Fish and Flag – Exploring Fluid-Structure Interaction during Undulatory Swimming in Fish

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Abstract— **Fish larvae swim by undulating their body. In order to understand the role of fluid-structure interaction on the shape of the body wave, we focus on the role of body stiffness in the swimming performance of larval zebrafish. In particular, we compare the wild type with a mutant called *stocksteif*. In this mutant, ossification is accelerated and affects all cartilage tissue of the vertebral column, causing the vertebrae to fuse into a stiff rod over the first 15 days of larval development. By comparing wild-type and *stocksteif* morphs, we studied how stiffening the vertebral column affects the shape of the body wave, and how this change in body wave kinematics in turn affects escape performance. We recorded escape responses from a top view at 1500 frames per second to determine swimming kinematics. At age 5 days—before the vertebral column shows significant ossification—the two morphs' body wave kinematics and escape performance are not significantly different. At age 15 days, the vertebral column of the *stocksteif* mutant is largely fused. This causes angular acceleration (of the posterior body relative to the anterior body) and peak curvature to be lower in the *stocksteif* mutant compared with the wild-type. Both body wave parameters affect the larva's escape performance: stocksteif larvae take longer to achieve peak translational accelerations. The increasing stiffness of the vertebral column seems to seriously limit the axial muscles' ability to bend and undulate the fish's body, which in turn deteriorates escape performance.**

Keywords— **Fish swimming, zebrafish, *stocksteif* mutant, vertebral column.**

I. Introduction

In order to swim, many fish bend their body. This body wave results from the interaction between the water surrounding the fish, the body's passive mechanical properties, and the muscle activation wave travelling down the fish body [1]. The body's passive mechanical behavior depends on the stiffness of such tissues as the vertebral column and skin, and the passive stiffness of the axial muscle tissue [1]. We know that muscle activation waves [2,3] and flow patterns [4,5] change with swimming style. Hence, the contribution of all three factors in establishing the body wave might vary with swimming style. We are particularly interested in how altering body stiffness might affect the relevance of muscle activation versus fluid-structure interaction in establishing the body wave during two swimming behaviors, C starts and cyclic undulatory swimming.

Fish respond to a startle with a C start. Usually, a C start is divided into three stages, stage 1 (preparatory phase), stage 2 and stage 3 (propulsive phases). During the preparatory phase, the fish activates only ipsilateral axial muscles, which bend the body into a C shape [3]. Curvature of the body and axial muscle activation are in phase [6]. This phase relationship is consistent with the hypothesis that the body wave is the result of the muscle activation wave. If muscle activation indeed dominates the shape of the body wave, then stiffening the body will increase the load on the axial muscles. Given the inverse relationship between contraction force and speed, a higher muscle load should lower the angular acceleration at the head and tail during the preparatory phase. This will increase the duration of the preparatory phase, which correlates negatively with the acceleration of the center of mass during the propulsive phase.

Several studies of cyclic swimming found evidence that fluid-structure interactions might be important in shaping the body undulations. First, combined EMG and kinematics measurements have found that the wave of muscle activation and the body's wave of curvature do not have a fixed phase relationship; indeed, phase changes along the body [2]. Second, experiments with physical models have shown that increasing stiffness increased rather than decreased the speed of the body wave, which led swimming speed to increase [7]. Third, experiments on trout swimming in a flow tank have shown that a body wave can form with little or no muscle activation [8]. These three observations suggest that the body wave is not simply the result of muscle contractions causing local bending of the body, but that the body wave is influenced significantly by fluid-structure interaction. If the body wave resembles a bending wave travelling down a beam or a waving flag, then we predict

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for cyclic swimming that increasing body stiffness should lead to a narrower body wave (lower curvature values) with a higher wave speed. Higher wave speeds mean higher tail beat frequencies, which in turn cause higher swimming speeds [9]. So, a fish with a stiffer body should swim faster.

For this study, we use two types of zebrafish, wild-type and a mutant called *stocksteif* [10]. *Stocksteif* mutants over-ossify their skeleton, developing a fused rather than an articulated vertebral column.

Our underlying hypothesis is that stiffness of the body is an important determinant of swimming performance. We therefore predict that altering the stiffness of the vertebral column will affect swimming performance of larval zebrafish. If on the one hand axial muscles dominate wave shape, then, during a C start, a stiffer vertebral column will lead to a lower body wave speed (i.e. lower angular acceleration of the head and tail during the preparatory phase) and lower acceleration of the center of mass during the propulsive phase. Similar predictions hold for cyclic swimming: a stiffer vertebral column should slow down the body wave and lower swimming speed. If on the other hand fluid-structure interactions dominate, then we expect that during cyclic swimming, a stiffer vertebral column will lead to a higher body wave speed and a higher swimming speed.

