



Swimming behaviors during diel vertical migration in veined squid *Loligo forbesii*

Seth F. Cones^{1,2,*}, Ding Zhang³, K. Alex Shorter³, Kakani Katija⁴, David A. Mann⁵, Frants H. Jensen^{1,6}, Jorge Fontes⁷, Pedro Afonso^{1,7}, T. Aran Mooney¹

¹Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

²MIT-WHOI Joint Program in Oceanography/Applied Ocean Science & Engineering, Cambridge, MA 02139, USA

³Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

⁴Research and Development, Monterey Bay Aquarium Research Institute, Moss Landing, CA 93940, USA

⁵Loggerhead Instruments, Sarasota, FL 34238, USA

⁶Biology Department, Syracuse University, Syracuse, NY 13244, USA

⁷Institute of Marine Sciences – Okeanos, University of the Azores, Rua Professor Doutor Frederico Machado 4, 9901-862 Horta, Portugal

ABSTRACT: Diel vertical migration (DVM) is a vital behavior for many pelagic marine fauna. Locomotory tactics that animals use during DVM define the metabolic costs of migrations and influence the risk of detection and capture by predators, yet, for squids, there is little understanding of the fine-scale movements and potential variability during these migrations. Vertical migratory behaviors of 5 veined squid *Loligo forbesii* were investigated with biologging tags (ITags) off the Azores Islands (central North Atlantic). Diel movements ranged from 400 to 5 m and were aligned with sunset and sunrise. During ascent periods, 2 squid exhibited cyclic climb-and-glide movements using primarily jet propulsion, while 3 squid ascended more continuously and at a lower vertical speed using mostly a finning gait. Descents for all 5 squid were consistently more rapid and direct. While all squid swam in both arms-first and mantle-first directions during DVM, mantle-first swimming was more common during upward movements, particularly at vertical speeds greater than 25 cm s⁻¹. The *in situ* variability of animal posture, swim direction, and gait use revealed behavioral flexibility interpreted as energy conservation, prey capture, and predator avoidance.

KEY WORDS: Diel vertical migration · DVM · Jet propulsion · Climb-and-glide · Bio-logging · Squid · Swimming behavior

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1. INTRODUCTION

Many pelagic marine fauna undergo diel vertical migration (DVM), whereby organisms spend day-time hours in deeper waters and return to the more productive euphotic zone at night when light intensity decreases (Hays 2003). DVM is believed to be the largest migration on earth in terms of biomass, and it is seen on daily time scales in both freshwater and marine ecosystems globally (Ringelberg 1995, Klevjer et al. 2016). Vertical movements during DVM,

or in response to prey DVM, are well-documented for many upper trophic level species, including sharks, cetaceans, and seabirds (Sims et al. 2005, Regular et al. 2010, Jensen et al. 2020, Braun et al. 2022). Yet, individual-level movements and swimming behaviors for middle to lower trophic levels are less known and difficult to monitor *in situ*, particularly at sub-second to hourly temporal scales.

Miniature animal-borne biologging tags provide an opportunity to investigate movement and behavior of pelagic fauna *in situ* (Rutz & Hays 2009, Chung et al.

2021). Accelerometers are widely integrated into these tags and allow for (1) identifying behaviors (Zhang et al. 2018), (2) estimating energy expenditure through proxies such as overall dynamic body acceleration (ODBA) (Halsey et al. 2009), and (3) creating 3-dimensional dead-reckoning paths of swimming animals (Zhang et al. 2019). Movement data from biologging tags have revealed important insights into animal physiology and ecology that would otherwise have been difficult or expensive to acquire, especially for cryptic marine species like squids (Whitford & Klimley 2019, Chung et al. 2021).

Squids are an abundant taxon and a key energy link in many ocean ecosystems (Clarke 1996, Smale 1996). Many squids, including muscular loliginids, are highly maneuverable swimmers, and their swimming mechanics have been well-studied in laboratory settings (O'Dor 1982, Hoar et al. 1994, Bartol et al. 2001, Stewart et al. 2010). Loliginids locomote using a combination of jet propulsion and finning. Jetting is pulsatile, and results from the intake of water into the mantle cavity and its expulsion out of a muscular funnel (Anderson & Grosenbaugh 2005), while thrust generated by finning is continuous, bidirectional, and tends to be used during low speed maneuvering (Anderson et al. 2001, Stewart et al. 2010). In practice, squid swimming gaits fall along a continuum between finning and jetting, but the relative use of different swimming gaits is largely unknown for free-ranging animals (Bartol et al. 2009, Stewart et al. 2010).

Squids face inherent bioenergetic shortfalls when compared to buoyancy-regulating fishes (O'Dor & Webber 1986). Jet propulsion is less efficient than caudal fin oscillation employed by most fishes, and because many squids are negatively buoyant, they must continually swim to maintain or attain their preferred depth (Hoar et al. 1994). O'Dor et al. (1995) found that jetting activity of veined squid *Loligo forbesii* was highest during periods of rapid upward swimming and estimated that its energetic cost was 2.6 and 3.1 times greater than horizontal swimming and hovering, respectively (O'Dor et al. 1995). Given their negative buoyancy, these squid used energetically costly jet propulsion during vertical ascents, but precise tactics such as finning activity, swim directions, and body orientations remain unknown. Such data could illuminate how these animals enact these costly but vital migratory behaviors.

Previous field studies of free-ranging Humboldt squid *Dosidicus gigas* used biologging tags (0.2–1 Hz sampling rate) to investigate the dynamics of daily vertical movements and the underlying movement

tactics (Gilly et al. 2006, 2012). The authors characterized many aspects of *D. gigas* swimming behaviors, including vertical swimming speeds and animal orientation. Notably, Gilly et al. (2012) showed that most *D. gigas* upward movements were cyclic climb-and-glide bouts, in which squid actively rose in the mantle-first (MF) orientation, followed by a passive descending glide at a lesser vertical speed. However, it remains uncertain why this tactic was used, how specific swimming behaviors (e.g. jetting and finning gaits) combined to enact these prominent cyclic movements, and whether other squid species also swim upward in intermittent bouts.

In this work, we examined the vertical swimming behavior of Azorean *L. forbesii* *in situ* using high-resolution (100 Hz) ITags (Mooney et al. 2015). *L. forbesii* are the largest of any loliginid squid, with dorsal mantle lengths (DMLs) up to 90 cm in the Azorean population (Martins 1982). They are nekto-benthic, primarily feed on smaller fishes (e.g. blue jack mackerel *Trachurus picturatus*), and are preyed upon by dolphins, sharks, and tunas, among others (Porteiro & Martins 1994). We used custom, high-resolution ITags to provide initial insights into *L. forbesii* diel habitat use variability, and at a finer scale, to quantify gait and swim direction during ascent and descent swimming. Additionally, we examined climb-and-glide swimming in detail to investigate underlying gait patterns and how those patterns relate to the ecophysiology of this species.

2. MATERIALS AND METHODS

2.1. Field deployments and ethics statement

Field deployments occurred on 3 days in May 2019 near Faial and Pico Islands, Azores archipelago (38.3003°N, 28.3522°W; Table 1). This study was completed under the Azorean Regional Government's International Recognized Compliance Certificate 01/2020 for access and use of natural resources for scientific purposes.

