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Support for the dimorphic niche hypothesis: Natural selection drives sexual eye dimorphism in ostracods.

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by

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Abstract

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Sexual dimorphisms usually arise in traits that define sexes, mainly primary and secondary sexual characteristics. Some instances of sexual dimorphisms do not fall directly into these categories and may not be a direct result of sexual selection. The dimorphic niche hypothesis states that sexual dimorphisms can arise when there are differences in the lifestyles in males and females because of differences in reproductive roles. In *Euphilomedes* ostracods, both males and females spend a majority of their lives in the sediment, they swim into the water column to mate. Males can likely mate many times, while females possibly only mate once, putting males at a greater risk of predation because they spend more time in the water. Males have two compound lateral eyes while females do not. In place of the compound eye, the females have an eye “rudiment”. We hypothesize male eyes are an adaptation to escape predation and tested the hypothesis that sexually dimorphic eyes in *Euphilomedes* have evolved through natural selection acting on the differing lifestyles of males and females two ways: direct experimentation and morphological measurements of optimality. We experimentally blindfolded two species of ostracods, *Euphilomedes chupacabra* and *Euphilomedes morini,* and compared the survival of the blindfolded and control ostracods in the presence of a predator. Blindfolded *Euphilomedes* had a significantly lower survival rate than controls. We also examined the spatial vision of male ostracods and determined distances at which they could resolve a predatory fish as well as at what distance a predatory fish could see an ostracod. These distances are very similar, suggesting that the *Euphilomedes* eyes are optimized for seeing predators. Additionally, the distance at which a male can see a conspecific female is so close that eyes cannot be used for long distance mate detection. Though we have not experimentally eliminated sexual selection as a cause for the sexually dimorphic eyes, our results indicate that the compound eyes in males may be a result of natural selection acting on the reproductive behavior differences between males and females and we believe we are presenting the first experimental evidence for the dimorphic niche hypothesis.

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Chapter 1: A review of the causes of sexual dimorphism.

Sexual dimorphisms are morphological or behavioral differences between the males and females of the same species. Though sexual selection is commonly considered to be the driving force behind sexual dimorphisms, natural selection can also play an important, if frequently overlooked, role. Here we review how the two types of selection can result in sexual dimorphisms.

Sexual Selection

Darwin (1871) first proposed the theory of sexual selection as competition (specifically between males) and mate choice (specifically by females) resulting in differences between the sexes. Though we know now that there can be female competition and male choosiness as well (Jennions and Petrie 1997), the aforementioned two modes are still what defines sexual selection. Two main types of differences can arise due to sexual selection: either sexual size dimorphisms (in overall body size or the size of an individual structure) or ornamentation dimorphisms (such as color variations); both can result from either competition, mate choice, or a combination of both (Andersson 1994). These characters are sometimes contrary to the demands of natural selection and seemingly reduce fitness because of energetic needs of creating dramatic ornamentation (eg. antlers, Ditchkoff et al 2001) or by making one sex more conspicuous or slow to react to predators (Andersson 1994). However, if this same character still results in greater reproductive success, it is considered to be a result of sexual selection (Hendrick and Temeles 1989).

Natural Selection

Natural selection, through ecological differences between the sexes, can also be a driving force for sexual dimorphism through three main modes: the bimodal niche hypothesis, the competitive displacement hypothesis, or the dimorphic niche hypothesis (Slatkin 1984, Hendrick and Temeles 1989). These three hypotheses do not necessarily act independently of each other and may actually reinforce each other in some cases. Unfortunately, most studies involving ecological causes of sexual selection are based on correlative data. It is often extremely difficult to experimentally manipulate or evaluate the origin of a sexual dimorphism and instead, the causes must be based on the behaviors and/or morphologies that can be measured.

Bimodal Niche

The bimodal niche hypothesis says that there are two possible optimal sizes for a trait (regardless of sex) in a species and that males and females have evolved separately to those optima (Slatkin 1984). There are few examples which support this hypothesis. In some *Anolis* lizard species, multiple body size optima may exist, depending on predation method (Schoener 1977 as cited in Slatkin 1984; Schoener 1969). Males may use one tactic while females may employ another to eat similar prey, leading to sexual dimorphism. This dimorphism may be further enhanced by reducing competition between the sexes, though the dimorphism may not have originated because of intraspecific competition. Though two optima may exist, Slatkin (1984) argued that this alone is an unlikely driving force for sexual dimorphism and this hypothesis and has been largely ignored in later reviews of ecological causes of sexual dimorphism (Hedrick and Temeles 1989; Shine 1989).

Competitive Displacement

The competitive displacement hypothesis argues that when there is competition between males and females for food, the two can evolve sexually dimorphic feeding structures instead of competing with each other (Slatkin 1984). There are numerous examples of sexually dimorphic trophic structures which are all correlative between the size and/or strength of a trophic structure and the type of food eaten. For example, male and female diamondback terrapins (*Malaclemys terrapin*) eat similar foods when they are young and similarly sized but as females grow larger heads than males, they eat different foods. Tucker et al (1995) argued that because of the dietary specialization between males and females as well as the habitats that the prey are found, the differences in head shape and size are due to resource partitioning between the sexes.

Dimorphic Niche

Finally, the dimorphic niche hypothesis argues that when there are behavioral or energetic differences due to reproductive behaviors, sexual dimorphisms can arise (Slatkin 1984). One such example of a sexually dimorphic behavior related to reproductive roles is sexual segregation. When a species is a sexually segregated, males and females occupy different habitats for some or all of their lives. In different habitats, there may be differences in resource quantity and/or quality or predation risk which may apply to each sex separately (Slatkin 1984). In cases where predation risk differs between the sexes due to segregation, it is important to distinguish whether the sexual segregation has led to the differences in predation risk or if other factors influencing predation risk have led to sexual segregation. Examples of both situations have been documented in nature, and we discuss a few below.

The sexual segregation of many species of ungulates including mountain sheep

(*Ovis canadensis*), caribou (*Rangifer tarandus*), huemel (*Hippocamelus bisulcus*), mule deer (*Ododoileus hemionus*) and red deer (*Cervus elaphus*) has been well studied and is likely an example of how differences in predation risks can lead to sexual segregation (Main et al 1996). Males do not give parental care and instead prepare their bodies for the next mating season through copious consumption of food. Males then choose to live in areas with ample nutritious vegetation but without shelter from predators. Because the male only has to look after himself, if a predator approaches he may have a good chance of escaping. However, calving females have to take care of their young, and tend to live in safer, but less resource rich areas. The predation risk hypothesis (also known as the reproductive strategy hypothesis) likely applies in this example, as differences in predation risk are thought to underlie sexual segregation in ungulates (Main et al 1996). Differing predation risks between males and females are also thought to cause sexual segregation in the guppy *Poecilia reticulata*. Females are larger than the males, which makes the males more vulnerable to predation. Males also tend to inhabit more shallow waters. Large predators have difficulty swimming into the shallow waters where males spend most of their time and therefore males are safer there (Croft et al 2006). There is not definitive evidence that the differences in predation risks led directly to the sexual segregation of these species, and it is theoretically possible that their other sexual dimorphisms may have evolved *after* sexual segregation occurred. It is also possible that sexual selection may have played a role in the evolution of these traits as well. However, it is very likely that these scenarios support the predation risk hypothesis.

