Mechanisms responsible for the enhanced pumping capacity of the in situ winter flounder heart (Pseudopleuronectes americanus)

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Mendonça PC, Genge AG, Deitch EJ, Gamperl AK. Mechanisms responsible for the enhanced pumping capacity of the in situ winter flounder heart (Pseudopleuronectes americanus). Am J Physiol Regul Integr Comp Physiol 293: R2112–R2119, 2007. First published August 29, 2007; doi:10.1152/ajpregu.00202.2007.—In situ Starling and power output curves and in vitro pressure-volume curves were determined for winter flounder hearts, as well as the hearts of two other teleosts (Atlantic salmon and cod). In situ maximum cardiac output was not different between the three species (~62 ml·min⁻¹·kg⁻¹). However, because of the small size of the flounder heart, maximum stroke volume per milliliter per gram ventricle was significantly greater (2.3) compared with cod (1.7) and salmon (1.4) and is the highest reported for teleosts. The maximum power output of the flounder heart (7.6 mW/g) was significantly lower than that measured in the salmon (9.7) and similar to the cod (7.8) but was achieved at a much lower output pressure (4.9 vs. 8.0 and 6.2 kPa, respectively). Although the flounder heart could not perform resting levels of cardiac function at subambient pressures, it was much more sensitive to filling pressure, a finding supported by pressure-volume curves, which showed that the flounder’s heart chambers were more compliant. Finally, we report that the flounder’s bulbus:ventricle mass ratio (0.59) was significantly higher than in the cod (0.37) and salmon (0.22). These data, which support previous studies suggesting that the flatfish cardiovascular system is a high-volume, low-pressure design, suggest a number of possible mechanisms through which this enhanced vis-à-tergo filling capacity might contribute to the high SV (per gram ventricle) is high in the winter flounder (resting SV 0.94 ml/g ventricle; maximum SV 1.5 ml·min⁻¹·g⁻¹ ventricle at 10°C), compared with other teleosts [see Table 3, (34)]. These authors suggest a number of possible mechanisms through which this elevated SV could be achieved: 1) the lack of compact myocardium and thus the presence of a highly compliant ventricle; 2) a more pronounced Starling response associated with enhanced vis-à-tergo and/or vis-à-fronter cardio filling; and 3) a reduced cardiac afterload, which could enhance ventricular emptying under conditions of maximal exercise. However, there are insufficient data on cardiovascular function in flatfishes and other teleosts to allow for a determination of which of these mechanisms is likely to be the most important.

To accurately determine the maximum pumping capacity of the winter flounder (Pseudopleuronectes americanus) heart and to identify the factors contributing to the high SV in this species, a comparative study was performed using the winter flounder and two other marine species, the Atlantic cod (Gadus morhua) and the Atlantic salmon (Salmo salar). These latter species were chosen because the cardiovascular physiology of cod (e.g., 1, 3, 8, 9) and salmonids (e.g., 17, 18, 21, 31, 43) has been extensively studied, and they have a morphology, physiology, and lifestyle very different from flatfishes. In situ perfused heart preparations (20) were used to determine sensitivity to filling pressure (i.e., Starling curves), and maximum SV, Q, and power output (P), for all three species. Pressure-volume curves were generated for the atrium, ventricle, and bulbus arteriosus (26) to examine whether differences in chamber compliance exist between species. Finally, the relative sizes of the heart chambers were measured to evaluate the contribution of heart morphology to pumping capacity and heart function.

MATERIALS AND METHODS

Experimental animals. Ethical approval was obtained from the Animal Care Committee at the Memorial University of Newfoundland and the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Cardiac Function in Winter Flounder

In situ heart preparations. Fish were anesthetized in seawater containing methane sulfonic acid of m-aminobenzoate (MS-222, 0.25 g/l), and then transferred to a surgery table, where their gills were cut to expose the gills containing methane sulfonic acid of m-aminobenzoate (MS-222, 0.25 g/l). In situ heart preparations were obtained for the salmon as described by Farrell et al. (21), with only minor modifications of this protocol required for cod. For example, the output cannula could not be secured in the ventral aorta of cod at a position between the 3rd and 4th gill arches due to the robustness of the cartilage in this area. Instead, the cannula was secured in place between the 1st and 2nd gill arches by tying directly to the ventral aorta, and the 3rd and 4th gill arches were subsequently occluded using cable ties. However, this is the first time that an in situ heart preparation has been used in the flounder, and thus, we briefly describe the procedure below.

