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Evolution of Cardiac Tissue and Flow Mechanics in Developing Japanese Medaka

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Abstract

The effects of pressure drop across cardiac valve cushion regions and endocardial wall strain triggering cardiac morphogenesis in a teleost species heart are poorly understood. In the presented work, we utilize microscale particle image velocimetry (μ PIV) flow measurements of developing medaka hearts from 3 to 14 dpf ($n=4-5$ at each dpf) to quantify the pressure field and endocardial wall strain. Peak pressure drop at the atrioventricular canal (ΔP_{AVC}) and outflow tract (ΔP_{OFT}) show a steady increase with fish age progression. Pressure drops when non-dimensionalized with blood viscosity and heart rate at each dpf are comparable with measurements in zebrafish hearts. Retrograde flows captured at these regions display a negative pressure drop. A novel metric, Endocardial Work (EW), is introduced by analyzing the ΔP_{AVC} -strain curves, which is a non-invasive measure of work required for ventricle filling. EW is a metric that can differentiate between the linear heart stage (< 100 Pa-%), cardiac looped chamber stage (< 300 Pa-%), and the fully formed chamber stage (> 300 Pa-%).

INTRODUCTION

Cardiac chamber formation and development in teleosts is a dynamic process where mechanical forces generated by the pulsating heart cause endocardial cells to migrate or reorganize, leading to cardiac remodeling. However, past studies investigating biomechanics have mainly probed into the flow wall shear stress (WSS) variation, with little to no attention to the pressure drop (ΔP) that induces the varying WSS profiles or the cardiac wall strain facilitating morphological changes in the endocardium. Pressure and strain data can potentially indicate the amount of endocardial work (EW) required for ventricle filling or ejection [1-6]. Standard indices of ventricle function do not measure myocardial work related to the metabolic demand. The EW has been shown in humans and higher vertebrates to directly reflect oxygen demand and

metabolic consumption. Hence it is a comprehensive metric for regional ventricle function that can be developed to distinguish between healthy and diseased ventricles [1, 2]. To our knowledge, no studies have evaluated in-vivo EW in teleost ventricles. Owing to the small size of the teleosts and limited spatial resolution of images, it is challenging to accurately calculate velocity gradients that contribute to calculations of pressure and strain.

Pressure measurements in previous studies were time-consuming. They were done with cannulae at a single point, with intrusive probes that provided limited information about the spatiotemporal pressure variation [7, 8]. Pressure gradients in the zebrafish (*Danio rerio*) heart were calculated using Laplace law [9] or from the knowledge of circumferential stress [10]. However, the accuracy of these measurements is limited since they neglected viscous forces in the heart. A non-invasive spatially and temporally resolved pressure field in a 48-hour post-fertilization (hpf) zebrafish heart was first calculated using micro-Particle Image Velocimetry (μ PIV) analysis by Dasi et al. [11]. The imaging was limited to the atrium only because the fish's eyes and head blocked a part of the ventricle.

Cardiac wall strain measurements of teleosts are affected by spatially under-resolved images that cannot accurately capture the wall motion. The recent introduction of high-frequency ultrasound probes designed for small animals enabled Hein et al. [12] to measure strain rate and strain (%) measurements of adult zebrafish suffering a myocardial injury. However, echocardiographic measurements are impossible to acquire from embryonic and larval fish due to the small size and difficulty of introducing a contrast agent. Lee et al. utilized the optical transparency of 48 hpf, 75 hpf, and 100 hpf zebrafish (both control and mutants) and used selective plane illumination microscopic imaging technique to report strain measurements [13].

Thus, an overview of the literature highlights the need to measure pressure and strain in teleost hearts accurately. To our knowledge, there is no baseline framework for embryonic vertebrate animal models that investigates the variation of EW along the developmental stages. In the current work, we selected Japanese Medaka (*Oryzias latipes*) as our teleost model because it is larger than zebrafish in both embryonic and larval stages. Additionally, medaka grows two times slower than zebrafish [14, 15] allowing capturing developmental landmarks during cardiac morphogenesis from embryonic to larval stages.

