Published in final edited form as:
Sci News. 2005 July 23; 168(4): .

Homing in on an alga's threat–and therapeutic promise

Janet Raloff

On a warm, sunny day, you can hear the presence of a “red tide” of toxic algae on popular Florida beaches, says Barbara Kirkpatrick. It's not the roar of coastal waves or the gurgle of flowing water, she explains, but “one continuous cough,” as thousands of sunbathers and swimmers respond to airborne irritants that the algae expel in the surf.

Kirkpatrick, manager of environmental health at the Mote Marine Laboratory in Sarasota, Fla., heard the sound last February when she visited Siesta Key Beach. Southwest Florida was in the throes of a red tide—in this case, a misnomer because the alga causing it, *Karenia brevis*, turns water yellow-green. Some traditional signs of the bloom were in evidence on beaches and in canals. Most obviously, manatees were dying. By the end of spring, officials had retrieved more than 4 dozen sea cow carcasses, weighing up to 1,000 pounds apiece.

As in the past, health officials had warned people not to eat local shellfish. It's long been known that their accumulated *K. brevis* toxins can cause vomiting, diarrhea, excessive urination, and a temporary reversal of temperature sensation—ice feels hot and near-boiling water feels frigid.

But new concerns are accompanying the latest *K. brevis* bloom because scientists have recently uncovered far wider health effects of the algal toxins.

Five years ago, in response to a rising incidence of red tides, the National Institute of Environmental Health Sciences began sponsoring research into how *K. brevis* toxins, called brevetoxins, function. The findings, announced this spring in more than a dozen papers and scientific talks, are largely disquieting. They show that the alga's poisons have several means of wreaking havoc beyond the neurotoxicity that had been previously recognized.

On a more positive note, this research has also revealed that along with its toxins, *K. brevis* produces counteracting agents. Scientists are working to harness these novel compounds as antidotes to brevetoxins or even as treatments for unrelated lung diseases, such as cystic fibrosis.

Quadruple trouble

Each *K. brevis* cell is a microscopic poison factory churning out 9 to 13 different toxins. Wave action readily ruptures the cells, turning the water along coastlines or in a tidal canal into a poisonous soup that can kill fish, sea turtles, and marine mammals such as manatees and dolphins. Birds can die after eating tainted prey. Filter-feeding shellfish, such as mussels,

William M. Abraham Department of Research Mount Sinai Medical Center 4300 Alton Road Miami Beach, FL 33140. Daniel G. Baden Center for Marine Science University of North Carolina, Wilmington 5600 Marvin Moss Lane Wilmington, NC 28409. Gregory D. Bossart Division of Mammal Research and Conservation Harbor Branch Oceanographic Institution 5600 US 1 North Ft. Pierce, FL 34946. Leanne Flewelling Florida Fish and Wildlife conservation Commission Fish and Wildlife Research Institute 100 8th Avenue, SE St. Petersburg, FL 33701. Elsa M. Haubold Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute 100 8th Avenue, SE St. Petersburg, FL 33701. Cynthia Heil Harmful Algal Bloom Program Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute 100 8th Avenue, SE St. Petersburg, FL 33701. Terrence P. Kane 1895 Floyd Sarasota, FL 34239. Barbara Kirkpatrick Environmental Health Program Mote Marine Laboratory 1600 Ken Thompson Parkway Sarasota, FL 34236. Society of Toxicology 1821 Michael Faraday Drive Suite 300 Reston, VA 20190 Web site: http://www.toxicology.org/.

accumulate brevetoxins without harm, but their flesh can poison animals, including people, that eat it.

The recent spate of research has clarified these neurotoxic effects. These studies trace to the toxins' effect on sodium channels in nerves, explains Daniel G. Baden of the University of North Carolina at Wilmington's Center for Marine Science. Normally, the channels open momentarily to pass a signal along a nerve cell.

Brevetoxins “act like little doorstops,” Baden says. “Once the sodium channel opens, they keep it open,” incapacitating the cell.

In March at the Society of Toxicology meeting in New Orleans, Baden reported that in the rodent brain, brevetoxins accumulate in the cerebellum, a region that affects cognitive function, breathing, and muscle control. If the same happens in manatees, he says, it might explain their disorientation and seeming inability to swim away from a red tide.

