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APPLICATION

The third dimension: a novel set-up for filming coelacanths in their natural environment

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Summary

1. Here, we describe a novel design to obtain three-dimensional data on the movements of aquatic organisms at depths of up to 140 m.

2. The set-up consists of two synchronized high-speed cameras fixed to two articulated arms.

3. The set-up was successfully used to film and quantify the locomotion of coelacanths *Latimeria chalumnae* living at a depth of about 120 m in Sodwana Bay, South Africa. As an example, the detailed motion of the dorsal fin is presented here.

4. This set-up can be used for any underwater applications that require synchronized video recordings of medium- to large-sized animals.

Key-words: 3D, behaviour, kinematics, locomotion, underwater filming

Introduction

The study of locomotion in terrestrial animals is often based on high-speed video recordings. Being able to quantify rhythmic activity based is essential to understand the motor control of locomotion. In the simplest case, the use of a single camera placed perpendicular to the axis of the motion can be sufficient to quantify the successive movements of the legs or body (e.g. Marey 1873; Hildebrand 1989; Hutchinson *et al.* 2006; Herrel, James & Van Damme 2007; Maes *et al.* 2008). However, this often does not allow the study of the detailed three-dimensional kinematics of the different limb segments. Despite the fact that for most applications two to three cameras suffice to accurately quantify movements in 3D, sometimes up to nine high-speed video cameras are used for the quantification of complex behaviours (Vidal *et al.* 2009; Revéret *et al.* 2011; Montuelle *et al.* 2012; Herrel *et al.* 2013; Herbin *et al.* 2016). Yet, these studies typically take place under standardized laboratory conditions.

For aquatic locomotion, one or two video cameras placed above and/or on the side of the aquarium often suffice in the case of laboratory studies (Gray 1939, 1968; Drucker & Lauder 2005; Standen & Lauder 2005; Liao 2007; Herrel *et al.* 2011). However, some animals cannot be maintained in animal housing facilities, and thus, videos must be obtained directly from animals moving in their natural habitat. Moreover, films of animals in their natural habitat allow the capture of more complex naturalistic behaviours that are often difficult to observe under laboratory conditions (Dunbar & Badam 1998; Cant, Youlatos & Rose 2001; Dunbar *et al.* 2004).

This is especially the case for the extant coelacanth *Latimeria*. These emblematic animals live at a minimal depth of about 100 m and cannot be kept alive once brought to the surface (Forey 1998a,b). Moreover, they are listed as critically endangered on the IUCN red list and any capture is prohibited, even for scientific purposes (Forey 1998a,b; Musick 2000; Nulens, Scott & Herbin 2011). Yet, understanding coelacanth locomotion is of prime importance as they are, together with lungfishes, the only extant sarcopterygian fishes (non-tetrapod sarcopterygians). Because of their close phylogenetic relationship to tetrapods (terrestrial vertebrates), an understanding of their locomotor patterns may provide insights into the evolution of motor control in vertebrates across the water-to-land transition. Moreover, the 3D modelling of the motion of their

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fins could provide a better understanding of the modifications that occurred in vertebrate fins/limbs across the water-to-land transition (Pierce, Clack & Hutchinson 2012). Although some aspects of lungfish locomotion are now understood (King *et al.* 2011), the locomotion of coelacanths remains poorly known. Previous studies on coelacanth locomotion in their natural environment have been based on observations made from a submersible (Fricke *et al.* 1987; Fricke & Hissmann 1992). Although highly informative, these recordings were obtained with a set-up causing distortions and perspective errors. Additionally, the low resolution and low frame rates of the videos prevented an analysis of the detailed kinematics needed to establish a comprehensive kinematic and hydrodynamic model of coelacanth locomotion. Finally, the presence of the submersible and the noise of its engines may result in stress and as such modify the natural behaviour of the coelacanths.

A series of dives during 2010 and 2013 offshore South Africa at a depth of 120 m demonstrated that the presence of the divers did not stress the coelacanths. As such, a new set-up was developed to film *Latimeria chalumnae* individuals in their natural habitat and to obtain the first 3D data on the undisturbed locomotion of these animals.