The objective of this work is to conduct a series of live experiments to image the Japanese medaka heart under a microscope followed by a subsequent μ PIV analysis and quantify the ΔP across the atrioventricular canal (AVC) and outflow tract (OFT), endocardial wall strain of the ventricle and consequently the EW required for ventricle filling across development in embryo/larva.

Results

Cardiac function and geometry

The progression of cardiac function and geometry parameters for this dataset have been described in detail in our previous analysis of wall shear stress (WSS) measurements done with this data [16]. The mean value of each parameter is presented in **Table 1**. HR represents heart rate in beats per minute (bpm), Re and Wo represent Reynolds number and Womersley number, respectively, and both are non-dimensional. “A” represents the ventricle area during peak diastole. EF represents the ejection fraction of the ventricle.

Table 1 : Cardiac function and geometry parameters

dpf	HR (bpm)	Re	Wo	A (μm^2)	EF (%)
3	78.5 $\pm$ 3.7	0.02 $\pm$ 0.003	0.021 $\pm$ 0.005	1143.3 $\pm$ 75.9	58.2 $\pm$ 4.7
4	85.5 $\pm$ 3.3	0.03 $\pm$ 0.007	0.022 $\pm$ 0.004	1433.3 $\pm$ 49.9	58.2 $\pm$ 7.9
5	93 $\pm$ 6.5	0.03 $\pm$ 0.008	0.023 $\pm$ 0.006	1610 $\pm$ 151.2	72.2 $\pm$ 4.8
6	93 $\pm$ 2.4	0.04 $\pm$ 0.005	0.023 $\pm$ 0.004	1466.7 $\pm$ 212.5	54.5 $\pm$ 7.4
7	106 $\pm$ 7.5	0.04 $\pm$ 0.008	0.024 $\pm$ 0.006	1857.5 $\pm$ 369.3	50.5 $\pm$ 9.3
8	132 $\pm$ 4.9	0.04 $\pm$ 0.003	0.027 $\pm$ 0.005	1604 $\pm$ 104.8	59.5 $\pm$ 10
9	150.5 $\pm$ 2.5	0.06 $\pm$ 0.011	0.029 $\pm$ 0.004	2660 $\pm$ 298.5	61.2 $\pm$ 11.9
10	143.3 $\pm$ 6.2	0.07 $\pm$ 0.009	0.028 $\pm$ 0.006	4065 $\pm$ 648.5	81.3 $\pm$ 3.4
11	161.3 $\pm$ 18.7	0.08 $\pm$ 0.027	0.029 $\pm$ 0.010	3796.7 $\pm$ 369.2	72.6 $\pm$ 3.1
12	145 $\pm$ 2.4	0.07 $\pm$ 0.007	0.028 $\pm$ 0.004	3531.2 $\pm$ 457.5	75.9 $\pm$ 6.7
13	173.6 $\pm$ 7.1	0.1 $\pm$ 0.034	0.031 $\pm$ 0.006	4884 $\pm$ 1095.5	76.3 $\pm$ 4.8
14	172.4 $\pm$ 7.1	0.1 $\pm$ 0.008	0.031 $\pm$ 0.006	3906 $\pm$ 1312.3	81 $\pm$ 5.5

Pressure contours across the AVC and the OFT

Pressure field variation across the atrium and ventricle along three phases of the ventricle filling cycle is plotted in **Figure 1** for a medaka heart at 3 dpf, 8 dpf, and 13 dpf. A zero-pressure boundary condition is applied at the ventricle outflow. Both the atrial inflow and the ventricle outflow remain closed throughout T_{dias} , which is the period of the ventricle filling cycle. At $0.3T_{dias}$ the peak pressure drop across the AVC is $800*(HR*\mu)$ at 3dpf, $1200*(HR*\mu)$ at 8 dpf,

Figure 1 : Pressure field across ventricle, atrium, and AVC during ventricle diastole at 3 dpf, 8dpf, and 13 dpf.

