**Update Report**

**Period 2/1/2013 - 1/31/2014**

**Project R/LME/N-3 - Alleviating Regulatory Impediments To Native Shellfish Aquaculture**

## STUDENTS SUPPORTED

Heare, Jake, jakeheare@gmail.com, University of Washington, SAFS, status new, no field of study, advisor Roberts, degree type MS, no degree date, degree completed this period No
Student Project Title none

Involvement with Sea Grant This Period research assistant

Post-Graduation Plans none

Jackson, Katie, k.e.jackson.1992@gmail.com, University of Washington, SAFS, status cont, no field of study, no advisor, degree type BS, no degree date, degree completed this period No
Student Project Title Genetic sample management and optimizing oyster relaxation

Involvement with Sea Grant This Period intern

Post-Graduation Plans none

Wear, Hannah, hannah.wear@hotmail.com, University of Washington, SAFS, status new, no field of study, no advisor, degree type BS, degree date 2014-06-01, degree completed this period No
Student Project Title none

Involvement with Sea Grant This Period intern

Post-Graduation Plans none

## CONFERENCES / PRESENTATIONS

Jackson, K., Vadopalas B., and S.R. Roberts. 2013. Putting an aphrodisiac to sleep. 67th Joint Annual Meeting of the National Shellfisheries Association, Pacific Coast Section, and the Pacific Coast Shellfish Growers Association. Sunriver, OR. September 30-October 3., public/profession presentation, 150 attendees, 2013-09-30
Vadopalas, B., Davis, J., Blake B., and S.R. Roberts. 2013. Local adaptation in Olympia oysters. 67th Joint Annual Meeting of the National Shellfisheries Association, Pacific Coast Section, and the Pacific Coast Shellfish Growers Association. Sunriver, OR. September 30-October 3., public/profession presentation, 150 attendees, 2013-09-30

## ADDITIONAL METRICS

| | |
|---|---|
| K-12 Students Reached | Acres of degraded ecosystems restored as a result of Sea Grant activities |
| Curricula Developed | Resource Managers who use Ecosystem-Based Approaches to Management |
| Volunteer Hours | HACCP - Number of people with new certifications |
| Cumulative Clean Marina Program - certifications | |

**PATENTS AND ECONOMIC BENEFITS**

No Benefits Reported This Period

**TOOLS, TECH, AND INFORMATION SERVICES**

| Description | | Developed | Used | Names of Managers | Number of Managers |
|---|---|---|---|---|---|
| Olympia oyster parentage assignment microsatellite panel | Actual (2/1/2013 - 1/31/2014) | 1 | 0 | | 0 |
| | Anticipated (2/1/2014 - 1/31/2015) | 0 | 0 | | |
| Olympia oyster anesthesia method | Actual (2/1/2013 - 1/31/2014) | 1 | 0 | | 0 |
| | Anticipated (2/1/2014 - 1/31/2015) | 0 | 0 | | |

**HAZARD RESILIENCE IN COASTAL COMMUNITIES**

| Name of coastal community | County | | Number of resiliency trainings / technical assistance services provided | Was community hazard resiliency improved (e.g., via changes in zoning ordinances)? |
|---|---|---|---|---|
| none | | Actual (2/1/2013 - | 0 | Yes |

| | |
|---|---|
| 1/31/2014) | |
| Anticipated (2/1/2014 - 1/31/2015) | 0 |

**ADDITIONAL MEASURES**

Safe and sustainable seafood

| | |
|---|---|
| Number of stakeholders modifying practices | Number of fishers using new techniques |
| Actual (2/1/2013 - 1/31/2014) | Actual (2/1/2013 - 1/31/2014) |
| Anticipated (2/1/2014 - 1/31/2015) | Anticipated (2/1/2014 - 1/31/2015) |

| | |
|---|---|
| Sustainable Coastal Development | Coastal Ecosystems |
| Actual (2/1/2013 - 1/31/2014) | Actual (2/1/2013 - 1/31/2014) |
| Anticipated (2/1/2014 - 1/31/2015) | Anticipated (2/1/2014 - 1/31/2015) |

