

Predation pressure, density-induced stress and tail regeneration: a casual-nexus situation or a bunch of independent factors?

Gerasimia Tsasi¹, Panayiotis Pafilis^{2,3*}, Chrysi Simou¹, Efstratios D. Valakos¹

Abstract. Caudal autotomy, the self-induced tail shedding from the body, is a common defensive strategy in lizards. Tail loss is followed rapidly by regeneration, revealing the importance of a fully functional tail. Predation pressure has been traditionally correlated with autotomy performance. However there is a lack of evidence regarding the impact of predation regime on tail regeneration. Another important factor that has been neglected is the population density. Though it is well established that crowding alters various life-history traits, the impact of density on caudal regeneration remains understudied. In this paper we compared three island populations of the Aegean Wall Lizard (*Podarcis erhardii*) that have evolved under different levels of predation pressure: whereas the Naxos population is exposed to numerous predators the Kopries and Daskalio islet populations experience low predation pressure. To evaluate the effect of density-induced stress on caudal regeneration, lizards were treated under two conditions of housing, in single and crowded (six individuals) terraria. Tail shedding occurred prior to this treatment following a standardised method. The length of regenerated tail was recorded weekly. Regeneration rate was higher in single terraria when compared to crowded, reflecting the negative impact of crowding. However we failed to detect any statistically significant difference between single and crowded terraria in the case of Naxos. We believe that the underlying reason must be the heavy predation pressure under which rapid tail regeneration, even under unfavourable conditions, is crucial for survival. It seems that the imperious need for regeneration counteracts density-induced stress.

Keywords: ecophysiology, insularity, Lacertidae, tail autotomy.

Introduction

Autotomy, the voluntary shedding of an expendable body part, is a typical antipredator mechanism occurring in numerous groups of invertebrates (Miller and Byrne, 2000; Beaumiller and Gahn, 2004) and vertebrates (Werner and Moravec, 1998; Shargal et al., 1999) in response to predator attack. Tail loss occurs in most lizard families (McConnachie and Whiting, 2003) and is very common among lacertids (Arnold, 1988). Shed tail thrash vigorously for prolonged time periods to distract a predator from the escaping lizard (Vitt, Congdon and Dickson, 1977; Dial and Fitzpatrick,

1984). In addition a thrashing tail increases predator's handling time, providing further get-away chances to lizard (Daniels, 1983; Dial and Fitzpatrick, 1983). However those benefits of caudal autotomy come with several important costs such as possible loss of caudal lipid deposits in those species that use their tail for fat storage (Fox and McCoy, 2000; Doughty, Shine and Lee, 2003) thus impairing reproductive effort and growth (Vitt and Cooper, 1986; Wilson and Booth, 1998), decreased social status (Fox and Rostker, 1982; Martin and Salvador, 1995), altered territorial behavior (Martin and Salvador, 1992; Martin and Avery, 1997) and increased overall mortality rates (Wilson, 1992). Among the most severe disadvantages of tail loss, diminished locomotory ability figures prominently (Downes and Shine, 2001; Lin, Qu and Ji, 2006). Autotomy has been shown to have a negative impact on numerous aspects (e.g., sprint speed, climbing ability, stamina) of locomotory performance (Chapple and Swain, 2002a), increasing predation risk or re-

1 - Section of Human and Animal Physiology, Department of Biology, University of Athens, Panepistimiopolis, 157-84 Athens, Greece

2 - School of Natural Resources and Environment, University of Michigan, Dana Building, 430 East University, Ann Arbor, MI 48109-1115, USA

3 - Modern Greek Program, Department of Classical Studies, University of Michigan, 2160 Angell Hall, 435 S. State, Ann Arbor, MI 48109-1115, USA

*Corresponding author; e-mail: pafman@umich.edu

ducing foraging success (Martin and Salvador, 1993; Chapple and Swain, 2002b). Lastly, tailless lizards suffer from an increased vulnerability to predation as shown in staged experimental encounters (Congdon, Vitt and King, 1974; Vitt and Cooper, 1986).

Because of these significant costs lizards regenerate their tail rapidly (Bellairs and Bryant, 1985). Indeed regenerated tails largely ameliorate the costs of autotomy (Brockes, 1997) and lizards regain most of their lost abilities (Vitt, 1981; Althoff and Thomson, 1994) while, at the same time, reduces the temporarily increased predation risk (Wilson, 1992; Downes and Shine, 2001). Nevertheless tail regeneration is quite expensive in terms of energy (Merino et al., 2004; Naya et al., 2007), characterized by large-scale cellular (Alibardi, 2000) and physiological activity (Bellairs and Bryant, 1985). In order to fuel this costly procedure, tailless lizards divert energy and nutrients from body growth and reproduction (Daniels, 1984; Vitt and Cooper, 1986) while exhibit higher metabolic rates (Naya and Bozinovic, 2006). The importance of tail as a valuable structure is mirrored in the diversion of energy from critical processes as reproduction, over which regeneration has energetic priority (Maiorana, 1977).

