# Why does the mudskipper *Boleophthalmus pectinirostris* form territories in farming ponds?

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The mudskipper *Boleophthalmus pectinirostris* maintained territories in farming ponds during the cold season between November and February. There were two types of territory, one was surrounded by mud-walls, and the other was without mud-walls. Both types of territory were entirely covered by shallow water. The area of walled territories was significantly bigger than that of non-walled ones. The nearest neighbour distance of walled territories was significantly shorter than that of non-walled territories. In January, fish surface activity occurred between 1200 and 1500 hours at which time the territorial water temperature reached a maximum, being significantly higher than that of the air, mud surface or deep burrow. The territorial sediments exhibited a significantly greater benthic microalgal biomass (chlorophyll-*a* and total chlorophyll-*a*) as compared to the non-territorial sediments. The present study indicates that *B. pectinirostris* forms a territory during the cold season to keep a pool of water that encourages diatom growth and enables the fish to engage in surface activity.

#### INTRODUCTION

Territoriality is a behavioral mechanism, which is linked to the phenomenon of restricted movement in the sense that it maintains individuals in a limited area for considerable periods (Gibson, 1982). Mudskippers are known to be territorial (Magnus, 1972; Brillet, 1975; Clayton & Vaughan, 1982). For example, the carnivorous Periophthalmus sobrinus in Madagascar may occupy either of two types of territory. One is centered on a single burrow and the other consists of two parts either linked by a subterranean passage or separated into a low tide (feeding) and a high tide (resting) territory (Brillet, 1975). In the herbivorous Boleophthalmus boddarti, territorial individuals live in well-defined, contiguous, polygonal mud-walled territories, and wall construction behaviour is density dependent and serves to reduce aggression in neighbouring territorial fish (Clayton & Vaughan, 1982, 1986, 1988; Clayton, 1987). Additionally, Clayton & Wright (1989) demonstrated that the mud walls are considered to play a secondary, indirect role in maintaining populations of diatoms within territories.

The mudskipper *Boleophthalmus pectinirostris* is mainly distributed throughout the coastal waters of the Indo-Pacific (Zhang et al., 1989; Murdy, 1989; Ishimatsu et al., 1998). It is an herbivorous and euryhaline species, inhabiting intertidal mudflats. The fish hides in a mud burrow during flood tide and leaves the burrow during ebb tide. The young and adult fish graze on the surface sediment with distinctive side-to-side movements of the anterior body and skim off the diatoms and other algae that are their predominant food items (Zhang & Zhang, 1988). This species is a commercially important fish in China. Farming of this species has been developed since 1960 in Taiwan and the 1980s in mainland China (Liao et al., 1973; Hong & Zhang, 2003). At present, this species is widely farmed in the south-eastern coastal waters of China. Farmers collect the *B. pectinirostris* fry from mudflats seasonally, and then introduce them into specially prepared farming ponds of 0.1–1.0 hectares at densities of about 50,000–60,000 fry per hectare. Farming ponds are built in the high tide zone within a sea wall, and the environmental conditions in the ponds are similar to those of the field, except that water is pumped into the farming ponds from the field during high tide.

We found that *B. pectinirostris* was a territorial animal during the cold season (from November to February) only in the farming ponds without artificial irrigation. This finding suggests the territories should confer some advantages for the fish to survive the cold season under farming conditions. The structure, niche and benthic microalgal biomass of the territories were investigated in order to illustrate the roles of the territories in the farming ponds.

## MATERIALS AND METHODS

## Study area

The study was carried out in Funing Bay ( $26^{\circ}53'N$   $120^{\circ}03'E$ ), Fujian, China, where annual air temperatures vary from  $2^{\circ}C$  in January to  $38^{\circ}C$  in July (Wang et al., 2004). Local farmers have cultured *Boleophthalmus pectinirostris* for 20 years and the farming area is ~1300 hectare. This bay maintains a *B. pectinirostris* fishery that takes ~60–70 tonnes of adult *B. pectinirostris* per year and is believed to hold one of the most important *B. pectinirostris* populations in China.