Bad as the neurotoxic effect might be, Baden's team unveiled at the meeting three new mechanisms of brevetoxin poisoning—detrimental changes in lung function, immunity, and DNA. Aspects of the first two might explain respiratory distress, which is the most prevalent symptom that the algae trigger in people.

As mucus clears from an animal's airways, it removes pollutants and debris. Small doses of the toxins reduced the clearance rate of mucus by 35 percent in sheep. This impairment lasted about 2 hours, notes research-team member William M. Abraham, a pulmonary physiologist at Mt. Sinai Medical Center in Miami Beach, Fla.

The scientists' studies also showed that the poisons attack the sheep immune system. White blood cells called neutrophils ordinarily protect the body by entering the lungs, gobbling up the poisons and the cells that they have damaged, and then quickly exiting. Abraham says that in sheep exposed to brevetoxins, however, neutrophils stick around, causing inflammation of the airways.

Another group of immune cells, macrophages, normally removes germs, damaged cells, and other debris from the lungs. In brevetoxin-exposed sheep, however, macrophages' destruction of trash fell by about 30 percent. This effect persisted for days.

Baden notes that his team's experiments in mice showed that brevetoxins can also depress an animal's response to infection by inactivating cathepsins, enzymes that immune cells use to break down debris as they attack it.

Finally, he and Abraham reported in March that brevetoxins induced significant DNA damage in lab cultures of human lymphocytes, which are white blood cells active in immunity.

Ticklish situation

“Most people in Florida have experienced what we call the red tide tickle,” says Cynthia Heil of the state's Fish and Wildlife Research Institute in St. Petersburg. “It's kind of a back-of-the-throat, just-beginning-to-get-a-cold feeling.”

People aren't alone in suffering from airborne algae. Heil and other researchers are studying the manatees killed by the recent *K. brevis* tide (see “Marine Mystery,” below).

Gregory D. Bossart, a veterinarian and pathologist at Harbor Branch Oceanographic Institution in Fort Pierce, Fla., studied a similar die-off of manatees in a 1996 red tide. By treating tissues

from dead animals with antibodies that stain the toxins, he and his colleagues pegged inhalation as a primary route of the poisons.

"We were able to trace [the brevetoxins] from their nasal cavities down to the lymph nodes and spleen," he says. "They were all loaded with toxin," and there were smaller amounts in the animals' brains and livers, says Bossart. In healthy people, Kirkpatrick notes, upper-airway symptoms are transitory. Indeed, she monitored some 28 healthy lifeguards before and after their work shifts. She examined the young men and women on 5 consecutive days during which the air was largely free of brevetoxins and on 5 consecutive days marked by moderate air concentrations—ranging from 3 to 27 nanograms of brevetoxins per cubic meter (ng/m^3).

In the May *Environmental Health Perspectives* (*EHP*), Kirkpatrick's team reports that the guards exhibited significantly more teary eyes, stuffy noses, and coughing on red tide days. However, the guards could still breathe deeply, even during 5 minutes of mild exercise on the beach. Moreover, their symptoms disappeared shortly after the guards entered an air-conditioned room.

On the other hand, red tide toxins can exert a choke hold on people with lung disease, Kirkpatrick's team reports in a second May *EHP* paper. The researchers evaluated lung function in 59 people who have asthma and take antiasthma drugs. Data were collected before and after these participants spent an hour on a beach with airborne brevetoxin concentrations of 3 to 36 ng/m^3.

These volunteers reacted far more strongly to the algae's lung irritants than did the lifeguards, none of whom had asthma. While on the beach, the people with asthma experienced not only the upper-airway effects seen in the guards, but also chest tightness, wheezing, and reductions in lung airflow.

Some participants with asthma contacted Kirkpatrick after the testing to report feeling even worse on the day following their hour at the beach. Abraham says that he isn't surprised. He's seen similar persistent effects in sheep induced to have a condition that serves as a model of human asthma.

In one study, Abraham administered 20 breaths of air laced with a brevetoxin to asthmatic ewes on 4 consecutive days. Air concentrations of the toxin were at the low end of what the recruits encountered in Kirkpatrick's beach tests.