**PARTNERS**

| |
|---|
| Partner Name Clam Fresh, LLC |
| Partner Name Fagergren Oyster Co., type Industry and Business, scale Local |
| Partner Name Fidalgo Marina, type Industry and Business, scale Local |
| Partner Name NOAA Manchester lab |
| Partner Name Puget Sound Restoration Fund |
| Partner Name Rock Point Oyster Company |
| Partner Name Swinomish Tribe |
| Partner Name Washington Department of Fish and Wildlife |

**IMPACTS AND ACCOMPLISHMENTS**

Title Washington Sea Grant investigates local adaptation, genetic diversity, and knock-out drops for Olympia oyster restoration

Type accomplishment

Description Relevance Restoring native Olympia oysters is a key goal of the Puget Sound recovery plan. But introducing cultured native shellfish can have both positive and adverse effects on the genetic makeup of nearby wild populations. Hatchery stock may threaten wild populations through inbreeding, weakening their ability to adapt to changing environmental conditions. Response With funding from a national strategic initiative, Washington Sea Grant-sponsored researchers are examining local adaptation in native Olympia oysters to help predict the impacts of culturing native shellfish species for restoration and commercial production. In collaboration with federal, state, tribal, and private partners, researchers have

cultured tested, genotyped and intermixed wild and cultured Olympia oysters from around Puget Sound to measure their breeding fitness. In that process, the team has also developed an effective anesthetic that induces the oysters to open their shells and allows nonlethal sampling. Results While culture activities and sampling were successful, analysis is still ongoing. Genetic testing suggests that the current breeding protocol for Olympia oysters used for restoration assures sufficient diversity.

Recap Washington Sea Grant-sponsored research establishes grow-out sites for testing local adaptation and the interbreeding of wild and cultured Olympia oysters, confirming that current breeding protocols protect genetic diversity. The research also develops a safe, effective oyster anesthetic.

Comments Primary Focus Area LME (SSSS) Secondary Focus Area LME (HCE) State Goal Support conservation and sustainable use of living marine resources through effective and responsible approaches, tools, models and information for harvesting wild and cultured stocks and preserving protected species (SSSS Supply; HCE Science).

Related Partners Clam Fresh, LLC, Fagergren Oyster Co., Fidalgo Marina, NOAA Manchester lab, Puget Sound Restoration Fund, Rock Point Oyster Company, Swinomish Tribe, Washington Department of Fish and Wildlife

**PUBLICATIONS**

Title Oyster genes Olympia oysters

Type Internet Resources, Topical Websites Publication Year 2013 Uploaded File none URL http //oystergen.es/olympia

Abstract Our approach is to simultaneously address local adaptation in three genetically differentiated populations of Olympia oysters by evaluating genotype-by-environment interactions. We will reciprocally transplant seed produced from wild parents collected from contrasting environments into all environments. This very large reciprocal transplant experiment can test for a home field advantage in survival, maturation and growth in Olympia oysters. The overall goals of this project are to increase our knowledge of local adaptation in Olympia oysters to address concerns that interbreeding between potentially maladapted cultured and wild stocks could negatively impact wild populations. Accordingly, in order to attain these goals, the specific objectives of this proposal are to 1) Evaluate fitness components and performance of seed from different origins in a reciprocal transplant experiment and 2) Characterize genetic and epigenetic markers associated with oysters from different origins in a reciprocal transplant experiment.

Citation http //oystergen.es/olympia

Copyright Restrictions + Other Notes

Journal Title none

Title Genomic resource development for shellfish of conservation concern.

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year 2013 Uploaded File none URL http //onlinelibrary.wiley.com/doi/10.1111/1755-0998.12052/abstract

Abstract Effective conservation of threatened species depends on the ability to assess organism physiology and population demography. To develop genomic resources to better understand the dynamics of two ecologically vulnerable species in the Pacific Northwest of the United States, larval transcriptomes were sequenced for the pinto abalone, Haliotis kamtschatkana kamtschatkana, and the Olympia oyster, Ostrea lurida. Based on comparative species analysis the Ostrea lurida transcriptome (41 136 contigs) is relatively complete. These transcriptomes represent the first significant contribution to genomic resources for both species. Genes are described based on biological function with particular attention to those associated with temperature change, oxidative stress and immune function. In addition, transcriptome-derived genetic markers are provided. Together, these resources provide valuable tools for future studies aimed at conservation of Haliotis kamtschatkana kamtschatkana, Ostrea lurida and related species.

