

A quick method for a rough estimate of the size of a small and threatened animal population: the case of the relict *Ichthyosaura alpestris inexpectata* in southern Italy

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Abstract. The size of an animal population can be rather accurately estimated by a mark-recapture method, but this may have a negative impact on survivorship of marked specimens. This should be avoided especially in the case of small endangered populations. We here propose a new method, the *species capture ratio*, for a quick and rough population size estimate, that can be used in the case of habitats shared by several species, one among which only is particularly endangered. The population size of a non-endangered species in this habitat may be estimated by random collecting and mark-recapture method, whereas specimens of the endangered species are just randomly collected along with them, but not marked, and the size of their population is roughly estimated through the ratio of the collected specimens of the two species. The efficiency of this method is illustrated with a case study of a mixed population of newts involving *Triturus carnifex*, *Lissotriton italicus* and *Ichthyosaura alpestris inexpectata* in a small lake of southern Italy. The results suggest that the latter taxon is at least “vulnerable”, under the IUCN categories of threat, and that the status of its populations should be ascertained more precisely, in order to be able to take action for their conservation. It is further suggested that the use and validity of the new method described here should be explored in other cases of small, endangered populations and taxa.

1. Introduction

A frequent way of estimating the size of animal populations is through the use of mark-recapture methods (MRC). Provided some methodological precautions are taken (Otis et al. 1978, Lebreton et al. 1992), they can allow a rather accurate estimate of a population size at a given date. However, the recourse to these methods encounters three major kinds of problems: (1) problems of sampling; (2) problems of calculation; (3) problems of impact of marking on animals. The latter kind of problem may be particularly worrying in the case of a small endangered population, and even more of a threatened species or subspecies having a very limited distribution and known to occur in only a few small populations. In such a case, it can be very useful, in particular for

taking appropriate decisions concerning the conservation of the taxon and/or of its habitat, to have an approximate evaluation of the size of these populations, but this should not be done at the cost of a negative impact on survivorship of some of the individuals. To address this problem, we devised a new method for a quick and rough estimate of the size of a population that does not require marking any individual, but simply to capture and count a random sample of them, before simply releasing them in the habitat. Estimate of the population size is made relatively to the size of another population of another species sharing the same habitat. We here describe the method and present a case study that we used as an example and an empirical test of the reliability of the method.

2. The case study: a threatened population of newts in southern Italy

We devised and tested the new method during a field survey of a population of newts in a mountain lake of Calabria (southern Italy).

The former European newt genus *Triturus* Rafinesque, 1815 (Amphibia, Urodela) was recently split in several genera (García-París et al. 2004; Dubois & Raffaëlli 2009). Three species of newts occur in southern Italy, and in particular in the locality where the study was carried out. These three species are now allocated to three distinct genera: (1) *Triturus carnifex* Laurenti, 1768 is still referred to the genus *Triturus* Rafinesque, 1815, corresponding to the former group of *Triturus cristatus* Laurenti, 1768; (2) *Lissotriton italicus* (Peracca, 1898) is now referred to the genus *Lissotriton* Bell, 1839, corresponding to the former group of *Triturus vulgaris* (Linnaeus, 1758); (3) *Ichthyosaura alpestris inexpectata* (Dubois & Breuil, 1983), or more shortly *Ichthyosaura inexpectata* if tentatively assigned the specific rank (Dubois 1998), should now be referred to the genus *Ichthyosaura* Sonnini & Latreille, 1801, a name of which *Mesotriton* Bolkay, 1927 is a junior synonym (Schmidtler 2004).

