Florida Sea Grant Project Synopsis

1. Report Status: Interim: Final: X
2. Date: 11/04/2010
3. Project Title: Carbon Fixation by Hard Clam Aquaculture in Florida
4. Project Number: PD-09-10
5. Investigators: Baker, Patrick; Baker, Shirley
6. Institution: University of Florida
7. Dates Covered: 09/01/2009 through 08/31/2010
8. PROJECT RESULTS:

A. Attainment of Technical Objectives:

Objective 1. Identification of significant processes in the sources and losses of long-term carbon fixation in Florida commercial clam culture.

This objective was met. The technical findings (processes + quantities) and conclusions are combined with those for objective 2 (below).

Objective 2. Quantification of important forms of carbon fixation across different mineralized and refractory organic materials produced by commercial clam culture in Florida.

This objective was met. The technical findings (processes + quantities) and conclusions are combined hereafter with those for objective 1 (above).

Northern hard clams, Mercenaria mercenaria, are commercially cultivated in Florida, USA. The shells of the clams mineralize carbon as calcium carbonate, providing a long-term sink for atmospheric carbon dioxide. In addition to the clams sold to the market, there are discarded or dead shells as a byproduct of the industry, plus the shells of associated fouling organisms such as oysters. Almost all shell material (>99%) over 5 mm was accounted for by marketable clams (76.2%), shells of dead Mercenaria (14.7%), and oysters (Crassostrea virginica and Ostrea equestris)(4.9 and 3.6%, respectively). The remaining shell fraction was dived among at least 39 additional species.

Six-month exposure studies of clam and oyster shells showed no significant dissolution, inferring (as suggested by the fossil record) that clam shells are stable form of carbon storage. Laboratory analysis of shells showed that cleaned, dried clam or oyster shell is about 96% calcium carbonate (the remainder is a protein matrix), and calcium carbonate is 12% carbon by weight. Using these values to adjust mineralized carbon values in samples, we estimated total carbon mineralization represented by each clam sold (2.93 g), carbon mineralization per hectare per year in Florida (8965 kg), and total mineralized carbon per year in Florida (534 metric tons in 2008).

B. Outcomes and Impacts:

C. Problems Encountered:

FORM I. Project Summary Information Input (Formerly NOAA Form 90-2)

INSTITUTION: Florida Sea Grant College Program
ICODE: 1200
TITLE: Carbon Fixation by Hard Clam Aquaculture in Florida

INITIATION DATE: September 1, 2009
COMPLETION DATE: August 31, 2010

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S.G. FUNDS: $4736 MATCHING FUNDS: $0

OBJECTIVES: 1. Identification of significant processes in the sources and losses of long-term carbon fixation in Florida commercial clam culture. 2. Quantification of important forms of carbon fixation across different mineralized and refractory organic materials produced by commercial clam culture in Florida.

METHODOLOGY: Sources of refractory organic carbon (shell proteins) and mineralized carbon ($CaCO_3$) in Florida hard clam aquaculture will be documented and quantified. Major processes or variables affecting long-term carbon sinks in shellfish aquaculture that may need further quantification will be identified. Element analysis will be used to identify possible non-carbonate mineral fractions of shells. Exposure experiments will be used to examine the

RATIONALE: Bivalve mollusk growers consider their industry to be environmentally sustainable in terms of water quality, but some preliminary analysis suggests that bivalve aquaculture may also address the issue of excess atmospheric carbon dioxide. Mollusks fix carbon in refractory proteins that are part of their shells but, more significantly, the shells themselves are 12% carbon by weight. These provide very long-term carbon sinks, much longer than any other agricultural products, and rates of fixation are comparable to softwood forests. Simply calculating the shell produced by marketable product, however, underestimates the carbon fixation rate, because of the fractions resident in non-harvested product and other organisms (other bivalves, barnacles, etc.) associated with the aquaculture process.

INTRODUCTION AND RATIONALE

The culture of bivalve mollusks (clams, oysters, etc.) is a sustainable agricultural practice on three levels. First, unlike for the production of most modern sources of animal protein, the bivalve grow-out process uses naturally occurring phytoplankton, rather than artificially harvested or harvested feeds (Castagna, 2001; Matthiessen, 2001). Second, populations of filter-feeding bivalves reduce seston levels in natural waters, which process generally improves water quality (Huang et al., 2005; Fulford et al. 2007).

