Electronic assessments of small fish health

URI Electrical Engineering Capstone Project

US Environmental Protection Agency Office of Research and Development

Atlantic Ecology Division Narragansett, RI

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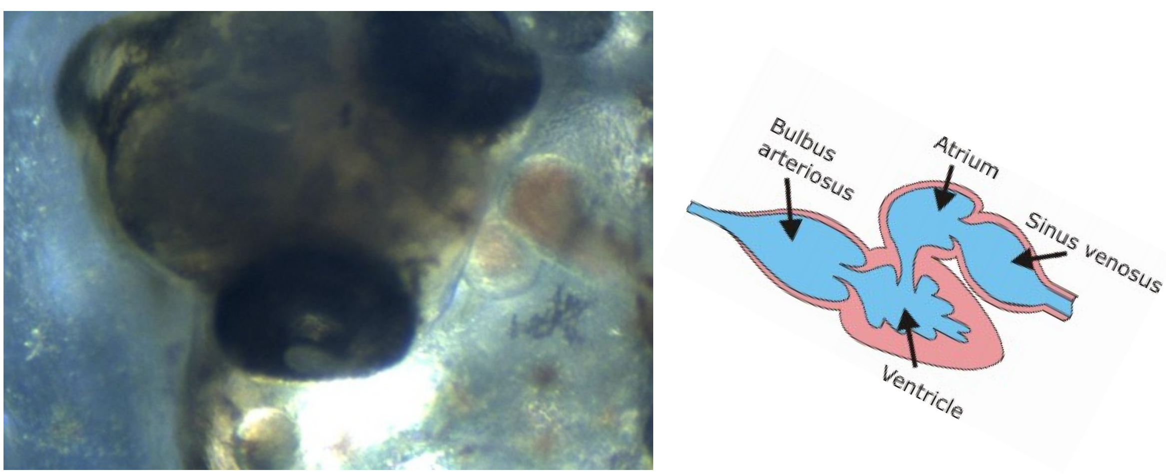
Overall Project: The US Environmental Protection Agency (EPA) is concerned with minimizing the adverse effects to human health and the environment of chemical pollution. At the Atlantic Ecology Division (AED) of the US EPA Office of Research and Development, ecological research includes optimization of methods to quantify subtle effects of pollution on aquatic species, often using laboratory-reared adults and early lifestages (embryos, larvae and juveniles) of small fish species. Recently developed computerized electronic approaches could facilitate determinations of such effects and serve as appropriate training opportunities for engineering students.

Activity 1: Monitoring embryonic and larval fish hearts electronically

Background: The hearts of embryonic and larval fish are sensitive indicators of the toxic effects of oil (Incardona et al. 2009), and that subtle alterations are correlated with later impairments such as reduced swimming speed (Incardona et al. 2014). The AED has being working with the Alantic killifish, *Fundulus heteroclitus*, a small estuarine fish species that serves as an important ecological indicator of environmental condition (e.g., Nacci et al. 2010). Of particular interest, this killifish produces transparent embryos whose developmental period lasts for about 14 days, providing a useful laboratory model to study the effects of environmental contaminants on the developing heart (Figure 1).

Figure 1: Microscopic image of embryonic killifish (10 days post fertilization) showing two chambered heart to right of head (dark eyes visible); blood engorged sinus venosus, atrium, and ventricle visible but bulbus arteriosus is obscured by head.



Fish photos: Ms. Rachel Struch, UC Davis http://esi.stanford.edu/circulation/circulation5.htm

There are two methods currently used to document embryonic fish heart condition and performance, each of which is promising but sub-optimal. AED uses the ‘video methods’ of Incardona and co-authors (2009, 2014) to produce recorded images of hearts (Figure 2) that document heart chamber size/shape (e.g., using shareware such as ImageJ); rate and rhythmicity are calculated by post-capture manual analysis. However, event marking software could be adapted from freely available software (such as JWatcher) to make these video analyses more quantitatively accurate or faster for the analyst through some degree of automation.