Both *T. carnifex* and *L. italicus* are widely distributed in southern Italy (Gasc et al. 1997) and cannot be considered as particularly threatened or endangered. Both are listed under the category “least concern” (LC) by the IUCN’s Global Amphibian Assessment (GAA; see www.iucnredlist.org). The species *I. alpestris* is also listed as LC by the GAA, but this database states that the subspecies

inexpectata is “threatened through significant alteration of the aquatic habitats”, and that “this subspecies would qualify as Vulnerable if qualified separately”. As a matter of fact, this taxon is only known from four lakes in Calabria (Giacoma et al. 1988) and in each of these localities the populations are small and completely isolated, except for two of them which are very close to each other and may probably be considered a single deme (Dubois 1983, 1993). This recently discovered taxon (Dubois & Breuil 1983), with a distribution restricted to a few localities at rather high altitude in the extreme South of Italy, most probably qualifies as a “post-glacial relict”.

Table 1 provides information on the number of newts of the three species captured on various occasions from 1982 to 1987 in three lakes of the Catena Costiera in Calabria: (1) Lago dei Due Uomini, near Fagnano Castello (39°33’N, 03°34’E Roma; altitude 1077 m); (2) Lago Trifoglietti (1048 m), very close to the former; (3) Laghiccello (39°25’N, 03°38’E Roma; altitude 1130 m), a small isolated lake near the road from Fuscaldo to San Benedetto Ullano. As can be seen from these figures, the number of specimens of *I. inexpectata* encountered on these occasions was always small (from 0 to 63), and it was thus considered useful to obtain an approximate estimate of the size of one at least of these populations, that of Laghiccello, which appeared the largest of the three.

The criteria of the IUCN for establishing the category of threat of a taxon (Anonymous, 2001) are based on several sets of data, an important one being the number of mature individuals in the known populations.

Table 1. Estimates of newt populations in three localities of Calabria (Italy) from 1982 to 1987. Lago dei Due Uomini, Lago Trifoglietti & Laghiccello. Total captures from 1982 to 1987 (except for Laghiccello in 1987: see next tables). *carn*: *Triturus carnifex* (Laurenti, 1768); *inex*: *Ichthyosaura inexpectata* (Dubois & Breuil, 1983); *ital*: *Lissotriton italicus* (Peracca, 1898). For each observation, the following figures are given: number of captured males / number of captured females (sex ratio in % = number of captured males / number of captured males and females).

Lake	Due Uomini	Due Uomini	Due Uomini	Due Uomini	Trifoglietti	Laghiccello	Laghiccello
Date	21-22.07.82	27-28.03.83	13.04.83	25-26.06.87	13-14.04.83	12.04.83	15.04.83
<i>carn</i>	0/5 (-)	12/9 (57.1 %)	13/24 (35.1 %)	5/3 (62.5 %)	1/0 (-)	95/69 (57.9 %)	-
<i>inex</i>	3/10 (23.1 %)	-	-	-	14/7 (66.7 %)	42/21 (66.7 %)	14/16 (46.7 %)
<i>ital</i>	1/2 (33.3 %)	5/3 (62.5 %)	6/11 (35.3 %)	5/2 (71.4 %)	36/64 (36.0 %)	5/7 (41.7 %)	3/5 (37.5 %)

Because of the supposed threat on this isolated newt population, any interference with these animals requires the greatest care, and we tried to use a method with little impact on the newts. This cannot provide a precise estimate of the population size, but at least an order of magnitude of the latter, which will be enough, as shown below, for the purpose of diagnosing the level of threat on the taxon *I. inexpectata*.

3. Material and field work

The study was carried out in the Laghicello from 28 to 30 June 1987. This small lake is completely isolated in the forest, no other water body being present over kilometres in the area. Every spring, the lake fills up with water, and every summer it progressively dries out (Dubois 1983). The newts then presumably seek refuge in subterranean rodent galleries, as the surface habitat of the forest at the end of the summer is completely dry and fully hostile to the survivorship of amphibians.

At the time of this study, the water level in the lake was low, allowing its complete walking through by persons equipped with waders. The lake was surveyed on three successive days by the two of us altogether, using narrow-mesh nets that did not allow for the newts of the three species to escape. Surveys were made at day time, during a standard period of two hours each day, from 12 to 14 h. The nets were moved quickly near the bottom of water, in all parts of the pond, whether with aquatic vegetation or without it. All newts taken in the nets were stored in plastic containers filled with water. They were sexed, counted and marked immediately after collection, then immediately released, at random spots all around the lake.

