Quantifying and explaining the decline in the occupancy of fire salamander populations

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Statement of authorship

I hereby declare that I have not used any other sources than those listed as references in the bibliography and that I am the only author of this master thesis. I further declare that this thesis was not submitted at any other institution in order to obtain a degree.

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Zusammenfassung

Der Verlust der Biodiversität prägt das Anthropozän. Ein wichtiger Teil des Biodiversitätsverlusts ist der Verlust von Populationen einer Art, denn dies ist der erste Schritt auf dem Weg zum Aussterben der Art. Dabei ist es auch wichtig zu wissen, ob die Verlustraten zunehmen, schwächer werden oder konstant sind. Dies kann man feststellen, wenn man die Verbreitung wiederholt zu mehreren Zeitpunkten untersucht. Neben der Quantifizierung und der Veränderung der Verlustraten ist es auch wichtig, die Ursachen für den Verlust zu untersuchen. Der Feuersalamander (*Salamandra salamandra*) ist eine Amphibienart, welche kürzlich unter einem grossen Populationsrückgang mit unbekannten Ursachen gelitten hat. Ich habe in dieser Arbeit neue Daten zur Verbreitung gesammelt und untersucht, ob der Rückgang weitergeht, wie stark der Rückgang ist und was die Ursachen sind. Die Verbreitung des Feuersalamanders wurde in den Jahren 2003 und 2004 in der Schweiz an 137 Bächen untersucht, indem Präsenz/Absenz Daten von Larven gesammelt wurden. Ich habe 115 Bäche in der Nordschweiz noch einmal besucht und Präsenz/Absenz Daten gesammelt, um die Besetzungs-, Aussterbe- und Kolonisierungsrate berechnen zu können. Die Resultate meiner Analyse zeigen, dass die Anzahl der besetzten Standorte weiterhin zurückgeht. Jedoch wurden auch an Orten, wo in den Jahren 2003/2004 keine Larven vorgekommen sind, Larven gefunden. Der Grund dafür könnte eine Wiederbesiedlung sein. Sowohl terrestrische als auch aquatische Faktoren haben die Besetzungsrate beeinflusst: In der ersten Aussterbeperiode – bis im Jahr 2003 – haben terrestrische Faktoren im weiteren Umfeld des Baches wie Totholz oder kleinere Strassen die Besetzungsrate am besten erklärt, während in der zweiten Aussterbeperiode – von 2003/2004 bis 2016 – aquatische Faktoren wie Charakteristika des Baches, Vorhandensein von Fischen oder die Art des Substrates für die Aussterberate entscheidend waren. Zusammengefasst lässt sich

sagen, dass Daten über das aquatische und terrestrische Habitat für die Verbesserung von Schutzprogrammen einbezogen werden sollten und dass der Trend des Rückgangs des Feuersalamanders mehr oder weniger unverändert weitergeht.

Abstract

Biodiversity loss is a major issue of the Anthropocene. A crucial part of biodiversity loss is the loss of populations of a species, which is the first step towards extinction. It is therefore essential to know whether extinction rates in- or decrease or remain constant. This can be seen by investigating the distribution repeatedly over many points of time. Next to the quantification and change of the extinction rates, it is crucial to investigate their causes. The fire salamander (*Salamandra salamandra*) is one of the amphibians which have recently suffered from a population decline from unknown causes. I collected new distribution data and investigated whether the population decline continued and the strength and causes of the decline. The distribution of the fire salamander has been investigated in 2003 and 2004 by collecting presence/absence data of larvae at 137 streams within Switzerland. In this study, I revisited 115 streams in Northern Switzerland and collected presence/absence data to determine occupancy, extinction and colonization rates. The results of my analysis show that the number of occupied sites is still declining. However, some larvae were found in previously vacant locations. This could be due to recolonization. Both terrestrial as well as aquatic variables influenced the occurrence of fire salamanders: In the first decline period – until the year 2003 – terrestrial variables of the wider steam environment such as deadwood and small roads explained the occupancy rate best, while in the second period – from 2003/2004 to 2016 – aquatic variables such as stream characteristics, presence of fish and substrate type were determinants for the extinction probability. In summary, the inclusion of aquatic as well as terrestrial habitat data is essential for

the improvement of conservation management programs and that the decline trend of the fire salamander remains more or less constant.

