrigerator in the wet lab in a lock box when not in use. ^{3}H 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 ^{3}H 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 and/or ^{3}H and ^{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 ^{3}H.
6. All stock solutions of $H^{14}CO_3^-$, ^{3}H-leucine, ^{3}H-thymidine and $H_3{}^{33}PO_4$ will be stored in separate containers in a locked Plexiglass box inside a refrigerator located within the wet lab. All ^{14}C and ^{3}H 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 ^{3}H 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 ^{3}H 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 incubated under natural light conditions, when samples will be placed in a radiation use only cooler plumbed with flowing seawater.

Solid Waste: All ^{3}H, ^{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 ^{3}H, ^{14}C and ^{33}P liquid waste will 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 ^{3}H, ^{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
^{3}H	Beta	0.02 ft	^{3}He	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	Amt Used	Liquid		Solid	Vials
			Activity	Vol.		
Primary Production	$H^{14}CO_3$	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-Thymi-dine	432 µCi	410 µCi	0.43 L	17.28 µCi	4.32 µCi
Bacterial Production	^{3}H-Leucine	432 µCi	410 µCi	0.43 L	17.28 µCi	4.32 µCi
Phosphorus Regeneration	$H_3{}^{33}PO_4$	900 µCi	855 µCi	18 L	36 µCi	9 µCi
Phosphorus Uptake	$H_3{}^{33}PO_4$	1000 µCi	950 µCi	20 L	40 µCi	10 µCi

Experimental Protocol

A.) $H^{14}CO_3$ Uptake (Primary Production)

1.) All manipulations of ^{14}C 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 *Trichodesmium* 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 ^{14}C labeled bicarbonate from a secondary stock solution of ^{14}C (made by placing a known amount of ^{14}C into ~25 ml sterile filtered seawater). Note: One secondary ^{14}C 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 ^{14}C liquid waste
   d. Total ^{14}C activity used = 144 bottles * 20 µCi/bottle = 2.88 mCi ^{14}C

3.) Remove triplicate initial samples (100 µl each) from the secondary stock ^{14}C 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 ^{14}C activity on filters will be ~4.8 µCi ^{14}C, 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 *Trichodesmium*.

1.) ~40-50 ml of concentrated *Trichodesmium* 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 uCi/bottle * 1 bottle/expt * 1 expt/day * 6 days = 150 uCi ^{14}C used

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 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 *Trichodesmium* 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.) ^{3}H Leucine Bacterial Production

1.) Bacterial activity of natural bacterial populations will be determined by measuring the uptake of L-[4,5-^{3}H] Leucine.

2.) A *Trichodesmium* 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 *Trichodesmium* sample in 50 ml centrifuge tubes

4.) 12 µCi of ^{3}H 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 ^{3}H liquid waste, 17.28 uCi in solid ^{3}Hwaste and 4.32 uCi in vials

c. Volume generated: 6 expts * 2 treatments/expt * 3 reps/treatment * 12 ml/rep * 1L/10^3 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.) ^{3}H Thymidine Bacterial Production
1.) Bacterial activity of natural bacterial populations will be determined by measuring the uptake of [methyl-^{3}H] Thymidine.
2.) All procedures and activities for ^{3}H-thymidine uptake are the same as for ^{3}H-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 ^{3}H Thymidine Use:
   a. 12 μCi of ^{3}H 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 ^{3}H liquid waste, 17.28 uCi in solid ^{3}Hwaste and 4.32 uCi in vials
   d. Volume generated: 6 expts * 2 treatments/expt * 3 reps/treatment * 12 ml/rep * 1L/10^3 ml= 0.432 ml/cruise

E.) ^{33}P uptake experiments
1.) All manipulations of ^{33}P 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 *Trichodesmium* 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 PO_4) additions ranging from 0 to 5 uM PO_4. (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 *Trichodesmium* and 4) killed DCM sample. Each control will have 2 reps with 100 ml/bottle. Each bottle will be inoculated with 5 μCi of ^{33}P from a secondary stock solution of ^{33}P (made by placing a known amount of ^{33}P into ~25 ml sterile filtered seawater). Note: One secondary ^{33}P 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. *Trichodesmium*:
      1. 8 P treatments * 2 reps/treatment * 100 ml/rep = 1600 ml
      2. 8 treatments * 2 reps/treatment * 5 uCi ^{33}P/rep = 80 uCi ^{33}P
   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 ^{33}P/rep = 80 uCi ^{33}P
   c. Controls:

