Quantification of a previously undescribed fast-start of larval clownfish targeting copepod prey: predatory posture, speed and acceleration from high-speed video, July 2015

Website: https://www.bco-dmo.org/dataset/750328 Data Type: experimental Version: 1 Version Date: 2018-11-28

Project

» The Drive to Survive: Copepods vs Ichthyoplankton (PreyEscape)

Contributors	Affiliation	Role
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Abstract

Posture, strike speed, and acceleration of clownfish larval attack on copepods, from high-speed videos, June-July 2015. Results of these data are published in Fashingbauer et al (in revision, J. Exp. Biol.) and Robinson et al (accepted, MEPS).

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Coverage

Spatial Extent: Lat:21.298 Lon:-157.8187 Temporal Extent: 2015-06-28 - 2015-07-31

Dataset Description

Posture, strike speed, and acceleration of clownfish larval attack on copepods, from high-speed videos, June-July 2015. Results of these data are published in Fashingbauer et al (in revision, J. Exp. Biol.) and Robinson et al (accepted, MEPS).

Methods & Sampling

Permits: All protocols and experiments, described below, followed institutional guidelines and were approved by the University of Hawaii Institutional Animal Care & Use Committee (IACUC protocol number 2099).

Experimental design: We set three larval fish age-classes of the clownfish, Amphiprion ocellaris (early: 1 to 5 days post-hatch [dph]; mid: 6 to 9 dph; and late: 11 to 14 dph) against three developmental stages of the copepod prey, Bestiolina similis (nauplii: NIII-NIV stages; copepodites: CII-CIII stages; and adults: CVI stage).

The choice of developmental stages provided a range in prey size (length: ~100 to 500 μ m; McKinnon et al., 2003), mechanosensitivity, and escape performance (Buskey et al., 2017). We designed the experiment to quantify how strike posture changed through larval development (and size: ~4 to 8 mm total length; Jackson and Lenz, 2016), while also assessing the effect of a prey's stage on its predator's posture.

Larval fish-rearing conditions: The experiments used larval clownfish lab-reared over their two-week planktonic phase. Their rearing, the culturing of copepod prey, the experimental apparatus and protocols, and the high-speed video recording and analysis software have been described previously (Robinson et al., accepted). Briefly, up to 200 recently hatched larvae were raised in a 30-L seawater aquarium kept at 24-26° C on a 12:12 L:D light cycle. They were fed twice daily on a mixed diet of rotifers (Brachionus plicatilis) and different developmental stages of another calanoid copepod (Parvocalanus crassirostris). Different prey were used for daily feeding than for experiments so that fish were exposed to a novel prey type during their trial, thus avoiding complications arising from learned feeding behavior and laboratory acclimation.

Behavioral observations and video set-up: For the experiments, two larvae that had been kept without food for 4 to 6 hours were placed into a circular observation chamber of 20 cm diameter, filled with seawater to a depth of 2 cm containing copepods at a density of 0.2 to 0.7 individuals ml-1. Experimental trials lasted for one hour or less and no fish larvae were used in more than one trial. Interactions between fish larvae and copepods were recorded at 500 frames per second (fps) using a Photron FastCAM SA4 video camera mounted above the observation chamber with dark-field illumination. The field-of-view of the camera was 35 x 35 mm with an image resolution of 1024 x 1024 pixels.

Data analysis: Predatory attacks were analyzed to characterize the temporal sequence up to and through the strike of fish larvae on different copepod developmental stages. We guantified the duration (in sec) of the approach phase of the fish, defined and described in Robinson et al. (accepted) as beginning when the tail stopped beating and started to bend to the left or right, and ending at t0. Thirty-seven successful captures (n = 37) by fish larvae were analyzed using the Fiji software package built on Imagel (v1.51) (Schindelin et al., 2012) to digitize the changing body shape of the fish as it prepared for and initiated the strike. For each interaction, we established a temporal reference (t0) as the image just prior to the opening of the fish's mouth. Twenty-five frames, including 12 frames before and 12 frames after t0, were then extracted and the posture of the fish larva was characterized frame-by-frame. Twelve frames (24 ms) before t0, labeled as t-24, was chosen as a standardized interval that captured the final approach phase of fish in our trials, which ranged from 28 to 1130 ms preceding t0. The median speed during the final frame of approach (t-2) was 0 mm s-1. Therefore, fish were most often motionless at this time interval, meaning that all motion involved in the acceleration of the strike occurred after t0. Twelve frames after t0, labelled as t+24, was chosen as a standardized interval as it always included peak strike speed, copepod capture, and subsequent deceleration of the fish. Starting with t-24 and continuing every other frame up to and including t+24, we digitized the x,y coordinates of 12 points along the central axis of the larva. Because its position with respect to the fish did not change, we used a small pigment spot located at the narrowest point between the eyes as a spatial reference for each frame (origin at x=0, y=0). The position of the copepod in each frame was also digitized to obtain the change in distance over time between fish and copepod.

