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SHORT COMMUNICATION

The First Record of Female Maturation of the Short-finned Eel, *Anguilla bicolor bicolor*, in the Coastal Waters of Thailand

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Abstrak: Objektif kajian ini adalah untuk memberikan informasi biologi pembiakan untuk perkembangan gonad belut *short-finned*, *Anguilla bicolor bicolor*, yang mendiami kawasan perairan pantai Thailand. Belut *short-finned* telah dikumpul daripada tiga kawasan pinggir laut di Provinsi Trang, di Selatan Thailand, dari September 2011 hingga Disember 2013. Gonad daripada 151 spesimen telah menjalani analisa histologi. Daripada pemerhatian histologi, betina pramatang dan matang dijumpai. Berpandukan oosit lanjut dalam keseluruhan bahagian ovari, ovari-ovari spesimen yang dikaji telah diklasifikasikan kepada tiga fasa matang: 1) fasa pramatang telah dikenal pasti dengan kehadiran ovari yang mengandungi oogonia dan oosit pertumbuhan primer, 2) fasa perkembangan telah dikenal pasti dengan kehadiran ovari yang mengandungi oosit peringkat vitellogenik awal bersama dengan oogonia dan oosit korteks alveolar dan adiposit yang banyak, dan 3) fasa vitellogenik lambat yang merujuk kepada ovari yang mempunyai hampir kebanyakannya oosit peringkat vitellogenik lambat. Kepadatan oosit yang berdampingan dengan matriks adipos dianggap mewakili darjah pembesaran gonad. Keputusan kajian ini boleh diaplikasi untuk takrif secara lanjut kawasan peneluran *A. bicolor bicolor* di kawasan Lautan Hindi.

Kata kunci: *Anguilla bicolor bicolor*, Kematangan Betina, Oogenesis, Thailand

Abstract: The objective of the present study was to provide reproductive biological information on the gonadal development of the short-finned eel, *Anguilla bicolor bicolor*, which inhabits the coastal waters of Thailand. Short-finned eels were collected from three coastal areas of Trang Province, southern Thailand, from September 2011 to December 2013. The gonads of 151 specimens were subjected to a histological analysis. The histological observations found both immature and maturing females. Based on the advanced oocytes within an entire ovarian section, the ovaries of the studied specimens were classified into three maturity phases: 1) the immature phase was defined by ovaries that showed oogonia and primary growth oocytes, 2) the developing phase was defined by ovaries that contained early vitellogenic-stage oocytes with some oogonia present along

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with cortical alveolar oocytes and many adipocytes, and 3) the late vitellogenic phase refers to ovaries that contained nearly entirely late-vitellogenic oocytes. The density of oocytes in juxtaposition to an adipose matrix is considered to represent the degree of gonadal development. The results of this study may be applicable in further defining the general spawning area of *A. bicolor bicolor* in regions of the Indian Ocean.

Keywords: *Anguilla bicolor bicolor*, Female Maturation, Oogenesis, Thailand

Anguillids, which belong to the family Anguillidae, are generally distributed in tropical and temperate seas and are occasionally found in the eastern Pacific and southern Atlantic. The life cycle consists of movements in two geographical directions, seaward from freshwater to spawn and landward during the early stages of development (Robinet & Feunteun 2002; Tsukamoto *et al.* 2011). The leptocephali then move landward to return to coastal areas, undergo metamorphosis and enter freshwater as elvers. The adults of the freshwater eel live in fresh water or in estuaries (Nelson 1994). However, there are minor differences between species regarding habitat use and distribution. Only true eels, *Anguilla* spp., inhabit inland freshwaters. Other taxa are either common in brackish water in estuaries or may even remain in coastal areas with high salinities. Some sub-adults may never inhabit freshwater for long periods (Miller & Tsukamoto 2004). Two *Anguilla* species are found in Thailand: the short-finned eel, *Anguilla bicolor bicolor* McClelland, and the long-finned marbled eel, *Anguilla marmorata* Quoy & Gaimard. Both *A. bicolor bicolor* and *A. marmorata* are also commonly found in the Indian Ocean along the coasts of India, Sumatra, Java, and northwestern Australia (Kottelat *et al*. 1993).

Reproductive information about a commercially exploited freshwater eel is important both for fishery management and aquaculture development. However, scientific reports on aspects of the reproduction of these species are limited. A variety of topics, from the onset of the coastal spawning migration to oceanic spawning activity, remain to be investigated. Robinet and Feunteun (2002) reported the first silver eels at Reunion Island. Robinet *et al*. (2003) then performed physiological and histological observations and characterised the advanced stages of sexual maturation occurring prior to marine migration in *A. bicolor bicolo*r and *A. marmorata*. Tsukamoto *et al*. (2011) described the oceanic spawning ecology of freshwater eels, *Anguilla japonica* and *A. marmorata*, in the western North Pacific. Although a substantial number of studies have reported the life history of freshwater eels from several regions, major questions remain, primarily in regard to reproductive biology (van Ginneken & Maes 2005).