1. Killed *Trichodesmium*:
   A.) 2 reps * 100 ml/rep = 200 ml
   B.) 2 reps * 5 uCi/rep = 10 uCi ^{33}P
2. Killed DCM:
   A.) 2 reps * 100 ml/rep = 200 ml
   B.) 2 reps * 5 uCi/rep = 10 uCi ^{33}P
3. Surface Filtered Seawater:
   A.) 2 reps * 100 ml/rep = 200 ml
   B.) 2 reps * 5 uCi/rep = 10 uCi ^{33}P
4. DCM filtered Seawater:
   A.) 2 reps * 100 ml/rep = 200 ml
   B.) 2 reps * 5 uCi/rep = 10 uCi ^{33}P

d. Totals/day:
   1. ^{33}P 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. ^{33}P 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 ^{14}C 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 ^{33}P activity. Place filters immediately into scintillation vials with scintillation fluid. For each experiment it is expected that the ^{33}P activity on filters will be ~2 µCi ^{14}C, 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 ^{33}P, 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.) ^{33}P regeneration experiments
1.) ^{33}P regeneration of 3 samples (water from surface and deep chl maximum and filtered surface seawater with *Trichodesmium* 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 expts/cruise * 3 samples/expt * 2 L/sample * 50 uCi/L = 900 uCi/cruise

b.) Volume generated: 3 expts/cruise * 3 samples/expt * 2 L/sample = 18 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 um (Poetics filter), <40 um (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 *Trichodesmium*

1.) ~40-50 ml of concentrated *Trichodesmium* sample will be transferred to filtered seawater in a 100 ml polycarbonate bottle.
2.) 25 µCi of $H_3{}^{33}PO_4$ will be added to the bottle.
   a.) 25 uCi/bottle * 1 bottle/expt * 1 expt/day * 6 days = 150 uCi ^{33}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 *Trichodesmium* 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 reps/time * 3 times/expt * 40 ml/rep=0.36 L/experiment + 50 ml in original incubation = 0.41 L water
   b.) 0.41 L waste/experiment * 1 experiment/day * 6 days =2.46 L ^{33}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

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: P. F. Kemp, B. F. Sherr, E. B. Sherr, J. J. Cole (eds), Handbook of Methods in Aquatic Microbial Ecology, Lewis Publishers, pp. 509-512.

O'Neil, J. M. 1998. The colonial cyanobacterium *Trichodesmium* as a physical and nutritional substrate for the harpacticoid copepod *Macrosetella gracilis*. Journal of Plankton Research, 20, 43-59.

Parsons, T.R., Y. Maita & C. M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, N.Y.

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

RADIOACTIVE MATERIALS LICENSE

Pursuant to Chapter 404, Florida Statutes, and Chapter 64E-5, Florida Administrative Code (F.A.C.), and in reliance on statements and representations heretofore made by the licensee designated below, a license is hereby issued authorizing such licensee to receive, acquire, possess and transfer the radioactive material(s) designated below and to use such radioactive material(s) for the purpose(s) and at the place(s) designated below. This license is subject to all applicable rules, regulations and orders of the state of Florida, Department of Health now or hereafter in effect and to any conditions specified below.

Licensee	
1. Name: **UNIVERSITY OF SOUTH FLORIDA**	3. License Number: **806-1** is hereby amended in its entirety, with reference to correspondence dated March 25, 2002.
2. Address: **Office of Research 4202 E. Fowler Avenue ADM 200 Tampa, FL 33620-5950**	4. Expiration Date: **12/31/2004** 5. Category: **3M(I)**