Materials and methods

Coelacanths were filmed using two synchronized high-speed, high-resolution cameras (Phantom MIRO M110; Vision Research, Elancourt, France) set at 200 or 500 frames per second. The cameras were positioned on a custom-made camera stand allowing for a fixed absolute positioning of the two cameras relative to one another. These conditions were essential to obtain three-dimensional positional data. With this experimental set-up, the diver had to approach the coelacanth and get the animal positioned at the centre of the set-up for filming.

CAMERA STAND DESIGN

The camera stand consisted of two articulated arms of 1 m, able to present a variable geometry. As such, the system could be set with an angle ranging from 45° up to 120° between the two arms (Fig. 1). A locking mechanism which could move along each arm permitted the cameras to be fixed at a desired angle (Fig. 1). This setting was performed before the dive such that the angle between the two cameras could not be changed during the dive. For locomotion trials, the set-up was adjusted to 120°, while for the attempts of the kinematic analysis of feeding, the arms were set at 90°. Each camera was installed in a housing positioned at the extremity of one of the arms. The camera dedicated as the master camera (which sends the impulse that synchronizes both cameras) was placed in the housing nearest to the diver while the slave camera was placed in the housing at the extremity of the other arm. Just above the master housing, two 5″ monitors (with their own 12-v battery supplies) were fixed, permitting the operating diver to visualize the images captured by each camera in real-time. The lighting was provided by one 40-W light fixed on each arm, running on two Ni Mh 13·2-v batteries. The batteries were fixed on each side of the master housing. The aiming of the cameras was facilitated by two underwater laser pointers of different colours (Innovam Lasers, Montreal, Canada) fixed on top of the camera housings (Fig. 1). The position of the animal in the set-up was deemed ideal for filming when it was positioned at the intersection of both lasers. At that instant, the animal was centred on both cameras, and was in the centre of the calibrated field of view.

TRANSPORT AND MANIPULATION OF THE SET-UP

It was important to be able to easily transport and manipulate the stand in the water as well as on land. On land and on the boat, the set-up was folded and put in a dedicated rigid box (124 × 38 × 42 cm). The total weight of the set-up and the box was 63 kg (32 kg for the set-up only). The stability of the stand in water was assured by two floats and ballasts fixed to the housing. A system of automatic floats was additionally fixed to one of the arms to ensure an automatic ascent in case of any problems. Moreover, two handles were fixed on the arm supporting the master housing to facilitate the manipulation of the stand. For efficient and rapid underwater displacement, the set-up was attached to an underwater scooter (Fig. 1). On site, at a depth of 110 m, the set-up was then deployed and the lights were turned on when the animal was positioned in between the cameras.

CAMERA CHARACTERISTICS

The selected cameras were monochrome Phantom M110 cameras (Vision Research), the monochrome version having a more sensitive CMOS sensor, resulting in the need for less light and thus less stress for the animals. The specifications of the camera CMOS sensor are as follows: 25·6 × 16 mm, a pixel size of 20 μm and a 1280 × 800 resolution. To optimize the set-up, we decided to use a high-speed internal DRAM memory buffer of 6 Go, and an onboard CineFlash storage drive of 240 Go. This configuration permitted to reduce the time spent saving the video. Thus, with the 6-Go buffer it was possible to obtain two consecutive sequences at 200 frames per second (or one at 500 frames per second) and transfer these videos to the 240-Go storage memory (maximum of 40 videos). The camera lens chosen was a 35-mm 1 : 2 D Nikon. The exposure time (shutter speed) was set to 1900 μs to have enough light while having adequate sharpness of the images. During the first recordings, we limited the sequence duration to 10 s, but subsequently, we extended it to 20 s (pre-trigger recording) to obtain 4000 frames (at 200 frames per second), a compromise between a sufficient number of frames and a short transfer time to the *CineFlash* storage (60 s). The cameras recorded and stored data continuously on the DRAM buffer of the camera. Once the camera was triggered, the recording process was stopped and the 4000 frames recorded before the trigger signal were sent to the CineFlash memory. Both cameras were synchronized by a BNC cable (3 m). The master camera drove the slave camera, and there was a maximum of 20 ns timing difference between both images. After the dive, the video folder was transferred either directly by Ethernet connection, or by the transfer of the memory card via a card reader. The cameras had two Sony BP-60 batteries (autonomy of 45 min) fixed inside each housing.

