

Facsimile Transmittal

To:	Ms. Janice H. Kirby Licensing Assistant NRC Region II	Fax:	404-562-4955
From:	Adam S. Weaver, RSO	Date:	June 18, 2001
Re:	Revision to Form 241	Pages:	8
For Review		Please Reply	

The University of South Florida, a State of Florida Agreement State licensee (SFRML 806-1), submits a revision to NRC form 241 (number assigned by NRC on 2/16/2001 was - 000476) to our 2/9/2001 initial request. We request reciprocity for using 5 millicuries of carbon-14 and 2 millicuries of hydrogen-3 on board a research vessel in offshore waters (west Florida shelf).

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, revision for 7/7- 7/13/2001 cruise 1 page
 - Research cruise plan for R/V F. G. Walton Smith. 5 pages
 - Layout of research lab 1 page

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

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)

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.

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., MDC 35
 Tampa, FL 33612 - 4799

4. LICENSEE CONTACT AND TITLE
 Adam S. 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 & Atmospheric Science
 Miami, FL 33149
 Vessel: R/V F.G. Walton Smith

9. ACTUAL PHYSICAL ADDRESS OF WORK LOCATION (Street and Number or other location. Give as complete an address as possible in directions as possible.)
 West Florida Shelf (-84.00°W 26.5°N)
 ~150km off Sarasota, Florida

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
7/7/2001	7/13/2001	7			000 476

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.)

Five (5) millicuries of Carbon - 14, biocarbonate
 1 millicurie of hydrogen-3, leucine 1 millicurie of H-3 labeled 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	STATE	EXPIRATION DATE
SFRML 806-1	FL	12/31/2004

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) SIGNATURE DATE

Adam S. Weaver, RSO ADAM S. WEAVER 6/18/2001

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

Janice H. Kirby and Title) SIGNATURE DATE TOTAL USAGE - DAYS TO DATE

Licensing Assistant *Janice Kirby* 6/18/01 7

Radiation Cruise Plan
R/V Walton Smith
Trichodesmium Research cruise
July 7-13, 2001

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 (-84.00°W, 26.5°N), ~150 km off Sarasota Florida. A surface drogue will be deployed at this location and followed over a 6 day period.

Vessel: R/V Walton Smith, University of Miami

Radiation Equipment Requested from R/V Walton Smith (UNOL's): Sole use radiation van on ship, scintillation counter, hood.

Project: *Fate of recently fixed N₂ 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	USF
Dr. Judith O'Neil	¹⁴ C	Univ. of Queensland, Australia
Dr. Margie Mulholland	³ H	Old Dominion University
Angela Michelle Watson	³ H	Old Dominion University

Project Summary:

The overall goal of this cruise is to quantify the contribution that the N₂ fixing cyanobacteria *Trichodesmium* spp. makes to bacterial and primary production and nitrogen dynamics as well as its effects upon phytoplankton and zooplankton community structure on the west Florida shelf. This will be accomplished by locating a *Trichodesmium* population on the shelf, deploying a radio-drogue within this *Trichodesmium* population and following the drogue for 6 days, with various biological, chemical and physical measurements taken throughout each day.

Summary of Radioisotope Use:

During this cruise, measurements of phytoplankton primary production will be measured by ¹⁴HCO₃⁻ uptake on water samples collected with Niskin bottles mounted on a rosette. 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 *Trichodesmium* collected by nets is labelled 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.

Additional measurements will also be made of bacterial productivity on water samples using both ^3H -leucine and ^3H -thymidine. It is expected that ~6 experiments (=measurements of bacterial production with 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 .

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

1. All isotope usage will be confined to the radiation van on the deck 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 and ^3H isotopes will be stored in a small refrigerator in the wet lab in a lock box when not in use. ^{14}C and ^3H liquid waste will be kept separated and stored in 20 L Nalgene carboys within drums lashed to the boat out of the way of daily operations.
2. A survey of the proposed usage area (rad van) 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. Upon return to the dock the radiation use area on board will be sealed until activity of swipes are read. If no activity above background is found, then the radiation use area will be reopened.
3. Only personnel authorized for the use of ^{14}C and/or ^3H by USF Division of Compliance Services will conduct the experiments and handle radioactive samples and waste. All authorized users will have provided USF Division of Compliance 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 or ^3H .
6. All stock solutions of $\text{H}^{14}\text{CO}_3^-$, ^3H -leucine and ^3H -thymidine will be stored in separate containers in a locked Plexiglass box inside a refrigerator located within the radiation van. All ^{14}C and ^3H stocks, solid and liquid waste and vial waste 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 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 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.
9. All ^3H and ^{14}C isotopes, waste (dry and liquid), samples etc will be kept separated from each other both on the cruise and upon return to the laboratory at the College of Marine Science, USF prior to disposal.