Alternatively, sexual segregation may originally occur for reasons other than predation risk but this segregation can ultimately lead to differences in predation risk between males and females. In the winter moth *Operophtera brumata*, females spend the majority of their lives on branches waiting for males to mate with them and conversely, males fly to find females (Meyer-Rochow and Lau 2008). These moths exhibit a dramatic sexual dimorphism in that females are functionally wingless and incapable of flight. Additionally, males have eyes with more and larger ommatidia and a smaller interommatidial angle than females. This allows males to have better spatial resolution and, likely, to be better able to escape predators and maneuver around objects while flying to find a mate (Meyer-Rochow and Lau 2008). Interestingly, another species of moth, *Acentria ephemerella*, has similarly winged males and wingless females, however the females have modified limbs for swimming (Lau et al 2007). These females actively swim, as opposed to their sedentary counterparts in *O. brumata* and spend most of their adult lives as aquatic insects. Males fly near the surface of the water to search for a mate, attracted to pheromones that females release. The eyes of the male and female *A. ephermerella* are much more similar in size, interommatidial angle, and number and size of ommatidia as compared to the eyes of male and female *O. brumata*. Because of the differences in the lifestyle of the female *A. ephermerella*, they are likely at a higher risk of predation and have a greater use for strong vision as compared to *O. brumata* (Lau et al 2007). It is likely that these differences in eye structure are a result of the different predation risks of the males and females of each species and the dimorphic niche hypothesis helps explain how these morphological sexual dimorphisms can arise.

The Combined Influence of Natural and Sexual Selection

Natural and sexual selection are not necessarily independent of each other. As mentioned earlier, sometimes natural and sexual selection will favor different traits, but other times, the two may favor similar traits and they can strengthen the influence of each other. It is also possible that sexual selection can reinforce sexual dimorphisms that arose through natural selection or vice versa. This seems to be the case with some examples of sexual size dimorphisms (Hendrick and Temeles 1989). It is important to consider that the two types of selection are not always discrete processes.

Chapter 2: Support for the dimorphic niche hypothesis: Natural selection drives sexual eye dimorphism in ostracods

Introduction

Sexual dimorphisms, prevalent in many animal taxa, are generally thought to consist of differences between male and female primary sexual characteristics, such as gonads, and secondary sexual characteristics, including colors and sizes. Most of these types of sexual dimorphisms are thought to be primarily a result of sexual selection. However, there are other types of sexually dimorphic traits where some of these differences may not be due to sexual selection. Males and females of the same species can, for example, differ in their visual capabilities and visually-influenced behaviors. These types of sexual dimorphisms may sometimes be due to different predation risks experienced by males and females and therefore may be driven by natural selection rather than sexual selection.

The dimorphic niche hypothesis (also known as the reproductive role hypothesis) explains one way sexual dimorphisms can arise due to natural selection, and not sexual selection (Slatkin 1984). According to the dimorphic niche hypothesis, morphological differences arise when males and females have different reproductive behaviors, such as sexual segregation (Slatkin 1984). Sexual segregation occurs when males and females of the same species occupy different habitats. Sexual segregation is exhibited by many diverse species. For example, female winter moths live sedentary lives on branches and winged males actively fly to find mates (Meyer-Rochow & Lau 2008). Female mountain ungulates and their calves live in safe, nutrition-poor areas while males live in more dangerous, nutrition-rich areas (Main et al. 1996). Among other differences, sexual segregation can lead to differing predation risks for males and females, which may in turn impact the evolution of many traits (Slatkin 1984). Regrettably, the dimorphic niche hypothesis is often difficult to test experimentally; instead hypotheses are made about the origins of the dimorphism based on the lifestyle of the animal. (eg. Ebenman 1986; Cowley & Atchley 1988)

Euphilomedes ostracod crustaceans possess unique sexually dimorphic traits and behaviors which are appropriate for testing the dimorphic niche hypothesis in laboratory experiments. Male and female *Euphilomedes* are sexually segregated for parts of their lives. Both males and females spend the majority of their lives burrowing in the sediment, but swim out of the sediment and into the water column to mate (Lum et al. 2008; Macquart-Moulin 1999). Males likely mate on multiple nights and possibly every night as adults, however in a related family of ostracods, there is evidence that females may mate only once (Cohen 1983; Kornicker 1978). Thus, the males likely spend a much greater portion of their lives exposed in the water as compared to females. Because of this, we hypothesize that males are at a greater risk from pelagic predators than females. Such differences in predation risk may explain why certain *Euphilomedes* ostracods (as well as many other species in the order Myodocopida) exhibit not only sexually dimorphic mating behavior, but sexually dimorphic eyes as well (Lum et al 2008, Macquart-Molin 1999, Kornicker and Harrison-Nelson 1997, Cohen and Morin 1990, Cohen 1983). Adult male *Euphilomedes* have two anteriodorsally located lateral compound eyes that, depending on the species surveyed, can have 20-33 ommatida (Figure 1a, d). Most females lack these eyes, possessing only eye “rudiments” which lack ommatidia (Figure 1b, c). The males also have a comparable, though larger, eye rudiment at the base of each compound eye (Figure 1d, e) (Rivera and Oakley 2009; Lum et al. 2008; Kornicker and Harrison-Nelson 1997).

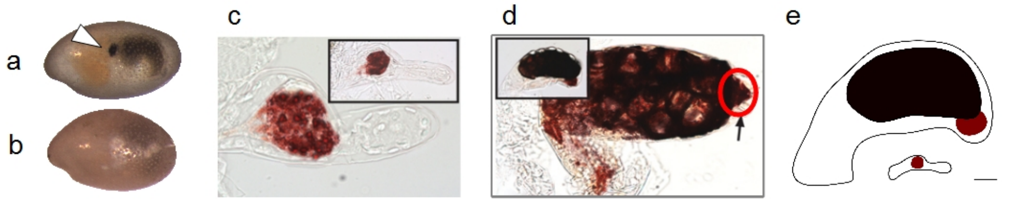

Figure 1. (a) Adult *E. morini* male with pointer indicating lateral compound eye. (b) Adult *E. morini* female. (c) Adult *E. carcharodontra* (a similar *Euphilomedes* species) female eye “rudiment” under cover slip (inset is whole-mount). (d) *E. carcharodontra* male compound eye under cover slip (inset is whole-mount). Pointer and circle indicate eye “rudiment” at far right. (e) Size comparison of male compound eye (brown) and “rudiment” (red) and female “rudiment”. Scale bar: 30 um. Images c-e from Rivera and Oakley 2009 with permission.

