Controlled ocular radiance in a diurnal fish looking at prey

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Abstract

Recent work on marine fishes revealed an association between the presence of red fluorescent eyes and a preference for small prey, an indirect suggestion that ocular radiance may be used for active detection, i.e., active photolocation. A recent study indeed showed that the ability to emit fluorescent light may help fish to capture more micro-prey. Here, we investigate two further predictions, namely that ocular radiance is modulated depending on (i) prey presence and (ii) background colour. We describe the "ocular spark" as a new behavioural mechanism of ocular radiance. Downwelling light is focused as a spot on the lower iris and redirected sideways. Our model species *Tripterygion delaisi* can switch between red or blue ocular sparks, or the off state, by subtle eye movement. We show experimentally that these sparks are used differentially depending on the background of the copepods: Blue ocular sparks were used more against red backgrounds, red ocular sparks more against blue backgrounds. This also occurred in the absence of copepods, albeit weaker. The data suggest that ocular sparks are modulated in response to the prey detection context. We discuss the implications for the active photolocation hypothesis.

Keywords:

Marine fishes, visual ecology, active sensing, prey detection

1. Background

Some animals enhance their sensory systems by actively sending out signals and perceiving the induced reflections of nearby objects. Well-studied examples of active sensing are echolocation with ultrasound in bats and dolphins, and electrolocation using electric fields in some fishes [1]. “Active photolocation”, where light is used for probing the environment, seems surprisingly rare [1]. This can in part be attributed to the risk of revealing one’s own position. Given that vision, or “passive” photolocation, is well developed across animals, bright structures may attract predators [2] and alert prey. Hence, if light were to be used for detection, it can be predicted to be subtle and controlled. As a consequence, it is also quite likely to be limited to the detection of specific target features under specific light conditions where the benefits of active photolocation exceed those costs.

Chemiluminescent deep-sea dragonfishes (Fam. Stomiidae), lanternfishes (Fam. Myctophidae) and flashlight fishes (Fam. Anomalopidae) are currently the only vertebrates assumed to use active photolocation [3-9]. They possess a chemiluminescent ocular light organ whose emission has recently been shown to support visual detection of prey [10]. Although the emitted light is dim, its anatomical location next to the pupil is optimal to induce eyeshine in nearby organisms [3]. Eyeshine can have different causes [11] but in most contexts refers to retroreflection by a focusing eye enhanced by the presence of a reflective layer at the back of the eye [12] (ESM 1). Its presence improves vision under dim light [13, 14]. In other cases, it can also play a role in camouflage [15]. Assuming a focussing eye, light backscattered or reflected by a distal layer in the eye is specifically directed back to the source following the reversibility principle of optical systems [16]. Consequently, reflective eyeshine is visible only to observers that radiate light from a source close to their own eye, as exemplified by dragonfishes, lanternfishes and flashlight fishes [17].

Interestingly, a similar configuration appears to be present in diurnal fishes as well, many of which possess conspicuously bright eyes (figure 1, ESM 2). Rather than involving chemiluminescence, however, light radiance originates from reflective or fluorescent (= photoluminescent) irides [18-21]. In a large comparative analysis, we

found that small fish feeding on small, eyed prey also have a higher probability to have red fluorescent irides [22], hinting at the possibility that light emission from the iris constitutes a visual aid in prey (or predator) detection [23]. A first experimental confirmation was obtained from one of these species, the benthic triplefin *T. delaisi*: It captures more prey when fed under "fluorescent-friendly" dim, blue light, simulating light at depth, compared to a broad ambient spectrum with identical brightness [24].

If ocular radiance is indeed used for detection, while at the same time being minimised to prevent detection of oneself, we predict that diurnal fish should actively modulate ocular radiance in response to the current context. Since up- and down-regulation of fluorescence is relatively slow [20, 25] and also hard to quantify in live individuals, we here shift our focus to the *ocular spark,* a new term we introduce to describe a bright point of downwelling light that can be seen in many fish just below the pupil (figure 1, 19-24). It is generated by the protruding spherical lens (figure 2a) which allows downwelling light to cross without entering the pupil. This can generate a bright focal spot on the lower iris or (rarely) the skin below the eye. Specular reflectors or diffuse pigmentation in the iris result in reflectance spectra limited to a range within the ambient spectrum (figure 2c) whereas a fluorescent iris shifts the emitted light to longer wavelengths (figure 2d). Although ocular sparks can readily be seen across many fish families in the field (figure 1, 19-24, own observations), we could not find a description for this mechanism in the literature. The major advantage of ocular sparks from an experimental perspective is that they represent a discrete behaviour. Small eye movements are sufficient to switched them "on" or "off" in an instant, facilitating quantification for the human observer.