IMAGE CALIBRATION AND ACCURACY OF MEASUREMENTS

Camera calibration is the first necessary step in 3D recordings to extract metric information from synchronized 2D videos. The images were calibrated from videos using a calibration grid (26 × 20 cm) that was moved across the entire field of view and at different orientations relative to both cameras. From these videos, intrinsic (internal camera geometric and optical characteristics) and extrinsic (position and orientation of the cameras) parameters can be extracted to obtain the 3D information from the 2D videos. A separate calibration must be

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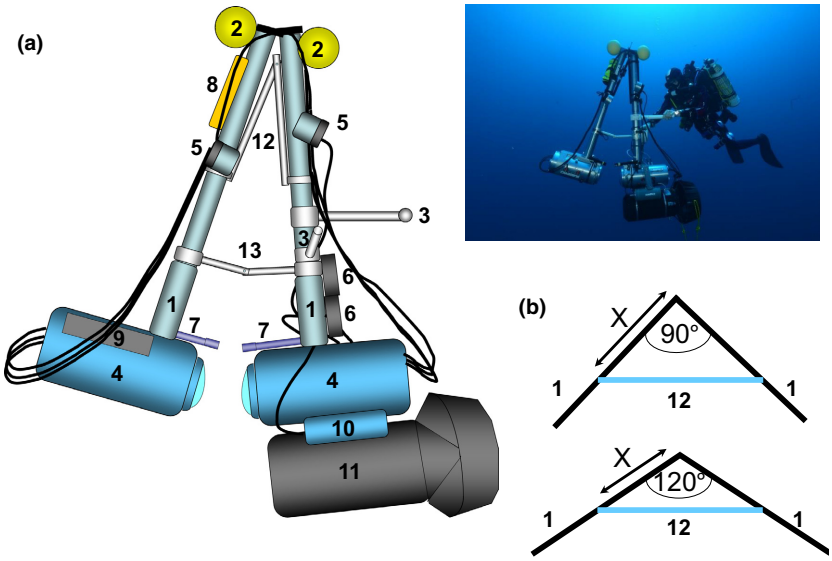

Fig. 1. (a) Schematic drawing of the set-up: (1) articulated arm, (2) float, (3) handle, (4) waterproof housing, (5) 40-Watt light (6) 5-inch monitor, (7) under-water laser pointer, (8) security float, (9) ballast, (10) batteries supplying the lights and both monitors, (11) under-water scooter, (12) locker of angle setting; its position on the arm defines the angle between both cameras, (13) locker holding the set-up in its folded position. (b) Examples of setting; for 90 °C, the distance *X* is 540 mm; for 120°, the distance *X* is 439 mm. The inset shows the set-up folded for transport (Photographs: L. Ballesta).

performed for both configurations, one at 90° and one at 120°. The correction of the image distortion, introduced by several refractive boundaries, is the second step for extracting the 3D coordinates from biplanar videos. For this purpose, a set of functions was developed in-house (*Loco program*) in a custom-written MATLAB routine (Camera Calibration Toolbox for MATLAB). The open-source MATLAB functions are available from www.vision.caltech.edu/bouguetj/calib_doc/. To calibrate and to correct image distortion, we tracked four points per frame (the four corners of the calibration grid) for 20 consecutive frames (for each camera) to cover the volume to be calibrated. This allowed to measure all objects or movements within this volume. Moreover, the position of the centre of this calibrated volume was identified by the intersection of both lasers.

Camera calibration was typically performed in the field during decompression stops required prior to ascent, but could also be performed before or after the dive in a pool as the position of the two cameras relative to one another is fixed. The fixed camera position allows a single calibration at a given angle between the two cameras thus minimizing the number of calibrations needed. The verification of the calibration and measures were made in the laboratory. First, to estimate the precision of the measures, we measured five distances on the same object (Fig. 2a) with our set-up and with a MicroScribe® G2 (Immersion, Bordeaux, France) with a precision of ±0·38 mm according to the manufacturer. Furthermore, to be sure that the calibration did not change between dives (with the same angle between the arms), we compared the measures of the same object using five calibrations obtained after five successive openings/closings of the set-up. The possibility to calibrate the set-up in a pool before or after a dive was particularly valuable when the environmental conditions inhibited diver to calibrate the cameras (e.g. strong currents or the presence of sharks;