and $2000*(HR*\mu)$ at 13dpf. The atrium experiences the highest pressure, and blood flows through the AVC from the atrium to the ventricle. At $0.6T_{dias}$ the AVC pressure drop reduces in magnitude while blood continues to flow in the same direction [$500*(HR*\mu)$ at 3dpf, $900*(HR*\mu)$ at 8 dpf and $1200*(HR*\mu)$ at 13dpf]. At $0.9T_{dias}$ the ventricle filling has almost ended with blood flowing from atrium to ventricle at 0.2 mm/s at 3dpf suffering a pressure drop of $200*(HR*\mu)$ at the AVC region. However, due to the absence of the valves in the AVC, there is a negative pressure drop across the AVC that causes retrograde flow with a velocity of approximately 1 mm/s from the ventricle to atrium [$-200*(HR*\mu)$ at 8 dpf and $-500*(HR*\mu)$ at 13dpf] (**Figure 1**).

The pressure field variation across the ventricle and the bulbus arteriosus (BA) along three phases of the ventricle systolic cycle is plotted in **Figure 2** for a medaka heart at 3 dpf, 8 dpf, and 13 dpf. A zero-pressure boundary condition is applied at the BA outflow. The narrow region joining the ventricle and the BA is referred to as the outflow tract (OFT). Both the atrial inflow

and the AVC inflow to the ventricle remain closed throughout T_{sys} , which is the period of the ventricle systolic cycle. At $0.3T_{sys}$ the peak pressure drop across the OFT is $1200 \cdot (HR \cdot \mu)$ at 3dpf, $550 \cdot (HR \cdot \mu)$ at 8 dpf, and $700 \cdot (HR \cdot \mu)$ at 13dpf. The ventricle experiences higher pressure, and blood flows through the OFT from the ventricle to the BA. At $0.6T_{sys}$, the OFT pressure drop almost stays constant in magnitude during 3 dpf and 8dpf. [3dpf : $1150 \cdot (HR \cdot \mu)$, 8dpf : $500 \cdot (HR \cdot \mu)$]. Interestingly for the 13 dpf heart, the OFT pressure drop increases substantially at $0.6T_{sys}$ to $1100 \cdot (HR \cdot \mu)$. At $0.9T_{sys}$ the ventricle outflow has almost ended with blood flowing from the ventricle to BA at 0.1 mm/s at 3dpf and 8dpf, suffering a pressure drop of $300 \cdot (HR \cdot \mu)$ at the OFT region. Nevertheless, due to the absence of the valves in the narrow OFT, a negative pressure drop of $-300 \cdot (HR \cdot \mu)$ at 13dpf causes retrograde flow with velocity 0.3 mm/s from the BA to the ventricle (**Figure 2**).

Figure 2 : Pressure field across ventricle, VB, and OFT during ventricle diastole at 3 dpf, 8dpf, and 13 dpf.

Time variation of pressure drop across AVC and OFT

Time variations of velocity magnitude and pressure drop across AVC (dimensional and non-dimensional) are plotted for a ventricle diastolic cycle in **Figure 3**. Similar variations of velocity magnitude and pressure drop across OFT are plotted for a ventricle systolic cycle in **Figure 4**. The pink shaded region between the minimum and maximum time variations in each dpf depicts the bounds within which the profiles across 4-5 samples fluctuate. The black line shows the median profile for each dpf. The peak values of the median profile are marked on each plot by a blue circle.

Figure 3 : Time variation profiles in the atrioventricular canal (AVC) for the flow parameters a) Velocity Magnitude (Vm_{AVC}) b) Pressure drop (ΔP_{AVC}) and c) Non-dimensionalized pressure drop $\frac{\Delta P_{AVC}}{HR * \mu}$. Black lines denote the median profiles while the pink shade denotes region between maximum and minimum profiles. The peak values of the median profile are marked on each plot by a blue circle. $n=3-5$ for each dpf.

