beam tuned to the resonant transition of the atoms, they imaged the overlapping region in absorption. Locations where the atomic density was large were imaged as darker regions and locations where the density was low were imaged as lighter regions. They observed such interference patterns. The spatial period of the interference pattern was 20 μm when the two condensates were 32 μm apart. By varying the distance d between the condensates, they could change the velocity ($v = d/t$) and prove that the spatial period of the interference pattern corresponded to the deBroglie wavelength λ. This is a clear demonstration that the Bose condensate has a definite phase and that the phase is robust.

In a photon laser there is a cavity resonator in which stimulated emission takes place and the photons are in phase. However for applications as a laser, it is necessary to bring out a fraction of the coherent photons out. In a laser, the mirror at one end of the cavity achieves this by partially transmitting the coherent beam of photons. If we want to build an atom 'laser' (i.e. a source of coherent atomic beams) we must devise a technique to couple out from the trap part of the coherent atoms in the condensate. The MIT group achieved this by using a suitable RF pulse[4]. The RF pulse couples the atoms in the magnetically trapped state of the condensate to other magnetic states which are not trapped. These atoms, originally derived from the coherent condensate, leave the trapped region. By an interference experiment, it was shown that a beam of such atoms coupled out of the trap is coherent. Thus the Bose condensate with a suitable output coupler has successfully resulted in an atom laser, albeit a primitive one.

With such an atom laser, one will be able to study phase coherence and superfluid behaviour over a range of particle densities not accessible with liquid ^{4}He. However for such applications one will have to increase the flux and simplify the design. The applications of such atomic beams will most probably be in precision measurements of fundamental constants, atomic clocks, etc.

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SCIENTIFIC CORRESPONDENCE

Glutathione-s-transferases activity in *Macrobrachium lamarrei lamarrei* during embryonic and larval development

Glutathione-s-transferases (GST, EC 2.5.1.18) are a group of multifunctional enzymes considered to play a vital role in the protection of cells against oxidizing metabolites. They enhance the nucleophilic attack of glutathione on the electrophilic centre of a wide array of lipophilic molecules[1,2] and are involved in the reduction of organic hydroperoxides (selenium-independent glutathione peroxidase activity)[3]. The GST activity is well documented in vertebrate species[4], amphibians[5] and fishes[6]. A limited number of studies have been made on the activity of GST in invertebrates[7,8]. The principle activity of this enzyme, in particular, during embryonic development remains uncertain. Since the young ones are fragile and extremely susceptible to any small change, they should have some intrinsic mechanism to overcome this change. Such a protective mechanism may include the expression of GST. Thus the detoxifying enzyme is quantified to understand how the aquatic animals develop to defend themselves against environmental evils right from the embryonic stage.

Many works are available on these detoxifying enzymes in hepatic and extra hepatic tissues of different animals including man, during foetal and postnatal development[9-12], but little is known about the level of this enzyme during embryo development and their possible changes occurring in the transition from embryonic to adult life[13-15]. The present investigation, therefore, was made to understand the specific activity of GST in the passage from embryonic to adult. In this regard the cytosolic fraction prepared from different stages of development such as egg, embryo, larvae and adult tissues of freshwater prawn *Macrobrachium lamarrei lamarrei* was studied.

The adult freshwater prawn *M. lamarrei lamarrei* was collected from Gundoor pond near Tiruchirappalli, India. The ovarian development and spawning were allowed to take place in the laboratory. The spawned mother incubated the eggs in their brood chamber. The developing eggs of *M. lamarrei lamarrei* were grouped into five different stages following the colour variations[16]. Cytosol preparation of developing eggs, embryo and freshly hatched larvae, adult hepatopancreas, gill and muscle were obtained from the homogenization of 1 g sample of each type of tissue. Six replicate samples from each tissue were suspended in 5 ml of 0.1 M sodium phosphate buffer at pH 7.0 and homogenized. The homogenates were centrifuged for 60 min at 105,000 g. The

Table 1. GST activity in the cytosol of unfertilized egg, fertilized egg, larvae and different tissues of *M. lamarrei lamarrei*

