Developing a Propagation Technique for Native Illinois River CWCP Mussels

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Abstract

Propagation of rare mussel species has become a viable restoration tool in some areas of the upper Mississippi River Basin. Propagation has proven difficult in Illinois due to accumulation of sediment in propagation cages. The goal of this project was to develop a captive propagation and rearing technique for *Ellipsaria lineolata* using The Nature Conservancy's wetland restoration sites. It was hoped that a successful propagation technique would become a critical tool used in species recovery on the Illinois River. Successful propagation of mussels relies on a number of factors including host fish survivability and productive mussel stockpiles. Transformer fitness is contingent on suitable site conditions, water quality parameters, and methodical care and placement of the animals. Our study demonstrated that host fish survivability increased with delicate collection, treatment of fungal disease, and by limiting time in the cage arrays only to critical days of the transformation process. Findings indicated that glochidial abundance within brood mussels increased when the stockpiled mussels overwintered in water depths of 2-3 meters, likely due to higher oxygen levels and less sedimentation build up. Finally, we found that the greatest challenge we faced was finding a location where suspended organic and inorganic solids would not fill the cages and suffocate the juvenile mussels. This challenge warrants future research on site placement and techniques best suited for choosing an experiment location with limited sediment deposition. The goal is to adapt these techniques and develop a reliable method for propagation and rearing of freshwater mussels in backwaters of the Illinois River.

Introduction

A greater proportion of freshwater mussels are considered "Species in Greatest Need of Conservation" (29 of 61; 48%) than any other taxonomic group; an additional 19 species are extinct or extirpated (Illinois Wildlife Action Plan, III. B, pp. 31-33). Approximately 49 species of freshwater mussels have been documented from the Illinois River, but fewer than half are still present. The butterfly mussel, *Ellipsaria lineolata*, formerly relatively abundant and widely distributed in the Illinois River, has nearly been extirpated from the river since the early 1900's. The species is of special concern in the state and is listed in the Illinois Wildlife Action Plan (Illinois Wildlife Action Plan, X Appendices-Appendix I, p. 295). The reported fish hosts (freshwater drum, green sunfish and sauger) for the butterfly mussel, necessary for completion of the unionid life cycle, are present in the Illinois River and are abundant in many sections of the river. This mussel's elimination from the Illinois River has been attributed to siltation and poor water quality. Recent studies indicate that some species of freshwater mussels have begun to recover as conditions in the Illinois River improve; however, those mussels still have source populations located in the Illinois River or its tributaries which can provide the individuals needed for recolonization.

Historic general distribution ranges for *E. lineolata* include the Mississippi River drainage from western Pennsylvania west to Minnesota, south to eastern Iowa, Kansas, and Oklahoma (Parmalee and Bogan 1998). *E. lineolata* once present in the Illinois River in significant numbers has in recent history been all but eliminated. Surveys of the Illinois River dating back to 1870 showed *E. lineolata* present from 1870 through 1912 and subsequent surveys in 1969 showed it as absent from the system (Starrett 1971). The most recent survey recorded only one individual at mile marker 48.4 in the Alton pool (Whitney et al. 1997). Interviews with resource professionals and literature review indicate no viable populations of *E. lineolata* remaining within the Illinois River or its tributaries. To reintroduce this species, or other extirpated species, to the Illinois River requires a means of reestablishing a source population. The source population should be a regional ecotype adapted to the water and climate of the area and also be of sufficient size to provide adequate numbers of individuals to establish a reproductively viable population in the river. With adequate water quality and the necessary fish host for completion of the mussel's life cycle, mussels which have been extirpated could be reestablished, restoring some of the mussel diversity formerly characteristic of the Illinois River. This project directly meets the following objective for the butterfly mussel: "Experimental propagation, modeled after *Lampsilis higginsii* (Higgin's Eye) efforts in the Mississippi, should be refined with other species in other systems…and evaluated as an effective conservation action." (Illinois Wildlife Action Plan, F: Research, Monitoring and Evaluation; p.101)

Objectives: The original objectives of the proposed research were to:

1) **Investigate the suitability of Spunky Bottoms for butterfly mussel propagation by reviewing water quality and food availability data which has already been collected on site.** An alternate site proposed would be The Conservancy's Emiquon Preserve. Since this grant proposal development, Emiquon has undergone extensive restoration activities and water has returned to the site. Suitable sites with dependable water levels have been identified and could be used if water levels diminish or water quality parameters fall outside parameters at Spunky Bottoms. Timeframe: May - June 2008. Estimated % of budget: 5

2. **Collect approximately 20-40 gravid female mussels from pool 19 of the Mississippi River.** Transfer mussels to Genoa national Fish Hatchery for host infestation procedure. Timeframe: July 2008. Estimated % of budget: 10.

3. **Propagate mussels with host fish from the Genoa, WI fish hatchery using two water quality treatments, aerated and unaerated, in 10 cages each at the TNC's Illinois River backwater restoration site at Spunky Bottoms near Meredosia, IL** TNC's Emiquon Preserve near Havana, IL may serve as a suitable alternate location for the project if needed. Timeframe: August 2008- June 2010. Estimated % of budget: 40

4. **Monitor and compare treatments of propagated mussels at project site (Spunky or Emiquon alternate).** Timeframe: August 2008-June 2010. Estimated % of budget: 30.

5**. Determine sites along the Alton reach of the Illinois River for mussel reintroduction using historic bed distribution.** Timeframe: April-May 2010. Estimated % of budget: 3.

6. **Reintroduce propagated mussels at determined sites along the Illinois River.** Timeframe: June 2010. Estimated % of budget: 2.

7. **Complete analysis of monitoring data to determine differences between propagation techniques.** Timeframe: June 1-October 1, 2010. Estimated % of budget: 5.

8. **Produce final recommendations and project report.** Timeframe: December 31, 2010. Estimated % of budget: 5.

Expected Results

The proposed propagation techniques have been successfully used on the Higgin's Eye mussel and the proposed project will test the success of applying the techniques to another species of mussel as required in the IWAP (p. 101). Currently conservation professionals within the Upper Mississippi River basin have experienced some success using similar propagation techniques within main stem river and connected floodplain areas. However, certain realities of these systems such as flooding, current, sedimentation, and exotic species have been limiting factors to widespread success. If the techniques developed through this project prove successful, the process could be a model for other mussel species propagation work in controlled backwater environments and will help restore mussel diversity to the Illinois River. Baseline information on growth and development of the butterfly mussel will be collected which can later be compared to historical data as well as provide a metric for future projects with this or other mussel species. Presentations on the methods and results of this research study will also be presented at scientific conferences.

This project will move Illinois closer to the statewide objective for mussels: maintain populations at all currently occupied locations and reestablish at 50% or more historic locations where suitable habitat persists or can be restored (Illinois Wildlife Action Plan, C; p.40). The Streams Campaign identified one of the priority actions as restoring populations of imperiled and extirpated aquatic animals, including reintroducing native species when decimating factors have been eliminated and natural recovery is unlikely (Illinois Wildlife Action Plan, Streams Campaign Action 4b; p. 63 and Illinois Wildlife Action Plan, F: Research, Monitoring and Evaluation; p.101).

Approach

Objective 1: **Investigate the suitability of the site for butterfly mussel propagation by reviewing water quality and food availability data which has already been collected on site.** Project collaborators will review water quality data, water levels, and algal community composition collected by TNC staff and Western Illinois University staff in previous monitoring activities. The previously gathered data is not reflected in the overall project budget. TNC's Emiquon Preserve may act as an alternate project site if Spunky Bottoms is deemed unsuitable because of low water levels or other variables such as dissolved oxygen and food availability. Emiquon may be suitable to use as a backup site also in the event of a catastrophic flood overtopping of the Spunky Bottoms river levee system. This event would be highly unlikely though given the fact that the current levee system is substantial and no flood overtopping has occurred at Spunky Bottoms since the levees were installed in the 1930's.

Objective 2: **Collect approximately 20-40 gravid female mussels from pool 19 of the Mississippi River.** Transfer mussels to Genoa national Fish Hatchery for host infestation procedure.

Female butterfly mussels will be collected from Pool 19 of the Mississippi River. There is a large population of these mussels at this location and the location is geographically similar, with approximately the same latitude and climate, to the proposed Illinois River propagation and release sites. The shell of *E. lineolata* is noticeably dimorphic with the females generally being smaller in relation to males as well as being more swollen and inflated posteriorly (Parmalee and Bogan 1998). From expert reports a substantial number of butterfly mussels can be found in shallow, off-channel sections of Pool 19 and they are easily located by "puddling". Mussels will be collected between June and August when the butterfly is typically gravid. Adult gravid females will be transported to the fish hatchery at Genoa, WI with hatchery tank equipment from IDNR Fisheries. As part of the Higgins Eye recovery plan, personnel at this site have successfully completed the parasitic glochidial phase of the mussel life cycle.

Project staff will assist IDNR staff at the Havana Fisheries Field Station in the construction of twenty floating cage systems. Plans and a prototype have been acquired from the Genoa Fish hatchery and will be used in construction of the cages.

Objective 3. **Propagate mussels with host fish from the Genoa, WI fish hatchery using two water quality treatments, aerated and unaerated, in 10 cages each at the TNC's Illinois River backwater restoration site at Spunky Bottoms near Meredosia, IL. .** Known hosts for *E. lineolata* are green sunfish, freshwater drum, and sauger (Fuller 1978). All these are locally abundant and also can be obtained from the hatchery as certified VHS free. These fish will be infested at the Genoa hatchery and then transported to TNC's Illinois River backwater restoration site at Spunky Bottoms near Meredosia, IL (or the Emiquon alternative). The infested fish will be placed in cages held in the backwater lake adjacent to the Illinois River. This provides a controlled environment where disturbance can be minimized and water quality conditions can be monitored. Ten cages will be placed in each of two treatments, aerated and unaerated. Cages will be secured by means of aircraft cable and up to six concrete anchors per cage. The aeration system will be secured similarly and buoyed by a PVC float. The areas chosen for the cage arrays is naturally isolated and secluded from strong winds by berms made from ditch spoil piled up from years of ditch maintenance.

After parasitic infestation and glochidial transformation is complete, which typically takes up to three weeks; host fish will be removed by project staff and released at the project site. The selected host species are already present at Spunky Bottoms and the proposed alternative site.

