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LUMINESCENT FLASH AVOIDANCE IN THE NOCTURNAL CRAB *PORTUNUS XANTUSII*

II. CARDIAC AND VISUAL RESPONSES TO VARIATIONS IN SIMULATED LUMINESCENT FLASHES

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Summary

Cardiac responses of *Portunus xantusii* (Stimpson) (Crustacea: Decapoda) to simulated luminescent flashes were similar to responses to real flashes produced by the sea pansy *Renilla kollikeri* (Pfeffer). Tethering the crabs did not significantly affect cardiac responses to light flashes and allowed for more control over stimulus presentation. Crabs were maximally responsive to wavelengths from 500 to 510 nm (175 nm width at half-maximum response). The wavelength of maximal sensitivity based on the electroretinogram was 510 nm. The critical fusion frequency for *P. xantusii* was 25.6 Hz. Pulse rate and train duration of light flashes were the most important features of the stimulus for eliciting maximal cardiac responses, with pulse duration and inter-pulse interval having minor effects on flash effectiveness. Pulse rates of 4–5 Hz within a train elicited the largest cardiac responses. Increases in train duration from 1 to 5 s resulted in a linear increase in heart response, but stimuli with train durations longer than 5 s did not elicit concomitant increases in heart response. Habituation to pairs of stimulus trains occurred if inter-train intervals were less than 8–10 min. The characteristics of the luminescent signals produced by benthic invertebrates are well matched to the characteristics of light stimuli that are most effective at eliciting physiological and behavioral responses from *Portunus xantusii*.

Introduction

Distinct behavioral responses have been reported for nocturnally active animals responding to the light signals of several groups of marine luminescent emitters. Flashlight fishes and ostracods respond to signals of their own species (Morin *et al.* 1975; Morin, 1986); copepods respond to the flashes produced by dinoflagellates (Buskey *et al.* 1983); and a variety of nocturnally active predators, including crustaceans, fishes and cephalopods, respond to the luminescence of benthic

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brittle-stars and sea pens (see references in Grober, 1990). Dinoflagellates and brittle-stars, as well as sea pens such as *Renilla*, *Stylatula* and *Acanthoptilum*, produce a characteristic luminescent signal that is a dominant signal type in coastal waters. This signal consists of one or more brief flashes that are produced upon mechanical stimulation; the intensity and duration of the flash are proportional to the strength of the stimulus (see Morin, 1976, 1983, for details). At least in dinoflagellates, brittle-stars and sea pens, the signal has been shown to be produced in response to a potential predator and elicits startle or avoidance behavior from the intruder. Morin (1983) suggested that the convergence in the form and function of coastal luminescent signals, in these and other emitters, has provided a class of signals, termed contact flash predator deterrents, that have specific characteristics and serve the general function of deterring visually orienting predators.

Although the overall features of these signals are well established (Morin, 1983), very little is known about the specific characteristics of the luminescent signals themselves that are responsible for eliciting the observed behavioral responses. By employing a simulated dinoflagellate flash, Buskey and Swift (1983) were able to show that the intensity, color and duration of the flash had significant effects on the responses of copepods and, in general, the copepods were maximally responsive to light flashes that mimicked natural dinoflagellate luminescence.

The luminescence produced by *Renilla kollikeri*, the sea pansy, elicits both behavioral and cardiac responses from the portunid crab *Portunus xantusii* (Grober, 1990), suggesting that the 'optocardial reflex' (Mislin, 1966) is involved in, or is an indicator of, the production of the behavioral responses to luminescence. Thus, this optocardial reflex can be used as a reliable bioassay for determining what specific aspects of the luminescent flash are important in eliciting both the physiological and behavioral responses. The purpose of this study was to determine the effects of variation in (1) wavelength, (2) train duration (total duration), (3) pulse duration, (4) pulse rate, (5) interpulse interval and (6) intensity of simulated luminescent flashes on the cardiac response in the crab *Portunus xantusii*. The spectral sensitivity of the electroretinogram (ERG) response was also determined. This allowed for a comparison of the spectral tuning in (1) the receptor organ itself and (2) a system that is both separated from the receptor organ by several levels of neural integration and is a reliable indicator of subsequent behavioral responses (the optocardial reflex, see Grober, 1990).

Materials and methods

Experimental animals and preparations

Specimens of *Portunus xantusii* (27–33 mm carapace length, both sexes) were obtained from Marinus Inc. and Pacific Biomarine (Los Angeles, CA, USA). The animals were held in aquaria kept at 15°C by a recirculating seawater system. All animals were fed twice each week on mussels and fish.

Heart activity was measured by implanting an impedance electrode on either side of the heart in accordance with the impedance technique (see Grober, 1990, for details). This technique allowed crabs to be monitored as they roamed freely about the aquarium. The output from the impedance leads was fed into the analog-to-digital converter of an Acorn computer data acquisition and analysis system (see Grober, 1990, for details). The computer was programed to sample heart rate every 0.05 s for either 30 or 60 s. The light stimuli were presented 10–15 s after recording started. After completion of a recording, heart rate was analysed for the 10 s periods immediately preceding and following the light flash. The ratio of these rates is the response ratio (Grober, 1990). All cardiac response experiments were conducted at 15±1 °C, in the same aquarium and under similar conditions to those described in the sensory stimuli experiments in Grober (1990).

Light stimulus and calibration

Luminescence was simulated using an 8 mm point source of light that could be varied with respect to the wavelength, intensity, duration and flash frequency. The light was produced by a high-intensity quartz–halogen bulb (Osman H3, 55 W/12 V) and was adjusted in intensity with neutral density filters or by varying the voltage to the bulb. The wavelength of the stimuli was controlled by passing the light through a grating monochromator (Bausch & Lomb, 1350 grooves mm^{-1}, 20 nm half band-width). The duration and pulse rate of the light stimuli were controlled by a Grass stimulator (S48) connected to an electronic shutter (Uniblitz model 214L) which, in turn, was connected to the exit slit of the monochromator. The stimulator was slaved to the cassette drive output of the computer so that the key pad could be used to close the cassette drive circuit and trigger the light stimulus. The data acquisition program stored the time of the stimulus trigger with each data file. A fiber-optic light guide (8 mm) was connected to the exit opening of the electronic shutter, and this light guide was then positioned 2 cm in front of one of the crab's eyes. The diameter and position of the light guide ensured that the entire eye was exposed to the light flash.

The intensity of simulated flashes was measured by placing the stimulus light guide 2 cm from a side window photomultiplier tube (RCA S4–102). The response of the photomultiplier was amplified through a Philbrick P2AU operational amplifier and displayed on an oscilloscope (Tektronix type 565). Since the photomultiplier is differentially sensitive to wavelength, the voltage response was multiplied by a 'sensitivity ratio' to provide a corrected stimulus intensity. The sensitivity ratio varied between 0.012 (675 nm) and 1.0 (400 nm) and was the ratio of the tube sensitivity at the test wavelength to the sensitivity at 400 nm (the wavelength of peak sensitivity – spectral sensitivity data were obtained from the manufacturer).