Previous studies have surveyed freshwater eel fisheries in Thailand (Piyavatee 1987; Laoprasert & Kaoeian 1995) and have attempted to collect elvers for growth experiments (Sihirunwong 1994; Kaoeian & Playlahan 2001). No previous reports have addressed the reproductive biology of freshwater eels.

The objective of the present study was to provide reproductive information on the gonadal development of the short-finned eel inhabiting a coastal area of Thailand.

This study was conducted in Trang Province on the southwest coast of Thailand. Trang Province is bordered by the Andaman Sea and has relatively large mangrove habitats (24,000 hectares) with a maze of winding creeks and rivers along the coast. These creeks and rivers may receive freshwater discharges from areas in the upper watershed, such as rubber plantations and oil palm plantations, and sewage from rubber and palm oil plants, shrimp farms and domestic sources. The lower parts of these creeks and rivers are covered by mangrove forests and are subject to tidal fluctuations of seawater associated with strong turbulence during the long rainy season (May to December) and with light turbulence during the dry season (January to April).

Specimens of the short-finned eel were collected from three coastal areas: the Sikao mangrove estuary, the Trang River estuary and the coastal swamps of Libong Island (Fig. 1). The Sikao mangrove estuary receives fresh water from several inland small creeks via the upper mangrove area and flows into Sikao Bay. The salinity of the water from which the eels were sampled ranged from 15–29 psu, and was measured using a refracto-salinometer (Atago, Japan). The Trang River estuary forms the mouth of the Trang River. It receives freshwater runoff from several inland areas of Trang Province. The fishing area is located near the upper creeks of the mangrove estuary. The salinity of these creeks was low in the rainy season and high in the dry season, ranging from 0–25 psu. The coastal swamps of Libong Island receive fresh water from the flooding that follows rainfall. The salinity was relatively low during the dry season because the mouth of the creek was closed by a longshore sand bank. The salinity in the fishing area ranged from 10–30 psu. The specimens were captured using a cage net and a hook on a long line. The collecting gear was operated during the night between September 2011 and December 2013. The eels were anaesthetised using a clove oil solution prior to transport to the Marine Biodiversity Laboratory (Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya) and preserved in a freezer at –4°C.

In the laboratory, the specimens were defrosted and identified at the species level on the basis of their characteristic morphology (Smith 1999; Robinet & Feunteun 2002). In particular, the coloration of *A. bicolor bicolor* is plain with no marbling on the back. A total of 151 specimens were examined. The standard length and body weight (BW) were measured for each specimen (to the nearest 1 mm and 0.1 g, respectively). The gonads were removed, weighed to the nearest 0.01 g and preserved in 10% buffered formalin. The gonadosomatic index (GSI) was calculated for each specimen as follows:

$$GSI = GW \times 100/BW, \text{ where GW = gonad weight.}$$

For histological examination, the middle portion of the gonad was dehydrated in ethanol and embedded in paraffin wax. The embedded gonad was serially sectioned into 6-μm-thick sections and stained with Mayer's haematoxylin and eosin. The developmental phases of the gonad and the stages of the oocytes were classified according to the categories and terminology in Lokman *et al*. (1998, 2003), Robinet *et al*. (2003) and Brown-Peterson *et al*. (2011). The phase of ovarian development was defined by the developmental stage of the most advanced oocytes within an ovary.

