The Journal of Experimental Biology **199**, 459–463 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0091

MUSCLE LENGTH CHANGES DURING SWIMMING IN SCUP: SONOMICROMETRY VERIFIES THE ANATOMICAL HIGH-SPEED CINE TECHNIQUE

DAVID J. COUGHLIN*, LEXIA VALDES AND LAWRENCE C. ROME†

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA and *Coastal Research Center, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA*

Accepted 6th October 1995

Summary

Recent attempts to determine how fish muscles are used to power swimming have employed the work loop technique (driving isolated muscles using their *in vivo* strain and stimulation pattern). These muscle strains have in turn been determined from the anatomical high-speed cine technique. In this study, we used an independent technique, sonomicrometry, to attempt to verify these strain measurements and the conclusions based on them.

We found that the strain records measured from sonomicrometry and the anatomical-cine techniques were very similar. The ratio of the strain measured from sonomicrometry to that from the anatomical-cine technique was remarkably close to unity (1.046±0.013, mean ± S.E.M., *N*=15, for transducers placed on the muscle surface and corrected for muscle depth, and 0.921±0.028, *N*=8, in cases where the transducers were inserted to the average depth of the red muscle). These measurements also showed that red muscle shortening occurs simultaneously with local backbone curvature, unlike previous results which suggested that white muscle shortening during the escape response occurs prior to the change in local backbone curvature.

Key words: *Stenotomus chrysops*, scup, sonomicrometry, red muscle, white muscle, fish swimming, muscle strain.

Introduction

Recent attempts to determine how fish muscles are used to power swimming have involved driving isolated muscle bundles through the length changes and stimulation patterns that the muscles undergo during swimming, and measuring the resulting power production (the work loop technique). In scup (*Stenotomus chrysops*), Rome *et al.* (1993) have found that most of the power required for steady swimming with red muscle comes from the posterior musculature, whereas relatively little comes from the anterior. The low power production of the anterior musculature is not an intrinsic deficiency of the muscle. On the contrary, the anterior muscle generates greater mechanical power than the posterior muscle when both are driven using identical conditions. The anterior muscle generates low mechanical power during swimming because of a combination of the small strain (percentage shortening) it undergoes and a relatively long stimulus duty cycle. This results in a small amount of work produced during shortening and a proportionally large amount of negative work generated during relengthening.

This observation was in contrast to generally accepted theories of fish swimming which proposed that most of the power required for swimming is generated by muscle in the anterior of the fish and that the posterior musculature serves primarily to transmit forces to the tail and in doing so performs net negative work (Videler and Hess, 1984; van Leeuwen *et al.* 1990). Because the results of work loop measurements depend heavily on the values of strain used, it is important to confirm the method of determining the strain that the muscle undergoes during swimming.

Muscle strain during swimming has previously been measured using a combination of anatomical and high-speed cine measurements (Rome *et al.* 1988; Rome and Sosnicki, 1991). This involves measuring the radius of curvature of the backbone in successive frames from high-speed film, and then determining sarcomere length (*SL*) from an anatomical calibration curve relating *SL* to backbone curvature. This calibration curve is obtained by bending freshly killed fish in positions that occur during locomotion, letting the muscle fibers go into rigor, freezing the fish, and then measuring *SL* from frozen sections. This anatomical-cine technique has indicated that muscles in the anterior portion of the fish undergo relatively small strains during swimming (Rome *et al.* 1992, 1993).

This technique, however, requires that the *SL*–backbone curvature calibration curve determined from dead fish accurately represents the *SL*–backbone curvature relationship

*Present address: Widener University, Science Division, One University Place, Chester, PA 19013, USA.
†Author for correspondence.

in swimming fish. The anatomical-cine technique also depends on the waveform of the backbone curvature change with time being discernible. In the anterior portion of the fish, however, the combination of only slight bending of the backbone, interference from the fins breaking the outline of the fish on the film, and the discontinuous nature of the record (frame-based), sometimes makes it difficult to discern the waveform accurately.

To verify the anatomical-cine technique and the conclusions regarding muscle power during swimming which are based upon it, we used an independent technique, sonomicrometry. Sonomicrometry has the advantage of not requiring the sometimes difficult procedure of tracing the outline of the fish and of being essentially continuous (i.e. length changes are determined at a much higher frequency, approximately 1.2 kHz).

