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Reversible frequency-dependent predation of a puffer, *Takifugu niphobles* (Pisces: Tetraodontidae), related to spatial distribution of colour-polymorphic prey

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The puffer *Takifugu niphobles* is a top predator of hard-shelled prey such as molluscs; its predatory tactics may affect the evolution of prey coloration. Two hypotheses concerning its foraging were tested: (1) *T. niphobles* shows frequency dependence in foraging colour-polymorphic prey, and (2) such dependence reverses in response to changes in prey distribution. Captive fish were provided with 70 artificial prey, coloured either dark brown or pale brown, at four frequencies (1 : 4, 2 : 3, 3 : 2, 4 : 1) and in two distribution patterns (uniform and aggregated). When prey were uniformly distributed, frequency and feeding rate significantly influenced colour preference: the common morph was consumed more. When prey were aggregated, frequency significantly affected preference only when the feeding rate was low, in which case the rare morph was consumed more. Thus both hypotheses were supported. The impact of *T. niphobles*'s frequency-dependent predation and its reversal on the colour evolution of prey species, especially molluscs, is discussed. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **81**, 197–202.

ADDITIONAL KEYWORDS: aggregated distribution – apostatic selection – intertidal zone – Mollusca – monomorphism – polymorphism – uniform distribution.

INTRODUCTION

Frequency-dependent selection has been studied mostly in terrestrial predator–prey interactions (such as the predation of land snails or moths by birds). Birds also exhibit reversed frequency dependence: disproportionately consuming a common morph when prey density is low (pro-apostatic selection) or consuming a rare morph when prey density is high (anti-apostatic selection) (Allen, 1972; Greenwood, 1985; Allen, Raison & Weale, 1998; Weale *et al.*, 2000). Pro-apostatic selection is a form of negative frequency-dependent selection that promotes polymorphism by favouring a rare morph, whereas anti-apostatic selection is a form of positive frequency-dependent selection that maintains monomorphism by removing rare variants (Allen, 1972; Horsley *et al.*, 1979; Greenwood, 1985). The reversal of frequency dependence predicts that predators can generate poly- and monomorphism in the same prey population, although such natural populations have not been found. Reversed frequency dependence has also been observed when the spatial distribution of prey shifts between being random and aggregated (Gianino & Jones, 1989; Church, Jowers & Allen, 1997).

Despite the existence of many polymorphic animals in marine ecosystems, few studies have directly addressed the frequency of predator food selection (Murdoch, 1969; Smith, 1975; Jormalainen, Merilaita & Tuomi, 1995). Colour polymorphism is especially ubiquitous in intertidal snails and clams, such as the Littorinidae and Donacidae. In littorinid snails, several selective pressures act to maintain polymorphism: camouflage (Reimchen, 1979; Atkinson & Warwick, 1983; Reid, 1987; Byers, 1990), linkage disequilibrium (Sokolova & Berger, 2000), and apostatic selection (Reid, 1987). Reid (1987) documented apostatic selection in the mangrove snail *Littoraria filosa* by the mark–recapture technique, but the selective agents remain unknown.

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Tetraodontidae and Diodontidae puffers are top predators, with jaws that are specialized to crush hard-shelled prey (Palmer, 1979). Many puffers forage in intertidal rocky shores, tidal flats and estuaries (Garrity, Levings & Caffey, 1986; Ortega, 1986; Duncan & Szelistowski, 1998). They have colour visual pigments (Munz, 1958; Schwanzara, 1967), and thus colour vision with which to search for prey. Therefore the puffer is a potential agent of the evolution of coloration in molluscan prey.

The purpose of this study was to investigate frequency-dependent predation and its reversal in the puffer *Takifugu niphobles* (Tetraodontidae), which is common along shores and in estuaries in Japan. To this end they were provided with two colours of artificial prey at four frequencies and in two distribution patterns (uniform and aggregated).