The Renilla *standard flash*

Before investigating the responses of crabs to variation in the characteristics of light flashes, it was necessary to determine whether simulated flashes and real

flashes elicited similar cardiac responses from crabs. In addition, since it was necessary to tether the crabs for the majority of the experiments, a standard flash was required to compare the cardiac responses of free-roaming and tethered individuals. For this reason, a standard flash was developed based on the intensity, duration, wavelength and pulse characteristics of the natural flashes produced by the sea pansy *Renilla kollikeri* (Anthozoa: Pennatulacea). This species was chosen because it co-exists with *Portunus xantusii*, and because the cardiac and behavioral responses of this crab species to *Renilla* luminescence have been established (Grober, 1990).

Based on the train duration of flashes produced by *Renilla* under semi-natural conditions (Grober, 1990), a 5 s train duration was used. The wavelength at peak emission (λ_{max}) of *Renilla* luminescence has been well established at 509 nm and a half band-width of 21–30 nm (Herring, 1983; Widder *et al.* 1983). The simulated flash had a peak of 510 nm and half band-width of 20 nm.

The intensity of natural flashes was determined by placing a dark-adapted *Renilla* 2 cm from the window of the photomultiplier and then mechanically stimulating the colony for 5 s. The maximum luminescence recorded over this period was corrected for the spectral sensitivity of the photomultiplier by assuming the flash had a wavelength peak of 510 nm. The corrected voltage responses from five individual *Renilla* colonies were averaged to give a *Renilla* standard flash intensity (=RSI) in relative units (volts) (7.05 ± 2.3 V, $\bar{x} \pm$ S.D.). All subsequent light stimuli were calibrated with reference to this standard. Estimates of the absolute intensities of the light stimuli were determined using a hexadecane–^{14}C light standard (Hastings and Weber, 1963).

The natural *Renilla* flash can range from a series of brief (100 ms) localized flashes, in response to weak stimuli, to longer waves (1500 ms) of luminescence that move across the colony in response to more vigorous stimulation (Buck, 1973). This variability in *Renilla* luminescence made selection of a *Renilla* standard pulse duration and flicker frequency somewhat arbitrary. A pulse duration of 250 ms was chosen on the basis of the most recent empirical determinations of pulse durations in *Renilla* (Morin, 1976). Based on this pulse duration and the results of the experiment that addressed the cardiac responses of *Portunus xantusii* to flashes of differing flicker rates, a standard pulse rate of 3.9 Hz was used (see Results: effects of flash pulse rate).

Spectral sensitivity and critical fusion frequency from the electroretinogram (ERG)

For the ERG determinations, each animal was wrapped in sea-water-soaked cheese cloth and then tethered to a glass rod that was connected to the lateral spines of the crab by a pair of stiff wires. The tethering rod was then connected to a ring stand that was situated in the center of a Faraday cage. The crab was positioned so that its venter was immersed in sea water. The cheese cloth on the ventral surface of the crab acted as a wick to keep the crab moist. The eye was immobilized by inserting modelling clay around the base of the eyestalk. ERG

recordings were made using two silver/silver chloride electrode wires. One electrode made contact with the illuminated eye *via* a saline-soaked cotton wick. The cotton wick was moistened with cold saline between stimuli and this maintained the temperature of the eye at 15±3°C. Care was taken to ensure that this electrode did not block the incoming light stimulus. Using a 30 gauge syringe tip, a hole was made in the ventral thorax and the reference electrode was inserted into the mid-ventral surface of the thorax. After electrode implantation, the stimulus light guide (8 mm diameter) was positioned 2 cm from one of the eyes, all lights were extinguished, and the crab was allowed to dark adapt for 1 h. Signals from the electrodes were amplified with a Tektronix 122 amplifier operated at a bandpass width of 0.2–1000 Hz, and displayed on an oscilloscope (Hitachi storage scope model V-134).

The spectral sensitivity of the ERG was determined, for five animals, in accordance with the methods of Goldsmith and Fernandez (1968). Single test flashes were of 200 ms duration, and 5 min elapsed between tests to ensure that animals were always completely dark-adapted. A 0.2 mV ERG output was used as a standard response. This output amplitude was elicited at each wavelength by adjusting the light intensity (by varying the voltage to the light source and using neutral density filters). The voltage to the bulb was measured with a digital voltmeter, and the relative intensity of test flashes was determined using the previously described calibration system. Wavelengths between 350 and 680 nm were presented at 20 nm increments, except between 480 and 520 nm where intervals were in 10 nm increments.

The critical fusion frequency (CFF) was measured for the same five crabs that had been tested for spectral sensitivity. CFF was determined by presenting trains of test flashes with increasing flicker frequency until the ERG no longer responded to each pulse of the stimulus. These test flashes were produced by a variable strobelight (General Radio Co. Strobotac 1531-A) whose beam was fed into the monochromator of the light stimulus apparatus (see above). Flashes had a duration of 10 ms, were at RSI, and had a spectral peak at 510 nm.

Cardiac responses to the Renilla *standard flash and the effects of tethering*

Cardiac responses to the *Renilla* standard flash were recorded for five free-roaming crabs and for five tethered crabs. Using methods similar to those described for the ERG recordings, crabs were tethered to a glass rod by connecting wires that were attached to the lateral spines of the carapace. However, since the crabs were totally submerged in sea water, no moistened cheese cloth was used. When tethered in this way, a crab could still move all its appendages, but it could not move around the aquarium. As a control, tethered crabs were tested in the same manner as described for the simulated flash, except that the power supply to the light source was turned off so that no flash was emitted from the light guide. Using an ANOVA, response ratios (ratio of heart rate 10 s before to that 10 s after stimulus) for tethered, non-tethered and control (no flash) trials were compared. Least significant difference tests (LSD) at an experiment-

wise error rate of 0.01 (Sokal and Rohlf, 1981) were used to assess differences between the three treatments.

Effects of varying flash parameters on cardiac response

Habituation

To quantify accurately the effects of variation in flash characteristics, it was necessary to determine the time required for crabs to recover completely from a stimulus event. Habituation (or its absence) to light flashes was determined by comparing the response ratios elicited by a pair of stimulus trains presented with inter-train intervals of 1–10 min (in 1 min increments). The 'habituation ratio' was calculated as the ratio of the response ratio of the first stimulus train to the response ratio of the second stimulus train. High habituation ratios result when the response to the second flash sequence is strongly inhibited by the response to the first flash sequence. Each of five tethered crabs was tested at each inter-train interval and their habituation ratios were calculated. Stimuli had a train duration of 5 s, a pulse duration of 200 ms, an interpulse interval of 50 ms, a pulse rate of 4 Hz, a λ_{max} of 510 nm, and were at RSI.