Materials and methods

Scup (*Stenotomus chrysops* L.; N=23, length 17.5–22.3 cm) were swum in a water treadmill at 80 cm s^{-1} at 20 °C (the maximum swimming speed that does not require recruitment of the white muscle). Muscle length changes were measured using the anatomical-cine and sonomicrometry techniques simultaneously in four locations named ANT-1, ANT-2, MID and POST (29 %, 40 %, 54 % and 70 %, respectively, of the distance from the snout to the tail) as in Rome *et al.* (1993).

For the anatomical-cine analysis, muscle length changes were determined over 4–5 tailbeats from measurements of backbone curvature from high-speed films (200 frames s^{-1}) and using the equation relating SL (in μm) to backbone curvature in scup:

$$SL = 2.10\,\mu\text{m} + A/R \times 1.36\,\mu\text{m}\,, \quad (1)$$

where A is the distance from the backbone and R is the radius of curvature (as in Rome *et al.* 1992). To obtain good length change records, the outline of the fish was traced and the curvature of the backbone determined from 75 individual film frames in each sequence. The length change records were digitally filtered to remove high-frequency noise (as in Rome *et al.* 1993). We used an eight-pole Butterworth filter with the half-amplitude set at 1.5 times tailbeat frequency for the ANT-1 and ANT-2 locations and set at 2.5 times tailbeat frequency for the MID and POST locations. The more extreme filtering in the ANT locations reflects the lower signal-to-noise ratio in this region due both to the lower strain and to the larger errors associated with tracing described above.

For sonomicrometry, a pair of 1 mm diameter transducers (Triton Technology) was attached to the red muscle approximately 1 cm apart in each of 23 fish. The transducers were oriented with their piezoelectric crystals directed toward each other along the longitudinal axis of the fish. For some measurements, the transducers were attached to the lateral margin of the red muscle. To minimize rotation of the transducers relative to one another, the leads to the transducers were sutured to the skin, allowing some slack between the suture and point of implantation to relieve the strain during fish bending. Because of concerns about the hydrodynamic drag associated with the transducer wires, only one pair of transducers was attached to a given fish (hence, sonomicrometry measurements were obtained for only one location of the fish at a time). Length changes between the transducers were measured using a sonomicrometry system (Triton Technology System 6). The sonomicrometry trace was recorded on computer using A/D software (Run Technology) and was synchronized to the film frames (to within 0.2 ms) using a digital synchronization device described elsewhere (Rome, 1995). The sonomicrometry records were shifted back in time by 5 ms to correct for an inherent time delay in the filtering system of the sonomicrometer (four-pole Bessel filter at 100 Hz). The length change measured using sonomicrometry was then corrected to account for the average relative depth of the red muscle at each position along the fish. Beam theory was used to obtain a correction factor (= distance from backbone to center of red muscle/distance from backbone to lateral margin of muscle). The mean (± S.E.M.) correction factors determined for five fish were 0.913±0.018, 0.913±0.015, 0.861±0.014 and 0.800±0.028, for the ANT-1, ANT-2, MID and POST positions, respectively. The change in the correction factor with position reflects the decreasing thickness of the fish along its length.

In an additional set of experiments, the transducers were inserted into the red muscle to the average depth of the red muscle at the ANT-1, ANT-2 and MID positions. These experiments were conducted because they did not require a correction for the depth of the red muscle and, in addition, might detect a potential deleterious impact of implanting the transducers in the muscle. The positions of the implanted transducers were verified by dissection after the completion of each experiment.

For consistency, the sonomicrometry records were digitally filtered in exactly the same manner as the length records from the anatomical-cine technique (see above). Filtering had very little effect on the sonomicrometry traces (Fig. 1).

Results

The data from anatomical-cine and sonomicrometer techniques were very similar (i.e. the length changes had the same amplitude and there was no phase difference between them; Fig. 1). Table 1 shows comparisons of the amplitudes of the length change using both techniques from the four different locations along the fish. Although muscle strain during swimming generally increases with posterior position along the length of the fish (Rome *et al.* 1988, 1990, 1992; Jayne and Lauder, 1995), the values obtained using each technique at a given location are very similar. The ratio of the values obtained using sonomicrometry to those obtained using the anatomical-cine technique was remarkably close to unity (1.046±0.013, mean ± S.E.M., N=15), although statistically different from unity (t-test). The magnitude of this difference from unity is too small to have a significant impact on power production as

