REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
19-03-2019	Final Report	1-May-2017 - 31-Oct-2018

4. TITLE AND SUBTITLE
Final Report: Quantifying Three-Dimensional Internal and External Deformations in Flexible Organisms during Locomotion

5a. CONTRACT NUMBER
W911NF-17-1-0234

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
70119-EG-RIP.6

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Eric Tytell
UU	UU	UU	UU		19b. TELEPHONE NUMBER
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Standard Form 298 (Rev 8/98)
Prescribed by ANSI Std. Z39.18

RPPR Final Report
as of 25-Mar-2019

Agency Code:

Proposal Number: 70119EGRIP **Agreement Number: W911NF-17-1-0234**

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DUNS Number: 073134835 EIN: 042103634
Report Date: 31-Jan-2019 Date Received: 19-Mar-2019
Final Report for Period Beginning 01-May-2017 and Ending 31-Oct-2018
Title: Quantifying Three-Dimensional Internal and External Deformations in Flexible Organisms during Locomotion
Begin Performance Period: 01-May-2017 **End Performance Period:** 31-Oct-2018
Report Term: 0-Other
Submitted By: Eric Tytell Email: eric.tytell@tufts.edu
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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 1 **STEM Participants:** 5

Major Goals: Many of the best-studied animal movements occur primarily in a plane. During walking, the limbs swing forward and back; during swimming, the tail undulates from side to side. The fact that these movements are primarily planar has allowed us to understand their basic mechanics and control on the basis of two-dimensional techniques like filming with a single camera. Most previous research has also focused on steady, linear locomotion on a smooth substrate or in laminar, smoothly flowing water. By reducing the variability and complexity of the environment, the basic movements become more planar and simpler to understand. These types of experiments have been particularly useful for identifying strategies that reduce the cost of long distance transport.

However, even these simple, nominally planar movements generally have substantial 3D components. During walking, a substantial portion of the energetic cost comes from small changes in lateral foot placement, which are needed to stabilize the side-to-side (out of plane) motion. Similarly, during steady swimming, fish tails twist around their long axes as they move from side to side, and the fin rays actively deform the fin's sur-face in complex 3D ways. These 3D movements are probably important for stability, because fishes are not passively stable, particularly in roll.

Moreover, such simple movements are rare. In reality, animals rarely move steadily and almost always move through complex, unsteady environments (Dickinson et al., 2000). Most fish, for ex-ample, spend most of their time turning, accelerating, or decelerating (Kramer and McLaughlin, 2001; Webb, 2011). These unsteady movements are almost always highly three dimensional, even those like escape responses (Butail and Paley, 2012) that have been long studied with planar views. To understand how fish and other soft or flexible organisms move, we must first understand how their bodies and fins deform in 3D.

When the body deforms in 3D, the internal musculoskeletal system also deforms in 3D. These 3D internal deformations may contribute to animals' versatile ability to shift rapidly from low speed, high force behaviors to high-speed movements. For example, the muscle fibers in many muscles are angled relative to the main axis of the muscle. As the fibers produce force and the overall muscle changes length, the angle also changes, which alters the effective mechanical advantage for the muscle. Such changes may contribute to internal instabilities, in which the mechanical advantage of the muscle rapidly changes to facilitate rapid movements. Moreover, the bodies of flexible organisms may have snap-through buckling transitions during rapid movements, again facilitating impulsive motion. We are currently exploring these hypotheses in an ARO-funded collaborative project. The importance of 3D deformations is especially important in fish axial musculature, because each muscle segment is surrounded by sheets of collagen, embedded with tendons and even bones; deformations in one direction can significantly affect the mechanical properties of the tissue in orthogonal directions. Additionally, muscle segments at one point along the body interdigitate with other segments, like cones nested inside each other, an anatomical arrangement that is thought to contribute to smooth movements in a flexible organism such as fish. Contraction in one muscle segment can affect the passive elements of up to 7 other segments, many joints removed from the site of muscle activity. Understanding how the body tissues change shape three-dimensionally as an animal moves in its natural environment can help us understand how active and passive muscle elements work together to control movement.

It is these anatomically complex segments, made up of fast-twitch white muscle, that power rap-id movements in fish. It is not known how this complex anatomical arrangement contributes to swimming performance, particularly during unsteady movements, but since swimmers with differ-ent swimming modes tend to have different white muscle anatomy, it is likely that the geometry has functional consequences. If the muscle fiber angles change during movement, as they do in salamanders, which have a similar, but simpler muscular architecture, then the mechanical advantage of the muscles could also change. Furthermore, while steady movements in fish are powered by an anatomically simpler strip of red muscle, the force from the red muscle must still be transmitted through the nested cones to the backbone in some way that remains poorly understood, aside from in a few specialized species of fishes.

