Sex, Food, and Dimorphism: Insights into the Trophic Ecology of *Euproctus platycephalus*

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Abstract.—*Euproctus platycephalus* is among the few urodeles with forced insemination, a behavior usually invoked to explain another peculiarity of this species: males are larger than females, and they have larger heads and longer hind limbs. We examined the diet in a population of *E. platycephalus* to evaluate if head size dimorphism is associated with trophic divergence between sexes. We analyzed the contents of the stomach of newts from one population in central Sardinia, taking into account their sex, age, and biometric features. Our data indicated that the newts were generalist benthic foragers, and that diets of males and females were similar. Head size dimorphism likely is influenced only by sexual selection.

Key Words.—diet; Sardinia; Sardinian Brook Newt; selection

Introduction

Sexual dimorphism, the differences in appearance between females and males of the same species, is widespread among amphibians (e.g., Shine 1979; Kupfer 2007; Wells 2007). Size dimorphism is usually female-biased, and fecundity selection (Hedrick and Temeles 1989) is generally invoked to explain it. Male-biased dimorphism of size, shape, and color are generally explained as the results of either sexual selection or ecological divergence (Hedrick and Temeles 1989; Shine 1989; Pincheira-Donoso and Hunt 2017). Generally, sexual selection occurs when individuals with enlarged traits have an advantage in competition for mating relative to other individuals of either the same sex (combat, display, etc.) or of the opposite sex (sequestration; see also Shine 1979; Halliday and Verrell 1986; Shine 1987). However, when sexes occupy different ecological niches, sexual dimorphism could result from ecological divergence (De Lisle and Rowe 2015). Possibly, more than one of the mechanisms mentioned above are involved in shaping sexual dimorphism of a species. Furthermore, more than one mechanism could act on the same body feature, which could be the case of the Sardinian Brook Newt, *Euproctus platycephalus*.

Euproctus platycephalus is endemic to Sardinia and is now restricted to the eastern side of the island (Sotgiu et al. 2010). Adults typically inhabit brooks and rocky ponds. Unlike most urodeles, males of this species have larger bodies, including larger heads and longer hind limbs than females (Bovero et al. 2003; Angelini et al. 2015). Moreover, *E. platycephalus* and its sister species, *E. montanus*, are among the few urodeles with forced insemination (Wells 2007). During a mating bout, males use their jaws, limbs, and tail to restrain a female, thus supporting the hypothesis that sexual selection is the cause for sexual dimorphism (Shine 1979). However, because sexual dimorphism of the head and, consequently, the feeding apparatus could mirror differences in trophic niche of males and females (e.g. Shine 1989; Malmgren and Thollesson 1999), we wondered if the larger head of males is associated with divergence of diet. We address this question by studying the trophic niches of males and females, while reporting on the trophic ecology of this endangered species of newt (Temple and Cox 2009). Only anecdotal data are available on diet of this species (Lecis 2007), apart from a preliminary analysis we presented, indicating that the species is a specialist predator and that sexes have different diets (Sotgiu et al. 2008). Here we reanalyzed the same dataset with more appropriate statistics and come to different conclusions. Furthermore, we discuss our findings in the framework of conservation of this endangered species.

Materials and Methods

We analyzed the stomach contents of 42 newts (25 males and 17 females). We caught newts between 22 and 25 July 1994 from one rocky pond approximately 30 × 4 m with a maximum depth of 2 m, located 600

m above sea level in central-eastern Sardinia. Newts belong to the sample studied by Bovero et al. (2003). After we captured newts and determined sex, we anesthetized individuals by immersing them for about 5 min in a 0.1% solution of MS222 Sandoz. We used a stomach flushing technique to collect the contents of the stomach and stored the contents in 70% ethanol.