Objective 4. **Monitor and compare treatments of propagated mussels at project site (Spunky or Emiquon alternate).** One numbered cage will be selected by random number generation for each treatment and juvenile mussel survival will be monitored at the beginning and end of the growing season in May and October. Growth rates will be determined based on shell length using calipers. Mussels will also be weighed in grams using small field suitable scales. Relative weights (Wr) will be calculated and recorded for comparison to known wild population Wr from literature. Survivorship and growth rates will be compared between treatments. Growth rates of the propagated mussels will also be compared to rates established from the parent population. This will be completed during the two propagation years of the project.

Water quality parameters such as dissolved oxygen, total dissolved solids, temperature, pH, and turbidity will be measured and recorded using two Yellow Springs Instrumentation long term deployment eco-sonde systems. These systems will be cleaned and data downloaded to a lap top computer once every other month for the duration of the project. Project staff will compile the water quality data into table form for analysis and storage.

Objective 5. **Determine sites along the Alton reach of the Illinois River for mussel reintroduction using historic bed distribution.** During the final propagation season T. Hobson along with D. Corgiat and D. Sallee will consult historic mussel bed distribution records and confer with other resource professionals such as the Illinois Natural History Survey's Long Term Monitoring Program staff to determine a suitable site for the propagated individuals to be reintroduced.

Objective 6. **Reintroduce propagated mussels at determined sites along the Illinois River.** Once shell length has reached approximately 30 mm (about 2 years growth) individuals will be uniquely marked and placed at determined sites within the Alton Reach of the Illinois River for subsequent monitoring during the next season in July. Placement of the mussels will be accomplished through direct over board distribution. All future monitoring will be conducted by TNC and IDNR staff outside the scope of this project. The site will be uniquely identified using hand held GPS in the field and recorded for return.

Objective 7. **Complete analysis of monitoring data to determine differences between propagation techniques.** Project collaborators will determine propagation success through analysis of data gathered throughout the project life. Success and or failure of the technique will be measured based on the number of individuals produced and their growth rates to determine differences between propagation techniques. Lessons learned through the project may ultimately benefit not only butterfly mussel on the Illinois River but other species of concern as well. Currently propagation success seen on the Mississippi River with Higgin's eye mussel and select others has not been able to be duplicated on the Illinois basically because of the lack of trials and investigation.

Objective 8. **Produce final recommendations and project report.** Denim Perry and Tharran Hobson along with assistance from project collaborators will compile data into paper and poster form for scientific presentation and review. The importance of this project is in determining the best approach to captive propagation in an isolated Illinois River backwater environment. If successful this approach can be duplicated

at one of three similar sites along the Illinois. Proper presentation and review is a key to understanding the process and disseminating information to conservation professionals throughout the region. Authors, contributors, and editors will include D. Perry, T. Hobson, D. Sallee, S. McClure, and Doug Blodgett. Paper presentations will be made at The Governor's Conference on Restoration of the Illinois River, the 70'Th Annual Fish and Wildlife Conference, and The Nature Conservancy's Central U.S. Region science conference.

Emergency contingency plans

The river levee system was constructed at Spunky Bottoms in the 1930's and has never been over topped during a flood event. If this unlikely scenario presents itself the cages can be salvaged and the mussels, host fish, and cages can be transferred via hatchery truck to the Emiquon Preserve less than one hour away. River levels within one foot of the levee top and forecast rise will trigger the emergency transfer.

Location

Nature Conservancy's Spunky Bottoms Preserve is located in Sections 5-8, T2S, R1W and Sections 12-14, T2S, R2W, in Brown County, Illinois and is in Congressional Districts 18 & 20. The Preserve lies between a high bluff locally known as Spunky Ridge and the Illinois River, and is across from the U.S. Fish & Wildlife Service's Meredosia National Wildlife Refuge(Map 1).

An alternate location, The Nature Conservancy's Emiquon Preserve is located in Sections 3,4,5,8,9,16,17, and 20, T4N-R4E, in Fulton County along the Illinois River(Map 2).

Original Project Schedule

2008

May T. Hobson & D. Perry spend 4 days @ Havana Field Station constructing 20 cages.

June T. Hobson, D. Perry & S. McClure to spend 2 days setting up the 20 cages at Spunky Bottoms Preserve.

June T. Hobson, M. Lemke & D. Perry spend 1 day to install water monitoring equipment.

July-August T. Hobson, D. Perry, S. McClure & M. Lemke of TNC and D. Sallee & D. Corgiat of IDNR spend 2 days collecting gravid female butterfly mussels on Pool 19 of the Mississippi River and transport to the Genoa hatchery. They will be assisted by volunteers from Western Illinois University (WIU)'s Kibbe Station and Rick Anderson of WIU.

August/September IDNR Hatchery truck picks up infested fish at Genoa fish hatchery and delivers to project site. T. Hobson, D. Perry, & S. McClure spend 1 day to place fish in cages at Spunky Bottoms Preserve.

October T. Hobson & D. Perry spend 1 day to release the host fish into project site waters.

November S. McClure & M. Lemke spend 1 day to download data, clean & calibrate monitoring equipment.

2009

January S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.

March S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.

May S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.
T. Hobson & D. Perry spend 1 day to record and assess total mussel population count, size, and weight measurements for a randomly selected cage.

July S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.

October S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.
T. Hobson & D. Perry spend 1 day to record and assess total mussel population count, size, and weight measurements for a randomly selected cage.

2010
January S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.

March S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.
T. Hobson reviews literature and expert analysis of best locations for mussel re-introductions. Grant project team meets to review project and select sites for mussel reintroduction.

May S. McClure spends 1 day to download data, clean & calibrate monitoring equipment.
T. Hobson spend 1 day to record and assess total mussel population count, size, and weight measurements for each cage.

June D. Corgiat of IDNR and T. Hobson, D. Perry & M. Lemke of TNC spend 2 days to mark and release mussels on selected historic bed sites on the Alton Reach of the Illinois River, located near the Spunky Bottoms Preserve.
June – December T. Hobson, S. McClure, D. Perry, Doug Blodgett will compile data and write final reports.

Contact Information

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ESTIMATED COSTS:

BY OBJECT CATEGORIES	**FEDERAL SHARE**	**STATE** IDNR MATCH	**SHARE** TNC MATCH	**TOTAL MATCH**	**TOTALS**
SALARIES AND WAGES	$1,900	$7,726	$10,500	$18,226	$20,126
FRINGE BENEFITS	$760		$4,200	$4,200	$4,960
TRAVEL			$1,000	$1,000	$1,000
EQUIPMENT					
SUPPLIES/COMMODITIES	$20,600				$20,600
CONTRACTUAL	$1,500				$1,500
CONSTRUCTION					
TOTAL DIRECT CHARGES	$24,760	$7,726	$15,700	$23,426	$48,186
INDIRECT CHARGES				$6,286	$11,238
F&A 20% TDC	$4,952				
F&A 25.02% TDC		$1,933			
F&A 23% TDC			$3,611		
Unrecovered F&A (20% v. 23%)			$742		
TOTALS	$29,712	$9,659	$20,053	$29,712	$59,424
	50%	16%	34%	50%	100%

RELATED GRANTS: none

Budget Justification

Salaries and Wages:

PROJECT PERSONNEL, FEDERAL SHARE
TNC Restoration Technician, Perry, estimate 20 days over the 3 year project period =$1900

PROJECT PERSONNEL, MATCH (STATE/TNC SHARE)
TNC project manager, Hobson, estimate 30 days over the 3 year project period =$4500
TNC project assistant, McClure, estimate 14 days over the 3 year project period =$1300
TNC aquatic ecologist, Lemke, estimate 14 days over the 3 year project period =$2400

TNCI IL River Program Director, Doug Blodgett, estimate 7 days over the 3 year project. period =$1700
TNC Illinois Director of Science, Herkert, estimate 3 days over the 3 year project period =$600
IDNR biologist, Corgiat & IDNR professionals, Sallee and Mick
3.3 days/year/person over the 3 year project period =$7726

IDNR staffs, Dan Sallee and Dean Corgiat are co-investigators on the project reviewing data for the project site and contributing professional experience. Sallee and Corgiat will also be involved in picking gravid female mussels on the pool 19 of the Mississippi. Sallee will accompany an IDNR hatchery truck in transporting host fish to the project site from Genoa Hatchery. Sallee and Corgiat will provide periodic review and oversight to the project as well as contributing to the final report paper.

Jim Mick and/or Havana field office staff will build cages. Mick will provide some project oversight and review.

Fringe Benefits: Federally approved fringe benefits rate for TNC is 40% of salaries.

Travel: ALL MATCH (TNC SHARE) - estimate $400 for Genoa hatchery trip, $300 for Mississippi River trip, and $300 for project meetings over the 3 year project period

Equipment: No funds are requested for purchase of equipment.

Supplies/Commodities: ALL FEDERAL SHARE – TNC will purchase the following supplies which are shown with their estimated costs

2 water monitoring sondes @ $4,000 each	8,000
oxygen bubbler	1,800
calipers, cables, weights, to make & keep the monitors operational	600
materials to build 20 fish cages	10,200
Total Supplies/Commodities	20,600

Contractual: ALL FEDERAL SHARE -
Funds are requested to pay for water sample analysis. Contractor TBD.

Compliance:
Dan Sallee has mussel collection permit for taking brood stock from Mississippi.

Genoa National Fish Hatchery will provide certified VHS free fish for transport into Illinois.

The IDNR will use its CERP (Comprehensive Environmental Review Process) as a tool to aid the Department in meeting NEPA compliance for the project outlined under this grant proposal. It is the Department's policy to require CERP applications for all land disturbing activities unless those activities are covered by CERP exemptions (see the enclosed Comprehensive Environmental Review Process documents).

All work identified in this proposal is believed to be addressed by categorical Exclusion 1.4B(1). If exceptions are identified or the scope of the work changes during the execution of the proposed projects, the Federal Aid Division of the USFWS will be contacted to determine if additional NEPA compliance actions are needed.

All planned activities will also be in compliance with the Endangered Species Act. All determinations and documentation will in accordance with the current established U. S. Fish and Wildlife Service protocols for Section 7.

All planned activities will be in compliance with the National Historic Preservation Act and the Council on Historic Preservation Act. All determinations and documentation will be in accordance with the terms of the Programmatic Agreement, as amended, effective September 23, 2002.

When applicable, those planned activities which involve a floodplain and/or jurisdiction wetlands will be done in accordance with Presidential Executive Orders 11988 and 11990.

When applicable, those planned activities which involve the use of pesticides, herbicides or other comparable chemicals wills be done in accordance with current state and federal regulations to assure the safe and legal application of those chemicals. All chemicals will be applied in accordance with the manufacturer's label instructions. All persons applying chemicals will be licensed by the Illinois Department of Agriculture as a chemical operator along with a licensed applicator, in accordance with Illinois state law.

