Twilight transitions in coral reef fish: the input of light-induced changes in foraging behaviour

SVETLANA RICKEL & AMATZIA GENIN
Department of Evolution, Systematics and Ecology, The Hebrew University of Jerusalem and
The Interuniversity Institute for Marine Sciences of Eilat

(Received 8 February 2004; initial acceptance 6 March 2004; final acceptance 13 October 2004; published online 10 May 2005; MS. number: 7998R)

The timing of twilight transition behaviour of diurnal reef fish is likely to be determined by the trade-off between food intake and risk of predation. However, the correspondence between fish behaviour and light-dependent feeding is poorly documented. We used flume measurements of prey detection and consumption by small reef-dwelling zooplanktivorous fish to examine the effects of light and prey flux on the fish’s in situ behaviour during twilight. Feeding rates increased from nearly nil to saturation as light level increased, corresponding to about 45 min of the morning twilight. Changes in feeding rates were mostly due to light-dependent changes in reactive distance. In the reef, fish emerged from their nocturnal shelter at lower light intensities than those at which they retreated at dusk. These light intensities at which the fish emerged and retreated in the field greatly exceeded the level required to detect prey. We examined whether the approach of measuring relative ratio of mortality risk to food gained can be applied to predict the observed patterns in the twilight transition behaviour of the fish studied. We suggest that both the times of emergence and retreat and the dawn–dusk asymmetry are the result of a direct response to temporal and spatial variations in predation risk; no evidence for fine-tuned adjustments to diel fluctuations in food availability by changes in the time of emergence and retreat was found.

© 2005 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Diurnal reef-dwelling fish have a well-defined sequence of behaviours that coincide with the twilight periods: emergence from nocturnal shelters at dawn, active feeding during the day, and retreat to shelter at dusk (Collette & Talbot 1972; Hobson 1972; McFarland et al. 1979). This pattern is highly predictable, occurring at distinct, species-specific light intensities (Hobson 1972; McFarland et al. 1979). That similar twilight behaviour is found among taxonomically remote fish in different habitat types suggests that the selective pressure imposed by twilight conditions is general (Helfman 1986).

Twilight is accompanied by very rapid changes in light intensity, spectral and polarization properties. During dusk, and vice versa during dawn, changes in light intensity reach about 50% per min, the spectral distribution shifts to shorter wavelengths, and polarization increases towards sunset (McFarland & Munz 1975). Such extreme changes are disadvantageous for diurnal fish, because they hinder prey detectability (Vinyard & O’Brien 1976; Confer et al. 1978; O’Brien 1987; Nicieza & Metcalfe 1997) and capture efficiency (Fiksen et al. 1998), reduce encounter rate and search volume (Link & Edsall 1996) and enhance escape probabilities for zooplankton prey (Giske et al. 1994). The decrease in light intensity during dusk also increases the fish’s own risk of predation, because the ability to detect predators is reduced (McFarland & Wahl 1996; but see De Robertis et al. 2003), whereas the activity of piscivorous predators increases (Hobson 1972; Helfman 1986; Danilowicz & Sale 1999; Holbrook & Schmitt 2002) and the predators’ vision improves (Munz & McFarland 1973).

Notably, most studies referring to the evolution of twilight behaviour consider the risk of predation as the main driving force (Hobson 1972; Helfman 1986; McFarland et al. 1999 and references therein). However, diel activity patterns might have evolved as a result of a complex trade-off between foraging and safety, which takes account of diel fluctuations in food availability, food capture efficiency and predation risk (Metcalfe et al. 1999).

If diurnal reef-dwelling fish experience predictable diel fluctuations in food availability and/or predation risk, an optimal feeding pattern is expected. The emergence from a refuge to a risky feeding ground should occur when the
ratio of mortality risk to food gained is minimized (Gilliam & Fraser 1987; Metcalfe et al. 1999), not necessarily when the feeding rate is maximized (Clark & Levy 1988).

Most of the diurnal fish in coral reefs are site-attached zooplanktivores, foraging for drifting prey while retaining their location near fixed shelters such as coral heads and perforated knolls. A feeding event by these fish consists of two steps: a quasistationary search, with the fish facing into the current waiting for prey to drift into its reactive volume, and a strike, including a body turn and rapid swim towards the intercepting point, followed by ram-jaw suction of the prey (Coughlin & Strickler 1990). Food availability for these fish is delimited by prey flux, the product of prey density and current speed (Kilfawi & Genin 1997). Food intake is thus dependent on both prey flux and the visual detection of a single prey, usually smaller than 1 mm in length (Hobson & Chess 1978; Hamner et al. 1988), which is expected to be light dependent (O’Brien 1987).

We combined in situ observations and controlled laboratory experiments to characterize the twilight foraging behaviour of three different zooplanktivorous coral reef fish and to examine the effect of light and prey flux on their emergence from and retreat to shelter.