Wavelength

The spectral sensitivity of the cardiac response was determined for a total of 10 tethered crabs. Test stimuli had a train duration of 5 s, a pulse duration of 200 ms, an interpulse interval of 50 ms, a pulse rate of 4 Hz, and only the spectral characteristics of the stimuli were varied. Crabs were allowed 10 min between stimulus presentations to avoid habituation (see results from habituation experiments, Fig. 2). The order of presentation of the different wavelengths was randomized, and each crab was tested five times at each wavelength. The spectral sensitivity was measured at RSI and 1 log unit below this intensity. A crab was arbitrarily considered to have responded to the flash if a 50 % decrease in heart rate was observed (i.e. response ratio ≥ 2.0). The number of crabs that responded to the flash was divided by the total tested ($N=10$) to give a value for the proportion responding. The mean proportion responding for the five trials at each wavelength was then plotted as a function of wavelength to determine the spectral sensitivity of the cardiac response.

Train duration

The effects of stimulus train duration (=total stimulus duration) on heart rate and duration of arrhythmia (altered heart rate) were determined by presenting crabs ($N=5$) with stimuli that varied from 1 to 15 s in train duration. Test stimuli had a pulse duration of 200 ms, an interpulse interval of 50 ms, a pulse rate of 4 Hz, a λ_{max} of 510 nm, and were at RSI. Response ratios and the total period of decreased heart rate (arrhythmia duration=the time between the reduction in heart rate and the return to pre-stimulus rates) were calculated for all trials.

Pulse rate

The effects of pulse rate (number of pulses per train) on heart rate were assessed in two ways. Five crabs were tested with each method. The first method (*a*) utilized a constant interpulse interval (5 ms), but allowed pulse duration to vary inversely with pulse rate. To do this, a stimulus train of 5 s duration was presented at pulse rates of 1 Hz and 5–35 Hz (in 5 Hz increments). Test stimuli had a λ_{max} of 510 nm, were at RSI, and were presented at 10 min intervals to avoid habituation (Fig. 2). The second method (*b*) utilized fixed pulse durations of 125 and 250 ms, but allowed the interpulse interval to vary inversely with pulse rate. Test stimuli had a train duration of 5 s, a λ_{max} of 510 nm, and were at RSI. For both methods, the response ratio was calculated and plotted as a function of pulse rate.

Pulse duration and interpulse interval

Although no experiments directly addressed the effects of pulse duration and interpulse interval on heart rate, an estimate of these effects was determined by rearranging the data obtained in the experiments on pulse rate (methods *a* and *b* above, see Results).

Stimulus intensity

The effect of stimulus intensity on heart rate was determined at the peak wavelength of crab sensitivity (510 nm) and at four different intensities. The response ratios from the spectral sensitivity experiments (five trials on each of 10 crabs) were used for stimuli at RSI and 0.1 times RSI. In addition, response ratio data were obtained for stimuli at 0.01 times RSI (five trials on each of four crabs) and at 10 times RSI (five trials on each of two crabs).

Results

Responses to simulated Renilla *luminescence and effects of tethering*

The response ratios to simulated luminescent flashes (=*Renilla* standard flashes) are in close agreement with the cardiac responses of *P. xantusii* to both natural *Renilla* flashes and mechanical stimulation (compare Fig. 1 to Grober, 1990, Fig. 4). The *Renilla* standard flash elicited significantly higher response ratios from both free-roaming and tethered crabs when compared to a control in which no flash was administered (Fig. 1). There was no significant difference in the mean response ratios for free-roaming and tethered crabs to simulated *Renilla* luminescence. This similarity suggests that tethering had little effect on the responsiveness of the study animals.

Effects of altering flash characteristics

Habituation

The results of the habituation tests demonstrated that there must be at least 8 min between two successive stimuli to avoid habituation (Fig. 2). Habituation

ratios were greater than 2 for inter-train intervals of 1–3 min, and gradually dropped to baseline values (dotted line=1.0) for intervals between 8 and 10 min.

Wavelength

The cardiac response varied with the spectral composition of the light stimulus (Fig. 3). Crabs were maximally sensitive at wavelengths between 500 and 510 nm.

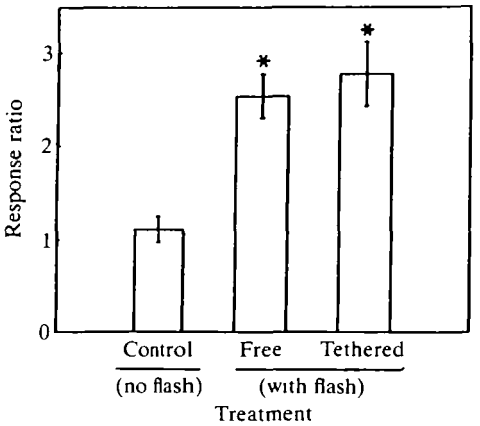

Fig. 1. Response ratios ($\bar{x}\pm$S.D.; ratio of heart rate 10 s before to that 10 s after stimulus) for simulated *Renilla* flashes and controls (no flash) in free-roaming and tethered *Portunus xantusii* (N=5). A response ratio of 1 indicates no change. All trials were conducted at 15±1°C. Flashes had a train duration of 5 s, pulse duration of 250 ms, interpulse interval of 5 ms, pulse rate of 3.9 Hz and wavelenth maximum (λ_{max}) at 510 nm, and were at *Renilla* standard intensity (see text for details). * P<0.01, ANOVA, LSD.

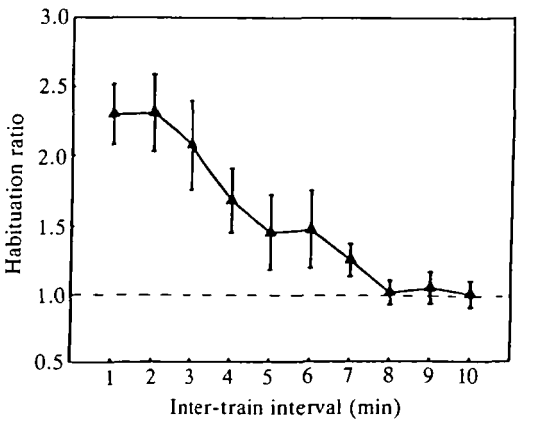

Fig. 2. The effects of inter-train interval on habituation ratio ($\bar{x}\pm$S.D.) in *Portunus xantusii* (N=5). Habituation ratio is the response ratio to the first train divided by the response ratio to the second train. All trials were conducted at 15±1°C. Flashes had a train duration of 5 s, pulse duration of 200 ms, interpulse interval of 50 ms, pulse rate of 4 Hz and λ_{max} of 510 nm, and were at *Renilla* standard intensity (see text for details).