6. Radioactive Material (element and mass number)	7. Chemical And/Or Physical Form	8. Maximum Quantity Licensee May Possess At Any One Time
A. Any radioactive material between Atomic numbers 3 and 83, inclusive, except as listed below	A. Any form	A. 250 millicuries each of any radioactive material between atomic numbers 3 and 83, inclusive, except as listed below. Total possession limit shall not exceed 2.5 curies.
B. Hydrogen 3	B. Any form	B. 20 curies
C. Nickel 63	C. Foils (U.S. Radium Corp. Model Number LAB-784 or NRD Model Number N-1001)	C. 5 foils not to exceed 15 millicuries each
D. Cobalt 57	D. Sealed source (E.I. DuPont Corp. Model Number NER-072)	D. 2 sources not to exceed 50 millicuries

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

6.	Radioactive Material (element and mass number)	7.	Chemical And/Or Physical Form	8.	Maximum Quantity Licensee May Possess At Any One Time
E.	Cesium 137	E.	Sealed source (3M Co. Model Number 4P6E, 4D6L, 4F68, or U.S. Nuclear Corp. Model Number 375, or Amersham Corp. Model Number X.9, X.8 or Industrial Reactor Lab Inc. Model Number 2-4, 2-10, or J.L. Shepherd and Associates Model Number 6810, or Isotope Product Type 255)	E.	1 source not to exceed 120 curies
F.	Polonium 210	F.	Any form	F.	50 millicuries
G.	Any radioactive material between Atomic numbers 3 and 83, inclusive	G.	Any form	G.	25 millicuries each of any radioactive material between atomic numbers 3 and 83. Total possession limit shall not exceed 75 millicuries.
H.	Radioactive material distributed to a general licensee per 64E-5.206(1) and (4), F.A.C.	H.	Sealed or contained source(s)	H.	No single source to exceed that quantity authorized for the general license
I.	Radioactive material distributed to a general licensee per 64E-5.206(6), F.A.C.	I.	Sealed or contained source(s)	I.	No single source to exceed that quantity authorized for the general license described in 64E-5.206(6), F.A.C.

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

6.	Radioactive Material (element and mass number)	7.	Chemical And/Or Physical Form	8.	Maximum Quantity Licensee May Possess At Any One Time
J.	Radioactive material distributed to a general licensee per 64E-5.206(7), F.A.C.	J.	Any form authorized by the general license described in 64E-5.206(7), F.A.C.	J.	See item 9.K. below
K.	Radioactive material distributed to a general licensee per 64E-5.206(8), F.A.C.	K.	Any form authorized by the general license described in 64E-5.206(8), F.A.C.	K.	See item 9.L. below

9. Authorized Use

A. - F. To be used for research and development as defined in 64E-5.101, F.A.C.; teaching, training, for use as components of analytical instruments and for calibration of instruments.

G. To be used at temporary job sites, such as research vessels and mobile laboratories, for the purpose of research and development as defined in 64E-5.101, F.A.C.; teaching, training, for use as components of analytical instruments and for calibration of instruments.

H. To be used in devices approved for receipt under general license provisions as described in Items 6, 7 and 8.

I. To be used as calibration and reference sources in accordance with the regulation and possession limits described in subsection 64E-5.206 (6), F.A.C.

J. To be used for In-vivo testing in accordance with the regulations described in subsection 64E-5.206 (7), F.A.C. Each laboratory shall not exceed the possession limits allowed under this general license.

K. To be used for In-vivo testing in accordance with the regulations described in subsection 64E-5.206 (8), F.A.C. Each laboratory shall not exceed the possession limits allowed under this general license.

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

CONDITIONS

10. A. Licensed material may be used and stored at the following University owned locations:

University of South Florida (USF) Tampa campus located at 4202 E. Fowler Avenue, Tampa, Florida 33620 in the following locations:

College of Art and Science:
Bioscience Facility (BSF)
Life Science Annex (LSA)
Physics Building (PHY)
Science Center Building A (SCA)
Social Science (SOC);

College of Engineering:
Engineering Research (ENG);

College of Public Health (CPH)

Florida Mental Health Institute:
Building H (MHH);

Hazardous Waste Facilities:
Hazardous Waste Building A (HWA)
Hazardous Waste Building B (HWB);

Medical Center Building (MDC) located at 12901 Bruce B. Downs Boulevard, Tampa, Florida 33612:
Med Center Laboratories (MLC)
Med Center Receiving (MDR)
Med Center Vivarium (MDV);