Our model species *Tripterygion delaisi* is a small, cryptobenthic triplefin with independent, high amplitude eye movement and strongly red fluorescent irides [21, 26] (figure 2). The iris also features 1-3 white-blue iridophore spots, the biggest one at the ventral edge of the pupil (figure 2b). On each of these spots, fish can produce a blue, reflective ocular spark (figure 2c). Relative to a diffuse white surface, ocular sparks are strong (ESM 3). While moving in its typical habitat of heterogeneously overgrown hard-substrate (figure 2e), *T. delaisi* frequently alternates between both ocular sparks and the "off" state by eye rotation and tilting while searching for prey

(ESM 4). Aiming and striking distances are in the 1-3 cm range. Because *T. delaisi* feeds opportunistically on a variety of benthic micro-crustaceans [27, 28], we use the benthic, lab-bred copepod *Tigriopus californicus* as an experimental prey target [29]. It is characterised by having small, but strongly reflecting ocelli, making it a potential target for active photolocation (ESM 4 and 5).

To test whether the use of ocular sparks correlates with the detection context, we carried out two replicate experiments in a 2x2 fully factorial design. In both, we tested whether red ocular sparks are used more when confronted with prey on a blue background, and *vice versa* for blue ocular sparks on red backgrounds The rationale was that these complementary combinations would maximise the contrast between prey reflection and background. Note that hues of natural substrates at depth can also be red due to fluorescence [18, 30]. We included prey presence/absence as a second factor in the first experiment. For confirmation, we replicated the first experiment (experiment 2), but now used satiation status (hungry or satiated) as the second factor, with copepods present in all trials. We predicted satiated fish to be more reluctant to show ocular sparks than hungry fish, assuming a trade-off between attracting predators and detecting food.

The results provide evidence for a differentiated modulation of ocular sparks in response to prey and background hue as predicted. We discuss whether this implies that ocular sparks can function as an active photolocation system and how to test this further in the future.

2. Material and methods

2.1. Model species *Tripterygion delaisi*

The triplefin *Tripterygion delaisi* (Fam. Tripterygiidae) is a 3-5 cm long benthic fish from the East-Atlantic Ocean and West-Mediterranean Sea. It is common in rocky coastal areas between 5 and 20 m, down to 50 m depth [31, 32] and is often found in or near shady areas and cracks in which they can retreat. When foraging, individuals often venture out into the brightly lit parts of the overgrown, complex substrate.

Preferred prey items are mostly small crustaceans, including harpacticoid copepods [33]. *T. delaisi* is highly cryptic, sitting motionless much of the time while scanning its immediate environment, interrupted by occasional forward hops over short distances (1-10 cm). Free swimming is rare. Potential prey is usually assessed for a few seconds, carefully approached in slow-motion, and then suddenly caught once the fish is within 1-3 cm from its target. *T. delaisi* moves its eyes independently and with high amplitude, similar to the sand lance *Limnichthyes fasciatus* [34]. Initial prey detection is mostly monocular, but actual striking is often binocular (personal observations by Ulrike Harant and N.K.M.).

T. delaisi has red-reflective irides that also fluoresce strongly in red with maximal excitation around 530 nm and peak emission at around 610 nm [20, 21]. During the breeding season (March-May), males have black heads and irides. Otherwise, all age and sex categories show the same cryptic colouration with bright red irides throughout the year, suggesting a function unrelated to adulthood or reproduction. Fluorescence brightness is under nervous control [20, 25]. The iris also features 1-4 blue-white coloured, irregularly shaped spots that consist of reflective iridophores. The biggest one is at the lower edge of the iris and is the one typically used for a blue ocular spark (figure 2). All other parts of the iris can be used for a red (reflective-fluorescent) ocular spark. Both ocular sparks have been seen in the field across our SCUBA diving range down to 30 m. Previous work already confirmed that *T. delaisi* can perceive its own fluorescence [21, 35] confirms that the spectral sensitivity of *T. delaisi* allows it to perceive its own red fluorescence. The blue ocular spark also falls well within *T. delaisi*'s spectral sensitivity, particularly that of the short-wavelength sensitive single cone with λ_{max} = 468 nm [21]. Spectral data about ocular sparks in the laboratory environment can be found in ESM 4.

2.2. Fish collection and housing

We collected *T. delaisi* either near the Station de Recherches Sous-marines et Océanographiques (STARESO) near Calvi, Corsica (France), or near the Hydra Centro Marino Elba in Italy (www.hydra-institute.com). Sampling took place under

the general sampling permit of these stations. *T. delaisi* is a non-threatened, non-protected, non-commercial, common species. Animals were transported individually in plastic fish bags filled with 50-100 ml purified seawater and pure oxygen. At the University of Tübingen, fish were kept in individual tanks (*L* x *W* x *H* = 24 x 35 x 39 cm^3). Black PVC dividers prevented visual contact between neighbouring fish. Coral sand and a piece of rock covered the bottom. All aquaria were interconnected to a flow-through filtering and UV-sterilisation system. The water was kept at a salinity of 35 ‰ at about 23°C. The room used for this study had 21 tanks and was illuminated by weak, diffuse, blue fluorescent ceiling lights (Osram L18W167 Lumilux Blue) for 10 h (0800 -1800) per day. From 0900 till 1400 each day, illumination was boosted with one blue LED point source per tank (LED-Fluter AV-LFL10B, Avaloid GmbH, Düsseldorf). Water quality was checked on a weekly basis. Fish were fed with a mixture of Tetramin (Hauptfutter für alle Zierfische, Tetra GmbH) and Mysis (Einzelfuttermittel, Aki Frost GmbH) every day.