An alternative or complementary method to assess heart activity is via electrocardiogram (ECG). Specifically, Dhillon et al. (2013) have developed instrumentation and methodologies to produce and analyze digitized ECG output for a freshwater fish species, the zebrafish. These authors analyze the digitized ECG signal using some proprietary software (LabChart) that interprets the ECG waveform as per the cardiac cycle (Figure 3), which is said to resemble that of mammals (Dillon et al. 2013). The use of non-proprietary software would make ECG data analysis more accessible. Optimally, combining both approaches might provide more comprehensive analyses of heart structure and functionality.

Figure 2: Videomicrography displayed as sequential images of killifish embryonic heart cycle.

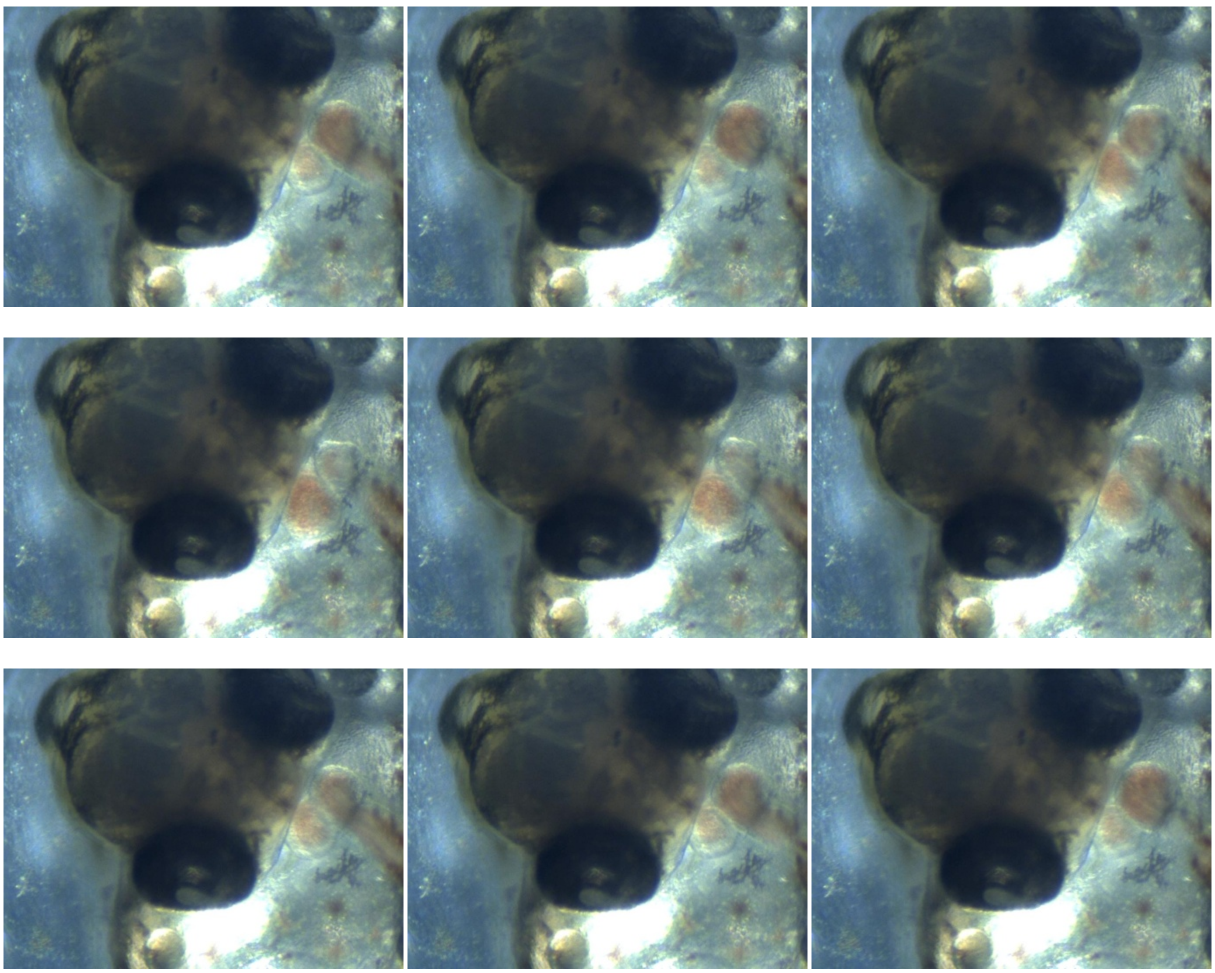
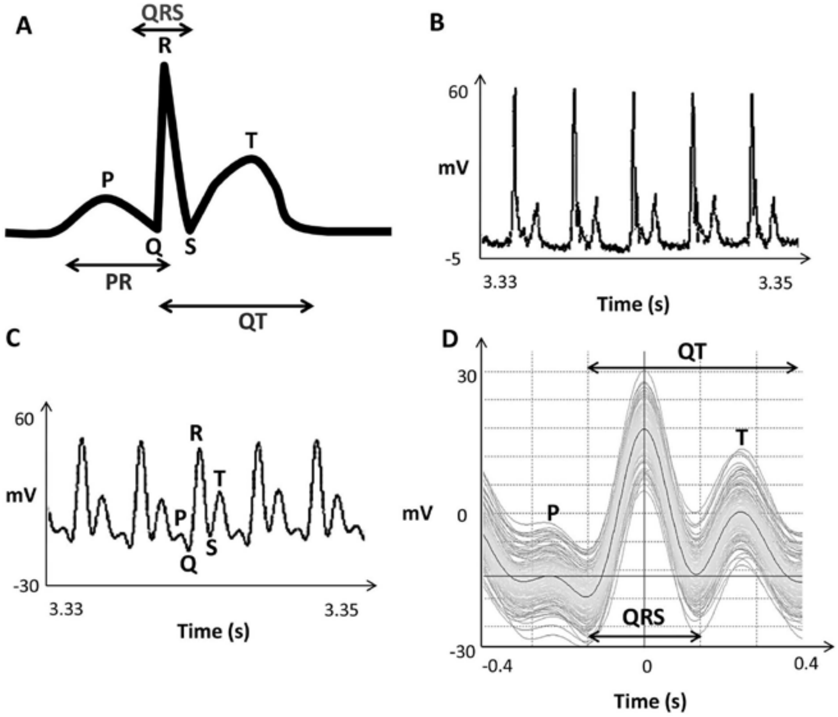