Stage/Tissues	Specific activity µmoles/min/mg(protein)	*t* value
Unfertilized egg	0.092 ± 0.0018	
Fertilized egg		
Stage I	0.114 ± 0.0047*	4.38
Stage II	0.157 ± 0.0021*	8.38
Stage III	0.256 ± 0.0079*	12.20
Stage IV	0.307 ± 0.0028*	6.10
Larvae	2.200 ± 0.028*	64.60
Aerated larvae	2.850 ± 0.016*	20.30
Adult		
Hepatopancreas	1.070 ± 0.031*	
Muscle	0.403 ± 0.002	21.40
Gill	0.249 ± 0.017	23.25

Each value is mean ± SEM of 6 determination.
* Statistically significant $P < 0.005$, $df = 5$.

supernatants were recovered for the measurement of enzymatic activity[12].

GST was assayed according to the method of Habig *et al.*[17] with 1-chloro-2, 4-dinitrobenzene as a substrate (CDNB is usually considered as a 'universal' substrate which conjugates most GSTs). The standard assay mixture contained 0.1 M potassium phosphate buffer pH 6.5, 1 mM EDTA, 1 mM 1-chloro-2, 4 dinitrobenzene and a suitable amount of enzyme source. The reference cuvette contained the complete assay mixture with the enzyme replaced by water. The rate of reaction was followed by measuring the increase in absorbance at 340 nm using UV-vis spectrophotometer. Specific activities were expressed as µmoles of GSH conjugated/min/mg protein. Protein concentration was determined by the method of Lowry *et al.*[18].

One-day-old larvae were maintained separately in two 5 l fibre glass tanks with dechlorinated tap water. One tank with larvae was aerated vigorously for 24 h and the dissolved oxygen was estimated at the end of 24 h. The other tank was maintained simultaneously without artificial aeration. The GST activity was estimated in both the experimental and control larvae after 24 h.

The results obtained were analysed by Student's *t* test and a *P* value less than 0.05 was considered to be statistically significant.

The GST activity values towards 1-chloro-2, 4-dinitrobenzene in *M. lamarrei lamarrei* at different stages of development and the *t* value between subsequent stages along with the level of significance are given in Table 1. During the course of egg and embryonic development, a gradual increase of GST activity was observed. The maximum activity was found at the stage IV embryo. Such increased GST level with the progress of embryonic development in *Bufo bufo* and *Salmo iridaeus* has also been reported by Ilio *et al.*[5]. The increased level of GST observed in *M. lamarrei lamarrei* embryo may be used to protect the cell against oxygen metabolites like organic hydroperoxides, i.e. GST has the ability to eliminate organic hydroperoxides[5]. A sudden increase of the GST activity in the hatched out *M. lamarrei lamarrei* larvae was about eight-fold higher than the enzyme activity measured at the previous embryo stage. A similar sudden spurt in GST activity was also observed in amphibian development and the reason for this trend was initially unknown[19]. Later it was suggested that in amphibians, this hike which occurred during the transition from the embryonic to the adult stage, induces the expression of GST to eliminate organic hydroperoxides; that a change from aquatic to terrestrial respiration may expose the animal to higher oxygen tension resulting in an increased formation of toxic metabolites[5]. Similarly the developing prawn larva within the egg receives oxygen for respiration through a permeable membrane. When the larva hatches out, it is exposed to a hyper oxygen condition. Thus there will be a chance for the production of toxic metabolites in the larvae and hence to combat this situation excess GST may be produced. To substantiate the obtained result of higher GST activity in the eclosed larvae, an aditional experiment was conducted with and without artificial aeration. A significantly enhanced level of GST was discernible in the prawn larvae exposed to hyperoxic conditions (artificially aerated and the saturated level was 12 mg/l dissolved oxygen) when compared to the control (Table 1). A similar hike in total glutathione due to increased aeration was recorded in rainbow trout[20].

GST activity in the cytosol of different tissues of the adult *M. lamarrei lamarrei* is presented in the Table 1. The maximum GST activity was observed in hepatopancreas when compared to its muscle and gill because hepatopancreas is the major site for generating the detoxifying enzymes[7]. Similarly, higher GST level of liver was reported in *Bufo bufo*[13] and *Salmo iridaeus*[19]. It is also interesting to note that a higher activity of GST was found in prawn larvae when compared to adult hepatopancreas. Aceto *et al.*[19] also reported the highest GST activity in the fry stage of *Salmo iridaeus* while compared to the activity in adult liver.