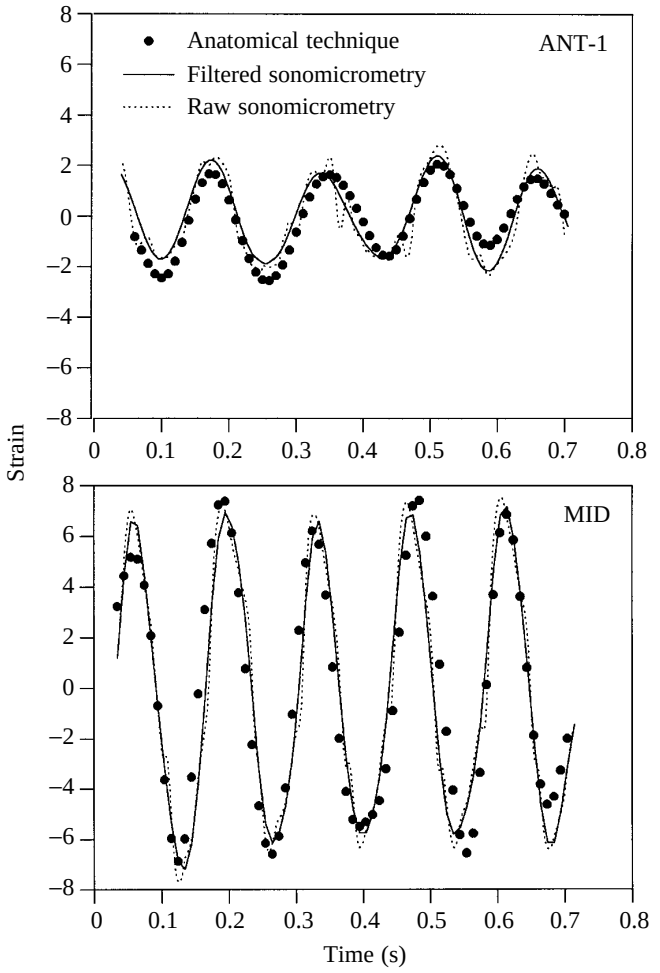

Fig. 1. Muscle length changes measured in the ANT-1 and MID positions using two techniques. The solid line in each case shows the percentage length change measured using sonomicrometry, the filled circles show the frame-based data from the anatomical-cine technique. The results from both techniques were digitally filtered to remove high-frequency noise as in Rome *et al.* (1993). The dotted line at each position represents the raw (unfiltered) sonomicrometer trace. There was very little difference between the filtered and raw traces. Data from both positions were determined from fish swimming at 80 cm s^{-1}; however, they are not from the same fish.

power production is relatively insensitive to very small changes in strain (a 5% difference for a 4% strain is only a 0.2% strain; Rome and Swank, 1992; Rome *et al.* 1993).

In addition, we observed no systematic phase difference between the length change records determined using the two techniques (i.e. the maxima of the length records occurred at the same time). For example, at the ANT-1 and ANT-2 locations, the mean phase differences between the two techniques were 0.2±3.6° (S.E.M., *N*=5) and 2.0±2.7° (S.E.M., *N*=4), respectively (1 tailbeat cycle = 360°). These values, as well as those in other regions of the fish, were small and not statistically different from zero.

Table 2 shows the results from additional experiments in which the transducers were implanted to the average depth of the red muscle. Similar to the results from experiments with the transducers attached to the lateral margin, the ratio of the values obtained using sonomicrometry to those from the anatomical-cine technique was very close to unity (0.921±0.028, mean ± S.E.M., *N*=8). This further validates the anatomical-cine technique.

The similarity of this ratio for the implanted transducers to that for transducers positioned on the lateral margin suggests that the corrections for depth used for transducers placed at the lateral margin were appropriate. This finding also suggests that these small transducers can be implanted in the red muscle without greatly affecting muscle length measurements.

Discussion

The close matching of measurements from the sonomicrometry and anatomical-cine techniques in this study suggests that the previous length changes measured by the anatomical-cine technique for scup red muscle during steady swimming (Rome *et al.* 1992, 1993) were accurate. This also indicates that the *SL*–backbone curvature relationship (equation 1), measured using frozen fish following rigor (on which the anatomical-cine technique is based), accurately reflects *SL* changes as a function of backbone curvature during swimming.

Importantly, our data suggest that the conclusion based on muscles driven using their *in vivo* length changes and stimulation pattern is also correct: that is, during steady swimming using red muscle in scup, most of the power is generated by the posterior musculature and relatively little by the anterior musculature of the fish. Not only does the magnitude of the strain measured by sonomicrometry agree with that measured by the anatomical-cine technique, but there was also no phase difference between the techniques; this removes one of the potential errors associated with imposing the correct stimulus phase (the timing of the stimulus with respect to the maximum length of the muscle) on the muscle during work loop experiments.