Project Modifications

1. **Requested and received a grant extension.**
   Due to river hydrology, grant timing, and mussel reproductive cycle an extension for the project was allotted until December 31, 2012.

2. **Change project location.**
   During the 2008/2009 propagation experiments, we concluded to move the site location to The Nature Conservancy's Emiquon Preserve. Above average precipitation coupled with high turbidity levels(Graph 2) at the Spunky Bottoms site influenced our decision to change locations to an area at Emiquon with more stable hydrological conditions(Graph 4).

3. **Alter target specie for propagation.**
   Brood butterfly mussels became difficult to locate within our time constraints. To continue with propagation experiments we chose to exchange butterfly mussels and freshwater drum host fish with plain pocketbook and largemouth bass host fish.

Methods

2008

Prior to the end of July 2008, Illinois River Program field staff Tharran Hobson, Denim Perry, Sally McClure, Dave Hedrick and Mark Jones worked to bring all the pieces to the complex propagation study project in line. Denim worked for a week with IDNR's Jim Walker constructing 20 cages and 4 floating racks at the Havana DNR Field Station (Picture 1). After the intricate aluminum fabrication and welding was complete Dave, Denim, and Tharran deployed the cage racks to two separate study arrays at the Spunky Bottoms Preserve (Picture 2(Map 1)). Mark had already spent part of a day reworking electrical wiring at the old pump station and the site was readied for installation of the oxygen bubbler system. The original experiment design involved attempting captive rearing of *E. lineolata* at Spunky Bottoms in two different locations at that preserve(Map 1). One control group would have no aeration system in place and the other set of cage arrays would have an aeration system suspended off the bottom substrate near the cages. The idea was to see if the aeration aided in keeping both host fish and/or transformed mussels alive in oxygen rich waters if anaerobic conditions were present.

On July 30th 2008 Denim and Tharran met up with the crew assembled to help find female butterfly mussel brood stock on the Mississippi. Experts from both the Illinois Department of Natural Resources and the Illinois Natural History Survey were on hand to assist the larger crew from Western Illinois University's Kibbe Field Station. Ten high school students from around the country participating in an Earth Watch training program through WIU made up the bulk of the enthusiastic crew along with Professors. All twenty-four of our search team boarded WIU's impressive river vessel to head out on the water (Picture 3). At the Devil's Island bed, once known for its diversity and high density of mussels, we saw some of the effects of 2008's devastating Mississippi River floods. Searching was difficult in the waist deep murky waters as flooding deposited a 4-6 inch layer of flocculent silt over the previously sandy bed. This day yielded but one of the elusive butterfly mussels giving credibility to the rare and threatened designation.

The next day was more fruitful as we headed up stream from Ft. Madison, IA to a promising bed along the Dallas City riverfront. The crew spent about six hours puddling the waist deep waters that extended out some 200 yards from shore. Puddling is essentially scouring the river substrate with your hands and feet feeling for mussels of any species. When one is discovered it's pulled from the substrate to be identified (Pic. 4). Many mussels were present as the site was spared the sediment deposition seen at Devil's Island. Twenty one different species in all were sampled including the black sand shell and finally the elusive butterfly mussel. The day saw us collect 57 butterfly mussels in all.

The success set the wheels in motion. Tharran got in contact with our IDNR partners and the search for the host fish, freshwater drum (*Aplodinotus grunniens)* was on. Two fisheries crews were dispatched to the Illinois River setting nets which would yield over 200 host fish by the next afternoon. Dan Sallee of IDNR drove a hatchery truck down from Peoria with the fish on Friday evening and Tony Brady of the USFWS drove eight hours from the Genoa National Fish Hatchery to join us. The inoculation process was overseen by Tony Brady and Dan Sallee, experienced propagators. The process began by carefully spreading or opening the mussel shell and inserting a rubber wedge to keep the mussel open (Pic. 6). The experienced eye now observes the mussels marsupium and looks to see if it is "inflated". If so a hypodermic needle containing water is carefully inserted into the marsupium and the water flushes the glochidia into a collecting dish(Pic. 11). The nearly microscopic glochidia can then be drawn up into a syringe and each fish inoculated by squirting the water across its gills(Pic. 12). One must be careful to not allow too many glochidia to infest the host fish gills as to many may stress the fish. Light spots on the fishes red gills are visible to the naked eye during the infestation however; to be sure successful inoculation takes place a representative host may be sacrificed and viewed under the scope(Pic. 8,9,11,12,and 13). An alternative method is to flush the glochidia as described into a holding tank with the fish in them(Pic.21). As the water passes over the host fish gills in the tank glochidia may attach. Things began to look down again though as one after one the mussels were checked for gravidity and only a lone female had glochidia present. We inoculated what fish we could with

the glochidia present and decided the other butterfly mussels should go into a stockpile for use at a later time when they did prove to be gravid(Pic. 6,7,8,9). This year's timing put us on the hunt for butterfly mussels at the one time in the season when they have discharged glochidia and are charging for the next breeding cycle. We knew this may be the case but were forced with flood events and grant award timing to search this time of the year. Our hope was that we would find enough females that had not fully expended glochidia for the summer.

To adapt to the realities of the situation we quickly worked with what we had and caged a few fish we had inoculated. Our new strategy was to get as much information from this season as possible, stock pile and monitor our brood mussels, document host fish longevity in captive environments. One big unknown about this captive rearing process is the ability to hold the host fish for the full mussel transformation phase which is between two and four weeks. The team decided to place drum in the holding cages to test survival rates in higher water temperatures of late summer and place our brood mussels into an over-wintering stockpile. We placed a few fish in select cages and monitored them in the two test situations; aerated and non-aerated cage arrays. With water monitoring devices in place we gathered data on Ph, dissolved oxygen, water temperature, and conductivity. Twenty four freshwater drum were placed in the rearing cages on 1 August 2008. Twelve drum were separated in four cages of three each in the two different arrays of aerated and non-aerated. During the two week time period diurnal water temperatures fluctuated between 26.8 C and 31 C. On 14 August 2008 we pulled the cages and found zero surviving drum in either treatment. Assumptions were made that due to high water temperature oxygen levels became too low, particularly at night, and the fish suffocated.
The team concluded that late summer is not conducive to captive mussel propagation due to the bradytictic female butterfly having discharged glochidia prior to the months of June and July. Also, it was found that high water temperatures preclude holding the host fish for a sufficient time period to allow transformation.

Even though this first attempt fell short of what we hoped for, Tony Brady who has been involved in mussel projects on the Mississippi for ten years reminded us that their first attempt to propagate Higgins Eye mussels in the mid 1990's yielded 3 mussels. The next year they raised 1,100 and estimates have them producing tens of thousands in following years. To our team the butterfly mussel is worth an equal effort. 57 butterfly mussels collected near Dallas City on the Mississippi were sent with Tony Brady of the Genoa National Fish Hatchery to be placed in a holding stockpile near Prairie du Chein, Wisconsin. All adult mussel brood stock was returned to the bed near Dallas City. In a measure to ensure we had a good back up plan in case the original stock of 57 butterfly mussels prove to not be gravid or go missing from the stockpile we decided to create a second stockpile at Spunky Bottoms. Water quality parameters continued to be monitored as part of the project. Denim Perry pulled the YSI units twice that summer to download data, clean, and calibrate. Rob Hilsabeck, IDNR began stocking adult freshwater drum into a holding pond at Banner Marsh. These fish would be used as hosts' spring 2009 and then returned to the river. This stockpile would give a better chance of locating drum for the project beyond the usual netting and electro-fishing in the river that often gives mixed results.

Later in August 2008 we accompanied Dean Corgiat (IDNR) and Missouri Department of Natural Resources staff and located another viable population of butterfly mussels near Hannibal, Missouri on the Mississippi River. This location even better matched latitude coordinates with our project site. In association with another contracted mussel survey we were able to pick up an additional 107 butterfly mussels. The mussels were brought to Spunky Bottoms and placed in a 4'x4' holding cage with substrate and dropped to the bottom in six feet of water. In the stockpile was a mix of mature male and female brood mussels that would most likely result in female gravidity by spring.

2009

On 24 February 2009 team members Dan Sallee (IDNR), Tony Brady (USFWS), and Tharran Hobson (TNC) met in East Moline, Illinois to review 2008 and discuss the upcoming propagation season strategy. Tony suggested using drum as a propagation host may be impossible because of their fragile nature. Tony reported Freshwater drum are the only know host for butterfly even though some report limited success with green sunfish or sauger (Fuller 1978). The team decided to modify the study slightly and include black sandshell mussel (*Elliptio dilatata*) whose host is walleye (*Stizostedion vitreum*). After inoculation, walleye would be put in a subset of cages in the two different treatments. The hardier walleye might give an indication of drum suitability for butterfly propagation if the walleye survived and drum did not. *E. dilatata* brood stock were available from near the Quad Cities and walleye host would be obtained from the Excellon hatchery also nearby. If the project has success in producing *E. dilatata* they would be relocated to Mississippi Poll 13 or 14 near their parental stock origin. Hatchery walleye stock will be destroyed after their use.

Rob Hilsabeck (IDNR) and Tharran Hobson accompanied a local commercial fisherman on 31 March 2009 to raise nets on Peoria Lakes. 56 drum were secured and transported to Jake Wolf Memorial Fish Hatchery and placed in a holding tank to condition. The fish were treated every third day with a 1% salt solution by Larry Willis to promote better health.

Tony Brady arrived at Jake Wolf on 6 April 2009 with 48 walleye and 8 brood *E. dilatata*. The butterfly mussels overwintered very well at Spunky and we pulled 55 gravid females for inoculation. Inoculation went as planned and we returned 48 infested walleye and 48 drum to separate holding tanks at the hatchery in 54 degree water(Pic. 11,12,13,14). Over the next 2 weeks water temperatures were slowly elevated to near 64 degrees for optimal glochidia development on the host fish.

Mussel propagation experts developed a technique for tracking glochidia transformation as it relates to water temperature and duration of parasitism. It is known that glochidia develop best at temperatures above 50 degrees with the optimum being in the range of 60-75 degrees. To adequately compile a transformation time period, propagators consider every degree of water temperature above 50 degrees for one day a “degree day”. Therefore, one day of infestation at 60 degree water temperature equates to 10 degree days (60-50=10, 10x1 day= 10 degree days). Two days at 60 degrees would be 20 degree days (60-50=10, 10x2 days= 20 degree days). Degree days compound by the day and temperature over 50 degrees. Through other propagation work such as that done by Genoa National Fish Hatchery it has been found that *E. dilatata* normally transform at around 350 degree days(Table 1). No good estimate was available for transformation period of *E. lineolata*.