The third reason bivalve aquaculture can be considered sustainable is that a significant fraction of the planktonic biomass removed is converted into calcium carbonate $CaCO_3$ (shell), which is 12% carbon by weight (or 44% carbon dioxide). The carbonate comes, in part, from dissolved atmospheric carbon dioxide. Bivalve shells comprise one of the various biogenic sources of limestone; in Florida, USA, limestone composed of bivalve shells is known as *coquina* (Bishop and Lee, 1961). Shells of cultured bivalves, therefore, represent a long term carbon sink. Long-term carbon sinks are of interest as means to offset carbon released from fossil fuels. Other carbon sinks, such as plant matter, have higher carbon content by weight (46% in cellulose, 65-70% in lignin), but these are non-mineralized, representing much shorter-term carbon sinks (excepting fossilization), and are mainly limited to terrestrial ecosystems.

The shells of commercial mussels (*Mytilus* or *Perna* species) are around 15-30 g dry weight, or about 1.8-3.6 g carbon per mussel within the mineralized portion (all shell masses from P. Baker, *unpubl. data*). The shells of hard clams (*Mercenaria mercenaria*) are about 20-40 g for "pasta" size clams and 25-60 g for "littleneck," or 2.4-7.2 g carbon. The shells of oysters (*Crassostrea* species) are highly variable, but are typically between 40 and 120 g (4.8-13.2 g carbon).

Carbon mineralization rates vary; for example, clams in Florida can produce commercial products in a single year, while the same species in some other regions may take three years to reach market size (Vaughan and Sturmer, 1994). In Florida, where hard clams culture is a significant industry, clams reach commercial size (pasta or littleneck) in about 1 to 1.5 years. Using numbers obtained from the industry (L. Sturmer, University of Florida Aquaculture Extension, *unpubl. data*), this works out to a range of about 0.9 to 8.4 metric tons of carbon per hectare fixed as harvested *Mercenaria* shell per year. This compares favorably with carbon fixation by southern pine forests (Binford et al., 2006), for example but, again, represents a potentially much longer-term sink than wood biomass. Additionally, clam culture is not associated with methane (a greenhouse gas) production, unlike the storage of carbon in wetlands (Whiting and Chanton, 2001).

No other food agricultural process is comparable to molluscan culture in terms of carbon fixation. Most farmed plants do not have mineralized skeletons. The mineralized skeletons of vertebrates (birds, mammals, fish, etc.) are composed of calcium phosphate, not calcium carbonate. Shrimp (crustaceans) have weakly mineralized exoskeletons, but the vast majority of carbon is in the form of long-chain carbohydrates (chitin).

Carbon mineralization by mollusks is well-understood, but the above numbers used as examples within bivalve culture tend are subject to variables that, in balance, tend to *underestimate* total levels of mineralization. There are unknown and variable rates of shell degradation and dissolution, but there are also additional forms of carbon mineralization. In Florida, clams are cultured in mesh bags in designated lease areas, which were chosen by the state of Florida because they were soft bottom areas that were otherwise unproductive for shellfish (Florida Department of Agriculture and Consumer Services, 2004). These mesh bags provide an environment for other shell-bearing marine organisms, including other mollusks and barnacles, both within and on the bags. There is always some such production of other species (Fajans et al., 2007) and, while it has not been quantified, it may approach that of the farmed clams themselves. Additionally, while there is some mortality of clams prior to harvest, the shells of these clams remain, even though they are not calculated as part of the harvest. In a preliminary study (P. Baker, *unpubl. data*), the mass of non-product shell material in and on clam bags averaged about 29% of the total shell per bag.

Another potential carbon sink among mollusks is the refractory protein that comprises portions of the shell. In bivalves, these include the periostracum (outer coating), ligament (on the shell hinge), and the organic matrix of the shell itself. In some taxa such as mussels, the byssus (attachment threads) may also be significant (Coan et al., 2000). Compared to living tissue, these protein components may also represent modest but long-term sinks; shell ligaments have been found in pre-Columbian shell middens in Florida (W. Arnold, Florida Fish and Wildlife Conservation Commission, *pers. comm.*). These protein components have lower mass than the shells, but contain a higher proportion of carbon (35-40% compared to 12% in $CaCO_3$).