The winter flounder’s abdominal cavity was exposed by cutting the abdominal wall along the lateral line. The gonads and this isolated portion of the digestive tract were removed to allow for access to the hepatic veins. The hepatic vein on the “eyed side,” and secured in place with 3-0 silk with a 1-0 silk thread (American Cyanamid, Pearl River, NY), and the gall bladder was drained. Umbilical tape (Baxter Healthcare, Deerfield, IL) was tied around the gastrointestinal tract, inferior to the liver. The gonads and this isolated portion of the digestive tract were removed to allow for access to the hepatic veins. The hepatic vein on the “blind side” was occluded using a 3-0 silk thread (American Cyanamid). Then an input cannula (2.2 mm OD; 1.7 mm ID; steel chromatography tubing) was introduced into the sinus venosus via the hepatic vein on the “eyed side,” and secured in place with 3-0 silk thread. At this point, the perfusate bottle was opened, and several gill arches were cut to prevent excessive pressure development by the ventricle. The height of the perfusate bottle relative to the heart arches were subsequently occluded using cable ties. However, this is the first time that an in situ heart preparation has been used in the flounder, and thus, we briefly describe the procedure below.

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To ensure that the input cannula was securely tied within the sinus venosus and that the in situ heart was isolated from the saline in the experimental bath, two final tests were performed. First, it was confirmed that Q rapidly fell to 0 ml/min when the input cannula was clamped off using a pair of hemostats. Second, with the hemostats still clamping the input cannula, the tubing that connected the output cannula to the heart was raised to 10 kPa to confirm that Q was maintained at 0 ml/min (i.e., there is no backflow of perfusate).

Pressure-volume curves. Fish were overanesthetized in seawater containing MS-222 (0.35 g/l) and transferred to a surgery table where the pericardial sac was exposed through a ventral incision. Then, the pericardium was cut and the heart was dissected free from the animal, being careful to include part of the sinus venosus and ventral aorta. The heart was placed in a chamber with ~3 cm of ice on the bottom to help maintain the temperature at ~8 °C. With the help of a microscope (Leica MZ 9.5, Leica Microsystems, Richmond Hill, ON, Canada) an input cannula (2.2 mm OD; 1.7 mm ID; steel chromatography tubing) was introduced into the atrium via the sinus venosus and secured in place with 0–0 silk thread. Steel chromatography tubing (1.0 mm OD) was then inserted via the ventral aorta into the bulbus arteriosus and was secured using 2–0 silk thread. Then a slightly curved micro-aneurysm clip (85 g pressure, 1 mm wide × 6 mm long; Harvard Apparatus, Holliston, MA) was placed at the junction of the atrium and ventricle.

To generate pressure-volume curves for the atrium, the atrium was filled with saline at 3.05 ml/h using a calibrated syringe pump (A-99 model, Razel Scientific Instruments, Stamford, CT), and pressure within the atrium was measured via a side arm in the input cannula, using a Gould Statham pressure transducer (Model P23 ID; Gould Statham, Oxnard, CA). Once the pressure-volume curve for the atrium was generated, a small incision was made in the atrium to drain the chamber, and the input cannula was advanced into the ventricle, and the atraumatic clamp moved to the ventricle:bulbar junction. In a similar fashion, pressure-volume curves were generated for the ventricle, and then for the bulbus arteriosus. For all chambers, maximum pressure and volume were taken at the point at which each chamber began leaking (i.e., pressure did not increase or began to fall with further increases in volume).

The determination of pressure-volume curves took about 90 min for each fish, and spontaneous cardiac contractions were often induced by the infusion of saline. Thus, chamber pressure was measured as diastolic pressure. During the measurements, the heart’s surface was kept moist by frequent applications of saline at 8–10°C. At the conclusion of each experiment, the heart chambers were separated, individually weighed, and atrial:ventricular (A:V) and bulbus:ventricular (B:V) mass ratios were calculated to examine whether the size...
of the heart chambers or their relative size might influence the shape of the pressure-volume curves.

**Data acquisition and calculations.** $P_{IN}$ and $P_{OUT}$ were measured using Gould Statham pressure transducers (Model P23 ID; Gould Statham). For the in situ experiments, these pressure transducers were calibrated daily against a static water column, where zero pressure (0 kPa) was set equal to the saline level in the experimental bath. Further, the recorded $P_{IN}$ and $P_{OUT}$ were corrected using predetermined calibrations (23) to account for the resistance in the tubing between the points of pressure measurement and the heart. For the pressure-volume curves, 0 pressure was set to the level of the heart in the humidified chamber.