The exposures rendered the animals' airways "more twitchy than normal, " says Abraham. When the animals inhaled an irritant, the airways constricted erratically, he reported in the May *EHP*. This heightened responsiveness to brevetoxins lasted a week.

In people, such airway hypersensitivity contributes to a feeling of chest tightness, he explains.

Help is coming

Terrence P. Kane, a Sarasota, Fla., pulmonologist, and his colleagues have their own way of knowing when red tide toxins have become airborne. Beach residents with asthma, emphysema, and bronchitis begin complaining that their prescribed drugs for those conditions aren't working. Kane says that the medications actually become overwhelmed by the onslaught of brevetoxins—"So, we may have to change their drug regimen."

The sheep studies have identified at least four classes of drugs as especially effective against the toxins' bronchial symptoms.

In the May *EHP*, Abraham and his colleagues report that albuterol was the most effective agent that they tested. With this inhalable antiasthma drug, airway constriction in the sheep was about a quarter of what it was with no drug. Even the least effective agent tested, the antihistamine diphenhydramine, prevented about half the constriction.

However, therapeutics that fight even more of brevetoxins' effects may be on the horizon. Their source could be *K. brevis* itself.

A couple of years ago, when Baden's team was isolating toxins from *K. brevis* cells, it turned up something new. In the January *Journal of Natural Products*, the researchers named the odd chemical brevenal. At the Society of Toxicology meeting, Baden reported isolating two additional variants of this chemical from the alga. What makes these brevenals interesting, says Baden, is that when administered to animals, all three prevent brevetoxins' damaging effects on nerves, lungs, and DNA.

These natural and strikingly potent antidotes bind to the same receptors on cells that brevetoxins target. “Receptor binding is dynamic,” Baden explains, with compounds continually attaching and then letting go. “It's like musical chairs,” the biochemist says, likening the receptors to the chairs. “So, the more brevenals you have relative to toxin, the less chance a toxin can get into one of those chairs” and harm cells.

Brevenals may also explain the decrease in the severity of health effects on people and animals in a coastal area during the course of a red tide. Algae tend to produce more of the antidotes as a bloom matures, Baden explains.

His team recently applied for a patent on brevenals, and “we're working with a local industry to move toward their clinical testing,” he says. The drug company, aaiPharma of Wilmington, N.C., foresees developing products to prevent the effects of the brevetoxins in people with compromised lungs. Workers, such as beach-cleanup or fishing crews, who endure regular exposure to red tide toxins might also benefit.

But Baden says that the company's primary interest in brevenals goes beyond concerns about red tides. People with cystic fibrosis and chronic obstructive pulmonary disease experience a slowdown of lung-mucus clearance similar to that in brevetoxin-exposed sheep. The company is designing drugs to restore normal mucus clearance from the lungs, as the brevenals do.

These algal agents or drugs derived from them might also find use combating algal toxins far beyond southwest Florida. Baden's group recently showed that an alga unrelated to *K. brevis* produces brevetoxins. The alga, currently known as *Chattonella cf. verruculosa*, was initially isolated in Delaware waters 5 years ago during a massive fish kill.

Scientists have recently identified *Karenia* species other than *K. brevis* in Japan and New Zealand. Although their toxicity is only beginning to be tested, Baden says that in experiments on asthmatic sheep, agents from at least one of the species induce bronchoconstriction and respiratory distress.

Such findings suggest that brevetoxins might pose health risks—and that brevenals may be useful to combat them—around the world.

Marine Mystery

Die-offs of manatees and dolphins in red tides prove perplexing

Manatees, the shy and docile mascots of towns around Florida's Gulf Coast, are experiencing a serious die-off. The current body count—55—is roughly 4 percent of southwest Florida's

population of these endangered marine mammals. Most of the deaths are being attributed to an ongoing red tide of *Karenia brevis*.

This bloom began last January in the Gulf of Mexico off Tampa. Currents moved it to coastal inlets, bays, and the mouths of rivers, where boaters began reporting dead manatees in March. *K. brevis* blooms in Florida can last from 30 days to 18 months.