Frequency of tail loss has been used traditionally as an indication of predation pressure (Pianka, 1970; Turner et al., 1982, but see Schoener, 1979; Jaksic and Greene, 1984). Capacity for autotomy is often correlated with predator-prey interactions (Dial and Fitzpatrick, 1981; Pafilis et al., 2009). Predation regime is considered as a crucial factor shaping various life-history characteristics of prey organisms (Losos, Schoener and Spiller, 2004) and can induce rapid changes on prey communities (Vervust, Grbac and Van Damme, 2007). Populations inhabiting environments with intense predation pressure show higher autotomy frequencies (Pérez-Mellado, Corti and LoCasio, 1997; Pafilis, Valakos and Fofopoulos, 2005). Although trade offs between predation

regime and autotomy performance have been thoroughly scrutinized, the relation between tail regeneration and predation pressure remains largely understudied. This is surprising given first, the increased risk from predation until regeneration is completed (Dial and Fitzpatrick, 1984; Oppliger and Clobert, 1997) and second, the essential role of regeneration for the restoration of lizard's fitness (Clause and Capaldi, 2006).

Lizards can occur in very dense populations on islands (Rodda and Dean-Bradley, 2002). In such populations food availability is greatly reduced making the energetically expensive tail regeneration process even more costly (Semlitsch and Reichling, 1989). High population density is known to affect various features of organism's fitness (Lochmiller, 1996; Oppliger et al., 1998; Sinervo, Svensson and Comendant, 2000; Pafilis, Pérez-Mellado and Valakos, 2008). Nonetheless there is no report, to the best of our knowledge, of the impact of crowding on caudal regeneration though past studies have evaluated the influence of food availability (a proxy variable of competition) on caudal regeneration (Naya and Bozinovic, 2006).

In this paper we worked with three populations of the Aegean wall lizard (*Podarcis erhardii*) deriving from three islands of the Aegean Sea. We tested two hypotheses: First, we presumed that regeneration rate should be lower under crowded conditions since high densities are known to induce stress, inhibiting various life-history traits (Svensson, Sinervo and Comendant, 2001; Rankin and Kokko, 2006). Second, we expected that regeneration would occur more rapidly in environments where sublethal predation pressure (which is usually associated with terrestrial predators) is high and consequently tail autotomy is more important as defensive strategy. Finally we attempted to clarify whether crowding and predation regime have a combined effect on tail tissue repair.

Materials and methods

Species and study area

The Aegean Wall Lizard, *Podarcis erhardii* (Bedriaga, 1876) is a medium-sized lacertid lizard (SVL up to 70 mm, body mass 7.6 ± 1.4 g) endemic to the southern Balkans, and which shows an impressive differentiation (which does not affect populations selected for this experimental procedure) throughout the Aegean archipelagos (Valakos et al., 2008). It preys on terrestrial arthropods, mainly insects, and population density varies considerably among islands (Valakos, 1986).

All lizards were captured by noose in the field during their non-reproductive period. Since predisposition to autotomy has been reported to differ depending the sex (Fox, Conder and Smith, 1998; Seligmann, Moravec and Werner, 2008) and age class (Arnold, 1984; Pafilis and Valakos, 2008), we worked exclusively with adult males with intact tails of comparative size (see table 2) to minimize gender and age induced biases. Captive animals were held in the laboratory facilities of the Biology Department at the University of Athens. Lizards were housed separately in single terraria (20 cm \times 25 cm \times 15 cm) on a sand substrate with bark pieces and stones providing shelter and basking sites. The temperature of the room was constant at 25°C and light was provided by fluorescent lamps in a photoperiod of 12 L:12 D. Incandescent lamps of 60 W kept on for a period of 8 hours daily (for behavioral thermoregulation) while additional special lamps provided UV radiation. Animals were fed with mealworms (two per individual dusted with multivitamin supplement, *TerraVit*) every other day and were supplied with water *ad libitum*.

None of the lizards was killed during the experimental procedure. Lizards were not anaesthetized as autotomy is neurologically controlled and shown only by conscious animals (Arnold, 1984; Downes and Shine, 2001). Blood loss is reduced by sphincters and valves in the caudal vessels (Winchester and Bellairs, 1977). All animals were controlled for parasites by scanning a stained, thin blood smear under 1000 \times magnification and infected individuals were excluded from experiments since blood parasitism has been associated with reduced tail regeneration (Oppliger and Clobert, 1997).

