Indigenous small fish in rural areas for sustainable use and management: growth and reproduction of *Esomus metallicus* in central Lao PDR

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The striped flying barb Esomus metallicus *is a small indigenous cyprinid found profusely in the plains of Lao PDR; it is an important edible protein resource in rural areas. However, its biological survey, relevant to future stock management and conservation for sustainable use, has been minimal so far. In this context growth and reproduction of* E. metallicus *in central Lao PDR were investigated via daily age analysis (using otoliths) and gonad analysis of the species collected during different temperature periods, i.e. low temperature periods (November 2009, February to March 2010) and high temperature periods (May to June 2010). Growth patterns in these three periods were each estimated on the basis of daily age (*t*) and size (*Lt*) relationships and were fitted by the following Gompertz curves;* $Lt = 46.00 \cdot exp[-2.19 \cdot exp(-0.026 \cdot t)]$ *in November,* $Lt = 40.11 \cdot exp[-2.34 \cdot exp(-0.029 \cdot t)]$ *in February to March and* $Lt = 41.72 \cdot exp[-2.46 \cdot exp(-0.057 \cdot t)]$ *in May to June, respectively. The formulae show that growth during the high temperature period was significantly faster than in the low temperature periods. Based on gonad analysis (gonad somatic index, percent) on females, the reproduction of* E. metallicus *was more active during high temperature periods than in low temperature periods. Further, differences in oocyte diameters in various size ranges suggested the maturation size of this species to be larger than 40 millimetres standard length with oocytes of approximately 0.5 millimetres in diameter.*

The simultaneous occurrences of various-sized/aged specimens regardless of periods and widely varied hatching periods indicate that this species breeds almost throughout the year.

Keywords: *Esomus metallicus*, otolith, growth, reproduction, Lao PDR

Introduction

The striped flying barb *Esomus metallicus*, locally called *Pa sieu* in Lao PDR, is a small cyprinid distributed widely in tropical and subtropical freshwaters. It originated in Lao PDR, Thailand, Myanmar, Cambodia, Viet Nam and northern Malaysia (Rainboth 1996; Kottelat and Whitten, 1996; Kottelat, 1998, 2001) but was introduced to Singapore (Ng *et al.* 1993). This species inhabits lowlands and is often found in rice paddies, reservoirs, swamps and irrigation canals (Kottelat 1998; Vidthayanon 2002) throughout the year. Its standard length reaches over 70 millimetres (Rainboth 1996). Because of its wide distribution and proximity to peripheral farmers, this species is an important edible protein resource in inland farming communities of the region often being dried or fermented, and occasionally eaten raw (Tomokawa *et al.* 2008). In recent years, however, there has been potential for stock decline because of environmental changes (for example urbanization and land exploitation for cropping). There is a need to obtain biological information on the

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species for future stock assessment and sustainable utilization. In addition, nearly 20 invasive exotic fish species have recently been reported as having established breeding populations in the Mekong River Basin (Phillips 2002; Welcomme and Vidthayanon 2003), a strong reason for conservation of the region's native/endemic fish species diversity, including *E. metallicus*. For fish diversity conservation as well as stock assessment relevant to sustainable utilization, information on the life history of this species, such as growth, sexual maturity and generation time, is a basic requirement, in addition to further considerations on phylogenic status within the genus and related groups. However, research on these aspects has been minimal so far for *E. metallicus*.

This study aimed to analyse growth and the size/timing/age at sexual maturation of *Esomus m etallicus* based on an analysis of otolith daily increments and gonads. In addition, observations on gut contents were made in order to obtain information on food supply at the collection sites.