The state retrieves each manatee carcass to study the cause of death. Because most bodies were recovered in areas hit by the algal bloom, observes Elsa M. Haubold of the State of Florida's Fish and Wildlife Research Institute, her team had little doubt what it would find: classic but nonspecific internal damage, such as tissues weeping blood. She notes that this occurs because the blood often doesn't coagulate in brevetoxin-poisoned manatees.

An antibody-based test for brevetoxins is confirming the poisons' presence in vital manatee tissues and body fluids. Physicians use similar antibody-based tests to diagnose various diseases in people.

Still, understanding how the algal compounds kill remains a challenge, notes veterinarian Greg Bossart of Harbor Branch Oceanographic Institution in Fort Pierce, Fla., who developed the brevetoxin test. He and other scientists used to think that disorientation triggered by nerve damage might be drowning the manatees. Bossart now reports that the algal poisoning isn't only neurological.

In many instances, chronic inhalation of brevetoxins contributes to the destruction of red blood cells, resulting in severe anemia. This can lead to lung hemorrhages during which the animals “drown in their own fluids,” Bossart says. He likens the condition to the human infection known as toxic shock syndrome.

However, inhalation is hardly the only route of poisoning. In 2002, months after a red tide had come and gone, nearly 3 dozen manatees died after entering their spring feeding grounds south of Sarasota, Fla. Some of them died abruptly, with their mouths still full of sea grass.

Leanne Flewelling of the state's Fish and Wildlife Research Institute headed a team that used Bossart's then-new antibody test to identify large amounts of brevetoxins adhering to sea grass the manatees had been eating.

The researchers' findings, reported in the June 9 *Nature*, might explain some manatee deaths this year. Several animals succumbed while eating sea grass.

The 2002 study also found high concentrations of brevetoxins in fish swimming in an area that was previously a red tide zone. “Until now,” the authors note, “it was uncertain whether live fish could accumulate and transfer [lethal amounts of] brevetoxins,” because even relatively low concentrations of these poisons usually kill fish.

The finding of brevetoxins in fish offers a dietary explanation for the deaths last year of 107 bottlenose dolphins off Florida's Panhandle. Flewelling and her colleagues propose that tainted fish poisoned the dolphins.