II. Materials and Methods

A. Animals

Zebrafish (*Danio rerio*) eggs were collected after mating two wild-type parents for the control group or two *stocksteif* heterozygous parents for the treatment group. Mutant larvae could be identified at age 5 dpf, when the abnormal development of the vertebrae can first be visualized with calcein. Calcein staining did not affect swimming performance in consecutive experiments. At age 5dpf, homozygous mutants show first signs of calcification in their anterior skeleton (skull and centra of the anterior vertebrae). Mutants exhibited severe over-ossification of their vertebral column during the first month of their development [10]. Excessive bone formation causes vertebrae to fuse; the vertebral column forms a stiff rod with few intervertebral boundaries.

Fish were fed *Paramecium* (age 5 to 6 dpf) and *Artemia* (age 7 dpf onward). Fish were bred and kept at 28°C.

B. Experiments

All experiments were conducted on zebrafish at age 5, 15 and 28 dpf . Individual fish were transferred to a test tank filled with water kept at 28°C. The recorded C starts comprise stage 1 (preparatory phase), stage 2 (propulsive phase) and stage 3 (cyclic swimming) of an escape response [11] and were elicited by lightly touching the fish with a horse hair. The cyclic swimming episodes analyzed for this study correspond to stage 3 of the escape response. We recorded up to seven swimming episodes from five fish per treatment at age 5, 15, and 28 dpf. All recordings were made from a dorsal point of view using a high-speed camera (Photron APX-RS, 1500 frames per second, 1024 × 1024 pixels, exposure time 1/8000 second) with a 105 mm Nikon lens.

C. Data Analysis

Recordings were analyzed using custom-made image analysis programs written in Matlab (Matlab 2007b, Mathworks). Fish outlines were detected automatically and tracked for as long as the complete fish was within the field of view or until the fish stopped moving. The automated tracker detects only the body of the fish, it omits pectoral fins and tail fin. From the area included in the body outlines, we estimated the fish's center of mass. By tracking center of area from frame to frame, we calculated the approximate displacement, swimming speed and translational acceleration of the fish's center of mass. Our error analysis showed that center of area of the fish's dorsal projection is at most 0.06 body lengths off the actual center of volume, which in turn is used to approximate center of mass [12]. From the tracked outlines, we determined the midline of the fish, which connects the snout to the tail. This midline is then used to calculate the following parameters describing the body wave: body curvature, speed of the body wave and tail beat frequency. All values are normalized by body length to facilitate comparison between different ages. For a full description of the motion analysis please refer to [12]. We selected only those sequences for analysis in which the fish is clearly responding to the stimulus rather than initiating a spontaneous swimming bout, and remains in full view for all three stages of the startle response.

III. Results

At age 5 dpf, the mutant vertebral column still has clear intervertebral boundaries. At 15 dpf, the number of intervertebral joints has dropped considerably. At 28 dpf, the vertebral column of several mutant fish has also developed permanent kinks, and the growth of the mutants is stunted [10].

Wild-type and homozygous *stocksteif* mutants respond to a tactile stimulus with a startle response. These C starts typically comprise all three stages [11], with the fish executing several tail beat cycles after stage 2. The fish begins to accelerate during stage 2; acceleration of the center of mass

peaks during stage 2 or 3. Fish do not maintain a constant swimming speed during cyclic swimming in stage 3.

During the preparatory phase (stage 1), wild-type and mutant fish at age 5 dpf show similar body curvatures and body wave speeds (Figure 1, Table 1). In the older fish, differences between wild-type and mutants are establishing: in the *stocksteif* mutant, angular acceleration in the posterior body and body curvature are lower (Figure 1, Table 1).