**METHODS**

**Study Sites**

We carried out the field study at the coral reef in front of the H. Steinizt Marine Biology Laboratory (MBL) of Eilat, and at the Underwater Observatory Marine Park (hereafter ‘Observatory’), Gulf of Aqaba, Red Sea (29°30’ N, 34°56’ E). A detailed description of the local reef community is found in Fishelson (1971) and Benyahu & Loya (1977); the local fish community was thoroughly studied by Brokovich (2001) and Khalaf & Kochzius (2002). Briefly, the local fringing reef is dominated by stony corals, living on a steep slope (10–30°) extending from the subtidal zone to more than 50 m depth. Among the ca. 260 species of fish inhabiting the local reefs the guild of zooplanktivorous fish is numerically the largest, comprising 42% of the total fish species counted by Brokovich (2001).

The Observatory is located near the southern edge of the Coral Beach Reserve of Eilat, about 400 m north of the MBL. The in situ section of the Observatory consists of a large underwater tower (hereafter, ‘underwater tower’) surrounded by a lusher reef community growing on a large circular platform which is attached to the tower at 6 m, about 5 m above the bottom. The tower is about 20 m seawards off the reef flat, reached from the shore via a bridge 100 m long. Large viewing windows allow an excellent view of the reef fish and other marine organisms in their natural habitat. The shore section of the Observatory includes several outdoor pools, inhabited by rich reef assembles, which we used for light-manipulation experiments (see below).

We observed twilight transitions of the fish at four sites: the reef at MBL (two sites), the Observatory underwater tower, and in two large mesocosms at the Observatory. At MBL, the observations were made over two natural reef knolls at 4 m depth and a soft coral reef at 10 m depth, dominated by Litophyton spp. but including also numerous live branching and soft corals.

**Study Fish**

Our study focused on two pomacentrids, the black-bordered dascyllus, Dascyllus marginatus Rüppell 1828, and Myr’s damselfish, Neopomacentrus miryae Dor & Allen 1977, and a serranid, the scalefin anthias, Pseudanthias squamipinnis Petters 1855. Pseudanthias squamipinnis is common throughout the Indo-Pacific Ocean, D. marginatus is endemic to the Red Sea, Gulf of Aden and Gulf of Oman, and N. miryae is endemic to the Red Sea north of Jeddah (Allen & Randall 1980). In the Northern Gulf of Aqaba, these three species are among the 10 most abundant (Rilov & Benyahu 2000; Brokovich 2001; Khalaf & Kochzius 2002); their biosociology, behaviour and ecology were described by Fishelson (1964), Popper & Fishelson (1973) and Fishelson et al. (1974). The fish are small (6, 11 and 15 cm in maximum total length, respectively, for D. marginatus, N. miryae and males of P. squamipinnis; Randall 1983), site-attached zooplanktivores, common at a depth of 2–25 m. The fish feed on zooplankton during the day and shelter during the night. The shelters of D. marginatus are live branching corals, mostly Stylophora spp. and Acropora spp., whereas N. miryae and P. squamipinnis seek nocturnal shelters in crevices and caves.

**In Situ Fish Behaviour**

Our observations consisted of four parts. First, we examined the effect of light intensity, current speed and zooplankton density on the timing of fish emergence and retreat throughout a year. Second, we tested interspecies differences in the times of emergence from, and retreat to, the reef, considering the behaviour of both whole and parts of fish groups as well as the extreme individuals: the first to emerge and the last to retreat. Third, we tested whether the fish’s transition behaviour was directly determined by light intensity, rather than by an internal biological clock. Finally, we compared the timing of twilight transitions between different habitats and depths. Table 1 summarizes all our observations, their settings and the fish species observed.

**Timing of emergence and retreat**

We observed the twilight transition behaviour of D. marginatus in situ, with an underwater, custom-made, video system. A high-resolution, black-and-white video camera contained in a watertight housing was deployed at MBL at 10 m depth and was connected with an underwater cable to a VCR (Panasonic AG-8700) at the shore laboratory. The camera was oriented to record a school of roughly 30 D. marginatus inhabiting a few colonies of the branching coral Stylophora pistillata at the soft coral reef.
A summary of the behavioural observations conducted, indicating the location (MBL = H. Steinitz Marine Biology Laboratory; LH = Light House beach, about 300 m south of the MBL), depth, period and the number of observations.