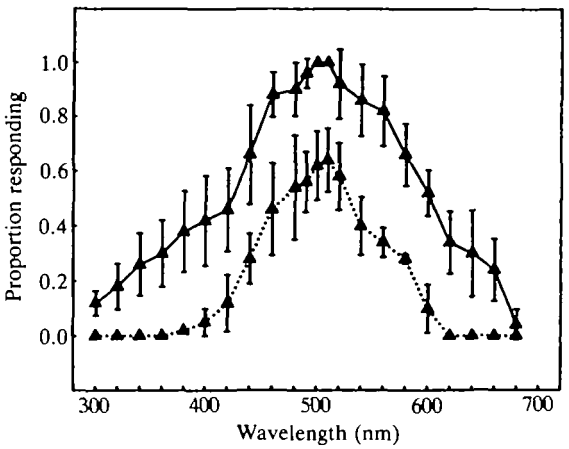

Fig. 3. The effect of stimulus wavelength on the proportion ($\bar{x}\pm$S.D.) of *Portunus xantusii* (N=5) that exhibited a 50% decrease in heart rate (response ratio $\geq$2) to flashes at *Renilla* standard intensity (upper, solid curve) and 1 log unit lower intensity (lower, dotted curve). λ_{max} was between 500 and 510 nm. All trials were conducted at 15±1°C. Flashes had a train duration of 5 s, pulse duration of 200 ms, interpulse interval of 50 ms and pulse rate of 4 Hz (see text for details).

Sensitivity dropped off evenly on either side of this maximum, and the spectral response curve had a width at half-maximum response of 175 nm. For stimuli at the wavelengths of maximum sensitivity, the proportion of crabs that produced response ratios greater than 2.0 dropped from 1.0 for standard *Renilla* intensity flashes to 0.62 for flashes 1 log unit below this intensity (Fig. 3). For the low-intensity flashes, no crab responded at wavelengths below 400 nm or above 600 nm.

Train duration

Increases in the train duration of the light stimuli from 1 to 5 s elicited concomitant increases in the response ratios from 1.3 to 2.2 (Fig. 4A). This relationship reached a plateau at a response ratio of 2.2 for train durations between 5 and 15 s (Fig. 4A). The duration of heart arrhythmia increased from less than 6 s to 10 s for train durations of 1–6 s (Fig. 4B). This relationship reached a plateau at approximately 10 s response durations for train durations between 6 and 15 s (Fig. 4B).

Pulse rate

When pulse duration was allowed to vary inversely with pulse rate (i.e. fixed interpulse interval, but varying pulse duration), maximal response ratios of about 2.5 were obtained for light stimuli with 5 Hz pulse rates (Fig. 5). Response ratio increased dramatically for pulse rates between 1 and 5 Hz, decreased slightly between 5 and 15 Hz, and showed a sharp decrease for pulse rates greater than 15 Hz (Fig. 5). Cardiac responses to pulses of fixed duration but variable

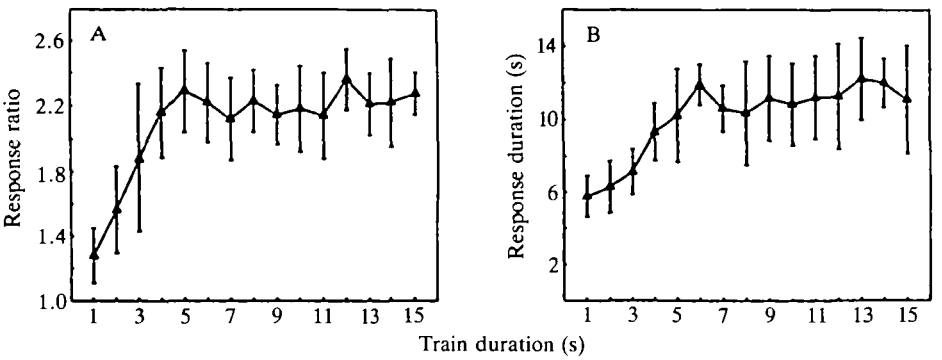

Fig. 4. The effect of flash train duration on the response ratio (A) and the duration of heart arrhythmia (B) in *Portunus xantusii* (N=5). All trials were conducted at 15±1°C. Flashes had a pulse duration of 200 ms, interpulse interval of 50 ms, pulse rate of 4 Hz and λ_{max} of 510 nm, and were at *Renilla* standard intensity (see text for details). Points and error bars represent $\bar{x}$±S.D.

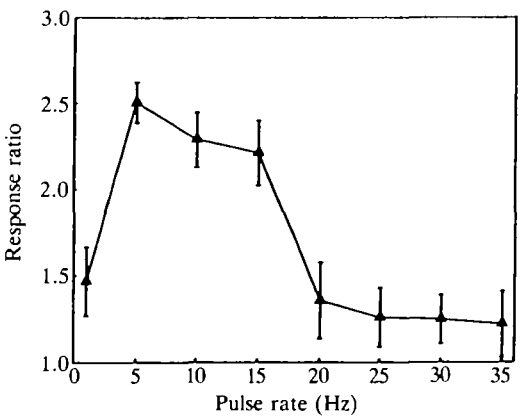

Fig. 5. The effects of pulse rate on the response ratio ($\bar{x}$±S.D.) in *Portunus xantusii* (N=5). All trials were conducted at 15±1°C. Flashes had a train duration of 5 s, interpulse interval of 5 ms, λ_{max} of 510 nm, were at *Renilla* standard intensity, and had pulse durations that varied inversely with pulse rate (see text for details).

interpulse interval showed a rapid increase for pulse rates between 1 and 2 Hz (for both 125 and 250 ms pulse durations; Fig. 6). Maximal responses were obtained for pulse durations of 4 and 5 Hz, respectively, for pulse durations of 250 and 125 ms, and the response ratio exhibited a relatively stable plateau for pulse rates between 2 and 8 Hz.

Pulse duration

To assess the effects of pulse duration on heart rate, pulse durations were calculated from the experiment involving variable pulse rates and a fixed

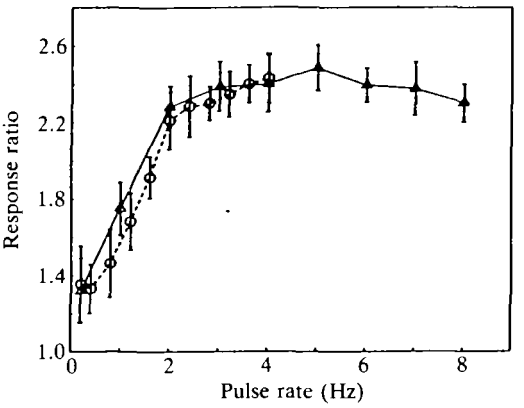

Fig. 6. The effects of pulse rate on the response ratio ($\bar{x}\pm$S.D.) in *Portunus xantusii* (N=5), for pulse durations of 125 ms (triangles and solid line) and 250 ms (circles and dotted line). All trials were conducted at 15±1°C. Flashes had a train duration of 5 s, λ_{max} of 510 nm, were at *Renilla* standard intensity, and had interpulse intervals that varied inversely with pulse rate (see text for details).