Materials and methods

A total of 238 *Esomus metallicus* individuals were used in the study (Figure 1). They were collected from a shallow reservoir of approximately 150 square metres and less than 30 centimetres in depth and from an irrigation canal of less than 30-centimetre depth both located in the Namxuang area (44 kilometres north of Vientiane City) and connected by agricultural irrigation canals to the Namgum River, a tributary of the Mekong River (Figure 2). Fish collections were made on 11 and 25 November 2009 (n = 58 and n = 20, respectively), 4 February (n = 19), 3 March (n = 41), 12 May (n = 74) and 3 June (n = 26) 2010. Samples were collected by a seine net (1.5 millimetre mesh, 10 metre width, 1 metre height). Based on the general pattern of monthly average temperature fluctuation (being higher in April to June [25->30°C +] and lower during November to February [20-25°C]) (Morioka *et a l.* 2009), fish samples were divided into two seasonal groups: the fish collected in May to June that had developed under higher temperature (the high temperature group [HTG]) and those collected in November and February to March under lower temperature (the low temperature group [LTG]). Fish samples were preserved in a 70 percent ethanol solution immediately after capture. Although teleost fishes have three pairs of otoliths (sagitta, lapillus and asteriscus), the lapillus was chosen for daily age analysis in the present study because of fragility and structural complexity in the sagitta of cyprinids (Hoff *et al.* 1997). After standard length (SL, in millimetres) measurement, the otoliths (lapilli) were removed under a dissecting microscope and mounted in epoxy resin on glass slides. They were ground (when opaque) using sandpaper (# 1 500) and lapping films (6 and 9 μm mesh). The ground otolith surfaces were occasionally etched by 0.1 N hydrochloric acid to emphasize increment contrast. Otolith increments were observed and counted under an optical microscope with transmitted light (× 200-1 000).

Figure 1. Adult striped flying barb, *Esomus metallicus* (42.0 mm SL) (courtesy Morioka *et al.*)

As otoliths were removed, gut contents were simultaneously observed for feeding habit observation. In addition, the ovaries of females (37 specimens in November 2009, 34 in February-March and 74 in May-June specimens) were removed and weighed as body weight (grams) for the gonad somatic index (ovary weight/body weight × 100 percent). For 22 females collected in June, the diameters of oocytes were measured, and the oocyte number per female was estimated by means of the number-weight relationship of oocytes, as follows: $WON = (WOW \times PON)/POW$, where WON, WOW, PON and POW were the whole oocyte number per female, whole ovary weight (grams) per female, partial oocyte number and partial ovary weight (grams), respectively.

Figure 2. Map of Lao PDR showing the collection site

Results

Characteristics of fish collection sites: The pond and irrigation canal from which the fish samples were collected contained water throughout the year. The shore and part of the bottom of the pond comprised leaf mould and fine mud, and the river bottom various-sized pebbles and sand; both had bank vegetation.

Age and growth: Daily increments were clearly observable in the otoliths (lapilli) (Figure 3). Daily ages estimated on the basis of otolith increment counts were 49-117 (26.3-43.1 millimetres SL, n = 78) in November 2009, 46-110 (27.5-47.4 millimetres SL, n = 78) in February to March 2010, and 39-118 (25.0-47.4 millimetres SL, n = 60) in May to June 2010, the former two being the LTG and the latter the HTG. Their growth patterns were fitted by the Gompertz formulae, as follows; $Lt = 46.00 \cdot \exp[-2.19 \cdot \exp(-0.026 \cdot t)]$ in November specimens, and $Lt = 40.11 \cdot \exp[-2.34 \cdot \exp(-0.029 \cdot t)]$ in February to March specimens and $Lt = 41.72 \cdot \exp[-2.46 \cdot \exp(-0.057 \cdot t)]$ in May to June specimens (Figure 4), where Lt and t denote the fish standard length and the age in days, respectively. These formulae indicated that the growth of the latter (the HTG) was significantly faster than the former two (the LTG) (F-test, $p < 0.001$), although the difference was not significant between the two formulae of the LTG.