<table>
<thead>
<tr>
<th>Observation period</th>
<th>Habitat</th>
<th>Location</th>
<th>Depth (m)</th>
<th>Period (month)</th>
<th>Species observed</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Soft coral reef</td>
<td>MBL</td>
<td>10</td>
<td>February–December</td>
<td><em>D. marginatus</em></td>
<td>19</td>
</tr>
<tr>
<td>Second</td>
<td>Underwater tower</td>
<td>Observatory</td>
<td>5</td>
<td>July</td>
<td><em>N. miryae, P. squamipinnis, D. marginatus</em></td>
<td>8</td>
</tr>
<tr>
<td>Third</td>
<td>Mesocosm I</td>
<td>Observatory</td>
<td>0.75</td>
<td>June–July</td>
<td><em>N. miryae, P. squamipinnis</em></td>
<td>3 (3)</td>
</tr>
<tr>
<td></td>
<td>Mesocosm II</td>
<td></td>
<td></td>
<td></td>
<td><em>N. miryae, P. squamipinnis</em></td>
<td>6</td>
</tr>
<tr>
<td>Fourth</td>
<td>Reef knoll I</td>
<td>LH</td>
<td>4</td>
<td>July–August</td>
<td><em>P. squamipinnis, D. marginatus</em></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Reef knoll II</td>
<td>MBL</td>
<td>4</td>
<td>April</td>
<td><em>P. squamipinnis, D. marginatus</em></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Soft coral reef</td>
<td>MBL</td>
<td>10</td>
<td>March–April</td>
<td><em>N. miryae, P. squamipinnis</em></td>
<td>8</td>
</tr>
</tbody>
</table>

Numbers in parentheses in the last two columns indicate the additional observations carried out under artificial illumination (see text).

(Table 1). A total of 19 dawn and 20 dusk recording sessions, each approximately 30 min long, were made between February and December 2002. We analysed the video recordings daily to obtain the time of emergence of the first fish at dawn and the time of retreat of the last individual at dusk. The video recordings were supplemented with concurrent measurements of light intensity, made with a HOBO–Light Intensity Logger (Onset Computer Corp., Pocasset, Massachusetts, U.S.A.), and current velocity, made with an Acoustic Doppler Current Profiler (ADCP; 600 KHz Workhorse, RD Instruments, San Diego, CA, U.S.A.), and current profile that the ADCP provided, we used only the data from 10 m depth, corresponding to the depth of the recorded fish. The ADCP was also used to obtain the backscatter intensity, which we used as an arbitrary index of zooplankton abundance as in Flagg & Smith (1989). Hence, the ADCP provided information on current speed and relative prey density, and thus on prey flux. For logistical reasons, ADCP data were available for only 14 of 39 daily observations on the fish’s twilight behaviour.

Interspecies differences

The observations were made during consecutive dusks (N = 9) and (N = 8) dawns on about 100 *P. squamipinnis*, 300 *N. miryae* and 12 *D. marginatus* inhabiting the Observatory underwater tower, about 3 m in front of the viewing windows (Table 1). We visually estimated the proportion of the group that was actively foraging or swimming for each species every 1 min. The observations started before dawn and lasted after dusk to cover the entire behavioural cycle between complete retreat at darkness to full foraging activity at daylight. Light intensity was measured with the aforementioned HOBO–Light Intensity Logger set to record downwelling light once a minute. The light meter was deployed at 6 m depth from a fixed position on the observatory bridge near the tower, tied to a cable about 10 m long. Each deployment started before our dusk observation and terminated after the completion of the following observations at dawn. The light meter was about 20 m from the observed fish. Simultaneous measurements 72 h long made with two identical HOBO light meters, one positioned at the site of the foraging fish and the other at the usual site next to the bridge, showed no significant difference in light intensity between the two locations (paired t test for dependent samples: t₀₅ = −1.519, P = 0.133).

Effect of light intensity

We tested the hypothesis that the fish’s transition behaviour is directly governed by light intensity, rather than an internal biological clock, by manipulating light intensity during twilight in an outdoor mesocosm reef at the Observatory (Table 1). The mesocosm reef was a large (280 × 120 × 80 cm) outdoor pool with glass walls containing a rich assembly of stony corals and several reef fish, including about 25 *P. squamipinnis* and 10 *N. miryae*. Piscivorous fish were absent except for a single moray eel, *Gymnothorax javanicus* Bleeker 1859. The mesocosm was continuously flushed with fresh sea water at a rate of about 60 m³/h supplying natural planktonic food for the fish. The experiment started with consecutive dawn and dusk records during 3 days under a natural light regime, followed by two sets of three observations (separated by ≥ 3 days) under conditions of artificially elongated evening or morning twilight. The observations consisted of visual recordings of the proportion of the groups foraging at 1-min intervals. Fish behaviour was recorded by an observer standing about 2 m away from the glass wall. Preliminary observations indicated that the presence of the observer had no effect on behaviour. Light intensity inside the mesocosm was concurrently measured once a minute with the aforementioned HOBO light meter. The light meter was placed facing upwards on a thin mooring line in the middle of the mesocosm.
The artificial light was generated with two metal halide lamps (MVR400 multivapor, light temperature 3700 °K, General Electric Lighting, Palm Desert, CA, U.S.A.), retaining a light level of approximately 1 ± 0.225 lx in the mesocosm. The lamps were turned on from 30 min before until 1 h after sunset and from 1 h before until 30 min after sunrise. At dawn, the two lamps were set to reach maximum light strength gradually in about 20 min, in part to imitate the increase in light intensity during dawn. As each lamp reached full emission in 5–7 min, the second lamp was turned on 10 min after the first one. Under natural conditions, the same increase in light intensity took about 25–30 min. We recorded the behaviour of different groups of *P. squamipinnis* and *N. miryae* in a nearby nonmanipulated pool (Mesocosm II in Table 1) in exactly the same way on the same days as a control for normal fish behaviour.