Various methods can be used to recognize or mark newts, either individually or collectively (Joly & Miaud 1990). Given the limited time available for the study, it was impossible to use methods that require spending several minutes on each specimen (photographs, tattooing, complex marking, electronic devices). We used a method which is quick to implement and presum-

ably has no significant impact on survivorship of the individuals. Because of cicatrization and regeneration abilities in newts, it is likely that our marks disappeared in the subsequent months, but this had no impact on our study, which lasted only three days. Newts were marked by small incisions on the upper or lower tail fin, as shown in Fig. 1: newts captured on the first day received mark M1, those captured on day 2 mark M2; those which had already been marked on day 1 received a double marking M1 + M2. On day 3 we also made a new marking M3 (two incisions on upper tail fin), but this was not used for population estimates as no recapture was made after 30 June.

4. Results

From day 1 to day 3, the numbers of newts captured went slightly decreasing (Table 2). However, in all three species, the sex ratio remained statistically constant during this period (Table 2).

Table 3 gives the complete data on the number of newts of each species and sex, either unmarked or already marked, collected over the three days of the study.

5. Population size estimates

The progressive decrease in the numbers of newts captured over the three days of the study suggests an increasing behavioural avoidance of the net by newts. However, the fact that the sex ratio remained constant in

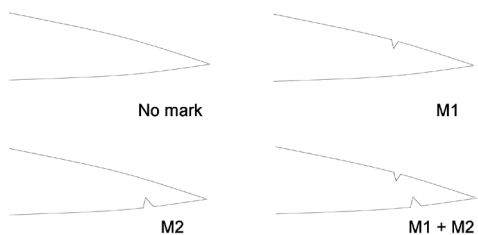


Fig. 1. Schematic representation of the tails of the newts studied in the lake Laghicello (Calabria, Italy) in June 1987, showing the position and importance of marks in upper and lower tail fins according to the day of capture: M1 for day 1, M2 for day 2.

Table 2. Laghicello. Total captures and sex ratio in June 1987. Chi-square test for significant differences in sex ratio for the three species over the three days of study. *carn*: *Triturus carnifex* (Laurenti, 1768); *inex*: *Ichthyosaura inexpectata* (Dubois & Breuil, 1983); *ital*: *Lissotriton italicus* (Peracca, 1898). Sex ratio in %: SR = number of captured males / number of captured (males + females). Significance of P (comparison of days 1-3): NS, P > 0.05.

	28.06.87 ♂ / ♀ (SR)	29.06.87 ♂ / ♀ (SR)	30.06.87 ♂ / ♀ (SR)	SR chi-square (P)
<i>carn</i>	89/118 (43.0 %)	35/63 (35.7 %)	49/51 (49.0 %)	3.584 (0.167 NS)
<i>inex</i>	11/23 (32.5 %)	1/4 (25.0 %)	2/4 (50.0 %)	0.326 (0.849 NS)
<i>ital</i>	2/13 (13.3 %)	1/0 (-)	1/6 (14.3 %)	4.969 (0.083 NS)

Table 3. Laghicello. Captures and recaptures on 28, 29 and 30 June 1987. *carn*: *Triturus carnifex* (Laurenti, 1768); *inex*: *Ichthyosaura inexpectata* (Dubois & Breuil, 1983); *ital*: *Lissotriton italicus* (Peracca, 1898). M1: captured unmarked on day 1, released with mark M1; M2: captured unmarked on day 2, released with mark M2; M3: captured unmarked on day 3, released with mark M3; M1 + M2: captured marked M1 on day 2, released with mark M2; M1 + M3: captured marked M1 on day 3, released with mark M3; M2 + M3: captured marked M2 on day 3, released with mark M3; M1 + M2 + M3: captured marked M1 + M2 on day 3, released with mark M3; ind.: individuals; capt.: captures.