We hypothesize that the compound eyes in male *Euphilomedes* are an example of a sexually dimorphic trait that is explained by the dimorphic niche hypothesis. We predict that these eyes are an adaptation that helps male ostracods detect and avoid predators during their increased time out of the sediment. We tested our hypothesis by examining whether vision allowed for increased survival rates of two species of *Euphilomedes* ostracods, *E. morini* from the California coast and *E. chupacabra* from the Caribbean, when they were confronted with a pelagic predatory fish. While the correlation between increased predation risk and increased visual abilities (Glazier and Deptola 2011) and more specifically, increased predation risk on one sex resulting in sexually dimorphic vision (Meyer-Rochow and Lau 2008; Lau et al. 2007) has been suggested before, we believe this is the first time that the dimorphic niche hypothesis has been tested experimentally, for any trait.

Methods

Collection of Euphilomedes

We collected male *E. chupacabra* from surface waters off of Isla Magueyes near La Parguera, Puerto Rico (17° 57’ 45.72” N, 67° 2’ 14.28” W) using an aquarium net. The water depth at the collection site was less than 1 meter. We collected ostracods in September 2010 at night during their most active period which was between the hours of 12:00 and 12:30 AM, approximately six hours after sunset. This is different than what was reported previously by Lum et al who found that males were most active approximately two hours after sunset (2008). The difference in the active period may be due to seasonal cues or another unknown factor. Ostracods were collected from a well-lit pier where the light level was 25 lux at the surface of the water. Ostracods were not found in the water column at unlit piers (< 1 lux). All lux readings were taken with a Milwaukee Smart Luxmeter SM700 (Milwaukee Instruments, Rocky Mount, NC). Only adult males were collected using these methods.

We collected *E. morini* from the sediment below Stearn's Wharf Pier in Santa Barbara, CA (34° 24′ 38.41″ N, 119° 41′ 14.5″ W) using an Ekman grab (Wildco, Buffalo, NY, USA) and a 500 micron sieve. The water depth at this location was between 5 and 7 meters, depending on the tide. At this site, we collected adult and juvenile males and females, though regardless of the time of year, the vast majority collected were brooding females. We separated *E. morini* by instar based on size and identified males by the presence of lateral eyes and separated them from females. For the phototaxis and predation trials, we collected ostracods between the hours of 9:00 AM and 3:00 PM from June 2009 through August 2010. For morphological measurements, we collected *E. morini* at mid-day on February 1, 2011.

Blindfolding of male Euphilomedes

To blindfold ostracods for predation experiments, we superglued squares of black ribbon (0.4mm^2) to both lateral sides of the carapace using the quick drying cyanoacrylate adhesive StickFast Black Flexible Glue (TMI Products, Peachtree City, GA). These blindfolds were carefully placed on the carapace over the compound eyes. As a control for the blindfolding procedure, we glued ribbon on the carapace over the posterior end (not covering the eye area) of a separate set of specimens. For both "blindfolded" and "control" specimens, we glued ribbon on one side of an ostracod at a time, and then allowed the glued ostracods to recover for several hours before gluing the opposing side. We then allowed the ostracods to recover for several more hours before using them in the predation trials that followed. Some ostracods did not survive the blindfolding process. These trials also included "unaltered" ostracods, which had no ribbon glued on their carapace and were used as an additional control for our experiments. (Figure 2)

Figure 2. Three treatments of male *Euphilomedes* used in phototaxis and predation experiments: unaltered (a), control (b) and blindfolded (c).

Phototaxis of male Euphilomedes

To determine whether unaltered adult male ostracods were negatively phototactic, we filled a bowl with seawater and lined it with sand. A Schott Ace Fiber Optic Light Source (SCHOTT North America, Elmsford, NY) was shone over the bowl. We covered half of the bowl with a piece of cardboard, producing a 50:50 light:dark setting. The illuminance on the light and dark halves of the bowl was approximately 24,000 and 130 lux, respectively. We then placed male ostracods in the center of the bowl at the light/dark interface. After one hour, we counted the number of ostracods in the light and dark halves of the bowl. We then moved the cardboard covering half of the bowl to the opposing half and left the bowl for another hour to ensure that the ostracods were actively moving away from the light. These experiments were conducted between the hours of 9:00 AM and 4:00 PM. The same procedure was performed for both *E. chupacabra* and *E. morini* and was repeated for the control and blindfolded male treatments to determine if their phototaxis changed with their respective treatments. The results from the first hour and second hour were examined separately.

Predation of male and female Euphilomedes

Predation experiments were performed for both *E. cupacabra* and *E. morini*. For the experiments with *E. chupacabra*, we used 38-liter aquarium tanks which were 50cm long, 10cm wide, and 30cm deep. We used seawater pumped from off Isla Magueyes which first passed through a 1µm mesh filter. The predation experiments were conducted outside, adjacent to the Isla Magueyes Marine Laboratory. There were outdoor lights outside of the building that provided an overnight illuminance of 5 to 15 lux, as measured from a location next to the tanks. We lined each tank with 3 liters of sand that had grains smaller than 500µm in diameter. We conducted the experiments from 6:00 PM to 8:00 AM in September 2010 when sunrise occurred around 6:15 AM and sunset occurred around 6:30 PM.

In each experiment conducted in Puerto Rico, we used three experimental *E. chupacabra* treatments: unaltered males, control males and blindfolded males. We placed 8 ostracods from each treatment (for a total of 24) in each tank, along with a juvenile fish that was either a silverside (*Menidia* sp.) or a cottonwick grunt (*Haemulon melanurum*). We chose these fish because we observed both feeding on ostracods in their natural environments; both species were collected using nets during the active period of *E. chupacabra*. We removed the fish at 8:00 AM the following morning and siphoned the sand out over a 500µm sieve in order to separate the ostracods from the sand. We then counted the number of surviving ostracods.

For the experiments with *E. morini*, we conducted the experiments in 120-liter seawater tanks which were approximately 60cm deep, 100cm wide and 20cm long. These tanks were housed inside the Marine Science Research Building at the University of California - Santa Barbara (UCSB). We obtained seawater through UCSB's offshore intake system which passes water through a rock-stone-gravel-sand media filter that removes particles larger than 20µm. The lighting included two Hamilton Technology 50/50 Actinic White and two Hamilton Technology Actinic Blue 70-inch bulbs. The bulbs all turned on at 8:00 AM and then turned off in 30 minute increments starting at 6:30pm to simulate sunset. At 7:30pm, three Lunar Link Moon White LED moonlights turned on and stayed on until 2:00 AM. We lined each tank with 5 liters of sand with grains smaller than 500µm in diameter. The sand was autoclaved prior to being put in the tank to remove any other living matter. We conducted the experiments from between 4:00 PM on the first day through 11:00 AM on the third day from November 2009 through August 2010.