2.3. Copepod prey

We obtained *Tigriopus californicus*, an intertidal, benthic, harpacticoid copepod (Crustacea) from the North-American Pacific coast, from an online retailer (http://www.hippozoo.de). As is true for most benthic copepods, *T. californicus* has a single nauplius eye on the anterio-dorsal part of the cephalothorax between the bases of the two antennae. The eye consists of 3 cup-shaped ocelli with an internal layer of reflective guanine platelets [29]. The two dorsal ocelli are directed anterio-laterally (one left and right) and are located above and slightly posterior to the single medial, ventral ocellus, which is directed in a slight anterio-ventral direction. In fully-grown adults, the diameter of the ocelli is 40 μm (dorsal) and 35 μm (ventral). Red pigmented cells fill the gap between the ocelli [29]. Coaxial illumination easily generates distinct eyeshine (ESM 5 and ESM 6). We cultured *T. californicus* in a climate-controlled chamber in 20 identical 12 L glass aquaria (30 x 20 x 20 cm^3) with artificial seawater (Preis Aquaristik Meersalz) at a salinity of 35 ‰ and 22 °C. The water level was kept at 8 cm to maintain a high surface-to-volume ratio for gas

exchange supplemented by bubbled air. Ceramic "biotube" cylinders were added to provide habitat structure and substrate for beneficial bacteria. Lighting consisted of fluorescent strip lights (Sylvania, T8, warm white) which were on 0700-1900 each day. Cultures were fed live phytoplankton, grown in four 4 L glass jars at 35 ‰ using a seed culture (http://www.hippozoo.de). Each aquarium was fed 500 ml of live phytoplankton initially. We maintained phytoplankton density in the copepod tanks by periodic feeding with additional phytoplankton as it was consumed. We regularly monitored water quality, performed water changes and seeded fresh tanks as needed.

2.4. Experimental setup

General design

The differential usage of ocular sparks was tested in a 2 x 2 design and took place in two replicate runs. Copepod background (blue or red) was the first factor. The second factor was copepod presence or absence in replicate experiment 1, and fish in satiated or hungry state in replicate experiment 2.

Experiment 1, with copepod presence or absence as 2nd factor

Each of 21 *T. delaisi* individuals from Elba was exposed to all four treatments in a randomised series. The response of fish to a block of 4 treatments was tested once per week for five weeks, seven individuals per day (Mon, Tue, Wed). We recorded data in the morning, followed by daily feeding in the afternoon. Four individuals showed no interest throughout the experiment and were excluded, resulting in a final sample size of $n = 17$.

The stimulus presented to the fish consisted of two microscopy slides (7.6 cm x 2.6 cm) glued together with a "U" shaped line of black, non-toxic, silicone (JBL, AquaSil Silicon), generating a flat, transparent chamber (ca. 25 x 20 x 2 mm) with a 5 mm opening at the top (ESM 3). Chambers were presented inside the tank at the bottom of the front windowpane facing upward in a 45° angle. For treatments with prey, five

live copepods were injected into the chamber using a pipette. Copepods occasionally escaped through the small opening into the aquarium, offering fish a small reward and encouraging them to pay attention. A piece of red fluorescent or blue-grey synthetic felt (Folia Paper, Bringmann, Ton in Ton Bastelfilz) was glued on the back of each chamber to generate the two background hues. Several copies of the chambers were produced and rotated throughout the experiment to balance out individual chamber effects.

Fish behaviour was recorded as a series of 100 still photos, one per 2 s for 200 s, using a GoPro Hero Black Edition camera with macro lens (Schneider optics, B+W macro lens +10) and a filter (LEE Filter 105 Orange) to facilitate distinction between blue and red ocular sparks under the dominant blue ambient light. The viewing angle was adjusted so that the copepod chamber was below the lower edge of the video recording. Thus, image analysis was blind for the treatment. Recording was started remotely when the fish entered the "active zone", an area within 15 cm from the tank front, indicated with a transparent plastic marker. Within this zone, we expected fish to be able to see the copepods inside the chamber.

Experiment 2, with fish satiation state as 2nd factor

We repeated experiment 1 using a new set of 21 *T. delaisi* from Corsica. This time, copepods were present in each trial. We replaced the "copepods present/absent" treatment by a satiation treatment: Fish were exposed to one satiated and one hungry phase per week. An individual started either as "satiated" or "hungry", alternated across aquaria. Normally, we fed *T. delaisi* one scoop of frozen *Mysis* (~3 shrimps) per day, 7 days per week. Fish in the satiated phase, however, received one scoop of *Mysis* at 10:00, 11:30, 13:00 and 15:00 on day 1, followed by two more at 8:20 and 08:50 just before trials on day 2 (= 6 scoops in ~36 h). On the contrary, fish in the hungry phase received nothing during this period, but were fed after trials on day 2 at 13.30 and 15:00. On day 3, none of the fish were fed. On days 4 and 5 the same feeding scheme as on days 1 and 2 was applied, but with treatments reversed. All fish received one scoop on day 6 and nothing on day 7, followed by day 1 again. This

regime was established during pilot trials and showed a clear difference in responsiveness to feeding in satiated (low response) and hungry (high response) individuals (unpublished results).