We used a digital calliper to measure snout-vent length (SVL, from the tip of snout to the anterior margin of cloaca), head width (HW, the maximum width of head), and head length (HL, from the tip of snout to the neck) to the nearest 0.01 mm. Because SVL includes HL, we calculated the distance from the neck to the anterior margin of cloaca (NVL) by subtracting HL from SVL. We excised one toe from a hind limb to determine the age of individuals. We clipped the same toe on all the newts captured in the same day to ensure that we did not process the same individual twice. After they recovered from anesthesia, we released the newts at their pond. We used skeletochronology to estimate age of newts (Francillon-Vieillot et al. 1990; Bovero et al. 2003). We analyzed data only from sexually mature newts. We were not able to estimate the age of one male; consequently, our sample size was 41 for analyses involving age. Analyses of head morphometry (HL, HW) were based on ANCOVA with NVL as covariate.

We used a binocular microscope to identify to family most prey items in the stomach contents. For statistical analyses, we primarily grouped prey items to family, but we did distinguish the larvae, pupae, and exuviae of chironomids, and we distinguished between larval and adult Dytiscidae. We measured the size of approximately 15% of the food items. To measure the food items, we photographed them under a binocular microscope with graph paper, and used morphometric-geometric software tpsDIG (Rohlf 2013). The size of prey in this subsample did not differ between sexes (ANOVA $F_{1,83} = 1.867$, $P = 0.177$, 11 prey groups), and within each family group prey size did not differ between sexes (nested ANOVA $F_{7,61} = 1.212$, $P = 0.321$, seven prey families eaten by both sexes). Thus, we assumed that sexes did not feed on prey of different size, and our analyses of prey items only considered differences in taxa. We compared the diets of males and females using Analysis of Similarities (ANOSIM, based on Bray-Curtis distance with 20,000 permutations) and the graphical method of Amundsen et al. (1996), which plots the frequency of occurrence of a given prey type (i.e., the relative number of stomachs in which that prey had been found) against its prey-specific abundance (i.e., the percentage of the prey items out of the total number of items found solely in the newts in which the given prey occurs). We evaluated whether the diversity of prey on which the newts fed, expressed with the Shannon diversity index (H'), depended on age and head size, and if and how they interacted with the sex of

individual, by using ANCOVA. To avoid redundancy, for H' analyses we used the residuals of multiple regressions of HL and HW as dependent variables against age with NVL as independent variables. All the variables were normally distributed, except age; thus, we log-transformed age to meet the assumption of normality. A preliminary test for the homogeneity of slopes did not reveal any interaction between the categorical variable sex and any of the covariates (data not shown). We used the software STATISTICA 7.0 (StatSoft, Inc.) for all the statistical analyses except ANOSIM, for which we used PAST 2.15 (Hammer et al. 2001).

RESULTS

Males had longer heads (mean = 16.00 ± 0.28 mm[SE]) than females (12.09 ± 0.29 mm; $F_{1,38} = 12.412$, $P = 0.001$), and larger heads (males: 11.78 ± 0.18 mm, females: 8.72 ± 0.15 mm; $F_{1,38} = 40.079$, $P < 0.001$). The mean ages of newts were not significantly different between sexes (males: 8.46 ± 0.7, females: 6.5 ± 0.4; $F_{1,39} = 87.68$, $P = 0.053$).