By quantifying the 3D structure of internal and external deformations, we will gain a much more complete understanding of the mechanics and control of natural, unsteady movements for soft and flexible organisms in complex environments. To accurately visualize the surface deformation of fish fins and bodies and avoid occlusions as the animal moves, we plan to develop a three-camera setup, using one of our current high-speed cameras and adding two more high-speed cam-eras. At the same time, we will simultaneously monitor both muscle activity, using electromyography, and the 3D internal deformations of tissues, using sonomicrometry. These measurements, along with ongoing work in our laboratory to characterize the complex 3D muscle anatomy of fishes, will lead to an improved understanding of how active and passive elements contribute to power production and stability in flexible organisms, especially during more realistic unsteady movements.

Objective: Identify strategies that flexible animals like fish use to maneuver effectively yet stably in an unpredictable 3D environment. Quantify the 3D external body and fin deformations, the internal musculoskeletal deformations, and the regional activation of different muscle groups during in situ bending, during steady locomotion with perturbations, and during unsteady locomotion.

Accomplishments: See attached PDF.

Training Opportunities: Nothing to Report

Results Dissemination: Work using the equipment has been or will be presented at several conferences, including at the Society for Integrative and Comparative Biology and the International Symposium on Adaptive Movement in Animals and Machines. These conferences do not have a published proceedings, so presentations are listed below.

Tytell ED. 2019. How body shape and mechanics interact for swimming performance in (physical models of) fishes: Volumetric flow visualization, forces, and power. In: Soc. Integ. Compar. Biol. Tampa, FL. Jan, 2019. 103–8.

Fath MA, Tytell ED. 2019. Using Perturbations to Study Locomotor Stability in the Bluegill Sunfish (Lepomis macrochirus). In: Soc. Integ. Compar. Biol. Tampa, FL. Jan, 2019. 34–4.

Pfeiffenberger JA, Tytell ED. 2019. Ontogenetic scaling of the viscoelastic mechanical properties of the body of the bluegill sunfish, Lepomis macrochirus. In: Soc. Integ. Compar. Biol. Tampa, FL. Jan, 2019. 8–4.

Scibelli A, Donatelli CM, Tidswell B, Tytell ED, Trimmer BA. 2019. SquMA Bot MKII: Squishable motor tendon actuated robot. In: Int. Symp. Adaptive Motion of Animals and Machines. Lausanne, Switzerland.

Donatelli CM, Shen TH, Khanna S, Tytell ED. 2019. The hydrodynamics of tail twisting during swimming in the American Eel (Anguilla rostrata). In: Soc. Integ. Compar. Biol. Tampa, FL. Jan, 2019. 103–2.

Mekdara PJ, Schwalbe MAB, Tytell ED. 2019. Tail synchronization of schooling giant danios is altered after lateral line system ablation and regeneration. In: Soc. Integ. Compar. Biol. Tampa, FL. Jan, 2019. 13-1.

Sullivan CM, Carr JA, Tytell ED. 2019. Muscle response to lengthening and shortening perturbations at various activation and perturbation phases. In: Soc. Integ. Compar. Biol. Tampa, FL. Jan, 2019. 68–4.

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

ARTICLES:

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Integrative and Comparative Biology
Publication Identifier Type: DOI Publication Identifier: 10.1093/icb/icy042
Volume: 58 Issue: 5 First Page #: 860
Date Submitted: 2/26/19 12:00AM Date Published:
Publication Location:
Article Title: Body stiffness and damping depend sensitively on the timing of muscle activation in lampreys
Authors: Eric D Tytell, Jennifer A Carr, Nicole Danos, Christopher Wagenbach, Caitlin M Sullivan, Tim Kiemel, Nc
Keywords: body mechanics, muscle, swimming, fish, stiffness, damping
Abstract: Unlike most manmade machines, animals move through their world using flexible bodies and appendages, which bend due to internal muscle and body forces, and also due to forces from the environment. Fishes in particular must cope with fluid dynamic forces that not only resist their overall swimming movements but also may have unsteady flow patterns, vortices, and turbulence, many of which occur more rapidly than what the nervous system can process. Has natural selection led to mechanical properties of fish bodies and their component tissues that can respond very quickly to environmental perturbations? Here, we focus on the mechanical properties of isolated muscle tissue and of the entire intact body in the silver lamprey, Ichthyomyzon unicuspis. We developed two modified work loop protocols to determine the effect of small perturbations on the whole body and on isolated segments of muscle as a function of muscle activation and phase within the swimming cycle. First, we examined how the m
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