During experimental trials, a fish was shown two chambers with 5 copepods in random order with either a blue or red fluorescent background. Each fish was tested for each background (red or blue) six times per satiation status (hungry or satiated). After 2 weeks of acclimation to the procedure, we collected data during the next 4 weeks. Photos were taken every 2 s for 300 s resulting in 150 pictures per fish and treatment. As in the first experiment, we evaluated 100 photos starting with the first photo in which the fish entered the active zone. One individual was extremely shy and showed up in the active zone in only 3 out of the first 16 trials (19%). It was ignored for the analysis, resulting in a sample size $n = 19$ for experiment 2.

2.5. Data acquisition

Spark type was assessed visually. Given that fish were free-roaming, each in its own aquarium, the variation in fish illumination, distance and orientation relative to the camera was large. Hence, automated scoring using fixed settings was not feasible. We implemented three measures to prevent observer bias:

(1) Following years of observation in the laboratory and in the field we derived unambiguous definitions for the two ocular sparks based on a combination of colour, shape, position on the iris, and orientation of the fish and its eye. An example of both sparks can be seen in ESM 5.

(2) Prior to image analysis, scoring persons were trained in correctly applying the criteria from (1) using picture series from the laboratory and the field.

(3) Picture analysis was entirely randomised and blind to the treatment.

We counted the number of photos showing (i) a blue ocular spark, (ii) a red ocular spark or (iii) neither. Photos where the fish was pointing away from the chamber were ignored.

2.6. Statistical analysis

Data were analysed using a Generalized Linear Mixed Effects Model in the lme4 package [36] of R v3.1.1 [37]. The number of photos showing either type of ocular spark over the total number of photos showing the fish in the active zone was modelled as a binomial (yes/no) response variable with logit link. For the first experiment, the fixed component of all initial models contained the main factors background (blue or red) and copepods (present or absent) as well as their interaction. We further included recording day and treatment order per day to correct for sequence effects on spark usage, and sampling depth to account for differences due to the depth of origin of our experimental fish. Models for the second experiment were constructed in an identical fashion, now with background (blue or red) and satiation status (yes or no) as the main factors and recording day, treatment order per day (the sequence in which each fish was exposed to the two backgrounds) and testing order (the sequence in which the different fish were tested on a given day) as the covariates.

In both models, the initial random component contained Individual ID with random slopes over the background and copepod or satiation treatments. This accounts for the repeated measurements per fish and captures variation that arises from differential individual treatment responses [38]. We further included an observation-level random factor to remove overdispersion [39]. We then performed backward model selection on the random and fixed components using the Bayesian Information Criterion (BIC) to find the most parsimonious model in terms of model fit penalized by the number of parameters [40]. We only report reduced final models, and provide proxies for the goodness-of-fit of the fixed component (marginal R^2) and the complete model (conditional R^2) [41, 42] as implemented in the pairwiseSEM package for R [43]. We used Wald z-tests to assess the overall significance of fixed effects. To explore the nature of statistical interactions between our main factors we implemented planned contrasts between the two background types and the presence or absence of copepods (experiment 1) or the satiation treatment within each background type (experiment 2).

Responsiveness of single fish under the two different satiation treatments in the second experiment (latency to reach, and time spent in, the active zone) were analysed using a mixed model with satiation treatment as a fixed factor and individual ID as a random intercept to account for the multiple measurements per fish and treatment using JMP v11.2.

3. Results

3.1. Experiment 1: Effects of background hue and prey presence

When confronted with copepods in front of a red or blue background, *T. delaisi* individuals produced blue and red ocular sparks non-randomly (figure 3, table 1): Blue ocular sparks were shown significantly longer against a red background, but red ocular sparks were on significantly longer against a blue background. Ocular sparks were also shown significantly longer for complementary combinations of ocular spark and background hues in the absence of copepods. Consequently, the interactions between the fixed factors "copepods" (presence/absence) and "background" (red/blue) were also significant.

3.2. Experiment 2: Effects of background hue and satiation status

The replicate experiment confirmed the effect of background colour on the differential use of ocular sparks seen in the first experiment (figure 4, table 2). Satiation status, however, showed no detectable effect, irrespective of background hue. The latency with which individuals appeared in the active zone after the start of a trial and the total time spent in the active zone also did not differ between satiation treatments (mixed model satiation treatment effects, $P = 0.63$ and $P = 0.13$).

4. Discussion

Our study introduces ocular sparks as a new mechanism of ocular radiance in fishes and shows that they are actively modulated in response to prey presence and background hue in a cryptobenthic triplefin. In the following, we discuss whether ocular sparks may represent a suitable system to test the plausibility of diurnal active photolocation in future studies.