We studied three lizard populations originating from three Cyclades islands (Central Aegean Sea, fig. 1) that differ substantially in several crucial features: Naxos is a large (448 km²) island hosting many predators (table 1) and is characterized by high food availability in comparison to the majority of Cyclades islands (Karamaouna, 1987); Kopries and Daskalio are small islets (0.138 km² and 0.015 km² respectively) without any terrestrial predators, visited only by aerial predators (table 1) and showing scarce food abundance. All lizards coming from Naxos were collected from the same location (Philoti, central Naxos) from dry-stone walls crossing a landscape characterized by olive trees, grasses and small bushes. In both Daskalio and Kopries the vegetation is comprised by phrygana (predominant species *Pistacea lentiscus*). Densities differed among the three populations; for Naxos population density was estimated in 220 lizards/ha while for Kopries and Daskalio the

equivalent value were 160 lizards/ha and 135 lizards/ha respectively. Density was evaluated using the transect count method (Burnham, Anderson and Laake, 1980).

Experimental design

In order to evaluate autotomy ease we simulated predator's attack adopting a simple technique proposed by Pérez-Mellado et al. (1997). Lizards were placed in a terrarium covered with cork substrate in order to provide traction. We used a pair of digital calipers, chosen to reduce pressure variation to grasp the tail 30 mm from the cloaca. We selected this rather distal position to avoid possible biases since most fat is aggregated in the proximal part of the tail and in order to preserve this fat storage lizards could be more reluctant to proceed in autotomy (Chapple and Swain, 2002b; Lin, Qu and Ji, 2006). Each trial lasted for 15 sec. If autotomy did not occur, tail was removed manually using forceps (Pafilis, Valakos and Foufopoulos, 2005).

Ease of autotomy is temperature dependent (Bustard, 1968; Daniels, 1984). Thus, prior to predation simulation lizards were allowed to thermoregulate to their preferred body temperature. For that purpose we used a specially out-fitted terrarium (1 m \times 20 cm \times 25 cm) providing a thermal gradient from 17°C to 55°C thanks to two incandescent heating lamps (100 and 60 W) at the one end and four ice bricks to the other (Van Damme, Bauwens and Verheyen, 1986).

Tail length was recorded weekly to the nearest second decimal, using a ruler. Measurements began the first week after autotomy and were taken for 17 weeks. The weight and the length of each autotomized tail were measured (Meyer, Preest and Lochetto, 2002).

To evaluate the effect of crowding-induced stress on caudal regeneration we artificially increased density following the example of previous captivity studies (Oppliger et al., 1998; Hawley, Lindström and Wikelski, 2006). Lizards were thus held under two different density treatments: some animals were held in single (only one individual) terraria (20 cm \times 25 cm \times 15 cm) and the rest in "crowded" (containing six individuals) terraria (30 cm \times 50 cm \times 25 cm) (see table 3 for the exact partition of lizards). We referred to from now on as noncrowded (NL) and crowded (CL) lizards respectively (*sensu* Oppliger et al., 1998). In both cases general holding conditions (lamps, food, photoperiod), aside terrarium size, were identical as the ones described above. Lizards were grouped in accordance to their origin. Thus for each one of the three populations we used two different housing treatments (NL and CL), so a total of six different groups was formed.

Males tend to protect their living space and fight with intruders (Lailvaux and Irschick, 2007; Schwartz, Baird and Timanus, 2007). Animals seemed to get accustomed after a while to the reduction of their niche and show higher tolerance against rivals. However aggressive males were expelled from crowded terraria and housed individually (though this occurred only once). Lizards were weighted weekly and in case of weight loss attenuated individuals were removed from the experimental procedure to follow special treatment.