In the predation experiments conduced at UCSB using *E. morini*, we used the same three experimental treatments that we used in Puerto Rico: unaltered males, control males and blindfolded males. We placed ten to fifteen male ostracods from each treatment (for a total of 30-45) in each experimental tank. While the number of ostracods varied slightly from experiment to experiment, we use an equal number of each treatment in each experiment. We placed one shiner perch (*Cymatogaster* *aggregata*), a known predator of *Euphilomedes* ostracods (Hobson and Chess 1986), in the tank as well. We removed the fish after two days and siphoned the sand out over a 500µm sieve in order to separate the ostracods from the sand. We then counted the number of surviving ostracods.

In addition, we repeated our experiments with *E. morini* brooding females (which lack compound eyes). For the “blindfolded” the females, the square of ribbon was superglued on the carapace over their lateral eye rudiment and for the “control”, the square of ribbon was superglued on the carapace over their brood. Unaltered females were used in these experiments as well. The experimental procedures for *E. morini* males were then repeated with *E. morini* brooding females and were performed between March and July 2010.

Statistics

For the phototaxis trials, we used one-tailed Fisher's exact tests to determine whether the phototaxis of *E. chupacabra* and *E. morini* deviated from the expected proportion of 0.5 in each light condition. We looked at the first hour and second hour separately to determine if there was an additional phototaxis effect after the light conditions reversed. We then repeated this test with the control and blindfolded males to determine if those processes altered the phototaxis.

We analyzed both the *E. chupacabra* and the *E. morini* predation trials with directed Fisher's exact tests (Rice and Gaines 1994), to compare survival versus predation in a small sample size. We compared control males with blindfolded males to determine whether the ostracods were using their lateral compound eyes to detect and avoid a predator. We then compared unaltered males with control males to determine if gluing a small piece of ribbon on the carapace affected the survival in the presence of a predator. We also analyzed the *E. chupacabra* predation trials with a one-tailed binomial test to determine if the number of trials in which more blindfolded than control males were eaten was significant. However, there were not enough trials with either the male or brooding female *E. morini* predation experiments to use a binomial test. Finally, to determine the predation risk on adult males versus brooding females, we compared the total number of surviving male versus female ostracods from all treatments using a two-tailed Fisher's exact test.

Attempted mating experiments with E. morini

To determine if the compound eyes in males improve the male's ability to find a mate, we attempted mating experiments. To do so, we had to raise females from a juvenile stage to ensure that they were virgins so that any embryos that developed were a result of mating during experimentation. Raising females was a time consuming process because of the long instar stages of a month or more and a high mortality rate before adulthood. Additionally, a small number of females raised in isolation from males from the adult minus two instar to the adult stage developed eggs within their carapace. We do not know if these eggs were fertilized, if the females mated as juveniles and stored the sperm until their adult stage, or if the embryos arose asexually. This posed an additional problem because if females can produce embryos without being exposed to males as adults, it would be difficult to determine if embryos produced after an experiment are due to mating during the experiment or not. We abandoned these experiments and could not experimentally test if males use their compound eyes in mating.

Eye morphology and visual acuity in male Euphilomedes morini

Specimens were fixed in a seawater-buffered 3.7% formalin solution for 4 hours, then rinsed and stored in sterile (autoclaved) phosphate buffered saline (PBS). Ostracods were sectioned with a Leica CM1900 cryostat microtome (Leica, Solms, Germany); sections were mounted on glass slides using Hydro-Matrix solution (MicroTech Lab, Graz, Austria). We viewed sections with either an Olympus (Melville, NY, USA) dissecting scope or the 10 or 40x objectives of an Olympus BX61 compound microscope. Images were obtained with a Microfire digital camera operated via PictureFrame software (Optronics, Goleta, CA, USA) and then processed using Helicon de-convolution software (Helicon Soft, Kharkov, Ukraine) and/or Picture Publisher (Micrografx Inc., Richardson, Texas). From our sections, we measured the angle between individual ommatidia to find $\Delta\varphi$, the sampling (or inter-ommatidial) angle for appostion compound eyes (Land and Nilsson 2002).

Results

Phototaxis experiments

Both *E. chupacabra* and *E. morini* adult males are negatively phototactic. This was confirmed with both the first hour of the phototaxis experiment as well as the second ($p < 0.001$ for both species in both the first and second hour, Figure 3, Tables 1 and 2). The blindfolded males displayed no significant phototaxis and this was also confirmed with the results from both the first and second hour ($p = 0.997$ for the first hour and $p = 0.419$ for the second with *E. chupacabra*, Figure 3a, Table 1, and $p= 0.500$ for the first hour and $p = 0.346$ for the second with *E. morini*, Figure 3b, Table 2). The control *E. chupacabra* males were negatively phototactic in the first hour ($p = 0.001$, Figure 3a, Table 1) but not in their second hour ($p = 0.115$, Figure 3a, Talbe 1). The *E. morini* males were not phototactic in the first hour ($p = 0.148$, Figure 3b, Table 2) but they were in their second hour ($p = 0.002$, Figure 3b, Table 2). These inconsistent results are likely the result of a low sample size and we proceeded with our experiments under the assumption that control males were negatively phototactic.