Keywords

Salamandra salamandra, fire salamander, decline, biodiversity loss, amphibians, conservation, Switzerland, site occupancy model

Introduction

Recent studies on species distribution state that amphibians are the most threatened of all vertebrate groups (Stuart et al., 2004). In the last decades, amphibians have undergone a dramatic global decline, which is mostly explainable by anthropogenic actions (Cushman, 2006). Altered water temperature, salinity or pH-values, which could be the consequence of modifications in land use or water pollution, could lead to an altered distribution pattern (Wyman & Hawksley-lescault, 1987) or even be lethal for amphibians (Fitzpatrick, 1973; Paul & Meyer, 2001; Egea-Serrano et al., 2012). While some anthropogenic actions might harm the environment especially over a short period of time, their consequences, such as habitat loss or fragmentation can affect nature over a long period of time (Stuart et al., 2004; Cushman, 2006). These long-term changes in the environment lead to three possible scenarios. The adaptation of animals to new conditions, migration out of an unsuitable habitat into a more favorable one or - if neither adaptation nor movements is possible - species can also go extinct in previously suitable habitats, resulting in a loss of biodiversity (Maggini et al., 2011; Vittoz et al., 2013).

It is often hard to identify which scenario occurs: one major problem when assessing species distribution is that data collection is mostly restricted to locations where the species is present while the absence of a species is rarely registered (Cruickshank et al., 2016). This limitation underlies the fact that one rarely knows with certainty where a species does not occur due to

imperfect detection (detection probability < 1) (Preston, 1979; Mackenzie et al., 2002; Cruickshank et al., 2016). By collecting additional data and comparing them with existing datasets, it would be possible to address species absence and make reliable statements about the situation where no individuals were detected: either the species was really absent (1/0/0) or the species was overseen due to imperfect detection or has recolonized (1/0/1) (Gu & Swihart, 2004). Also, the size of the area where data is collected is crucial for determining the status of a species because its absence in a location due to local extinction could be compensated by colonization at another location (Hecnar & M'Closkey, 1997). Therefore, to reliably assess the status of a species and get an overview over the population dynamics, it is inevitable to take into account its dispersal rate and to visit the locations repeatedly (Hecnar & M'Closkey, 1997; Kéry, 2002).

In this study, I investigated the recent population decline and its causes in the fire salamander (*Salamandra salamandra*), an amphibian species lately categorized as "vulnerable" (VU) in the Swiss Red List: (Houlahan et al., 2000; Schmidt & Zumbach, 2005). Previous investigations revealed that especially amphibians living in stream systems, such as fire salamanders, are very sensitive to small-scale changes in their habitat (Davic & Welsh, 2004). This sensitivity is mostly explainable by their permeable skin, which makes them susceptible for infections, chemicals and injuries (Pessier, 2002; Burns et al., 2016). However, the reasons for the recent population decline remain unclear (Schmidt & Zumbach, 2005). In 2003 and 2004, the occurrence of fire salamanders was investigated by taking presence/absence data in over a hundred locations in Switzerland, where they had occurred in previous years (information provided by karch, the Swiss Amphibian and Reptile Conservation Program). Since then, further information about the situation of these *S. salamandra* populations is missing and thus, numbers relating to the occupancy rate in 2003/2004 up to this date do not exist. Therefore, more visits

in the same locations are needed in order to compare them to existing datasets (Kéry, 2002) and to make statements about how the decline trend in the two time periods – until 2003 and from 2003/2004 to 2016 – has changed. Up to this date, only very few studies have been conducted on quantifying the change of decline rates (see Hoffmann et al., 2010).

In my master's thesis, I revisited 115 of the locations that had been investigated in 2003/2004 and collected presence/absence data of fire salamander larvae. In addition, I also recorded information on various environmental aspects to find out more about their impact on the distribution of *S. salamandra*. The main emphasis of the study lied in identifying potential variables, which negatively affect the number of populations of the fire salamander, as well as identifying how strongly the populations in Switzerland have been affected over the last years. Specifically, I wanted to answer the following questions:

- How many and which of the previously occupied locations were still inhabited by the fire salamander in 2003/2004 and in 2016?