Care was taken during capture and handling of animals to limit physical stress. Squid were caught by jigging, which is a minimally invasive means of capture since it reduces damage to the fragile epidermal layer (Boletzky & Hanlon 1983). Once at the surface, animals were transferred to a padded table equipped with constant seawater flow to ventilate the gills. Only large squid with DMLs greater than 45 cm were selected for tagging. Squid eyes were covered during tagging to reduce light and

Table 1. Date, sex, morphology, and summary data from ITag deployments on veined squid *Loligo forbesii*. DML: dorsal mantle length

ID	Date (2019)	Sex	DML (cm)	Deployment duration (h)	Max. depth (m)	Min. depth (m)	Max. temperature (°C)	Min. temperature (°C)
Squid 1	22 May	Unknown	64	19.6	350	5	16.6	13.2
Squid 2	27 May	Female	58	23.5	373	18	16.4	12.0
Squid 3	27 May	Female	57	19.9	376	101	15.8	13.2
Squid 4	28 May	Male	51	23.9	389	88	15.7	12.3
Squid 5	28 May	Male	47	23.5	399	88	16.2	12.9

visual stress (Gonçalves et al. 2009). Tagged squid were immediately released over their capture site within 6–11 min, with the exception of Squid 1, which was held in an aerated cooler for 30 min before tagging.

2.2. Tag specifications

The ITag (12.5 × 2.6 × 2.7 cm; Fig. 1) is a custom-built biologging tag designed for soft-bodied invertebrates (Mooney et al. 2015, Fannjiang et al. 2019, Flaspohler et al. 2019). It contains a triaxial inertial measurement unit (IMU) with an accelerometer, gyroscope, and magnetometer sampled at 100 Hz (TDK Invensense MPU9250), and pressure, temperature (Keller 7LD), and light sensors (Inter-sil ISL29125) sampled at 1 Hz. The ITag body in the present study was designed specifically for squid, with a hydrodynamic shape to limit additional lift and drag forces exerted on the tagged animal. Tags were placed between 5 and 10 cm from the posterior mantle tip with the IMU x-axis, or surge direction, aligned with the squid mantle (Fig. 1). The tag was secured on top of the mantle cavity using a 3D-printed (Formlabs Durable resin) 1 mm thin base that the tag could release from using a nichrome burn wire. The base plates were affixed with dissolvable surgical sutures to the dorsal surface of each animal near the posterior mantle tip. Together, the 2 components were neutrally buoyant, with a positively buoyant electronics portion of the tag and a negatively buoyant base. The ITags were programmed to release from the squid and float to the surface where they were located using a

1 Hz VHF beacon (218–220.999 MHz, Advanced Telemetry Systems F1835B).

2.3. Orientation and swim direction estimation

Tag orientation (roll, pitch, and yaw) was estimated via the combination of accelerometer, gyroscope, and magnetometer data using the filtering technique proposed by Madgwick (2010). Squid can swim bidirectionally in arms-first (AF) and MF orientations, and although we were unable to directly measure swim direction in the wild, it was estimated by using measurements of animal pitch and vertical speed. Flaspohler et al. (2019) demonstrated that captive *Loligo forbesii* swim at a positive and negative pitch during

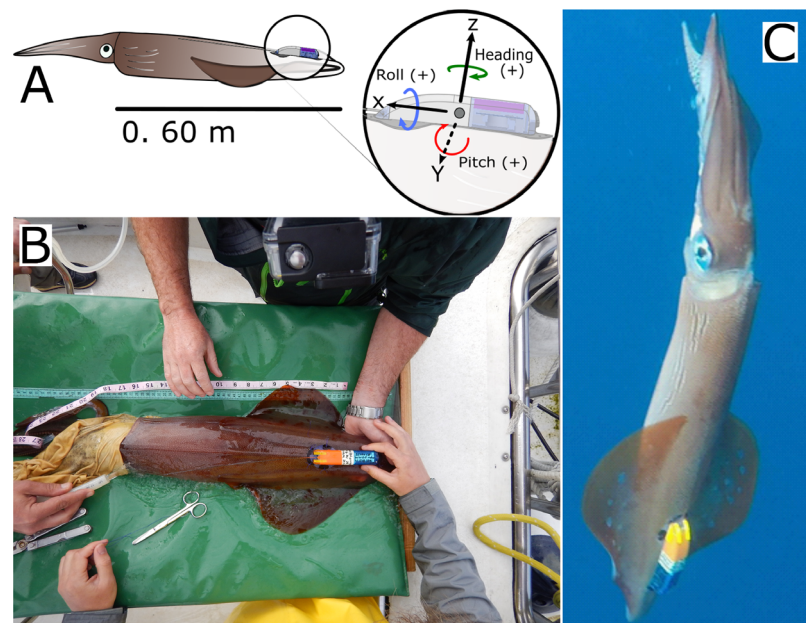


Fig. 1. Tag schematic and tagging methods used for veined squid *Loligo forbesii*. (A) ITag was affixed to the dorsal side near the tip of the mantle. Red, blue, and green arrows represent positive Euler angles of pitch, roll, and yaw (heading), respectively. (B) Tagging took place on a padded table, while squid gills were oxygenated with ambient seawater. (C) After tagging, squid were carefully placed in the water in a location near the capture location

AF and MF swimming, respectively. A similar method was used to classify swim direction *in situ*. Additionally, because the ocean environment allows for greater vertical movements than an aquarium, a depth change threshold (5 cm s^{-1}) was added to our classifier (Fig. 2). If squid ascended at vertical speeds $>5 \text{ cm s}^{-1}$, movements were classified as AF or MF if pitch was $>5^\circ$ or $<-5^\circ$, respectively. If squid were descending faster than 5 cm s^{-1} , movements were classified as AF or MF when pitch was $<-5^\circ$ or $>5^\circ$, respectively.

2.4. Movement and gait classification

ODBA was used for gait classification and as a general activity metric. To distinguish between high-frequency animal movements (dynamic acceleration) and slower changes in orientation, a low-pass filter was applied to the accelerometer data (see Flaspohler et al. 2019 for a detailed description). Animal movement intensities were classified into 3 levels (low, medium, and high) based on ODBA values and 2 empirically defined thresholds from previous captive tagging experiments: $<0.06 \text{ g}$ (low) and $>0.18 \text{ g}$ (high). The correspondence between the 3 ODBA intensity levels (low, medium, and high) and the 2 gait categories (finning: low, medium; jets: high) were made accordingly (Flaspohler et al. 2019). Time periods in which peak ODBA was $<0.18 \text{ g}$ were classified as finning. If peak ODBA within a 0.8 s window surpassed 0.18 g , it was considered a jetting event. A threshold of 0.18 g was deemed conservative because it is a statistical outlier for the peak ODBA distribution of the finning gait (Flaspohler et al. 2019), and the 0.8 s window size was selected by considering the maximum jetting frequency of *Illex* (Webber & O'Dor 1986) and *Doryteuthis* (Anderson & Grosenbaugh 2005) squids.