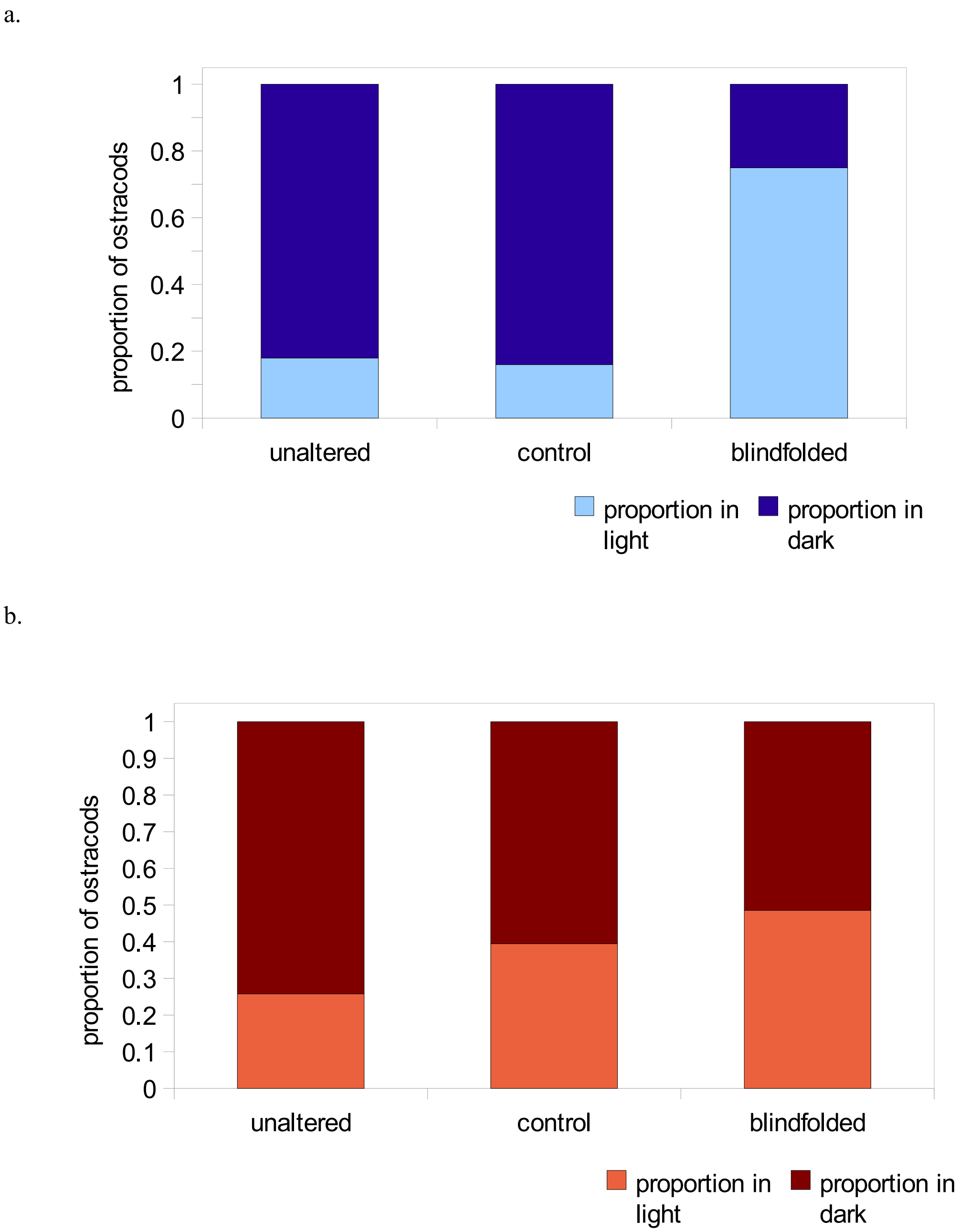

Figure 3. Both *E. chupacabra* and *E. morini* unaltered males are negatively phototactic ($p < 0.001$ for both species). Blindfolded males are not phototactic in both *E. chupacabra* $p = 0.997$ (a) and *E. morini* $p = 0.500$ (b). Control males were negatively phototactic in *E. chupacabra* $p < 0.001$ (a) but not in *E. morini* $p = 0.148$.

Table 1. Phototaxis of *E. chupacabra*

	First Hour: Light	First Hour: Dark	Second Hour: Light	Second Hour: Dark
Normal Unaltered Males	18	82	20	80
Control Males	4	21	9	16
Blindfolded Males	18	6	11	13

Table 2. Phototaxis of *E. morini*

	First Hour: Light	First Hour: Dark	Second Hour: Light	Second Hour: Dark
Normal Unaltered Males	36	104	12	128
Control Males	13	20	8	25
Blindfolded Males	16	17	15	18

Predation experiments

In the presence of predatory fish, blindfolded *E. chupacabra* males had a lower survival rate than control males. We recovered a significantly smaller number of blindfolded males after being exposed to a predator as compared to control males ($p_{dir} = 0.016$, Figure 4a, Table 3), whereas there was no significant difference between the number of recovered unaltered and control males ($p_{dir} = 0.630$). There were also significantly more *E. chupacabra* trials in which more control males were recovered than blindfolded males ($p = 0.006$; Figure 4a, Table 3). Blindfolded *E. morini* males also had a lower survival rate in the presence of a predator than control males ($p_{dir} = 0.043$, Figure 4b, Table 4). One trial was not included in the analysis because the experimental predator gave birth to six live fry during the experiment, therefore increasing the number of predators. In this excluded trial, 9 of each treatment of male were used and 3 blindfolded males, 1 control male, and 7 normal unaltered males were recovered at the end of this experiment. There was no significant difference in the number of surviving unaltered *E. chupacabra* recovered as compared to control males ($p_{dir} = 0.630$, Figure 4a, Table 3), however there was a significant difference in the number of surviving unaltered *E. morini* males as compared to control males ($p_{dir} < 0.001$, Figure 4b, Table 4).

