Phototactic behaviour and the nature of the shadow response in larvae of the estuarine crab *Sesarma catenata*

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The phototactic behaviour of the first zoea of the estuarine crab *Sesarma catenata* (Brachyura, Grapsidae) was studied in the laboratory. Light and dark-adapted larvae showed only positive phototaxis when stimulated horizontally with light intensities ranging from 10^{-7} W m^{-2} to 1 W m^{-2}. Peak percentage response was at 10^{-1} W m^{-2} and 10^{-4} to 10^{-2} W m^{-2} in light and dark-adapted larvae respectively. This pattern differs from that common to other brachyuran larvae in the absence of negative phototaxis at low light intensities in light-adapted larvae. The shadow response in *S. catenata* first zoea was studied in a natural light field. The nature of the shadow response is discussed according to larval responses in the horizontal plane. An interpretation of the mechanics of the shadow response is proposed which does not involve negative phototaxis. The consequences of the phototactic behaviour of larvae for dispersal in the Swartkops estuary are briefly discussed. According to their phototactic pattern, larvae would migrate to the top section of the water column and aggregate there during the day. This would increase their chances of being exported out of the estuary with the ebb tides.

Die fototaktiese gedrag van die eerste zoea van die estuariese krap *Sesarma catenata* (Brachyura, Grapsidae) is in die laboratorium bestudeer. Lig- en donker-aangepaste larwes het slegs positiewe fototaksis getoon wanneer gestimuleer met ligintensiteite wat varieer van 10^{-7} W m^{-2} tot 1 W m^{-2}. Spitsreaksie-persentasie was onderskeidelik by 10^{-1} W m^{-2} en 10^{-4} tot 10^{-2} W m^{-2} in lig- en donker-aangepaste larwes. Hierdie patroon verskil van dié wat algemeen voorkom by ander Brachyura-larwes in die afwesigheid van negatiewe fototaksis by lae ligintensiteite in lig-aangepaste larwes. Die reaksie op skaduwee deur *S. catenata* se eerste zoea is in 'n natuurlike ligveld betsudeer. Die aard van die reaksie op skaduwee word bespreek na aanleiding van larwale reaksies in die horisontale vlak. 'n Interpretasie van die meganisme van reaksie op skaduwee wat nie die negatiewe fototaksis betrek nie word voorgestel. Die uitwerking van fototaktiese gedrag op die verspreiding van larwes in die Swartkops-monding word kortliks bespreek. Na aanleiding van hul fototaktiese patroon sal larwes migreer na die boonste deel van die waterkolom en daar versamel gedurende die dag. Dit sal dus hul kanse verbeter om uit die Swartkops-monding uitgevor te word gedurende die ebgetye.

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Like all other estuarine planktonic organisms, *Sesarma catenata* (Decapoda, Brachyura) larvae face exportation and loss from the estuaries owing to a net seaward flow of water. To maintain its population, a mechanism must be present either to allow larvae to avoid being exported from the estuary, or in cases when they are exported, to return to the estuary from the sea.

Both mechanisms have been described in larvae of brachyuran crabs (Sandifer 1975; Scheltema 1975; Dittel & Epifanio 1982; Cronin 1982; Provenzano, McConnaugha, Phillips, Johnson & Clark 1983). These are based on a behavioural pattern of depth regulation in response to environmental stimuli. The larval behaviour of several species in relation to light, gravity, and hydrostatic pressure has been studied in detail (reviewed by Sulkin 1984) allowing the formulation of descriptive models of dispersal and recruitment with some predictive value (Cronin & Forward 1981; Cronin 1982; Sulkin & van Heukelem 1981; Sulkin, van Heukelem & Kelly 1983; Kelly, Sulkin & van Heukelem 1982).

In attempting to formulate a dispersal and recruitment model for the population of *S. catenata* inhabiting the Swartkops estuary salt marshes, the behaviour of larvae towards light, gravity and hydrostatic pressure was studied in the laboratory. This paper reports findings on the phototactic behaviour of the first zoeal stage. This stage was chosen for experimentation for two reasons.

Firstly, phototactic behaviour of brachyuran larvae does not change significantly during ontogeny (see discussion by Sulkin 1984). Secondly, preliminary data on tidal flux at the mouth of the Swartkops estuary (Pereyra Lago 1986) suggests that the volume of water in the lower estuary basin (which includes the salt marshes) is totally exchanged every few tidal cycles. The first zoeal stage of *S. catenata* lasts 6–7 days when reared in the laboratory at 20°C (Pereyra Lago 1987). This time is probably longer than that needed for a total exchange of water in the lower estuary. Accordingly, any phototactic response presumably aimed at avoiding export must be functional within the first stage life-time.