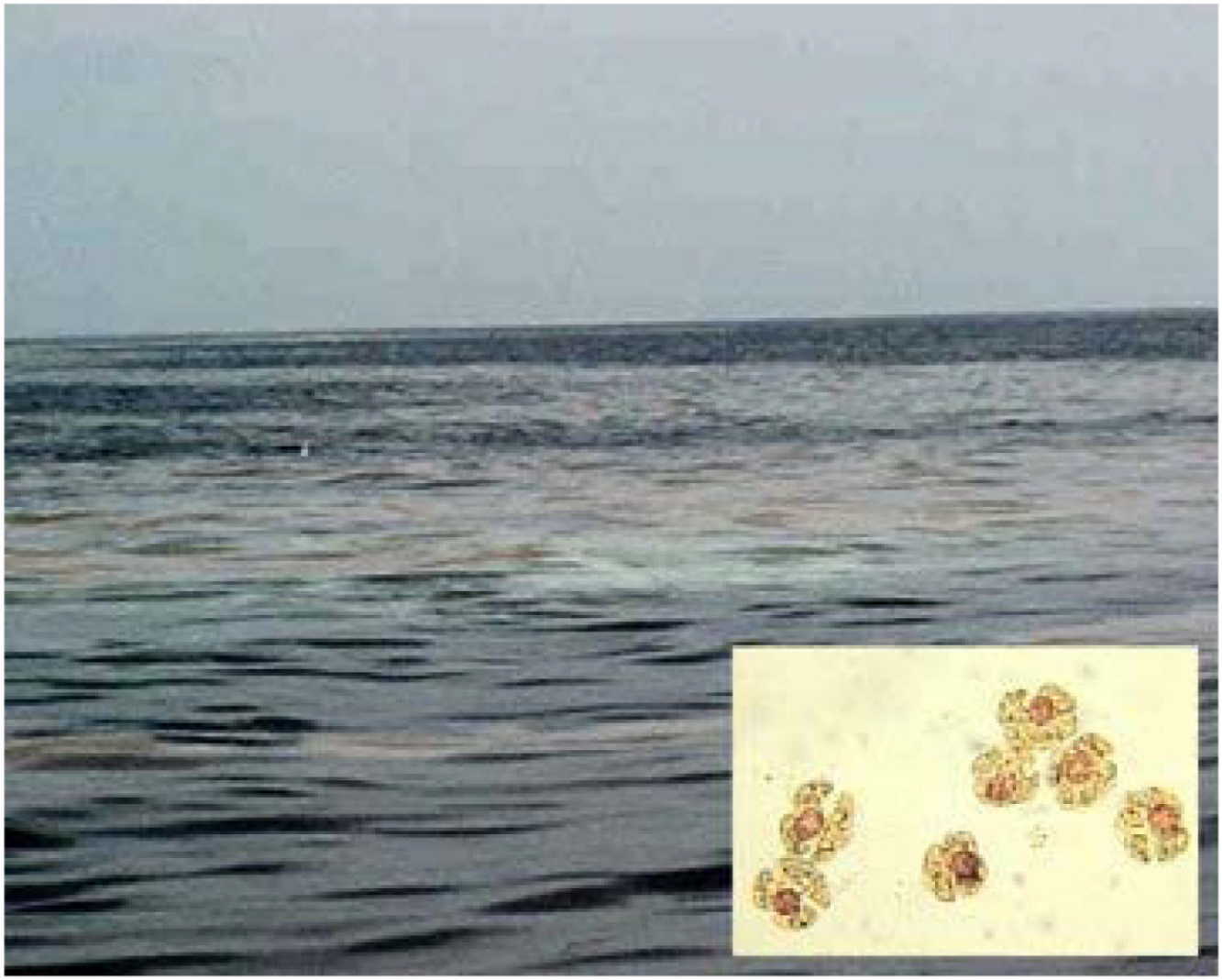
References

1. Abraham, WM.; Baden, DG. Effects of inhaled brevetoxins in allergic airways: Toxin-allergen interactions and pharmacologic intervention; Environmental Health Perspectives. 2005. p. 632-637.Available at http://ehp.niehs.nih.gov/members/2005/7498/7498.html