2.5. Dead reckoning and motion entropy

A dead-reckoning track was constructed for all tagged animals to both visualize their paths in 3D and quantify the unpredictability, or entropy (H), of their motion. The direction of motion (AF or MF) and orientation of each animal were estimated as described in Sections 2.3 and 2.4. Forward speed for high pitch angles ($|\text{pitch}| \geq 20^\circ$) was calculated by dividing the vertical speed derived from the pressure sensor by the sine value of the pitch angle (Gilly et al. 2012). When the animal had a small pitch angle ($|\text{pitch}| < 20^\circ$), a naive approach was taken to estimate forward speed of the animal. Accelerometer data were used to bin movement intensity as low, medium, or high. Speed and movement intensity observed from animals tagged in captive settings were then used to assign a constant speed for the identified movement intensity: (low = 0.1 m s^{-1} , medium = 0.3 m s^{-1} , high = 0.6 m s^{-1}). Gaussian noise (mean 0, standard deviation 0.1) was then added to the constant speed to include uncertainty for the estimated forward speed of the animal.

The dead-reckoning track was used to quantify the unpredictability of an animal's motion by calculating the next-frame positional entropy. Dead-reckoning tracks are prone to errors due to cumulative positional drift. To circumvent drift errors, only H for 1 s periods were calculated, and since start position is arbitrary in our model, drift was significantly reduced (Gunner et al. 2021). For each time instance, the next-frame (1 s in the 'future') relative position of the animal was calculated with respect to its current position and direction of motion. Unpredictable movements would have a wide distribution in the next-frame relative position, while predictable motion would have a narrow distribution. H was quantified using Shannon's equation (Shannon 1948; see Moore

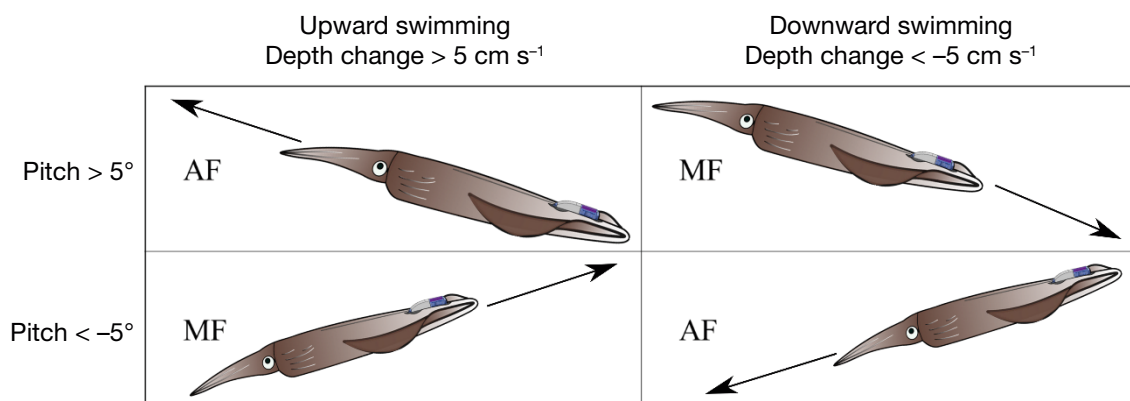


Fig. 2. Arms-first (AF) and mantle-first (MF) swim direction classification criteria during upward and downward swimming

et al. 2017 for a detailed description). First, an individual's surroundings were discretized into 2500 cubes (0.1 m sides), and the frequency of next-frame positions that landed in each volume was counted (denoted as p_i for the i^{th} cube).

$$H = -K \sum_{i=1}^n p_i \log(p_i) \quad (1)$$

$K = 0.1278$ was then used to scale the entropy H from 0 to 1. Deviations from fixed orientation and animal speed were positively correlated with entropy. An H value of 0 implied that the next-frame position was absolutely certain, and an H value of 1 implied that the next-frame position was fully random and unpredictable. Using this framework, the path of the squid was assumed to be linear, and a squid moving at a constant speed and orientation would result in 0 entropy. An animal that had an equal probability of occupying all 2500 positional cubes had an entropy of 1 because the next position was most difficult to predict.

2.6. Statistics

Differences in swim direction, gait use proportionality, and vertical speeds were tested using the non-parametric Mann-Whitney U -test. This test was selected since normality could not be properly assessed with our small sample size, and there was an uneven number of ascents ($n = 5$) and descents ($n = 4$), making the alternative paired-tests not applicable. Jetting events were treated as discrete events modeled with a Poisson distribution, whereafter a z -test was used to compare jetting rates among conditions. The non-parametric Kruskal-Wallis test was implemented to compare ODBA distributions during different behavioral states because ODBA distributions were non-normal. Heading data were treated as circular data, and differences in circular heading variance were tested using the Watson-Williams test.

Lastly, differences in movement entropy were tested using a permutation test. For all ascent data, H data were averaged over 30 s periods and were categorically assigned to either the climb-and-glide or continuous ascent mode (see Section 3.2). Data were aggregated across individuals, and the difference in H between the 2 modes was used as the empirical difference in movement entropy. The ascent mode assigned to each dead-reckoning track segment was then randomized by permuting the categorical vector; thus, the amount of H samples was kept fixed, but the order was randomized. This permutation was

done 10 000 times, with a permuted difference in entropy between modes calculated each time. Finally, the p -value was calculated as the number of permuted mode differences that were equal to or more extreme compared to the empirical difference in entropy divided by the total number of permutations.

3. RESULTS

Ten *Loligo forbesii* were affixed with ITags, with data totaling 133 h (Table 1). Three tags released prematurely and 2 were programmed for shorter durations to test the tag. Five deployments lasted the preprogrammed duration (ca. 24 h), and these data sets were analyzed to provide an initial characterization of *L. forbesii* *in situ* movements.

3.1. DVM

Upon release, all individuals immediately descended to 200–300 m water depth, the approximate depth of capture. All squid spent substantial time (6–9 h) at depth, then migrated toward the surface at dusk. Pressure data showed that all squid exhibited DVM, with daytime depths (mean $\pm$ SD, 278.6 ± 65 m) more than twice those of nighttime (119.7 ± 37 m, Fig. 3). Daytime depth distributions were bimodal, with peaks at 275 and 375 m, while nighttime depths were less variable, with over 60% of time spent between 100 and 150 m. Upward and downward migratory movements were roughly linked with sunset and sunrise, respectively (Fig. 3C), although patterns of vertical movement differed.

3.2. Ascent swimming

Two swimming tactics, i.e. 'continuous' and 'climb-and-glide,' were employed during upward migrations (Fig. 4). These tactics had distinct differences in vertical speed, swim direction, jetting rate, and heading variance. Climb-and-glide ascents consisted of cyclic MF maneuvers and were highly stereotyped with 3 distinguishable sections: Phases I, II, and III (Fig. 4B). Phases I and II were 2 successive upward movements of differing vertical speeds, followed by a slow descent (Phase III). Phase I had a vertical speed of 0.061 ± 0.05 m s⁻¹ and roughly a -10° pitch angle. The transition to Phase II was marked by a more rapid vertical speed (0.25 ± 0.14 m s⁻¹) and steeper pitch (ca. -40°). The average estimated speed for