Tony Brady transported three infected walleye and one drum to his lab at Genoa and placed them in an observation aquarium to monitor degree days and transformation period. *E. dilatata* transformed off walleye at around 400 degree days. *E. lineolata* transformation came at about the same time period. An estimated 3,850 transformers developed from 3 walleye.

The project team decided the best approach was to hold the infected fish at Jake Wolf Hatchery until the mussels were close to transformation. A concern was holding fish in the confined rearing cages at Spunky for too long a time period and this might lead to higher mortality. At the hatchery the fish could be kept in cleaner water and their health monitored closely.

On 17 April 2009 one deceased walleye and one drum were examined by Larry Willis (IDNR) using a 40x-100x pathology scope. Examination detailed heavy infestation with as many as 6 encysted glochidia per single gill filament(Pic. 13). Through examination it was estimated that a single infested fish may be hosting as many as 2,000 encysted glochidia. Once again on 23 April, 2009 a deceased walleye was examined and similar results were found. In a single 40x field of view 32 encysted glochidia were observed. At this time measurements were performed and the developing glochidia size ranged from 250-300 microns.

On 22 April 2009 Larry Willis and Tharran Hobson performed treatment on the drum for a fungal condition that was affecting 8 fish. Hydrogen Peroxide (H2O2), a common anti-fungal used in hatcheries was chosen for the treatment. Literature search revealed a study performed to determine H2O2 tolerance thresholds of

encysted glochidia on large-mouth bass. The study revealed tolerance levels to 100 parts per million before encysted glochidia slough off occurred. On Larry Willis's recommendation we started with a small sample size of 3 drum at 100 ppm H2O2. The 3 fish showed loss of equilibrium at the first ten-minute interval and they were removed to fresh water. As suspected, drum as a species, are not as tolerant to chemical treatment as large-mouth bass. Three additional fish were later tested using a concentration of 50ppm. At the first ten-minute interval the fish appeared unaffected by the chemical. After one hour the three fish were removed from the treatment. All fish were treated for one hour with a 50 ppm solution of H2O2 and it appeared effective in removing the fungus. The next day (23 April, 2009), one of the worst fungal infected fish died and its gills were examined. No sign of glochidia slough-off was observed.

Water temperature for calculating degree days was observed and documented for the next week. Based on Tony Brady's findings on degree-day transformation it was determined all of the fish should be moved to rearing cages at Spunky by 7 May 2009 when they would be near 300 degree-days (Table 1). On 30 April 2009, with the assistance of Rich Lewis (IDNR) the team decided to move 12 drum and 15 walleye to rearing cages at Spunky rather than wait too long and possibly miss a transformation window with all the fish. On 7 May, 2009 the remaining hatchery fish were moved with 22 drum and 33 walleye going to rearing cages. In total 34 drum and 48 walleye were now distributed among 20 cages at Spunky Bottoms (Table 2).

Ten cages in one array are aerated and the remaining ten at another array were not. A Yellow Springs Instrumentation water monitoring device was stationed at each array. The YSI units' measure dissolved oxygen, temperature, total dissolved solids, and Ph. The units were deployed early April of 2008 and were cleaned and calibrated every three weeks.

All host fish remained in cages until 27 May, 2009. Based on continued temperature monitoring it was determined the fish had passed 750 degree days, well beyond typical transformation time (Table 1). Walleye obtained from the hatchery were removed and destroyed while drum obtained from the Illinois River were returned to the river. No walleye hosts were lost during cage confinement time. 5 out of 34 drum were lost to mortality during cage confinement.

Next steps for the project team were to continue to clean and calibrate monitoring equipment and sample cages. In late October 2009 two cages selected at random from each treatment array will be examined by sifting the substrate with a fine mesh sieve. If practical all juvenile mussels would be counted and a subset measured to begin tracking growth rates. The team anticipated two summer growing seasons will be needed before any mussels produced will be of sufficient size to mark and place in river beds.

Periodic review of water quality parameters were made during scheduled calibration and cleaning of sondes. The sondes recorded these parameters continuously every 15 minutes except during calibration or maintenance. Dissolved oxygen ranges showed typical diurnal fluctuations for backwater wetlands with daytime levels between 5-10 milligrams per liter and nighttime lows occasionally going as low as 1.5 mg/liter. Data gathered at Emiquon during the same time period showed similar diurnal patterns (Graph 1). There was no data indicating prolonged low oxygen events in the water column adjacent to the cage arrays. Turbidity levels showed abnormally high spikes periodically in July and August. Turbidity measured in late July showed peek concentrations of over 750 NTU's (Graph 2).

On 6 October 2009 a team of Hobson, Lewis, Perry, Sallee, Herndon, and Brady assembled to pull the cages for sampling. Both racks of cages were pulled at each treatment location and transported to shore for sampling. A three stage sieve provided by Brady was used to sift the substrate for any juvenile mussels. The sieve system is used extensively for sampling mussel rearing cages at Genoa Hatchery and other locations on the UMR. It is designed to filter substrates down to 1 mm(Pic. 15,16,17). Based on growth patterns of juvenile mussels the team estimated any mussels present should be in the 5-20 mm range.

During sieving it was noted that all cage floor sand substrates were covered completely with a flocculent sediment layer of up to 8 cm in thickness(Pic. 16,17). This dark green/black sediment layer lacked the consistency

of silt and was suspected to be predominantly organically derived from phyto-plankton fallout. No individuals of *E. lineolata* or *E. dilatata* mussels were found during the sampling nor were any shell fragments observed. One juvenile giant floater*, Pyganodon (Anodonta) grandis,* 25mm was found in the substrate of cage 1B. Two other giant floaters (25mm) were located in cage 4B. These two cages also contained two black bullheads each that probably entered through small openings around the lid. The bullhead may have been the host fish for the giant floaters or their presence in the cages disturbed the flocculent sediments enough to allow the floaters to survive.

Water samples taken once monthly from July through September at each cage location were examined by Dr. Susan Meiers and Dr. Larry O'Flaherty of Western Illinois University for presence and abundance of phyto-plankton suspected to have contributed to the high turbidity readings. The samples from Spunky Bottoms did show high abundances of centric diatoms as compared to samples taken from the Emiquon Preserve during the same time frame. In most samples, centric diatoms were 3-4 times more concentrated per milliliter than Emiquon. Pinnate diatom taxons were found to be 2-3 times more abundant per milliliter in Spunky samples as well.

Three small streams feed into the wetland at Spunky Bottoms and abnormally high rainfall lead to run-off events of high magnitude during late summer. Elevated phyto-plankton concentrations coupled with clay sediment particles suspended in the water column as a result of heavy rain events in July and August was the likely source of high turbidity NTU's recorded by the sampling sondes. Dr. O'Flaherty and Prof. Meiers also investigated a sample of the flocculent sediments deposited on the cage substrate and determined the source of the sediment to be predominantly fine clay particles with some organic composition. Most notably, the sample contained high concentrations of bacteria, euglenophytes, and protozoans. Meiers and O'Flaherty's professional opinion indicates the flocculent sediments contained high enough concentrations of micro-organisms to reduce oxygen levels within the sediment layer enough to suffocate developing juvenile mussels.

Total dissolved oxygen levels recorded in milligrams per liter at each cage array treatment were similar enough to not warrant the use of an aeration system to aid in the propagation of mussels at the Spunky Bottoms site(Graph1). Average DO levels observed maintained within ranges sufficient to support mussel survival (Table 3). Turbidity NTU's measured during the July-September time frame were abnormally high and indicative of high levels of total dissolved solids in the water column (Graph 2). These inorganic and organic particles settled out of the water column at a rate high enough to accumulate an 8cm thick layer in the course of just three months. This rate of sedimentation is likely too high for survival of juvenile butterfly and black sandshell mussel species. 2009's abnormal precipitation events may have to be enough of an anomaly to say that given a normal year, mussel propagation may be possible in the isolated backwaters of Spunky Bottoms. However, the 2009 summer season at Spunky Bottoms was not conducive to propagating butterfly mussels. Denim Perry prepared and presented a paper on our initial project results at the 70th annual Midwest Fish and Wildlife Conference in December. . A feature story on the project was highlighted in The Nature Conservancy in Illinois' Annual report for 2009. There are also links from Nature.org/Illinois to stories about the project.

2010

With one more growing season left in the project cycle the team decided to attempt propagation in Thompson Lake at the Emiquon Preserve. Emiquon was included in the original project scope as a back-up site in case conditions were not good or a flood occurred at Spunky. Continuous water quality monitoring since 2005 has shown adequate DO concentration levels and moderate to low turbidity NTU readings at Emiquon(Graph3,4). Lower total suspended solids in the water column at Emiquon as compared to Spunky indicate sedimentation rates should be significantly lower.

Tharran proposed moving at least one cage array to an isolated location near the pumphouse at Emiquon by spring 2010. The location is in a deep ditch off the main lake near one of our YSI water monitoring

stations(Map 2). Similar protocol would be followed and inoculated fish should be placed in the cages by the end of May.

To accomplish this next round of propagation trials Tharran requested a slight modification to the remaining project budget (Table 1). Tharran asked that a portion of remaining dollars in the supplies category be moved to cover personnel and contracted items. Tharran did not anticipate using all the supply funds allocated in the budget. The additional contract funds would be spent on yearly fees for hosting real time water quality data on the web that is collected from existing YSI Econet water quality monitoring systems at Emiquon and Spunky. Currently the fees are $840 per system each year. Having real time DO, temperature and turbidity data would allow the project team to better track propagation conditions and progress(Graph 3,4). Additional contract funds could also be used to fund a professional diver in April or May on the Mississippi to help collect butterfly brood stock. Last year Tharran accompanied a contract dive team and that is where most of our original stock came from. The dive was funded through a separate survey project and it did not require funds from this budget. Tharran did not anticipate any similar opportunity to get free assistance the following season. A contract dive may cost in the range of $800 to $1,000. With these requested changes the team would be prepared for another attempt at propagating mussels in a controlled backwater environment.