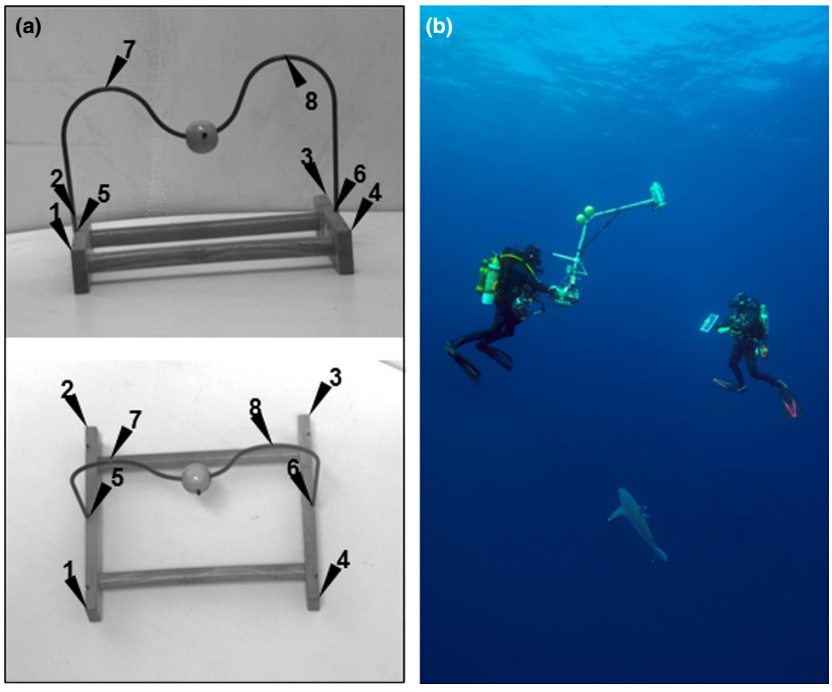

Fig. 2. Calibration of the set-up. (a) Views of the different markers used to calculate the accuracy of the set-up. The distances measured were between markers: 1–3; 2–4; 5–6; 5–8; 6–7. From the top, view from master camera and from the slave camera. (b) Calibration of the cameras in the field.

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Fig. 2b). The 3D reconstruction of the movements of an animal requires real-world coordinates to correct for the movement of the animal relative to its surroundings and the movement of the set-up by the divers. Alternatively, one can define a coordinate system fixed on the animal allowing to calculate movements of the fin, for example, relative to the body of the animal. As real-world coordinates, natural fixed landmarks visible on both camera views during the sequence can be used and allow to quantify the movement of the animal relative to its surroundings. However, in our case we were interested in movements of the fin relative to the animal and as such a reference point on the animal was chosen at the base of the dorsal fin.

Results

MANIPULATION OF THE SET-UP

Although cumbersome on land, the set-up is relatively easy to manipulate and transport under water. An underwater scooter facilitated transport of the set-up to the coelacanth habitat. The deployment of the set-up lasted no more than 1 min, thanks to the different handles facilitating the manipulation of the arms (Fig. 3). At the end of the dive the set-up was folded up again. During the first decompression stop, the set-up remained with the diver. However, at the decompression stop at 40 m (after calibrations) the set-up was brought to the surface by a technical diver after a calibration was performed.