Day of capture	1	2	2	3	3	3	3	1-3
Mark	M1	M2	M1 + M2	M3	M1 + M3	M2 + M3	M1 + M2 + M3	Total ind./capt.
♂ <i>carn</i>	70	32	3	48	1	0	0	150/154
♀ <i>carn</i>	98	57	4	45	2	2	2	200/212
♂ <i>inex</i>	3	4	0	9	0	1	0	16/17
♀ <i>inex</i>	13	12	3	28	1	1	0	53/58
♂ <i>ital</i>	1	3	0	2	0	0	0	6/6
♀ <i>ital</i>	8	5	0	8	1	1	0	21/23

the three species during the study suggests that the effect of avoidance was probably random among individuals, as it did not affect differently the proportions of captured individuals of the two sexes in each species. Although we have no way to demonstrate it, we assume that it did not affect differently the individuals of the three species in the lake. We also assume that during the short span of the study there was no mortality, emigration or immigration of newts. The terrestrial habitat around the lake at those dates was dry and quite inappropriate for amphibians, and we assume that newts remain in water until the lakes become completely dry at the end of the summer (Dubois 1983).

The data on mark-recapture of Table 3 allow estimation of the sizes of the three populations of newts, treating males and females separately. For this, we used three standard models: models M(0) and M(t) of Otis et al. (1978), and model M(t) of Chao (1989).

These estimates (Table 4) concern the population size N and its 95 % interval. Because of the absence of recaptures of males of *Lissotriton italicus*, two of the methods used do not allow calculation of a population size estimate. Although variable, the results provide rather consistent figures, with a *Triturus carnifex* population around 1500-2000 individuals of each sex, a *Lissotriton italicus* population of about 20 males and 60-80 females, and an *Ichthyosaura inexpectata* population of about 50-100 males and 150-200 females. According to these data, and given the extreme isolation of the lake, the Laghicello populations of the two latter species at least should be considered vulnerable (see below). In the case of *L. italicus*, which is widely distributed in southern Italy, this does not result in a threat on the taxon itself, but the situation is much more worrying for *I. inexpectata*, as these data concern one of the only four populations of the taxon so far known,

Table 4. Laghicello. Population size estimates of *T. carnifex*, *I. inexpectata* and *L. italicus* by method of captures-recaptures (MRC) over 3 successive days of captures-recaptures. M(o): model M(o) of Otis et al. (1978); M(t): model M(t) of Otis et al. (1978); M(c): model M(t) of Chao (1989); n: number of captured specimens; NMRC: estimate of population size (N) by MRC method.

Species	Sex	n total capt.	n total recapt.	Model	N _{MRC}	Standard dev. N _{MRC}	95 % confid. interval N _{MRC}
<i>carn</i>	♂	150	4	M(o)	1938	930	836 - 4816
				M(t)	1863	888	809 - 4608
				M(c)	1513	652	711 - 3467
<i>carn</i>	♀	200	12	M(o)	1463	431	859 - 2621
				M(t)	1407	413	831 - 2517
				M(c)	1909	693	997 - 3870
<i>inex</i>	♂	16	1	M(o)	86	75.3	29 - 406
				M(t)	72	57.7	27 - 311
				M(c)	48	28.7	23 - 160
<i>inex</i>	♀	53	5	M(o)	212	82.5	115 - 467
				M(t)	196	73.7	109 - 423
				M(c)	163	56.8	96 - 338
<i>ital</i>	♂	6	0	M(o)	-	-	-
				M(t)	-	-	-
				M(c)	17	14.7	8 - 85
<i>ital</i>	♀	21	2	M(o)	80	48.0	36 - 259
				M(t)	77	43.9	36 - 238
				M(c)	60	29.3	32 - 165

Table 5. Laghicello. Simultaneous captures on 28, 29 and 30 June 1987. *carn*: *Triturus carnifex* (Laurenti, 1768); *inex*: *Ichthyosaura inexpectata* (Dubois & Breuil, 1983); *ital*: *Lissotriton italicus* (Peracca, 1898).