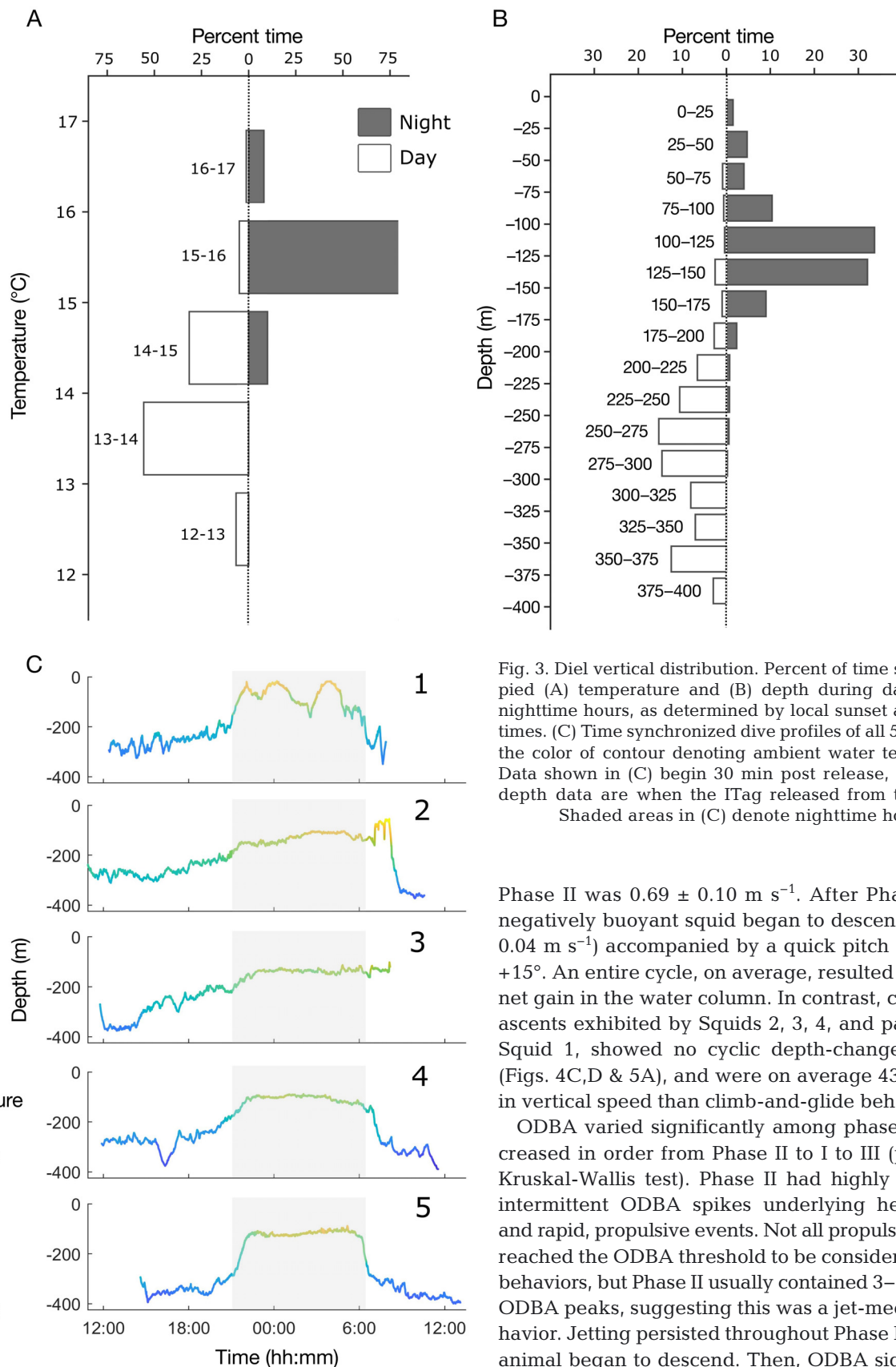


Fig. 3. Diel vertical distribution. Percent of time squid occupied (A) temperature and (B) depth during daytime and nighttime hours, as determined by local sunset and sunrise times. (C) Time synchronized dive profiles of all 5 squid with the color of contour denoting ambient water temperature. Data shown in (C) begin 30 min post release, while final depth data are when the ITag released from the animal. Shaded areas in (C) denote nighttime hours

Phase II was $0.69 \pm 0.10 \text{ m s}^{-1}$. After Phase II, the negatively buoyant squid began to descend ($0.066 \pm 0.04 \text{ m s}^{-1}$) accompanied by a quick pitch change to $+15^\circ$. An entire cycle, on average, resulted in a 3.1 m net gain in the water column. In contrast, continuous ascents exhibited by Squids 2, 3, 4, and partially by Squid 1, showed no cyclic depth-change patterns (Figs. 4C,D & 5A), and were on average 43% slower in vertical speed than climb-and-glide behaviors.

ODBA varied significantly among phases and decreased in order from Phase II to I to III ($p < 0.001$, Kruskal-Wallis test). Phase II had highly repetitive intermittent ODBA spikes underlying heightened, and rapid, propulsive events. Not all propulsive events reached the ODBA threshold to be considered jetting behaviors, but Phase II usually contained 3–10 distinct ODBA peaks, suggesting this was a jet-mediated behavior. Jetting persisted throughout Phase II until the animal began to descend. Then, ODBA significantly

decreased, and values only rose when the animal reached the start of the next cycle. Lastly, ODBA was greater during climb-and-glide than continuous swimming ($p < 0.001$, Kruskal-Wallis test, Fig. 4E).

The average inter-jet interval (IJI), or time between subsequent jets, during Phase II of climb-and-glide ascents remained consistent throughout the entirety of an ascent but differed between individuals. In this analysis, all distinct ODBA peaks greater than 0.1 g

were considered jets to ensure that all propulsive events were included. Squid 1 had an IJI of $2.16 \pm 0.08\text{ s}$ and Squid 5 had an IJI of $1.56 \pm 0.09\text{ s}$. When omitting the highest and lowest IJI, Squid 1 and Squid 5 IJI range did not exceed 0.33 s over the entire climb-and-glide ascent period.

Jetting rate was significantly greater during climb-and-glide ($1.92\text{ jets min}^{-1}$) than during continuous ascents ($0.142\text{ jets min}^{-1}$, $p < 0.001$, $Z = 15.41$, Fig. 5A).

