Complete regeneration of ablated eyestalk in penaeid prawn, *Penaeus monodon*

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Ablation of one eyestalk is generally practised in all commercial prawn hatcheries to induce gonad maturation and spawning. An observation was made that the ablated eyestalk of spent females of the tiger prawn *Penaeus monodon* was completely regenerated in less than 6 months and assumed the shape, size, structure and pigmentation of the unablated eye. Significance of this observation in the context of captive broodstock development and the need for detailed examination of the regeneration process and the functional aspects of the regenerated eyestalk in inducing gonad maturation and spawning are highlighted.

CONTROLLED breeding of penaeid prawns under captivity became a necessity as the demand for quality prawn seed (post-larvae) increased tremendously, due to large-scale expansion of prawn farming activities all over the world. This was made possible by the pioneering work of Panouse[1] on the endocrine regulation of reproduction in female *Palaemon serratus*, in which he demonstrated that ovarian maturation can be accelerated by ablating the eyestalk. The eyestalk ganglia and the brain/thoracic ganglia are considered to be the main neuroendocrine centres which play the dual role of inhibition and stimulation of reproduction and moulting in crustacea[2–6]. The X-organ along with the associated sinus gland (X-organ sinus gland system) is the most important ganglionic formation within the eyestalk of the crustacea. In a recent review, Subramoniam[7] has discussed the role of various endocrine hormones in the reproduction of crustacea and has given future directions on this aspect of research.

Partial arresting of the gonad inhibiting hormone (GIH) by removing or ablating a single eyestalk of the female prawn is the simple method generally practised in all commercial shrimp hatcheries, for inducing gonad maturation and spawning. Earlier attempts on eyestalk ablation of the tiger prawn *Penaeus mondon*[8,9] did not yield fertilized eggs although they induced maturation and ovulation processes. It was Santiago Jr.[10], who succeeded in inducing maturation and spawning by ablating the eyestalk of *P. monodon* that produced fertilized eggs with > 90% survival. Since then this method has been successfully tried out on more than 20 species of penaeid and non-penaeid prawns all over the world[7]. However, there is no report available on any aspect of regeneration of the ablated eyestalk in penaeid prawns. Complete regeneration of ablated eyestalk in *P. monodon* is reported here.

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In the pilot-scale prawn hatchery at the National Institute of Oceanography (NIO), Goa[11], various studies related to gonad maturation, reproduction, larval rearing and related aspects are being carried out. Different species of penaeid prawns have been successfully induced to gonad maturation and spawning by unilateral eyestalk ablation. Although different techniques are being used for ablating the eyestalk, the one followed at NIO hatchery is by tying the base of the eyestalk tightly with a surgical thread and cutting the eyestalk just above the knot with sterilized sharp scissors. The uncut eye is tagged with coloured rubber rings to identify the females (Figure 1).

During December 1998, unilateral eyestalk ablation was performed on 15 females of *P. monodon*. Out of these, 13 females attained full gonad maturity and spawned for 3–5 times within 7 to 35 days. After completing the spawning cycle, 8 spent females and 5 males were transferred to the maturation tank for re-maturation studies by the end of January 1999. They were fed a combination of meats and beef liver once a day. Water exchange was made daily at the rate of 150–200%. With the help of an underwater torch, prawns were closely observed for gonad development. The ovaries in all females attained either stage I or II, but no further development was observed until the end of June 1999.

By the end of June 1999, two females were found dead. While removing the dead females, it was observed that the ablated eye of both females had completely regenerated. On seeing this interesting phenomenon, the remaining six live females were also carefully removed from the maturation tank and observed for eyestalk regeneration. Except in one female, in which the regeneration was partial, the ablated eyestalk of all other females were completely regenerated (Figure 1). Since regeneration of eyestalk was not expected and only gonad development was closely monitored, the sequence of the regeneration process could not be followed.

Figure 1. Regenerated eyestalk of *Penaeus monodon*; RR, rubber ring; FR, fully regenerated eye; PR, partially regenerated eye.

The appearance, shape, size, structure and pigmentation of the regenerated eyestalk exactly resembled those of the unablated one. This is an interesting observation, as no report is available on this aspect in penaeid prawns. This may also have important practical application in captive broodstock development as concerted efforts are being made all over the world, to develop techniques for production of selective and superior quality broodstock of penaeid prawns under captivity (see Browdy[12]).

Earlier studies on regeneration of crustacean appendages and eyestalk did not yield encouraging results, although the regeneration triggered precocious moulting[13,14], but later studies indicated the possibility of regeneration of some appendages[15–18]. Kao and Chang[19] were able to regenerate the walking legs of the crabs on transplanting tissues of the limbs in the empty sockets of the same crabs. The only report available, on the possibility of eyestalk regeneration in crustacea, that too based on circumstantial evidence, is by Lyla and Khan[20] who have observed different sizes of eyestalk in natural population of the hermit crab *Clibanarius longitarsus* and remarked that the finding has taxonomical implication. However, there are some reports available on regeneration of eyes in some species of molluscs[21,22].

The present observation becomes more important as it has significant implications on the gonad maturation and captive breeding of penaeid prawns, which are being considered as thrust areas of research in prawn aquaculture[12]. However, it is important to clearly understand the mechanism of regeneration and functional aspects of the regenerated eyestalk. We propose that regeneration of the ablated eyestalk may also be activating GIH production, slowly in the initial stages and actively later, which may be responsible for reducing fecundity in successive spawns and completely stopping maturation of gonad after a certain period.

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Erratum

The programme of cell death in plants and animals – A comparison

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The authors inform that: "In the above article a small error has crept in on page 1172. Apaf-1 is actually a cytosolic factor and not a factor released from mitochondria as stated in the text. The readers should ignore this while reading".