$Q$ was measured using a Model T206 small animal blood flowmeter in conjunction with a precalibrated in-line flow probe (2N, Transonic Systems, Ithaca, NY). Pressure and flow signals were amplified and filtered using a Model MP100A-CE data acquisition system (BIOPAC Systems, Ithaca, NY). The signals acquired during the in situ protocol were analyzed and stored using Acqknowledge 3.7.2 Software (BIOPAC Systems), installed on a 300-MHz Toshiba laptop computer.

Cardiovascular function during the in situ experiments was continuously monitored by measuring $Q$, $P_{IN}$, and $P_{OUT}$. Although data were continuously collected, cardiac function was only analyzed at specific intervals during each experiment. The $P_{IN}$ required to maintain resting in vivo $Q$ was measured prior to obtaining the Starling curve. In addition, all cardiac parameters ($Q$, $S_V$, $f_H$) were measured at each level of $P_{IN}$ during the Starling curve and at each level of $P_{OUT}$ during the Max. Power test. Heart rate ($f_H$) was measured by counting the number of systolic peaks on the $Q$ recording during a 30-s interval, and $S_V$ (ml/kg) was calculated from $Q$ (ml/min $\cdot$ min $^{-1}$)/$f_H$; $S_V$ per gram of ventricular mass ($S_V$, ml/g ventricle) was calculated by dividing $S_V$ (ml) by ventricular mass (g), and power output of the heart ($P_H$, mW/g) was calculated as $[Q$ (ml/min)/60] $\times$ [(POUT $-$ PIN) $\times$ 0.098]/ventricle wet mass (g).

Pressure values obtained during the generation of the pressure-volume curves were also analyzed and stored using Acqknowledge 3.7.2 software (BIOPAC Systems, Santa Barbara, CA), installed on a Sea3ix computer. Chamber volumes were calculated based on the delivery rate of the syringe pump (3.05 ml/h) used to fill the heart chambers. Plots of ventricle, atrium, and bulbus arteriosus volume vs. pressure were used to describe the hemodynamic characteristics of each of the heart chambers, and maximum compliance values for each of the chambers were determined by calculating the slope of sections (>0.2 ml) of the mean volume-pressure relationships (see Fig. 2) at which the compliance was greatest (i.e., the slope of each relationship was at a minimum). Finally, maximum distensibility was determined, by dividing maximum compliance by the initial volume for each chamber (i.e., the volume at the lower end of the range used to calculate maximum compliance).

**Statistical analyses.** After latransforming the data for the Starling and pressure-volume curves, ANCOVA was used to test for homogeneity of slopes between species ($P < 0.05$; SPSS Software). Maximum power values were obtained by fitting 3rd-order regressions (SigmaPlot Software) to the pressure-flow data of each fish. Differences in maximum $P_H$, $Q$, $S_V$, $S_V$, maximum pressure and volumes, chamber masses, and A:V and B:V mass ratios between species were assessed by ANOVAs followed by pairwise Tukey post hoc tests (SPSS Software, $P < 0.05$).

**RESULTS**

**In situ heart preparation.** In situ heart rate was significantly lower in the cod, compared with the salmon and flounder, when the hearts performed at resting levels of cardiac performance. Although there was no significant difference in resting $S_V$ between species (Table 1), the cod and salmon hearts could generate in vivo resting levels of $Q$ at negative $P_{IN}$ values, whereas the flounder heart required a positive $P_{IN}$ of 0.04 ± 0.02 kPa (Fig. 1A).

In situ maximum $Q$ was not significantly different between the three species, averaging 62 ml min $^{-1}$ kg $^{-1}$. However, because the relative ventricular mass (RVM) of the flounder was 40% less than for the other two species, the maximum $S_V$ (per gram of ventricle) achieved by the winter flounder was significantly higher (2.3 ± 0.1) compared with the Atlantic cod (1.7 ± 0.2) and Atlantic salmon (1.4 ± 0.1) (Fig. 1A; Table 1). In addition to having a higher maximum $S_V$, the flounder heart was much more sensitive to increases in filling pressure, and fewer increments in $P_{IN}$ were required by the flounder heart to achieve elevated levels of $S_V$ (Fig. 1A). For instance, to achieve a $S_V$ of 1.4 ml/g ventricle (the maximum $S_V$ for the salmon heart), the flounder heart only needed a $P_{IN}$ increase of 0.19 kPa, whereas cod and salmon hearts required $P_{IN}$ increases of 0.29 and 0.69 kPa, respectively.