Two main functions have been postulated for photic responses in brachyuran larvae. One is the control of depth distribution during the day. The other is the display of the shadow response (Forward 1976, 1977, 1986) which is an escape response of larvae from large passive predators (e.g. Ctenophores, Medusae) based on the perception of the shadow these predators cast on the water. This paper discusses the nature of the shadow response in *S. catenata* first zoea.

Material and Methods

Maintenance of crabs and larvae in the laboratory

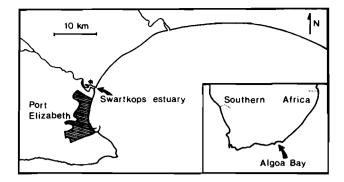*Sesarma catenata* ovigerous females were collected in

Figure 1 Situation of the Swatkops River estuary. The asterisk indicates the locality of collection of *S. catenata* ovigerous females.

the Swartkops estuary salt marshes near Port Elizabeth (Figure 1) and kept in the laboratory in standard culture water (SCW; 35 ppt salinity, 20°C, millipore filtered and UV irradiated sea water), under a 12 : 12 h light/ dark cycle. Upon hatching 300 to 500 larvae were placed in 500 ml glass culture dishes filled with SCW and kept under similar illumination. Larvae were unfed and used for experimental purposes within 48 h after hatching. The effects of starvation were considered negligible since brachyuran larval development may continue independently of food supply until intermoult (Anger 1983, 1984; McConnaugha 1982). All experiments were initiated 6 to 8 h after the onset of the natural light period to avoid interference owing to rhythmic changes in the responsiveness of larvae (Forward & Cronin 1980).

Responses of larvae to light intensity

The apparatus used was comparable to that previously used by other workers (Latz & Forward 1977; Forward & Cronin 1980). A rectangular chamber (65 × 5 × 5 cm) constructed of transparent lucitte was divided into three equal sections by two removable perspex partitions. A slide projector pointing into the chamber along its long axis provided the light stimulus. The excessive heat was dissipated by means of a fan. Light intensity was controlled by the use of neutral density filters with a transmission coefficient of 1 : 10 arranged to give attenuations of 10^{-1}, 10^{-2}, 10^{-3},...10^{-7} of the original light intensity.

The phototactic response of light and dark-adapted larvae was tested. Larvae were light-adapted by placing them under a 100 W sun-blue tungsten filament lamp in addition to room lights for at least 1 h prior to testing. Dark-adapted larvae were kept in total darkness also for 1 h before testing. These adaptation times were chosen following Forward (1976) and Cronin & Forward (1980), although 30 min is probably enough to achieve the adaptation of larvae to any one light intensity (Forward & Cronin 1978).

The observation chamber was filled with SCW and an average sample of 30 sibling larvae (range 28–32) were pipetted into the middle section of the chamber and allowed to disperse for 1 min in total darkness. The partitions were raised and the projector switched on for

1 min after which the partitions were replaced and the number of larvae in each section of the chamber counted. Control tests were run in total darkness in the same manner. Three replicates were performed at each light intensity using larvae from three different broods.

Eight light intensities, from 1×10^{-7} W m^{-2} to 1 W m^{-2} were used in each run, together with one control test. In each case the movement of larvae from the middle section of the chamber into the section nearest the light source or furthest away from it was compared with that of the control. A phototactic response was assumed if the number of larvae swimming in either direction was greater than the control. Responsiveness of light- and dark-adapted larvae was compared using one-way ANOVA (Sokal & Rohlf 1969) after an *arcsin* transformation of the data.

Presence of the shadow response

The shadow response of newly hatched larvae was examined by placing animals in a 4-l beaker which was painted matt-black externally except for an observation window, and filled with SCW. Uniform illumination was provided from above by a 60 W sun-blue tungsten lamp shining through a frosted glass (Figure 3). Light intensity at the bottom of the beaker was 0,1 W m^{-2} as measured with a Li-Cor Quantum Meter. Under this illumination larvae remained swimming just beneath the surface, more or less evenly distributed over the beaker area. A plastic 'ping-pong' ball was then floated on the water and moved backwards and forwards by gently directing an air current at it without disturbing the water surface. The response of larvae to the shadow cast by the ball in the water column was observed by eye.