Ocular sparks show several suggestive features that fit predictions for diurnal active photolocation developed in the introduction: Their increased use in the presence of prey suggests that their use is associated with prey detection. This is reminiscent to e.g. increased pulse rate observed in echolocating predators when prey has been detected [44, 45]. Moreover, the complementary adjustment of spark hue to background hue is suggestive of contrast optimization. Given that ocular spark hue was also adjusted to the background in the absence of copepods, fish seem to adjust baseline sparking activity, even if prey have not yet been detected, but in a setting where they have seen copepods before. Contrary to our expectations, hungry fish did not show an increase in ocular sparking relative to satiated fish (experiment 2). Although satiated fish showed a reduced interest in the standard food supply, live copepods may have revived their interest. Combined, our findings suggest that ocular sparks are adapted to a prey detection context as predicted by the active photolocation hypothesis.

However, whether *T. delaisi* actually perceives reflections or even eyeshine from the copepods cannot be inferred from this study. This requires follow-up experiments and visual modelling that includes the optical properties and spectral sensitivity of *T. delaisi* [21], the reflective properties of natural prey species, as well as exhaustive spectral measurements of natural, ambient irradiance and substrate radiance in *T. delaisi* environments. In order to acquire experimental evidence, a physical manipulation of the ability of *T. delaisi* to emit light (either by blocking light re-emission or controlling the ambient light) as well as the reflective properties of the target will be required.

Are ocular sparks too weak?

Even weak, diffuse reflectors can work as light sources and induce perceptible reflections in reflective targets such as eyes, assuming short distances and small-scale light gradients (e.g. ESM 7, 8). Given the short striking distance of 1-3 cm typical for benthic micro-predators such as *T. delaisi* and its complex, patchy light microhabitat (figure 2e), a fish exposed to the sun might indeed be able to induce perceptible reflections in prey hiding in the shade nearby. Although we argue that eyes may be the prime targets for detection because of their reflective properties, this does not need to be the case. Depending on the exact conditions, even less reflective body structures may become detectable with the application of some extra light. As exemplified by chemiluminescent flashlight fishes [3, 10], however, the close anatomical association between light radiance and pupil is very suggestive of a primary adaptation to emit coaxial light, which is a specific requirement to detect retroreflective eyeshine [3, 23, 46]. For most other types of reflective target structures, a close vicinity of source and pupil in the detector is not required.

If active photolocation by means of ocular sparks can be confirmed for *T. delaisi* in future studies, it is quite likely to be a ubiquitous mechanism used by many species. Own observations over 7 years in the Mediterranean Sea, Red Sea and Indo-Pacific region indicate that ocular sparks and other forms of ocular radiance are strikingly common (figure 1), as already demonstrated for red fluorescent irides [19, 22]. Until now, we have emphasized prey detection. Yet, ocular sparks may also function in a context of predator detection given that many cryptic predatory fish feature strong (retro)reflective eyeshine and large eyes [3]. Moreover, the selective pressure not to overlook a predator may be stronger than the cost of overlooking a food item.

What are the advantages of ocular sparks over other forms of ocular radiance?

Even when assuming ocular radiance is useful, why should it be in the form of an ocular spark? It does not radiate more light than reflection off the full iris could, but merely concentrates it into a small spot. Many fish species have silvery or golden irides that reflect light from most or all of their surface (figure 1). One answer may be

that light concentrated onto a small spot is less conspicuous to a predator than a full, bright iris. Seen from a distance, an ocular spark will fall below the spatial acuity of most observers, becoming a point source. Any further increase in distance would result in an exponential decrease in radiance [47]. As a result, ocular sparks may be less conspicuous than a complete iris at distance. This may allow micro-predators to hunt successfully over very short ranges, while remaining cryptic over the detection distances used by their own predators. Second, only ocular sparks allow for accurate and immediate swapping between two hues with minimal eye movement. This appears particularly useful for fish foraging on substrates characterised by complex spatial and temporal patterns of different hues. Non-cryptic fish feeding against a homogeneous background (e.g. the open water) may not need such a "filter wheel". Finally, by focusing light onto the lower edge of the pupil, ocular sparks approximate a fish's equivalent of an ophthalmoscope. Given that its retinal *area centralis* is positioned in line with this bright, reflective edge and the target (unpublished data), this coaxial arrangement is ideal to highlight eyeshine, even if the source may seem weak.

Alternative functions of ocular sparks

As a non-exclusive, alternative explanation, ocular sparks might act as lures and *attract* copepods. A similar mechanism has been proposed for the fluorescent tentacles of hydromedusae that are assumed to attract fish [48, 49]. This however, seems unlikely for prey with poor visual acuity as is true for benthic copepods with just three ocelli [29]. It also does not explain why fish would alternate between two hues in response to the background of the copepod rather than that of the fish itself. Moreover, *T. delaisi* does not lure or ambush approaching prey, but actively searches for prey. Finally, zooplankton shows an escape response when confronted with flashes of light [50, 51]. Hence, although light-emitting lures exist, it does seem to be a likely function for ocular sparks.