MATERIAL AND METHODS

Collection and husbandry of *T. niphobles*

From 22 to 25 October 2002, 23 *T. niphobles* puffers (mean standard length, 11.8 mm) were collected by fishing along the shore near the Seto Marine Biological Laboratory, Wakayama Prefecture, Japan (33°41′N, 135°20′E). Each *T. niphobles* was identified by the dot pattern on its back. They were kept in two tanks (semitransparent, 100 × 70 × 70 cm) with running sea water in a greenhouse and fed krill (*Euphausia superba*) twice a week. They were acclimatised to the tanks and the artificial prey for about ten days. After acclimatisation, all fish were fed artificial prey on plastic plates (see next section).

Prey presentation and predation trials

The artificial prey was steamed fish paste (fish paste, egg albumin, starch and other seasonings) that was formed into 5 mm cubes and dyed with food colouring either dark brown ([3 g red + 2 g green dye]/15 mL water) or pale brown ([1 g red + 0.67 g green dye]/15 mL water).

Plastic plates (70 × 100 cm) were covered with polychromatic stones (black, grey, brown, green, white; about 5 mm in diameter) and then artificial prey. To attach the prey, 70 toothpicks (5 mm in length) were fixed on each plate in one of two distribution patterns: uniform (toothpicks were placed at the cross-points of a 10 cm^2 lattice) or aggregated (ten toothpicks were placed at each of seven points of a 10 cm^2 lattice, with at least 30 cm between each point). A total of 70 artificial prey were placed on the toothpicks in four proportions of dark brown : pale brown prey (2 : 8, 4 : 6, 6 : 4, or 8 : 2 for the uniform distribution and for each of the seven patches in the aggregated distribution).

In each trial, one *T. niphobles* was moved from a holding tank to the experimental tank (the same type as the holding tank) and was observed from a distance until feeding stopped, usually after 5–6 min. The number of prey remaining at the end of the trial was counted.

Trials were conducted over six sunny days from 10.00 h to 14.00 h to standardize light conditions. All fish participated in one trial each every day. The trials were planned to standardize patterns provided for each individual so as to eliminate individuality or other factors affecting preference: (1) each individual experienced six different patterns; (2) four of eight patterns were provided per day. A single trial took 10 min from attaching to counting prey, and trials were performed continuously.

Statistical tests

The deviation of the colour proportion of actual from expected consumed prey was evaluated as relative risk θ, following Weale *et al.* (2000):

$$\theta = \ln\{R_D/R_P\},$$

where R_D is the risk for the dark morph (number of dark prey [N_D] eaten/N_D provided) and R_P is the risk for the pale morph. Trials in which fish ate all or nothing of one colour prey were rejected from analyses (Fig. 1). Frequency was analysed by log transforming the relative frequency of the dark morph (N_D provided/number of pale prey [N_P] provided). Although θ represented which colour was consumed more often relative to the proportion provided, hereafter this index is called fish 'preference'. Feeding rate per trial was defined as the total number of prey eaten as a proportion of the number provided.

RESULTS

Trial duration (D) and feeding rate were not related (feeding rate = –0.32 D + 0.70, $n = 71$, $t = 0.50$, $P = 0.62$). About half (78/138) of the trials (41 uniform and 37 aggregated) were used to calculate θ. The feeding rate in these trials increased with dark-morph frequency in both distribution patterns (Fig. 1; uniform: y = 0.064x + 0.61, $t = 2.06$, $P < 0.05$; aggregated: y = 0.13x + 0.56, $t = 4.75$, $P < 0.001$). Regressions of θ to feeding rate were significant for both distributions (Fig. 2; uniform: y = –2.81x + 2.63, $t = 7.98$, $P < 0.001$; aggregated: y = –2.29x + 2.18, $t = 3.97$, $P < 0.001$). The results of multiple regressions of θ on frequency and feeding rate are shown in Table 1. When prey were uniformly distributed, both variables were significant, as suggested in Figure 3. When prey were aggregately distributed, feeding rate was significant but frequency was not. Figure 3 shows changes in slope with feeding