Day	♂ <i>carn</i>	♀ <i>carn</i>	♂ <i>inex</i>	♀ <i>inex</i>	♂ <i>ital</i>	♀ <i>ital</i>
1	89	118	11	23	2	13
2	35	63	1	4	1	0
3	49	51	2	4	1	6
1-3	173	232	14	31	4	19

and furthermore apparently the largest of the four. It would appear that this taxon is indeed threatened with extinction, and that actions should be undertaken to protect it seriously (Dubois 1983, 1993, 1998).

Beside the standard methods mentioned above, we used another, new method to try and obtain an estimate of the population size of *I. inexpectata*. This relies on the respective numbers of specimens of the three species collected simultaneously on the three days

of capture. These figures are given in Table 5. Using these data, it is possible to calculate the ratio of the number of specimens of each sex of the two rarest species (*I. inexpectata* and *L. italicus*) to the number of specimens of the same sex of the commonest species (*T. carnifex*). We treat the sexes separately in order to obtain independent estimates of the population size for each sex, also considering that sexes may have different behaviours and distributions in the lake, and different behaviours of avoidance of the net. On the

Table 6. Laghicello. Simultaneous captures and species capture ratio to carnifex on 28, 29 and 30 June 1987. *carn*: *Triturus carnifex* (Laurenti, 1768); *inex*: *Ichthyosaura inexpectata* (Dubois & Breuil, 1983); *ital*: *Lissotriton italicus* (Peracca, 1898). Species capture ratio in % for each capture day: $SCR_n = (\text{number of captured sp.} / \text{number of captured carnifex})$ for day *n*. Significance of *P* (comparison of days 1-3): NS, $P > 0.05$; *, $0.01 < P < 0.05$. Species ratio in % for all 3 capture days pooled: $SCR_{1-3} = (\text{total number of captured sp.} / \text{total number of captured carnifex})$ for the 3 days.

Species	Sex	28.06.87	SCR_1	29.06.87	SCR_2	30.06.87	SCR_3	SCR chi-square (P)	28-30.06.87	SCR_{1-3}	
<i>inex</i>	♂	11/89	(12.4 %)	1/35	(2.9 %)	2/49	(4.1 %)	3.871	(0.144 NS)	14/173	(8.1 %)
	♀	23/118	(19.5 %)	4/63	(6.3 %)	4/51	(7.8 %)	6.035	(0.049 *)	31/232	(13.4 %)
<i>ital</i>	♂	2/89	(2.2 %)	1/35	(2.9 %)	1/49	(2.0 %)	0.061	(0.970 NS)	4/173	(2.3 %)
	♀	13/118	(11.0 %)	0/63	(-)	6/51	(11.8 %)	6.909	(0.032 *)	19/232	(8.2 %)

Table 7. Laghicello. Comparison of population size estimates of *I. inexpectata* and *L. italicus* by MRC and by species capture ratio to *T. carnifex* population size estimate over 3 successive days of captures-recaptures. *carn*: *Triturus carnifex* (Laurenti, 1768); *inex*: *Ichthyosaura inexpectata* (Dubois & Breuil, 1983); *ital*: *Lissotriton italicus* (Peracca, 1898); *sp*: species; *n*: number of captured specimens. N_{MRC} : population size estimated by mark-recapture methods; SCR_{1-3} : ratio $n\ sp / n\ car$ over 3 successive days; M(o): model M(o) of Otis et al. (1978); M(t): model M(t) of Otis et al. (1978); M(c): model M(t) of Chao (1989); N_{SCR} : population size estimated by species capture ratio method.