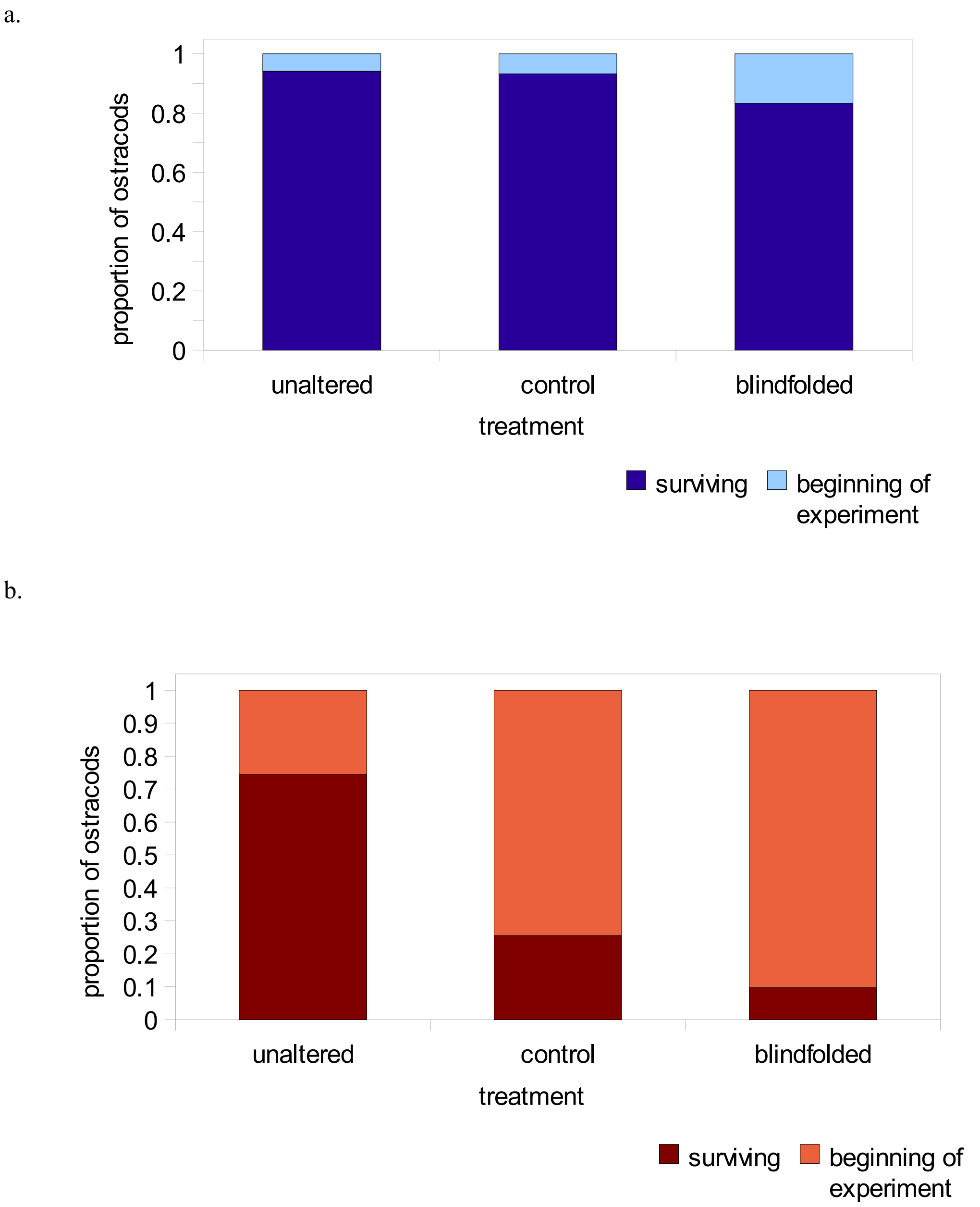

Figure 4. Equal numbers of unaltered, control and blindfolded males were placed in a tank with one predatory fish. Surviving ostracods were counted. Blindfolded males had a greater risk of predation as compared to control males in both *E. chupacabra* $p_{dir} = 0.016$ (a) and *E. morini* $p_{dir} = 0.043$ (b). There was no difference in the number of surviving unaltered *E. chupacabra* recovered as compared to control males ($p_{dir} = 0.630$), however there was a difference in the number of surviving unaltered *E. morini* males as compared to control males ($p_{dir} < 0.001$).

Table 3. Predation experiments with *E. chupacabra* males.

Trial No.	Total number of ostracods in each treatment	Unaltered males recovered (Number Missing)	Control males recovered (Number Missing)	Blindfolded males recovered (Number Missing)
1[1]	8	7 (1)	7 (1)	7 (1)
2[1]	**8**	**2 (6)**	**4 (4)**	**2 (6)**
3[1]	8	8 (0)	8 (0)	8 (0)
4[1]	**8**	**8 (0)**	**7 (1)**	**6 (2)**
5[1]	**8**	**8 (0)**	**8 (0)**	**6 (2)**
6[1]	**8**	**8 (0)**	**8 (0)**	**7 (1)**
7[1]	**8**	**8 (0)**	**8 (0)**	**6 (2)**
8[1]	**8**	**8 (0)**	**7 (1)**	**6 (2)**
9[2]	**8**	**8 (0)**	**8 (0)**	**7 (1)**
10[1]	**8**	**8 (0)**	**8 (0)**	**7 (1)**
11[1]	8	8 (0)	8 (0)	8 (0)
12[1]	8	8 (0)	8 (0)	8 (0)
13[1]	**8**	**8 (0)**	**8 (0)**	**7 (1)**
14[1]	**8**	**8 (0)**	**8 (0)**	**7 (1)**
15[2]	**8**	**8 (0)**	**7 (1)**	**8 (0)**
Total	120	113 (7)	112 (6)	100 (20)

[1] Trials which used *Menidia sp.* as a predator.

[2] Trials which used *Haemulon melanurum* as a predator.

Bolded trials are those in which the number of recovered blindfolded males was different from the number of recovered control males.

Table 4. Predation trials with *E. morini* males.

Trial No.	Total number of ostracods in each treatment	Normal unaltered males recovered (Number Missing)	Control males recovered (Number Missing)	Blindfolded males recovered (Number Missing)
1	10	6 (4)	1 (9)	0 (10)
2	14	10 (4)	4 (10)	0 (14)
3	15	10 (5)	8 (7)	5 (10)
4*	9	7 (2)	1 (8)	3 (6)
5	12	12 (0)	0 (12)	0 (12)
Total	60	45 (15)	14 (46)	8 (52)
Total without 4*	51	38 (13)	13 (38)	5 (46)

*Trial in which the predatory fish gave birth to six live fry during the trial.

There was no significant difference between the survival of blindfolded and control *E. morini* females ($p_{dir} = 0.630$, Figure 5, Table 5). However, there was a significant difference between the unaltered females and the control females ($p_{dir} < 0.001$, Figure 5, Table 5). These results suggest that the act of gluing ribbon on the ostracod did reduce survival in brooding females; however the different locations on the carapace where the ribbon was glued did not significantly influence survival rates for female ostracods. Finally, we found that *E. morini* brooding females are, in general, at a lower predation risk than males. We found that a significantly larger proportion of unaltered females than unaltered males survived in the presence of a predator ($p < 0.0001$, Figure 6, Table 6).

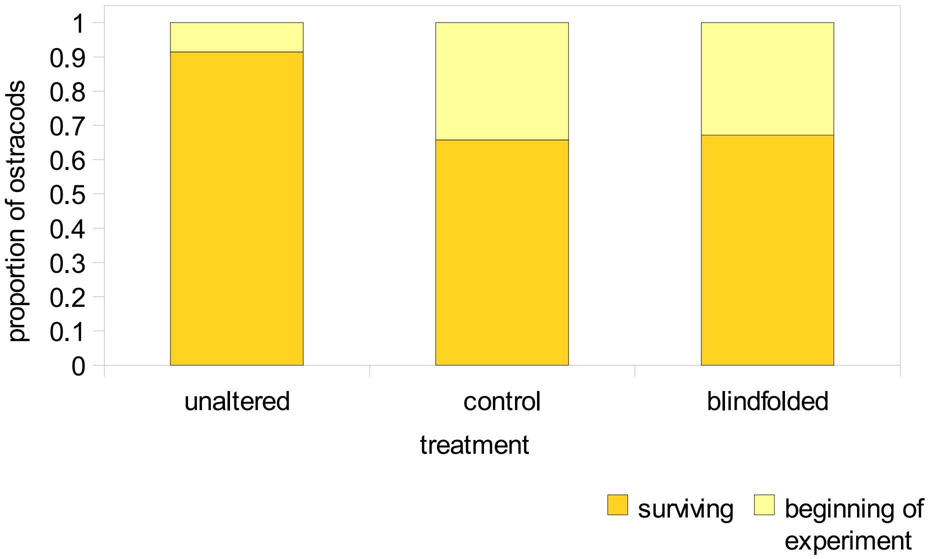

Figure 5. Equal numbers of unaltered, control and blindfolded brooding females were placed in a tank with one predatory fish. Surviving ostracods were counted. There is no difference in the predation risk between control and blindfolded brooding *E. morini* $p_{dir} = 0.630$.