Recent studies by Johnson *et al.* (1994) are in agreement with this conclusion regarding the location of power production during swimming, showing that red muscle in the posterior musculature generates positive net work. This observation contrasts with studies on white muscle power production during steady swimming (Altringham *et al.* 1993), which suggest that power is generated by the anterior musculature while the posterior musculature performs net negative work. However, unlike the red muscle of scup, whose cross section increases from the ANT-1 to the MID positions and then declines somewhat further posteriorly, the white muscle cross section decreases dramatically moving posteriorly from the ANT-2 position (G. Zhang, D. Swank and L. C. Rome, in preparation). This might make it advantageous to generate most of the power anteriorly, where most of the white muscle is located.

This apparent difference between the functions of red and white muscle is supported by a basic difference in the timing

Table 1. *Comparison of muscle strain (percentage length change) measured using the sonomicrometry and anatomical-cine techniques*

	ANT-1			ANT-2			MID			POST		
	Sono	Anat	Ratio	Sono	Anat	Ratio	Sono	Anat	Ratio	Sono	Anat	Ratio
	2.65	2.46	1.08	4.20	4.12	1.02	6.41	6.36	1.01	6.43	6.36	1.01
	2.65	2.61	1.01	5.74	5.55	1.04	4.03	3.96	1.02	5.15	4.97	1.04
	2.32	2.20	1.05	3.75	3.14	1.20	7.10	6.54	1.09	4.69	4.61	1.02
	3.50	3.14	1.11	2.99	2.98	1.00				5.85	5.69	1.03
Mean	2.78	2.60	1.06	4.17	3.95	1.06	5.85	5.62	1.04	5.53	5.41	1.02
S.E.M.	0.67	0.62	0.02	0.57	0.58	0.04	0.92	0.83	0.02	0.38	0.39	0.01

Sono, sonomicrometry technique; Anat, anatomical-cine technique.

For each of the four positions along the length of the fish (ANT-1, ANT-2, MID and POST), the strain measured using each technique and the ratio of the values obtained using the sonomicrometry to the anatomical-cine technique are given.

In this set of experiments, the sonomicrometer transducers were placed on the lateral margin of the red muscle and the values were corrected for the average depth of the red muscle (see text).

of local backbone curvature relative to muscle shortening in the two muscle types. Previous sonomicrometry measurements on the white muscle of fish during the escape response (Covell *et al.* 1991) suggest that muscle shortening may occur *prior* to the change in local backbone curvature and that the length change of the muscle in one region of the fish causes bending of the backbone in more caudal regions. This is consistent with the notion that muscle forces and length displacements in the anterior portion of the fish may be transmitted through tendons to more posterior regions and the tail. By contrast, the present experiments clearly show that red muscle length changes occur *simultaneously* with local changes in backbone curvature. This indicates that red muscle in scup is not anatomically arranged such that muscle shortening in one region of the fish can cause backbone bending in a position situated *far* posterior.

Finally, it should be stressed that both sonomicrometry and anatomical-cine techniques are useful and each has specific advantages. Because of the size of the sonomicrometer transducers and their leads, the number that can be implanted in one fish is limited by the size of the fish, whereas using the anatomical-cine technique permits simultaneous measurements of muscle strain at numerous positions in fish of all sizes. Simultaneous measurements at several positions are necessary to determine the speed of the bending wave moving down the fish or to measure the total power generated by the fish during swimming. More generally, we believe that muscle strain can be most reliably measured by using both techniques simultaneously. Because muscle length change is the direct result of anatomical changes with time (e.g. relative movements of muscle origins with respect to their insertions), it would seem difficult to assess the potential errors associated with sonomicrometry (for example, due to damage to the muscle during implantation of transducers, misalignment of transducers with fibers or changes in the orientation of transducers during shortening), without having this anatomical and kinematic information. Likewise, using only the anatomical-cine technique might result in errors due to

Table 2. *Comparison of muscle strain (percentage length change) measured using the sonomicrometry and anatomical-cine techniques*

	ANT-1			ANT-2			MID		
	Sono	Anat	Ratio	Sono	Anat	Ratio	Sono	Anat	Ratio
	2.04	2.11	0.97	3.77	3.81	0.99	3.74	4.15	0.90
	2.09	2.23	0.94	1.81	2.22	0.82	5.17	5.09	1.02
	1.96	2.46	0.80						
	1.80	1.91	0.94						
Mean	1.97	2.18	0.91	2.79	3.01	0.90	4.46	4.62	0.96
S.E.M.	0.06	0.12	0.04						

Sono, sonomicrometry technique; Anat, anatomical-cine technique.