**Figure 1.** Territories of *Boleophthalmus pectinirostris* in farming ponds. (A) Aerial view of the territories. w, walled territory; nw, non-walled territory; nt, non-territory; (B) walled territory; (C) non-walled territory. Arrows indicate the primary opening of burrow. Scale bars: A, 50 cm; B,C, 5 cm.

#### Structure of the territories

Images of the two types of territory were taken using a digital camera (Nikon 4300, Nikon Corporation, Tokyo, Japan) as shown in Figure 1. Territorial data, such as the distance between the boundary and primary burrow opening, the area of territory and the nearest distance between the boundaries of neighbour territories of the same type were measured using ADOBE ACROBAT 6.0. The primary burrow opening was distinguished by the traces of pectoral fins. Mud-walls were considered to be the boundary of a walled territory. The edge of the shallow water was considered to be the boundary of a non-walled territory, since the *B. pectinirostris* seldom slid beyond the shallow water.

## Biomass of the benthic microphytobenthos assemblage

Sediment samples in each of walled and non-walled territories were collected from a 60 cm<sup>2</sup> area using a sampler



**Figure 2.** Nearest neighbour distances and territorial area comparison between walled territories and non-walled territories. Data presented as means ±SD of eight replicate territories. \*, Significant difference (*P*<0.01) between walled territories and non-walled territories.



**Figure 3.** Temperature changes in the air, territorial water, mud surface and deep burrow from sunrise (0600 h) to sunset (1800 h) on 10 January 2006. Data presented as means ±SD of six replicates.

that allowed the sediment surface to remain undisturbed during sampling. The sampler, a glass cylinder, was pushed into the sediment to a depth of 3 cm. A shovel was inserted under the frame and the sampler lifted up together with the enclosed sediment. The sediment was then placed in a container and the shovel removed, leaving the surface of the sediment undisturbed. Six sediment samples were collected at intervals of 10 m. Six sediment samples from non-territorial areas without water (i.e. the mudflat surface) were collected as controls.

A modified syringe was used as a corer (1.6 cm<sup>2</sup>) to take two mud cores from each sediment sample. Cores were frozen prior to laboratory analysis. In the laboratory, the top 1 cm of mud core was removed and transferred to a centrifuge tube. Ten millilitres of 90% aqueous acetone, neutralized with magnesium carbonate, were added and the tube sealed. The tube was shaken vigorously and allowed to stand overnight at 4°C in the dark. After 24 h, the samples were centrifuged at 4000 rpm for 15 min and the supernatant collected. Chlorophyll-a and phaeophytin-a were determined spectrophotometrically following a method modified from that of Lorenzen (1967). Absorbance of the supernatant was measured at 750 and 665 nm in a spectrophotometer before and after acidification with two drops of 1 M HCl. The two measurements allowed a correction to be made for absorption by phaeophytin so that 'active' chlorophyll could be distinguished from that in detrital material, which was abundant in the mud. The reading obtained at 665 nm before and after acidification, corrected for the 750 nm reading, was entered in the equation given by Lorenzen (1967). We multiplied the result by the depth of mud core (0.01 m). Chlorophyll-a and phaeophytin-a data, as surrogate estimates of benthic microalgal biomass, are reported as biomass per unit surface area (mg m<sup>-2</sup>).

#### Measurement of temperature

Temperatures of territorial shallow water, non-territorial mudflat surface, burrow mud and air were measured with a thermometer. Burrow mud temperatures were taken by



**Figure 4.** Variation at the different sites in the abundance of benthic microalgae. (A) Chlorophyll-*a* (mg m<sup>-2</sup>); (B) phaeophytin-*a* (mg m<sup>-2</sup>); (C) total chlorophyll-*a* (mg m<sup>-2</sup>); (D) the ratio of phaeophytin-*a* to chlorophyll-*a*. Data presented as means  $\pm$ SD of six replicate sediments. Statistically significant differences (*P*<0.01) between the sites are indicated by different letters.

inserting a thermometer to a depth of 30 cm. Air temperatures were taken in the shade 5 cm above the surface of the mudflats near the mouth of the burrow. All temperature measurements were made at intervals of 4 h between 0600 and 1800 hours.