Table 5. Predation trials with *E. morini* brooding females.

Trial No.	Total number of ostracods in each treatment	Normal unaltered females recovered (Number Missing)	Control females recovered (Number Missing)	Blindfolded females recovered (Number Missing)
1	12	12 (0)	12 (0)	12 (0)
2	15	14 (1)	8 (7)	5 (10)
3	15	12 (3)	3 (12)	4 (11)
4	15	15 (0)	13 (2)	15 (0)
5	13	11 (2)	10 (3)	11 (2)
Total	70	64 (6)	46 (24)	47 (23)

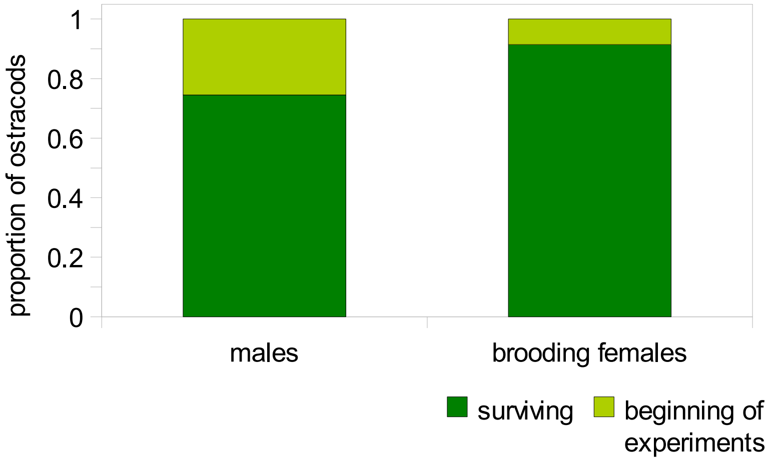

Figure 6. Unaltered male and brooding female *E morini* were placed in separate tanks with one predatory fish. Surviving ostracods were counted. *E. morini* males had a greater risk of predation thank brooding females $p_{dir} = 0.021$.

Table 6. Predation risk on male and brooding female *E. morini*.

	Number Recovered	Number Missing
Males	58	97
Brooding Females	157	53

An estimate of ostracod visual acuity

The inter-ommatidial angle ($\Delta\varphi$), a measure of the smallest region of space sampled by a single ommatidium, gives an estimate of the image resolution potentially provided by apposition compound eyes. The smallest $\Delta\varphi$ we estimated for *E. morini* compound lateral eyes was 8° (Figure 7), which suggests that this species has, at best, a visual resolution of about 8°. Following the logic provided by Land and Nilsson (1990) in their analysis of the compound eyes of the deep-sea ostracod *Macrocypridina castanea,* we can predict that *E. morini* can only detect conspecifics (1.6mm in height, 1.1mm in height, Kornicker and Harrison-Nelson 1997) at distances of about 1 cm or less. We can predict, further, that *E. morini* can spot predators that are 5-10 cm in length, such as the juvenile fish used in the predation experiment described above, at distances as far away as 35 - 70 cm. Here, we have made major assumptions: first, that *E. morini* can spot objects with an angular size as small as the smallest value of $\Delta\varphi$ estimated for its eyes and second, that object detection is taking place under perfect conditions (brightly lit, perfectly clear water).

Thus, the spotting distances we provide here are likely overestimates of those that occur under natural conditions, particularly given that *E. morini*, like other *Euphilomedes* species (Lum et al 2008), are likely nocturnal and that they inhabit shallow coastal environments that are often highly turbid.

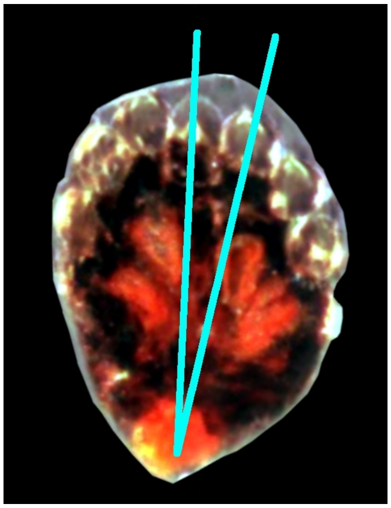

Figure 7. The cross section of an *E. morini* adult male eye. Blue lines indicate the angle between two ommatidia.

Discussion

Here we present evidence that sexually dimorphic eyes in *Euphilomedes* ostracods are an adaptation to allow male ostracods to detect and escape pelagic predators they encounter while out of the sediment seeking mates. We hypothesize that this adaptation is a result of natural selection, not sexual selection, acting on the differing reproductive habits of male and female *Euphilomedes*. Males spend more time out of the sediment searching for a mate leaving them more vulnerable to pelagic predators than females, which spend most of their time in the sediment. We examined this hypothesis through two approaches of testing natural selection: experimentation, through manipulation of a trait, and optimality, through measuring a trait and examining how it may function.

Blindfolding reduces male, but not female, survival rates in the presence of a predator

First, we examined our hypothesis experimentally. We directly tested whether spatial vision helps males avoid predators. Males regularly leave the safety and shelter of the sediment at the bottom of the ocean to mate. Once in the water, they are vulnerable to pelagic predators. Other than their compound eyes, they have extremely limited, or possibly no, defenses against predation. We blindfolded males and found that these males suffered higher predation rates than that of both control and unaltered males. We thus believe that male ostracods use their compound lateral eyes for predator detection and escape. Our experiments attempted to simulated the predatory environment that *Euphilomedes* encounter on a nightly basis. However, these simulations were by no means exact replicas of the conditions in the wild. Little is actually known about the life history of *E. chupacabra* and especially *E. morini* and many of our assumptions are based on the knowledge of other closely related species (Lum et al 2008, Macquart-Molin 1999, Kornicker and Harrison-Nelson 1997, Cohen and Morin 1990, Cohen 1983). For example, *E. chupacabra* males have been observed during their active period at night (Lum et al 2008) and we assume *E. morini* is similarly active at night.

As predicted, unaltered brooding *E. morini* females experienced a lower predation risk than unaltered adult males, likely because these females spent more time in the sediment at the bottom of the experimental tanks than males. As brooding females have no immediate need to leave the sediment to mate, they likely have little motivation to swim out of the sediment. Our interpretation is supported by evidence that females, unlike males, rarely leave the sediment. In some species of Myodocopid ostracods, the swimming appendages of females break off shortly after mating, suggesting the females never swim out of the sediment again (Kornicker 1978). Additionally, brooding females have been found with sperm stored in their bodies, possibly to rear another brood suggesting that they would not need to swim out of the sediment and mate again (Cohen 1983). Finally, we used two sampling methods to collect ostracods: sampling from sediment and sampling from water. When we collected ostracods using sediment samples, the vast majority of those collected were females and when we sampled the water column, the vast majority of those collected were males, which is similar to the findings of previous studies (Cohen and Morin 1990; Baker 1977). It is likely that the sex ratio at birth is 1:1, but the number of males decreases as they are preyed on during their time out of the sediment (Baker 1977).