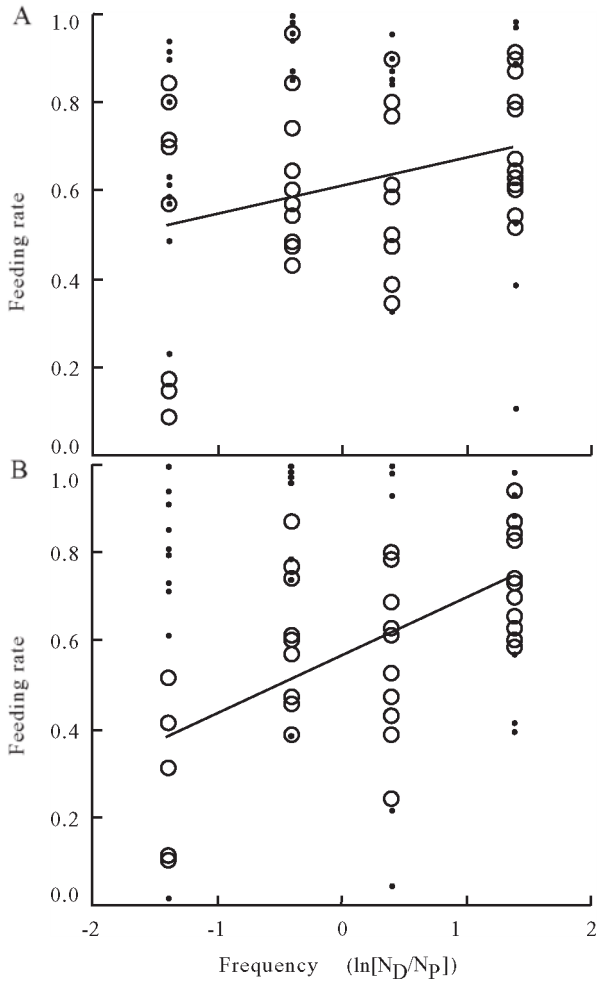

Figure 1. Relation between frequency and feeding rate when prey were (A) uniformly distributed and (B) aggregated. Analysed data (○) and rejected data (•) are indicated. Regression lines were significant in both distributions: uniform: $y = 0.064x + 0.61$, $R^2 = 0.075$; aggregated: $y = 0.13x + 0.56$, $R^2 = 0.38$.

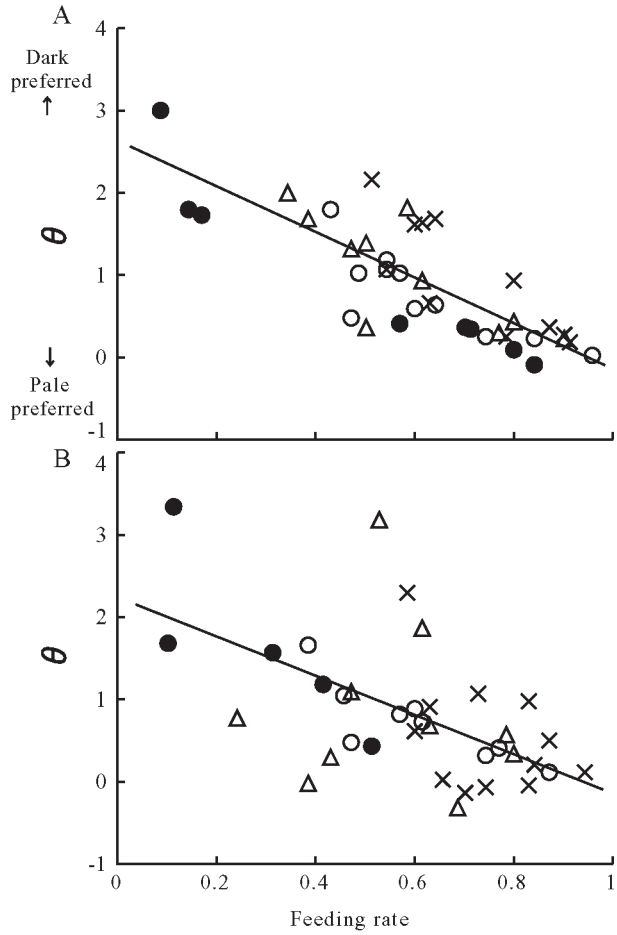

Figure 2. Relation between feeding rate and preference index (θ) for uniform (A) and aggregated (B) distribution patterns at four different dark (D) : pale (P) prey colour frequencies (● = 2D : 8P; ○ = 4D : 6P; △ = 6D : 4P; × = 8D : 2P). Regression lines were significant for both distributions: uniform: $y = -2.81x + 2.63$, $R^2 = 0.61$; aggregated: $y = -2.29x + 2.18$, $R^2 = 0.29$.