A more plausible alternative function is that ocular sparks are used for intra-specific signalling, as already suggested for the chemiluminescent flashes of *Anomalops*

katroptron [3]. *T. delaisi* usually forms loose groups of adults and juveniles. It is likely that they can see each other's ocular sparks – irrespective of whether their own sparks induce eyeshine in their conspecifics or not. Most behavioural interactions between individuals are, however, characterised by fin-related behaviour such as dorsal fin flicking, push-ups and tail-flicks [52]. Whether ocular sparks supplement this repertoire is as yet unknown, but even if this were the case, it cannot explain ocular spark modulation in solitary individuals in response to prey and background hue.

Conclusions and outlook

To summarize, our model species *T. delaisi* radiates light from its eyes in a controlled way by means of ocular sparks in response to prey presence and background hue. This pattern is suggestive of controlled light emission for diurnal, active photolocation. Given the prevalence of ocular radiance in numerous fish species, we see great potential for a phenomenon currently known from deep sea or nocturnal fishes only.

Ethics statement

For the experiments, animals had been well habituated to their "home" aquaria for 6 months. To prevent fish from being distracted by increased human activity in the room, we covered the front of all tanks with a black PVC panel for the duration of the study. Animal husbandry was in accordance with German animal welfare legislation (Tierschutzgesetz of 7 August 2013, §2 and §11) and is checked regularly by independent, regional authorities (Dr. S. Wheeler, Landkreis Tübingen, Veterinärwesen und Lebensmittel-Überwachung, Wilhelm-Keil-Str. 50, 72072 Tübingen, Germany). Data collection relied exclusively on the spontaneous curiosity of fish for the stimulus provided in their home aquarium. Fish were never handled in any way. Consequently, our design did not involve pain, suffering or injury to the animals. According to §7 (2) of the German animal welfare act (Tierschutzgesetz) of 7 August 2013 this implies that a formal application for an ethics permit is not

required, as confirmed by the institutional animal welfare officer, Dr. Annette Denzinger (Fachbereich Biologie, Universität Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany).

Data accessibility

The main data files are provided as ESM 9 and ESM 10.

Competing interests

The authors declare no competing interests.

Author information

Contributions

The idea for active photolocation was brought to us by C.B.J. in 2009. Concept and experimental design were developed by N.K.M. and M.G.M. with assistance from V.S., N.K. and N.A. Spectrometry was developed during student projects under supervision of N.K.M. and M.G.M. and carried out by N.K.M. and A.M. for the copepods (ESM 5). Behavioural experiments were carried out by V.S. (experiment 1) and N.K. (Experiment 2). Data analysis was done by N.A. and N.K.M. The paper was written by N.K.M. All authors approved the final version of the manuscript.

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TABLES

Table 1. Best models from a series of hierarchical GLMM testing the effect of the presence of copepods and background colouration on the total duration (*n* images) of red (A) and blue (B) ocular sparks.

(A) Red ocular spark				
Parameter	**Estimate** (logit-scale)	**SE**	***z***	***P***
Intercept	-1.476	0.198	-7.449	< 0.0001
Copepods	0.671	0.165	4.066	< 0.0001
Background	-1.313	0.232	-5.661	< 0.0001
Treatment order	0.132	0.057	2.324	0.0201
*Copepods*Background*	-0.987	0.255	-3.830	0.0001
(B) Blue ocular spark				
Parameter	**Estimate** (logit-scale)	**SE**	***z***	***P***
Intercept	-2.895	0.169	17.104	<0.0001
Copepods	-0.189	0.237	-0.796	0.4263
Background	1.549	0.227	6.809	<0.0001
*Copepods*Background*	0.721	0.316	2.281	0.0225

Marginal R^2: 17.1 % for red and 17.2 % for blue ocular sparks. The sequence order in which treatments were offered had an effect in (A) and was therefore maintained in the model to compensate for its effect. All other parameters that were initially in the model were removed because of their insignificant contribution (See Methods 2.7). Parameter significance is assessed using Wald *z*-tests.

Table 2. Best models from a series of hierarchical GLMM testing the effect of satiation status and background on the total duration (*n* images) of red (A) and blue (B) ocular sparks.

(A) Red ocular spark				
Parameter	**Estimate** (logit-scale)	**SE**	***z***	***P***
Intercept	0.022	0.482	0.047	0.963
Satiation status	-0.297	0.287	-1.033	0.302
Background	-1.963	0.308	-6.369	<0.0001
Observation day (1-8)	-0.147	0.047	-3.124	0.002
*Satiation status * Background*	0.121	0.436	0.277	0.781
(B) Blue ocular spark				
Parameter	**Estimate** (logit-scale)	**SE**	***z***	***P***
Intercept	-1.319	0.247	-5.325	<0.0001
Satiation status	0.216	0.193	1.115	0.265
Background	0.808	0.235	3.439	0.0005
Fish testing order	-0.026	0.012	-2.173	0.0298
*Satiation status * Background*	-0.223	0.271	-0.824	0.4099

Marginal R^2: 13.8 % for red and 3.3 % for blue ocular sparks. Significant confounding factors were kept in the model to compensate for their effect (Variables "observation day rank number" for red ocular sparks and "daily fish testing order" for blue ocular sparks). All other parameters that were initially in the model were removed because of their insignificant contribution (See Methods 2.7). Parameter significance is assessed using Wald *z*-tests.