**Effect of habitat and depth**

We observed groups of a few tens of *P. squamipinnis* sheltering in crevices in two shallow reef knolls (about 4 m deep) and a few individuals of *D. marginatus* inhabiting nearby live heads of *S. pistillata*, while snorkelling above the reef during dawn and dusk. Reef knoll I (Table 1) was located about 300 m south of the MBL reef at Light House beach; reef knoll II (Table 1) was a few metres off the soft coral reef, described above. Consistent with our other observations, an observer floating motionless on the sea surface recorded the time of emergence of the first individual at dawn (or retreat of the last individual at dusk). No interference by the floating observer was apparent. Light intensity was concurrently measured with a moored HOBO light meter as described above. To observe whether twilight transitions at the shallow reef knoll and at the deep soft coral reef located at the same site occur at the same time and/or under the same light levels, we deployed a video camera at 10 m, as described above. The camera was oriented on the foraging space of a group consisting of hundreds of *N. miryae* and *P. squamipinnis*. Thereby, we were able to use simultaneous records of fish at depths of 4 and 10 m to examine a possible effect of depth and at two sites at 4 m to examine an effect of site on the timing of fish emergence and retreat.

**Flume Experiments**

**Experimental set-up**

We carried out a series of controlled flume experiments to quantify the effect of light intensity on fish feeding rates and on prey detection. The design of the recirculating flume and the experimental procedure followed those described by Kiflawi & Genin (1997). Briefly, the flume was 330 litres in volume, 200 cm long and 30 × 30 cm in cross-section. Two plastic-coated fencing screens (1 × 1 cm mesh size) placed at the downstream end of the flume delimited a 30-cm-long experimental arena where an individual fish was held. A marine propeller regulated by an electric speed controller generated water movement within the flume. A frame consisting of 12 fluorescent lamps (36 W, 115 cm long) hung from the ceiling above the flume. We changed light intensity by changing the height of the lamps above the flume (20–85 cm) and by changing the number of lamps turned on (1–12). Light intensity in the water was measured with the aforementioned HOBO–Light Intensity Logger.

**Experimental subject and prey**

For logistical reasons we carried out the flume experiments only with *N. miryae*. This species was selected for the clear visualization of its feeding strikes, involving pronounced ram-jaw suction. The fish (40–55 mm SL) were collected, with hand nets, at the reef (about 8 m depth) and transferred to individual holding tanks (8 litres) for 1–2 weeks acclimation before introduction into the flume. A bare skeleton of small branching coral was placed in the tank to provide shelter for the fish. Each fish was allowed 5–7 days acclimation in the flume before the experiments. During the acclimation, the fish were held in a 12:12 h light: dark cycle and fed ad libitum with nauplii of *Artemia salina* (24–36 h old; $\overline{X} \pm SD = 0.60 \pm 0.07$ mm, $N = 50$). Nauplii are comparable in size and general shape to the fish’s natural food (cyclopoid and calanoid copepods; Fishelson et al. 1974). Flow speed and light intensity were changed several times a day to prevent the fish from becoming used to feed at one particular illumination or flow speed. All fish were released into the sea after the experiment.

**Effect of light on feeding rate**

We measured feeding rates at nine light intensities: 515, 255, 125, 62.5, 43, 21.5, 10.75, 0.55, and 0.25 lx. These intensities corresponded with those prevailing during the morning hours (from 15 min before sunrise to 3 h later) at the depth (about 10 m) from which we collected the fish for the flume experiment. The fish were allowed to feed for 1 min under each light intensity and at a constant prey flux of 18.9 prey/m² per s, combined by either low flow speed–high prey density (3 cm/s × 630 prey/m³) or by high flow speed–low prey density (9 cm/s × 210 prey/m³). Each combination of light intensity–prey flux was replicated four times with five *N. miryae*. Feeding rates of the first three individuals were measured at six of the nine light levels with the three additional intensities (43, 21.5 and 10.75 lx) added later.

We obtained the exact number of prey by collecting individual live nauplii under a dissecting microscope into a plastic syringe. Before an experimental trial, the prey were gradually introduced into the flume while the flow was running at 20 cm/s and the fish chased into shelter (to prevent their feeding while prey were being released into the flume). The time of prey introduction matched the time for one complete revolution of the recirculating water. The fish were never observed to feed while prey were released. The trial started at the second the fish exited the shelter and commenced feeding. A trial lasted exactly 1 min, after which we recovered the remaining prey by filtering the water through a 100-μm plankton net placed inside the flume, upstream of the fish. The net was mounted on an aluminium frame tightly fitted in the flume’s cross-section. The number of prey recovered was...
subtracted from that introduced to give the total number of prey eaten. Twenty-eight control runs with no fish in the flume revealed a mean ± SD efficiency of 98 ± 2% in terms of recapture success.