rate; these equations suggest that the multiple regression was unable to detect the influence of frequency because it does not assume changes in slope. Simple regressions of θ on frequency were conducted, with θ divided into feeding rates of 0–0.5 and 0.5–1.0. Frequency was significant at the lower feeding rate but not at the higher feeding rate when the error rate was adjusted ($\alpha' = 0.025$) (0–0.5: $y = -0.79x + 0.82$; 0.5–1.0: $y = -0.055x + 0.67$) (Table 2).

DISCUSSION

The results suggest that *T. niphobles* puffers forage using visual cues and that they exhibit frequency-dependent foraging as well as its reversal, consuming rare morphs in aggregated prey distributions and common morphs in uniform prey distributions. However, over-consumption of prey in this experiment may have created statistical artefacts by reducing sample size. Such reduction occurred mainly in the low dark-morph proportion/high feeding rate because of a frequency-independent preference for the dark morph. This preference resulted in a positive relation between frequency and feeding rate. Even if frequency did not directly affect preference, their positive association might be due to feeding rate. Although this finding was mitigated as much as possible by considering the effect of feeding rate in regression analyses, some effect might have remained.

The frequency-independent preference for dark morphs could have been caused by similarity in colour

Table 1. Results of multiple regression analyses for each uniform and aggregated prey distribution patterns

Distribution/ Variable	Slope	t	P
Uniform			
Frequency	0.21	3.06	<0.01
Feeding rate	–3.14	–9.29	<0.001
Aggregated			
Frequency	–0.014	–0.016	0.93
Feeding rate	–2.25	–2.99	<0.01

Table 2. Simple regression analysis of preference at low feeding rates (0–0.5) and high feeding rates (0.5–1) in aggregated prey distribution

Feeding rate	Slope	t	P
Low	0.79	–2.91	0.017*
High	0.055	0.29	0.77

*Significant in adjusted error rate (Dunn-Sidak method, $\alpha' = 0.025$).