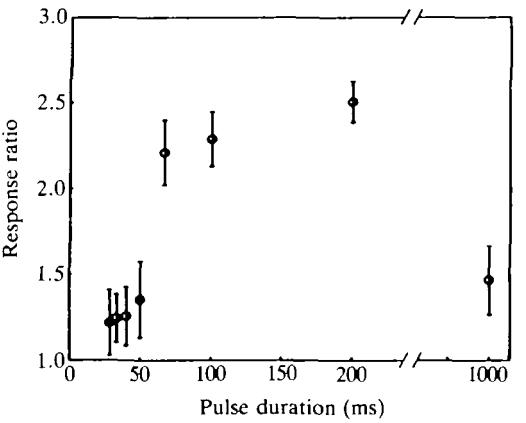

Fig. 7. The effects of pulse duration on the response ratio ($\bar{x}\pm$S.D.) in *Portunus xantusii* (N=5). All trials were conducted at 15±1°C. Flashes had a train duration of 5 s, interpulse interval of 5 ms, λ_{max} of 510 nm, were at *Renilla* standard intensity, and had pulse rates that varied inversely with pulse duration (see text for details).

interpulse interval (method *a*) and plotted against the response ratio results (Fig. 7). Response ratios were relatively low (less than 1.5) for pulse durations between 5 and 50 ms, increased to more than 2.0 for 66 ms pulse durations, reached a maximum at a pulse duration of 200 ms, and decreased to 1.5 for the 1 s pulse duration.

The effects of pulse duration can also be determined by comparing the results of the experiment involving variable pulse rates but fixed pulse durations (method *b*;

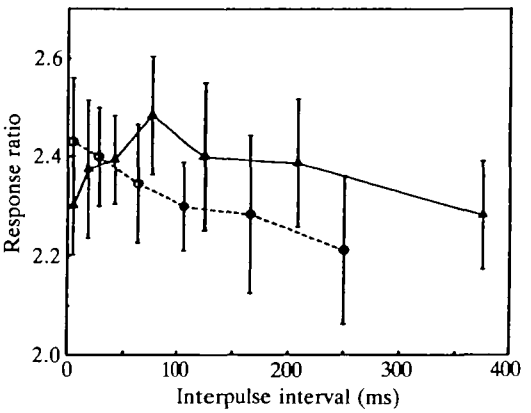

Fig. 8. The effects of interpulse interval (IPI) on the response ratio ($\bar{x}\pm$S.D.) in *Portunus xantusii* (N=5), for pulse durations of 125 ms (triangles and solid line, IPI_{max}=72 ms) and 250 ms (circles and dotted line, IPI_{max}=5 ms). To expand the segments of the curves that show maximal responses, only stimuli that elicited response ratios above 2.0 are shown. Both curves continue to decrease for stimuli with longer interpulse intervals. All trials were conducted at 15±1 °C. Flashes had a train duration of 5 s, λ_{max} of 510 nm, were at *Renilla* standard intensity, and had pulse rates that varied inversely with interpulse interval (see text for details).

Fig. 6) for the two different pulse durations (125 and 250 ms). This comparison shows that cardiac responses are almost identical for pulse durations of 125 and 250 ms, even over a wide range of pulse rates, and is in accordance with the suggestion that these short pulse durations are most effective at eliciting cardiac responses, as long as they are presented at rates well below the CFF of the crab eye.

Interpulse interval

The effects of interpulse interval (IPI) can be derived from the results of the fixed pulse duration and variable pulse rate experiment (method *b*). In general, stimuli with short interpulse intervals elicit the greatest cardiac responses (Fig. 8). This result was the case for stimuli with a pulse duration of 250 ms, where response ratio was maximal for stimuli with the shortest IPI (5 ms, Fig. 8). For stimuli with 125 ms pulse durations, maximal response ratio was obtained with a 72 ms IPI (Fig. 8). Once again, it is important to point out that both IPI and pulse rate were varied in this experiment, so individual effects of either stimulus parameter are confounded. However, the observations that peak pulse rates, for stimuli with 125 and 250 ms pulse durations, have similar values (4 and 5 Hz), whereas peak interpulse intervals are less similar (5 and 72 ms), suggest that maximal cardiac responses can be elicited with a wider range of interpulse intervals (i.e. responses are more narrowly tuned with respect to pulse rate than IPI).

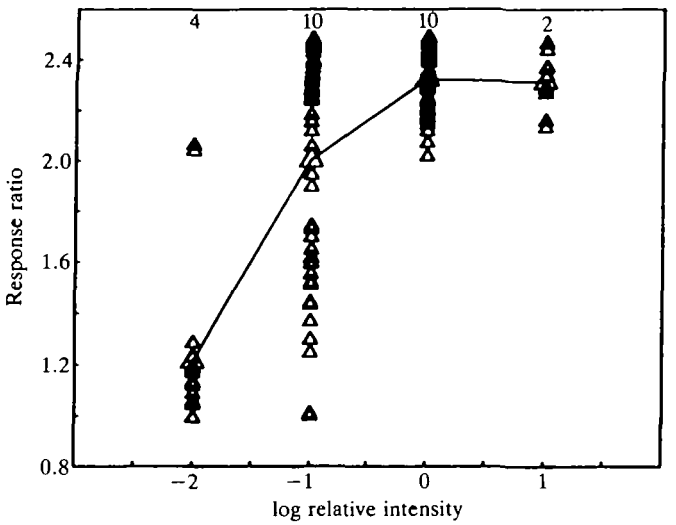

Fig. 9. The effect of stimulus intensity on response ratio in *Portunus xantusii*. Intensity is expressed as the logarithm of the *Renilla* standard (see text for details). The symbols represent trials on individual crabs (five trials per crab, number of crabs is shown), and the curve connects the mean values (large triangle) at each intensity. All trials were conducted at 15±1°C. Flashes had a train duration of 5 s, pulse duration of 200 ms, interpulse interval of 50 ms, pulse rate of 4 Hz and λ_{max} of 510 nm.

Stimulus intensity

The response ratio increased as a function of stimulus intensity and saturated at *Renilla* standard intensity (Fig. 9). The response ratio at 0.1 *Renilla* intensity was the same as the threshold response ratio value of 2.0 that I chose for crab responses in the spectral sensitivity experiment. The *Renilla* standard flash had an intensity of 7×10^8 quanta $cm^{-2}s^{-1}$, based on the light standard of Hastings and Weber (1963).

Critical fusion frequency and spectral sensitivity

The critical fusion frequency based on the results of the ERG responses ranged from 22 to 29 Hz and averaged 25.6±2.5 Hz ($\bar{x}\pm$S.D., N=5). The spectral sensitivity of the ERG response provided a broad sensitivity curve with a λ_{max} of 510 nm (Fig. 10). The decrease in relative sensitivity was more pronounced for longer wavelengths than for the short-wavelength end of the spectrum. If the log sensitivity values for the ERG responses are converted to arithmetic values (Fig. 11), they can be plotted as a proportion of maximal sensitivity. This curve has a width at half-maximal sensitivity of 144 nm, which is narrower than that for the cardiac response (172 nm) (Fig. 11).