Summary of Radiation Use and Storage

All isotope use will be restricted to the radiation van, except when samples need to be incubated under natural light conditions, when samples will be placed in a rad-use only cooler plumbed with flowing seawater.

Solid Waste: All ^3H and ^{14}C solid waste will be stored under bench in radiation van in large plastic bags provided by USF Radiation Compliance

Liquid Waste: All ^3H and ^{14}C 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 and ^{14}C in vials will be stored within radiation van

Isotope Storage: all isotopes will be stored in locked box within refrigerator in radiation van.

Table 1. Summary of Isotope Use, expected waste activity

	Isotope	Amt Used	Liquid	Solid	Waste
Primary Production	H^{14}CO_3	2.88 mCi	2736 uCi	115.2 uCi	29.952 uCi
Zooplankton Grazing	H^{14}CO_3	150 uCi	142.5 uCi	6.0 uCi	1.5 uCi
Bacterial Production	^3H -Thymidine	432 uCi	410.0 uCi	17.28 uCi	4.32 uCi
Bacterial Production	^3H -Leucine	432 uCi	410.0 uCi	17.28 uCi	4.32 uCi

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* colonies (picked from tows) have been added. 4x200 ml samples for each treatment will be added to 250 ml glass Wheaton bottles, with 2 of the 4 bottles incubated in 100% light and 2 in 0% light. Each bottle will be inoculated with $\sim 20 \mu\text{Ci}$ of ^{14}C labelled bicarbonate from a secondary stock solution of ^{14}C (made by placing a known amount of ^{14}C into $\sim 25 \text{ ml}$ 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=28.8 L ^{14}C liquid waste
 d. Total ^{14}C activity used = 144 bottles * 20 $\mu\text{Ci}/\text{bottle}$ = 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 $\sim 4.8 \mu\text{Ci}$ ^{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 (~ 29 L).
 - 6.) Particulate samples will be counted immediately using a scintillation counter on the R/V Walton Smith provided by the University of Miami.
 - 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.) It is anticipated that 6 experiments examining uptake will be conducted during the cruise
 - 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.
- B.) Zooplankton grazing on ^{14}C labeled *Trichodesmium*
- 1.) ~ 40 -50 *Trichodesmium* colonies isolated from plankton tows 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 $\mu\text{Ci}/\text{bottle}$ * 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 'hot' ^{14}C labeled colonies to a beaker of 'cold' filtered seawater to wash off any unincorporated ^{14}C , then the labeled colonies 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
 - b. 0.36 L waste/experiment * 1 experiment/day * 6 days = 2.16 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.) *Trichodesmium* colonies will be isolated from plankton tows and transferred to filtered seawater.
- 3.) Treatments will consist of 12 mls of seawater with and without added *Trichodesmium* colonies in 50 ml centrifuge tubes
- 4.) 12 μ Ci 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
- 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 μ Ci 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
 - a. 432 uCi used, with 410.4 uCi in ³H liquid waste, 17.28 uCi in solid ³Hwaste and 4.32 uCi in vials

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.

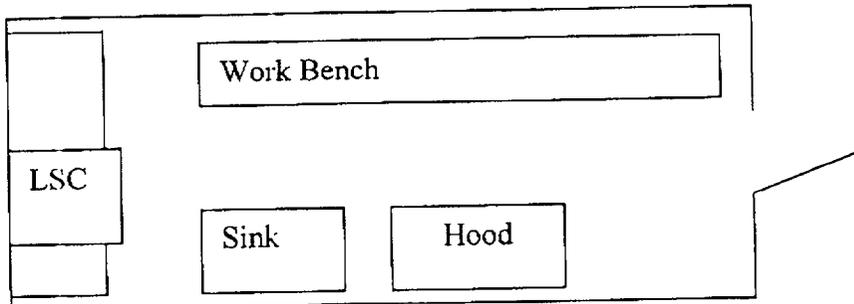
O'Neil, J. M. 1998. The colonial cyanobacterium *Trichodesmium* as a physical and nutritional substrate for the harpacticoid copepod *Macrosetella gracilis*. Journal of Plankton Research, 20, 43-59.

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

R/V F.G. Walton Smith
USF cruise 7/2001

Shipboard/Mobile Radiation Research Van:

Survey date: _____ by: _____



Survey results:

Action levels:

- LSC wipe (open/wide mode) 200 cpm [net] – clean and resurvey
- Survey meter – 2X background – clean and resurvey