We quantified the relative curvature of the body and caudal fin during the larva's initial lunge, frame-by-frame from t0 to t+8 (8 ms, i.e., 4 frames after t0). To do so, we used the oval tool in Fiji to place a circle within the curl of the tail. The "fit circle" and "measure" commands were then used to calculate the area (A) of the circle. From the circle's area, we derived the reciprocal radius, $r - 1 = \sqrt{(\pi / A)}$ as a metric of relative curvature of the larva's caudal fin, with greater values being more curved and lesser values being less curved. The measurement of reciprocal radius became less reliable as an estimate of curvature after t+8 because the tails of most fish began to curve in the opposing direction, making the inscribed circle too large to measure. We therefore employed another relative measure of curvature, the straight-line distance between the tip of the tail and the narrowest point between the eyes (chord length), divided by the length of the fish when its body was straight (fish length), also measured between the tip of the tail and origin/pigment. These normalized distances (chord length-to-fish length ratio, or CVF) approached 1 when the fish was straight and were decreasing fractions of 1 when the fish was increasingly bent into a J-like posture. To measure speed of the fish during its predatory lunge (in mm s-1), we tracked the changing position of the pigment between its eyes from t-4 to t+20 (4 ms, i.e., 2 frames before t0 and 20 ms, i.e., 10 frames after t0, respectively) and divided the frame-byframe distance travelled by the time elapsed between frames. In addition, the distance from the spot between the eyes and the tip of the mouth was measured at t0 and t+8 to determine the contribution of jaw extension to prey capture.

BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- re-formatted date from m/d/yyyy to yyyy-mm-dd

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Data Files

File clownfish_posture.csv(Comma Separated Values (.csv), 14.64 KB) MD5:5583afed81257b2baedf3d6cd29031b3

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Primary data file for dataset ID 750328
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Related Publications

Buskey, E. J., Strickler, J. R., Bradley, C. J., Hartline, D. K., & Lenz, P. H. (2017). Escapes in copepods: comparison between myelinate and amyelinate species. The Journal of Experimental Biology, 220(5), 754–758. doi:10.1242/jeb.148304

Methods

Fashingbauer, M. C., Tuttle, L. J., Robinson, H. E., Strickler, J. R., Hartline, D. K., & Lenz, P. H. (2019). Predatory posture and performance in a precocious larval fish targeting evasive copepods. Journal of Experimental Biology. https://doi.org/<u>10.1242/jeb.191411</u> *Results*

Jackson, J. M., & Lenz, P. H. (2016). Predator-prey interactions in the plankton: larval fish feeding on evasive copepods. Scientific Reports, 6(1). doi:<u>10.1038/srep33585</u> *Methods*

McKinnon, A. D., Duggan, S., Nichols, P. D., Rimmer, M. A., Semmens, G., & Robino, B. (2003). The potential of tropical paracalanid copepods as live feeds in aquaculture. Aquaculture, 223(1-4), 89–106. doi:10.1016/s0044-8486(03)00161-3 https://doi.org/10.1016/S0044-8486(03)00161-3 https://doi.org/10.1016/S0044-8486(03)00161-3 https://doi.org/10.1016/S0044-8486(03)00161-3 https://doi.org/10.1016/S0044-8486(03)00161-3

Robinson, H., Strickler, J., Henderson, M., Hartline, D., & Lenz, P. (2019). Predation strategies of larval clownfish capturing evasive copepod prey. Marine Ecology Progress Series, 614, 125–146. doi:<u>10.3354/meps12888</u> *Methods*

, Results

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods, 9(7), 676–682. doi:<u>10.1038/nmeth.2019</u> *Methods*