Psychiatric Hospital (PSH) located at 3515 E. Fletcher Avenue, Tampa, Florida 33613;

USF Sarasota Campus located at 5700 Tamiami Trail, Sarasota, Florida 34243-2197, in the following locations:
Hansen Building (HAN)
Selby Building (SEL);

USF St. Petersburg Marine Science and Bayboro Campus located at 830 1st Street South, St. Petersburg, Florida 33701 in the following location:
Receiving Department
Knights Oceanographic Research Building (KOR)
Marine Science Building A (MLS);

Children's Research Institute (CRI), 420 6th Avenue S., St. Petersburg, Florida 33701; and

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

10. A. Non University owned locations listed below:
All Children's Hospital, (ACH) and Children's Health Clinic (CHC), 824 4th Street, South, St. Petersburg, Florida 33701 to include the following:
General Receiving Department
Research labs located: ACH 2nd floor room 2NE and CHC 5TH and 6TH floors
All children's Hospital (ACH) to include:
Shipping/Receiving Department located at 801 6th Street South, St. Petersburg, Florida 33701

Archbold Biological Station, located near, Lake Placid, Florida on Old State Road 8, 2 miles south of County Road 70, 33862
Moffitt Cancer Research Hospital (MCC), 12902 Magnolia Drive, Tampa, Florida 33612
Moffitt Cancer Research Center (MRC), 13131 Magnolia Drive, Tampa, Florida 33612
Shriners Hospital (SHC), 12502 North Pine Drive, Tampa, Florida 33612-9449
Tampa General Hospital (TGH), Davis Island, Tampa, Florida 33606
USF Dialysis Center, 13101 Bruce B. Downs Boulevard, Tampa, Florida 33612.

B. All records will be kept at the physical address located at 3500 E. Fletcher Ave., Suite 523, Tampa, Florida 33613.

C. Licensed material described in Items 6, 7, 8, Subitem H shall be used and stored on research vessels and mobile laboratories at temporary job sites throughout the State of Florida in accordance with the correspondence dated September 1, 1988.

D. This condition does not prohibit use in other agreement states and states under the jurisdiction of the U.S. Nuclear Regulatory Commission (NRC) under reciprocity that has been approved by an agreement state or the NRC.

11. Failure to comply with the provisions of this license is a felony of the third degree pursuant to section 404.161, Florida Statutes. Also, violations may warrant an administrative fine of up to $1,000.00 per violation per day, pursuant to section 404.162, Florida Statutes.

12. A. Licensed materials shall be used by, or under the supervision of, individuals designated by the University of South Florida Radiation Safety Committee, Bruce G. Lindsey, Ph.D., Interim Vice President of Research. Records of such designations shall be made available for inspection by the department.

B. The radiation safety officer is Adam Weaver, CHP.

13. The licensee shall comply with the provisions of Chapter 64E-5, F.A.C., Part IX, "Notices, Instructions and Reports to Workers; Inspections" and Part III, "Standards for Protection Against Radiation."

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

14. The licensee shall not transfer possession or control of radioactive material, or products containing radioactive material as a contaminant except:

    A. By transfer to a specifically licensed recipient; or

    B. As provided otherwise by specific provision of this license pursuant to the requirements of Chapter 64E-5, F.A.C.

15. Radioactive material transported on public thoroughfares shall be packaged, prepared for shipment and transported in accordance with Title 49, Code of Federal Regulations and Chapter 64E-5, F.A.C.

16. A. The licensee shall assure that each sealed source is tested for leakage or contamination and follow the appropriate actions as required by section 64E.5.1303, F.A.C. Licensed material shall be tested at least every 6 months. The test sample (smear) shall be taken by the licensee using an approved leak test kit. Analysis of the test sample shall be performed by licensee, individuals who are licensed by the department, NRC, agreement state, or licensing state to provide these services. The licensee is required to retain leak test records containing the manufacturer's name, model and serial number of each sealed source tested, identity of each sealed source radionuclide and its estimated activity, the measured activity of each test sample expressed in microcuries, the date of the test and signature of the radiation safety officer or designee. The records shall be maintained for 3 years for inspection by the department.

