DISCUSSION

The study shows that strong anti-GTH II β (LH) labeling gonadotrophs were found in sand goby (*Oxyeleotris marmoratus*) 's pituitary gland in all stages by means of specific staining with anti-GTH II β. GTH II (LH) gonadotrophs are typical basophils also found in other fishes such as bluefin tuna (*Thunnus nathynnus*) (Kagawa *et al*, 1998). Most basophils show immunoreactivity to anti-GTH II β. Alteration of gonadotrophs was observed in the animal reproductive cycle. The number of anti-GTH II β (LH) labeling gonadotrophs increases towards the mature stage and reaches its maximum in the gravid stage. This explains the large size of PPD in the gravid stage seen in gross morphological examinations. In the immature stage, a great number of vacuoles was observed in areas of the PPD normally occupied by gonadotrophs. In the immature and mature stages, the GTH II β (LH) immunoreactivity is found inside some vacuoles. In the gravid stage, the number of vacuoles was markedly reduced, because most of the vacuoles are fully filled with the LH hormone, suggesting that the gonadotrophs start to produce LH hormone in the immature stage and the producing hormone fully occupied most vacuoles in the gravid stage. This was confirmed by histological checks. In all stages, strongly immunoreactive gonadotrophs often showed cytoplasmic extension that contacted other gonadotrophs. The anti-GTH II β (LH) labeling gonadotrophs exhibited patterns of activity correlated with ovarian maturity. Interestingly, the anti–GTH Iβ (FSH) labeling gonadotroph was not found in any stage, suggesting that LH may cover all ovarian regulation functions in the sand goby. This result is also found in the primitive teleosts such as the European eel *(Anguilla anguilla)* (Querat *et al.*, 1990), chinook salmon (*Oncorhynchus tschawytsha*) (Breton *et al*, 1978), tilapia, *(Orechromis mossambica)* (Farmer & Papkoff, 1977), and the African catfish (*Larias gariepinus)* (Koide *et al.*, 1992; Schulz *et al.*, 1997) in which only a single gonadotroph is present. This contrasts with the situation in relatively modern species e.g chum salmon (*Oncorhynchus keta)* (Suzuki *et al.*, 1988 a,b; Kawauchi *et al*, 1989), coho salmon (*Oncorhynchus kisutch)* (Swanson *et al.*, 1991) and Japanese eel (*Anguilla japonica)* (Yoshiura *et al.*, 1999), in which two gonadotrophs: GTH I (FSH) and GTH II (LH) are identified. It would be interesting to study the gonadotropic cell type in the male sand goby, as GTH I (FSH) may be present at early stages of gonadal development. Our data show that all maturity stages of ovary are found throughout the year, but only the gravid stage is identified in November, the rainy seasons wettest month in

Southern Thailand. The results correspond to the work of Boonyoung et al. (2003), who demonstrated that the gonadosomatic index and spawning period of the female sand goby (*Oxyeleotris marmoratus*) are highest in November. The environmental factor may thus affect reproductive physiology and endocrinology. Furthermore, it has been reported that treatment with testosterone increases the number of cytologically mature gonadotrophs and pituitary gonadotropin II in juvenile male African catfish (*Clarias gariepinus*) (Cavaco *et al.*, 2001) and in juvenile female striped Bass (*Morone saxatilis*) (Claire *et al.*, 1998). Therefore, a further research is planned to study the factors affecting or stimulating pituitary gonadotrophs and the initiation of puberty in sand goby.

In summary, the present results show that only a single type of gonadotrophs is present in female sand goby (*Oxyeleotris marmoratus*), namely anti-GTH II β (LH) labeling gonadotrophs. The anti–GTH IIβ (LH) labeling gonadotrophs exhibited patterns of activity correlating with ovarian maturity, thus it is likely that LH may cover all functions requiring ovarian regulation.