A small pressure drop between 3dpf and 5dpf drives the low velocity flow into the ventricle when the AVC is not clearly defined. At 6dpf peak values of both Vm_{AVC} and ΔP_{AVC} increase considerably owing to narrowing of the AVC region after cardiac looping. At 7dpf, the peak median Vm_{AVC} and the peak median $\frac{\Delta P_{AVC}}{HR * \mu}$ reduce a little. As the fish prepares to hatch between 8 dpf and 9 dpf, there is a rise in peak flow velocity and pressure drop. After hatching, they eventually plateau from 10 dpf to 14 dpf. At 3 dpf, unlike the AVC, the peak flow velocity and pressure drop through OFT is high, which reduces at 4 dpf and 5 dpf. At 6dpf, there is a substantial rise in the peak median Vm_{OFT} . Moreover, the peak median $\frac{\Delta P_{OFT}}{HR * \mu}$ that can be attributed to the narrowing of OFT after cardiac looping. These parameters decrease subsequently at 7 dpf and 8 dpf. After hatching, the peak median Vm_{AVC} and the peak median $\frac{\Delta P_{AVC}}{HR * \mu}$ increase at 9 dpf and fluctuates within a small range from 9 dpf to 14 dpf.

Figure 4: Time variation profiles in the atrioventricular canal (OFT) for the flow parameters a) Velocity Magnitude (V_{m_OFT}) b) Pressure drop (ΔP_{OFT}) and c) Non-dimensionalized pressure drop $\frac{\Delta P_{OFT}}{HR * \mu}$. Black lines denote the median profiles while the pink shade denotes region between maximum and minimum profiles. The peak values of the median profile are marked on each plot by a blue circle. $n=3-5$ for each dpf.

Strain measurements of the ventricle wall

Endocardial wall strain (%) is extracted during a heart cycle for each sample at each dpf. **Figure 5a** shows the 13 dpf ventricle during peak diastole. The red boundary has been drawn manually to outline the ventricle. The wall locations on the pericardium and the endocardium are colored by the % strain experienced at each location. **Figure 5b** shows the time variation of endocardial strain along a heart cycle. A black line represents the median profile across 5 samples at each dpf. The pink shaded region between the minimum and maximum time variations in each dpf depicts the bounds within which the profiles across 5 samples fluctuate. The peak strain during ventricle diastole of the median profile is marked on each plot by a blue circle. The peak diastolic strain evolution with age progression is low at 3 dpf (19.5%) and 4 dpf (22%). But 5 dpf onwards, the median peak does not follow a specific trend with age progression. It fluctuates between 29% and 55%.

EW measurement from ΔP_{AVC} -strain loop

Pressure drop across AVC (ΔP_{AVC}) is plotted across endocardial wall strain for a ventricle diastolic cycle in a medaka heart at each dpf in **Figure 6**. The endocardial work (EW) represents

Figure 5: Strain measurement of the ventricle wall along a heart cycle. a) Pericardial and endocardial strain overlapped with velocity vectors on the raw image of a 13 dpf medaka heart at peak diastole. V stands for ventricle. b) Variation of strain time history across medaka age progression.

the area under this ΔP_{AVC} -strain curve. EW obtained from this will have the unit Pa-% instead of the conventional unit of N-m. The three curves in a subplot are selected according to a minimum (black curve), maximum (red curve), and median EW (blue curve) measured at each dpf. The y-axis limits for the subplots in each column are identical. As seen in 3 dpf to 5 dpf, a smaller area curve denotes that the contractility of the endocardial wall is lower than the larger area curves (6 dpf to 14 dpf). The curve shapes are noisy and inconsistent owing to two reasons: a) the reference (end of ventricle systole) time may have been slightly different in each sample. b) the velocity gradients calculated from the vectors of μ PIV analysis are susceptible to 10% error for 1% error in velocity measurements. These errors may show up as “noisy” plots. The maximum strain occurs when the ventricle expansion is the largest, while the maximum pressure drop occurs after the peak flow velocity crosses the AVC.

Peak EW, peak ΔP at AVC, OFT, and peak diastolic strain measurement

The peak values of EW, ΔP_{AVC} , ΔP_{OFT} , and diastolic strain have been plotted against developing medaka age progression in **Figure 7**. The mean across 4-5 samples in each dpf is plotted, with the standard deviation being displayed by the error bars. The (***) over each dpf shows that the

Figure 6 : Pressure drop (ΔP_{AVC}) vs. endocardial wall strain plots from 3 dpf to 14 dpf. Three curves in each dpf denote minimum EW (black curve), median EW (blue curve), and maximum EW (red curve). The y-axis limits for subplots in each column are identical. The x-axis limits for all subplots are the same.