The Atlantic salmon hearts achieved a much higher maximum $P_H$ (9.7 ± 0.5 mW/g) than the other two species, and could maintain significant flow at pressures in excess of 10 kPa (Fig. 1B). When comparing the power output curves of the flounder and cod, two things became apparent. First, maximum $P_H$ was surprisingly similar in the two species (cod, 7.8 ± 0.6 mW/g; flounder, 7.6 ± 0.3 mW/g). Second, the power output curve for the flounder was shifted considerably to the left. This resulted in maximum $P_H$ being achieved at a $P_{OUT}$ of 4.89 ± 0.17 kPa in the flounder, compared with 6.24 ± 0.18 kPa in the cod (Fig. 1B; Table 1).

**Pressure-volume curves.** In several preparations one of the chambers leaked due to damage during surgery or advancement of the cannula, and thus, the data were not used. This resulted in different numbers of pressure-volume curves for each chamber (Table 2, Fig. 2). Only two ventricles were used to obtain the salmon pressure-volume curves, due to

<table>
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<th>Resting</th>
<th>Maximum</th>
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<td></td>
<td>RVM % $Q_{ml-min^{-1}kg^{-1}}$ $S_V$ ml/kg $S_V$ ml/g ventricle $f_H$ bpm $Q_{ml-min^{-1}kg^{-1}}$ $S_V$ ml/kg $S_V$ ml/g ventricle $P_H$ mW/g</td>
<td></td>
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<tr>
<td>S. salar</td>
<td>0.07 ± 0.002 $^a$ 73.1 ± 1.9 $^a$ 16.3 ± 0.36 0.22 ± 0.01 0.33 ± 0.01 66.8 ± 1.4 $^a$ 63.8 ± 1.9 0.96 ± 0.05 $^a$ 1.4 ± 0.08 $^a$ 9.7 ± 0.51 $^a$</td>
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<td>G. morhua</td>
<td>0.07 ± 0.004 $^a$ 58.4 ± 1.22 $^b$ 16.8 ± 0.23 0.29 ± 0.01 0.40 ± 0.02 51.3 ± 0.77 $^b$ 62.3 ± 2.8 1.2 ± 0.07 $^b$ 1.7 ± 0.16 $^b$ 7.8 ± 0.63 $^b$</td>
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<tr>
<td>P. americana</td>
<td>0.05 ± 0.001 $^a$ 68.4 ± 2.9 $^b$ 14.3 ± 0.28 0.21 ± 0.04 0.44 ± 0.09 54.0 ± 1.5 $^b$ 60.3 ± 4.1 1.1 ± 0.07 $^b$ 2.3 ± 0.14 $^b$ 7.6 ± 0.33 $^b$</td>
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Values represent means ± SE (n ≥ 7). $^{a,b}$Dissimilar letters indicate a significant difference ($P < 0.05$) between species for a given parameter. Relative ventricular mass (RVM), heart rate ($f_H$), cardiac output ($Q$), stroke volume ($S_V$), and power output ($P_H$).
The pressure-volume relationships for the atrium and ventricle in all species generally had a characteristic J-shape, whereas those of the bulbus arteriosus were more sigmoidal (Fig. 2). Furthermore, with the exception of the salmon ventricle (which could be filled to a pressure approaching 10 kPa), the heart chambers of the three species attained similar maximum pressures; averaging 0.36, 5.3 (cod and flounder), and 10.6 kPa for the atrium, ventricle, and bulbus, respectively (Table 2; Fig. 2). Although the shape of the curves and the maximum pressure values obtained were similar between species, the pressure-volume curves show that all of the flounder’s chambers are significantly more compliant and distensible (see Table 2), and, in general, fill to significantly higher volumes (atrium: 0.65 ± 0.03 ml; ventricle: 0.80 ± 0.06 ml; bulbus: 0.50 ± 0.05 ml) compared with the cod (atrium: 0.34 ± 0.03 ml; ventricle: 0.46 ± 0.04 ml; bulbus: 0.33 ± 0.03 ml) and the salmon (atrium: 0.28 ± 0.03 ml; ventricle: 0.75 ± 0.01 ml; bulbus: 0.30 ± 0.03 ml).

There were small or no differences in the atrial or ventricular masses (g), the relative atrial or bulbar masses (in % body mass), or the A:V mass ratio between species. However, the flounder’s bulbar mass and B:V mass ratio (0.21 ± 0.03 g, 0.59 ± 0.06 g) were significantly greater than measured in the cod (0.14 ± 0.01 g, 0.37 ± 0.03) and salmon (0.11 ± 0.01 g, 0.22 ± 0.01 g) (Table 3). This enlarged bulbus may work in concert with the bulbs’ higher compliance to keep systolic blood pressure low despite the large stroke volume delivered to the circulation.