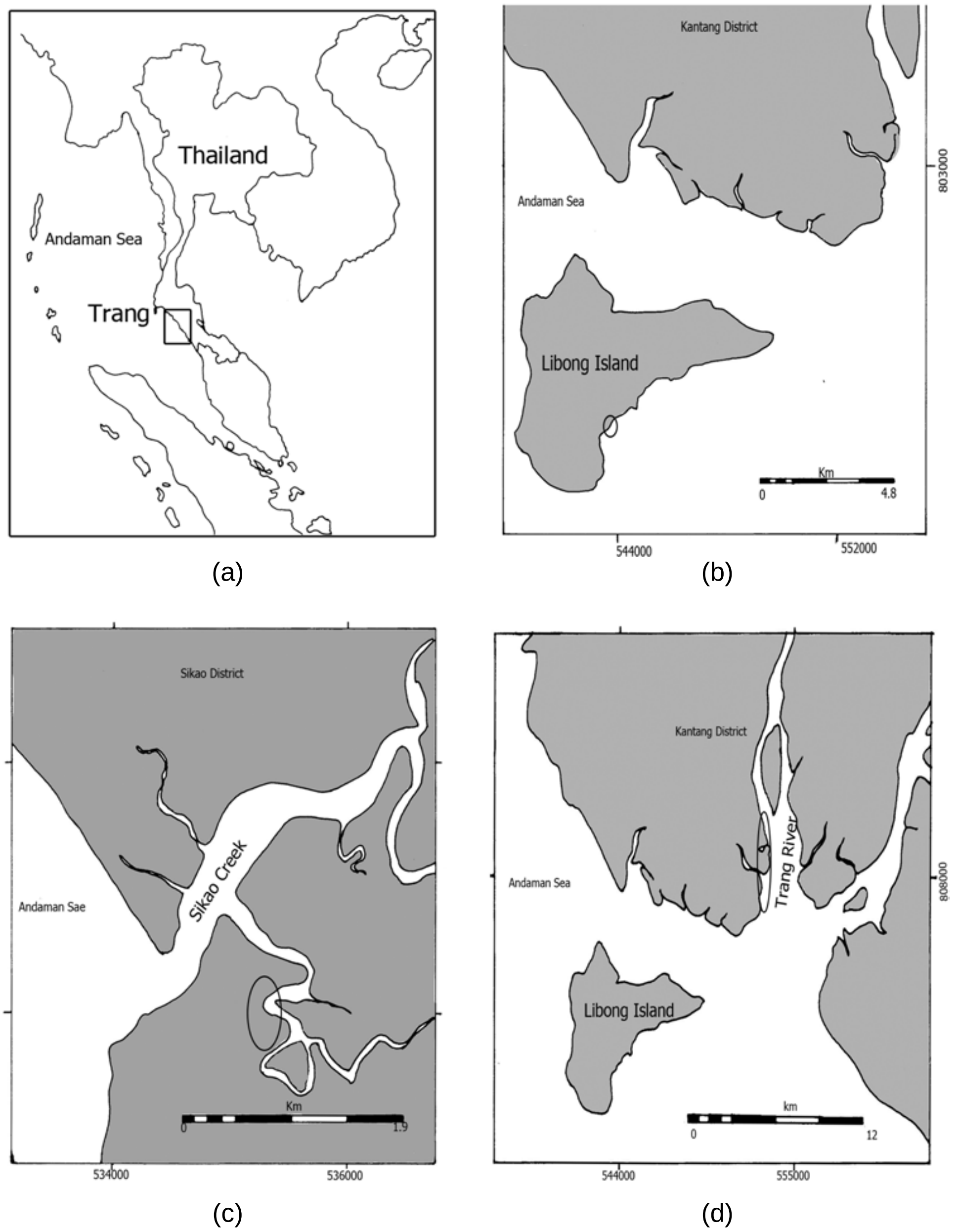

Figure 1: (a) Map showing sampling area in Thailand; (b) coastal swamp of Libong Island; (c) Sikao mangrove estuary; (d) Trang River estuary.

Note: Elliptical symbol indicates fishing area.

In this study, 151 individuals of the short-finned eel were used to examine gonad development. The standard length (SL) of these specimens ranged from 240–885 mm. The wet BW ranged from 22.57–1,117.39 g. Histological observations revealed that the samples from each study site included specimens representing several stages of gonadal development (Table 1). The sex of 136 specimens was determined. A total of 15 additional samples were unsexable due to low gonadal development. Based on the advanced oocytes within an entire ovarian section, the ovaries of the specimens examined were classified into three maturity phases: 1) the immature phase was defined by ovaries that contained oogonia and primary growth oocytes (Fig. 2 [a] and [b]); 2) the developing phase was categorised by ovaries that contained early vitellogenic-stage oocytes with some oogonia and cortical alveolar oocytes (these oocytes were surrounded by a thick adipose matrix, Fig. 2 [c] and [d]); and 3) the late vitellogenic phase refers to ovaries that nearly exclusively contained late vitellogenic oocytes (Fig. 2 [e] and [f]). Therefore, the female specimens could be classified into immature females and maturing females. The immature specimens were 302–742 mm (SL), weighed 50.03–793.08 g, and had gonadosomatic indices ranging from 0.07 to 1.87. The microscopic observations of the immature females showed that the entire sectioned ovary possessed oogonia and primary growth oocytes (perinucleolar stage) (Fig. 2 [a] and [b]). For the maturing female, the microscopic observations indicated that the fish entered the spawning cycle, the period of gonadal growth and the period of oocyte development prior to the beginning of the spawning season. The length (SL) of the specimens that were in the developing phase ranged from 421 to 885 mm, and their wet body weight ranged from 138 to 1,117.39 g; the gonadosomatic indices ranged from 0.09 to 3.18. Only one specimen was found in the late vitellogenic phase; the length (SL) of this specimen was 575 mm, and the wet body weight of the specimen was 187 g. The gonadosomatic index was relatively high, 8.42, with individual oocytes visible macroscopically. These histological observations showed that the late vitellogenic oocytes developed synchronously (Fig. 2 [e] and [f]). The size of late vitellogenic oocytes diameter ranged from 659 to 957 µm (preserved oocytes measurement, n = 125).