mean is statistically significantly different when compared to the mean value at 3 dpf. In **Figure 7c**, the peak strain during ventricle diastole ($Strain_{dias}$) is low at 3dpf and 4 dpf. It fluctuates within a broad range from 5 dpf and 14 dpf, with high variability in each dpf. So, it is not a metric that can accurately distinguish ventricle wall remodeling stages. In **Figure 7b**, peak values of ΔP_{AVC} and ΔP_{OFT} show a well-defined increase from pre-hatch (3dpf - 8 dpf) to post-hatch stages (9 dpf – 14 dpf). The variability across the samples is high in the post-hatch stages. **Figure 7c** shows a well-defined progression of EW with developing fish age. EW at 3 dpf and 4 dpf are below 100 Pa-%. EW at 5 dpf and 6 dpf are below 300 Pa-%, while the EW at 9 dpf, 10 dpf, 12 dpf, and 13 dpf are above 300 Pa-%. The sudden reduction of EW and ΔP_{AVC} at 11 dpf with little variability can be attributed to unknown developmental changes occurring at AVC.

A larger sample size is required to substantiate this observation. Overall, EW is the metric that best demarcates between the linear heart stage (< 100 Pa-%), cardiac looped chamber stage (< 300 Pa-%), and the fully formed chamber stage (> 300 Pa-%).

Discussion

Non-invasive pressure drop measurements are reported for the first time across the AVC and OFT regions of a developing medaka during the linear heart tube stages, cardiac looping end stages, and fully developed chamber stages. Retrograde flows were observed in pressure contour

Figure 7 : Variation of a) Peak EW, b) peak ΔP at AVC and OFT, and c) peak diastolic ventricle strain with medaka age progression. The * in the plots denote statistical significance compared to the control values in 3 dpf. *** denote $p < 0.0001$, ** denote $p < 0.001$, and * denote $p < 0.05$.

and velocity vector plots at AVC during 8 dpf and 13 dpf and at OFT during 13 dpf (**Figure 1**, **Figure 2**). The pumping mechanics at the AVC and OFT during 3dpf are significantly different, as observed from the pressure contours. The AVC is not narrow at 3dpf, due to which the pressure drop across the chambers are small. With age progression, the AVC starts narrowing, and the ΔP_{AVC} increases. The OFT is initially positioned such that during ventricle systole, a strong suction force draws blood directly from the atrial inlet. Due to this large ΔP_{OFT} , a high flow velocity is observed at 3dpf. At 6dpf, the appreciable increase in both ΔP_{AVC} and ΔP_{OFT} can be attributed to similar filling mechanics of flow into a compressed closed vessel, like a balloon. This increase was observed in peak-WSS values in the previous work with this data [16]. The balloon-filling mechanics continues in the successive embryonic ages. After hatching, ΔP_{AVC} and ΔP_{OFT} are appreciable higher than their pre-hatch measures. Due to decompression of the organs, the ventricle rapidly increases in size after hatching. This leads to a stronger pressure drop when the ventricle expands (ΔP_{AVC}) or contracts (ΔP_{OFT}). The non-dimensional pressure drop is useful to compare across stages within the same species and with other species where the length and time scales are different [11]. The pressure measurement technique can also be used for three-dimensional images. When combined with physical pressure readings, this can predict the absolute pressure distribution in the medaka heart in future studies.

Strain measurements have not been explored earlier in medaka heart. Our measurement technique avoids the lack of repeatability associated with manual segmentation of cardiac walls. Instead, using velocity gradients to detect wall edges circumvents the expertise required to differentiate between pericardium and endocardium in a strain calculated from ultrasound scans. The results show that the ventricle deformation is much less in the linear heart tube stage at 3 dpf and 4 dpf. This is attributed to the lack of cardiomyocytes that start populating significantly after 5 dpf [17]. The peak diastolic strain measured earlier in the normal zebrafish heart was around 40% at 50 hpf, 75 hpf, and 100 hpf [13] which could not differentiate between the three developmental stages. The %-strain is still a useful metric to compare the contractility in different segments of the cardiac wall and can identify disease regions on the cardiac wall.