Figure 3. Otolith (lapillus) of *Esomus metallicus* (32.4 mm SL). Scale bar: 100 µm
Source Morioka *et al.*

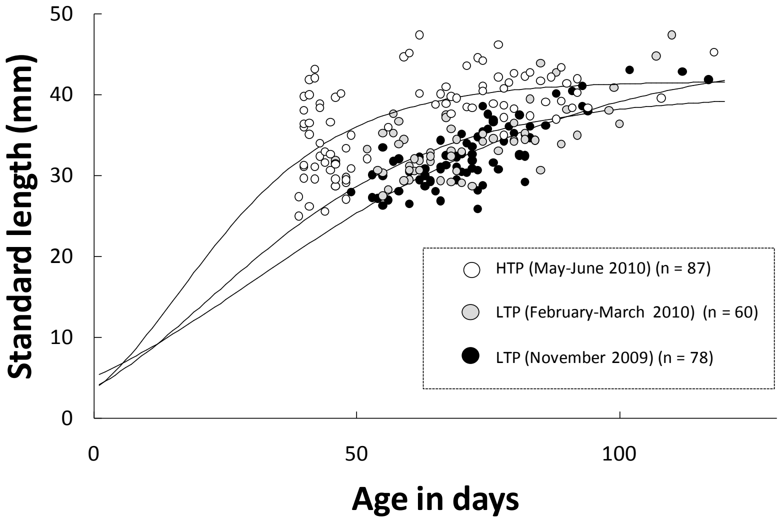

Figure 4. Growths of *Esomus metallicus* occurring in different periods

Source: Morioka *et al.*

Feeding habits: Most specimens collected during high and low temperature periods had considerable gut contents; the major organisms found in the gut were phytoplanktonic algae, including green algae (*Cosmarium* spp.), blue-green algae (*Stigonema* spp.) and diatoms (*Achnanthes* spp.); detritus of unknown species were also often found. However, the animal contents (e.g. zooplankton and insect larvae/adults) were very low. This result suggested that *Esomus metallicus* is herbivorous in the collection sites of the present study.

Gonadal development: The gonad somatic index (GSI, percent) in the fish samples collected in November 2009 (the LTG) was low in all size ranges from 25.0 to 45.0 millimetres standard length (Figure 5). The GSI subsequently increased slightly in the fish samples collected in February to March 2010 (the LTG), and further increased in the fish samples collected in May to June 2010 (the HTG) in which the GSI reached approximately 15 percent or more in specimens larger than 40 millimetres standard length (Figure 5). In addition, comparison between oocyte diameters in different size ranges of fish showed that the larger oocytes (approximately 0.5 millimetres in diameter) were found in fish larger than 40 millimetres standard length and the smaller ones were found in fish smaller than 40 millimetres standard length (approximately 0.2-0.4 millimetres in diameter) (Figure 6a);

the relationship between standard length (millimetres) and oocyte diameter (millimetres) being logarithmically deduced by the following formula: $D = 0.43 \cdot \mathrm{Ln}(L) - 1.1$ ($R^2 = 0.79$, $n = 22$) (Figure 6b), where D and L denoted the oocyte diameter and standard length, respectively. These results suggested the maturation size of *Esomus metallicus* as being over 40 millimetres standard length for those with oocytes of approximately 0.5 millimetres in diameter. The oocyte number per fish tended to increase with fish size, the relationship being linearly deduced by the following formula: $O = 129.1 \cdot L - 3142$ ($R^2 = 0.79$, $n = 22$) (Figure 6c), where O and L denote oocyte number and standard length, respectively.