Citation Timmins-Schiffman EB* Friedman CS, Metzger DC, White SJ and Roberts SB. (2013) Genomic resource development for shellfish of conservation concern. Molecular Ecology Resources 13(2) 295-305. doi 10.1111/1755-0998.12052



Journal Title Molecular Ecology Resources

**OTHER DOCUMENTS**

No Documents Reported This Period

**LEVERAGED FUNDS**

Type influenced Period 2013-09-30 2013-10-02Amount $150

Purpose Travel award to Katie Jackson to attend the 67th Joint Annual Meeting of the National Shellfisheries Association, Pacific Coast Section, and the Pacific Coast Shellfish Growers Association. $150. Sept 30-Oct 2, 2013

Source National Shellfisheries Association, West Coast Section

**UPDATE NARRATIVE**

Uploaded File Roberts_6976_update_na....2.pdf

**R/LME/N-3 - Alleviating Regulatory Impediments To Native Shellfish Aquaculture**
**Progress Report: February 2013 – January 2014**

The overall goals of this project are to increase our knowledge of local adaptation in Olympia oysters to address concerns that interbreeding between potentially maladapted cultured and wild stocks could negatively impact wild populations. This reporting period marks the ***deployment and monitoring of oysters in our reciprocal transplant experiment***. This field work will be the basis of our characterization for a home field advantage in survival, maturation and growth in Olympia oysters. In addition, over the past year we have continued ***genetic assessment of restoration aquaculture practices*** and experimented with **anesthesia in mature oysters**.

1) ***Deployment and monitoring of oysters in reciprocal transplant experiment***
Adult oysters were collected from three locations in Puget Sound (Fidalgo, Dabob, and Oyster Bays) during November and December 2012 (Figure 1). Mass spawning of oysters occurred in June 2013 following several months of conditioning. Larvae were raised in flowing seawater and fed microalgae. Following setting on microcultch, juveniles were cultured in flowing seawater in Port Gamble. In August 2013, 480 oysters (5-10 mm) from each population were planted at Fidalgo, Oyster Bay, Dabob, and Manchester Bays**.** At each site, oysters from each population were placed into four 0.61M X 0.61M growout trays (120 each). In each tray, oysters were equally distributed in four 10x7.5cm mesh (1475 micron) bags with 24 oysters glued to ceramic tiles. Trays were anchored into substrate using using rebar stakes. In late autumn trays at Fidalgo Bay, Oyster Bay, and Manchester were transferred from substrate to a midcolumn hanging system tied to a floating docks. At each site, a HOBOlogger temperature logger (OnSet, USA) was deployed to monitor temperature every 15 minutes.

*Site Monitoring and Growth*
Survival and growth were assessed at all sites in December 2013, Dabob in January 2014, and Fidalgo, Manchester, and Oyster Bays in February 2014. Survival was measured by counting all live animals remaining in each tray for each population. Growth was measured via size and weight measurements taken directly from live subsamples collected in the field and measured in the lab. In the lab each of the sampled oysters was measured using calipers for hinge to bill length and weighed with shell to determine whole body weight.

