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Published in:
Bioinspiration and Biomimetics
DOI:
10.1088/1748-3190/abf031
Publication date:
2021
Citation for published version (APA):
Fetherstonhaugh, S. E. A. W., Shen, Q., & Akanyeti, O. (2021). Automatic segmentation of fish midlines for optimizing robot design. Bioinspiration and Biomimetics, 16(4), [046005]. https://doi.org/10.1088/1748-3190/abf031
AUTOMATIC SEGMENTATION OF FISH MIDLINES FOR OPTIMIZING ROBOT DESIGN

SUPPLEMENTARY INFORMATION

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**Supplementary Figure 1.** Segment growing method to obtain a multi-segment model that can describe fish midlines accurately (i.e., model error should be lower that the error threshold indicated by the horizontal dashed line). Segment error increases exponentially as it gets longer (its respective joint moves along the body). The black lines indicate the portion of the curves with error values lower the error threshold and the grey lines indicate the portion of the curves with error values above the threshold. The first segment ($S_1$) extends from the most anterior midline point to the first joint ($J_1$), the second segment ($S_2$) from $J_1$ to $J_2$, the third segment ($S_3$) from $J_2$ to $J_3$ and the last segment ($S_4$) from $J_3$ to the most posterior midline point. Vertical dashed lines indicate the final joint positions after segment growing stopped. The fish silhouette and final joint positions (black filled circles) are shown on the top. Horizontal lines below the fish (solid black) indicate growing segments.
Supplementary Figure 2. Evaluating the performance of the segment model. a. Grey color map shows the amplitude difference between the segment model and actual fish midlines along the body (horizontal axis) and over time (vertical axis); dark colors (low amplitude difference) and light colors (high amplitude difference). b. Series of fish (dashed grey) and model (solid black) midlines over a half tail beat (grey vertical bar indicates the selected time interval). Black circles (filled) indicate the joint positions of the model. c. Mean amplitude difference (averaged over time) along the body. Maximum mean amplitude difference is used to arrive at the final model performance (i.e., model error).
**Supplementary Figure 3.** Genetic algorithm to calculate the optimal joint positions of a multi-segment model for a fixed number of segments (four segments in this example). 

**a.** Initial population of random solutions (25 out of 200 solutions are shown). Each solution corresponds to a model with three joints, $J_1 - J_3$, and four segments, $S_1 - S_4$ (the first solution is labelled). Filled circles depict joints; $J_1$ (black), $J_2$ (dark gray) and $J_3$ (light gray). 

**a(ii).** Improved solutions after four generations; all models converge on same joint locations. 

**b.** Mean joint positions over 10 generations. Horizontal lines indicate the standard deviation of the mean. 

**c.** Mean fitness (model error) over 10 generations; the smaller the error, the higher the fitness. The error gradually
decreases up to the fifth generation and then plateaus at 0.009 $L$. Vertical lines indicate the standard deviation of the mean.
Supplementary Figure 4. Comparison between segment growing method and genetic algorithm in estimating the joint positions of multi-segment models (both trout and multi-species datasets).

a. Linear regression analysis ($y = 1.01x - 0.01$, $R^2 = 0.98$, $p < 0.001$). Regression (solid) line and 95% confidence interval (gray shaded area) are plotted. b. Bland-Altman plot. Horizontal lines indicate the mean difference (solid) and 95% limit of agreement (dashed), respectively. In both figures, empty circles correspond to individual data points.
Supplementary Figure 5. Multi-segment model versus fish midlines (four variable-length segments calculated by the segment growing method). Vertical bars show the maximum perpendicular distance between predicted and actual midline points.
Supplementary Figure 6. Multi-segment model versus fish midlines (six equal-length segments). Vertical bars show the maximum perpendicular distance between predicted and actual midline points.
Supplementary Figure 7. Multi-segment model versus fish midlines (four equal-length segments). Vertical bars show the maximum perpendicular distance between predicted and actual midline points.
Supplementary Figure 8. Single segment model versus fish midlines. Vertical bars show the maximum perpendicular distance between predicted and actual midline points.
Supplementary Figure 9. a. Mean joint positions in multi-segment models with increasing number of segments (up to 10 segments) in trout dataset. Horizontal error bars indicate the standard deviation from the mean. b. Relative segment lengths for the same models.
Supplementary Figure 10. Head segment length decreases with increasing body width in multi-species data set ($y = -2.68x + 0.64$, $R^2 = 0.75$, $p < 0.01$).
Supplementary Figure 11. Analysis of fish midlines (multi-species dataset). a. Amplitude envelope. b. Phase envelope.
| Species                  | L (cm) | U (L s⁻¹) | tbf (Hz) | tba (L) | ha (L) | λ (L) | C (L⁻¹) | Cp (L) |
|-------------------------|--------|-----------|----------|---------|--------|-------|---------|--------|
| Florida gar             | 36.9   | 1         | 2.82     | 0.13    | 0.01   | 0.78  | 5.33    | 0.83   |
| Northern barracuda      | 33     | 2.6       | 5.95     | 0.12    | 0.02   | 0.77  | 3.51    | 0.9    |
| Clown knifefish         | 19.4   | 1.4       | 2.3      | 0.13    | 0.01   | 0.7   | 3.91    | 0.87   |
| Rainbow trout           | 23     | 3.4       | 3.76     | 0.16    | 0.03   | 0.9   | 3.85    | 0.9    |
| Mangrove snapper        | 23     | 3.2       | 4.11     | 0.18    | 0.06   | 0.98  | 3.34    | 0.83   |
| Indo-Pacific tarpon     | 23     | 2.7       | 4.51     | 0.21    | 0.05   | 1.1   | 3.3     | 0.83   |
| Sheepshead              | 32     | 1.8       | 2.99     | 0.15    | 0.05   | 0.88  | 4.17    | 0.83   |
| Pinfish                 | 28     | 2.9       | 4.29     | 0.16    | 0.07   | 0.9   | 3.46    | 0.83   |
| Tomtate                 | 20     | 1         | 4.13     | 0.19    | 0.03   | 0.95  | 2.97    | 0.87   |
| Crevalle jack           | 45.5   | 3.1       | 4.61     | 0.16    | 0.05   | 0.96  | 3.49    | 0.83   |

Supplementary Table 1. Kinematics parameters (multi-species dataset): $L$ (body length); $U$ (swimming speed); $tbf$ (tail beat frequency); $tba$ (tail beat amplitude); $ha$ (head amplitude), $\lambda$ (wavelength); $C$ (maximum mean curvature); $C_p$ (maximum curvature point along the body).
| Parameter | S1  | S2  | S3  | S4  | S5  |
|-----------|-----|-----|-----|-----|-----|
| tbf (Hz)  |     |     |     |     |     |
| tba (L)   |     |     | 0.68| 0.74|     |
| ha (L)    | -0.68|   | 0.67| 0.71|     |
| λ (L)     |     | 0.64| 0.65| 0.79|     |
| C (L⁻¹)   |     |     |     |     | -0.65|

**Supplementary Table 2.** Spearman rank correlation coefficients between kinematic parameters and segment lengths (multi-species dataset). Only coefficients with significance (p < 0.05) are shown.