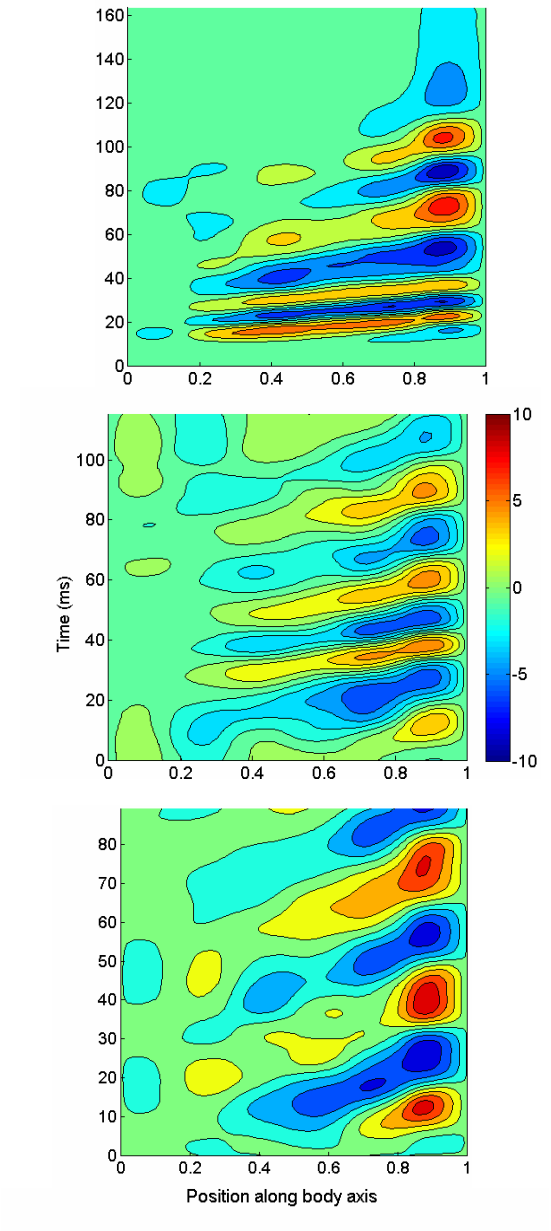

Fig. 1 Curvature profiles for mutant (age 5, 15 dpf) (top, middle panel) and wildtype zebrafish (15 dpf) (bottom panel). The color scale represents body-length specific curvature

We see similar differences in stage 2 of the propulsive phase. At age 5 dpf, mutants accelerate as well as their wild-type siblings, reaching translational accelerations of nearly 3×10^3 body lengths s^{-2} and swimming speeds of nearly 70 body length s^{-1} (Table 1). Older mutants reach similar or higher accelerations compared with their wild-type siblings, but they take longer to do so; mutants also take longer to reach a given magnitude of translational acceleration (Table 1). This is consistent with a lower angular acceleration during stage 1.

During stage 3 of the startle response, the cyclic swimming phase, we see again no differences between wild-type and mutant fish at age 5 dpf: both have similar curvature profiles, similar maximal curvatures, body wave speeds and swimming speeds (Figure 1, Table 1). Older mutants reach similar swimming speeds as their wild-type siblings, but again take longer to do so. Wild-type fish have higher curvatures than mutants. In the oldest mutants, the anterior body remains relatively straight and a significant body wave develops only in the posterior half of the body; however, permanent kinks in the body distort the curvature pattern (not shown).

Table 1 Summary of kinematic parameters for wild-type (wt) and *stocksteif* (stkf) zebrafish at 3 ages (*b* angular acceleration near the tail; *f* tail beat frequency; *c* body wave speed; *U* swimming speed and [Δt time from beginning of C start to maximum value]; *a* acceleration and [Δt time from beginning of C start to maximum value])

Age	b ($10^5\times$ rad s^{-1})	f (s^{-1})	c (s^{-1})	U(s^{-1}) [Δt (ms)]	a ($10^3\times s^{-2}$) [Δt (ms)]
5 wt	2.5	73	87	51 [30]	1.5 [32]
	3.2	40	38	77 [11]	2.9 [13]
	1.9	34	46	80 [7]	2.7 [10]
5 stkf	1.2	41	47	21 [23]	0.4 [17]
	1.6	41	43	47 [9]	1.3 [22]
	2.7	35	43	68 [12]	2.5 [8]
15 wt	9.3	30	33	12 [21]	2.0 [14]
	6.0	28	44	17 [35]	2.4 [14]
	1.8	51	57	55 [14]	1.9 [11]
15 stkf	1.3	28	30	23 [103]	3.0 [79]
	1.2	35	56	27 [73]	4.5 [69]
	1.5	43	35	34 [36]	6.8 [40]
28 wt	0.5	23	23	11 [32]	1.2 [17]
	3.4	15	25	12 [44]	0.8 [22]
	4.6	24	27	22 [29]	2.6 [19]
28 stkf	0.2	11	10	5 [106]	0.2 [101]
	0.3	19	25	16 [34]	1.7 [27]
	0.9	23	18	12 [64]	0.9 [20]

IV. Discussion

The goal of this study was to explore how changing the passive mechanical properties of the fish's body affects its body wave and swimming performance. We used a mutant called *stocksteif* to study how stiffening the vertebral column affects body curvature and center-of-mass kinematics.