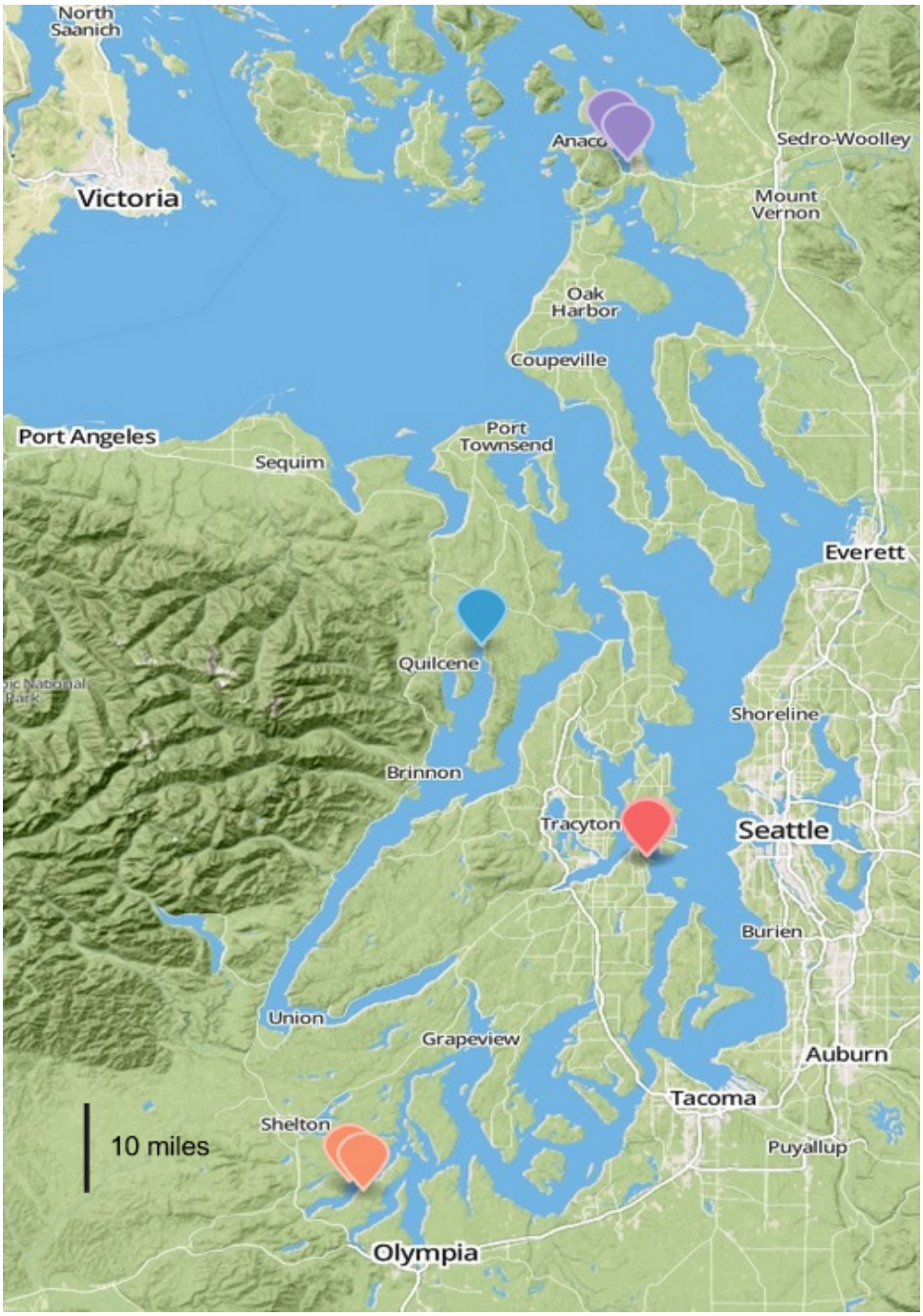


**Figure 1.** Map of oyster outplanting sites. Field sites include Fidalgo Bay (purple), Dabob Bay (blue), Manchester (red), and Oyster Bay (orange).