The EW is a metric that we have introduced through this work in the teleost cardiac mechanics literature. The EW has shown sufficient demarcation (**Figure 7a**) between the three main developmental stages of the medaka heart but needs to be validated with a higher number of samples at each dpf. A lower EW suggests a stiffer ventricle with low flowrates through the AVC. The shape of the ΔP_{AVC} -strain curve at different stages offers insight into the pumping mechanism and time scales of ventricle expansion, relaxation, and contraction. These can be quantified in future studies with more samples. A flat-topped curve (**Figure 6**) indicates that the ΔP_{AVC} is caused by the positive pressure in the atrium only with no contribution from the ventricle expansion. A bell-shaped curve, on the other hand, includes the contribution of ventricle expansion to the ΔP_{AVC} . The EW calculated from these curves is indirectly related to the metabolic demand of the heart and can be further developed into a diagnostic tool that can differentiate normal hearts from diseased hearts in humans.

Limitations

The main limitation of this study is that there are only four/five samples per dpf. Including more samples would require the investment of sufficient time and resources but would increase accuracy. The limited spatial resolution near the endocardial wall introduced errors in the velocity gradient measurements that propagated in both pressure and strain calculations. The orientation of the heart during imaging can be different for different samples at each dpf. In some orientations, the out-of-plane-velocity vectors are larger in number at the AVC. This may again bias the final pressure and strain calculations. The EW is calculated from an open ΔP_{AVC} -strain

loop because the pressure cannot be calculated when the AVC is closed. Despite all these limitations, both the pressure drop at AVC, OFT, and the EW show a gradual increase with developing medaka age progression that has not been reported yet.

Methods

Details about the Japanese Medaka Husbandry, Data Acquisition, and μ PIV analysis in the medaka heart have already been discussed in the previous work using this data [16]. The fish maintenance and experimental protocols were approved by Purdue Animal Care and Use Committee (PACUC) (Protocol Number 1702001545). The study was performed in accordance with the ARRIVE guidelines. All methods were performed in accordance with relevant guidelines and regulations as approved by PACUC.

Velocity reconstruction across the AVC and the OFT

The medaka heart has two small connector regions that reduce the area as the fish ages from 3 dpf to 14 dpf. One is the AVC [diameter: 6-10 μ m] joining the atrium and ventricle, and the other is the OFT [diameter: 10-14 μ m] connecting the ventricle and the BA. The high velocities at AVC and OFT at certain heart cycle phases could not be accurately captured due to increased noise or signal dropout in those frames. So, using the velocity information of a neighboring region in the ventricle/atrium and applying the conservation of mass principle as shown in **Equation 1**, the velocity vectors were reconstructed in these areas.

$$\nabla \cdot \mathbf{u} = 0 \quad \text{Equation 2}$$

Pressure analysis of the cardiac chambers

The pressure field in the cardiac chambers for a heart cycle was obtained by integrating **Equation 3** for the known phase averaged velocity field. Velocity gradients were calculated using a high accuracy gradient calculation technique, namely the Compact-Richardson method [18] in accordance with second-order polynomial fitting done in previous studies [11, 19].

$$\nabla P = -\rho \left(\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) + \mu (\nabla^2 \mathbf{u}) \quad \text{Equation 4}$$

The pressure was then integrated along multiple line paths in the cardiac field, assuming a zero value at a fixed node on the boundary. Integration paths were averaged to smooth out

uncertainties in the velocity field. The average value was updated in successive iterations until the pressure residual converges to a value of 1×10^{-3} . A second-order trapezoidal rule was used for spatial integration. The dependence of this omnidirectional method on grid resolution, sampling rate, velocity field error levels, and off-axis recording was explored extensively for PIV measured velocity fields in previous work from our lab [20]. Blood dynamic viscosity (μ) was calculated for each sample at each dpf using the hematocrit and shear rate information as described in previous work [16]. Owing to small Re ($\ll 1$) and Wo ($\ll 1$), the first two terms on the right-hand side of equation X are negligible. So pressure (P) is non-dimensionalized by the product of heart rate and blood dynamic viscosity ($HR \cdot \mu$) as shown in [11]