Finally, we examined predation risk in brooding females alone. As stated earlier, brooding females have a lower predation risk than males, likely because they spend dramatically more time in the sediment. As almost exclusively interstitial animals, they have minimal use for complicated sensory organs. Because the females are at a lower risk of predation than males, eyes may be of little use and it may be more optimal for them to lack eyes altogether. In our predation experiments with females, we found no difference in the number of surviving blindfolded *E. morini* females versus control females. Females do not have compound eyes, only eye rudiments and our results suggest that the eye rudiments do not influence the ability of females to detect and avoid predators in the same way that compound lateral eyes do for males. The lack of eyes may be a tradeoff for a slightly larger body size and for developing eggs.

Male ostracods have eyes optimized for predator detection, not mate detection

Second, we tested whether ostracod vision is optimized to detect predators or mates. We argue that males can detect conspecific females only from distances less than 1cm. At this close range, the male compound eye is likely not useful for anything other than detection alone. By detection, we mean that males can simply determine that an object the size of a conspecific female (approximately 1mm) is within that range, but may not necessarily detect finer details, such as the orientation of the female, which would require a higher resolution eye. However, the resolution of the male eyes is useful to spot potential fish predators (5-10cm long) from distances less than 35-70cm depending on the size of the predator. Interestingly, fish similar in size to the predators used in our experiments appear to have the ability to spot ostracods from a similar distance. We are assuming that our fish have an optical resolution of about 0.07°, which is the value given for similarly sized fish: goldfish (Nicol 1989 as cited in Land and Nilsson 2002) and juvenile (10cm) roach and yellow perch (Wanzenböck et al 1996). With this optical resolution, the 5-10cm fish used in our experiments can spot a 1mm ostracod at a distance close to 80cm. This is very close to the distance at which a *Euphilomedes* can spot a fish, which may suggest that these prey only need to spot their predators at the distance that the predators can spot them in return. Because *Euphilomedes* are so much smaller than the predatory fish, they may still be successful in detecting predators with a low resolution eye.

It is important to note that the values mentioned thus far imply ideal conditions including perfectly clear water and a high contrast between the object being spotted (a *Euphilomedes* spotting a predator or vice versa) and the background. In reality, *Euphilomedes* often live in turbid water and are active in low light conditions. These environmental conditions can and will further decrease the distance at which *Euphilomedes* as well as the predatory fish can spot each other. The *Euphilomedes* eyes are small, low-resolution, apposition compound eyes, which have a high sensitivity to light, whereas fish have relatively large, high-resolution, camera eyes with a much lower sensitivity. Eyes with higher sensitivity can gather more photons per receptor and therefore can function more effectively in low light conditions. As stated earlier, the ostracods and fish may be able to spot each other from similar distances under ideal conditions, but the higher sensitivity of the ostracod eyes will give them an advantage in dimmer and cloudier water.

Ostracod eyes are an example of a sexual dimorphism driven by natural, not sexual, selection

Our results are particularly interesting because they may explain a poorly studied but potentially widespread cause of sexual dimorphism. Most sexual dimorphisms are considered, sometimes incorrectly, to be a result of sexual selection. However, it seems that the sexually dimorphic eyes of *Euphilomedes* are rather a result of natural selection acting differently on males and females because of their differing reproductive behaviors. Because males likely leave the sediment to mate more often than females, males are at a greater risk of predation and strongly benefit from an adaptation which allows them to detect and avoid predators, whereas that adaptation may not be as useful in the less active females. It is interesting that such a dramatic and important sexual dimorphism may be the result of natural and not sexual selection. However, given our current experiments, we cannot completely rule out the possibility that sexual selection has some influence on the sexual dimorphism of ostracod eyes. We were unsuccessful in attempts to test that hypothesis. Though our spatial acuity measurements suggest that the compound eyes of male *Euphilomedes* are not useful in finding females from great distances, we do not have experimental evidence to support this argument.

The dimorphic niche hypothesis may explain some sexual dimorphisms that are a result of natural selection. Many instances of the dimorphic niche hypothesis are difficult or impossible to test experimentally and instead hypotheses are based on behaviors and physical attributes, but remain untested. The sexual size dimorphism in birds of prey, where females are typically larger than males, is one such example. This is hypothesized to be because females protect the nest while males forage and these behavior differences may account for the size differences (Slatkin 1984). Sexually dimorphic trophic structures, such as sex-specific differences is head sizes in terrapins (Tucker et al 1995) and head shapes in cottonmouth snakes (Vincent et al 2004) are often likely due to competitive displacement, where males and females evolve different trophic structures to minimize intraspecific competition through natural selection. There are numerous other examples of sexual size dimorphism that may be a result of natural selection (eg. Jormalainen and Tuomi 1989, Son and Hughes 2000). Unfortunately, it is nearly impossible to experimentally test hypotheses like these in a laboratory or field setting. Altering the size of a body or a body part of an animal is often unachievable and if it can be done, it may have drastic consequences. In our experiments however, we were able to reduce the vision in *Euphilomedes* and then empirically test our hypothesis about the dimorphic niche hypothesis with appropriate controls. To the best of our knowledge, this is the first time a species has been manipulated while alive to test the dimorphic niche hypothesis as well as to suggest that natural selection, not sexual selection, is the driving force for a sexual dimorphism. It is important to note that sexual and natural selection are not necessarily two discrete, independent processes. The two can have independent influence on the evolution of a trait, they may work together, or one may be influenced by the other. In our case the sexual segregation is likely due to sexual selection. This behavior is what causes the increased predation risk in males. We argue that predation on males is what is driving the sexually dimorphic eyes. Though the effects of predation typically fall within the realm of natural selection, because the predation is related to reproductive behaviors, this could be considered indirect sexual selection.

Conclusion

In conclusion, our data demonstrate that the presence or absence of spatial vision has a dramatic influence on the survival rates of male *Euphilomedes* ostracods. Experimentally, we found that fewer blindfolded male ostracods survived than control males and that fewer females, which naturally lack compound lateral eyes, survived than sighted males, but there was no difference between control and blindfolded females. We also calculated the spatial acuity in male *E. morini* and determined that their eyes are optimized for detecting and avoiding predators. While a correlation between sexually dimorphic visual abilities and implied predation risk is not a new idea (Meyer-Rochow and Lau 2008; Lau et al. 2007), *Euphilomedes* ostracods have provided a rare opportunity to experimentally test the hypothesis that sexually dimorphic vision has likely evolved through natural, rather than sexual, selection because of differing predation risks between males and females.

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