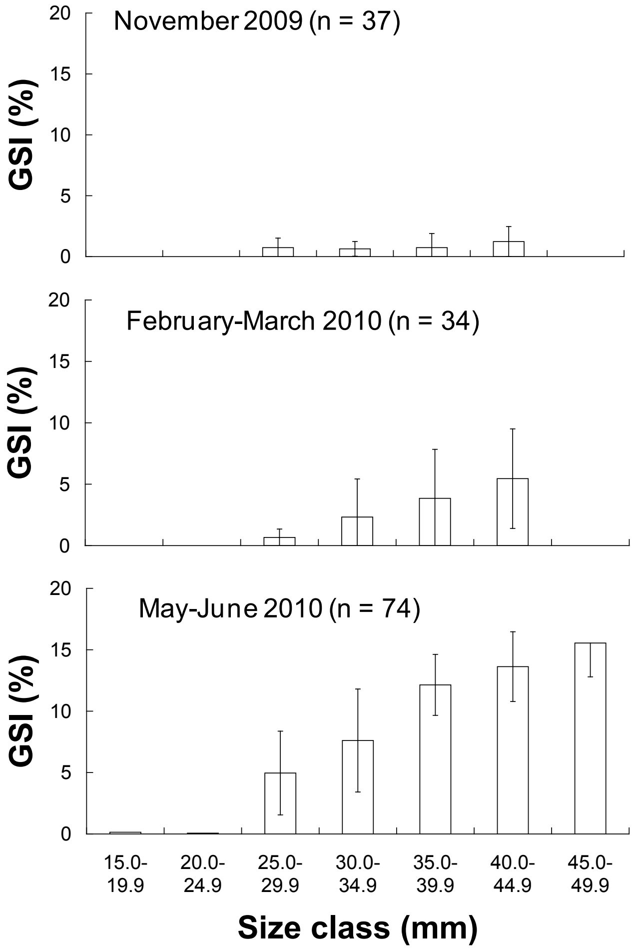

Figure 5. Gonad somatic index (GSI, percent) of female *Esomus metallicus* in different temperature periods

Source: Morioka *et al.*

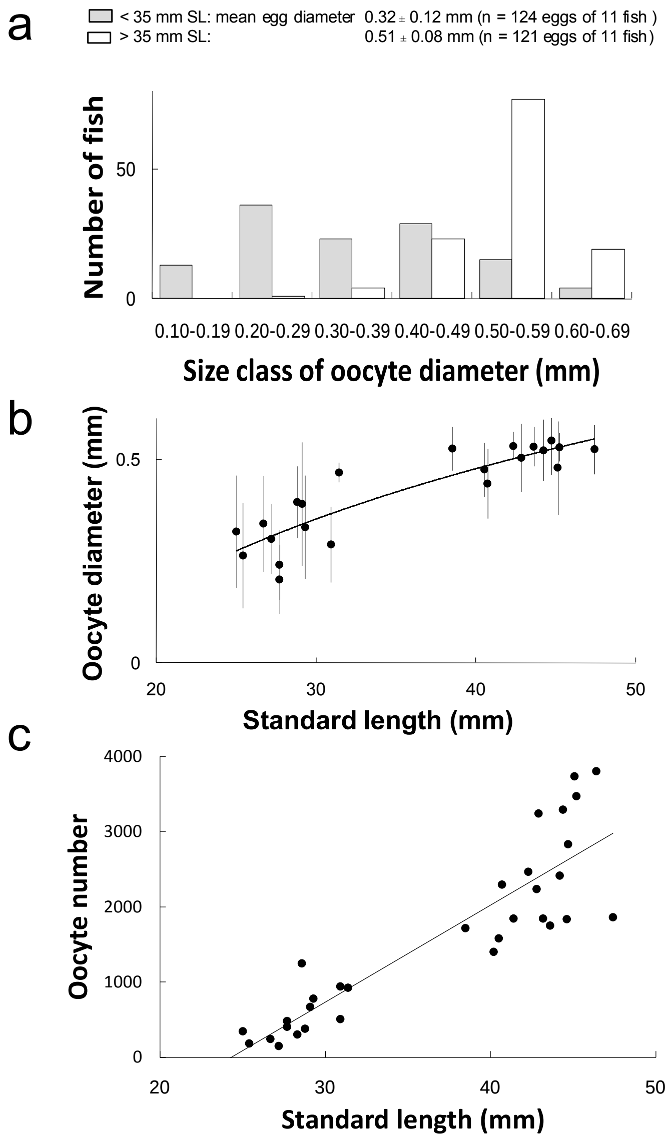

Figure 6 . (a) Frequency di stributions o f oo cyte di ameter i n di fferent si ze class of *Esomus metallicus*. (b) Relationship between standard length and oocyte di ameter. (c) Relationship bet ween st andard l ength and ooc yte number

