High-speed videos of larval clownfish, Amphiprion ocellaris, predators and copepod prey

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Data Type: experimental
Version: 2
Version Date: 2018-11-26

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

Contributors	Affiliation	Role
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Abstract
High-speed videos of larval clownfish, Amphiprion ocellaris, predators and copepod prey.

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Coverage

Spatial Extent: **Lat**:1.298 **Lon**:-157.8187
Temporal Extent: 2015-06-28 - 2015-08-03

Dataset Description

High-speed videos of larval fish predators and copepod prey will be made available from this dataset landing page once the videos are transferred to BCO-DMO (~1 TB). Please contact us at info@bco-dmo.org for access.

This dataset includes a summary of the high-speed video clips of clownfish larvae predators and copepod prey with dates, fish age, copepod stage, clip ids, clip start and end frames, and calibration values, and links to video clips. URL's to the compressed files are provided.

These data are published in the following papers. See the publications section, below, for full citations:
Fashingbauer et al (2019)
Robinson et al (2019)
Tuttle et al (2018)

Please contact the PI for further details and questions.

Methods & Sampling

METHODS, EXCERPTED FROM ROBINSON ET AL (2019):

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).

Larval fish rearing protocol:
All experiments were performed on A. ocellaris fish larvae from two consecutive broods from one breeding pair, hatched in June and July, 2015. Late-stage eggs of A. ocellaris attached to the inside of a clay flower pot were obtained from a local fish breeder (K. Brittain) the afternoon prior to hatching and transported in an aerated container to the laboratory. Fish larvae hatched the same evening, within two hours of the transfer to the laboratory. The clay pot and any unhatched eggs were removed the next morning within 12 hours of hatching. Up to 200 fish were transferred to a rectangular aquarium (51 x 28 x 33 cm, length x width x height) containing 30 L of seawater, which was gently aerated and on a 12:12 h light:dark cycle from two 20W fluorescent bulbs located above the tank. Water temperature was kept between 24–26° C. Daily maintenance included siphoning detritus and dead fish from the bottom of the tank each morning and exchanging 10% of the water in the tank with UV sterilized seawater. Ammonia and nitrates/nitrites were monitored three times per week using a saltwater test kit (Mardel) and remained at 0–5 ppm throughout the experiments.

Cultivation of copepods and rotifers:
All larval fish were fed live prey, which was added to the rearing tank twice daily. Larval fish were fed two prey species: rotifers (Brachionus plicatilis), and mixed developmental stages of a calanoid copepod, Parvocalanus crassirostris. A different copepod species was used for daily feeding (P. crassirostris) than for the experiments (Bestiolina similis) to provide novel prey during experiments. Both calanoid species were originally isolated from Kaneohe Bay, Hawaii, Oahu, and have been in continuous culture since 2008 (VanderLugt & Lenz 2008, VanderLugt et al. 2009, Jackson 2011). Rotifers (B. plicatilis) were obtained from stock cultures maintained by K. Brittain (Kaneohe, HI). Copepods and rotifers were cultured in 21- L containers under similar temperature, salinity, and light:dark conditions as the fish. Copepod and rotifer cultures were fed every other day by adding 100 to 300 mL of mature cultures of Tisochrysis lutea (106–108 cells per mL) (formerly known as Isochrysis galbana Tahitian strain). Nitex™ sieves were used to isolate B. plicatilis (>20 µm), P. crassirostris nauplii (<80 µm), copepodites (80 µm < x <123 µm), and adults (>123 µm) from cultures to feed the larval fish as needed. The proportion of each prey species, and stage, provided to the fish was adjusted with fish age to accommodate their growing nutritional needs as follows: 300 prey per day at 1 day post-hatch (dph) and up to 1,000 prey per day at 14 dph. Fish consumption rates were monitored twice per day by counting the prey in a 25 mL water sample from the rearing tank. Prey were then added to maintain a target concentration, and were delivered to the rearing tank through an array of four feeding tubes that reduced agitation of the water.