Shellfish aquaculture practices vary with region and species, but they almost always involve the production (usually unwanted) of additional shell-bearing invertebrates, and some level of pre-harvest mortality of the target species. We propose to examine these additional forms of carbon mineralization using the successful Florida hard clam industry as a model, with the goal of identifying significant losses or gains of mineralized carbon in the shellfish culture process, and providing preliminary quantitative data that can be used to develop a broader study of this topic across shellfish aquaculture and fisheries.

GOAL AND OBJECTIVES

The underlying goal is to increase our understanding of the role of refractory carbon - especially shell - produced by marine and aquatic invertebrates, in the geochemical carbon cycle. Of the many possible topics within this, we have chosen clam aquaculture because of the quantitative opportunities it provides, and because it is an example of a practice that humans can clearly and easily modify.

Our intermediate goal, upon the successful completion of the proposed project, is to develop a large-scale research program to examine shellfish aquaculture in greater depth. We plan to compare several types of aquaculture (clams, oysters, mussels) in several regions (Florida, Pacific Northwest, New England) and to include medium-term studies that examine the fate of mineralized carbon over time, when exposed to weathering or biological processes. We first, however, need to understand what the important processes are, what the appropriate questions are to ask, and what sort of experimental variability we will be dealing with.

The short-term objectives of this study are as follow.

1. Identification of significant processes in the sources and losses of long-term carbon fixation in Florida commercial clam culture.
2. Quantification of important forms of carbon fixation across different mineralized and refractory organic materials produced by commercial clam culture in Florida.

PROJECT DESIGN

The investigator will work closely with clam growers and processors to estimate long-term carbon reservoirs in the clam-farming process. The project will be divided into two phases: farm observations and experimental studies. Farm observations will collect data on total shell biomass and the fate of shells. Experimental studies will quantify the various components of refractory carbon and short-term post-mortality changes in these refractory carbon components.

Study Area

Farm observations will be conducted in the Cedar Key area, the center of the Florida hard clam culture area. There are several state-designated clam culture lease areas in the vicinity, with a well-documented range of environmental conditions (Florida Department of Agriculture and Consumer Services, 2004-2005).

Farm Observations

Catch statistics available from University of Florida Shellfish Aquaculture Extension program will be used to determine quantities of harvested hard clams, *Mercenaria mercenaria*. These values, combined with shell mass estimates (from prior calculations by these researchers or lab studies, below) will be used to estimate baseline calcium carbonate production, per year per unit area, from *M. mercenaria* aquaculture. Baseline data will be adjusted by considering the following additional sources of carbon, based on observations from the pilot study.

- shells from *M. mercenaria* that died or were lost *prior to* processing but remained in clam culture bags
- shells from *M. mercenaria* that died or were lost *during* processing
- shells from other $CaCO_3$ shell-producing taxa *within* clam bags
- shells from shell-$CaCO_3$ shell-producing taxa *attached to* clam bags

Samples will be collected in the field, directly from the leases in cooperation with growers, because of the possibility of material loss on the way to the processor. Shell samples collected from farms will be mechanically cleaned, either by scraping or boiling and scraping, to remove tissue but not refractory organic matter (periostracum, shell ligament, etc.). Dried shells including this refractory organic matter will be termed *raw* for the purpose of this study. For large quantities of hard-to-remove or small specimens, subsamples will be collected from randomly selected portions of the clam bags. This process will require destructive sampling of the clam bags themselves, and clam growers will be reimbursed.

During the farm-based phase of this project, we will also look for different potential sources of carbon fixation or loss, or sources of error, not considered above. If these cannot be reasonably addressed in the field, they will be noted for additional study at a later date.

The number of clams harvested will be compared to the number of clams initially stocked, per bag. The difference will be compared to the number of dead clams recovered per bag. This will provide an estimate of shell loss from sampling through shell destruction (e.g. by predator crushing or holes in the bag) but, until we have evidence otherwise, it will be hypothesized that this material is lost to the carbon fixation process (e.g. through dissolution of small fragments).