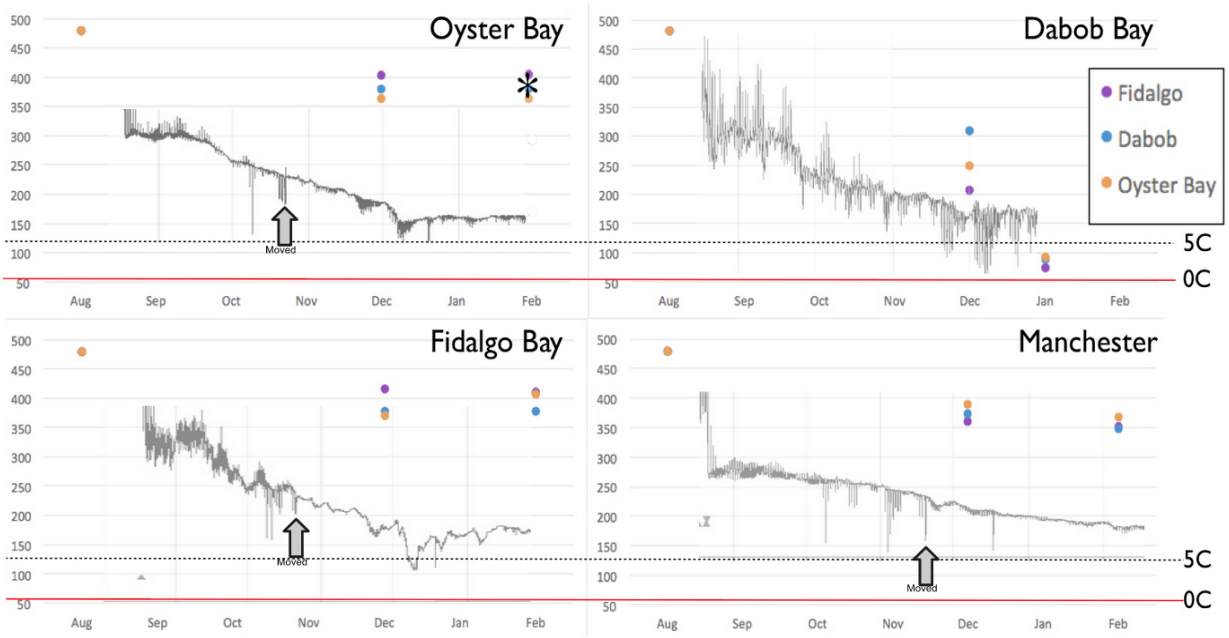


**Figure 2**. Number of surviving oysters at each field site. Circle color reflects originating broodstock population. Temperature data is displayed to scale with 0C and 5C indicated. Grey arrows indicate when oysters were relocated from substrate to suspension. Asterisk denotes estimate as some trays were inaccessible at time of sampling.

Samples for genetic testing were collected at outplanting in August 2013 for baseline data. Samples were also taken in December 2013. These samples included approximately 32 animals per population at Fidalgo, Manchester, and Oyster Bays. At Dabob we collected 43 from the Fidalgo population, 50 from the Oyster Bay population, and 59 from the Dabob population due to the high mortality. We visited Dabob again in January to collected another 22 from the Fidalgo population, 33 from the Oyster Bay population, and 39 from the Dabob population.

***2) Genetic assessment of restoration aquaculture practices***

Olympia oyster seed produced from wild broodstock in our restoration hatchery from two different years (2010 and 2011) were compared. Wild broodstock were collected from five locations in in the south subbasin of Puget Sound in both 2010 and 2011. Each year, approximately 500 broodstock were randomly assigned to each of 20 breeding groups. In 2010, the larvae produced by each breeding group were collected and reared separately as a seed group (n=20). In 2011, the larvae produced on a given day from all breeding groups were reared separately as a seed group (n=20). A tissue subsample from 10 individuals from each seed group was taken in 2013 and stored in 70% EtOH. From 10 randomly selected seed groups from each year, DNA was extracted from each sampled individual following standard protocols, and each

individual was genotyped at 7 microsatellite loci. Raw genotype data, binned into length classes using TANDEM (Matschiner & Salzburger, 2009), were used to calculate pairwise relatedness using the maximum likelihood estimator of Queller and Goodnight (1989) as implemented in GenALEx (Peakall and Smouse 2006, 2012). Mean pairwise relatedness values within each seed group ranged from 0.07-0.28 (2010) and 0.09-0.33 (2011) (Fig. 3, open circles). Mean pairwie relatedness among 100 pairs over all seed groups (100 bootstrap replicates) did not differ significantly from zero for 2010 or 2011 ($P<0.05$; Fig. , solid circles). *This result suggests that the partial matrix breeding design as implemented incorporates sufficient genetic diversity to obviate undo relatedness, assuming equal contribution from each breeding group*. Future work will broaden comparisons to include the restoration seed, commercially produced seed, and wild groups. We will conduct allele count rarefaction (HP-Rare, Kalinowski 2005), apply the more powerful groupwise maximum likelihood relatedness estimator of Jones & Wang (2009), and test for shifts in allele frequency among groups.