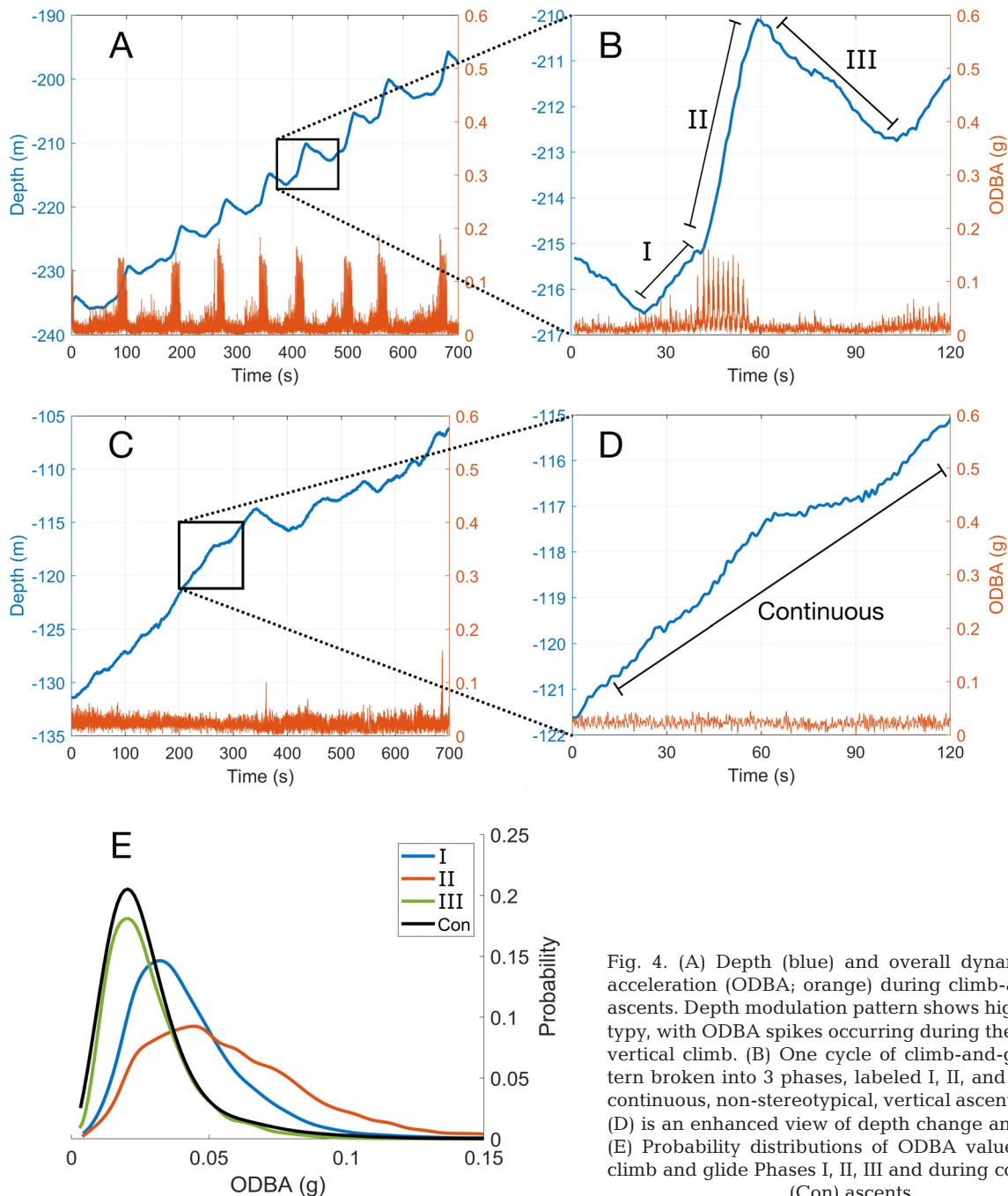


Fig. 4. (A) Depth (blue) and overall dynamic body acceleration (ODBA; orange) during climb-and-glide ascents. Depth modulation pattern shows high stereotypy, with ODBA spikes occurring during the steepest vertical climb. (B) One cycle of climb-and-glide pattern broken into 3 phases, labeled I, II, and III. (C) A continuous, non-stereotypical, vertical ascent. Subplot (D) is an enhanced view of depth change and ODBA. (E) Probability distributions of ODBA values during climb and glide Phases I, II, III and during continuous (Con) ascents

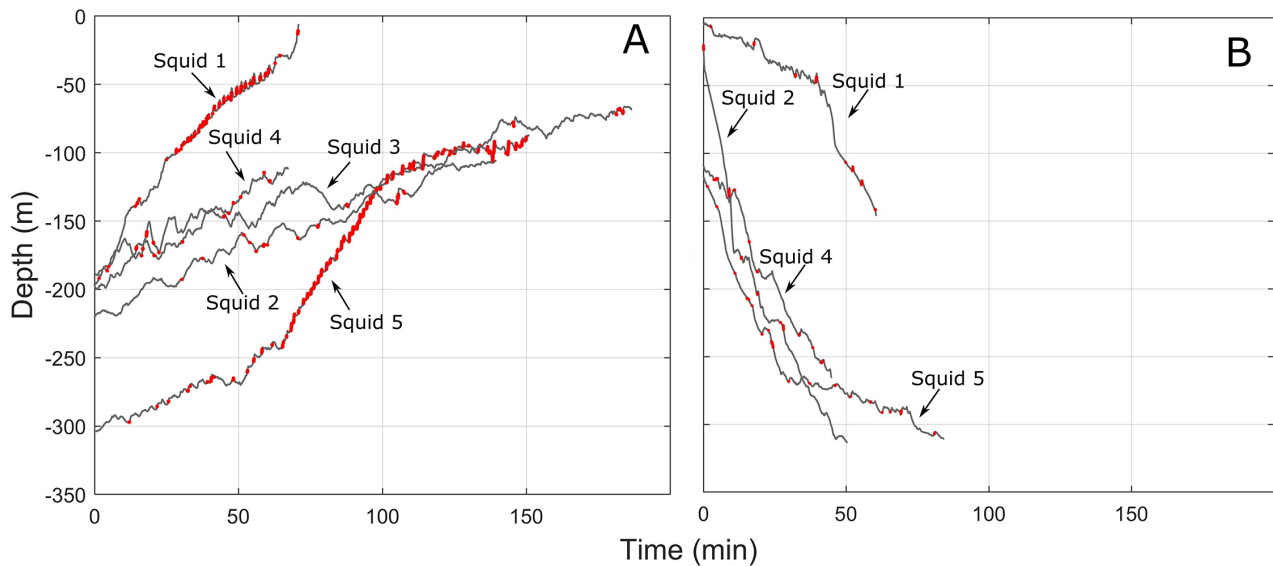


Fig. 5. Overlaid (A) ascent and (B) descent depth profiles of all squid. Red dots denote jetting events, where animal overall dynamic body acceleration exceeded 0.18 g

Jets during climb-and-glide ascents only resulted in an upward movement, unlike jets during continuous ascents which frequently occurred during brief downward movements.

Heading variance differed by ascent tactic. Squid ascending by climb-and-glide showed a more consistent heading, contrasting squid ascending continuously ($p < 0.001$, Watson-Williams test, Fig. 6). The interquartile ranges for climb-and-glide and continuous ascents were 70° and 176° , respectively (Fig. 6F).

Next-frame positions during climb-and-glide ascents ($H = 0.485$) were significantly less predictable than during the continuous ascents ($H = 0.435$, $p < 0.0001$, permutation test, Fig. 7). Heading variance was greater during continuous ascents, suggesting more turning behaviors. Despite less horizontal turning, positional estimates during climb-and-glide ascents were still more entropic (Fig. 7B,C). Heavily jet-mediated climb-and-glide movements caused a greater positional range in the surge direction contributing to the greater entropy.

3.3. Descent swimming

Descent periods were shorter in duration than ascent periods, underlying greater vertical swimming speeds during descents ($0.0628 \pm 0.113\text{ m s}^{-1}$) than ascents ($0.0198 \pm 0.11\text{ m s}^{-1}$, $p = 0.0159$, Mann-Whitney U -test, Fig. 5B). Descents contained long stretches of constant downward movements of up to 130 m. However, similar to ascent swimming, animals

did not move unidirectionally throughout the descent period. There were many nested upward movements within the overall descent period, and these were times of relatively high jetting activity. On average, squid swam upward toward the surface for 25% of descent periods, and these periods contained 58% of total jets during downward migrations.