Sample sizes will be based on the standard errors (relative variability) of data for each of at least two culture areas to the east and west of Cedar Key (e.g. Dog Island, Gulf Jackson). At least three replicate bags will be processed in a given sample, from which will be generated means and standard deviations for shell mass and species-specific fractions thereof. Sufficient sampling for this project will have been achieved when the standard errors over repeated samples have stabilized. In the event that this stabilization is never achieved (e.g as a result of seasonal variability), twelve samples of three replicate bags each will be collected from each of at least two areas.

Experimental Studies

Raw shell material (cleaned and dried but not bleached, above) will be further processed in the lab to separately remove two additional forms of non-mineralized carbon: external refractory organic material and internal refractory organic material, both of which may persist in fossil material (Goodfriend and Weidman, 2001; Sarashina et al., 2008).

External refractory organic material (periostracum, ligament) will be removed by treatment in sodium hypochlorite (NaClO) for up to 21 days, after Sikes et al. (1998), depending on preliminary trials, and the shell will again be dried and weighed.

Internal refractory material (the protein matrix within which the crystalline structure resides) will be removed by grinding followed by standard ashing techniques (Brower et al., 1990), and the material will again be dried. This material will then be analyzed chemically by the UF geology minerals testing lab to determine trace element content, primarily to determine the chance capture of other particular matter by rapidly growing shells. These efforts will focus on *M. mercenaria*, the dominant species, and on the two oysters, *Crassostrea virginica* and *Ostrea equestris*. The results will be used to calculate absolute carbon content within the calcium carbonate fraction of shells (null hypothesis: all mineral content = $CaCO_3$), and the carbon content in various refractory components of shells.

The fine fragments that will form a part of all samples will likely contain siliceous sand as a contaminant. Following all other treatments, these fractions of the samples will be dissolved in 5% hydrochloric acid and decanted, and the residue dried and weighed as non-carbonate material.

Samples of shells of important taxa will also be exposed under different conditions for a period of six months, to examine dissolution of calcium carbonate (or potential crystallization). Pre-weighed shell samples, consisting of about 1 kg of intact shells in each of six replicates, will be held in plastic mesh bags. Exposure treatments, based on typical fates of aquaculture shells, include: seawater (seawater tables at the University of Florida lab in Cedar Key), above-ground shell mounds, shallow burial (e.g. as road matrix), and control (laboratory dry storage). Samples will be treated as above for refractory organic material and compared to pre-exposure samples, to estimate change in mineralized carbon content (null hypothesis = no change).

PARTICIPANTS AND CO-SPONSORS

We will work closely with commercial clam growers in Florida; a letter of support is appended.

We will also be cooperating with Leslie Sturmer, University of Florida/Institute of Food and Agricultural Sciences Shellfish Aquaculture Extension; Ms. Sturmer will be coordinating assistance with shellfish growers.

EXPECTED RESULTS, APPLICATIONS AND BENEFITS

This project will document a beneficial and timely side-effect of shellfish aquaculture: the removal of carbon dioxide through long-term fixation in mollusk shells. This should help the shellfish aquaculture industry promote itself as a "green" industry to an increasingly environment-conscious public and government.

Upon completion of this developmental project, we will have the data required to propose a large-scale and longer-term study on the role of shellfish aquaculture in long-term carbon sinks. We will be able to identify the appropriate hypotheses to study, and have the preliminary data we will require to design robust experiments. We also will have the outline of a story with which to persuade policy-makers to pay more attention to this value-added process in aquaculture. Student research will be a critical component of this future full-scale proposal.

LINKS TO OTHER PROJECTS

University of Florida researchers, in collaboration with other institutions, have an ongoing program to further develop and improve hard clam culture in Florida. Currently, there are twp USDA-funded projects to investigate hybrid clams, to increase productivity and, incidentally, carbon mineralization. These projects are as follow:

- Ensuring Sustainability of the Hard Clam Aquaculture Industry in Florida through Evaluation of Stock Hybridization and Stocking Densities, and Initial Assessment of Soil Characteristics (Univ. Florida and Harbor Branch Oceanogr. Inst.). Aquaculture, FL, CA, TX Special Research Grants Program PM 2006-06021.
- Assessment of F1 Hybrids Back Crossed with *Mercenaria mercenaria* to Improve Hard Clam Crop Production during the Summer Months in Florida (Univ. Florida). USDA CSREES Aquaculture, FL, CA, TX Special Research Grants Program.