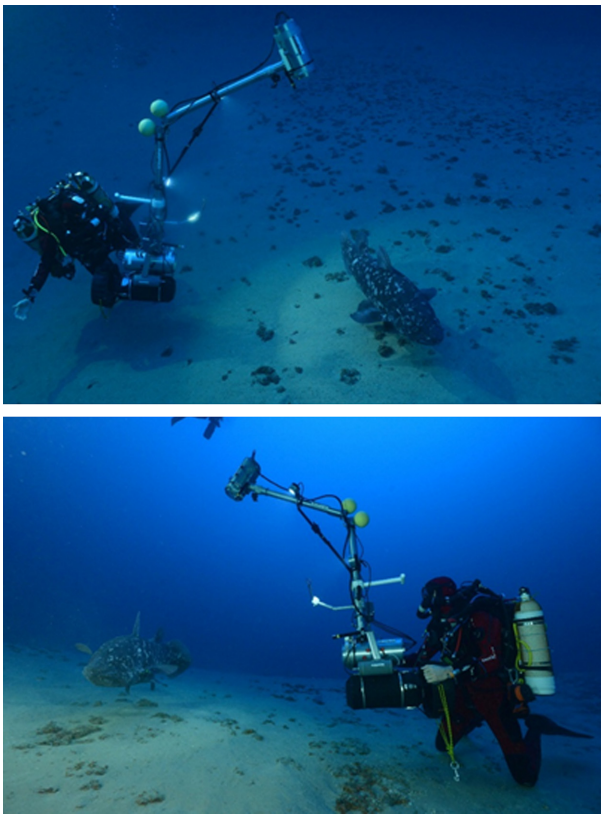

Fig. 3. The set-up being manipulated by a diver at a depth of 120 m. The set-up is deployed at 120° to capture images for the analysis of locomotion (Photographs: L. Ballesta).

CALIBRATION AND ACCURACY OF MEASUREMENTS

The comparison of the measures of five distances of a complex object with our set-up and with the MicroScribe gave a mean difference of 0·79% (SD = 0·13) of the measured distance (Fig. 4). Similarly, the measures of the same distance after five openings/closings of the set-up gave a mean maximal difference of 1·58% (SD = 0·38), and a mean minimal difference of 0·29% (SD = 0·27). These results confirmed the accuracy and the precision of our set-up, and allowed for the possibility to calibrate before or after a dive, provided that the angle between arms of set-up had not been changed.

DATA

Dive duration at a depth of about 110 m is restricted to 20–25 min to maintain decompression times as low as possible. As such, the data collection was limited due to time restraints. Over four dives dedicated to obtaining data on the fin kinematics of the coelacanth, the operating diver was able to collect a total of 14 well synchronized sequences (6-0-7-1) from three different individuals allowing us to identify the trajectories of the fins in both camera views. Animals could be easily identified by the distinctive pattern of the white scales on their body. During one dive, the coelacanth did not move outside its cave, and thus, we were unable to obtain videos. A total of 58 000 frames were collected for each camera (total of 116 000 frames), for a total memory of 155 gigabytes. Once on land, each sequence was visualized. The images obtained were bright and sharp enough to be used for the kinematic study (Fig. 5). The synchronized calibration videos were analysed using the custom-written *Loco* routine allowing calibration of the videos from each camera and allowing us to quantify the movement of the animal in 3D.

Kinematics of the second dorsal fin

A preliminary quantification of the movements of the second dorsal fin of one coelacanth based on one sequence (one for each camera) was performed to validate this approach. The 200 frames per second film selected to test our set-up corresponds to a slow forward swimming in open water. The slow movement of the second dorsal fin during this sequence allowed us to use only one of every 10 frames. This subsampling allowed us to accurately quantify the movement of a landmark on the fin. The tracked point was located on the anterior fin margin, at the base of the lepidotrichia at the level of the bone named 'pièce radial préaxial 3' by Millot and Anthony (1958). The reference point chosen to quantify the movement of the fin relative to the animal was located the base of the second dorsal fin. The results of the analysis are presented in Fig. 6 and permit visualization of the large excursion and complex movement of this lobed fin. One fin cycle was analysed by tracking two points over 120 frames. The cycle duration was 6 s, trajectory length was 0·64 m, and the amplitude of the stroke was 0·421 m. The maximum speed of the fin relative to the body of the animal was 0·179 m s^{-1}, and the