#### Statistical analyses

Mean values were compared using Student's unpaired *t*test. All statistics were performed with the statistical software SPSS for windows (SPSS, Chicago, IL, USA).

### RESULTS

As shown in Figure 1, there were two types of territory, one surrounded by either complete mud-walls or incomplete mud-walls, and the other without mud-walls. Both types were entirely covered by shallow water.

The longest distances between the primary openings to boundaries in walled and non-walled territories ranged from 23 to 51 cm and 20 to 35 cm, respectively; and the shortest distances, from 0 to 16 cm and 0 to 10 cm, respectively. Heights of the mud walls ranged from 0.5 to 6 cm. The areas of walled territories (296.35  $\pm$ 53.21 cm<sup>2</sup>) were significantly bigger than those of non-walled territories (181.21  $\pm$ 53.69 cm<sup>2</sup>) (*P*<0.01). The nearest neighbour distances of walled territories (2.0  $\pm$ 1.5 cm) were significantly shorter than those of non-walled territories (4.9  $\pm$ 1.2 cm) (*P*<0.01) (Figure 2). Temperature changes in the air, territorial water, mud surface and deep burrow from sunrise (0600 h) to sunset (1800 h) on 10 January 2006 are shown in Figure 3. The maximum air temperature (14.5  $\pm 0.9^{\circ}$ C) was attained at 1400 h and the minimum temperature (4.0  $\pm 0.1^{\circ}$ C) occurred at 0600 h. The mud surface temperature also reached the maximum (15.8  $\pm 0.36^{\circ}$ C) at 1400 h, which was higher than the air temperature at the same hour. The deep burrow temperature remained relatively stable, varying between 10.0°C and 12.7°C. The minimum territorial water temperature (8.0  $\pm 0.32^{\circ}$ C) occurred at 0600 h, and reached its maximum (21.7  $\pm 0.25^{\circ}$ C) at 1400 h, which was significantly higher than the temperatures of air (14.5  $\pm 0.9^{\circ}$ C), mud surface (15.8  $\pm 0.36^{\circ}$ C) and deep burrow (13.2  $\pm 0.2^{\circ}$ C) (*P*<0.01).

Figure 4 shows that the concentrations of chlorophyll-*a* were significantly greater in the walled (147.35 ±43.08 mg m<sup>-2</sup>) and the non-walled (94.67 ±3.43 mg m<sup>-2</sup>) territorial sediments as compared to the control, non-territorial sediments (75.60 ±12.35 mg m<sup>-2</sup>). The chlorophyll-*a* degradation product, phaeophytin-*a*, was recorded in all sediments sampled. The concentrations of phaeophytin-*a* were significantly greater in the non-walled territorial sediment (99.46 ±6.40 mg m<sup>-2</sup>) compared to the walled territorial sediment (87.47 ±18.23 mg m<sup>-2</sup>) and non-territorial sediment (77.89 ±11.90 mg m<sup>-2</sup>). Total chlorophyll-*a*, uncorrected for phaeopigments, was present at all sites. Total chlorophyll-*a* was significantly

greater in the walled territorial sediment (234.82 ±58.96 mg m<sup>-2</sup>) compared with the non-walled territorial sediment (194.13 ±6.89 mg m<sup>-2</sup>) and non-territorial sediment (153.49 ±19.33 mg m<sup>-2</sup>). The ratio of phaeophytin-*a* to chlorophyll-*a* in the walled territorial sediment (0.62 ±0.12) was significantly less than that in the non-walled territorial sediment (1.05 ±0.08) or non-territorial sediment (1.05 ±0.21).

## DISCUSSION

Unlike *Periophthalmus sobrinus* and *Boleophthalmus boddarti*, territories of *Boleophthalmus pectinirostris* only existed in the cold season. Non-reproductive territoriality has been described in many species of intertidal fish from most of the main families, but there is considerable variation between species in the degree to which territorial behaviour is exhibited (Gibson, 1982). The functions normally ascribed to non-reproductive territoriality are partitioning of the habitat to ensure efficient use of resources such as food and shelter and consequently as a means of controlling population size (Gibson, 1982).