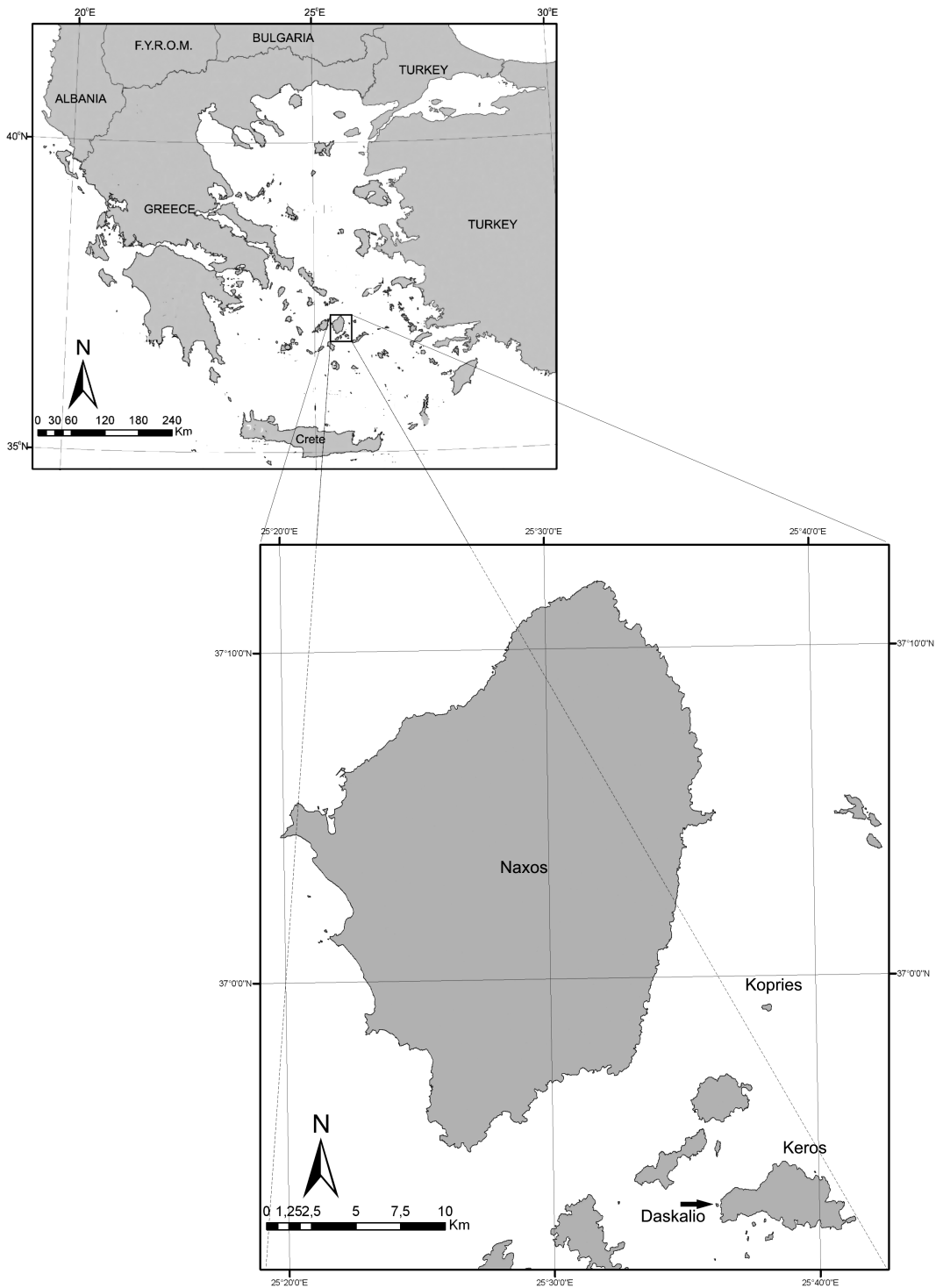


Figure 1. Map of the study islands in the Naxos Archipelago (Cyclades Islands, Central Aegean Sea, Greece, NE Mediterranean Basin).

Table 1. Geographic origin of the animals used in this study (map localities given in fig. 1). Main lizard predators (species codes on the end of the table) of each island with supporting references.

Population	Sample size	Predators	Reference
Naxos Island	20	Birds: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 Snakes: 1, 2, 3 Mammals: 1, 2	Watson, 1964; Magioris, 1987; Valakos, 1990; Magioris, 1995; Handrinos and Akriotis, 1997; Valakos et al., 2008
Kopries islet	25	Birds: 2, 4, 7, 8, 9, 10, 11	Magioris, 1987, 1995; Handrinos and Akriotis, 1997
Daskalio islet	23	Birds: 2, 7, 9, 10	Magioris, 1987, 1995; Handrinos and Akriotis, 1997

Birds: 1. *Athene noctua*. 2. *Buteo buteo*. 3. *Buteo rufinus*. 4. *Circus gallicus*. 5. *Corvus corax*. 6. *Corvus corone*. 7. *Falco eleonorae*. 8. *Falco naumanni*. 9. *Falco peregrinus*. 10. *Falco tinunculus*. 11. *Hieraeetus pennatus*. 12. *Lanius senator*.
Snakes: 1. *Elaphe quatuorlineata*. 2. *Telescopus fallax*. 3. *Vipera ammodytes*.
Mammals: 1. *Martes foina*. 2. *Erinaceus concolor*.

Table 2. Mean snout vent length (mm) and mean length of autotomized tails (mm) of the examined lizards and *t*-test results for comparisons between single and crowded hosted animals. NL: animals hosted individually (noncrowded lizards), CL: animals hosted in group (crowded lizards). *N*: number of individuals, mean is shown as millimeter.