We found 1,276 prey items in 42 stomachs, which were assigned to the lowest taxonomical rank to 18 families and one order of invertebrates: Insecta (67.5%, of which 89.6% were Diptera), Crustacea (31.3%), Gastropoda and Arachnida (Table 1). Three newts fed on eggs of *E. platycephalus*. The average number of prey per individual was 30.38 ± 4.03, ranging from 1 to 129 items, and the average number did not differ significantly between sexes ($F_{1,40} = 0.001$, $P = 0.995$). From the inspection of the Amundsen's graph (Fig. 1), the diets of males and females appear similar, as confirmed by ANOSIM ($r = -0.04$, $P = 0.864$). The graph shows that the species is a rather generalist predator, i.e., all prey-categories are located in the lower part, meaning that the average contribution of each prey taxon to the stomach contents was low. Only Limnadidae, especially for males, and larval chironomids, might be considered as a relatively important prey. Furthermore, few males were specialized on Notonectidae. Individual H' ranged between 0–1.91 (1.2 ± 0.06); it did not change with age of a newt ($r_p = -0.17$, $P = 0.272$), HL residuals ($r_p = 0.23$, $P = 0.144$), or HW residuals ($r_p = -0.08$, $P = 0.601$), and it did not differ significantly between sexes (F1,39 = 0.201, P = 0.656). H' was similar between sexes also after controlling for HL ($F_{1,38} = 0.00$, $P = 1.00$), HW ($F_{1,38} = 0.599$, $P = 0.443$), and age ($F_{1,38} = 0.687$, $P = 0.412$).

DISCUSSION

Our results mirrored the trend of intersexual size and shape differences reported in Bovero et al. (2003). Despite head shape differences between sexes,
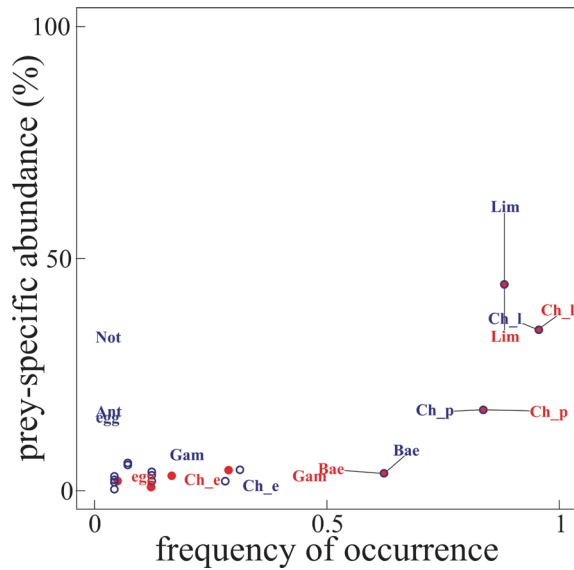
FIGURE 1. Feeding strategy of the Sardinian Brook Newt, *Euproctus platycephalus*, in central Sardinia represented by the Amundsen's graph. Codes have been used only for most important prey groups (Ant: Anthomyiidae; Bae: Baetidae; Gam: Gammarida; Lim: Limnadiidae; Not: Notonectidae; egg: *Euproctus*' eggs; Chironomidae codes are followed by the initial of exuviae [Ch_e], larvae [Ch_l] or pupae [Ch_p]); blue color is for males, red for females; double color circles for the four most important preys represent their relative positions after merging data from males and females.

ANOSIM analysis, Amundsen's graph inspection, and the study of relations of the *H′* index with individual features indicate that male and female *E. platycephalus* have similar trophic niches. Thus, we have no evidence for an ecological causation for head dimorphism. On the other hand, larger male heads possibly increase the capability of restraining females during copulation, and to restrain larger females, resulting in higher reproductive success. Consequently, only sexual selection seems to act on head shape, although this has to be tested in future studies. Furthermore, head size dimorphism apparently has not resulted in trophic niche divergence of the sexes. We previously reported that *E. platycephalus* specializes on Chironomidae and that the sexes have rather different diets (Sotgiu et al. 2008). Thus, our current analyses contradict our previous hypotheses from the same dataset (Sotgiu et al. 2008). However, we previously arranged the prey items only by taxa; whereas, for the current study we have considered the ecology of different life-stages of Chironomidae and Dytiscidae, which is a more realistic approach when studying the trophic ecology of a predator. Furthermore, the investigation of diet difference between sexes was based only on indices; whereas, for the current study, we use more proper tests of comparisons, such as ANOSIM and ANCOVA.