CONFERENCE PAPERS:

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Soc Integ Compar Biol
Date Received: 18-Mar-2019 Conference Date: 05-Jan-2019 Date Published: 05-Jan-2019
Conference Location: Tampa, FL
Paper Title: Ontogenetic scaling of the viscoelastic mechanical properties of the body of the bluegill sunfish, Lepomis macrochirus
Authors: Janne Pfeiffenberger, Eric D. Tytell
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Soc Integ Compar Biol
Date Received: 18-Mar-2019 Conference Date: 07-Jan-2019 Date Published: 07-Jan-2019
Conference Location: Tampa, FL
Paper Title: How body shape and mechanics interact for swimming performance in (physical models of) fishes: Volumetric flow visualization, forces, and power
Authors: Eric D. Tytell
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 4-Under Review
Conference Name: 9th Int. Symp. Adaptive Motion Animals Machines
Date Received: 18-Mar-2019 Conference Date: 20-Aug-2019 Date Published: 20-Aug-2019
Conference Location: Lausanne, Switzerland
Paper Title: More than Skin Deep: Crawling Soft Robots with Functional Skin
Authors: Cassandra M. Donatelli, Anthony Scibelli, Ben Tidswell, Eric D. Tytell, Barry A. Trimmer
Acknowledged Federal Support: **Y**

DISSERTATIONS:

Publication Type: Thesis or Dissertation
Institution: Tufts University
Date Received: 18-Mar-2019 Completion Date: 9/25/17 7:41PM
Title: Biological Beam Bending: How Lamprey Muscles Can Change the Viscoelastic Properties of Their Bodies
Authors: Chris Wagenbach
Acknowledged Federal Support: **Y**

Accomplished

We purchased all of the types of equipment in the original proposal, including a sonomicrometry system for measuring muscle fiber lengths in vivo, highly sensitive amplifiers and data acquisition hardware, and two high resolution, high speed cameras. Both the high speed cameras and the data acquisition system were substantially less expensive than the original quote, even though we purchased the same or very similar items. We purchased Phantom Miro 340 cameras, as originally proposed, but the price had dropped to $24,610 each rather than $55,815 each, as in the original quote. Additionally, after demoing the proposed system (AD Instruments Octal BioAmp and PowerLab; original quoted at $19,925) and several others, we conclude that Dagan amplifiers with National Instruments data acquisition hardware worked best (total price $12,307).

As a result, following approval from the program officer, we were able to purchase further equipment in line with the objectives of the project. To quantify muscle activation, we purchased two electrical stimulators, which were needed for the in vitro and in vivo muscle activation experiments. For both sets of experiments, we also found that custom machined components were important. We therefore purchased a CNC milling machine. Finally, we found that measuring forces in the experiments was crucial. For that reason, we purchased a miniature force transducer and infrared laser, used to quantify fluid flow patterns without interfering with animal behavior. See Table 1 below for a full list of purchased items.

All of the equipment is assembled and working. We have currently published one journal article using the equipment, which includes results from a Master's thesis, and we have presented work using the equipment at several international conferences, including the Society for Integrative and Comparative Biology and the International Symposium on Adaptive Movement in Animals and Machines. We anticipate at least six journal publications using the equipment over the next few years.

Table 1. Equipment purchased during the reporting period.

Item	Source	Model	Unit price	units	total price
Sonomicrometry system	Sonometrics		25,610	1	25,610
Amplifier	Dagan	EX4-400	5,000	2	10,000
Data acqusition hardware	National Instruments	USB-6361	2,308	1	2,308
Computer for data acquisition	Dell		2,630	1	2,630
High resolution high speed camera	Vision research	Phantom Miro 340	24,999	2	49,998
Computer for high speed cameras	Dell		2,338	1	2,338
Miniature high speed cameras	Basler	acA 1440	369	2	738
		acA 1300	443	1	443
Stimulator	A-M Systems	4100	2,970	1	2,970
	Aurora	701C	4,489	1	4,489
Animal holding tank	Iwaki		11,649	1	11,649
Force transducer	ATI	Mini-40	7,162	1	7,162
CNC milling machine and installation	Tormach	PCNC 440	15,354	1	15,354
Infrared laser	Crystalaser	DL808	5,475	1	5,475
Parts for 3D printer	Stratasys		5,002	1	5,002
Other optical components			4,928	1	4,928
Lenses, filters, and other camera equipment	Graftek		8,286	1	8,286
	B&H		2,944	1	2,944
Other miscellaneous parts			1,309	1	1,309
Total					*$163,633*