Population	Snout-Vent Length			Length of amputated tail		
	NL Mean ± SD; <i>N</i>	CL Mean ± SD; <i>N</i>	<i>t</i> -test	NL Mean ± SD; <i>N</i>	CL Mean ± SD; <i>N</i>	<i>t</i> -test
Naxos	64.8 ± 7.2; 8	59.5 ± 3.5; 12	<i>t</i> = 1.93, df = 18 <i>p</i> = 0.07	75.8 ± 12.8; 8	75.6 ± 12.3; 12	<i>t</i> = 0.02, df = 18 <i>p</i> = 0.98
Kopries	62.2 ± 3.4; 13	61.0 ± 3.3; 12	<i>t</i> = 0.84, df = 23 <i>p</i> = 0.41	76.6 ± 14.6; 13	74.1 ± 13.4; 12	<i>t</i> = 0.41, df = 23 <i>p</i> = 0.69
Daskalio	61.3 ± 2.3; 11	63.4 ± 4.0; 12	<i>t</i> = 1.35, df = 21 <i>p</i> = 0.19	78.1 ± 6.1; 11	79.2 ± 9.1; 12	<i>t</i> = 1.27, df = 21 <i>p</i> = 0.78

Table 3. Mean caudal regeneration rate (mm/day) and length as percentage of original tail length (%) of tail replacement during the 8th week after autotomy. NL: animals hosted individually (noncrowded lizards), CL: animals hosted in group (crowded lizards). *N*: number of individuals.

Population	Mean growth rate		% replacement of tail tissue	
	NL Mean ± SD; <i>N</i>	CL Mean ± SD; <i>N</i>	NL Mean ± SD; <i>N</i>	CL Mean ± SD; <i>N</i>
Naxos	0.68 ± 0.10; 8	0.61 ± 0.12; 12	51.8 ± 11.4; 8	45.7 ± 9.8; 12
Kopries	0.70 ± 0.13; 13	0.52 ± 0.17; 12	51.7 ± 6.8; 13	40.6 ± 14.7; 12
Daskalio	0.57 ± 0.07; 11	0.32 ± 0.08; 12	41.2 ± 4.8; 11	22.9 ± 5.6; 12

Statistical analysis

We used one-way ANOVAs to test differences on the SVL among populations. Two-way ANCOVAs followed by post-hoc analysis (LSD test) were used for comparisons between the examined traits, using housing treatment and population as factors. We also used *t*-test for population traits comparison, using once more housing treatment and population as factors. Fisher exact test was used to compare autotomy performance among the examined populations. Normality of each trait was assessed using the Kolmogorov-Smirnov test.

All test were two-tailed (*a* = 0.05). Statistical analysis followed Zar (1999).

Results

There were no statistically significant differences in SVL among the examined samples, either in NL (one-way ANOVA, $F_{2,29} = 1.266$, *p* = 0.59) or CL (one-way ANOVA, $F_{2,33} =$

2.68, $p = 0.08$). The length of shed tail did not differ significantly among samples for both NL (one-way ANOVA, $F_{2,29} = 0.085$, $p = 0.92$) and CL (one-way ANOVA, $F_{2,33} = 0.495$, $p = 0.61$). No differences were detected between treatment groups when compared for SVL and shed tail length (t -test, $p > 0.05$) (table 2).

Laboratory rates of tail loss varied among samples. Lizards from Naxos had the highest autotomy rates (55%, 11 individuals autotomized their tail out of 20), followed by Kopries (36%, 9 individuals out of 25) and Daskalio (26%, 6 individuals out of 23) populations. Pairwise comparison indicated that there were statistically significant differences in autotomy performance between the animals from Naxos and those from Daskalio ($Z = 1.940$, $p = 0.02$) but not between the two islets ($Z = 0.764$, $p = 0.2$) nor between Kopries and Naxos ($Z = 1.275$, $p = 0.1$).

The length of regenerated tail was plotted against time, giving a sigmoid curve (fig. 2). Three distinct growth periods are evident from this plot, regardless of treatment group: following an initial period (0-3 weeks) of low regeneration associated with wound healing, tail re-growth accelerated dramatically during the second period (4-8 weeks). The third period (9-14 weeks) was characterized by a plateauing rate of growth. The principal part of regeneration is accomplished within the second period.