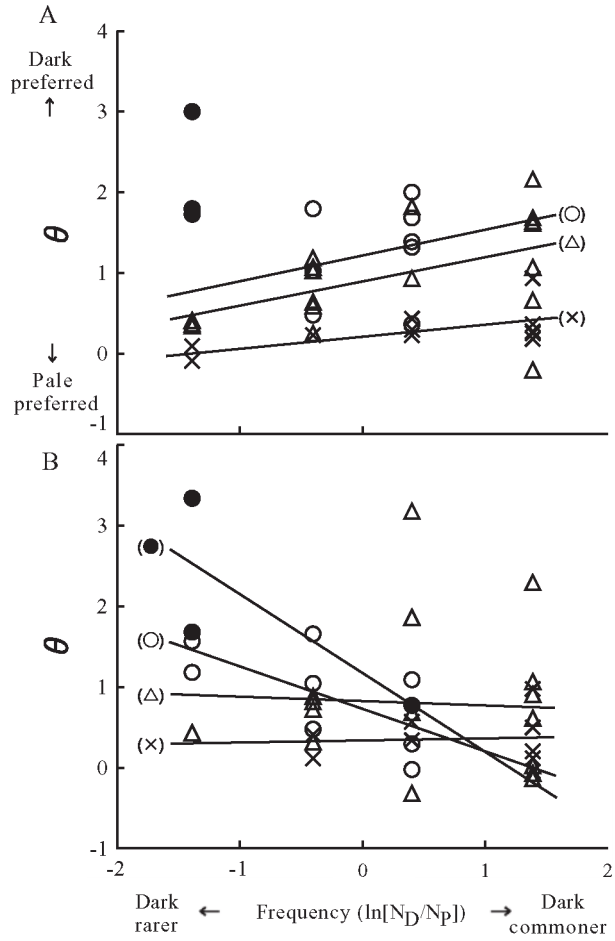

Figure 3. Relation between frequency and preference index θ for uniform (A) and aggregated (B) distribution patterns. Regression lines were generated for each feeding rate (● = 0–0.25; ○ = 0.25–0.5; △ = 0.5–0.75; × = 0.75–1.0), suggesting an effect of both frequency and feeding rate.

to natural prey, the flavour of the artificial dye, or conspicuousness against the background. This preference could have driven the relation between feeding rate and morph preference, because θ must be reduced by a shortage of dark morphs when the feeding rate increases.

When the prey were uniformly distributed, regression analysis showed a significant effect of frequency on preference. Dark prey were consumed more as their proportion increased. Although frequency-independent preference for the dark morph ($\theta > 0$) prevented the reversal of an ultimate preference for the pale morph ($\theta < 0$), preference did change toward the more common morph.

In aggregated prey distributions, on the other hand, frequency dependence was not detected in multiple regression analysis. Equation slopes did vary with feeding rate (Fig. 3), however, which suggests that regression analysis was unable to detect the effect of frequency. Additive regression analysis of low feeding rate (0–0.5) data showed the significant influence of frequency on preference: fish preferred the rare morph in aggregated prey distributions, in contrast to their preference in uniform prey distributions. This result was not found for high feeding rate (0.5–1.0) data, probably due to difficulty in detecting preference as θ approaches zero.

Because aggregated distribution provides higher density locally than does uniform distribution, the above relations between frequency-dependent predation and prey distribution correspond to those found for birds, where birds prefer rarer morphs when prey is dense but commoner morphs when it is scarce (Allen, 1972; Greenwood, 1985; Allen *et al.*, 1998; Weale *et al.*, 2000). When prey is scarce, foraging for a common morph is predicted by optimal foraging theory (Giraldeau, 1997). On the other hand, a predator seeking unique, conspicuous prey in a swarm leads to antiapostatic foraging (Ohguchi, 1978; Wilson, Allen & Anderson, 1990). In many animals (birds, fish and insects), either pro- or antiapostatic selection, but not both, has been observed (Allen, 1988; Sherratt & Harvey, 1993). Only the passerine bird *Turdus merula* (Allen, 1972) and the puffer *T. niphobles* (this study) have shown evidence of both types of frequency-dependent selection. Whether this

reversal is common remains unknown, although bird community suggests it (Allen *et al.*, 1998; Weale *et al.*, 2000).

Colour polymorphism in Littorinidae is probably maintained by fishes rather than birds or crabs (Hughes & Mather, 1986; Johannesson & Ekendahl, 2002), and a mark–recapture experiment has shown that *L. filosa* is subject to proapostatic selection in mangroves (Reid, 1987), where puffers frequently forage at high tide. The present study suggests that the puffer may be a selective agent of polymorphism in these mangrove snails.

Few studies have investigated the relationship between prey density and polymorphism. The change of predator preference has only been reported for the polymorphic beach clam *Donax faba* (Smith, 1975). The most common colour had the highest predation pressure (from crabs) when clam density was low and the second-most common colour when clam density was high; this finding may have been related to reversed frequency-dependent foraging (Smith, 1975). On the other hand, density and degree of polymorphism is positively related in populations of land snails and beach clams (Owen, 1963; Greenwood, 1969; Smith, 1971), as well as in *L. filosa*, as shown in its seasonal change of density (Reid, 1987). This trend appears to be the opposite of that expected by the reversal of frequency-dependent foraging. To explain this trend, Smith (1975) has proposed that predators allow polymorphic prey to be dense because polymorphism decreases the rate of predation (Croze, 1970). Frequency-dependent predation, foraging pressure, and prey density need to be explored in the context of polymorphism in natural populations.

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