Results

Phototactic response of larvae

S. catenata first zoea showed a consistent positive phototactic response to most light intensities tested in both treatments (Figure 2). The percentage of larvae displaying negative response was less than that attributable to random movement in both cases. Light-adapted larvae showed a positive response peak at moderate to high light intensities (Figure 2a). Dark-adapted larvae were more responsive to lower light intensities, showing a plateau of response between 1×10^{-4} W m^{-2} and 1×10^{-2} W m^{-2} (Figure 2b). One-way ANOVA revealed that positive responses in light- and dark-adapted larvae were significantly different from each other at all light intensities ($P < 0,1$) whereas no significant difference was found between negative response in both treatments ($P > 0,01$). The lower sensitivity threshold in light-adapted larvae lay close to the 1×10^{-6} W m^{-2} intensity level (Figure 2a) but it was not clearly defined in dark-adapted larvae (Figure 2b).

Display of the shadow response

Larvae reacted to the shadow cast by the ball in the water by swimming actively or sinking passively away from it. Of 136 observations of larvae which showed a clear response, 15% displayed passive sinking to the
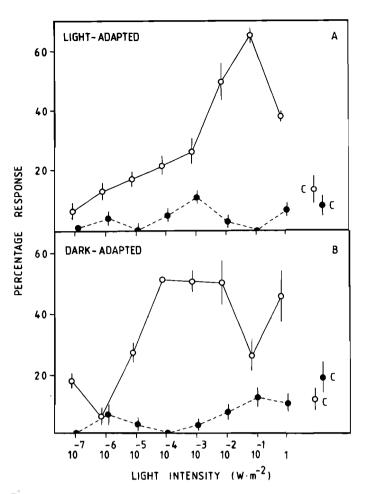
Figure 2 Percentage response of (A) light-adapted and (B) dark-adapted *S. catenata* first zoeae to light intensity. Open circles-continuous line = positive response. Solid dots-broken line = negative response. C = control experiments. Vertical bars represent 1 *SD*. See text for details.

Figure 3 Experimental apparatus used to investigate the shadow response in *S. catenata* larvae. Shading patterns are based on high contrast photography and darker shading represents lower light intensity. Further details in text.

bottom of the beaker, returning to their original position when the shadow disappeared. Eighty-one per cent of larvae actively swam away from the shadow in an apparently random direction between 0° and 60° from the horizontal (Figure 3). Only 4% were observed to swim vertically downwards (60° to 90° from the horizontal).

Discussion

Phototactic behaviour of *S. catenata* first zoea

In most brachyuran larvae the sign of phototaxis depends on previous light adaptation. Dark-adapted larvae are positively phototactic through a wide range of light intensities and most responsive at high intensities. Light-adapted larvae show positive phototaxis to high intensities and negative phototaxis at low intensities (Herrnkind 1968; Forward 1974, 1977). The phototactic behaviour of *S. catenata* first zoea differs from this general pattern in the absence of negative phototaxis to low light intensities in light-adapted larvae (Figure 2a).

Forward (1974) suggested that these differences in response of light- and dark-adapted larvae provide a regulatory mechanism for vertical migration aimed at -
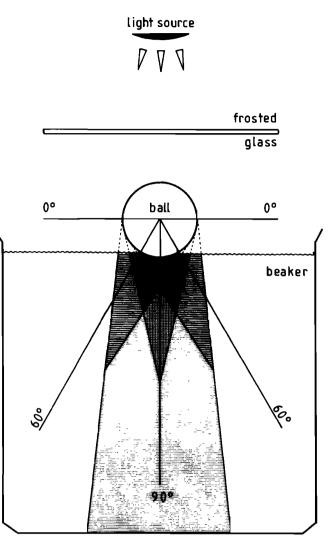
avoiding export from the estuaries. Later evidence (Cronin 1982; Forward & Cronin 1978, 1980) showed that light intensity was not involved in timing of vertical migration, but provided instead a barrier for upward migration of larvae during the day (Forward, Cronin & Stearns 1984).

An alternative interpretation is that the negative response to low light intensities in light-adapted larvae is related to the presence of the shadow response (Forward 1976, 1977). This is a predator-avoidance response in which the larvae sink when light intensity is reduced by 0,5 to 1,0 log units from the original intensity, followed by negative phototaxis when intensity decreases below an absolute level, and a third phase of negative geotaxis which returns larvae to the depth of previous light adaptation (Forward 1986).

Recently Forward *et al.* (1984) and Forward (1986) found that light-adapted *Rhithropanopeus harrisii* (Brachyura, Xanthidae) larvae stimulated in a natural light field (i.e. reproducing the angular distribution of underwater light) lacked the positive response to

medium and high light intensities they exhibited when illuminated horizontally. They showed instead a consistent negative phototaxis at low as well as high light intensities, and Forward (1986) concluded that positive phototaxis in light-adapted larvae was an experimental arctifact of the unnatural lighting conditions. *R. harrisii* larvae still displayed the main phases of the shadow response in a natural light field as they do when stimulated horizontally, the cue for the initial phase of sinking response being the rate of light intensity decrease (Forward 1986).