Table 1: Number of individuals and maturity of *A. bicolor bicolor* collected from each study site.

Study site	No. of specimens	Unsexable	Male	Female	Immature female	Maturing female
Coastal swamps of Libong Island	44	6	3	35	30	5
Sikao mangrove estuary	10	1	–	9	3	6
Trang River estuary	97	8	3	86	69	17

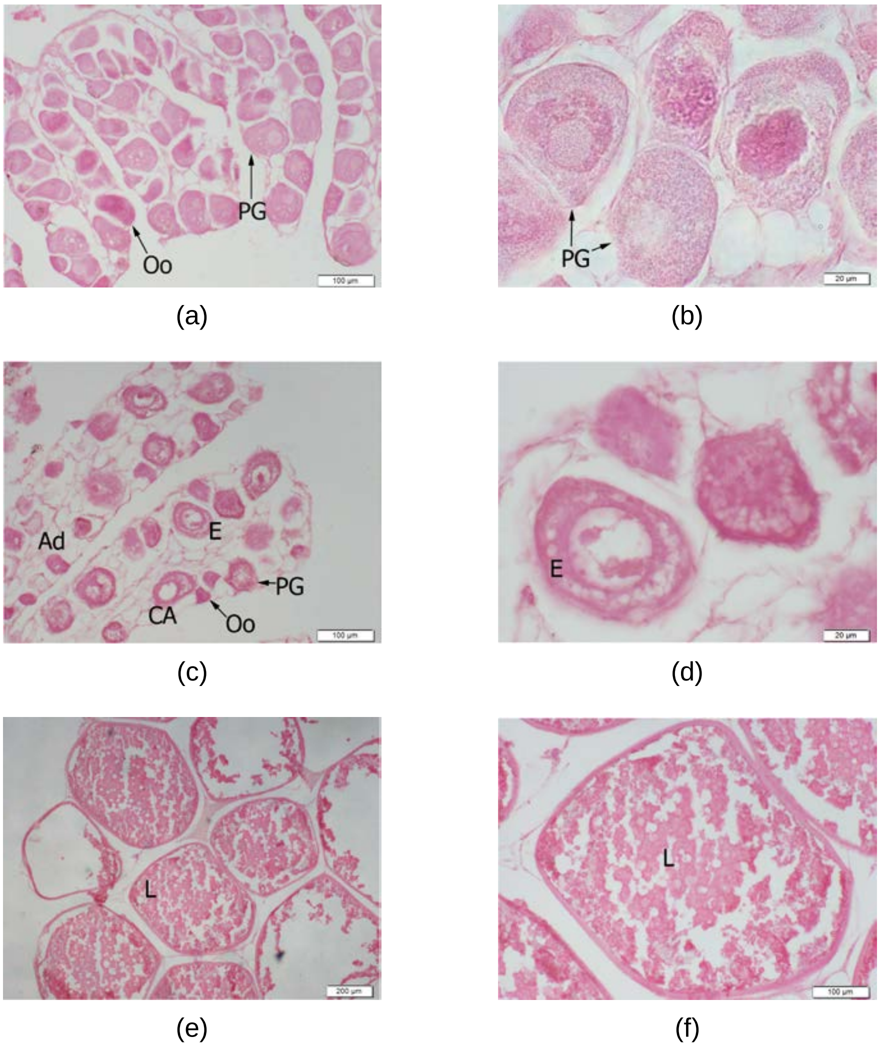

Figure 2: Photomicrographs of ovarian histology of female *A. bicolor bicolor*: (a) and (b) immature phase; (c) and (d) developing phase; (e) and (f) late vitellogenic phase.

Notes: PG = primary growth oocyte; Oo = oogonia; CA = cortical alveolar oocyte; E = early vitellogenic oocyte; Ad = adipocyte; L = late vitellogenic oocyte.

In these specimens, histological evidence showed that lipid accumulation occurred prior to the spawning migration to a deeper marine environment. Our observations are similar to those previously made for other *Anguilla* species (Lokman *et al*. 1998; Robinet *et al*. 2003); however, our observations revealed several more degrees of gonad development. It was previously suggested (not based on scientific evidence) that the spawning ground of tropical eels was located in the Eastern Indian Ocean. Aoyama *et al*. (2003) analysed information about the distribution of the larvae (leptocephali) of several species of tropical eels (but not for *A. bicolor bicolor*) in the Celebes Sea and hypothesised that these species may perform a short-distance migration from the shore to spawn in areas near their freshwater habitat. The results of the present study may be applied to further define the general spawning area of the short-finned eel, *A. bicolor bicolor*, in a portion of the Indian Ocean.

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