From this experiment it is propounded that the significantly elevated level of GST may be a self protection against the toxic effect of compounds derived from oxygen metabolism.

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Vertical transmission of white spot baculovirus in shrimps – A possibility?

White spot syndrome has caused high mortalities and severe damage to shrimp culture industry in China[1], Thailand[2], Japan[3], Taiwan[4], Indonesia and India[5]. The etiology of white spot syndrome has been well studied and confirmed to be a baculovirus called by different names as hypodermal haematopoietic necrosis baculovirus (HHNBV)[1] systemic ectodermal and mesodermal baculovirus (SEMBV)[2], penaeid rod-shaped DNA virus (PRDV)[6] and white spot baculovirus (WSBV)[7].

Histopathological studies conducted in our laboratory (Figure 1) and elsewhere[2,4,8] have consistently demonstrated that only ectodermal (cuticular epidermis, fore and hindgut epithelium, gills, nervous tissue) and mesodermal (lymphoid organ, antennal gland, connective tissue, haematopoietic tissue) origin tissues as the target organs for viral replication. Endodermal origin tissues (hepatopancreas and midgut) are not affected. Basophilic intranuclear inclusion bodies in the target organ cells are the diagnostic feature of WSBV infection. The specificity of WSBV to ectodermal and mesodermal origin tissues has been further confirmed by the recently developed highly sensitive diagnostic tools like DNA hybridization[9,10] and PCR[7,11,12].

While examining large numbers of white spot syndrome-affected shrimps histopathologically, we have noticed interesting changes in the gonadal tissues in a small number of severely affected subadult tiger shrimp (*Penaeus monodon*). The ovary of the severely affected shrimps had atrophied oocytes and also had inclusion-like bodies inside the affected oocytes (Figure 2) and in the connective tissue. Basophilic intranuclear inclusions were also observed in the epithelial cells lining the lumen of vas deferens in severely affected males (Figure 3).

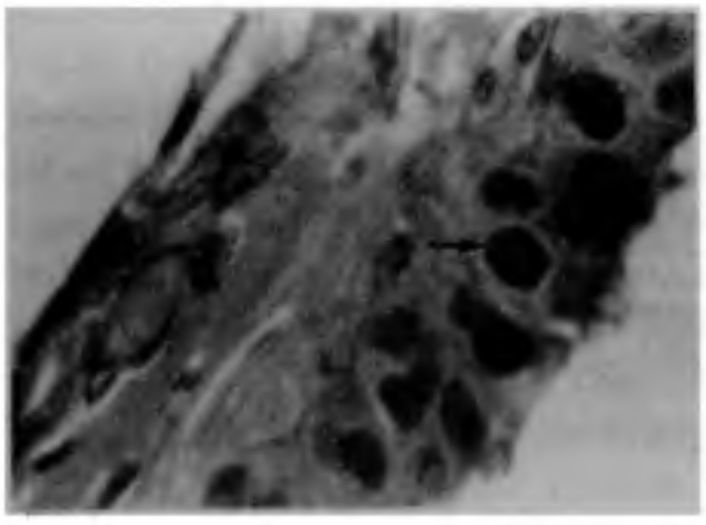

Figure 1. Intranuclear inclusion bodies (arrow) in the hypertrophied nuclei of cuticular epidermal cells. Haematoxylin and eosin (H&E), × 1000.