3.4. Swim direction

There was no difference in the proportion of both AF and MF swimming among ascents versus descents ($p = 0.103$, randomization test, Fig. 8A), and all squid utilized both swim directions to ascend (MF: 63–82%; AF: 18–37%) and descend (MF: 35–77%; AF: 23–65%) in the water column. Next, the proportion of AF versus MF swimming was compared during ascents and descents individually to test if squid predominately swam in one direction during upward and downward migrations. Squid preferred to ascend MF ($p = 0.008$, Mann-Whitney U -test), but showed no swim direction preference during descents ($p = 0.343$, Mann-Whitney U -test). However, when considering only ascents or descents at vertical speeds $\geq 25\text{ cm s}^{-1}$ in magnitude, MF swimming was more frequent (ascent: $p = 0.032$, descent: $p = 0.029$, Mann-Whitney U -test, Fig. 8B), showing that MF swimming was preferred at higher vertical speeds.

Jetting behavior was examined during AF and MF swimming in 3 ways. (1) First, the total numbers of MF versus AF jets were counted during both ascent and

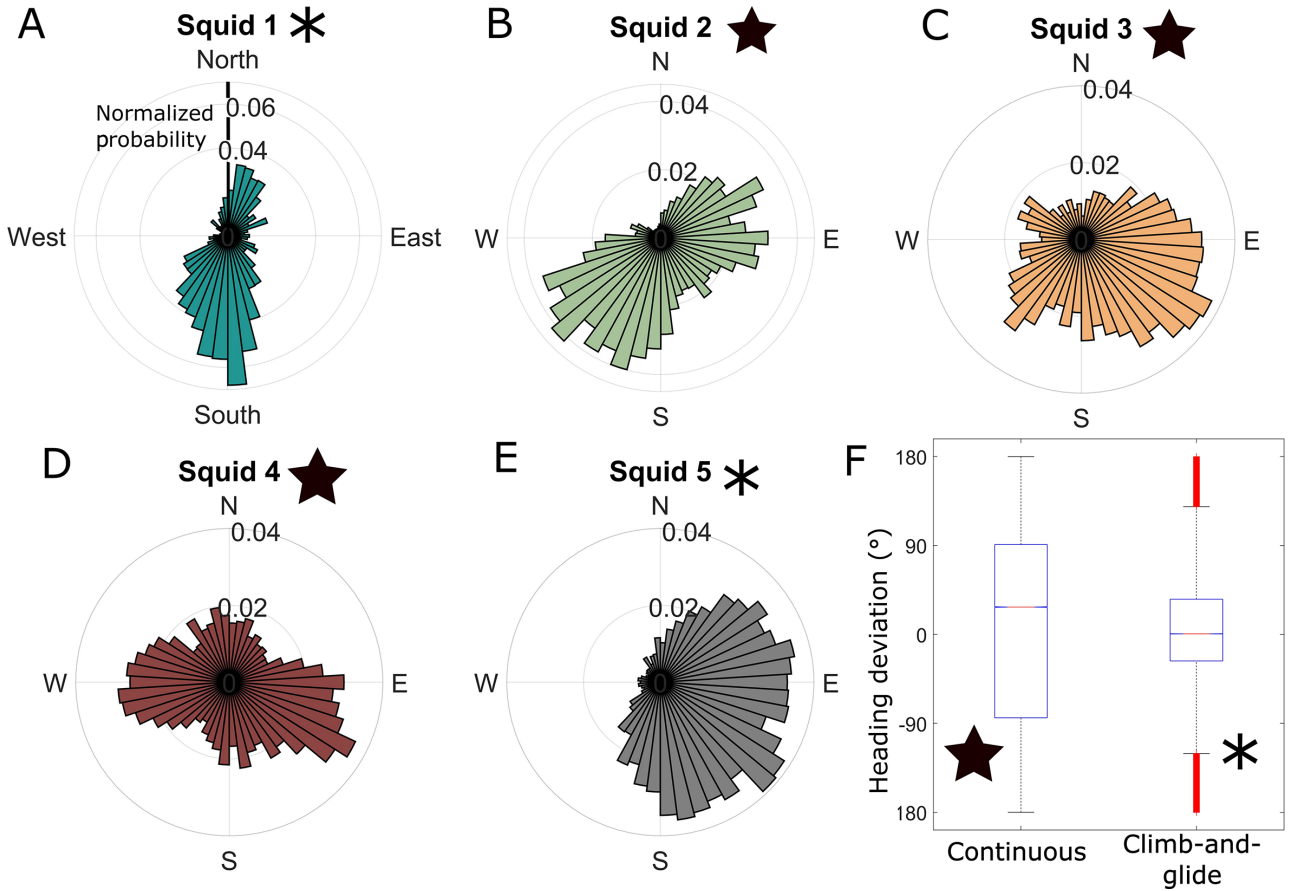


Fig. 6. (A–E) Squid headings during their respective ascents. The histograms are projected onto a compass and the lengths are normalized counts in the respective direction. Stars and asterisks denote squid predominately ascending by continuous and climb-and-glide swimming, respectively. (F) Animal heading during all continuous (left) and climb-and-glide (right) ascents. Red markings denote heading variances that were statistical outliers. Mean heading values for each individual animal were centered around a derived 0° position so that data could be combined into categories for statistical comparison. For each box-plot, the central line marks the median value, and bottom and top edges mark the 25 and 75 percentiles, respectively. Red markings denote heading variances that were statistical outliers

descent periods together. Overall, squid jetted in the MF direction ($n = 317$, 92 %) more frequently than in the AF direction ($n = 27$, 8 %, $p = 0.032$, Mann-Whitney *U*-test). (2) Next, MF versus AF jets were compared at positive vertical speeds irrespective of whether jets were during ascent or descent periods. Upward jetting events were significantly more common in the MF ($n = 304$, 98 %) than AF ($n = 6$, 2 %) swim direction ($p = 0.008$, Mann-Whitney *U*-test). Upward jets were most common during climb-and-glide swimming, and Squids 1 and 5 jetted upward in the MF orientation 75 and 183 times, respectively. (3) Lastly, AF versus MF jets occurring at negative vertical speeds were compared, again combining ascent and descent periods. Squid showed no swim direction preference during downward jets (MF = 21 jets [70 %], AF = 9 jets [30 %], $p = 0.90$, Mann-Whitney *U*-test).

4. DISCUSSION

In this study we used high-resolution kinematic sensors to measure squid movement and behavior, resulting in novel data on gait, swim direction, body orientation, and movement trajectories. These results reveal new aspects of squid swimming behavior in the context of a prominent and ecologically meaningful phenomenon, the DVM of marine animals.

