

Details of Materials and Methods

Suction is kids play: extremely fast suction in newborn seahorses

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Serial sectioning. One specimen (1.87 mm head length) was fixed in a 4% buffered and neutralized formalin solution and stored in alcohol 70%. Prior to sectioning, it was decalcified with Decalc 25%, dehydrated through a graded alcohol series, and embedded in Technovit 7100 (Heraeus, Kulzer). Next, semi-thin sections (2 μm) were cut using a Leica Polycut SM 2500 sliding microtome equipped with a wolframcarbide coated knife, stained with toluidine blue, mounted with DPX, and covered.

Anatomical 3D-reconstructions. Computer-generated 3D-reconstructions were made to visualize musculoskeletal topography (using histological sections). Images of the histological sections were captured using a Colorview8 digital camera mounted on a Reichert-Jung Polyvar light microscope and controlled by analySIS 5.0 software (Soft Imaging System GmbH Münster, Germany). These images were imported into Amira 3.1 (Template Graphics Software Mérignac, France). Alignment of the histological sections and tracing of the elements was done manually by superimposition. Each element was separately rendered and smoothed. The volume of the epaxial muscles, needed to scale the calculated power output to muscle mass, was obtained from these reconstructions using Amira 3.1, and was assumed to be neutrally buoyant with respect to seawater.

Kinematics. Kinematic data were obtained from high-speed videos of 0 to 3 day old *H. reidi* ($N = 11$, one video clip per individual, head length between 1.68 and 2.13 mm) recorded at 8000 Hz using a Redlake M3 camera. Only videos with adequate sharpness and precise lateral-view position of the seahorse were selected for further analyses out of over 40 recorded feeding strikes. First, anatomical landmarks were digitized using Didge (A. Collum,

Creighton University, USA) and kinematic profiles of neurocranial rotation and hyoid rotation were calculated. Next, in order to assess velocities and accelerations, the high-frequency noise results from manual tracking of anatomical landmarks was removed. To do so, a fourth-order Butterworth low-pass filter was used. Finally, numerical differentiation by means of a first-order, central difference method yielded angular velocities and accelerations. The time-averaged (time = 0 to 3 ms) position of the axis of neurocranial rotation was determined for each feeding sequence, the average position of which was used for modelling (see further). Several dorsal view videos were recorded for volume modelling purposes.

Volume modelling. Contours of the head in lateral and dorsal view were traced manually, and converted into a 3D closed surfaces using Rhinoceros 3.0 (McNeel Europe SL Barcelona, Spain). This data was obtained for video frames prior to the start of feeding, and at maximal head expansion. The resting volume of the mouth cavity was inferred from the video images in lateral view. The head tissue volume was assumed to remain constant when reconstructing the mouth cavity volume after expansion. A second estimate of the volume increase of the head in newborn seahorses during feeding was performed using the method of Drost & van den Boogaart (1986). This implied that the head was modelled as a series of 21 elliptical cylinders based on the contour tracings of a different set of lateral and dorsal images than used for the first estimate.

Moment of inertia of the head. The hollow, external head volume prior to the start of feeding was exported from the 3D-model generated using Rhinoceros 3.0, and meshed using a tetrahedral volume fill functions of Tgrid 5.0.6.(Ansys, Lebanon, USA). Next, the mesh was imported into Fluent 6.3.26 (Ansys, Lebanon, USA), where a custom-written function calculated the moment of inertia of the head around its average axis of rotation assuming a uniform sea-water density of $1024.75 \text{ kg m}^{-3}$.

Computational Fluid Dynamics (CFD). CFD was performed using Fluent 6.3.26 on a finite-volume mesh consisting of 790.459 tetrahedral cells. The model assumptions and setting correspond closely to the ones outlined in Van Wassenbergh & Aerts (2008). In order

to capture the entire flow generated by the pipefish, the flow domain around the seahorse head was modelled as a box with a width of 2 mm, a height of 4 mm and a length of 3.5 mm. The surfaces of the pipefish model were meshed with a uniform triangle size of 0.027 mm, while a larger spacing between the nodes of the mesh was chosen for the outer boundary surface of the flow domain (triangle size of 0.31 mm).

The unsteady rotation of the pipefish head was described using a Fluent user-defined function (UDF). First, the following function was fitted to the time-averaged velocity profile of head rotation measured for newborn *Hippocampus reidi*:

$$\omega = 265 \sin(2100 t - 1.5708) + 265$$

where ω is the instantaneous angular velocity of head rotation (radians s^{-1}), and t the time (s). This angular velocity was implemented in a DEFINE-CG-MOTION UDF, and compiled using Microsoft Visual Studio 2005. This motion profile was then assigned to the surfaces of the seahorse head model.

Numerical algorithms of Fluent 6.3.26 automatically update the mesh after each time step relative to the pipefish motion. To do so, the spring-based smoothing method was used, in which the edges between nodes are considered as a network of interconnecting springs. To smooth the mesh, a value of 1.0 was used for spring constant factor and 1.0 for boundary node relaxation factor, while a standard value of 0.001 was used for the convergence tolerance.

The flow was assumed to be laminar because the critical Re for transition to turbulent flow (2×10^5 for smooth cylinders; Hoerner 1965; Schlichting 1979) is not likely to be reached during head rotation in newborn seahorses. Using a velocity of 1.5 m s^{-1} (higher than the measured peak velocities of the mouth) and a characteristic length of 2 mm (approximate head length), Re was calculated to be 2.85×10^3 . Properties of seawater with a salinity of 35 g kg^{-1} at 20° C were assigned to the fluid: a constant density of $1024.75 \text{ kg m}^{-3}$ and a dynamic viscosity of 1.08 mPa s (Fofonoff 1962).

The no-slip wall condition was enforced at the rotating seahorse head surface, which is the default condition for models of viscous flow in Fluent 6.3.26. The open boundary surface of the flow domain was modeled as a pressure-outlet where a gauge pressure of zero applies (i.e. no changes in pressure due to head rotation is assumed at this boundary) and a backflow normal to the boundary. A steady flow simulation with a considerably larger flow domain around the seahorse showed that this boundary condition was realistic.

The pressure-based solver (chosen to obtain fast-converging solutions) was used with a node-based Green–Gauss gradient treatment. The latter treatment achieves higher accuracy in unstructured tetrahedral grids compared to the cell-based gradient treatment. The first-order implicit unsteady formulation option was used in the simulation because moving mesh simulations (see above) currently only work with first-order time advancement. The standard pressure discretization scheme was used for the pressure calculation and a second-order upwind scheme was used for the momentum equations. The pressure-velocity coupling was solved using the SIMPLE scheme. The latter is a discretization method that uses a relationship between velocity and pressure corrections to enforce mass conservation and to obtain the pressure field. A fixed time step size of 0.01 ms was used in the calculations. A maximum of 60 iterations per time step was sufficient to reach a converged solution.

The reported moments exerted by the surrounding water on the seahorse head from are always the sum of pressure moments (resulting from pressure forces) and viscous moment (resulting from wall shear forces) on the external surfaces of the seahorse.

References:

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