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Parameters

Parameter	Description	Units
TRIAL_DATE	date the experimental observations took place formatted as yyyy-mm-dd	unitless
BIRTHDATE	birthdate of fish; when the fish hatched formatted as yyyy-mm-dd	unitless
DPH	larval fish age; days post hatch	days post hatch

FISH_AGE_CLASS	larval age-group of Amphiprion ocellaris: early (1-5 dph [days post-hatch]); mid (6-9 dph); or late (11-14 dph)	unitless
PREY_STAGE_CLASS	developmental stage-class of Bestiolina similis: nauplii (NIII-NIV stages); early copepodites (CII-CIII stages; called just "copepodites" in hard-drive folders); late copepodites (CV stage); adults (CVI stage)	unitless
STAGE_CATEGORY	predator-prey age group combination	unitless
CLIP_ID	two-letter identifier in TIF file name - separating clips	unitless
CLIP_START	first frame in clip; numbered with reference to other clips in the same data folder	unitless
CLIP_END	final frame in clip; numbered with reference to other clips in the same data folder	unitless
CLIP_DURATION	length of a clip	# of frames
PIXEL_TO_MM	calibration ratio of the number of pixels per millimeter; unique for each trial date	pixels per millimeter
BODY_LENGTH	body length; measured as total length	milllimeters
FRAME_APPROACH_START	frame number when final approach began (per definition set by Robinson et al. (accepted))	frame #
APPROACH_DURATION	duration of the final approach (per definition set by Robinson et al. (accepted))	seconds
FRAME_T0_STRIKE	frame number at t0; moment when mouth began to open	frame #
STRIKE_DISTANCE_PIXEL	distance at t0 between edge of fish mouth and rostrum of copepod (or center of copepod if rostrum not visible)	pixels
STRIKE_DISTANCE_MM	distance at t0 between edge of fish mouth and rostrum of copepod (or center of copepod if rostrum not visible)	milllimeters
TIME_FROM_T0_TO_CAPTURE	time from t0 until capture (copepod completely in fish mouth)	milliseconds
PEAK_STRIKE_SPEED	maximum speed attained by clownfish from t-2ms to t22ms	millimeters/second
STRIKE_SPEED_T_2ms	speed of fish during attack at time = -2 milliseconds	millimeters/second
STRIKE_SPEED_T1ms	speed of fish during attack at time = 1 millisecond	millimeters/second
STRIKE_SPEED_T3ms	speed of fish during attack at time = 3 milliseconds	millimeters/second
STRIKE_SPEED_T5ms	speed of fish during attack at time = 5 milliseconds	millimeters/second
STRIKE_SPEED_T7ms	speed of fish during attack at time = 7 milliseconds	millimeters/second
STRIKE_SPEED_T9ms	speed of fish during attack at time = 9 milliseconds	millimeters/second
STRIKE_SPEED_T11ms	speed of fish during attack at time = 11 milliseconds	millimeters/second
STRIKE_SPEED_T14ms	speed of fish during attack at time = 14 milliseconds	millimeters/second
STRIKE_SPEED_T18ms	speed of fish during attack at time = 18 milliseconds	millimeters/second
STRIKE_SPEED_T22ms	speed of fish during attack at time = 22 milliseconds	millimeters/second
TIME_TO_PEAK_ACCELATION	time from t0 until peak acceleration is reached	milliseconds
PEAK_ACCELERATION	maximum acceleration from t0 to t20ms	millimeters/second^2
ACCELERATION_T0	acceleration of fish at t0	millimeters/second^2
ACCELERATION_T2ms	acceleration of fish at t2ms	millimeters/second^2
ACCELERATION_T4ms	acceleration of fish at t4ms	millimeters/second^2
ACCELERATION_T6ms	acceleration of fish at t6ms	millimeters/second^2