As epipelic diatoms are the most important contributors to primary production and dominant components of the microbial film in soft sediment systems, chlorophylla concentration of the mud has been assumed to reflect the biomass of diatoms rather than that of macroalgae, flagellates, or cyanobacteria (Yang et al., 2003). In this study, the territorial sediments exhibited a significantly greater benthic microalgal biomass (chlorophyll-a and total chlorophyll-a) as compared to the sediment without water, possibly due to the shallow water within the territories. In B. boddarti, there were no significant differences in diatom density between territorial and non-territorial areas (Clayton & Wright, 1989). This result is not consistent with the present study. It could be due to the regular tidal irrigation in B. boddarti habitats. The ratio of phaeophytin-a to chlorophyll-a provides an indication of the physiological state of benthic microalgal populations, where high values correspond to stressed or declining populations (Light & Beardall, 1998). The results of the present investigation showed that the walled-territory had a lower value of phaeophytin-a to chlorophyll-a ratio, indicating that the benthic microalgal population in the walled-territory was vigorous. Therefore, it can be inferred that one of the functions of territorial water is to promote diatom growth within territories.

Temperature is of great significance in the ecology of intertidal fish and the ranges to which mudskippers are exposed are among the highest encountered by any fish (Clayton, 1993). Since the temperature sense in fish is acute (Fry & Hochachka, 1970), there is a clear relationship between temperature and activity of mudskippers especially with respect to emergence from their burrows (El-Zaidy et al., 1975; Colombini et al., 1995). The emergence of Periophthalmus koelreuteri and B. boddarti from their burrows required a minimum surface water temperature of between 10 and 11°C and basking in the warm pool water, in Kuwait (29°N) (Tytler & Vaughan, 1983). Further north in Japan (33°N), deep burrow temperature in the cold season dropped to 5.0-5.6°C and Periopthalmus modestus remained in their burrows from November to March, only emerging when the air temperature reached 18°C. A surface pool of water did not cover the burrow opening of this species (Kobayashi

emergence of *B. pectinirostris* from their burrows can be seen between 1100 and 1500 h, and the highest density of the fish appeared between 1200 and 1400 h, possibly due to the highest territorial water temperature  $(21.7 \pm 0.25^{\circ} C)$  during that time. When the fish emerged from their burrows, they remained motionless and basked for a period, before starting feeding excursions. When feeding on diatoms, the fish always kept the posterior part of their bodies in the territorial water. Since the absorption of solar energy of water is high, the other function of territorial water is to provide suitable temperature for the fish so they could feed on the mud surface. Additionally, concentration of chlorophyll-a on mudflats has been documented to be positively correlated with temperature (Underwood & Paterson, 1993) and therefore it is likely that, the pool temperature may have a positive influence on soft sediment microphytobenthos.

et al., 1971). In the present, more southerly location, the

In *B. boddarti*, the occurrence of wall building is density dependent and a contiguous mosaic of territories is only produced at high fish densities, and the mud wall acts as a visual barrier and reduces aggression between neighbouring territorial fish (Clayton & Vaughan, 1982, 1986; Clayton, 1987). In non-walled territories, B. pectinirostris grazed at the edge of the territorial surface sediment and skimmed off the algae and diatoms. If neighbouring fish detected this behaviour and considered it as aggression, an agonistic encounter could occur. In addition, during the feeding process, the fish are effectively eating the mud and thus lowering its height so that eventually the water pool would gradually expand. When the area of the territory is extended in this way, the nearest neighbour distance would be reduced. As a result, the neighbouring fish could build a mud wall to act as a visual barrier to avoid the increased likelihood of confrontation.

In conclusion, this study has demonstrated that *B. pectinirostris* living in farming ponds form territories during the cold season to retain pools of water. These pools facilitate diatom growth and enable the fish to engage in surface activity. Building a territory by *B. pectinirostris* in the farming ponds is a strategy for the fish to survive the cold season.

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