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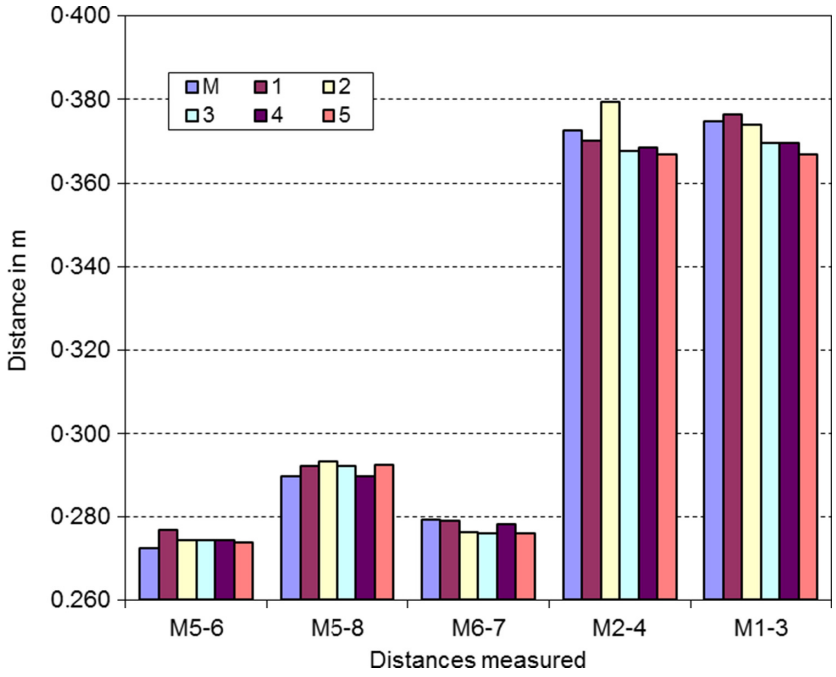

Fig. 4. Comparison of the different measures of the objects with our set-up during the five trials (1, 2, 3, 4, 5) during which the set-up was redeployed and with the Microscribe (M, in blue). The distances M5–6, M5–8, M6–7, M2–4, M1–3 correspond to the distances between different markers on the object (see Fig. 2a for details).

Fig. 5. Sample of synchronized images from a sequence captured at 200 frames per second detailing the fins of the coelacanth in lateral and dorsal views. Notable are the quality and brightness of the images. In each image, the light from the laser pointer is visible on the side of the animal. The same points on the body of the animal viewed by both cameras allow the reconstruction of the movements in 3D.

acceleration and deceleration were respectively, 0·604 and $-0{\cdot}533$ m s^{-2}. A second stroke was analysed and the values were close to the previous with a trajectory length of 0·71 m, a stroke amplitude of 0·498 m, a maximum speed of 0·235 m s^{-1}, an acceleration of 0·404 m s^{-2} and a deceleration of $-0{\cdot}515$ m s^{-2}.

EFFECTS OF THE VIDEO RECORDING ON COELACANTH BEHAVIOUR

All coelacanths were filmed in open water outside of their caves and at a sufficient distance from the reef. Although filmed during daytime, these nocturnal animals did not show any evidence of modification of their behaviour due to the proximity of the divers, the set-up or the light. Filmed coelacanths displayed no obvious external signs of stress or abnormal behaviour. The divers could easily swim near the coelacanth. However, when a coelacanth was cornered by the divers it made a fast u-turn. Typically, animals swam away slowly after a few minutes of filming.

Discussion

One of the major aims of our study was to assess a new protocol for analysing the kinematics of fin movements in coelacanths in their natural environments. Typically, studies on swimming animals are complex and performed under standardized conditions (Wilga & Lauder 1999; Standen & Lauder 2005). Recording videos for a three-dimensional analysis of the kinematics of fin movements and swimming in large animals at water depths of more than 100 m is extremely difficult and has never been performed before. However, a better understanding of the locomotion of coelacanths in their natural environment is now possible. Thanks to the combination of autonomous deep diving, which allows direct observations of the animals while avoiding the stress generated by submersibles,