4.1. Vertical organization

All squid in the present study inhabited shallower waters at night, but the scale and rate of vertical migration varied, potentially as a function of external cues. Variability in overall ascent rate and swimming

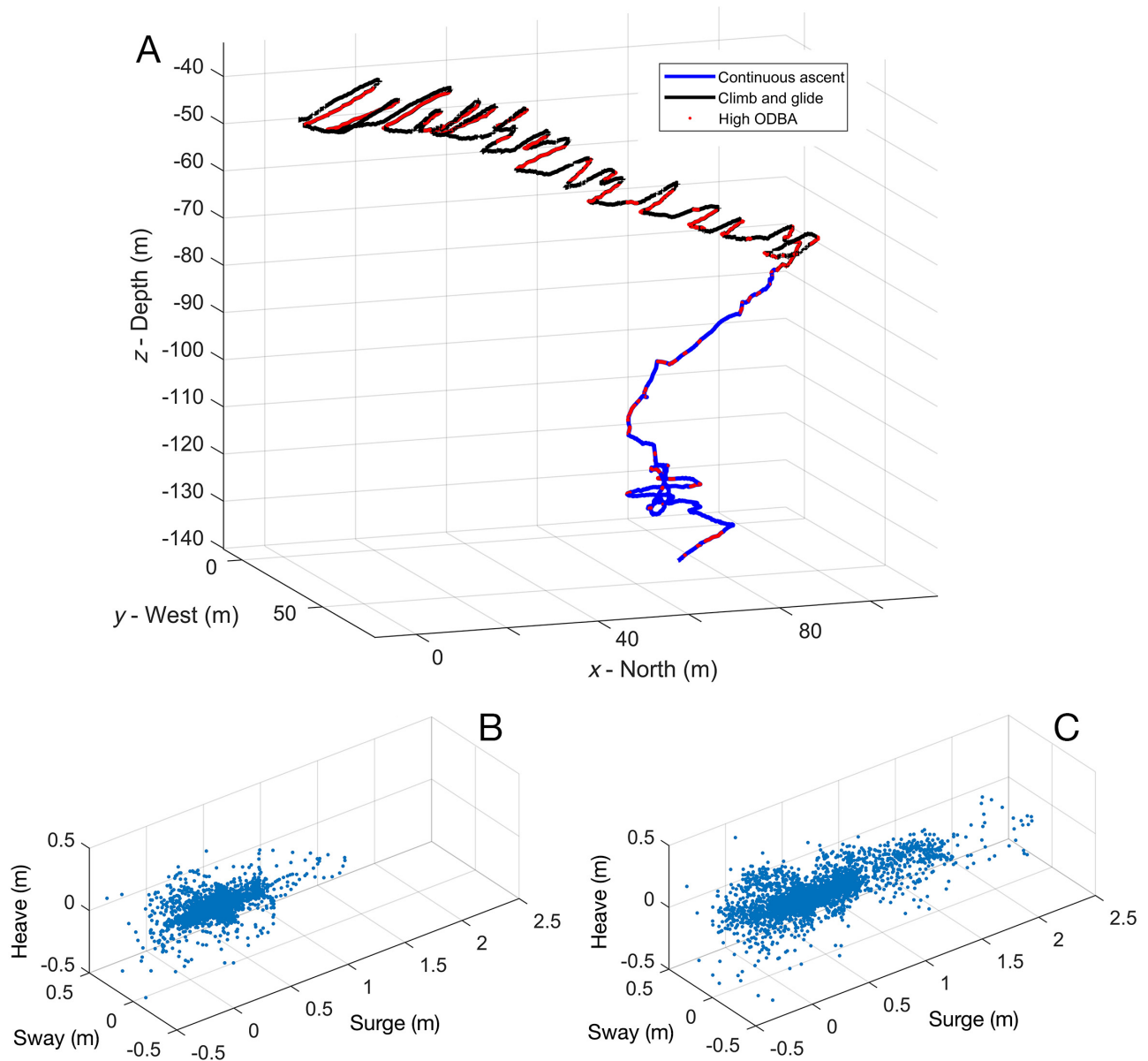


Fig. 7. Visualization of (A) a partial 3D dead-reckoning track from Squid 1 that was composed of both continuous ascent (blue) and climb-and-glide ascent (black), with high overall dynamic body acceleration (ODBA) events marked red. The clusters of next-frame positions corresponding to the continuous ascent and climb-and-glide ascent are shown in (B) and (C), with entropy values of 0.435 and 0.485, respectively

tactic suggests there may be other proximate factors, abiotic or biotic, that influenced the timing and magnitude of *Loligo forbesii* vertical migration. It is evident that light irradiance is integral to migrator depth-modulation (Bollens et al. 1992, Benoit-Bird et al. 2009, Benoit-Bird & Moline 2021). Isolume tracking, in which organisms preferentially adjust their vertical position to stay within a narrow illumination bin, is exhibited by many species, influencing ascent rates and timing (Hays 2003). Although we did not

have ambient light data for our squid, given the similarity of squid depth profiles tagged on the same day (Squid 2/3 and Squid 4/5), we speculate that light irradiance influenced their vertical migrations at least to some degree, either directly or indirectly through movements of prey and predators.

Similar to other taxa, variation in body condition may have caused variability in DVM swimming. Migratory behavior of the copepod *Metridia pacifica* is linked to its oil sac volume (energy reserves), and

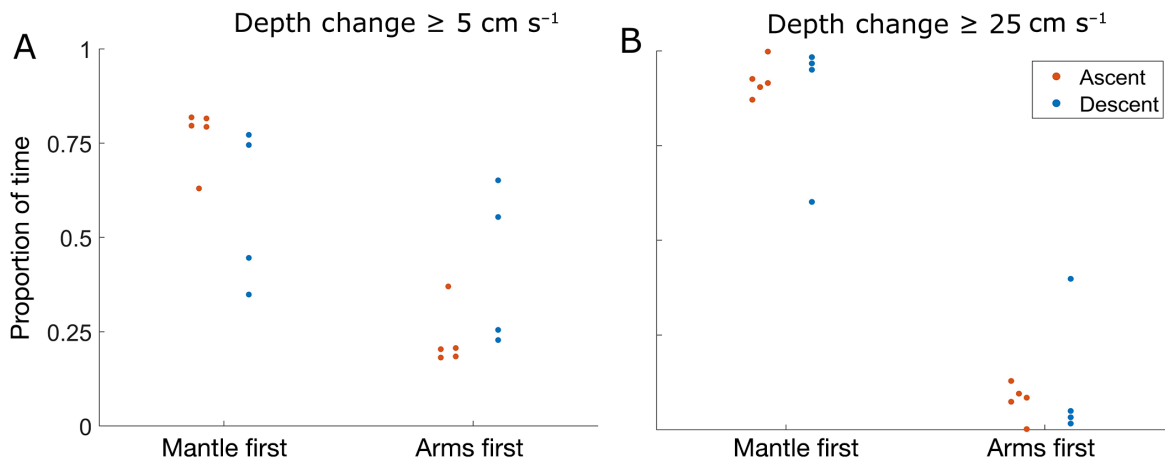


Fig. 8. Pitch and swimming direction during ascent and descent for all 5 squid. Proportion of arms-first or mantle-first swimming during ascent and descent for vertical speeds (A) $\geq 5 \text{ cm s}^{-1}$ and (B) $\geq 25 \text{ cm s}^{-1}$

individuals with large oil sacs are more likely to forego dusk ascents (Hays et al. 2001). All individuals in the present study inhabited shallower waters at night, but previous data (O'Dor et al. 1995) demonstrated that this *L. forbesii* population may forego DVM and remain in deeper waters at night. However, we suspect gut fullness to be insignificant in modulating *L. forbesii* DVM behavior. First, squid have high metabolic rates compared with similarly sized taxa, and exhibit the highest energy turnover of all marine invertebrates (Pörtner 2002). As such, there is likely a physiological incentive to feed during both day and night periods (Pörtner & Zielinski 1998). Furthermore, there is likely an evolutionary incentive to maintain a high feeding rate, since it is positively correlated with growth (Sakurai et al. 1993), and larger squid tend to have more reproductive success (Hanlon & Messenger 2018).