2. Abraham, WM.; Baden, DG. Airway responses to aerosolized brevetoxins in an animal model of asthma; American Journal of Respiratory and Critical Care Medicine. 2005. p. 26-34.Abstract available at http://ajrccm.atsjournals.org/cgi/content/abstract/171/1/26
3. Backer, LC.; Kirkpatrick, B.; Baden, DG. Occupational exposure to aerosolized brevetoxins during Florida red tide events: Effects on a healthy worker population; Environmental Health Perspectives. 2005. p. 644-649.Available at http://ehp.niehs.nih.gov/members/2005/7502/7502.html
4. Baden, DG., et al. Natural and derivative brevetoxins: Historical background, multiplicity, and effects; Environmental Health Perspectives. 2005. p. 621-625.Available at http://ehp.niehs.nih.gov/members/2005/7499/7499.html
5. Baden, D. Florida red tides: Molecular therapeutics from a sea of red; Society of Toxicology 44th Annual Meeting and ToxExpo; New Orleans. March 6-10; 2005.
6. Benson, JM.; Bossart, GD.; Baden, DG. Inhalation toxicity of brevetoxin 3 in rats exposed for twenty-two days; Environmental Health Perspectives. 2005. p. 626-631.Available at http://ehp.niehs.nih.gov/members/2005/7497/7497.html
7. Bourdelais, AJ.; Baden, DG. A new polyether ladder compound produced by the dinoflagellate *Karenia brevis*; Journal of Natural Products. 2005. p. 2-6.Available at http://pubs.acs.org/cgi-bin/sample.cgi/jnprdf/2005/68/i01/abs/np049797o.html
8. Bourdelais, J.; Benson, J., et al. Use of metabolomics to identify biomarkers for brevetoxin exposure; Society of toxicology 44th Annual Meeting and ToxExpo; New Orleans. March 6-10; 2005.
9. Bourdelais, AJ.; Baden, DG. Brevenal is a natural inhibitor of brevetoxin action in sodium channel receptor binding assays; Cellular and Molecular Neurobiology. 2004. p. 553-563.Abstract available at http://dx.doi.org/10.1023/B:CEMN.0000023629.81595.09
10. Bourdelais, AJ.; Baden, DG. New fish-killing alga in coastal Delaware produces neurotoxins; Environmental Health Perspectives. 2002. p. 465-470.Available at http://ehp.niehs.nih.gov/members/2002/110p465-470bourdelais/bourdelais-full.html
11. Cheng, YS.; Baden, DG. Concentration and particle size of airborne toxic algae (brevetoxin) derived from ocean red tide events; Environmental Science & Technology. 2005. p. 3443-3449.Abstract available at http://pubs.acs.org/cgi-bin/abstract.cgi/esthag/2005/39/i10/abs/es048680j.html
12. Fleming, LE.; Backer, LC.; Baden, DG. Overview of aerosolized Florida red tide toxins: Exposures and effects; Environmental Health Perspectives. 2005. p. 618-620.Available at http://ehp.niehs.nih.gov/members/2005/7501/7501.html
13. Fleming, LE.; Kirkpatrick, B.; Abraham, W.; Baden, DG. Initial evaluation of the effects of aerosolized Florida red tide toxins (brevetoxins) in persons with asthma; Environmental Health Perspectives. 2005. p. 650-657.Available at http://ehp.niehs.nih.gov/members/2005/7500/7500.html
14. Gibson, JE.; Baden, DG. The effect of brevenal on DNA damage in human lymphocytes induced by brevetoxins 2 and 3 (PBTX-2 or 3); Society of Toxicology 44th Annual Meeting and ToxExpo; New Orleans. March 6-10; 2005.
15. Gold, EP.; Bourdelais, AJ.; Baden, DG. Brevenal, a natural brevetoxin antagonist: Competitive antagonist or allosteric modulator; Society of Toxicology 44th Annual Meeting and ToxExpo; New Orleans. March 6-10; 2005.
16. Naar, J.; Flewelling, L.; Baden, DG. A competitive ELISA to detect brevetoxins from *Karenia brevis* (formerly *Gymnodinium breve*) in seawater, shellfish, and mammalian body fluid; Environmental Health Perspectives. 2002. p. 179-185.Available at http://ehp.niehs.nih.gov/members/2002/110p179-185naar/naar-full.html
17. Flewelling, LJ.; Baden, DG.; Bossart, GD.; Haubold, EM.; Heil, CA., et al. Brevetoxicosis: Red tides and marine mammal mortalities; Nature. 2005. p. 755-756.Abstract available at http://dx.doi.org/10.1038/nature435755a
18. Milius, S. Many cyanobacteria make a neurotoxin; Science News. 2005. p. 285Available to subscribers at http://www.sciencenews.org/articles/20050430/note13.asp
19. Raloff, J. Taming toxic tides; Science News. 2002. p. 344-345.Available to subscribers at http://www.sciencenews.org/articles/20021130/bob8.asp
20. Raloff, J. Rain of foreign dust fuels red tides; Science News. 2001. p. 207Available to subscribers at http://www.sciencenews.org/articles/20010929/note17.asp

21. For up-to-date information on red tides in U.S. coastal waters, go to http://www.marinelab.sarasota.fl.us/~mhenry/rtupdate.phtml.

22. The Harmful Algae Page from the National Office for Marine Biotoxins and Harmful Algal Blooms at Woods Hole Oceanographic Institution is available at http://www.whoi.edu/redtide/



1. .
RED IN NAME ONLY. Because Karenia brevis cells (inset) aren't red, substantial blooms of the microscopic algae create a “red tide” that's actually a muddy yellow-green or brown. Fish and Wildlife Res. Inst., Fla. Fish & Wildlife Conserv. Comm.; (inset) Mote Marine Laboratory

2. .
TRAPPED BY TOXINS. Manatees often can't evade brevetoxins when red-tide algae infest the warm haunts that the marine mammals need to survive during the winter.
Fish and Wildlife Res. Inst., Fla. Fish & Wildlife Conserv. Comm.