LITERATURE CITED

Binford, M.W., Gholz, H.L., Starr, G., and Martin, T.A. 2006. Regional carbon dynamics in the southeastern U.S. coastal plain: Balancing land cover type, timber harvesting, fire, and environmental variation. *Journal of Geophysical Research* 111 D24S92, doi:10.1029/2005JD006820.

Bishop, E.W. and Lee, L.L.. 1961. *Rocks and Minerals of Florida. A Guide to Identification, Occurrence, Production and Use*. Florida Department of Natural Resources, Spec. Publ. 8. Tallahassee, Florida, USA. 41 pp.

Brower, J.E., Zar, J.H., and von Ende, C.N. 1990. *Field and Laboratory Methods for General Ecology*. Wm. C. Brown Publishers, Dubuque, Iowa, USA. 237 pp.

Castagna, M. 2001. Aquaculture of the hard clam, *Mercenaria mercenaria*. 675-699. **In** Kraeuter, J.N. and Castagna, M. (eds.). *Biology of the Hard Clam*. Elsevier, New York, New York, USA.

Coan, E.V., Valentich Scott, P., and Bernard, F.R. 2000. *Bivalve Seashells of Western North America*. Santa Barbara Museum of Natural History, Santa Barbara, California, USA. 764 pp.

Colson, S. and Sturmer, L. 2000. One shining moment known as Clamelot: the Cedar Key story. *Journal of Shellfish Research* 19: 477-480.

Fajans, J., Sturmer, L., Baker, S., Hulen, K., and Cassiano, E. 2007. What's in the clam bag? Shellfish Aquaculture Extension, University of Florida, Gainesville, Florida, USA. [http://shellfish.ifas.ufl.edu/clambag/ accessed April 22, 2009]

Florida Department of Agriculture and Consumer Services. 2004. Florida's aquaculture lease program. Division of Aquaculture, Florida Department of Agriculture and Consumer Services, Tallahassee, Florida, USA. http://sondes.floridaaquaculture.com/sondes/sonde_maphome.htm [accessed April 30, 2009]

Florida Department of Agriculture and Consumer Services. 2004-2005. Real-Time Live Data Sondes. Division of Aquaculture, Florida Department of Agriculture and Consumer Services, Tallahassee, Florida, USA. http://sondes.floridaaquaculture.com/sondes/sonde_maphome.htm [accessed April 30, 2009]

Fulford, R.S., Breitburg, D.L., Newell, R.I.E., Kemp, W.M., and Luckenbach, M. 2007. Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. *Marine Ecology Progress Series* 336: 43-61.

Goodfriend, G.A. and Weidman, C.R. 2001. Ontogenetic trends in aspartic acid racemization and amino acid composition within modern and fossil shells of the bivalve *Arctica*. Geochimica et Cosmochimica Acta 65: 1921-1932.

Huang, S.-C., Jeng, S., and Hsieh, H.-L. 2005. The function of filter-feeding bivalves in aquatic ecosystems: using Tapeng Bay as an example. *Journal of the Fisheries Society of Taiwan* 32 (1): 17. [abstract]

Matthiessen, G. 2001. *Oyster Culture*. Fishing News Books/Iowa State University Press, Ames, Iowa, USA. 162 pp.

Sarashina, I., Kunitomo, Y., Iijima, M., Chiba, S., and Kazuyoshi Endo, K. 2008. Preservation of the shell matrix protein dermatopontin in 1500 year old land snail fossils from the Bonin islands. *Organic Geochemistry* 39: 1742-1746.

Sikes, C.S., Wheeler, A.P., Wierzbick, A., Dillaman, R.M. and de Luca, L. 1998. Oyster shell protein and atomic force microscopy of oyster shell folia. *Biological Bulletin* 194: 304-316.

Vaughan, D.E. and Sturmer, L.N. 1994. The development of clam aquaculture through demonstration projects on the Gulf Coast of Florida. *Journal of Shellfish Research* 13: 323. [abstract]

Whiting, G.J. and Chanton, J.P. 2001. Greenhouse carbon balance of wetlands: methane emission versus carbon sequestration. Tellus. Series B, Chemical and physical meteorology: 53: 521-528.