Species (<i>sp</i>)	Sex	SCR_{1-3}	Model	Estimated N_{MRC} for <i>sp</i>	Estimated N_{SCR} for <i>sp</i>
<i>inex</i>	♂	0.081	M(o)	29 - 86 - 406	68 - 157 - 390
			M(t)	27 - 72 - 311	66 - 151 - 373
			M(c)	23 - 48 - 160	58 - 123 - 281
<i>inex</i>	♀	0.134	M(o)	115 - 212 - 467	115 - 196 - 351
			M(t)	109 - 196 - 423	111 - 189 - 337
			M(c)	96 - 163 - 338	134 - 259 - 519
<i>ital</i>	♂	0.023	M(o)	-	19 - 45 - 111
			M(t)	-	19 - 43 - 106
			M(c)	8 - 17 - 85	16 - 35 - 80
<i>ital</i>	♀	0.082	M(o)	36 - 80 - 259	70 - 120 - 215
			M(t)	36 - 77 - 238	68 - 115 - 206
			M(c)	32 - 60 - 165	82 - 157 - 317

other hand, we assume that, during the short span of the study, no mortality, emigration or immigration has taken place, and that the avoidance behaviour has been similar in the different categories of newts (species, sex).

For each of the sexes of the rarest species, we calculate a “species capture ratio” to *T. carnifex*, which is:

$SCR_n = (\text{number of captured specimens of species } sp. / \text{number of captured specimens of } carnifex)$ for day *n*.

Table 6 provides the values of this ratio for the four categories of newts considered and for each of the three days of the study. Chi-square tests show that the figures are not significantly different among the three days of capture for the males of both *I. inexpectata* and *L. italicus*, which suggests that the results of the three days can be pooled. Let us call SCR_{1-3} the value of SCR computed using the pooled capture data over the three days.

The situation is different for the females, which suggests that there may exist indeed

behavioural differences between the species along time and this does not allow the pooling the data over the three days.

For the males, these data allow to reasonably hypothesize that the relative proportions of the three species in the lake have remained stable over the three days of the study. We have an estimate N_{MRC} of the population size of *T. carnifex* by standard methods of capture-recapture. This can allow computing an estimate of the population sizes of the other two species by multiplying the mark-recapture estimate of the population size of *carnifex* (N_{MRC}) by the ratio SCR_{1-3} for the species and sex considered. We can do this both for the mean estimate of N_{MRC} and for the extreme values of its confidence interval. Table 7 and Fig. 2 give the results of these

computations for the males, but also for the females, although these computations are not warranted according to the data discussed above.

6. Discussion

For the proper use of mark-recapture data, a number of conditions must be respected (Otis et al. 1978): the population must be closed during the study (without migration, recruiting of new individuals or loss of individuals), and there must exist an equal catchability among individuals, sexes and species. For the reasons explained above, we think these conditions were fulfilled in this study. We consider the population size estimates by mark-recapture methods for the