These findings raise questions about the validity of the interpretation of the results in this work, and about the nature of the shadow response itself. Firstly, is the positive phototaxis observed in light-adapted *S. catenata* larvae in the horizontal plane, an experimental arctifact? Secondly, is the negative phototaxis at low light intensities common to light-adapted brachyuran larvae, a pre-requisite for the display of the shadow response?

When stimulated in a natural light field *S. catenata* larvae remained in a position just beneath the water surface swimming upwards. This indicates that larvae were not negatively phototactic, as they would have aggregated at the black-painted bottom of the beaker. It cannot be concluded, however, whether the upward swimming of *S. catenata* larvae was due to positive phototaxis towards the light above or to negative geotaxis. But since light-adapted larvae behaved similarly at high light intensities (i.e. $0,1$ W m^{-2}) in a natural light field as when stimulated horizontally, it must be assumed that positive phototaxis of light-adapted larvae (Figure 2a), (a) is not an experimental arctifact, and (b) should occur in natural conditions.

In Figure 3 the cone of shadow cast by a spherical object when illuminated from above is schematicaly represented. A brachyuran larva situated anywhere along the axis of such a cone and swimming *away* from it in any direction below the horizontal $0°–0°$ (Figure 3) would necessarily swim towards an area in which a higher light intensity exists. Thus, larvae swimming away from the shadow would be displaying positive phototaxis and not negative phototaxis. Negative phototaxis on the contrary, would direct larvae towards the area of lower light intensity which, according to Figure 3, is situated right beneath the predator. The direction of larval swimming during the shadow response in *S. catenata* is not caused by negative phototaxis but by a change in the direction of the light gradient. This gradient which in normal conditions is vertical and upwards (from low to high intensity) becomes horizontal and outwards when larvae fall within the cone of shadow cast by a predator. Accordingly, no negative phototaxis at any absolute level of light intensity seems necessary for the display of the shadow response since, as with *S. catenata,* positive phototactic larvae would still move away from the predator's shadow both when actively swimming and when sinking passively.

S. catenata larvae swam or sunk but not sequentially. The highest proportion of larvae swam away from the shadow in a direction from $0°–60°$ from the horizontal.

Gelatinous passive predators such as ctenophores and medusae which may induce the shadow response, swim primarily in the vertical plane (Mackie 1980; Mackie, Larson, Larson & Passano 1981). Ctenophores such as Mnemiopsis (Buskey, Mann & Swift 1986) and the siphonophore *Diphyopsis* sp. (unpubl. pers. obs.) swim with their oral end forwards while feeding. Intuitively, the swimming direction most frequently shown by *S. catenata* seems to be within the area in which larvae could move away from the shadow *and* the object causing it while swimming the shortest possible distance. In this way larvae would avoid coming into contact with the predator itself and also escape from its feeding path in the water column. Possibly, visual recognition of the predator and its position in space may serve as an orienting stimulus.

Consequences of phototactic behaviour for dispersal

The phototactic behaviour pattern of *S. catenata* first zoeae provides a positive feed-back system by which larvae would migrate to the top regions of the water column during the day and remain there. As hatching takes place at high tide shortly before dawn (Pereyra Lago 1986) larvae should be dark-adapted at the moment of entering the water column. The intensity of light projected by a cloudy night sky (minimun light available) is in the order of 10^{-4} W m^{-2} (Clarke 1970). Strong positive phototaxis of dark-adapted larvae at this intensity (Figure 2a) should stimulate upward migration. As light intensity increases larvae become progressively light-adapted and the peak of positive phototactic response shifts to higher intensities (Figure 2b). This should induce larvae to a further upward migration and to maintain their position near the surface.

Light penetration at the mouth of the estuary is relatively high. Secchi disk readings on spring tides (highest turbidity) vary between $0,44$ and $2,3$ m during normal summer conditions (Pereyra Lago 1986). The water column is vertically uniform and thermoclines or haloclines are usually absent. There is, therefore, enough light at all depths during the day to elicit larval phototaxis and no physical discontinuities to interrupt larval vertical migration. An estuarine planktonic organism in suspension in the top section of the water column has the highest probability of being exported out of the estuary with the ebb tide. The phototactic behaviour of *S. catenata* first zoea therefore appears to facilitate the export of larvae out of the Swartkops estuary into Algoa Bay.

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