**Effect of light on reactive distance and volume**

We recorded feeding motions of five *N. miryae* (two of them also used in the previous experiment) for up to 10 min as they fed under combinations of two levels of flow (3 and 9 cm/s) and seven levels of light (515, 255, 125, 62.5, 43, 21.5 and 10.75 lx). As our video camera did not allow qualitative video recordings under 0.55 and 0.25 lx, we excluded these levels from this experiment. Prey density in the flume was kept low (<200 prey/m³) to extend the search interval between consecutive strikes. Two high-resolution, ultra-compact video cameras (chassis type) were used to record the fish motions in 3D. The cameras were positioned perpendicular to each other and provided information on fish movements across the X–Y (side view) and X–Z (top view) planes. Video Splitter (Color Quad Processor, M.J. Electronics, Mt Vernon, NY, U.S.A.) was used to record simultaneously the images from the two cameras on a single tape. Video recordings were made with a VCR (Sony, 8 mm digital high-resolution) connected to a 30-cm (12-inch) monitor (Sony Trinitron). The video records were processed with image analysis software (Image-Pro Plus version 4 for Windows version 4.0; Media Cybernetics, Silver spring, MD, U.S.A.). Three intervals, each, 120 s long, were randomly selected from the total 10-min record of each fish for each light–flow combination. We measured all strikes during each interval by digitizing the 3D coordinates of the position of the fish at the initiation of a strike (defined by a clear motion of the eye) and its position at prey suction (clear protrusion of jaws). We used the coordinates to calculate the reactive distance, defined as the distance between the fish and prey at the time of strike initiation, and the critical strike angles, defined as the maximum strike angle beyond which no strikes in the upstream direction were expected (Kiflawi & Genin 1997). These parameters were used to calculate the reactive volume:

\[
V_r = 2/3 \pi L_r^3 (1 - \cos \theta_c)
\]

where \(V_r\) is a conical space around the fish within which encountered prey elicit a strike, \(L_r\) is the reactive distance and \(\theta_c\) is the critical strike angle. We analysed 7–10 strikes for each fish at each combination of light and flow.

Since *Artemia* nauplii were too small to be resolved with our video system, we used an indirect method to measure the fish’s reactive distance. This method was based on measuring the distance between the positions of the fish at the initiation and completion of a strike plus the distance the prey was swept with the flow during the respective strike time.

**Spectral Characteristics of the Artificial Light**

To measure the spectral properties of the artificial light, we used an Ocean Optics USB2000 spectrometer (Ocean optics, Dunedin, FL, U.S.A.), attached to a UV-Vis transmitting optic fibre 600 μm in diameter and 10 m long, calibrated for both spectra and intensity and equipped with a submersible measuring head that allowed underwater measurements. The measurements were both electrically dark corrected and corrected for the measurements taken in total darkness. Light provided by the two metal halide lamps was very similar in colour and spectrum to the natural twilight, with only a slightly stronger emission in green and orange-red (Fig. 1). The fluorescent lamps emitted light reduced in the short-wavelengths range, mainly in UV (Fig. 1).

**Statistical Analysis**

We used repeated measures ANOVA to test the effects of light and prey flux on feeding rate, following a test for verification that the sphericity assumption was not violated (Rao 1998). Feeding rates obtained at light intensities of 10.75, 21.5 and 43 lx were not included in the analysis because they were replicated with only two of the five fish. The number of strikes analysed for each fish was not the same in the different light intensities, resulting in an unbalanced data set. Therefore, we analysed reactive distances and critical strike angles with ANOVA models on the mean values scored by each fish. All statistical analyses were carried out with Statistica (version 6.0 for Windows, StatSoft, Tulsa, OK, U.S.A.).

**RESULTS**

**In Situ Fish Behaviour**

At 10 m depth at MBL, the time the first *D. marginatus* emerged from shelter at dawn and the time the last individual retreated to shelter at dusk were highly synchronized with sunrise and sunset (Fig. 2a). Emergence occurred around sunrise (± 4 min), while the time of retreat always occurred 2–10 min before sunset. That is, emergence at dawn occurred under significantly lower light intensity than the retreat at dusk (t test: \(t_{17} = -7.046, P < 0.001\); Fig. 2b). Neither current speed nor relative zooplankton abundance had a significant effect on the time of emergence or retreat (Table 2; Fig. 2c, d).

At 6 m depth, at the reef outside the underwater observatory, the emergence of the first individuals from shelter began a mean ± SD of 12 ± 1 min (N = 8) before sunrise, at a light intensity of about 0.24 lx. The time of retreat relative to sunset was more variable, but complete sheltering typically occurred 4–5 min after sunset at a light level of about 1.0 lx (Fig. 3). No interspecific difference was found in the time of emergence and retreat (ANOVA: effect of fish species: \(F_{1,2} = 0.002, P = 0.998\)). The mean length of time ± SD it took groups of *D. marginatus*, *N. miryae* and *P. squamipinnis* to emerge at dawn, from the time the first fish had emerged until the entire group was foraging outside the shelter, was 10 ± 2, 7 ± 3 and 8 ± 4 min (N = 8), respectively, while the corresponding
retreat at dusk took 3 ± 4, 4 ± 2 and 5 ± 4 min, (N = 9), respectively (Fig. 3).