Discussion

The cardiac responses of *Portunus xantusii* to simulated *Renilla* flashes are

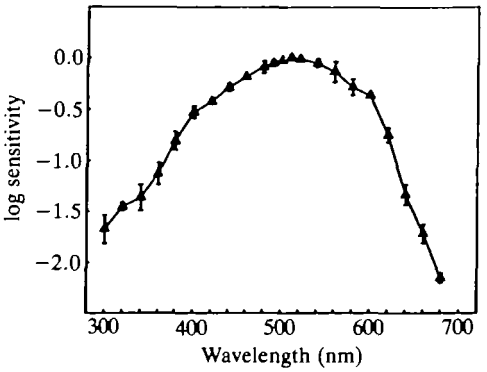

Fig. 10. Spectral sensitivity of the dark-adapted eye of *Portunus xantusii* derived from electroretinogram recordings (N=5). Data are expressed as the mean±S.E. The ordinate (log sensitivity) represents the reciprocal of the relative intensity of the stimulus that elicits a constant response of 0.2 mV. Flash duration was 200 ms. Temperature of the eye was maintained at 15±3°C.

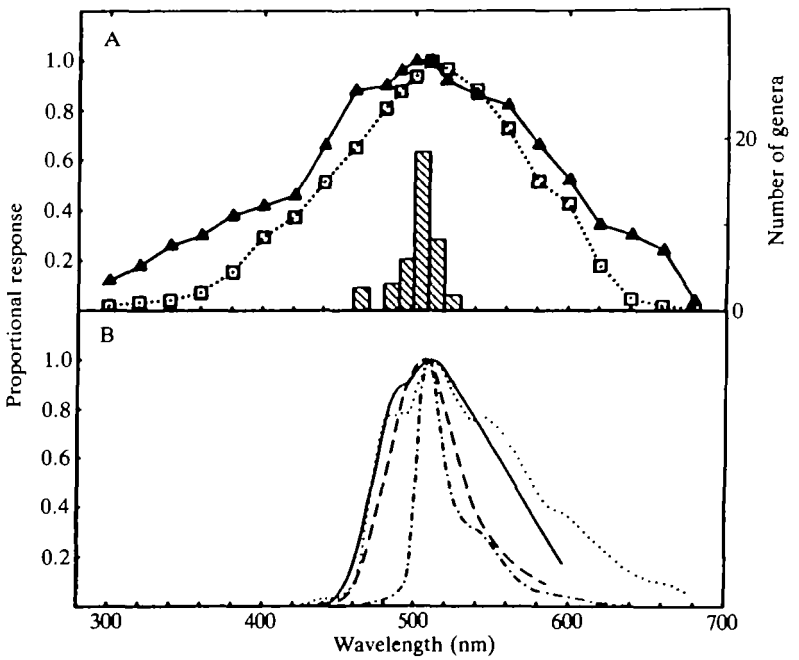

Fig. 11. Summary figure showing (A) the spectral sensitivity of the cardiac (solid triangles, from Fig. 2) and electroretinogram responses (open squares, from Fig. 6 – replotted on an arithmetic ordinate) for *Portunus xantusii*; and the estimated frequency distribution of bioluminescence emission maxima from coastal (benthic or demersal) genera (hatched bars, from Hastings and Morin, 1990); (B) representative emission spectra of luminescence produced by several coastal species [cnidarian: *Renilla kollikeri*, –·–; polychaetes: *Eunoe* sp., ——, unidentified syllid, – – –; ophiuroid: *Ophiopholis longispina*, ····· (modified from Herring, 1983; Widder *et al.* 1983)].

similar to their responses to natural *Renilla* luminescence or gentle mechanical stimulation (Fig. 1 and Grober, 1990, Fig. 2). Since luminescent stimuli also elicit specific behavioral responses from crabs (see references in Grober, 1990), cardiac activity can be used as a reliable bioassay of behavioral responses to light stimuli. The similarity of the response ratios to real and simulated luminescence (Fig. 1 and Grober, 1990, Fig. 1) suggests that the parameters of the standard *Renilla* flash provide the necessary components to elicit the behavioral responses. However, maximal cardiac responses were only elicited over a specific range of values for any given component of the stimulus. My data on *Portunus xantusii* indicate that this nocturnal crustacean predator (1) has a broad visual spectral sensitivity function that is most sensitive between 500 and 520 nm, (2) responds maximally to stimuli with train durations of 5 s or more, (3) shows increased responses to pulse rates of 2–15 Hz with maximum responses between 4 and 5 Hz, (4) shows greatest responses to pulse durations of 125–250 ms, (5) responds to interpulse intervals between 5 and 100 ms, with the greatest response being to shorter intervals, and (6) can respond to stimuli that are 2 log units less intense than the *Renilla* standard and saturate at about the *Renilla* standard intensity. These response characteristics can be used to assess the tuning of nocturnal crustacean vision to important aspects of both biotic (luminescence) and abiotic sources of ambient light.

The spectral tuning determined from the cardiac response in *P. xantusii* is similar to the results obtained from studies of spectral variation in movement perception by *Carcinus* (Horridge, 1967). Both spectral curves peak between 500 and 510 nm, but the spectral sensitivity curve for *Carcinus* was more narrowly tuned in comparison to my results for *Portunus*. The wavelength at peak sensitivity from the ERG in *P. xantusii* was much higher than the λ_{max}, obtained with the microspectrophotometer (MSP), of 483 nm in *Portunus spinimanus* (Cronin and Forward, 1988). This large difference in peak sensitivity could be due to differences in (1) the experimental method used (MSP *versus* ERG data), or (2) the latitudinal distribution of collection sites (*P. xantusii* was collected from a temperate environment whereas *P. spinimanus* came from tropical waters). However, the λ_{max} in *P. spinimanus* was very low in comparison to the other 22 species of coastal and estuarine crabs studied by Cronin and Forward (1988). The absorption maxima obtained from the 23 species in their study ranged from 483 to 515 nm and the average absorption maximum across all coastal and estuarine species was 497 nm. The crab *Callinectes* has a peak sensitivity of its ERG response at 505 nm (Goldsmith and Fernandez, 1968) and microspectrophotometric analysis of the photopigment showed sensitivity maxima at 508 and 440 nm (see references in Cronin, 1986). The wavelength of maximum sensitivity in the lobster *Panulirus argus* was 515 nm on the basis of intracellular ERG recordings, 500 nm on the basis of extracellular ERG recordings, and 510 nm on the basis of MSP (Cummins *et al.* 1984). The maximum sensitivity of the digitonin extract of the photopigment in *P. argus* was about 504 nm (Fernandez, 1965). The lobster *Homarus* shows maximum sensitivity at 515 nm for both difference spectra analysis (see Waterman,

1961) and MSP (Bruno *et al.* 1977). The general trend that can be derived from these data is that nocturnally active crabs and lobsters are maximally sensitive to wavelengths in the range 500–515 nm.