    B. Each sealed source fabricated by the licensee shall be inspected and tested for construction defects, leakage, and contamination prior to use or transfer as a sealed source. If the inspection or test reveals any construction defects or 0.005 microcurie or greater of contamination, the source shall not be used or transferred as a sealed source until it has been repaired, decontaminated and re-tested.

17. The licensee shall conduct a physical inventory and inspection at least every 12 months to account for all sealed sources received and possessed under this license as required by section 64E-5.1304, F.A.C. Inventory records shall be maintained for 3 years from the date of the inventory for inspection by the department, and shall include the manufacturer's name, model and serial numbers of each sealed source, the identity of each sealed source radionuclide and its estimated activity, the location of each sealed source, the date of the inventory and the signature of the radiation safety officer or designee.

18. Detector cells containing nickel 63 shall only be used in conjunction with a properly operating temperature control mechanism which prevents the temperature from exceeding 360 degrees Celsius.

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

19. The licensee shall not use radioactive material in or on human beings nor in field applications where radioactive material is released to the environment, except as provided otherwise by a specific provision of this license.

20. Animals and plants administered radioactive materials, and their products, shall not be used for human consumption.

21. Radioactive material shall not be used in or on human beings, nor in products distributed to the public.

22. The licensee shall notify the Bureau of Radiation Control at least 48 hours in advance of shipping its low-level radioactive waste to a commercial treatment, storage, or disposal facility. Notification shall consist of either calling (407) 297-2095 or writing the Bureau of Radiation Control, Department of Health, Post Office Box 680069, Orlando, Florida 32868-0069.

23. The following conditions pertain to device(s) received under general license provisions as described in item 6, Subitem I:

A. Sealed sources containing radioactive materials authorized for distribution under a general license shall not be opened or removed from their source holders by the licensee.

B. Installation, relocation, maintenance, repair, removal from service and initial radiation survey of devices containing radioactive material and installation, replacement and disposal of sealed sources containing radioactive material used in devices shall be performed only by persons specifically authorized by the NRC, a licensing state, or an agreement state to perform such services.

C. The licensee shall maintained record showing date of receipt, site of use and date and method of disposal.

23. D. At intervals not to exceed 12 months, an inventory and inspection of all devices containing radioactive material shall be conducted which determine, where applicable, at least the general physical condition of the device, proper shutter operation and adequate posting of radiation caution signs. Records shall be maintained for inspection by the department and shall include the date and name of the individual taking the inventory, the location and identification of the devices, the quantity, and kinds of radioactive material, and the findings of the physical inspection.

E. Required testing for leakage and contamination of the sealed sources containing radioactive materials shall be performed by the licensee, persons specifically authorized by the NRC, a licensing state, or an agreement state to perform such services.

STATE OF FLORIDA
DEPARTMENT OF HEALTH
BUREAU OF RADIATION CONTROL

24. A. Except as specifically provided otherwise by this license, the licensee shall possess and use licensed material described in Items 6, 7, 8, and 9 of this license in accordance with statements, representations and procedures contained in the licensee's application dated April 13, 1999, signed by George R. Newkome, Ph.D., Vice President of Research, and correspondence dated:

August 13, 1999, signed by George R. Newkome, Ph.D., Vice President for Research;
October 25, 1999, signed by Robert B. Vomacka, Radiation Safety Officer;
December 1, 1999;
November 16, 2000, both signed by George R. Newkome, Ph.D., Vice President for Research; and
March 25, 2002 (new package delivery procedure for Tampa campus), signed by Bruce G. Lindsey, Ph.D., Interim Vice President of Research, Professor of Physiology and Neuroscience.

B. The licensee shall comply with all applicable requirements of Chapter 64E-5, Florida Administrative Code, and these regulations shall supersede the licensee's statements in applications or correspondence, unless the statements are more restrictive than the regulations.

For the Bureau of Radiation Control:

Issuance Date: APR 0 1 2002

Joy Stephenson
Environmental Specialist
Bin #C21
4052 Bald Cypress Way
Tallahassee, FL 32399-1741
(850) 245-4545