Strain analysis of the ventricle wall

Velocity field measurements are used to calculate the deformation gradient tensor F for the entire ventricle. The end of ventricle systole is used as the reference time (or frame number) against which the deformation of the ventricle at other time points in the heart cycle is calculated. The Strain is then calculated from F according to the equation below

$$F = \begin{bmatrix} \frac{\partial u}{\partial x} & \frac{\partial u}{\partial y} \\ \frac{\partial v}{\partial x} & \frac{\partial v}{\partial y} \end{bmatrix}; \text{ Strain} = \frac{1}{2} F^T F - I \quad \text{Equation 5}$$

Where T denotes the transpose of a matrix, and I is the identity matrix. Endocardial wall locations were detected from the velocity gradient images by using the Canny edge detection scheme to identify the coordinates of peak gradient locations. The lagrangian strain was extracted at the ventricle wall locations to show the spatial variation of strain along the ventricle wall (**Figure 5a**). At each time point, three maximum strain values were further extracted and averaged to generate peak endocardial wall strain variation along a cardiac cycle (**Figure 5b**).

EW measurement of the ventricle

The endocardial work (EW) is measured by calculating the area under the curve of a ΔP_{AVC} -strain plot, as shown in **Figure 6**. The area calculation is performed in Matlab by implementing a numerical trapezoidal integration technique. The ΔP_{AVC} -strain plot is not a closed loop because the ΔP information is available only for the diastolic cycle of the ventricle when the AVC is open. The EW measure reflects the combined effect of changes in ΔP_{AVC} and strain on the effective contractility of the endocardial wall.

Conclusion

To our knowledge, this study is the first to report peak pressure drop values in the AVC and OFT of a developing Japanese medaka from 3 dpf (onset of blood circulation) to 14 dpf. The viscosity used in the pressure calculations was calculated at each dpf instead of assuming a constant value. Flow reversals observed for the first time in the valveless narrow cushion regions of the medaka ventricle inflow and outflow are validated by negative pressure drop towards the end of ventricle diastole during 8 dpf and 13 dpf. A non-intrusive metric EW is introduced to differentiate between the linear heart tube stages, end of cardiac looping stages, and the fully developed chambers in the post-hatch stages.

Longitudinal studies of cardiovascular development relating the pressure drop to cardiac developmental landmarks are scarce in all vertebrates. An important insight gained from this longitudinal analysis of flow and tissue mechanics variation with age is the different pumping mechanisms of the heart at different dpf. At 3dpf, the pumping mechanics at the AVC was different from that of the OFT. This baseline framework can be further tested by probing the flow-tissue mechanics evolution in higher vertebrate models.

Author Contribution

SC-Conception and design of study, Imaging data curation, μ PIV analysis, methodology and validation, writing original draft, review & editing.

MSS- Conception and design of study, Writing - review & editing.

PPV-Conception and design of study, Writing - review & editing.

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Figures

Figure 1

Pressure field across ventricle, atrium, and AVC during ventricle diastole at 3 dpf, 8 dpf, and 13 dpf.

Figure 2

Pressure field across ventricle, VB, and OFT during ventricle diastole at 3 dpf, 8dpf, and 13 dpf.

Figure 3

Please see the Manuscript PDF file for the complete figure caption.

Figure 4

Please see the Manuscript PDF file for the complete figure caption.

Figure 5

Strain measurement of the ventricle wall along a heart cycle. a) Pericardial and endocardial strain overlapped with velocity vectors on the raw image of a 13 dpf medaka heart at peak diastole. V stands for ventricle. b) Variation of strain time history across medaka age progression.

Figure 6

Pressure drop (ΔP_{AVC}) vs. endocardial wall strain plots from 3 dpf to 14 dpf. Three curves in each dpf denote minimum EW (black curve), median EW (blue curve), and maximum EW (red curve). The y-axis limits for subplots in each column are identical. The x-axis limits for all subplots are the same.

Figure 7

Variation of a) Peak EW, b) peak ΔP at AVC and OFT, and c) peak diastolic ventricle strain with medaka age progression. The * in the plots denote statistical significance compared to the control values in 3 dpf. *** denote $p < 0.0001$, ** denote $p < 0.001$, and * denote $p < 0.05$.

Supplementary Files

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