To produce copepods of a specific developmental stage for behavioral experiments, cohorts of B. similis were raised from eggs. Approximately two thousand adults were sieved from the stock culture and transferred into 2 L of aerated, UV-sterilized seawater in a container with 40 mL of mature T. lutea stock culture. After 4 hours, the contents of the container were sieved (123 µm) to remove the adults, and eggs were allowed to hatch and cohorts were cultured as described above. These cohorts were harvested according to the copepod developmental stage needed for an experiment: nauplii at 24 hours (NIII–NIV); early copepodites at 120 hours (CII–CIII); and late copepodites after 165 hours (mostly CV). Adult copepods (CVI) were sieved from stock cultures using a 156 µm mesh to remove earlier developmental stages. Prior to the introduction of copepods to the observation chamber, harvested copepods were checked and photographed under the microscope to confirm developmental stages.

Behavioral observations:
Video set-up:
A system for tracking freely-swimming fish was used to obtain high-speed, high-resolution recordings (500 frames per second – fps; 1024 x 1024 pixels) of both larval fish (3–8 mm length) and individual copepods (100–500 µm length). The observation chamber consisted of an acrylic ring (20 cm inner diameter, 2.5 cm height) sealed onto a glass plate with aquarium cement. The glass plate was held in place by a metal frame supported by four posts fastened to an optical table. A high-speed camera (Photron FastCAM SA4) was mounted on a vertical optical rail to view organisms from above. The high-speed camera was fitted with a Nikon micro-NIKKOR 60 mm lens and a 36 mm extension tube, providing a field of view of 35 x 35 mm. A video monitor connected to the camera provided feedback to allow manual tracking of individual fish in the aquarium and focus adjustment. The experimental chamber was illuminated by a dark field, ring light (Fiber-Lite MI-150 high-intensity illuminator, Dolan-Jenner) positioned below the aquarium. Thus, the relative position of camera and light was fixed, and they moved as a single unit. This unit (vertical rail, camera, and light) was

mounted on a manually-operated, linear positioning slide (Automation Gages Inc.) affixed to the optical table. The same aquarium background paper surrounding the rearing tank was used around the experimental chamber.

Experimental protocol:
Two types of predator-prey experiments were conducted during the planktonic phase of the fish larvae, which lasts for approximately 14 days with first feeding starting at 1 dph (Wittenrich & Turingan 2011). A preliminary feeding experiment was conducted to determine at what age fish attempted to capture adult B. similis copepods. A previous study by Jackson & Lenz (2016) using a similar copepod species (P. crassirostris) found that A. ocellaris did not capture adults in mixed copepod assemblages until 8 dph. To determine this milestone for B. similis, feeding experiments were conducted with fish between the ages of 1 and 14 dph. One hundred adult copepods were isolated under the microscope with a pipette and transferred into 100 mL of UV-sterilized seawater in a cylindrical, glass dish (6.5 cm inner diameter). Then two fish that had been removed from the rearing tank and left without food for 4–6 hours were transferred into the glass dish and allowed to forage for 1 hour. After removal of the fish, the remaining copepods were counted. All feeding experiments were conducted at 25 °C under 8 W m-2 illumination with experimental containers surrounded by background paper that was also used around the rearing aquarium.

During behavioral experiments, four different developmental stages of B. similis were used: NIII–IV (nauplii), CII–CIII (early copepodites), CV (late copepodites), and CVI (adults). Nauplii were tested with fish ages 1 to 9 dph, early copepodites with fish ages 1 to 12 dph, and adult copepods with fish ages 6 to 14 dph (Fig. 2). Late-stage copepodites were tested with fish larvae ages 3, 6 and 9 dph. No observations were made at 10 dph. On each day of the behavioral experiments, 12–16 fish were removed from the rearing tank before morning feeding, transferred into a 7- L clear plastic container with UV-sterilized seawater without food for 4–6 hours. For each trial, the experimental chamber was filled with 700 mL of UV-sterilized seawater (2.2 cm depth) and 0.2–0.7 copepods mL-1 of a single developmental stage class of B. similis: nauplii (ca. 500), early copepodites (ca. 250), late copepodites (ca. 250), or adult copepods (ca. 100). Subsequent mention of copepodites will refer to early- stage copepodites; late copepodites will be specified. Two of the isolated clownfish were placed into the chamber and allowed to acclimate and feed for 10 minutes. During the subsequent observation period, the camera was manually positioned to keep the tracked fish in the field of view and focused. When a fish-copepod interaction was observed, the high-speed camera was triggered and recorded video was saved onto a computer as a sequence of digital images. Both successful and unsuccessful fish strikes were recorded. An experimental trial was terminated after six interactions were recorded, or after one hour. Experimental trials were run between 1200 and 1900 h.

