

# Cannibalistic phenotype of the larvae of the Oita salamander *Hynobius dunni*

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## INTRODUCTION

The larvae of many temperate salamanders develop under conditions of high density, low productivity with associated low abundance of prey, and therefore strong competition (Wells, 2007). This has sometimes resulted in the evolution of facultative cannibalistic phenotypes, whereby some larvae develop morphology enabling them to consume conspecifics, at the cost of increased risk of disease (Pfennig et al., 1991). This is best studied in ambystomatid salamanders, particularly the *Ambystoma tigrinum* group (Hoffman & Pfennig, 1999), but has also been reported in other taxa (see Wells, 2007 and below). *Hynobius dunni* Tago, 1931 is distributed in lowland central Japan and breeds in early spring following snow-melt; its larvae develop in ponds where competition may be fierce (Kaneko & Matsui, 2004). This provides conditions under which cannibalistic morphs are likely to evolve, and cannibalistic larval phenotypes have been reported in allied taxa (see below). However, this adaptation has not been reported in *H. dunni*. The presence of this feature in *H. dunni* was investigated using a captive colony of salamanders.

## METHODS

Two pairs of *H. dunni* genetically originating in Beppu Shi, Oita Prefecture, Japan, were maintained in captivity and environmentally cycled throughout 2014-2015 to induce reproduction in early April 2015. One resulting larval clutch was maintained at an initial density of c.0.5 larvae/L (Low Density; LD; initial N = 42), and the other at an c.1 larva/L (High Density; HD; initial N = 73). Larvae were housed in mature, pond-style aquaria between 12 and 19°C and fed on aquatic invertebrates ad libitum. Developmental stages follow Iwasawa & Yamashita (1991). Larvae were photographed against a scale and measured using ImageJ (<http://imagej.nih.gov/>). Statistical analyses were performed in SPSS 22.0 for Windows.

## RESULTS AND DISCUSSION

Fifteen (26%) of HD larvae developed a cannibalistic phenotype (Fig 1) and there were no LD cannibalistic morphs. Measuring a subset of five larvae of each phenotype at Stage 51, cannibal larvae had wider heads

proportionately for their Snout-to-Vent Length (SVL) (mean maximum head width/SVL = 0.44) than normal larvae (mean maximum head width/SVL 0.35) and less pointed snouts (mean head width at eyes/max head width = 0.70 in normal larvae and 0.80 in cannibals). Larval duration, total length and mortality rates of HD and LD larvae are reported in Table 1. The cannibalistic phenotype differentiated at Stage 50 and such larvae were observed consuming conspecifics on three occasions; the corpses of the other 13 lost HD larvae were not found and these are presumed to have been cannibalised as well. Although only five of the 15 cannibals were measured, these were drawn randomly and were morphologically representative of the group as a whole. It was not practical to monitor the growth rates of larvae and so the developmental trajectory

Feature	High Density Larvae	Low Density Larvae
Larval duration (days post hatching)	74-113	70-84
Mean (N; SD) TTL at metamorphosis (mm)	72.3 (57; 4.0)	73.9 (41; 3.8)
Proportion of larvae with cannibalistic phenotype (%)	26	0
Mortality (no. dead/total)	16/73	1/42

**Table 1.** Larval duration, Total Length at metamorphosis, proportion of cannibalistic phenotype larvae and mortality in High- and Low-Density larvae of *Hynobius dunni*.

of cannibal and normal phenotypes in this species is not known.

Cannibalistic larval morphs have been reported in *H. retardatus* and *H. nebulosus tokyoensis* (Kusano et al., 1985; Nishihara, 1996; Michimae & Wakahama, 2001); more complete data for comparison are available for the former species. Irrespective of phenotype, *H. retardatus* larvae have proportionately broader heads (head width at eyes/max head width = 0.90 in cannibals and <0.86 in normal larvae, both at Stage 51; Michimae & Wakahama, 2001) than recorded here for *H. dunni*. However, there is a smaller disparity in this ratio between cannibal and normal larvae (difference = c. 0.04 in *H. retardatus* compared with c. 0.1 in *H. dunni*); i.e. *H. dunni* cannibals in this captive population are proportionately more distinct from normal



**Figure 1.** *H. dunni* larvae on 28/5/2015 (day 56 post hatching; Stage 51 sensu Iwasawa and Yamashita (1991)), showing normal (left) and cannibalistic (right) morphs.

larvae. The proportion of cannibalistic HD *H. dunni* larvae was within the range found in *H. retardatus* (Michimae & Wakahama, 2001). Like in *H. retardatus* (Nishihara, 1996), cannibalistic *H. dunni* larvae did not have the elongated vomerine teeth evident in *Ambystoma* cannibals (e.g. Lannoo & Bachman, 1984). Unlike in *H. retardatus*, there were no intermediate morphs and larvae conformed clearly to one of the two morphologies shown in Fig. 1.

Although unreplicated, these observations suggest that the development of cannibalistic morphs was triggered by higher stocking density, as is the case in *H. retardatus* and *H. tokyoensis* (Kusano et al., 1985 - describing *H. tokyoensis* as a subspecies of *H. nebulosus*; Nishihara, 1996; Michimae & Wakahama, 2001). HD larvae metamorphosed smaller (two-sample  $t_{96} = 1.933$ , 1-tailed  $p = 0.028$ ) and later (two-sample  $t_{96} = 8.82$ , 1-tailed  $p < 0.0001$ ) and mortality was substantially higher (see Table 1). This is indicative of density-induced larval stress in *Hynobius* (Kusano, 1981) and so is consistent with the development of cannibalistic larvae in response to competition. A single larva of the high density clutch maintained in isolation in 2L of water was able to grow to 86mm TTL at metamorphosis, suggesting that a simple genetic/maternal effect factor (as exists in *H. retardatus*; Michimae et al., 2009) is unlikely to alone have led to smaller size in the HD clutch. The cannibalistic phenotype developed despite larvae being fed ad libitum and so direct competition for food is unlikely to have been important; indeed in *H. retardatus*, cannibals develop in larvae held and fed separately from, but within the same aquarium as, conspecifics (Nishihara, 1996; Michimae & Wakahama, 2001), suggesting a chemical trigger may be involved.

Although at Stage 51 *H. dunni* cannibals are overall similar in their morphological modifications to those of other *Hynobius* species, these traits had disappeared by Stage 68 (metamorphosis) and all individuals looked alike, which is also documented in *H. retardatus* (Nishihara, 1996). It was not possible to follow individuals and so the effect of cannibalistic phenotype on size at metamorphosis and larval duration in *H. dunni* is unknown but the two distinct morphological groups evident in the larvae were no longer present. It is possible that by the end of

larval development, increased temperatures and higher productivity in nature, as well as reduced larval density and slowing larval growth rates at this point in development (Kusano, 1981), alters the balance between the benefits and presumed costs (Pfennig et al., 1991) so that it is not advantageous to maintain the cannibalistic phenotype later in development.

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