TABLE 1. Prey detected in stomachs of the Sardinian Brook Newt (*Euproctus platycephalus*) in central Sardinia. The total number of prey belonging to each taxon is followed, in round brackets, by the number of stomachs in which that taxon was found.

Taxa		Males (n = 24)	Females (n = 17)
Arthropoda, Malacostraca	Gammaridae	10 (4)	9 (8)
Arthropoda, Branchiopoda	Limnadiidae	198 (18)	164 (14)
Arthropoda, Arachnida	Araneidae	2 (2)	
Arthropoda, Insecta	Dytiscidae larvae	11 (7)	3 (3)
	Dytiscidae adults	3 (2)	
	Anthomyiidae larvae	3 (1)	
	Chironomidae exuviae	12 (8)	7 (4)
	Chironomidae larvae	256 (19)	198 (15)
	Chironomidae pupae	135 (17)	101 (15)
	Culicidae larvae and pupae	6 (6)	7 (5)
	Dolichopodidae larvae	1 (1)	
	Stratiomyidae larvae	3 (3)	
	Syrphidae larvae	1 (1)	
	Tabanidae larvae	1 (1)	
	Tipulidae larvae	1 (1)	1 (1)
	Baetidae nymphs	33 (13)	16 (9)
	Lestidae nymphs	1 (1)	
	Corixidae adults	2 (2)	2 (2)
	Notonectidae adults	4 (1)	
	Trichoptera	1 (1)	
Mollusca, Gastropoda	Ancyliidae	1 (1)	
Mollusca, Gastropoda	Planorbidae	3 (3)	
Chordata, Amphibia	amphibian eggs	3 (1)	2 (2)

Euproctus platycephalus tend to be a generalist predator, feeding mostly on benthic or necto-benthic prey, but also on active swimmers (e.g., Dytyscidae, Corixidae, Notonectidae) or prey that lay under the water surface (Culicidae). This suggests that *E. platycephalus* is an active forager that primarily seeks prey on the pond floor, but that also feeds within the entire water column, even pursuing swimming prey. Adults *E. platycephalus* also scratch prey, such as Ancyliidae, from the substratum. Cannibalism on eggs is rarely reported in amphibians (interestingly, it is reported also for *E. montanus* by Salvidio and Sindaco 2007); however, eggs seem to be of little importance for the diet of *E. platycephalus* compared to other pond-dwelling

newts (Denoël and Andreone 2003; Cicort-Lucaciu et al. 2005).

The aquatic diets of adults of the closely related stream-dwelling species *E. montanus* (Salvidio and Sindaco 2007) and *Calotriton asper* (Montori, A., and G.A. Llorente. 2014. Tritón pirenaico – *Calotriton asper*. Available from http://www.vertebradosibericos. org/anfibios/calasp.html [Accessed 6 September 2016]) are rather different from our population, because they fed mostly on prey typical of stream habitat. However, a lacustrine population of *C. asper* mostly fed on larval Diptera, similar to our pond population. This suggests that the diet of these species depends on habitat, and we expect that investigations on the diet of brook populations of *E. platycephalus* will result in more typical stream diet. Whatever the case, most Sardinian streams experience drought and are fragmented in a sequence of ponds during summer, and we regard our findings as informative for the feeding ecology of many populations of *E. platycephalus*, at least during the warm season. Furthermore, we also think our findings are useful for the protection of the species. In fact, even though predation by fishes is unlikely (Bovero et al. 2005), trophic competition with fishes in such isolated ponds is possible, especially with salmonids and eels, whose niches partially overlap the *E. platycephalus* (Pomini 1940). Moreover, competition can be exacerbated by the global warming and water catchments that cause more severe and prolonged summer droughts, as well as by inappropriate fish restocking, including introduction of allochtonous fishes. More generally, we stress the importance of information on the feeding habit of any endangered species when planning conservation measures and managing natural resources which concern their habitat.

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