Our results show that during the preparatory phase of the C start, the generation of the body wave is dominated by muscle forces rather than fluid-structure interactions. Stiffening the body reduces curvature and angular acceleration, which in turn leads to lower accelerations of the center of mass during the propulsive phase.

During cyclic swimming, we expected to see fluid-structure interactions to dominate. Yet, stiffening the vertebral column did not lead to the expected increase in body wave speed, tail beat frequency and swimming speed. Instead, mutants with a stiff vertebral column exhibit a similar body wave speed and swimming speed as wild type. In the oldest mutants with almost completely fused vertebral columns, tail beat frequency and body wave begin to drop. We conclude that stiffening the vertebral column does not lead to the same effects as observed in model experiments [7].

V. Conclusions

We have not measured by how much the vertebral column of mutant zebrafish is stiffer than that of wild-type fish. Measurements of the stiffness of axial skeletons in adult fish have yielded values between 1 and 10 MPa [1, 13]. If we assume that a completely fused vertebral column has a similar stiffness to that of bone, then the *stocksteif* mutant has a vertebral column with a stiffness that is three orders of magnitude higher at roughly 1 to 10 GPa [14]. In the model experiments of McHenry et al. [7], stiffness increases of less than 50% were sufficient to generate the observed differences in swimming performance. Muscle activation increases the stiffness of muscle tissue by up to two orders of magnitude from 10 MPa to 1 GPa [15]. *Stocksteif* mutants might have a body that is too stiff for optimal swimming performance, possibly even too stiff for the axial muscles to generate effective body undulations. We will conduct experiments with *stocksteif* larvae between the ages of 5 and 15 dpf in order to illuminate how an increase in axial body stiffness by an estimated three orders of magnitude manifests in the body wave.

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References

1. Cheng J-Y, Pedley TJ, Altringham JD (1998) A continuous dynamic beam model for swimming fish. Phil Trans R Soc London B 353:981–997
2. Wardle CS, Videler, JJ and Altringham, JD (1995) Tuning in to fish swimming waves: body form, swimming mode and muscle function. J Exp Biol 198:1629–1636
3. Tytell ED, Lauder GV(2002) The C-start escape response of *Polypterus senegalus*: bilateral muscle activity and variation during stage 1 and 2. J Exp Biol 205:2591–2603
4. Müller UK, Stamhuis EJ, Videler JJ (2002) Riding the waves: the role of the body wave in undulatory fish swimming. Intergr Comp Biol 42:981–987
5. Fish FE, Lauder GV (2006) Passive and active flow control by swimming fishes and mammals. Ann Rev Fluid Mech 38:193–224
6. Spierts ILY, van Leeuwen JL (1999) Kinematics and muscle dynamics of C- and S-starts of carp (Cyprinus carpio L.) J Exp Biol 202:393–406
7. McHenry MJ, Pell CA, Long JH Jr (1995) Mechanical control of swimming speed: stiffness and axial wave form in undulatory fish models. J Exp Biol 198:2293–2305
8. Liao JC, Beal DN, Lauder GV et al. (2003) Fish exploiting vortices decrease muscle activity. Science 302:1566–1569
9. Müller UK, van Leeuwen JL (2004) Swimming of larval zebrafish: ontogeny of body waves and implications for locomotory development. J Exp Biol 207:853–868
10. Spoorendonk KM, Peterson-Maduro J, Renn J et al. (2008) Retinoic acid and Cyp26b1 are critical regulators of osteogenesis in the axial skeleton. Development 135:3765–3774
11. Weihs D (1973) The mechanism of rapid starting of slender fish. Biorheology 10:343–350
12. Fontaine E, Lentink D, Kranenbarg S et al. (2008) Automated visual tracking for studying the ontogeny of zebrafish swimming. J Exp Biol 221:1305–1316
13. Long JH Jr, Koob-Emunds M, Sinwell B et al. (2002). The notochord of hagfish, *Myxine glutinosa*: viscoelastic properties and mechanical functions during steady swimming. J Exp Biol 205: 3819–3831
14. Currey JD (1999) The design of mineralized hard tissue for their mechanical functions. J Exp Biol 202:3285–3294
15. Collinsworth AM, Zhang S, Kraus WE et al. (2002) Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation. Am J Physiol Cell Physiol 283:C1219–1227

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