Mean growth rates at the end of the second regeneration period (8th week after autotomy) are given in table 3. We detected statistically significant differences between the examined samples and between housing treatment (NL and CL) but not in the interaction of the two factors (two-way ANCOVA with SVL as covariate: populations $F_{2,61} = 17.06$, $p = 0.00001$; housing treatment $F_{1,61} = 29.09$, $p = 0.00001$; interaction $F_{2,61} = 2.24$, $p = 0.11$). Post-hoc analysis (LSD test) indicated that there were significant differences between NL and CL in the cases of Daskalio ($p = 0.0005$) and Kopries ($p = 0.00001$) but not in the case of Naxos ($p = 0.16$). Moreover post-

hoc analysis revealed that mean growth rate for Daskalio differed from Kopries and Naxos both in NL (Daskalio-Kopries: $p = 0.008$; Daskalio-Naxos: $p = 0.02$) and CL (Daskalio-Kopries: $p = 0.0002$; Daskalio-Naxos: $p = 0.0001$). To the contrary we failed to find any statistically significant difference between NL and CL from Kopries and Naxos (for NL: $p = 0.75$, for CL: $p = 0.12$).

Differences in mean regeneration rate were reflected in the proportion (%) of tail replacement (table 3). We found statistically significant differences between the examined populations and the housing treatment but not in the interaction of the two factors (two-way ANCOVA with SVL as covariate: populations $F_{2,61} = 18.89$, $p = 0.00001$; housing treatment $F_{1,61} = 21.83$, $p < 0.05$; interaction $F_{2,61} = 1.97$, $p = 0.11$). Post-hoc analysis indicated differences between NL and CL in Daskalio ($p = 0.0005$) and Kopries ($p = 0.01$) but not in the case of Naxos ($p = 0.77$). Post-hoc analysis shown also significant differences in tail replacement between animals from Daskalio and those from the other two populations, both in NL (Daskalio-Kopries: $p = 0.001$; Daskalio-Naxos: $p = 0.00001$) and CL (Daskalio-Kopries: $p = 0.0001$; Daskalio-Naxos: $p = 0.00001$). The corresponding comparisons between the animals from Naxos and Kopries did not show any significant difference (for NL: $p = 0.85$; for CL: $p = 0.24$).

Discussion

Rapid caudal regeneration following tail loss is an effective strategy to offset the majority of costs related to autotomy (Bellairs and Bryant, 1985). Lizards are known for high regenerative potentials (Alibardi, Gibbons and Simpson, 1992) and prioritize caudal tissue repair over other important life history processes (e.g., reproduction, growth, immunological defense) (Bernardo and Agosta, 2005). During regeneration period lizards are more susceptible to predator's aggressions (Downes and Shine,