Figure 3: Detection and analysis of fish electrocardiogram, from Dhillon et al. 2013. doi:10.1371/journal.pone.0060552.g001



Activity description: Hard- and software will be adapted from published literature that will permit the quantitative analysis of embryonic and larval heart cycling of the estuarine fish *Fundulus heteroclitus*. Optimally, digitized ECG patterns will be synchronized with video imaging to permit visualization of the heart cycle, and coordinated measurements of the size and shape of heart components.

Task to be completed:

- Living embryo tasks:
    - EE task: Adapt from zebrafish systems (Dhillon et al. 2013) simple ECG hardware for embryos and larvae of the estuarine fish *Fundulus heteroclitus* that is robust to saltwater and student operators.
    - CE task: Produce digitized ECG output and analyses of output consistent with human clinical patterns, as per Dhillon et al. 2013.
- Video analyses:
    - CE task: Synchronize digitized ECG output with video imaging of the heart, which will quantify the timing and magnitude of events in the heart cycling and can be displayed as an overlay to microvideoimaging.
    - CE task: As complement or alternative to above method, develop or adapt non-proprietary post-video analysis software such as J-Watcher to partially automate the 'marking' of defined events of the cardiac cycle, and then analyze the timing of these events to document heart rate and rhythmicity.

Constraints: *Fundulus heteroclitus* is a summer-spawning fish species; therefore, the availability of living embryos may be limited through the fall and winter. While AED will try to manipulate laboratory conditions to ensure year-round availability, some tasks that do not require living embryos (i.e., video analyses, adult endpoints) may be prioritized as necessary.

Minimal system requirements: System operates safely and reproducibly, is compatible with microvideo imaging equipment operated through standard PC system, using non-proprietary software.

Citations:

Dhillon SS, Doro E, Magyary I, Egginton S, Sik A, Muller F. 2013. Optimization of embryonic and larval ECG measurements in zebrafish for quantifying the effect of QT prolonging drugs. PLOS One e60552.

Incardona JP, Carls MG, Day HL, Slaon CA, Bolton JL, Collier TK, Scholz N. 2009. Cardia arrthmia is the primary response of embryonic pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. Environ. Sci. Tech. 43: 210-207.