The wavelength of maximal sensitivity in nocturnal crustaceans provides a close match to the wavelength of peak emission in coastal, benthic luminescent organisms (Fig. 11). Both spectral curves peak between 500 and 520 nm. This peak also corresponds to the color of downwelling light that predominates during twilight in temperate marine communities (Munz and McFarland, 1977). Conversely, moonlight and starlight have wavelength peaks that are shifted to longer wavelengths (550–600 nm, Munz and McFarland, 1977). Furthermore, the visual pigments of nocturnal and crepuscular fishes are maximally sensitive around 500 nm (Hobson *et al.* 1981) and most closely match the spectral characteristics of twilight or bioluminescence rather than moonlight or starlight. Hobson *et al.* (1981) consider this spectral match a special case of the sensitivity hypothesis (Lythgoe, 1966) whereby visual sensitivity is improved when absorption of photopigments matches ambient light. Hobson *et al.* (1981) propose that the greater match with twilight is due to the greater risk of predation from crepuscular predators. Forward *et al.* (1988) compare their downwelling irradiance spectra to the λ_{max} data from Cronin and Forward (1988) and conclude that the sensitivity hypothesis is not supported for crustaceans. This conclusion is based on the fact that midday maximal irradiance is in the region of 570–700 nm, whereas the λ_{max} data for the crabs range from 483 to 516 nm. However, the vast majority of crab species are active primarily at night or twilight (see Morin, 1983) and take refuge during the day. A more ecologically relevant comparison would be to look at λ_{max} *versus* either maximal irradiance at twilight or peak emission of bioluminescent species (a primary source of light at night). The maximal underwater downwelling irradiance at twilight determined by Forward *et al.* (1988) was in the range 490–520 nm (very similar to the data of Munz and McFarland, 1977), which is in close correspondence to the wavelength at peak emission of coastal luminescent species, and is well matched to the λ_{max} values obtained by Cronin and Forward (1988). Moreover, the importance of spectral matching should vary inversely with total ambient irradiance such that at midday there would be sufficient numbers of photons at all wavelengths to accomplish even the most detailed visual tasks. However, when available photons become limited, during twilight and throughout the night, spectral matching would allow for maximal light capture and may confer a critical selective advantage. Thus, it appears that the data of Cronin and Forward (1988) do support the sensitivity hypothesis when interpreted in accordance with the visual ecology of the study species.

The close match of λ_{max} in nocturnally active animals to bioluminescence may also be important because when a fish or crustacean moves through the water at night it stimulates luminescent dinoflagellates and, thereby, produces a luminous wake that advertises its position to prospective predators or prey (Hobson *et al.* 1981; based on the burglar alarm hypothesis of Burkenroad, 1943). Hobson *et al.* (1981) go on to show that the maximal wavelength of bioluminescence from a

dinoflagellate (λ_{max} 475 nm), which is then filtered by coastal water (transmission spectrum based on coastal water type I, Jerlov, 1968), would result in an emission spectrum peaking at 500–510 nm. Thus, these fish could be maximally sensitive to the luminescent signals that are associated with movements of other animals through coastal waters. The similarity in the spectral sensitivity of nocturnally active fishes and decapod crustaceans (this study; Waterman, 1961; Fernandez, 1965; Horridge, 1967; Goldsmith and Fernandez, 1968; Bruno *et al.* 1977; Cummins *et al.* 1984; Cronin, 1986; Cronin and Forward, 1988) from coastal environments suggests that decapods are similarly maximizing their sensitivity to luminescent signals.

The responses of the neritic copepod *Acartia hudsonica* to varying wavelengths of light flashes (Buskey and Swift, 1983) result in a broad spectral curve with a maximum sensitivity at 525 nm. Based on these results, Buskey and Swift (1983) suggest that the visual system of the copepod is tuned to the wavelengths that are best transmitted in coastal waters and not to the peak wavelengths of dinoflagellate luminescence (λ_{max} 475 nm). They conclude that dinoflagellate luminescence would be most effective as a deterrent to grazing copepods in the open ocean where peak transmission is near 475 nm. However, they do not consider the differential attenuation of light in coastal waters (see discussion of Hobson *et al.* 1981, above), which results in a wavelength shift from 475 to 500–510 nm (for the peak emission of a dinoflagellate), and this corresponds well with their determination of spectral sensitivity in *Acartia*. Moreover, their results show that the copepods are effectively deterred when the system is functioning under the conditions that would be encountered in coastal waters, the natural habitat for these herbivores. Thus, it appears that spectral tuning in these copepods provides a close match to both the wavelength of maximal transmission in coastal waters and the wavelength of dinoflagellate emission that is incident on the eye of the copepod.

The mechanism of light production in cnidarians and ophiuroids suggests that at least the spectral characteristics of luminescent signals are also under strong selective pressures. The light-emitting system in these taxa involves energy transfer from a molecule that emits at 440–460 nm to a green fluorescent protein complex (Morin, 1974; Shimomura, 1986) that emits light between 500 and 510 nm. The observation that the initial bioluminescent reaction in the majority of luminous emitters produces a blue light, which can then be altered in a variety of ways (for a review, see Hastings and Morin, 1990), suggests that the spectrum of luminescence in benthic cnidarians and brittle-stars has been tuned by selection to match the color of light that both penetrates furthest through coastal waters and coincides with the maximal spectral sensitivity of both vertebrate and invertebrate predators from this environment. Alternatively, the close match between the emission spectra of cnidarians, brittle-stars and polychaetes (Fig. 11B) and the peak spectral sensitivity of predators could be fortuitous. Luminescent spectra can range from 460 to 520 nm in coastal waters and, at *Renilla* standard intensities, any of these wavelength maxima should provide a stimulus that is above the threshold

Table 1. *Range of temporal characteristics of coastal benthic luminescent organisms and the simulation model eliciting maximal responses from* Portunus xantusii

Category	Pulse duration (ms)	Pulse frequency (Hz)	Train duration (s)	Reference[b]
Cnidarians				
Hydroids	60–100	6–17	1–2[a]	Morin, 1974
Sea pens	100–1500	1–3	1–4.5[a]	Morin, 1974
Polychaetes				
Polynoids	63–573	1–25	1–10[a]	Bassot, 1979[c]
Syllids	100–400	1–10	3–8	Bassot, 1979[d]
Ophiuroids	50–150	7	1–10[a]	Brehm, 1977
Emitter range	50–1500	1–25	1–10	–
Mean of emitter range	75–545	2–14	1–7	–
Model maxima	72–200	2–15	4–15	This study

[a] The train duration in these species is proportional to the stimulus intensity.
[b] In addition to the primary reference shown, other references can be found in Morin (1983).
[c] The data in this row were derived from Figs 1–3.
[d] The data in this row were derived from Plate 3.

for the startle response in nocturnally active decapod crustaceans within contact distance of the emitter.