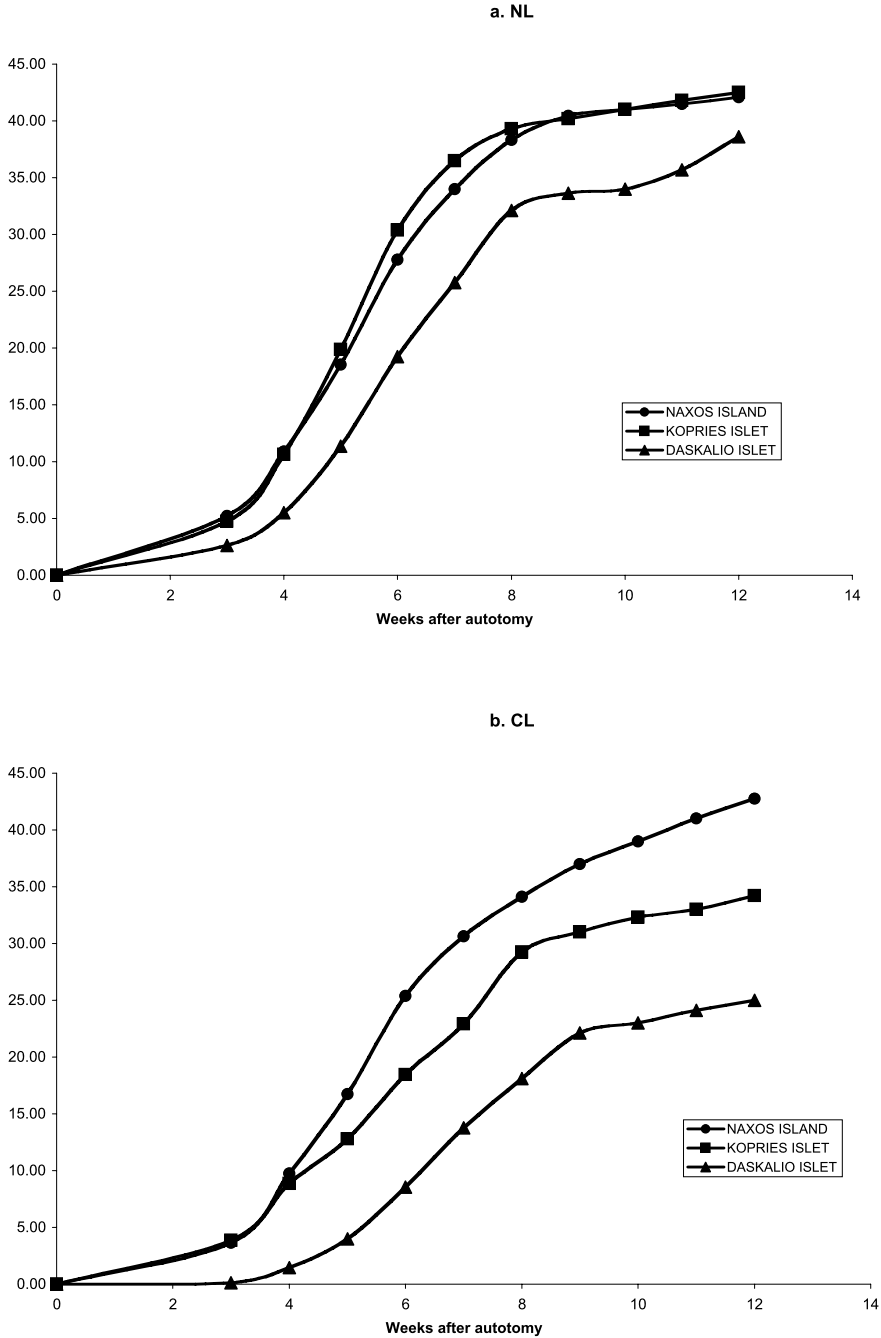


Figure 2. Tail regeneration in animals hosted (a) individually (NL: noncrowded lizards) and (b) crowded (CL: crowded lizards) in the three examined populations.

2001) since tail shedding is responsible for dramatic alterations on locomotory capacities (Chapple, McCoull and Swain, 2004). Hence rapid regeneration should be crucial for lizards,

especially in high predation pressure environments.

Regeneration proceeded in a pattern model in all studied populations and could be divided

into three distinct periods: during the first (0-3 weeks after autotomy) tissue repair was slow, in the second (4-8 weeks after autotomy) tail stump lengthened swiftly while in the third (10-14 weeks) growth stabilized (fig. 2). Our results are in agreement with previous studies (Vitt, Congdon and Dickson, 1977; Meyer, Preest and Lochetto, 2002), indicating that during the first period following autotomy, lizards are recovering from the trauma of tail loss and cells dedifferentiate at the injury site to form the regenerative blastema (Bryant, End and Gardiner, 2002). The histochemical basis of regeneration has been described in detail and appears to be invariant among various lizard species (Bellairs and Bryant, 1985). The conservative nature of this process is to be expected given its importance for the organism's survival.

Although the general regeneration pattern was similar for all groups, regardless of density treatment, regeneration rate differed among them. In crowded lizards (CL) regeneration rate was lower in comparison to noncrowded lizards (NL). During the second "rapid" period, tail growth's average rate for CL varied from 0.31 mm/day (Daskalio islet) to 0.61 mm/day (Naxos island) while in the case of NL the respective values were 0.57 mm/day and 0.70 mm/day (table 3). These regeneration rates are comparable to those from other lizard (0.92 mm/day for *Hemidactylus mabouia*, 0.73 mm/day for *Hemidactylus garnotii*, 0.7 mm/day for *Coleonyx variegatus*, 0.44 mm/day for *Eumeces gilberti* and 0.35 for *Eumeces skiltonianus*: Congdon, Vitt and King, 1974; Vitt, Congdon and Dickson, 1977; Meyer, Lochetto and Preest, 2002). The underlying reason for regeneration rate depression in CL is the artificially increased density that causes stress and, likely, deceleration in regeneration. Other studies have shown that when animals are crowded survival rates and other life-history traits are affected (Lochmiller, 1996). Previous captive studies where density conditions were experimentally increased yielded higher levels of corticosterone (a stress induced hormone), which influence

various features of organism's fitness (Knapp and Moore, 1995; Oppliger et al., 1998). The reduced growth rates we observed in CL may be the result of elevated corticosterone levels due to crowding though more studies are needed to show this or to elucidate other possible explanations (e.g., lizards competing for basking places may have suboptimal body temperature and thus suffer from decreased metabolic rate and consequently low tail growth).

We also evaluated the cost of tail repair by quantifying the length of the regenerated tail in comparison to the original tail length. The proportion of the new regenerated tail compared to the original is lower in CL than in NL (table 3). Regenerated tails have been reported to differ in numerous ways from the original (Meyer, Preest and Lochetto, 2002; Simou et al., 2008) and usually they are shorter than the intact tail before autotomy (Arnold, 1988). We found that under conditions of high density the strong competition exacerbate this tendency to produce shorter regenerated tails.