**DISCUSSION**

In situ heart preparations have been used for the past two-and-a-half decades to investigate aspects of cardiac function without any physical disturbance to the heart (e.g., 4, 11, 20–22, 25). However, this was the first time that an in situ cod heart preparation had been used to investigate flatfish or cod cardiac function. Although the in situ cod heart preparation was relatively easy to obtain, the dorsoventrally compressed body morphology of adult flounder made the perfused heart the difficulty in positioning the cannula in the centre of the small lumen of the salmon’s heart chambers; however, the data for these two ventricles were very similar and were incorporated for comparative purposes.

**Table 2. In vitro maximum pressures and volumes recorded when generating pressure-volume curves for the heart chambers of the three species (Atlantic salmon, Atlantic cod, and winter flounder)**

<table>
<thead>
<tr>
<th>Chamber</th>
<th>S. salar</th>
<th>G. morhua</th>
<th>P. americanus</th>
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<tbody>
<tr>
<td>Atrium</td>
<td>0.39±0.07</td>
<td>0.39±0.07</td>
<td>0.29±0.06</td>
</tr>
<tr>
<td>Ventricle</td>
<td>11.3</td>
<td>5.1±0.13</td>
<td>5.5±0.66</td>
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<tr>
<td>Bulbus</td>
<td>11.0±0.35</td>
<td>10.3±0.20</td>
<td>10.5±0.18</td>
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<tr>
<td>Maximum pressure, kPa</td>
<td></td>
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<tr>
<td>Maximum volume, ml</td>
<td>0.28±0.03</td>
<td>0.34±0.03</td>
<td>0.65±0.03</td>
</tr>
<tr>
<td>Maximum volume, ml/kg</td>
<td>0.52±0.05</td>
<td>0.76±0.04</td>
<td>1.1±0.07</td>
</tr>
<tr>
<td>Maximum volume, ml/g chamber</td>
<td>3.6±0.29</td>
<td>4.7±0.34</td>
<td>9.4±0.47</td>
</tr>
<tr>
<td>Maximum compliance, ml/kPa</td>
<td>1.2</td>
<td>1.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Maximum distensibility, fold change kPa</td>
<td>25.0</td>
<td>43.0</td>
<td>164.0</td>
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</table>

All chambers were filled at a rate of 3.05 ml/hr, and pressure was recorded through a side-arm in the input cannula. Values represent means ± SE (n = 7, except for the salmon ventricle, where n = 2). a,b Different letters indicate a significant difference (P < 0.05) between species for each chamber. Values for maximum compliance and distensibility were calculated from the mean values presented in Figure 2 (see text).
surgery exceptionally difficult and resulted in a low surgical success rate (~35%). Despite this, however, the flounder in situ heart proved to be a tractable preparation for examining cardiac function independent of hormonal and/or nervous control mechanisms.

**Interspecific differences in cardiac performance.** The mass-specific (per kilogram body mass) values for Q and SV in the flounder are comparable to those measured for the cod and salmon, two considerably more active pelagic species. Moreover, the maximum in situ SV (2.3 ± 0.14 ml/g ventricle) that we recorded for the winter flounder is the highest ever reported for fish, even considerably higher than that measured in the Antarctic fish *P. bernacchii* (1.4 ml/g ventricle at 0°C; Ref. 2). This in situ evidence for an enhanced pumping capacity of the winter flounder heart is supported by the pressure-volume curves (Fig. 2), which show that the flounder ventricle is able to fill to a maximum volume of ~2.1 ml/g ventricle (~0.8 ml).

The high maximum Q (60.3 ± 4.1 ml·kg⁻¹·min⁻¹) and SV (1.1 ± 0.07 ml/kg) reported for the flounder heart may seem surprising, considering the winter flounder’s benthic and relatively inactive lifestyle (24, 41) and the low values reported for aerobic capacity in flatfishes (14, 37, 46). However, flatfish are also reported to have lower hematocrit levels (~20%) (24, 51, 53, 54) compared with other teleosts (e.g., 24–30% for cod and salmonids) (13, 30, 39, 45). Thus, it is likely that this enhanced cardiac function offsets the effects of reduced hematocrit on blood oxygen transport and thus allows these fish to achieve moderate levels of activity [critical swimming speed of 0.73 bl/s at 10°C; (34)]. Further, the large SV in flounder may be advantageous during severe hypoxia (e.g., 20% O₂ saturation), a situation where fH is reduced by 41% of normoxic conditions (unpublished observation). This latter point may be particularly important for coastal flatfishes like the flounder, which can periodically face hypoxia due to high nutrient loading (44, 49) and can be found buried several centimeters (12–15 cm) into the substrate (24).