The team planned to re-start the project in the spring of 2010 and relocate the cages to Thompson Lake at the Emiquon preserve(Map 2). A contract diver was located and plans were made for Dean Corgiat and Tharran Hobson to lead the diver to a location south of Quincy on the Mississippi River to gather host mussels where Dean had sampled butterfly mussels in the past. Continued high water and currents on the Mississippi through July lead the team to cancel host gathering and pushed the project into another growing season year. Shawn Canady, Grants Specialist for The Nature Conservancy applied for a project extension in June. As soon as water levels allowed, the team planned to gather host mussels and over-winter them in a holding cage at Emiquon. This method has proven successful in the past as the team was able to hold host mussels through the winter at Spunky Bottoms.

In early July of 2010, Tharran and Denim moved two sets of cage racks to Emiquon to prepare for the next season. By April of 2011 the team would be ready for another attempt at propagation. Jake Wolf Hatchery personnel and IDNR Fisheries Biologists were prepared to assist with fish host gathering and mussel inoculation in April of 2011.

2011

The Mississippi River hydrology voided attempts to make real significant progress this season. River levels were often high with swift currents. Water and weather monitoring equipment (YSI), were cared for and deployed at both sites in hopes to gain more understanding of the sites and there suitability for mussels rearing locations. An extension was applied for and approved to move the grant completion date from December 31, 2011 to December 31, 2012. A collaboration of partners including Iowa DNR, TNC and USFWS collected brood stock in September 2011. Twenty five butterfly mussels and ten black sandshell mussel brood mussels were collected on the Mississippi river near Muscatine, Iowa and were shared between the programs(Pic. 18). Plans were to continue over-winter them at the Emiquon preserve. Mixed sex stock were placed in holding cages shortly after gathering and submerged in a secure area for over-wintering(Pic. 19). The holding cage would then be pulled in spring of 2012 and gravid females removed for host fish inoculation at either Genoa or Jake Wolf Hatcheries. This over-wintering method proved successful for us in supplying gravid females in 2009. Keeping the brood stock in a secure area precludes the uncertainty of spring flooding on the river. Genoa agreed to provide VHS free certified freshwater drum hosts for the inoculation. This required a project staff member to transport fish to Emiquon and Spunky for placement into cages. This would be completed by June 2012. Host fish would be kept and monitored in the cages for a length of time to

allow mussel transformation. Regularly scheduled cage monitoring would be initiated and followed through the fall until adequately sized juveniles can be released in the Illinois River in winter 2012.

2012

April 4, 2012, TNC and IDNR staff pulled the mussel stockpile from Emiquon. All of the butterfly mussels were accounted for. A gravidity check indicated that only a few females were still holding glochidia and none of those were over 30% charged. Our theories as to lack of gravidity was the unusually warm winter caused the females to expel glochidia earlier than normal or the animals experienced low oxygen stressors. Tharran transported the butterfly mussels to Cordova to meet with Nathan Eckert from Genoa National Fish Hatchery. He took possession of the black sandshell we over-wintered for them and also our butterfly mussels. The plan was to do inoculation the next day at Genoa with hatchery raised freshwater drum. Tharran received a call the next day from Nathan reporting that there was not enough glochidia present in the butterfly mussels to do an inoculation. Nathan agreed to return our butterfly stock to near the Fairport site in Iowa.

Even after this most recent set-back, staff and partners wanted to move forward with testing captive mussel propagation techniques in isolated backwater environments. The team made a decision to use a surrogate mussel species in the trials after missing the window of opportunity with the short term brooding butterfly mussel. Plain pocketbook (*Lampsilis cardium),* is an abundant species found at the Fairport bed in Iowa. It is a longer term brooder and tends to be a hardier species that can tolerate marginal growing conditions better than most. All other propagation techniques would remain the same and the site at Emiquon with long term water quality data sets would be used.

On April 12, 2012 Tharran and Denim Perry returned to Fairport with USFWS and Iowa DNR Fisheries to search for plain pocketbook mussels and additional black sandshell (for USFWS). The puddling effort yielded several pocketbook and 9 of those individuals were fully charged females(Pic.20). We also picked up and additional 10 butterfly females, of which none were gravid. We returned the butterfly back to the bed. The 9 female plain pocketbook were transported back to Emiquon and placed in a holding bag in the lake until largemouth bass hosts could be secured. On May 9, 2012, Rob Hilsabeck provided 31 wild, largemouth bass ranging in size from 70 to 280 mm. The next day Tharran, Denim, and Sally were able to extract large quantities of glochidia from four plain pocketbook mussels using a small syringe(Pic. 21). The glochidia were washed into a plastic tub containing approximately 5 gallons of water. An oxygen stone was used to keep the glochidia suspended in the water and bass were introduced in groups according to similar size. Per Nathan Eckert's instruction, approximately every 5 minutes fish were randomly removed from the tub and gills were inspected with a magnifying glass to check for infestation levels(Pic. 22). After 15 minutes most of the fish were found to have sufficient glochidia parasitic coverage on the gill filaments. As we completed the infestation on different size groups we removed to fish to holding cages in the floating arrays(Pic. 23(Table 3)). Nathan Eckert suggested from experience we should leave the fish in cages at the site until after June 15th. This should provide adequate time for transformation.

Water quality parameters such as Total Dissolved Oxygen, Ph, Turbidity, Temperature, Total Dissolved Solids, and Conductivity were continually monitored from a remote sensing sonde adjacent to the propagation site. Staff cleaned and calibrated the equipment on a regular schedule. Two sondes are due to be sent to YSI for routine maintenance and updating this summer. Other sondes in TNC stock were used while the maintenance is performed(Graph3,4).

On October 15, Hobson, Perry, Hilsabeck, and McClure sampled cages(Pic.24,25). Two cages (3D, 3B) were pulled and sieved through the multi-grade sieve. Cage 3D produced 171 specimens and cage 3B produced 18. All specimens were measured for total length and ranged from 5-17 mm. Specimens from 3D were placed into 3C and specimens in 3B were placed in 3A to simply ease cage sampling later. A couple specimens were taken back to the University of Illinois-Springfield, Therkildsen field station, to be properly

identified. After further observation the specimens didn't appear to be *Lampsilis cardium*, but rather appeared to be *Musculium sp.*, commonly known as fingernail clams(Pic. 26,27,28). Plans were made for Hobson, Sallee, and McClure to pull all the cages the next day to confirm findings and look for species of interest. All cages were pulled, contents sieved and several representative specimens were collected to identify in a lab. Then all the cages were combined into one clean cage with fresh sand substrate. Identification of the collected specimens confirmed *Musculium sp.,* were present, however we decided to continue with propagation techniques until further maturation next spring 2013 when all specimens will be more easily identified.

A poster on the propagation project was presented at The Nature Conservancy's Science and Stewardship Conference in Aurora, Nebraska on February 21-23, 2012. The same poster was also presented at University of Illinois-Springfield's annual Emiquon Science Symposium in March 2012.

Conclusions

Although a few preliminary conclusions can be made, it proves difficult to draw any conclusive reports based on concrete evidence at this point. One reason for this is that the final survey of mussels in the propagation cages has not yet been completed. In the spring of 2013 the propagation cage will be surveyed for target species transformers (*Lampsilis cardium*). Results from the 2009 propagation efforts indicated that heavy sedimentation in the systems subsequently inundating the propagation cages resulted in poor conditions for mussel transformers(Graph 2). This is supported by Tony Brady's success in transforming black sandshell mussels and butterfly mussels in the lab from a subset of our inoculated host fishes in 2009. The locations chose for the experiment vary in water quality seasonally due to many contributing factors including precipitation, run-off, wind fetch and algal growth. It has been suggested that prior to continued propagation experimentation, a preliminary study may be performed by placing cage arrays in different locations throughout the preserves to identify areas with less turbid conditions and subsequent sediment fall out.

It was determined that decreasing host drum mortality was possible by careful collection and reducing parasitic disease by treating fish with H2O2 at rates not exceeding 50 ppm. Treatment practices were developed at the Jake Wolf Memorial Fish Hatchery by Larry Willis and Tharran Hobson. Findings showed that 100 ppm of H2O2 reduced drum equilibrium but 50 ppm of H2O2 reduced black fungal infection while not affecting infested glochidia or threatening the host. Holding the fish in a closely controlled environment until required proved a valuable method. Walleye and largemouth bass require less effort to ensure survivability while freshwater drum are more susceptible to mortality due to high water temperature, reduced oxygen, and handling. Additionally we found that gravidity of *E. lineolata* varied from reported documentation. We found that the reported time frame of July-August was much too late to expect gravid *E. lineolata.* During the span of our experiment, the first of June was the end of the expected gravidity period. Another observation we made occurred while over wintering the brood mussels. We found that stockpiled brood mussels over wintered better when stored at a shallower depth of 2-3 meters. Less sediment was observed in the cages and gravidity was higher in the shallower environment.

Future Plans

As mentioned spring 2013 will begin by sampling the remaining cage at the Emiquon Preserve for transformed plain pocketbook mussels. If transformed pocketbooks are identified then the next phase of the experiment begins. That is holding juvenile mussels until they reach 30-50mm before transferring them to a historic mussel bed on the Illinois River.

If we do not identify any transformed *Lampsilis cardium*, we will continue to refine the process by locating more suitable locations within the preserves that experience lower sedimentation and higher DO rates. With the infrastructure associated with the grant project we plan to continue to modify experimental design and adapt propagation techniques to better suit parameters with successful propagation as a future goal.