Regeneration rate in NL varied among populations: in Naxos and Kopries it was considerably higher than in Daskalio. We believe that predation regime plays a decisive role. Naxos hosts various predators (table 1), including *Vipera ammodytes*, a specialized lizard predator (Pafilis et al., 2009) and this intense predation regime is mirrored in the high laboratory autotomy rates (Results). Autotomy costs decrease as lizards repair their tail and, most important, regenerated tail can be used again as defensive mechanism since locomotory performance is regained (Chapple and Swain, 2002a) and ability for autotomy is once more available (Vitt, 1981; Arnold, 1988). The risk of increased predation is greater for longer periods of regeneration (Oppliger and Clobert, 1997). Hence the need for rapid replacement of the lost tail becomes imperative under the intense predation pressure environment of Naxos (65% of injured tails in the field, P. Pafilis, unpubl. data). To the contrary Daskalio's relaxed predation pressure (table 1) makes the necessity of a quick tail re-

pair less exigent. Lizards will certainly regenerate their tail, but not in the high rates of Naxos.

Surprisingly Kopries share the same rapid regeneration pattern with Naxos. This may be explained by the ecological particularities of Kopries. A common practice of stock farming in the Aegean region is the release of free-range goats onto small islets for grazing. This practice results in the large-scale destruction of the islets' vegetation. 14 goats were found at the time of sampling in Kopries, overgrazing the islet and let only few bushes that could be used as shelters by lizards. Grazing has been reported to alter vegetation providing less cover from avian predators, increasing thus predation risk (Vervust, Grbac and Van Damme, 2007). Under these circumstances even a small number of avian predators may exert severe pressure on the resident lizard population. It seems that lizards from Kopries regenerate their tail swiftly since the defensive role of the tail is important in this environment of high predation.

Another factor that could be responsible for the variable regeneration rate is food availability. Tail regeneration requires energy, often at the expense of other functions (Clause and Capaldi, 2006). Lizards, during the procedure of tail repair, show higher metabolic rates (Naya and Bozinovic, 2006) and their energetic requirements increase considerably (Chapple, McCoull and Swain, 2002). Scarce food availability, common in Mediterranean islands (Brown and Pérez-Mellado, 1994), constrains nutrient income for lizards, which thus may have less energy available to invest in tail regeneration. Under these unfavorable conditions it would be expected for the costly regeneration to decelerate. Naxos, being much bigger than Kopries and Daskalio (3.246- and 29.866-folds respectively), encompasses a variety of productive ecosystems offering a high diversity and abundance of arthropods (Karamaouna, 1987). Hence food scarcity is less pronounced without being a limiting factor.

When the two treatment groups were compared, both islets showed the same pattern:

higher regeneration in NL and lower in CL. Interestingly no penalty for crowding was found for Naxos (table 3). Predation pressure may be responsible for the lack of reduced growth under crowded conditions. In a high predation environment rapid regrowth of a regenerating tail is particularly advantageous since a full tail aids locomotory maneuvers and can be sacrificed in order to escape a direct predator attack. Lizards in Naxos are continuously jeopardized and cannot afford a prolonged period of regeneration during which predation risk would be even higher. They struggle to reduce tail loss costs by repairing their tail as soon as possible. In addition a high and reliable food availability, as observed on Naxos, makes the evolution of frugal life history strategies, including a slowly regeneration, less urgent.

Rapid caudal regeneration may take precedence, in energy terms, important life-history traits (Maiorana, 1977). According to Oppliger and Clobert (1997) "the decision to allocate energy in tail regeneration must depend on the importance of allocating energy to other processes". We believe that the importance of density-induced stress (and the eventual energy demands) is minimized by the immediate need for regeneration. Crowding has a negative impact on tail growth, but only under conditions of relaxed pressure. Living in environments of intense predation regime is compromised by high autotomy frequencies (Cooper, Pérez-Mellado and Vitt, 2004; Pafilis, Valakos and Foufopoulos, 2005). We believe that regeneration in such environments should occur more rapidly to provide lizards with an effective antipredatory mechanism and a valuable auxiliary limb. Hence, in places like Naxos, it seems that lizards cede absolute priority to regeneration, even under conditions of crowding, in order to survive the harsh predation pressure.

In conclusion both crowding and predation pressure affected regeneration rate and length of regrown tail. Artificially induced increased density had a negative impact on tail regeneration of CL in comparison to NL, verifying our

first hypothesis. However in the last treatment group predation pressure had a catalytic impact accelerating tail repair, confirming that way our second work hypothesis. The relation between the two factors was clarified under the heavy predation regime of Naxos where the priority was to regain the lost tail regardless the cost. Density-induced stress, which was the determinant for the delayed regeneration in CL, gave place to the rapid caudal tissue repair to compensate for high predation. Answering the question we posed, predation pressure seems to predominate over crowding and selective pressure favored tail regeneration, at least in our study system.

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