4.2. Vertical swimming behavior

At vertical speeds between 5 and 25 cm s^{-1} , squid preferred MF swimming during ascents, but showed no swim direction preference during descents. Interestingly, MF direction was almost exclusively used when vertical speeds surpassed 25 cm s^{-1} . The propensity for MF swimming at high speeds is also evident in other species (e.g. *L. brevis*, Bartol et al. 2001) in laboratory experiments. Animals exercised in a flow-through system will swim both AF and MF until a transitional speed, and then at higher speeds, squid only swim MF, suggesting that there may be a biomechanical advantage of swimming MF at higher animal speeds. However, Bartol et al. (2016) com-

pared movement efficiency of AF and MF swimming of *L. brevis*, and found no significant difference in swimming efficiency across all measured animal speeds ($0\text{--}6.6 \text{ DML s}^{-1}$). However, there are mantle and fin morphology differences between *L. brevis* and *L. forbesii*, and individuals in the laboratory experiment were only swimming horizontally, in contrast to the vertical movements we observed *in situ*.

Despite preferentially selecting MF during ascent periods, there were long periods in which squid enacted AF swimming bouts. MF swimming has been associated with intermediate and high-speed swimming, escape jetting, and prolonged migratory swimming (O'Dor 1988, Bartol et al. 2001, Hanlon & Messenger 2018). Gilly et al. (2012) noted that *Dosidicus gigas* swam upward almost exclusively in the MF direction, but did display brief AF vertical movements. AF is employed during foraging, low-speed maneuvering, and antagonistic interactions (Bartol et al. 2001, Hanlon & Messenger 2018). We show that *L. forbesii* swam AF for 18–37% of the total duration during ascent migratory swimming. Although AF swimming is significantly less frequent than MF swimming, AF swimming is likely ecologically or biomechanically important in their migratory behavior. We suspect that AF swimming was enacted in part due to the heightened ability, relative to MF, to detect predators and prey. When *L. forbesii* swam vertically using AF orientation, their pitch was positive with their head angled upward, which is likely beneficial for detecting diving predators and prey.

Being negatively buoyant, *L. forbesii* expend more energy during upward than downward swimming (O'Dor et al. 1995). Thus, it is not surprising that descent periods were more rapid, as animals did not

have to contend with gravity. In fact, there were several long periods during descents in which animals moved downward tens of meters with low activity (i.e. no jetting events). These periods could enhance movement efficiency as animals glide down to their preferred daytime depths. Despite long periods of low activity, we recorded nested upward swimming bursts with high jetting rates, suggesting *L. forbesii* were reactive to their surroundings, potentially foraging or avoiding predation.

4.3. Climb-and-glide swimming

Many marine and aerial species move intermittently, potentially as an energy-saving strategy (Gleiss et al. 2011). Gleiss et al. (2011) compared ODBA during continuous and intermittent movements in southern elephant seals *Mirounga leonina* and found that animals expended less energy during intermittent locomotion across all measured speeds. Previous studies on the movement efficiency of squid have solely been conducted in laboratory flumes (Bartol et al. 2001), and due to the limited size of such systems, no direct analysis of climb-and-glide efficiency has been completed. Squids 1 and 5 employed climb-and-glide swimming for over 40 min, in which vertical speeds, jetting activity, and ODBA were higher compared to continuous ascents, which all suggests that a higher energetic cost was incurred during climb-and-glide swimming. However, the ability to rest for 30–60 s during gliding phases before another bout upward may allow squid to limit overall energy expenditure and increase efficiency (Finke et al. 1996).

Mechanistically, climb-and-glide swimming by *L. forbesii* varied slightly from that of *D. gigas*. Climb-and-glide swimming by both species were entirely MF, but *D. gigas* vertical climbs were slower (0.35 m s^{-1} , 0.46 DML s^{-1}) and covered less distance (2.2 m) than those of *L. forbesii*, which commonly climbed 6 m and occurred at almost twice the velocity (0.69 m s^{-1} , 1.25 DML s^{-1}). Animal pitch also differed, with *D. gigas* climbing at a steeper angle (-51°) than *L. forbesii* (-40°). Pitch angles during gliding also differed; *D. gigas* glided horizontally with a 0° animal pitch, albeit with notable variability, while *L. forbesii* frequently glided at $10\text{--}20^\circ$ pitch. These posture and speed differences could be attributable to differences in morphology, size, or habitat, or a combination thereof.

Interestingly, Squid 1 (63.5 cm DML) had a shorter IJI than Squid 5 (47.0 cm DML) during Phase II of climb-and-glide swimming. Squid DML has pro-

found impact on swimming mechanics and efficiency of the finning and jetting gaits, although the most substantial changes occur during the transition from paralarvae to juveniles (Bartol et al. 2008). However, differences in squid morphology (i.e. fin area, mantle volume) could be influencing the observed difference in IJI. Indeed, the DML of Squid 1 was 16.5 cm longer, and laboratory studies have demonstrated that smaller individuals rely more heavily on jet propulsion (Thompson & Kier 2001); however, this trend has not been studied in squid of this size.

4.4. Movement entropy

Intermittent locomotion is not only important from a physiological perspective, but also ecologically. In other species, pauses in movement have been shown to increase perception of sensory field (i.e. decreases in 'velocity blur'), decrease detectability, and aid in predator evasion (Kramer & McLaughlin 2001). Squids 1 and 5 consistently glided (Phase III) at a $10\text{--}20^\circ$ pitch posture during climb-and-glide ascents, with their head angled upward. Squid could be angling upward during glides to decrease velocity blur and watch for predators simultaneously. Extended low-acceleration gliding periods would also decrease the chaotic flow around squid epidermal hair cells, which serve as a lateral line analog and are known to aid in predator evasion (Budelmann & Bleckmann 1988, York & Bartol 2014). Moving passively and pitching at 15° might therefore aid both visual and lateral-line detection of predator movements and pressure differentials (Mooney et al. 2010, Nilsson et al. 2012). Of particular note, pitching at 15° is also favorable for passive horizontal movement (Weihs 1973, O'Dor 1988), but these 2 ideas are not mutually exclusive.

Risso's dolphins *Grampus griseus*, and other cetacean and teleost predators, forage at dusk when squid migrate upward (Baird et al. 2001, 2002, Jensen et al. 2020). During these time periods, climb-and-glide was 17 % more unpredictable than continuous ascents and consisted of significantly more high-acceleration jetting events. We were unable to definitively assert why either of the 2 tactics was enacted, whether for a biomechanical advantage, predator aversion technique, or a soaring behavior in conjunction with currents. However, free-ranging squid have been shown to alter their behavior in the presence of predators (Bollens et al. 1992, Benoit-Bird et al. 2017, Benoit-Bird & Moline 2021). Intended or unintended, climb-and-glide likely decreased pre-

dation probability during high-risk vertical migration through more unpredictable positional estimates (Moore et al. 2017).

4.5. Conclusion

Our work demonstrates that high-resolution bio-logging tags can be used to elucidate the ecology and swimming mechanics of large squid (e.g. gait, swim direction, posture, DVM). High resolution, individual-level movement data have been elusive for many soft-bodied invertebrates and lagged behind other taxa including fishes, marine mammals, and seabirds (Chung et al. 2021). The *in situ* variability of animal posture, swim direction, and gait use revealed behavioral flexibility, potentially as energy conservation, prey capture, and predator avoidance strategies. Although our study is the first to quantitatively measure swim direction and jetting and finning gait use in wild squid, more work is needed to determine causes of the observed variability in movement tactics.

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