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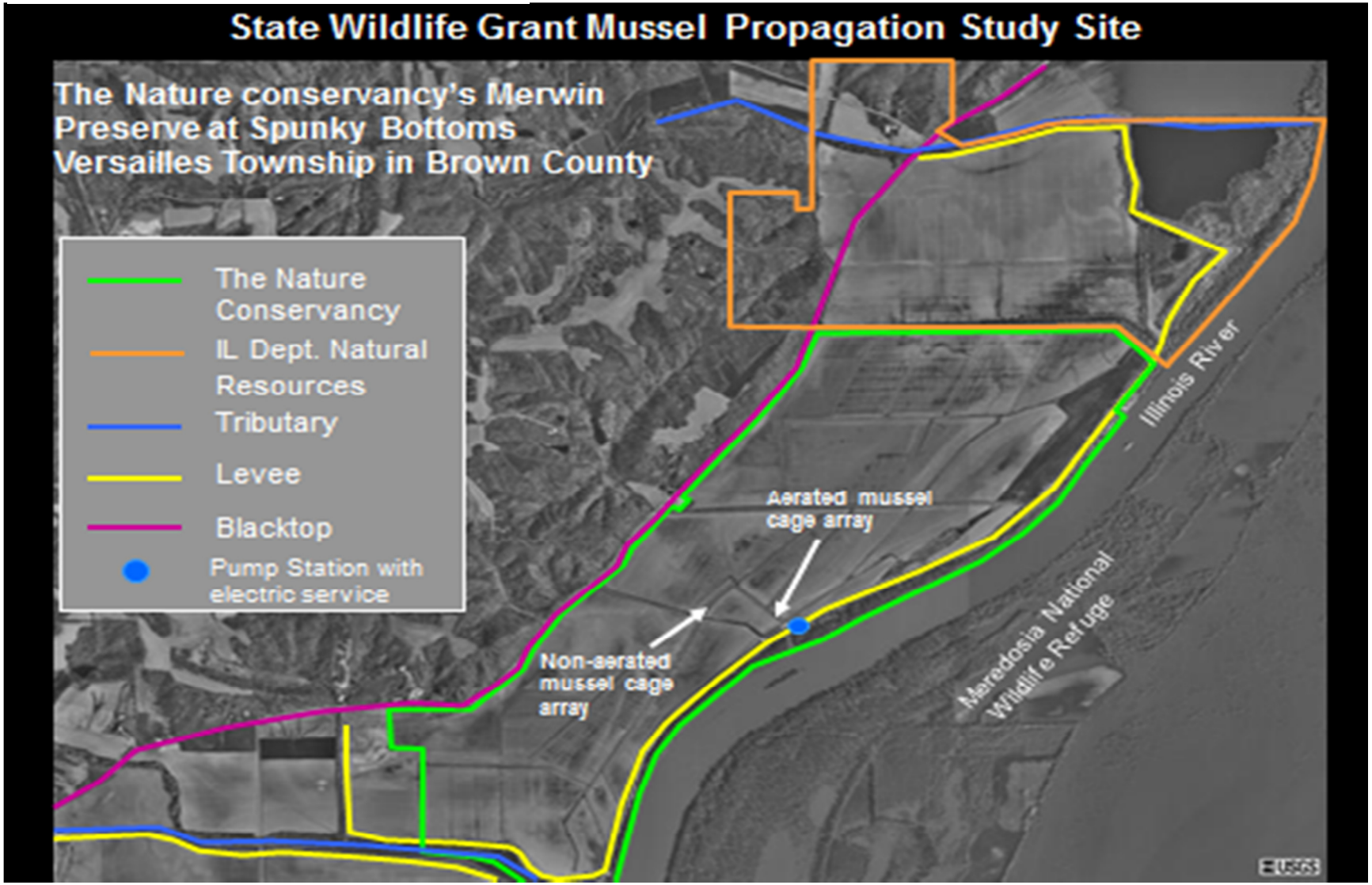
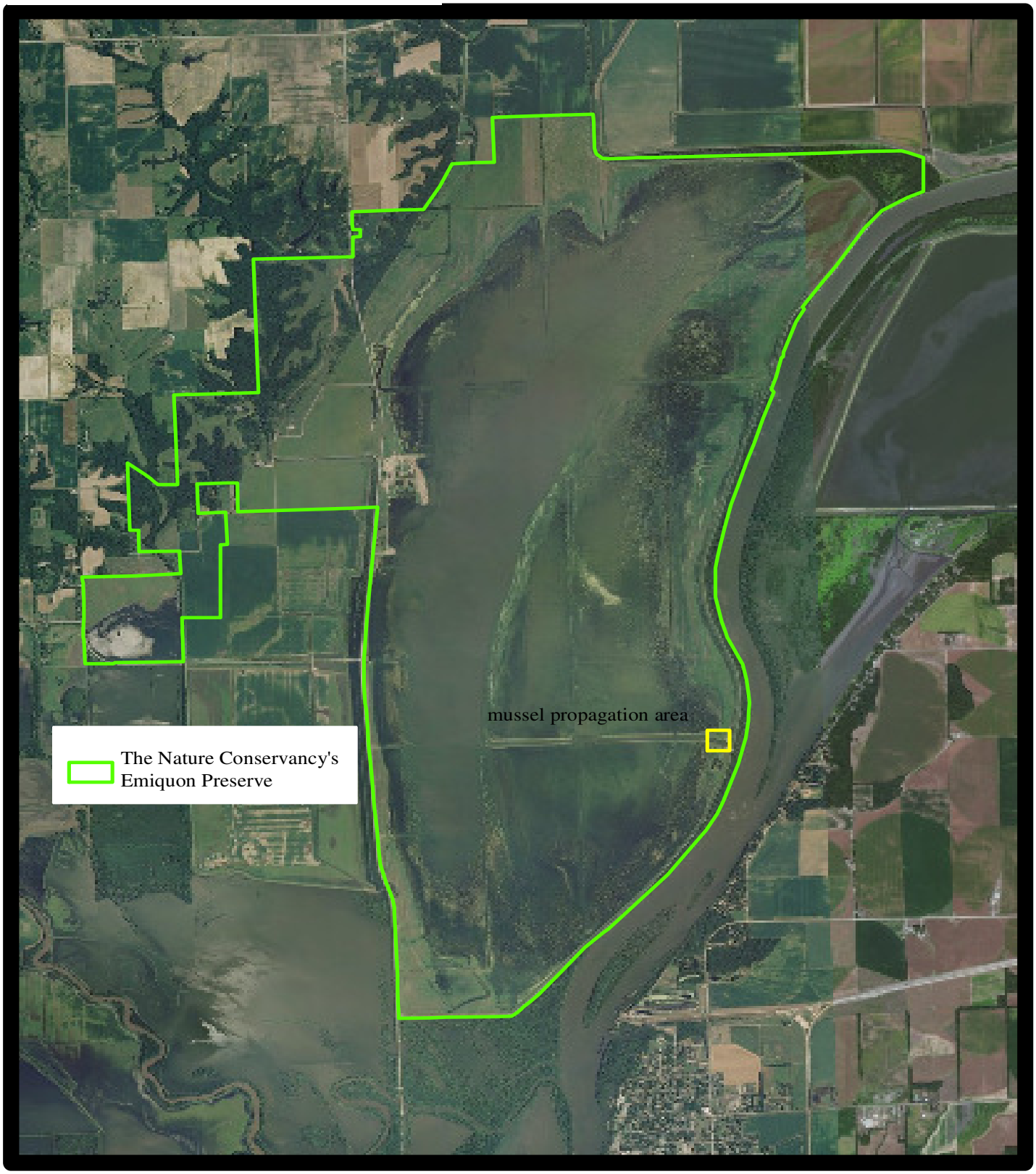
Map 1. The Merwin Preserve at Spunky Bottoms.




mussel propagation area
The Nature Conservancy's
Emiquon Preserve

PICTURES and GRAPHS

Picture 1. Completed cage array containing 4 propagation cages receiving sand substrate.

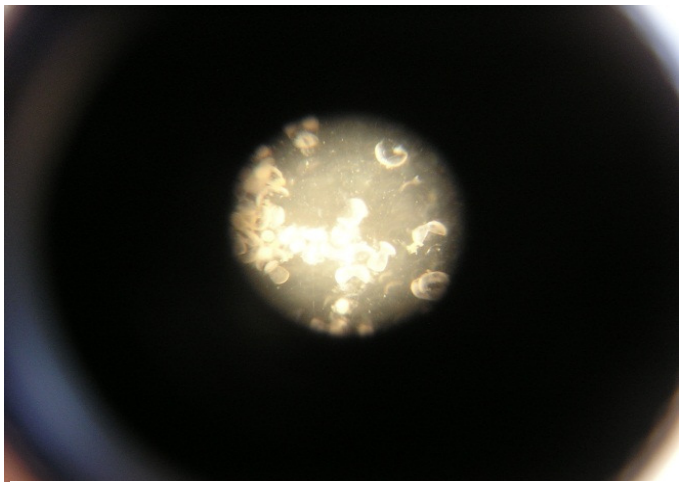
Picture 3. WIU research vessel headed toward Devil's

Picture 4. Collection of E. lineolata on the Mississippi River.

Picture 5. Close view of collected *E. lineolata*.

Picture 6. Wedge inserted in to specimen to better view marsupium and aid in glochidia collection.

Picture 7. 2008 propagation trial at Spunky Bottoms.

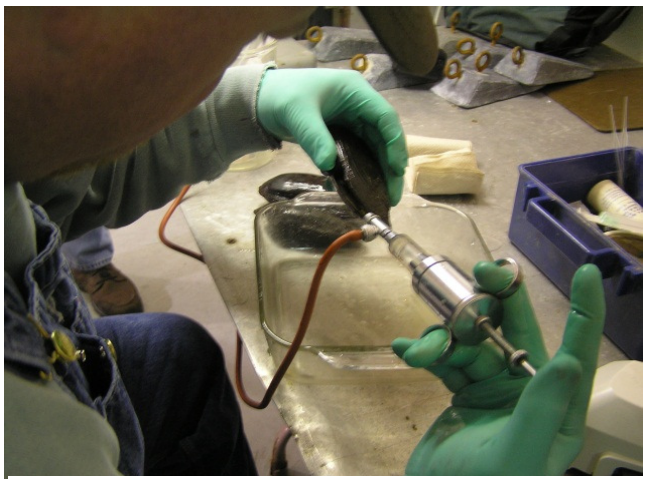


Picture 8. Expelled glochidia in petri dish under magnification.

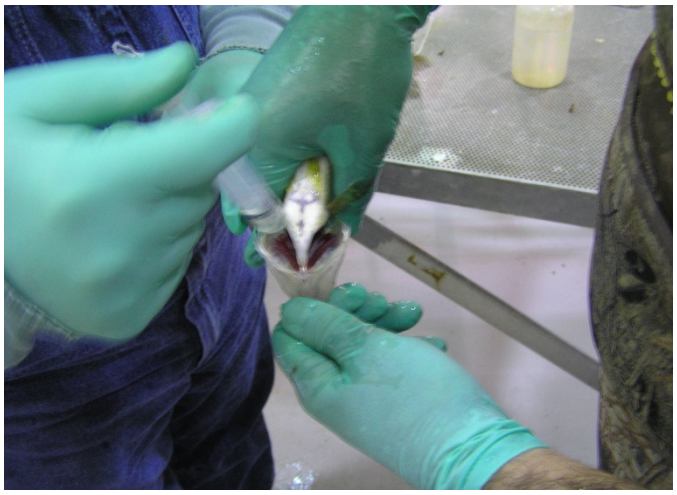
Picture 9. 2008 inoculation at Spunky Bottoms.