FIGURES 1 through 4

(included here, will be submitted as separate files in better quality in case of acceptance)

Figure 1. Bright eyes in fish can be caused by luminescent mechanisms such as chemiluminescence in nocturnal fish (1) or photoluminescence (fluorescence) in diurnal fish (2). Many more species show forms of reflection off the iris (3-16) or cornea (17-18). This study focuses on so-called "ocular sparks", a mechanism where fish focus downwelling light onto their own iris (19-22, see also figure 2) (species names in supplementary material, data **S**1, photos by N.K.M.).

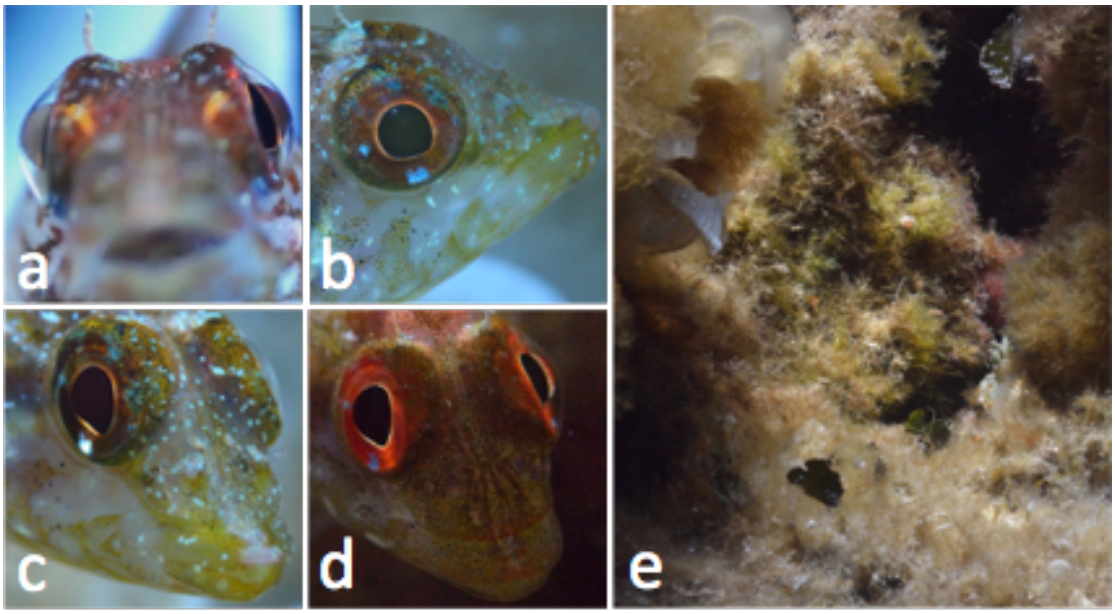

Figure 2. Ocular radiance by ocular sparks in the triplefin *Tripterygion delaisi*. Downwelling light is focused by the laterally protruding lens of the eye (**a**, frontal view) onto the lower iris, which features red fluorescent and blue-reflective areas (**b**). By tilting and rotating the eye, fish can position the focusing spot on a blue iridophore area resulting in a blue ocular spark (**c**) or a red reflective/fluorescent area, resulting in a red ocular spark (**d**). This is hypothesized to generate reflections in nearby target species in shady parts of the natural light environment (**e**). Note that in (**c**) the blue ocular spark is overexposed, and therefore brighter in reality (see also supplementary material, video S2). Pictures taken in the field by N.K.M.