Previous authors have reported that maximal values for Q in rainbow trout, determined using the in situ perfused heart preparation, are within ~20% of the highest in vivo Q values measured during prolonged swimming (e.g., 7, 21, 50). Our in situ Q max values for the cod and Atlantic salmon (~63 ml·min⁻¹·kg⁻¹) are within 2 and 29% of those obtained for these two species when swum to exhaustion [Atlantic salmon, 63.8 ml·min⁻¹·kg⁻¹ (13); cod, 44.5 ml·min⁻¹·kg⁻¹ (L. H. Petersen and A. K. Gamperl, unpublished observation)], and thus our data are consistent with that for the rainbow trout. In contrast, the maximum Q (60.3 ml·min⁻¹·kg⁻¹) and SV (2.3 ml/g ventricle) measured in this study are more than 50% higher than the values reported for the winter flounder by Joaquim et al. (34) during a critical swimming speed (U crit) test at 10°C (39.2 ml·min⁻¹·kg⁻¹ and 1.51 ml/g ventricle, respectively). The reason(s) for the discrepancy is/are unknown; however, the difference may be related to the inability of the flounder heart to deliver blood to the circulation at high pressures. First, while maximum P H values for the cod and the salmon hearts were measured at ~6 and 8 kPa, respectively, the flounder ventricle was unable to completely empty at arterial pressures above 4.8 kPa. Second, arterial pressures (afterload) increase considerably (by 25 to 65%) when fish are forced to swim at or near maximal speeds (3, 36), and Joaquim et al. (34) showed that cardiac parameters in the flounder were at maximal levels at slow swimming velocities and remained constant until U crit. Thus, these data suggest that the flounder heart cannot deal with the high-pressure demands of continuous exercise and that flounder are unable to fully exploit the flow potential of their hearts while swimming.

Atrial filling in fish is achieved by *vis-à-fronte* and *vis-à-tergo* mechanisms. In *vis-à-fronte* filling, the energy of ventricular contraction creates a subambient intrapericardial pres-
sure, and consequently, a negative atrial transmural pressure gradient that is used to distend the atrium and thus assist in its filling (15, 16, 19). In contrast, vis-à-tergo filling of the atrium is dependent on central venous pressure, as well as potentially contraction of the sinus venosus. It was suggested, for many fish species with a rigid pericardium that vis-à-fron
te filling was the primary determinant of Q under resting conditions and that at higher SV there was a transition from vis-à-tergo to vis-à-tergo (venous pressure) filling (18, 19). In contrast, recent work by Minerick et al. (40) proposes that, at least for the rainbow trout, vis-à-tergo filling is the primary determinant of cardiac filling and that vis-à-fron
te filling is only important in situations, such as high-intensity exercise in which elevated cardiac output is required. Our research does not add to the debate about which of these two mechanisms is dominant at rest or during situations demanding elevated cardiac performance in salmonids. However, it supports the present dogma that vis-à-fron
te filling is only present in active (nonbenthic) species (19) and strongly suggests that vis-à-fron
te filling is not a requirement for achieving high values of SV. In this study, resting SV could be achieved at subambient filling pressures in the cod and Atlantic salmon (both active pelagic species), whereas a positive P_in of 0.04 kPa was needed for resting Q values in the winter flounder (Fig. 1A). This requirement for a positive input pressure to achieve resting Q in flounder is consistent with studies that report that in situ eel (A. dieffenbachia) (12, 28), sea raven (H. americanus) (20), and ocean pout (M. americanus) (22) hearts are unable to maintain resting levels of Q at subambient filling pressures. In these previous studies on benthic (inactive) teleosts, maximum values for SV and Q were 0.6 ml/kg and 20 – 30 ml · min⁻¹ · kg⁻¹, and thus it appeared that vis-à-fron
te filling was required for the high cardiac performance exhibited by more active teleosts such as the trout (maximum values ~1.0 ml/kg and ≥ 50 ml · min⁻¹ · kg⁻¹ at similar temperatures) (7, 18, 50). Clearly, our results for the in situ flounder heart suggest that this is not the case and that high cardiac outputs can be achieved by vis-à-tergo filling mechanisms alone.