An important determinant of the duration of the luminescent signals produced by the majority of coastal emitters is the strength of the mechanical stimulus (Morin, 1983). Larger or more voracious predators or intruders will produce more vigorous mechanical stimuli, resulting in the production of longer and more intense luminescence (M. S. Grober, in preparation). For *P. xantusii*, longer and more intense light stimuli result in a greater cardiac response and a greater probability of producing a startle or avoidance response. This simple luminescence control mechanism is based upon stimulus facilitation (Morin, 1976), yet it provides for both economic flash production and effective deterrence of a wide size range of potential predators. Gauging flash duration and intensity to match disturbance intensity is important both from an energetic standpoint and because the production of excessively long signals could be strongly selected against since they might advertise the position of the emitter to a variety of other potential predators. In fact, many coastal emitters produce maximum flash train durations, in response to single stimuli, that range from 1 to 10 s (Table 1). Based on the close proximity of the predator to the emitter (close enough for contact), the inverse square law of light attenuation in water and the present study (Table 1), this maximal duration should provide effective deterrence while minimizing the risk of attracting other predators from a distance.

The strength of a mechanical stimulus also has important effects on the intensity of the luminescence that is produced by benthic coastal emitters (Morin, 1983). For this reason, nocturnally active and visually orienting species will be exposed to

a wide range of signal intensities. The low level of responsiveness to the lowest intensity stimuli (0.01 *Renilla* standard, Fig. 9) suggests that these stimuli were close to the visual threshold for *P. xantusii*. Based on the absolute intensity of the *Renilla* standard, the resulting threshold intensity would be between 7×10^5 and 7×10^6 quanta $cm^{-2}s^{-1}$. This compares favorably with behavioral estimates of threshold sensitivity of 4×10^5 quanta $cm^{-2}s^{-1}$ from a shrimp (*Palaemonetes*) and 4×10^6 quanta $cm^{-2}s^{-1}$ from deep-water plankton (see references in Waterman, 1961).

Buskey and Swift (1983) found that copepods responded to simulated luminescent flashes that were 2 log units lower in intensity than the natural flashes produced by dinoflagellates. *P. xantusii* also responded to light stimuli that were 2 log units less intense than *Renilla* standard flashes, as long as the flash had a peak wavelength at about 500 nm (Fig. 9). In both systems, the emitters produce *more than enough* light to elicit both the behavioral and the physiological responses. Although the excess light production could be interpreted as an inefficient component of the predator deterrence system (Buskey and Swift, 1983), it may also be an evolutionary response to the high variability in the illumination of coastal waters. During periods of low water quality (e.g. storm seasons), it may be necessary to produce high intensities of light to ensure sufficient transmission to elicit predator avoidance. Also, since the peak wavelength of the luminescent signals can be shifted by 30–35 nm with respect to the maximal spectral sensitivity of the common predators (Fig. 11A), high-intensity flashes may be necessary to yield sufficient numbers of quanta at the peak for the particular predator's spectral sensitivity.

The increase in cardiac sensitivity to stimuli with pulse rates of 2–15 Hz (Fig. 5) suggests that vision in decapod crustaceans has evolved to maximize detection of a broad range of pulsing or flickering stimuli. The observation that the response ratio does not reach the baseline (1.0) for non-flickering stimuli (Fig. 5) shows that crabs do respond to constant stimuli, but that flicker enhances cardiac responsiveness. This result is in agreement with the suggestion by McFarland and Loew (1983) that the detection of flicker has been a major driving force in the evolution of visual receptors in both invertebrates and vertebrates. The low response to very short-duration (5–50 ms) high-frequency (>15 Hz) pulses may be the result of pulse fusion, since these pulses were produced at frequencies that were near or above the CFF (25.6) for *P. xantusii*. The similar results for very short-duration pulses and long pulses (e.g. 1 s) suggest that (1) short-duration, rapidly pulsed stimuli appear as one long pulse, and (2) pulses with durations that are longer than a few hundred milliseconds are less effective at eliciting cardiac responses. McFarland and Loew (1983) showed that maximal responses to flickering stimuli occur at flicker rates that are 10–20 % of the photopic CFF. Maximal responses in *P. xantusii* occurred at about 22 % of the CFF (4–5 Hz). Although this represents a higher than expected rate of flicker for maximal responsiveness, it provides an explanation for the very low response to stimuli that are of higher frequency, yet still below the CFF (e.g. 20 Hz).

For any given train of light flashes the pulse rate, pulse duration and interpulse interval are all inter-related. In *P. xantusii*, pulse rate appears to be the most critical of these three factors and has a peak value of 4–5 Hz. This conclusion is based on two observations. First, given fixed pulse durations of 125 and 250 ms there was no substantial difference in cardiac response to a wide range of pulse rates, and the maximum pulse rate for both pulse durations was very close (Fig. 6). Second, the interpulse interval that elicited maximum responses increased by a factor of 14 for pulse durations of 125 and 250 ms (Fig. 8). The duration and interpulse interval of the signal are also important, but both factors have broader maximal response ranges than pulse rate. Pulse duration can vary between about 125 and 250 ms and interpulse interval can vary between about 1 and 100 ms without causing an appreciable change in the response.

The duration of the light pulses from luminescent coastal emitters can range from 50 to 1500 ms (Table 1), but usually ranges between 50 and 600 ms in response to brief mechanical stimuli. This range overlaps the range of maximal pulse duration sensitivity in *P. xantusii* (Table 1). The pulse rate of coastal luminescent signals ranges from 1 to 25 Hz and has an average value of about 8 Hz (Table 1). This range corresponds well with the pulse rate of maximal sensitivity in *P. xantusii*, and predicted maxima of a wide range of coastal predators (McFarland and Loew, 1983). Finally, the interpulse intervals for ophiuroid and hydroid signals range from 10 to 25 ms and 5 to 20 ms, respectively (Brehm, 1977; Morin, 1974). These ranges also correspond favorably with the interpulse intervals that elicit a maximal response in *P. xantusii* (Fig. 8).

In summary, it is apparent that coastal luminescent emitters have converged upon a class of signals (contact flash predator deterrents – Morin, 1983) that effectively elicit avoidance or startle behavior from a variety of predators (Buskey and Swift, 1983, 1985; Grober, 1988). Wilkens *et al.* (1974) proposed that a command fiber system was responsible for the coordinated responses of the heart and gill bailers to a variety of sensory stimuli in the crab *Cancer magister*. Since the command system is triggered by rapid and intense stimuli to a number of different senses (visual and/or mechanical), it represents a general response track for 'startling' signals. Similarly, fishes have a startle response that is mediated by command fibers and is elicited by both visual and mechanical stimuli (see Eaton and Bombardieri, 1978). These results for both crustaceans and fishes suggest that selection has favored the production of luminescent signals whose characteristics take advantage of the pre-existing command systems that drive startle and avoidance behavior and are found in the majority of nocturnally active predators.

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