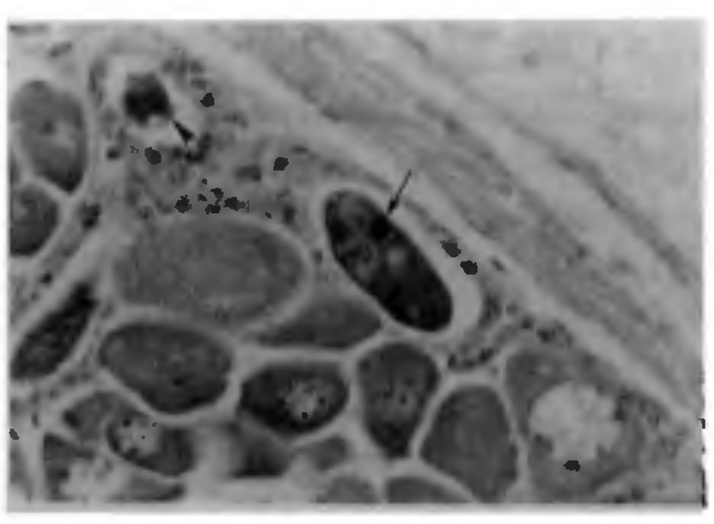

Figure 2. Atrophied oocyte (arrowhead) and intranuclear inclusion-like bodies (arrow) in the affected oocyte. H&E, × 200.

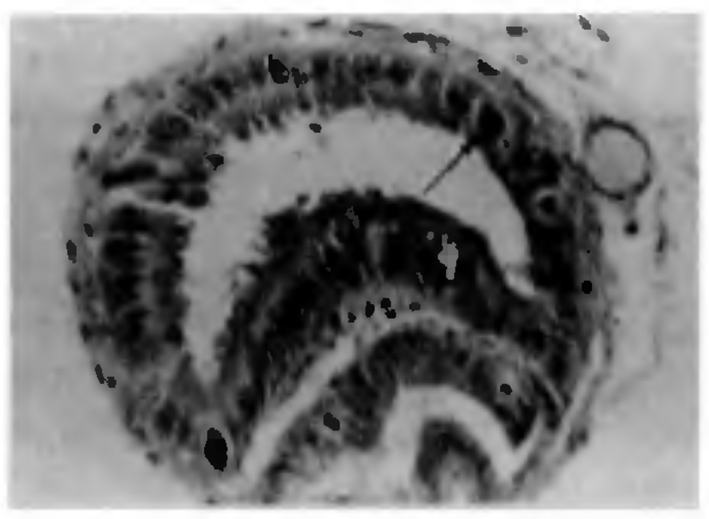

Figure 3. Intranuclear inclusion bodies (arrow) in the epithelial cells lining the lumen wall of vas deferens. H&E, × 400.

Epidemiological analysis of white spot syndrome affected areas along west coast of India over the last two years has indicated mainly two distinct forms of the disease. Shrimp culture areas with previous history of the disease usually experienced acute outbreaks with heavy shrimp mortality. This may be largely because of the likely high viral load in the water. However, in new shrimp culture areas with no previous history of the disease, a chronic form was observed, wherein the disease, when present, developed slowly with delayed or no mortality. The possibility of the virus entering into new areas through seed and taking a longer time for reaching infectious dose in the water through the disintegration of infected dead shrimps cannot be ruled out. Interestingly, cases of crop loss were observed due to acute outbreaks of white spot disease in such culture systems during subsequent crops.

Horizontal transmission through water and feeding of infected shrimps have been suggested as the probable route for the spread of WSBV. Since the target tissues of WSBV are not prone to shedding, the virus dissemination would occur through disintegration of infected shrimps following structural damage or death. Non-involvement of tissues of endodermal origin rules out shedding and spreading through faeces as seen in the case of hepatopancreatic viral infections like monodon baculovirus (MBV)[13] and baculovirus penaei (BP)[14].

Till date there have been no reports of vertical transmission of WSBV. The presence of intranuclear viral inclusions in the gonadal tissues of mesodermal origin strongly suggests the possibilities of vertical transmission of WSBV. Vertical transmission of virus in cultured shrimps has already been documented in the case of infectious hypodermal and haematopoietic necrosis virus (IHHNV) for which gonad is also one of the target tissues[15]. IHHNV is quite similar to WSBV and affects cells of ectodermal and mesodermal origin in shrimp causing eosinophilic intranuclear inclusion bodies.

The presence of WSBV intranuclear inclusions in the gonads, epidemiological clues for introduction of WSBV to newer culture areas through seed and confirmed reports of vertical transmission of IHHNV, strongly support the likelihood of WSBV transmission vertically.

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