Picture 10. Divers and puddlers searching for mussels on the Mississippi River.

Picture 11. Successful mussel collection.



Picture 11. Flushing glochidia from *E. dilatata.*



Picture 12. Infesting host fish in the lab.

Picture 13. Gill filaments infested with glochidia.

Picture 14. Host fish holding tanks at Jake Wolf Memorial Hatchery.

Picture 15. Removing holding cage following expected transformation at Spunky Bottoms.

Picture 16. Base of holding cage (note sedimentation).

Picture 17. Sieving cage contents, looking for transformers.

Picture 18. 2011 overwintered specimens that proved to not be gravid.

Picture 19. Preparing cage for overwintering mussels, 2011.

Picture 20. Collected mussels for 2012 propagation. *E. lineolata* were not gravid.

Picture 21. Flushing *L. cardium* glochidia, 2012.

Picture 22. View of host fish gills after inoculation, 2012.

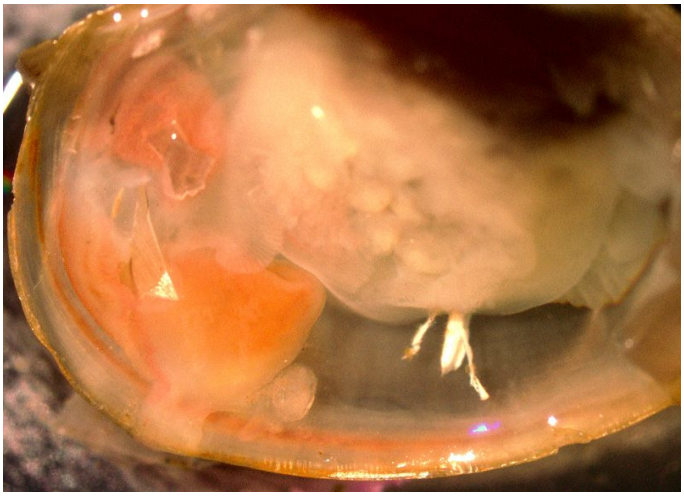
Picture 23. Placing host fish in cage array, 2012.

Picture 24. Looking for transformed mussels, 2012.

Picture 25. Sieving cage contents, looking for transformers 2012.

Picture 26. Calipers on specimens found in cages, 2012.

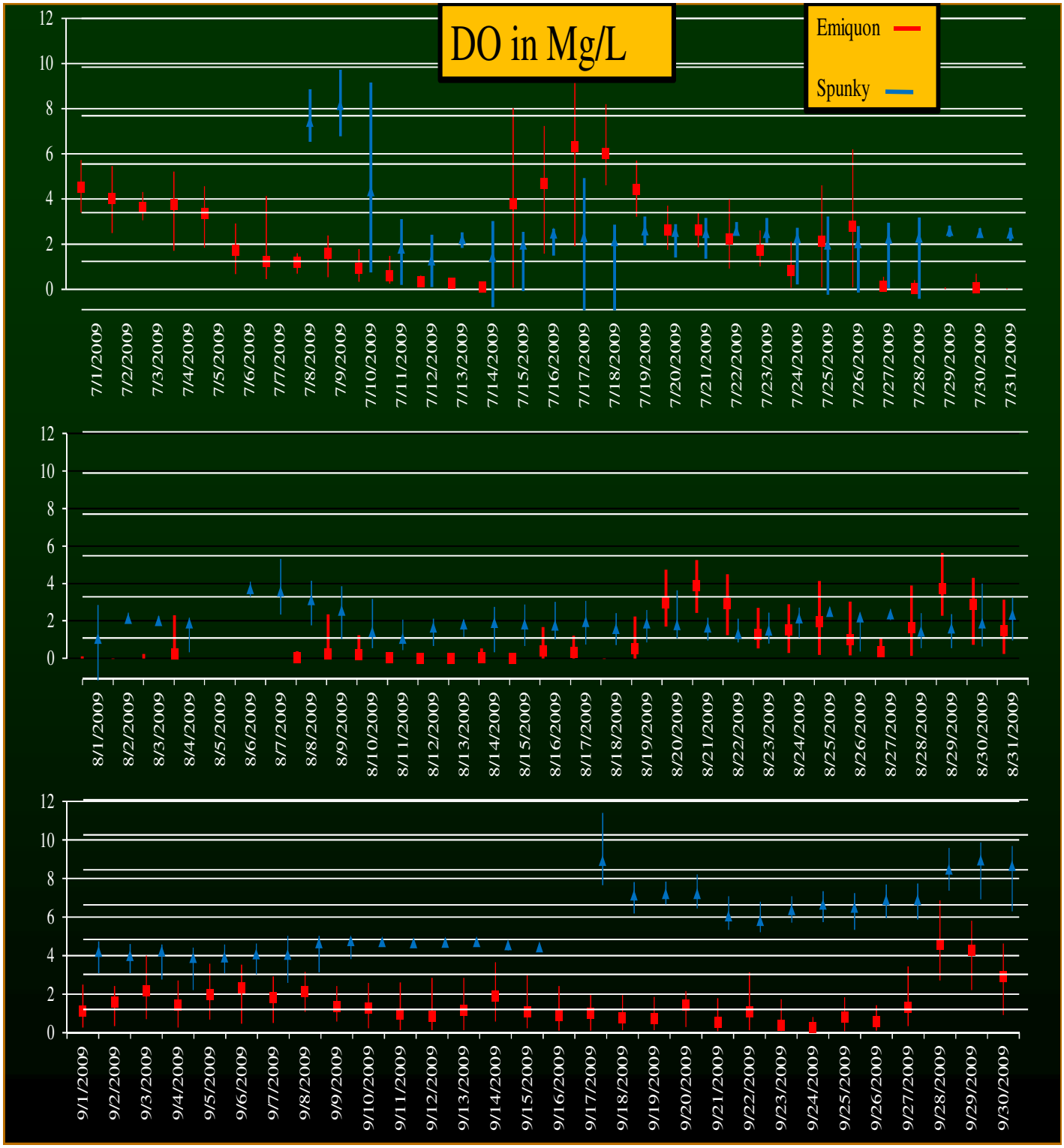
Picture 27. Specimens identified as *Musculium sp.,* 2012.



Picture 28. Collected *Musculium sp.* (note young) 2012.

Graph 1

Graph 1.


DO in Mg/L
Emiquon
Spunky
12
10
8
6
4
2
0
7/1/2009
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7/3/2009
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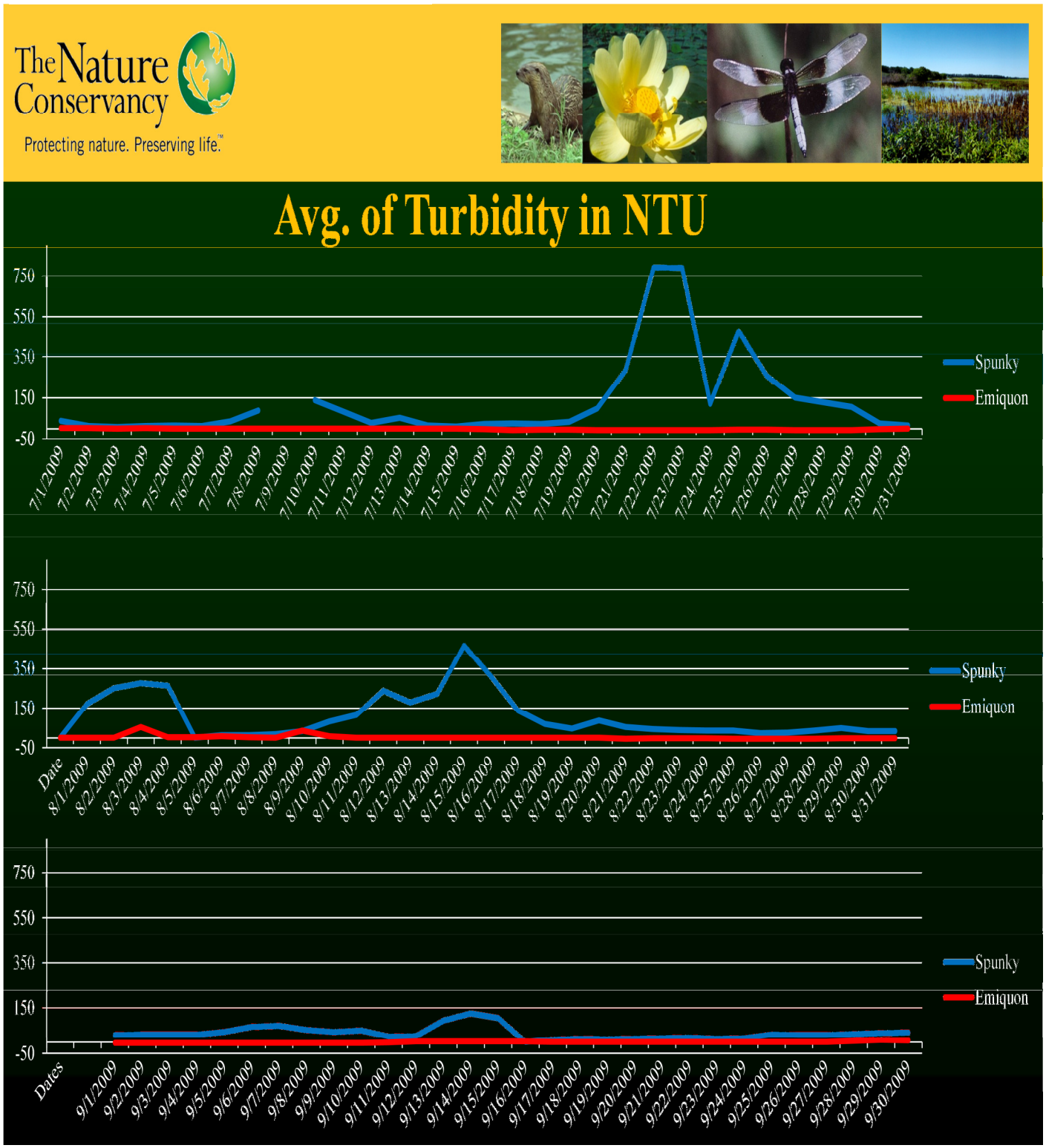
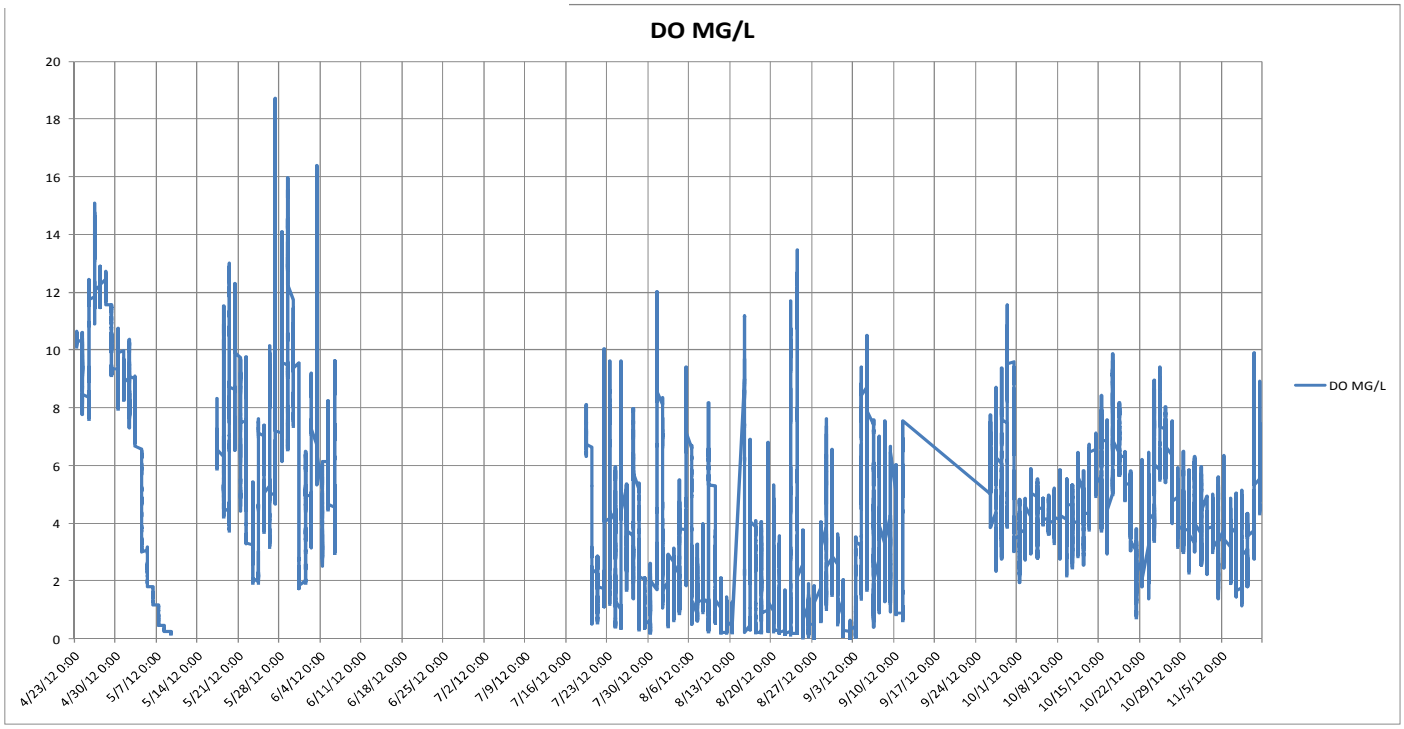
Graph 2.