Data Processing Description

Video analysis: Digital image sequences (TIFF format) of predator-prey interactions were viewed in Fiji (ImageJ) (Schneider et al. 2012). Kinematics of fish and copepod behavior were quantified using the software packages Fiji and Tracker (version 4.9.1, Open Source Physics, Douglas Brown). Calibration was set using an image of a ruler in the field of view.

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
- groups of video .tif files were compressed for serving; grouped by date and developmental stage
- replaced data version 1 (2018-11-26) with version 2 (2019-01-31) which contains the paths to video clips [2019-10-24]

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Data Files

File	
video_clips_links.csv	(Comma Separated Values (.csv), 119.28 KB) MD5:f3d95c299f4bdfdd2f0f7a142fcac412
Primary data file for dataset ID 747926	

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Supplemental Files

File
Sample image of fish predator about to capture copepod. filename: RA002299.tif (Octet Stream, 1.00 MB) MD5:e94432d0369b343077feb1775bed93cd This .TIF file is an example of one of the million-plus high-resolution images of larval clownfish predators and copepod prey resulting from this project.

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Related Publications

Jackson, J. M. (2011). Larval clownfish Amphiprion ocellaris predatory success and selectivity when preying on the calanoid copepod Parvocalanus crassirostris (Doctoral dissertation, [Honolulu]:[University of Hawaii at Manoa],[August 2011]). https://hdl.handle.net/10125/101569
Methods

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

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:10.3354/meps12888
Methods

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089
Software

Tuttle, L. J., Robinson, H. E., Takagi, D., Strickler, J. R., Lenz, P. H., & Hartline, D. K. (2019). Going with the flow: hydrodynamic cues trigger directed escapes from a stalking predator. Journal of The Royal Society Interface, 16(151), 20180776. doi:10.1098/rsif.2018.0776
Results

VanderLugt, K., & Lenz, P. H. (2008). Management of nauplius production in the paracalanid, Bestiolina similis (Crustacea: Copepoda): Effects of stocking densities and culture dilution. Aquaculture, 276(1-4), 69–77. doi:10.1016/j.aquaculture.2008.01.041
Methods

VanderLugt, K., Cooney, M. J., Lechner, A., & Lenz, P. H. (2009). Cultivation of the Paracalanid Copepod,Bestiolina similis(Calanoida: Crustacea). Journal of the World Aquaculture Society, 40(5), 616–628. doi:10.1111/j.1749-7345.2009.00282.x
Methods

Wittenrich, M. L., & Turingan, R. G. (2011). Linking functional morphology and feeding performance in larvae of two coral-reef fishes. Environmental Biology of Fishes, 92(3), 295–312. doi:10.1007/s10641-011-9840-0
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
FISH_AGE_CLASS	larval age-group of Amphiprion ocellaris: early (1-5 dph); 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
PAIR_ID	numerical identifier; unique to an experimental fish pair	unitless
CLIP_ID	two-letter identifier in TIF file name; separating clips	unitless
NEW_CLIP_ID	three-letter identifier; unique to a clip (referenced in outside analyses)	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	number of frames
PIXEL_TO_MM	calibration ratio of the number of pixels per millimeter; unique for each trial date	pixels per millimeter
NOTES	additional description of clip ("na" if no additional comment made)	unitless
cal_file	calibration file name	unitless
filename	video clip file name	unitless
clip_link	full path to access the video clip	unitless

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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.

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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)	OCE-1235549

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