ACCELERATION_T8ms	acceleration of fish at t8ms	millimeters/second^2
ACCELERATION_T10ms	acceleration of fish at t10ms	millimeters/second^2
ACCELERATION_T1point5ms	acceleration of fish at t12ms	millimeters/second^2
ACCELERATION_T16ms	acceleration of fish at t14ms	millimeters/second^2
ACCELERATION_T20ms	acceleration of fish at t16ms	millimeters/second^2
CVF_T0	chord length-to-fish length ratio at t0	dimentionless
CVF_T2ms	chord length-to-fish length ratio at t2ms	dimentionless
CVF_T4ms	chord length-to-fish length ratio at t4ms	dimentionless
CVF_T6ms	chord length-to-fish length ratio at t6ms	dimentionless
CVF_T8ms	chord length-to-fish length ratio at t8ms	dimentionless
CVF_T10ms	chord length-to-fish length ratio at t10ms	dimentionless
CVF_T12ms	chord length-to-fish length ratio at t12ms	dimentionless
CVF_T14ms	chord length-to-fish length ratio at t14ms	dimentionless
CVF_T16ms	chord length-to-fish length ratio at t16ms	dimentionless
CVF_T18ms	chord length-to-fish length ratio at t18ms	dimentionless
CVF_T20ms	chord length-to-fish length ratio at t20ms	dimentionless
INVERSE_RADIUS_T0	reciprocal radius of inscribed circle in fish's tail-bend; r-1 = $v(p / A)$ at t0	per millimeter
INVERSE_RADIUS_T2ms	reciprocal radius of inscribed circle in fish's tail-bend; r-1 = $v(p / A)$ at t2ms	per millimeter
INVERSE_RADIUS_T4ms	reciprocal radius of inscribed circle in fish's tail-bend; r-1 = $v(p / A)$ at t4ms	per millimeter
INVERSE_RADIUS_T6ms	reciprocal radius of inscribed circle in fish's tail-bend; r-1 = $v(p / A)$ at t6ms	per millimeter
INVERSE_RADIUS_T8ms	reciprocal radius of inscribed circle in fish's tail-bend; r-1 = $v(p / A)$ at t8ms	per millimeter

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Instruments

Dataset- specific Instrument Name	high-speed video camera
Generic Instrument Name	high-speed camera
Dataset- specific Description	A Photron FastCAM SA4 high-speed video camera with a Nikon micro-NIKKOR 60 mm lens and 36 mm extension tube was used to record predator-prey interaction. The experimental chamber was illuminated by a dark field, ring light, Fiber-Lite MI-150 high-intensity illuminator, Dolan-Jenner. The camera was mounted on a manually-operated, linear positioning slide (Automation Gages Inc.)
Generic Instrument Description	A high-speed imaging camera is capable of recording rapid phenomena with high-frame rates. After recording, the images stored on the medium can be played back in slow motion. The functionality in a high-speed imaging device results from the frame rate, or the number of individual stills recorded in the period of one second (fps). Common video cameras will typically record about 24 to 40 fps, yet even low-end high-speed cameras will record 1,000 fps.

Project Information

The Drive to Survive: Copepods vs Ichthyoplankton (PreyEscape)

Coverage: Pacific

Description from NSF award abstract:

This study will experimentally elucidate the dynamics of predator evasion by different species and life stages of copepod responding to a model larval fish predator. The PIs will use standard and high-speed videographic and cutting-edge holographic techniques. Predator-prey interactions within planktonic communities are key to understanding how energy is transferred within complex marine food webs. Of particular interest are those between the highly numerous copepods and one of their more important predators, the ichthyoplankton (the planktonic larval stages of fishes). The larvae of most fishes are planktivorous and heavily dependent on copepods for food. In general, evasion success increases with age in copepods and decreases with the age of the fish predator. How this plays out in detail is critical in determining predatory attack outcomes and the effect these have on predator and prey survival. To address this problem, different copepod developmental stages will be tested against several levels of predator competence, and the results examined for: 1) the success or failure of attacks for different combinations of predator and prey age class; 2) the kinematics (reaction latencies and trajectory orientation) for escape attempts, successful and unsuccessful, for different age classes of copepod; 3) the hydrodynamic cues generated by different ages and attack strategies of the predator and the sensitivity of different prey stages to these cues; and 4) the success or failure of the predatory approach and attack strategies at each prey stage. The data obtained will be used to inform key issues of zooplankton population dynamics. For the prey these include: predator-evasion capabilities and importance of detection ability, reaction speed, escape speed, escape orientation, and trajectory irregularity; for the predator they are: capabilities and importance of mouth gape size, stealthiness, hydrodynamic disturbance production, and lunge kinematics.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1235549</u>

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