In the Observatory mesocosm (0.75 m deep), under natural light conditions, *N. miryae* and *P. squamipinnis* displayed transition behaviour similar to that in situ but with earlier emergence and later retreat. At dawn, the first individual emerged from shelter a mean ± SD of 28 ± 0 min, (N = 3) before sunrise (0.013 ± 0.0007 lx), with the emergence of the whole group completed 8 min later. Retreat at dusk began a mean ± SD of 17 ± 1 min, (N = 3) after sunset and was completed within 5 ± 1.5 min (0.97 ± 0.025 lx), with no interspecific difference.

Under artificial extension of light conditions after dusk, the fish in the mesocosm kept foraging outside as long as the light remained on, retreating to their shelter immediately after the light was turned off (Fig. 4). At dawn, however, when the artificial light was turned on an hour before sunrise, only two individuals emerged earlier (by about 10 min) than ‘normal’ (Fig. 4), and even these fish appeared confused, repetitively re-entering and re-emerging from their shelter for about 30 min. The rest of the group (23 fish) emerged about 20 min later than normal and their emergence took 15 min longer than normal to complete (Fig. 4). The fish in the nonmanipulated, ‘control’ mesocosm did not change their time of emergence and retreat during the days of our light-manipulation experiment.

The timing of twilight transitions all over the observed sites was virtually the same for *N. miryae* and *P. squamipinnis*, whereas *D. marginatus* usually emerged later and retreated sooner (Fig. 5). The light level at the time of transitions was different at different sites, with fish in the Observatory’s mesocosms emerging and retreating at much lower light intensities, thereby having a 30–45-min longer foraging day than their conspecifics in the natural habitats (Fig. 5). No apparent trend was found with regard to depth or habitat type.

**Flume Experiments**

Both light intensity and speed–density combinations of the prey flux significantly affected the feeding rate of *N. miryae* in the flume (ANOVA: light effect: $F_{5,60} = 85.63$, $P < 0.001$; speed–density effect: $F_{1,60} = 1800.7$, $P < 0.001$; Fig. 6a). The effect of light was most pronounced at the range of dim illumination, with a two-fold enhancement of the feeding rate as light intensity increased from 0.25 to 10.75 lx, reaching saturation at stronger levels. Significant interaction was found between light intensity and speed–density combination (ANOVA: $F_{5,60} = 23.61$, $P < 0.001$), indicating that the 3 cm/s treatment is much closer to the asymptote than is the 9 cm/s treatment at high light levels (Fig. 6a). Of the two speed–density combinations of the prey flux, feeding rates at the combination of low speed–high prey density were approximately double those under the combination of high speed–low density (Fig. 6a).

The effect of light intensity on reactive distance was highly significant (ANOVA: $F_{5,55} = 21.62$, $P < 0.001$), whereas that of flow speed was not ($F_{5,55} = 3.48$, $P = 0.068$; Fig. 6b). In contrast, critical strike angles were not affected by light intensity ($F_{5,55} = 1.18$, $P = 0.335$), but differed significantly between the two flow speeds ($F_{5,55} = 268.9$, $P < 0.001$; Fig. 6c). There was no significant interaction between light intensity and flow speed in terms of their effect on reactive distance ($F_{6,55} = 0.96$, $P = 0.453$) or critical strike angle ($F_{6,55} = 0.54$, $P = 0.744$).
The relation between reactive distance and light intensity was best described by the logarithm regression:

\[ L_r = 1.1 \times \ln(I_t) + 4.3 \tag{1} \]

where \( L_r \) is the reactive distance (cm) and \( I_t \) is light intensity (lx) (log-linear regression: \( F_{1,3} = 130.5, P < 0.001, R^2 = 0.92; \) Fig. 6b). The reactive distance, calculated from equation (1) and the mean critical strike angles, obtained empirically, served as estimates of the parameters \( L_r \) and \( \theta_c \) used to calculate the reactive volume. The relation between reactive volume and feeding rate was tested with an ANCOVA (homogeneity of slopes design), treating the mean feeding rate by each fish under each combination of light intensity and flow speed as the dependent variable and the estimated reactive volumes as the independent (continuous) predictor. Flow speed was used as the categorical predictor variable. The results revealed a significant effect of reactive volume (\( F_{1,68} = 127.4, P < 0.001 \)) and flow speed (\( F_{1,68} = 93.8, P < 0.001 \)), with no significant interaction between the two factors (\( F_{1,68} = 0.57, P = 0.452 \)). Changes in the reactive volume induced by changes in light intensity and flow speed explained 91% of the variance in the fish feeding rates (\( F_{3,68} = 223.7, P < 0.001, \) adjusted \( R^2 = 0.91 \)).