At present, we do not have an explanation for why the flounder heart is not capable of filling through vis-à-fron
te mechanisms. First, the flounder pericardium is not sac-like, is relatively rigid, and is closely associated with the body wall and musculature. Thus, it is morphologically similar to the pericardium found in salmonids and not benthic species such as the eel (19, 28). Second, Farrell and Jones (19) indicate that for the atrium and sinus venosus to act as variable-volume reservoirs within a rigid (semirigid) pericardium, thus facilitating vis-à-fron
te filling, the maximum end-diastolic volume of these chambers must be equal to the maximum stroke volume plus the difference between resting and maximum stroke volume (26). Although we did not construct pressure-volume curves for the flounder’s sinus venosus, the maximum diastolic volume of the flounder atrium is equal to maximum stroke volume (1.1 ml/kg), and in the trout, maximum diastolic volume of the sinus venosus is 75% of that for the atrium (26). Thus, it is likely that the combined maximum diastolic volumes of the flounder’s sinus and atrium are sufficient to meet maximum pumping demands.

As with most active fish, the salmon ventricle is composed of a spongy layer and an outer compact layer of myocardium (30 – 45% of myocardial mass) (13, 47), the latter generally considered to be important for the enhanced cardiac performance required by active fish species. Thus, it was not surprising that the salmon had a significantly higher maximum P_H (9.7 mW/g) when compared with the cod (7.8 mW/g) and the flounder (7.6 mW/g). It was somewhat unexpected that the cod (a demersal species) and the flounder (a benthic and relatively sedentary species) would have similar values for maximum P_H. However, the hearts of both of these species are composed entirely of spongy myocardium, and Fig. 1B shows that the flounder heart reaches maximum P_H at a significantly lower output pressure (4.9 kPa) than the cod (6.2 kPa). Thus, the similarity in P_H values is due to the enhanced flow capacity of the flounder heart.

Mechanisms allowing for enhanced cardiac function in flounder. Through this study, we have begun to elucidate how a species with a RVM 30–50% smaller than most salmonids and other pelagic species (3, 30, 50) can achieve comparable levels of body mass-specific SV and Q. First, we show that the flounder heart has a more pronounced Starling curve, meaning that it needs smaller increases in preload to achieve similar, or even higher, values of SV. This greater sensitivity of the flounder heart to filling pressure undoubtedly reflects the high distensibility/compliance of its chambers (Fig. 2, Table 2) and the fact that in vivo end-systolic volume is normally zero at physiological output pressures (27). For example, the flounder atrium only requires 18–23% of the in vitro input pressure required by cod and salmon to reach equivalent diastolic volumes, and flounder ventricular pressures at the cod’s maximum diastolic volume (0.46 ml) are only ~30% of that recorded in the other two species. This increased distensibility/compliance may, in fact, compensate for the lack of vis-à-fron
te filling by still allowing the flounder to rapidly attain large end-diastolic volumes, and increase SV in situations demanding elevated cardiac performance.

Second, we report that there are several features of the flounder’s bulbus arteriosus that would allow the heart to effectively deliver its enhanced end-diastolic volume into the circulation. The primary function of the bulbus arteriosus is to depultate the blood ejected from the ventricle, permitting an

Table 3. Body and heart morphometrics, RVM, RAM, RBM, A:V, and B:V ratios recorded for 8–10°C acclimated Atlantic salmon, Atlantic cod, and winter flounder

<table>
<thead>
<tr>
<th>Body Weight, g</th>
<th>Ventricle Mass, g</th>
<th>Atrium Mass, g</th>
<th>Bulbus Mass, g</th>
<th>RVM %</th>
<th>RAM %</th>
<th>RBM %</th>
<th>A:V</th>
<th>B:V</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. salar</td>
<td>568.3 ± 14.1b</td>
<td>0.44 ± 0.02</td>
<td>0.08 ± 0.004</td>
<td>0.11 ± 0.01a</td>
<td>0.08 ± 0.003a</td>
<td>0.01 ± 0.001ab</td>
<td>0.019 ± 0.001b</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>G. morhua</td>
<td>492.6 ± 25.6a</td>
<td>0.37 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.14 ± 0.01a</td>
<td>0.08 ± 0.01a</td>
<td>0.02 ± 0.001a</td>
<td>0.026 ± 0.001a</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>P. americanus</td>
<td>650.3 ± 30.8b</td>
<td>0.39 ± 0.03</td>
<td>0.07 ± 0.004</td>
<td>0.21 ± 0.03b</td>
<td>0.05 ± 0.003b</td>
<td>0.01 ± 0.001b</td>
<td>0.034 ± 0.004b</td>
<td>0.22 ± 0.01</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (n ≥ 7). **Dissimilar letters indicate a significant difference (P < 0.05) between species for a given parameter. RAM, relative atrial mass; RBM, relative bulbar mass; A:V, atrium:ventricular mass ratio; B:V, bulbus:ventricular mass ratio.
almost continuous blood flow in the ventral aorta and gills, and to minimize increases in ventral aortic pressure that are associated with ventricular ejection (17, 35). The bulbar pressure-volume relationship is shifted downward for the winter flounder compared with the other two species at all chamber volumes, and the flounder’s bulbus is most compliant over a range of pressures from ~2.5 to 5 kPa, compared with ~5 to 8 kPa for the cod and salmon (Fig. 2C). Further, the winter flounder has a B:V ratio (0.59) ~3 times larger than the salmon (0.22) and ~2 fold larger than that of cod (0.37) (Table 3).