Source: Morioka *et al.*

Discussion

In the present study, the growth of *Esomus metallicus* during the HTP was significantly faster than that during the LTP (Figure 4). As most of the collected specimens had considerable amounts of food material in their guts, regardless of periods and fish sizes, nutritional conditions were sufficiently stable in collection sites almost throughout the year. These results indicate that the differences in growth rates by periods are attributable to the difference in temperature. Similar phenomena were reported in the sympatric fishes in different taxa, for instance *Brachygobius mekongensis* (Gobiidae, see Morioka and Sano 2009) and *Parambassis siamensis* (Ambassidae, see Okutsu *et al*. 2011). On the basis of both the growth patterns (Figure 4) and the maturation size of females more than 40 millimetres standard length, *E. metallicus* tended to grow relatively faster both in the high and low temperature periods before sexual maturation; the growth rate slowed after sexual maturation (Figure 6a, b) at 60 to 70 days during the HTP and approximately 100 days during the LTP. These results coincided with earlier reports showing that higher temperature causes faster maturation and growth within the species (Dotsu 1982; Kon and Yoshino 2002; Morioka and Kaunda 2005). These short maturation times indicate occurrence of plural generation alternations within a year, also reported in other sympatric fish such as *B. mekongensis* and *P. siamensis* (Morioka and Sano 2009; Okutsu *et al*. 2011). The oocyte number increased with fish size and the maximum number was estimated at approximately 3 000 to 4 000 oocytes per female in the present study (Figure 6c). However, as the maximum size of the species is reported at over 70 millimetres standard length (Rainboth 1996), maximum fecundity is probably more than that estimated here. Furthermore, the simultaneous presence of small/young and large/aged specimens regardless of periods suggests that *E. metallicus* breeds throughout the year, although the breeding peak is observed during the HTP (Figure 5). Such prolonged breeding in subtropical and tropical areas is often reported in various taxa, for example the aforementioned *B. mekongensis* (Gobiidae), *P. siamensis* (Ambassidae), *Anabas testudineus* (Anabantidae, see Morioka 2009), *Corynopoma riisei* and *Corydoras aeneus* in Trinidad (Characidae and Callichthyidae, respectively, see Alkins-Koo 2000).

Esomus metallicus was observed to be highly herbivorous (mainly phytoplanktonic algae) at the collection sites of the present study, this observation coinciding with analysis of the stable isotope of the species (A. Mori, unpublished data). In contrast, the sympatric fish *Parambassis siamensis*, often collected simultaneously with *E. metallicus*, was reported to be highly zooplanktivorous and insectivorous (Okutsu *et al.* 2011). This difference in feeding habits illustrates clear species segregation on the basis of food items among these two sympatric fishes. However, according to an earlier description (Rainboth 1996), *E. metallicus* is planktivorous/insectivorous. Considering these different observations, *E. metallicus* may be omnivorous (adaptable from algae to zooplankton/insect feeding) and its feeding habit can vary by fauna and flora in the habitat, although further investigation is needed.

In addition, Tomokawa *et al.* (2008) reported that consumption of raw small-/middle-sized cyprinids, including *E. metallicus*, causes a risk of parasitic infection (*Opisthorchis viverrini*) in south-central Lao PDR; however they also indicated that *Esomus metallicus* was an important edible protein resource in rural areas. Thus there is a need to publicize such infection risk caused by eating raw fish and the manner of preparation (cooking and process methods) as well as stock management and sustainable use. Some cyprinids (*Parachela siamensis* and *Rasbora tornieri*) contain high vitamin A and contribute to public health improvement in rural societies (Roos *et al.* 2007); more detailed investigations on various fishes in this context are required.

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