**Figure 2.** Time of emergence/retreat of the first/last *Dascyllus marginatus* as a function of (a) the time of sunrise/sunset, (b) light intensity, (c) ambient current velocity and (d) backscatter echointensity from an Acoustic Doppler Current Profiler (AU = arbitrary units). Each symbol indicates a single day of observation either at dawn (○) or at dusk (●). Horizontal error bars in c and d indicate the range from the minimal to the maximal values, measured 30 min before or after the above emergence and retreat, respectively.

**Table 2.** ANCOVA model of the effect of ambient light intensity, current speed and relative zooplankton abundance on the time of transition behaviour of *Dascyllus marginatus* at MBL at 10 m depth

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light intensity</td>
<td>1</td>
<td>0.0004</td>
<td>6.286</td>
<td>0.033</td>
</tr>
<tr>
<td>Current speed</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>0.046</td>
<td>0.836</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>0.659</td>
<td>0.438</td>
</tr>
<tr>
<td>Dawn-Dusk</td>
<td>1</td>
<td>0.082</td>
<td>1284.644</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The transition behaviour in the site-attached reef-dwelling planktivorous fish we studied was asymmetric with regard to light intensity. The emergence at dawn occurred at lower light levels than the retreat at dusk, equivalent to a difference of 6–10 min. Fish in the reef mesocosms had a longer feeding time than in situ, as they emerged from, and retreated to, their shelter at lower light levels. Our manipulation experiment indicated that the time of retreat at dusk was directly controlled by light, but the factors determining the time of emergence at dawn remained unclear. Prey density and current speed had a significant effect on the fish’s feeding rates; however, neither parameter affected the time of emergence or..
retreat. Effects of light on the fish’s reactive distance and feeding rate were observed mostly at low light levels, in the range typical for twilight.

In general, the fish we studied in Eilat emerge from shelter at dawn and retreat at dusk at slightly lower light levels than those reported by McFarland et al. (1999) and Hobson (1972) for pomacentrids (*Chromis* and *Dascyllus* spp.) in Caribbean and Hawaiian reefs. Our flume experiments (Fig. 6a) showed that the fish were capable of visual feeding under light intensities dimmer than the level at which they emerged from and retreated to shelter in the field (Fig. 2). However, their feeding efficiency at these transition times was about 50% of the maximal rates found in the flume under saturating light levels (Fig. 7). Hence, emergence and retreat occurred at light intensities much greater than the threshold for prey detection. In the flume, the strongest light-induced changes in the feeding rates occurred over a narrow range of light intensities, corresponding to a short period of the visual day (about 45 min). Compared to that of light, the effect of flow speed and prey density on feeding rates was consistent over the whole range of light levels (Fig. 6a). Thus, prey flux, as a main physical variable to shape the functional response of site-attached planktivorous fish (Kiflawi & Genin 1997), might have a potential adjusting effect on the timing of transition behaviour. In fact, such an effect of prey flux was not found (Fig. 2c, d).

By applying experimentally measured feeding rates to the fish feeding performance in the field, we have made several simplifying assumptions regarding fish spectral sensitivity and retinomotor performance. Since good correlations were found between the photosensitivity of fish eyes and the spectral transmission properties of their habitats (reviewed in Lythgoe 1984; Partridge & Cum- 
mings 1999; Marshall et al. 2003), the potential bias associated with using UV-absent artificial light in the flume experiment (Fig. 1) deserves further discussion. Several zooplanktivorous fish in coral reefs are known to have UV vision, and many more are very likely to have it (Losey et al. 1999, 2003). UV vision is thought to help in

---

**Figure 3.** In situ foraging activity of *Dascyllus marginatus*, *Neopomacentrus miryae* and *Pseudanthias squamipinnis* with respect to the time relative to sunrise and sunset and light intensity. The symbols indicate the mean percentage ± SE of the group swimming in the water column (out of shelter) during the observation time. The observations were carried out at the underwater observatory at 6 m depth.

**Figure 4.** The effect of artificial illumination (○), turned on for 1 h before sunrise or left for 1 h after sunset, on the time of emergence and retreat, respectively, of *Pseudanthias squamipinnis* and *Neopomacentrus miryae* in the Observatory mesocosm. Each symbol indicates the mean percentage of the fish foraging in the water column (out of shelter). The activity of the fish (percentage of the fish foraging in the water column) at the Observatory tower (— — —) and at the mesocosm (●) under natural light conditions are presented for comparison.
prey detection by enhancing contrast between the prey and its background (Browman & Hawryshyn 1992; Loew et al. 1993; Browman et al. 1994; Losey et al. 1999). Browman et al. (1994) showed that the feeding efficiency of juvenile trout, *Oncorhynchus mykiss*, and pumpkinseed sunfish, *Lepomis gibbosus*, on zooplankton improved with the addition of UV radiation to the visible spectrum. Rocco et al. (2002), however, failed to find this effect with rainbow trout, which casts doubt on the ecological significance of UV in enhancing feeding performance in the natural environment. Nevertheless, it is likely that threshold light intensities are specific regarding the spectral sensitivity of the fish. We have therefore probably underestimated the absolute feeding rates of the fish in the field.