The Nature Conservancy
Protecting nature. Preserving life.™
Avg. of Turbidity in NTU
750
550
350
150
-50
Spunky
Emiquon
Date
Dates

Graph 3.
Dissolved Oxygen at Emiquon 4-11/2012



Graph 4.
Turbidity in NTU at Emiquon 4-11/2012

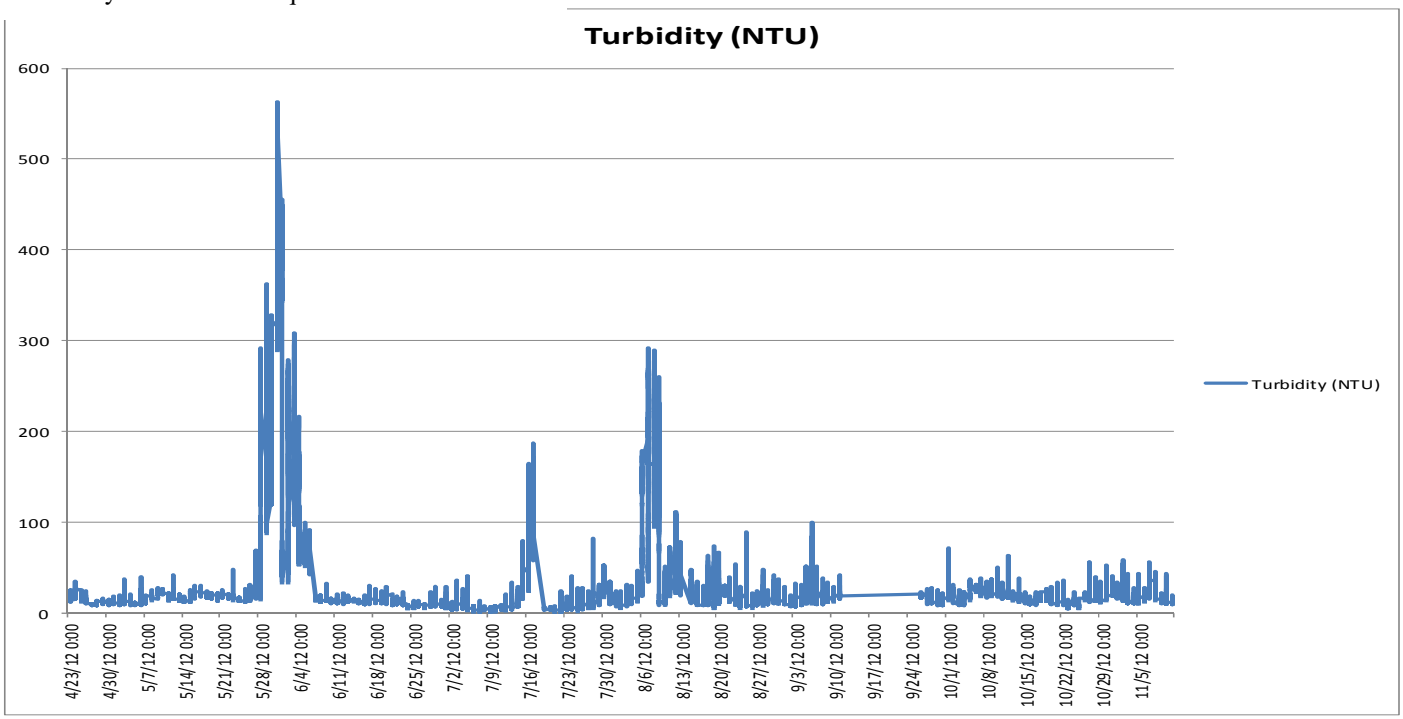


Table 1
2009 transformation estimation sheet

Date	Water Temperature at Jake Wolf Fish Hatchery °F	Water Temperature at Spunky Bottoms °F	Growing degrees over 50	Total Growing Degree days	Notes/Comments
6-Apr-09	54		4	4	Fish inoculated
7-Apr-09	54		4	8	
8-Apr-09	54		4	12	
9-Apr-09	54		4	16	
10-Apr-09	54		4	20	
11-Apr-09	54		4	24	
12-Apr-09	54		4	28	
13-Apr-09	54		4	32	
14-Apr-09	54		4	36	
15-Apr-09	57		7	43	
16-Apr-09	57.5		7.5	50.5	
17-Apr-09	57		7	57.5	
18-Apr-09	57		7	64.5	
19-Apr-09	60		10	74.5	
20-Apr-09	58.5		8.5	83	
21-Apr-09	63		13	96	
22-Apr-09	62		12	108	
23-Apr-09	61		11	119	
24-Apr-09	64		14	133	
25-Apr-09	64		14	147	
26-Apr-09	64		14	161	
27-Apr-09	64		14	175	

28-Apr-09	64		14	189	
29-Apr-09	64		14	203	
30-Apr-09	64		14	217	
1-May-09	64	68	14	231	
2-May-09	64	68	14	245	
3-May-09	64	68	14	259	
4-May-09	64	68	14	273	
5-May-09	64	70	14	287	
6-May-09	64	70	14	301	
7-May-09		70	20	321	Moved fish to Spunky
8-May-09		70	20	341	
9-May-09		70	20	361	Estimated transformation of sandshell
10-May-09		70	20	381	
11-May-09		70	20	401	
12-May-09		70	20	421	Estimated transformation of butterfly
13-May-09		70	20	441	
14-May-09		72	22	463	
15-May-09		72	22	485	
16-May-09		72	22	507	
17-May-09		72	22	529	
18-May-09		73	23	552	
19-May-09		73	23	575	
20-May-09		73	23	598	
21-May-09		73	23	621	
22-May-09		73	23	644	
23-May-09		73	23	667	
24-May-09		73	23	690	
25-May-09		73	23	713	

26-May-09		73	23	736	
27-May-09		73	23	759	Removed fish from cages

Table 2
2009 propagation cage array, host fish distribution.

Fresh Water Drum	Cage Numbers																			
Date	**1A**	**1B**	**1C**	**1D**	**1E**	**2A**	**2B**	**2C**	**2D**	**2E**	**3A**	**3B**	**3C**	**3D**	**3E**	**4A**	**4B**	**4C**	**4D**	**4E**	**Totals**	**Notes**
04/30/09	3	3									3	3									12	Stocked Fish
05/07/09						3	2	2	2	2						2	2	3	2	2	22	Stocked Fish
																					34	
05/27/09	3	2				2	1	2	2	1	3	3				2	2	3	1	2	29	Pulled Fish

Walleye	Cage Numbers																			
Date	**1A**	**1B**	**1C**	**1D**	**1E**	**2A**	**2B**	**2C**	**2D**	**2E**	**3A**	**3B**	**3C**	**3D**	**3E**	**4A**	**4B**	**4C**	**4D**	**4E**	**Totals**	**Notes**
04/30/09			8										7								15	Stocked Fish
05/07/09				9	8									8	8						33	Stocked Fish
																					48	
05/27/09			8	9	8								7	8	8						48	Pulled Fish

* Cage sets 1 and 2 are not aerated.
* Cage sets 3 and 4 are aerated.

Table 3
2012 propagation cage array, host fish distribution.

West cage array: cage #	**Number of fish**	**Size range**
3A	7	240-280 mm
3B	5	240-280 mm
3C	6	240-280 mm
East cage array: cage #		
3D	3	240-280 mm
3E	13	70 mm
Water temp. 19.8 C		