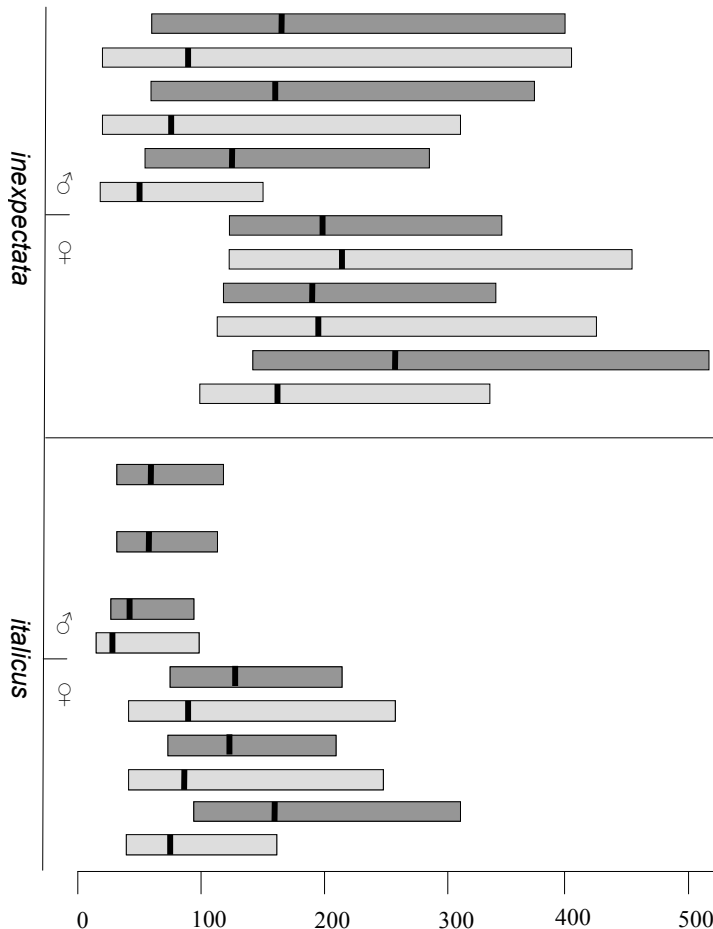


Fig. 2. Estimates of population sizes of males and females *Ichthyosaura inexpectata* and *Lissotriton italicus* by three different mark-recapture methods (MRC) (light grey) and by the species capture ratio method (SCR) (dark grey). (From the data of Table 7).

three species in the lake as rather accurate, at least providing reliable orders of magnitude regarding the overall sizes of the three populations.

Although indirect and rough, the estimates obtained by the method of the species capture ratio (SCR) are of the same order of magnitude as that obtained by the standard methods of mark-recapture (MCR), and this is true both for males and for females. As shown in Figure 2, over 10 such estimates, in only one case the mean SCR estimate falls outside the 95 % confidence interval of the MCR estimate. Interestingly, the new method allows to obtain a rough estimate of the population size even with very low numbers of captured individuals and no recapture, i.e., under conditions which do not allow for an estimate using some MCR methods (two cases for the males of *L. italicus*). This empirical confirmation of the rough estimate of the population size is encouraging. It suggests that if we only had the SCR data (i.e., if the *L. inexpectata* and *L. italicus* specimens had not been marked during this study), we would have obtained a reliable estimate of the order of magnitude of the size of these two populations. We think this method should be tested in other cases, in other populations of other taxa, and possibly incorporated in routine methodologies of population studies in cases of mixed populations of related species with similar ecologies, one of which is large and not endangered while the other one is small and appears to be threatened.

Regarding the *Ichthyosaura inexpectata* population of Laghicello, according to this study it counts roughly between 210 and 300 individuals (Table 4). Four natural populations of this newt are known (Giacoma et al. 1988). According to the incomplete data of Table 1 and those provided by Giacoma et al. (1988), the other three populations are apparently much smaller than the one of Laghicello. The total number of mature individuals in the four populations is probably much below four times the estimated size of the Laghicello population, i.e., between 840 and 1200 specimens. According to criterion D1 of the IUCN (Anonymous 2001), with a population size estimated to a number prob-

ably fewer than 1000 mature individuals, this taxon should be considered as “*Vulnerable*”. It is known from less than five populations and its occupancy is below 20 km², thus fulfilling also criterion D2. The taxon is certainly fragile because very few reproductive sites exist and none is in a National Park. These four lakes completely dry out during summer and one of them (Trifoglietti) is already in an advanced process of invasion by permanent vegetation (bushes and trees). If the mean temperature on earth continues to rise because of global climate change, the period with water in these habitats may become too short for breeding and larval development of these newts. These recently discovered animals, isolated survivors from the last glaciation period, could disappear quite rapidly.

7. Conclusions

This preliminary study suggests that further data would be worth gathering on two different aspects: (1) the method of *species capture ratio* for a quick and rough estimate of the population size of an endangered taxon sharing a habitat with an unthreatened taxon should be investigated further and in more detail, in other taxa and situations, and possibly implemented in field studies dealing with sensible species; (2) the status of the populations of the newt *Ichthyosaura (alpestris) inexpectata* should be studied in more detail, and appropriate measures of conservation of this taxon, including protection of its habitat, should be taken before it is too late.

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