The dawn–dusk asymmetry was shown by the three species we studied but differed from the symmetric behaviour reported for zooplanktivorous fish in St Croix (see figure 5 in McFarland et al. 1999) and Kona, Hawaii (Hobson 1972). Deciphering the causes for the asymmetric behaviour can help our understanding of the factors that determine the twilight transition in these fish. Twilight changes in light properties, including intensity (Fig. 3), spectrum (Nielsen 1963) and polarization (N. Shashar, unpublished data), are symmetric (mirror images) during dawn and dusk. Hence, the asymmetry in fish behaviour is apparently related to biological processes that differ at dawn and dusk, such as time courses of light-to-dark versus dark-to-light adaptation in the retina, differences in the level of hunger, competition for shelter, or asymmetric behaviour of predators.

Light-to-dark adaptation in the retina occurs along the range of light changes from 10 to 0.001 lx (Blaxter 1970), equivalent to the changes in light intensity at dusk (McFarland et al. 1999). The course of dark adaptation usually takes 30–60 min, with faster adaptation for cones and slower for rods (Lythgoe 1979; Ferwerda et al. 1996). This is longer than the fall of light at dusk, which might lead to short periods of night blindness (Ali 1959). The dark-to-light adaptation, on the other hand, is much more rapid (Ferwerda et al. 1996), keeping pace with the change of light at dawn. These differences in light-to-dark adaptations may lead to sheltering at higher light levels at dusk.

The level of hunger can affect the timing of emergence from, and retreat to, shelter. The emergence at dawn takes place several hours after feeding, with the fish being more
hungry and likely to take greater risks (Dill 1983; Goticetas & Godin 1991; Höjesjö et al. 1999), compared with the retreat at dusk, which follows a day-long interval of feeding. A dawn–dusk asymmetry in the level of hunger can therefore induce the observed asymmetry in fish behaviour.

Predation by piscivorous fish is also likely to affect the timing of transition behaviour. Piscivorous fish are abundant in most coral reefs and, in some cases, show a remarkable twilight asymmetry in their foraging activity (Holbrook & Schmitt 2002). Predation of coral-dwelling pomacentrid fish (Dascyllus spp.) by jacks, groupers and snappers in Moorea (French Polynesia) was most intense during the first 2 h after sunset, with sporadic attacks during the remainder of the night and morning (Holbrook & Schmitt 2002). Furthermore, intragroup competition for better shelter results in the displacement of late-arriving fish to more risky sheltering locations (Holbrook & Schmitt 2002). Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.

Admittedly, the ecological significance of a threshold \( m/f \) value is unclear. Since \( f \) depends on prey availability, the fish would be expected to prolong/shorten foraging during dawn (dusk). As a proxy for \( m \) we used a published relation between the time relative to sunrise/sunset (a correlate of light levels) and the rate of attacks by a piscivorous fish (leopard grouper, Mycteroperca rosacea) when preying on schooling fish (Harengula thrissina) in the Gulf of California (Hobson 1972). The results (Fig. 8) suggest that (1) emergence from and retreat to shelter occurs at \( m/f \) ‘threshold’ values higher than the minimum and (2) these threshold values are the same at dusk and dawn but the former occurs when the ambient light intensity is stronger.
activity in response to fluctuations in the prey flux. At our study site, two to three fold changes in zooplankton density during the course of a week are common (A. Genin, unpublished data). Considering such changes in prey density, the corresponding change in p/μ would be comparable to a ±10 min difference in the timing of emergence or retreat. Our observations indicate that the time of emergence and retreat did not vary by more than 2 min (Fig. 2). Hence, it seems that reef-dwelling zooplanktivores ignore their feeding rates when deciding whether to begin or to end foraging. The marginal fitness return from adding several minutes of feeding to the 10–14 h daily foraging time is apparently negligible compared to the added risk of predation. The more likely scenario is that the fish respond mostly to the perceived risk of predation.

Acknowledgments

We thank Moty Ohevia for fabricating the illumination sources used both in the flume and at the Observatory, designing video cameras, helping with the video recordings and extensive technical help during the study; R. Holzman and M. Kilfawi for productive discussions, ideas, and comments on the manuscript; and O. Ben-Tzvi, D. Cohencius, S. Eckstein, B. Farstey, R. Goldshmid, R. Kent, S. Sabah, R. Yahel and G. Yahel for invaluable help with observations on fish behaviour at the Observatory. This work would not have been possible without assistance of the people at the Underwater Observatory Marine Park and at the Interuniversity Institute for Marine Sciences of Eilat. This study was supported by the Israeli Science Foundation funded by the Israel Academy of Science and Humanities.

References


RIKKEL & GENIN: TWILIGHT BEHAVIOUR IN REEF FISH 143