For each of three positions along the length of the fish (ANT-1, ANT-2 and MID), the strain measured using each technique and the ratio of the values obtained using the sonomicrometry technique to the anatomical-cine technique are given.

In this set of experiments, the sonomicrometer transducers were implanted into the muscle at the average depth of the red muscle, thereby making a correction for depth unnecessary.

unaccounted for tendon compliance, changes in fiber orientation or changes in muscle moments.

The authors thank Dr Frank Carey and Dr Mark Grosenbaugh for helping to organize our stay at the Woods Hole Oceanographic Institution. We thank Dr Dale Leavitt, Dr Bruce Lancaster and Dr Bruce Tripp from the Coastal Research Center at WHOI for facilitating our work there. We thank Dr Fred Thurburg of the Bureau of Marine Fisheries for the loan of the water treadmill. This work was supported by an NIH Postdoctoral Fellowship (to D.C.) and by NIH AR38404 and NSF IBN92-05397 grants to L.C.R. The sonomicrometry equipment was purchased with a grant from the University of Pennsylvania Research Foundation.

References

ALTRINGHAM, J. D., WARDLE, C. S. AND SMITH, C. I. (1993). Myotomal muscle function at different locations in the body of a swimming fish. *J. exp. Biol.* **182**, 191–206.

COVELL, J. W., SMITH, M., HARPER, D. G. AND BLAKE, R. W. (1991). Skeletal muscle deformation in the lateral muscle of the intact rainbow trout *Oncorhynchus mykiss* during fast start maneuvers. *J. exp. Biol.* **156**, 453–466.

JAYNE, B. C. AND LAUDER, G. V. (1995). Speed effects on midline kinematics during steady undulatory swimming of largemouth bass *Micropterus salmoides*. *J. exp. Biol.* **198**, 585–602.

JOHNSON, T. P., SYME, D. A., JAYNE, B. C., LAUDER, G. V. AND BENNETT, A. F. (1994). Modeling red muscle power output during steady and unsteady swimming in largemouth bass. *Am. J. Physiol.* **267**, R481–R488.

ROME, L. C. (1995). A device for synchronizing biomechanical data to cine film. *J. Biomech.* **28**, 333–338.

ROME, L. C., CHOI, I., LUTZ, G. AND SOSNICKI, A. A. (1992). The influence of temperature on muscle function in fast swimming scup. I. Shortening velocity and muscle recruitment during swimming. *J. exp. Biol.* **163**, 259–279.

ROME, L. C., FUNKE, R. P., ALEXANDER, R. M., LUTZ, G., ALDRIDGE, H. D. J. N., SCOTT, F. AND FREADMAN, M. (1988). Why animals have different muscle fibre types. *Nature* **355**, 824–827.

ROME, L. C. AND SOSNICKI, A. A. (1991). Myofilament overlap in swimming carp. II. Sarcomere length changes during swimming. *Am. J. Physiol.* **260**, C289–C296.

ROME, L. C. AND SWANK, D. (1992). The influence of temperature on power output of scup red muscle during cyclical length changes. *J. exp. Biol.* **171**, 261–281.

ROME, L. C., SWANK, D. AND CORDA, D. (1993). How fish power swimming. *Science* **261**, 340–343.

ROME, L. C., FUNKE, R. P. AND ALEXANDER, R. M. (1990). The influence of temperature on muscle velocity and sustained swimming performance in swimming carp. *J. exp. Biol.* **154**, 163–178.

VAN LEEUWEN, J. L., LANKHEET, M. J. M., AKSTER, H. A. AND OSSE, J. W. M. (1990). Function of red axial muscle of carp (*Cyprinus carpio* L.): recruitment and normalised power output during swimming in different modes. *J. Zool., Lond.* **220**, 123–145.

VIDELER, J. J. AND HESS, F. (1984). Fast continuous swimming of two pelagic predators, saithe (*Pollachius virens*) and mackerel (*Scomber scrombius*): a kinematic analysis. *J. exp. Biol.* **109**, 209–228.