Incardona JP, Garner LD, linbo TL, Brown TL, Esbasugh AJ, Mager EM, Steiglitz LD, French BL, Labenia JS, Laetz CA, Tagal M, Slaon CA, Elizur A, Bennetti DD, Grosell M, Black BA, Scholz NL. 2014. Deepwater Horizon crude oil impacts the developing hearts of large predatory pelagic fish. www.PNAS.org/cgi/doi/10.1073/pnas.1320950111

Nacci D, Champlin D, and Jayaraman S. 2010. Adaptation of the estuarine fish *Fundulus heteroclitus* to toxic pollutants. Estuaries and Coasts 33(4): 853-864.

Activity 2: Assessing body condition of larval and adult killifish

Background: Body composition (i.e. lipids, energy density) is an important indicator of the overall health or condition of fish. Simple non-destructive methods that have been used to assess condition of some species of adult fish take advantage of bioelectrical impedance and ultrasound technologies; these methods are also potentially useful for adults and the early lifestages of small fish.

Bioelectrical impedance analysis (BIA) is a commonly used method for estimating body composition, and in particular body fat for mammals and fish. BIA measures opposition to the flow of an electric current through body tissues which can then be used to calculate an estimate of total body water, which can be uased as a proxy for body fat (http://en.wikipedia.org/wiki/Bioelectrical_impedance_analysis). Specifically, BIA has been used for the rapid, cost-effective, accurate, and non-lethal measure of proximate body composition (i.e., lipids, energy density) of fish collected in the field or laboratory; however, this method has typically been applied to moderate- to large-sized adult fish (Figure 4). Several reports provide detailed descriptions of BIA systems that could serve as models for small adult and early lifestages of fish (Hafs 2011; Hooks et al 2012).

Less frequently used in aquacultural applications, ultrasound methods have also been used successfully to assess properties such as fat content for the food industry (Halim et al. 2013), and might serve as useful approaches to assess the condition in living small fish. Whether the ultrasound technology is feasible for ecological applications is yet to be determined.

Figure 4:

(http://www.glerl.noaa.gov/res/Task_rpts/2005/epludsin05-3.html)

Activity description: Published hard- and software technologies will be evaluated that will permit the quantitative analysis of embryonic, larval and adult body condition and/or fat content in living *Fundulus heteroclitus*. Feasible approaches will be adapted and tested for their suitability for this purpose.

Task to be completed:

- CE and EE task: Evaluate available scientific and technical literature that describes methods to assess fish condition/fat for its usefulness with small living fish; after considering technical and logistical properties work with mentor to select appropriate method, such as bioimpedance or ultrasound.
- EE task: Adapt from systems used on other fish species, e.g., Hafs 2011; Hook et al. 2012) a simple bioimpedance or ultrasound method (Halim et al. 2013) for larvae, juvenile and adult *Fundulus heteroclitus* that is robust to saltwater and student operators.
- CE task: Produce digitized output that is interpretable and can be tested for validity using samples (supplied by mentor) that will be measured by chemical analysis (by mentor) for fat content et al.

Constraints: *Fundulus heteroclitus* adult and juvenile fish are available year-round from the AED laboratory.

Minimal system requirements: System operates safely and reproducibly, is compatible with use on living small fish in saltwater system.

Citations:

Hafs AW, 2011, Dissertation West Virginia University, Bioelectrical Impedance Analysis Methods for Prediction of Brook Trout Salvelinus fontinalis Percent Dry Weight (http://www.bemidjistate.edu/academics/departments/biology/Hafs,%20A_%20W_%202011_%20Bioelectrical%20impedance%20analysis%20methods%20for%20prediction%20of%20brook%20trout%20Salvelinus%20fontinalis%20percent%20dry%20weight.pdf

Halim et al. 2013. The Use of Ultrasound As a Fat Measurement Sensor For the Food Industry: a Review. Proc. of the IEEE International Conference on Smart Instrumentation, Measurement and

Applications (ICSIMA). 26-27 November 2013, Kuala Lumpur, Malaysia
http://ieeexplore.ieee.org/stamp/stamp.jsp?arnumber=6717974

Hook T et al. 2012. NOAA report: Development of Bioelectrical Impedance Analysis (BIA) for Rapid Assessment of Fish Condition http://www.glerl.noaa.gov/res/Task_rpts/2005/epludsin05-3.html