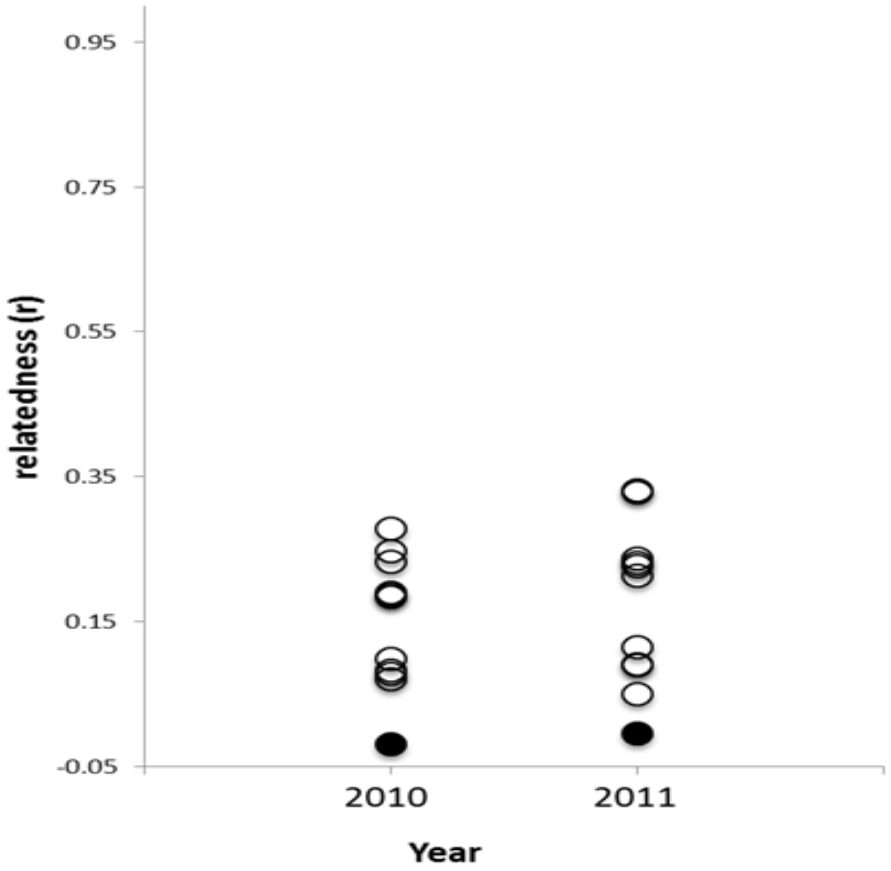


**Fig. 3.** Mean pairwise relatedness values for offspring from ten breeding groups of Olympia oysters, *Ostrea lurida*, in 2010 and 2011 (open circles) and from pairs randomly sampled from each year (closed circles).

**3) Anesthesia in mature oysters**

In shellfish research, there are many situations in which tissues of individual animals need to be sampled multiple times, necessitating nonlethal sampling methods. In particular, an accurate way to effectively measure fecundity, one of the primary metrics of fitness in our project, would be highly beneficial. We needed a nonlethal method to remove broods from female Olympia oysters to enable direct measurement of fecundity, and so we explored the possibility of oyster anesthesia. Although relaxation methods have been developed for a number of oyster species, none were directly applicable or effective for the Olympia oyster, *Ostrea lurida*. We optimized an anesthetization method to induce gaping in the Olympia oyster. We tested concentrations of 25-100 g/L $MgSO_4$ and exposure durations of up to three hours on adult Olympia oysters, and measured response, recovery, and survival. To stimulate opening of the valves to ensure exposure to the anesthetic, we also tested temperature increase and ambient air exposure as pre-treatments. *We found the optimal concentration to be 85 g/L in 50% seawater, which resulted in 45% of the animals anaesthetized in two hours*; the remaining oysters did not open their shells and were therefore not exposed. We also found pre-treatment with 30 minutes of air exposure and a temperature increase of 10°C to increase the proportion of oysters that opened their shells for exposure to the treatment. We observed no adverse effects of treatment on subsequent recovery or survival. Future work will assess the influence of microalgae presence on the proportion of open oysters, efficiency of brood removal, and the effect of brood removal on survival. We are planning to use this method to measure fecundity and success in conservation efforts. In addition, this method can accommodate nonlethal tissue sampling.