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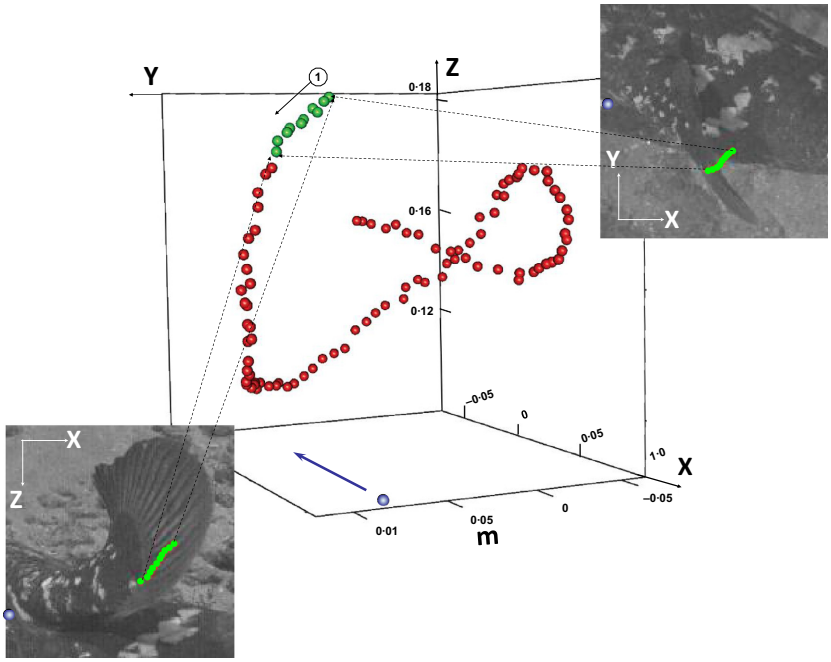

Fig. 6. 3D trajectory of the second dorsal fin of the coelacanth. The position and trajectory of one landmark on the fin was recorded in three dimensions for 6 s using the two-camera system (200 Hz) at 110 m deep. The 1 indicates the beginning of the trajectory, and the arrow indicates the direction. The insets show the image from a dorsal view (top right) and from a lateral view (bottom left). The trajectory of the first 10 points is indicated in green in the 3D graph and on both insets. The red points show the entire 3D trajectory of the fin. Units are in meters. The blue point indicates the reference point at the base of the fin, and the arrow indicates the postero-anterior axis of the animal.

with technological advances in cameras and underwater housings (i.e. high-speed and high-definition cameras recording multiple synchronized views in low light conditions), we were able to obtain unique video recordings of coelacanth fin movements. The set-up conceived for this purpose has proven to be fully appropriate for this type of kinematic study in a deep-water environment. However, the set-up described here is easily customizable according to the aim of the experimenter (type of lenses, recording speed, etc.), so that it can be used to quantify other events and behaviours in 3D for a variety of aquatic species.

During swimming, the fins (or limbs) assume several functions including propulsion, stabilization and the control of direction. However, the complete set of videos must be now analysed to understand both the coordination of the different limb pairs and the three-dimensional movements of the fins (or limbs). These unique data on living coelacanth will allow the study of the patterns of fin coordination during different locomotor behaviours observed throughout the dives. The collected data set on the three-dimensional movements of the fins (Fig. 6) will be coupled with virtual three-dimensional models created based on microCT and MRI scans of adult *Latimeria* specimens housed in the collections of the MNHN (Herbin *et al.* 2010; Dutel *et al.* 2013, 2015). This will allow us to quantify the kinematics of the skeletal elements through superimposition of anatomical data obtained from μCT or MRI data in the body envelope of the moving animal obtained as defined by our high-definition video recordings (Revéret *et al.* 2011). Subsequently, the use of computational methods will allow us to explore the role of the fin pairs (pectoral and pelvic fins) and unpaired fins (second dorsal and anal, and caudal fin) in generating propulsion in *Latimeria*. Computational fluid dynamic models will allow us to study the hydrodynamics of fin movements during locomotion at different speeds (e.g. during station holding, slow movements, rapid forward locomotion, manoeuvring). Together with muscle, cartilage and bone, physical properties obtained from the dissection of a specimen housed in the MNHN collection, multibody dynamic analyses (Curtis *et al.* 2011) will allow us to simulate the movements and to calculate the forces acting on the fins and girdles during swimming, and thus predict muscle activity. In an evolutionary context, this will allow to better understand the functional implications of the changes in body shape and fin morphology observed in the evolutionary history of coelacanths, as well as of fossil sarcopterygian fishes.

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Data accessibility

Video data (Fig. 5 was extracted from those HD high-speed video) and coordinates of the pectoral fin used to generate the 3D trajectory (to reproduce Fig. 6) are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.111qc.

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