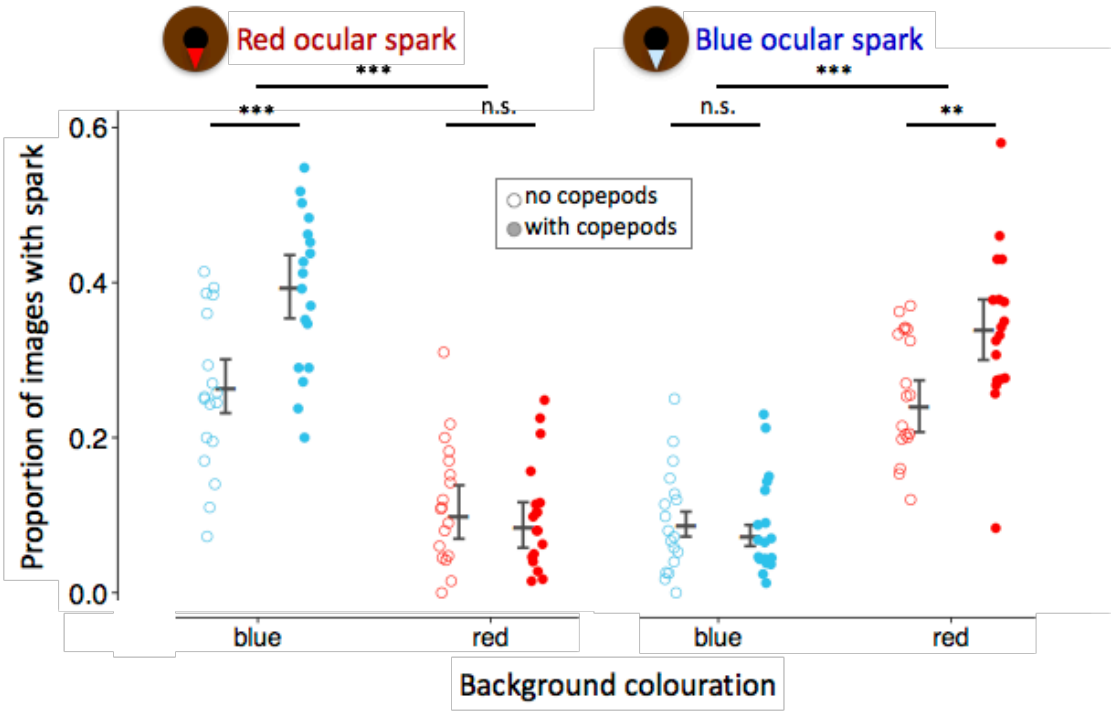

Figure 3. Proportion of observations as a measure of total time during which red (left) and blue (right) ocular sparks were in the "ON" state in the absence (open points) or presence (filled points) of copepod prey against two backgrounds (blue or red). Data points represent averages for each of 17 individuals. Vertical error bars indicate model prediction (central line) with 95% credibility interval (flags) for each treatment group. Significance labels (n.s. = not significant, ** $P < 0.01$, *** $P < 0.001$) indicate planned contrasts between backgrounds, and between copepod treatments within backgrounds.

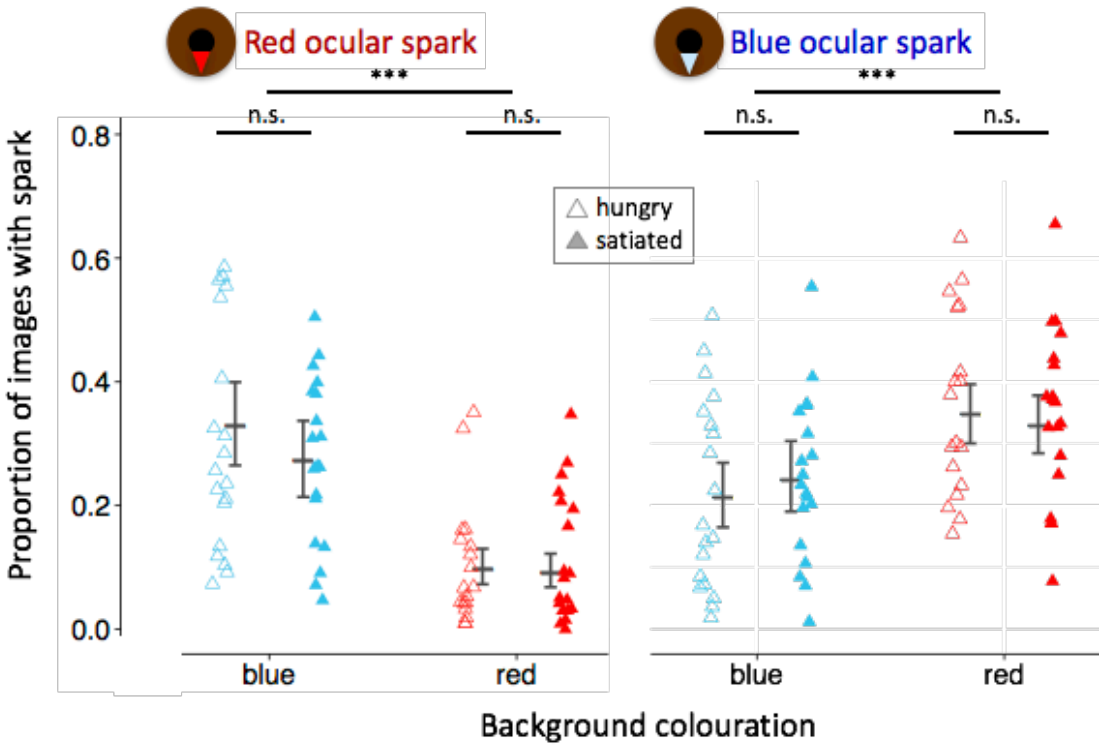

Figure 4. Proportion of observations as a measure of total time during which red (left) and blue (right) ocular sparks were in the "ON" state in hungry (open triangles) and satiated (filled triangles) fish against blue or red backgrounds. Data points represent averages for each of 19 individuals. Vertical error bars indicate model prediction (central line) with 95% credibility interval (flags) for each treatment group. Significance labels (n.s. = not significant, *** $P < 0.001$) indicate planned contrasts between backgrounds, and between satiation treatment within backgrounds.