Thus, it appears that the flounder’s bulbus has adapted, both in terms of compliance/distensibility and size, to permit large stroke volumes at pressures that do not become limiting to ventricular function. This conclusion is consistent with Clark and Rodnick (10), who proposed that alterations in ventricular function should be matched by morphofunctional changes in the bulbus and suggested that a disjunct between ventricular and bulbar function in mature male rainbow trout led to hypertension and promoted ventricular hypertrophy. In addition, this would at least partially explain why flounder have arterial pressures of ~3 kPa (5, 6, 54), considerably less than measured in most other teleosts (~3.9 to 5.3 kPa (19)).

Interestingly, fH rate decreased by 14 beats/min (21%) in the flounder compared with 12% and 8.6% in the cod and salmon, respectively, between resting and maximum levels of cardiac contractility/myofilament Ca²⁺ sensitivity, and during non-exhaustive exercise. On the basis of the research presented here, one might have expected a larger decrease in fH in the flounder, as its heart is considerably smaller compared with the other two species, and thus equivalent increases in Sv (ml/kg) would result in greater myocardial stretch compared with the cod and salmon. However, this difference in fH responsiveness does not explain why maximum Sv was greater in the flounder or that this species had a steeper Starling curve. For example, while the flounder’s fH was 19% lower than measured in the salmon at maximum Q, the flounder’s maximum Sv (ml/g ventricle) was 65% higher. In addition, fH at maximum Q was not significantly different between the cod and flounder (Table 1, Fig. 1).

Although this study provides some information on how the flounder heart achieves such high stroke volumes, further investigation is required to 1) ascertain the cellular basis behind the steepness and extension of the flounder Starling curve and 2) determine whether alterations in myocardial contractility/myofilament Ca²⁺ sensitivity, in addition to bulbus morphophysiology and low arterial blood pressures, permit the flounder ventricle to deliver such large stroke volumes into the circulation. On the basis of the research presented here, one could hypothesize that for a specific length, flounder myocytes have a decreased passive tension and an increased active tension compared with the trout and cod. Indeed, the enhanced distensibility of the flounder’s heart chambers, at least when compared with the cod heart, which also lacks a compact myocardium, may well be related to changes in myocardial connective tissue (e.g., titin and collagen) content or isoform (33, 55). Further, research suggests that low resting tensions and length/stretch-dependent increases in myofilament Ca²⁺ sensitivity are important cardiomyocyte features in fish species such as the trout, which are capable of large increases in stroke volume as compared with mammals (48). However, such an adaptation in myocyte physiology may not be required in the flounder, compared with the trout. The trout/salmon heart has a distinct lumen, and an outer layer of compact myocardium that is largely responsible for its enhanced pressure generating capacity (29). Thus, the Law of Laplace would apply to the whole trout heart and make it difficult for this species to develop enough wall tension at very large stroke volumes, so that zero or minimal end-systolic volumes are maintained. In contrast, the flounder heart has essentially no lumen and is composed entirely of spongy myocardium. Because the radius of the lacunae comprising this spongy myocardium is small, Laplacian relationships dictate that pressure may be generated at “considerable mechanical advantage compared with hearts consisting solely or partially of compact myocardium” (19).

Overall, this work shows that the Sv measured in the winter flounder (per gram of ventricle) is extremely high and that this high Sv is related to 1) a pronounced and extended Starling curve; 2) more compliant heart chambers; and 3) a high bulbus:ventricle mass ratio. Our data support the in vivo data of Joaquim et al. (34), which showed that the cardiovascular system of flatfish is a high volume, low-pressure design. However, it also raises several questions whose answers may lead to significant advances in our understanding of fish cardiac physiology. Thus, we plan to perform single cardiac myocyte length-tension curves to examine the passive (between contractions) and active (during contraction) properties of flounder cardiac muscle and thus determine whether these cells possess unique physiological adaptations that allow for easy ventricular expansion (filling), yet the development of substantial contractile force.

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