I expected that since the years 2003/2004, there were a lot less locations occupied by fire salamanders because as the reason for the decline is unknown (Schmidt & Zumbach, 2005), specific conservation management seems to be difficult to carry out.

- Which environmental variables could be responsible for the change in occupancy?

To investigate the second question, it is important to take into account the habitat and life cycle of the fire salamander. As *S. salamandra* changes its habitat from aquatic to terrestrial during the life cycle (Baumgartner et al., 1999), its occurrence might be affected by variables from both environments (Denoël & Ficetola, 2008; Manenti et al., 2009; Ficetola et al., 2011). In this study, I examined the influence of several aquatic as well as terrestrial variables and considered dispersal by taking into account landscape-scale variables in order to find out more about the occupancy of the fire salamander in Northern Switzerland.

Material & Methods

Study area

I resurveyed locations (streams) that were surveyed in 2003 and 2004 for the update of the 2005 Swiss Amphibian Red List (Schmidt & Zumbach, 2005). In the dataset of the years 2003/2004, only locations which had positive salamander records between the years 1900 and 2002 (see Figure 1) were surveyed. I resurveyed the locations in the lowland of Switzerland north of the Alps (see Figure 2) and excluded those from Ticino.

Study species

Fire salamanders live in a terrestrial environment during most of their life cycle but in the breeding season, females search for streams to give birth to juveniles (Schmidt et al., 2005; Schmidt et al., 2007). After the ovoviviparous birth process between February and May, the larvae stay in the aquatic habitat for up to a year but normally change to a terrestrial environment after about four months (Kopp & Baur, 2000). The ecological impact of fire salamanders on the environment and vice-versa has not been extensively investigated so far (Nery & Schmera, 2016). What is known is that they – both as predators and prey - serve as a keystone species, which stabilises the balance of the ecosystem community (Davic & Welsh, 2004). Fire salamander larvae prefer small head water streams with calm spots and low water flow rates, while the adults live in the surrounding forest areas (Baumgartner et al., 1999). In these often fishless stream systems, they can take over the role as top predators and thereby also serve as ecosystem engineers by preying on leaf-litter decomposing animals (e.g. the freshwater amphipod *Gammarus fossarum*) (Baumgartner et al., 1999). Adult fire salamanders play a similar role in the terrestrial habitat (Baumgartner et al., 1999; Davic & Welsh, 2004). This means that through the presence of *S.*

salamandra, the equilibrium of food resources for other species is maintained. The loss of fire salamanders would thus have substantial consequences for the surrounding environment (Davic & Welsh, 2004).

Datasets

I collected presence/absence data of larvae in order to compare it to data previously collected employing similar methods. In order to analyse the population trends and calculate the occupancy rate, I focussed on two time periods – from before 2003 to 2003 and from 2003/2004 to 2016:

- **Before 2003:** This expression refers to a historical dataset where all the investigated locations were occupied by fire salamanders (median year of the last proof is 1988, see Figure 1). This information was provided by karch.

- **2003/2004:** In 2003 and 2004, karch collected data for the revision of the Swiss Red List (Schmidt & Zumbach, 2005) with the help of volunteers. To do so, 137 streams, which had fire salamanders before 2003 and were situated in the vicinity of participating volunteers were investigated. Between April and June 2003/2004, volunteers would visit a stream section during the day, preferably one which was located in the forest, three times and observe it for no longer than an hour to determine the presence/absence of fire salamander larvae each time (Schmidt & Zumbach, 2004). This dataset was provided by karch and incorporates the presence/absence data of fire salamander larvae within that section, dates of three visits to the same stream section as well as the coordinates of the area that was surveyed (see Table A1 in the Appendix).

- **2016:** This year refers to my own data collection with the help of an intern. We collected presence/absence data, noted the date of the visit and the coordinates of the observed

stream part and measured various additional variables which are listed below. Raw data can be seen in Table A1 in the Appendix.

Data collection

My fieldwork was conducted from mid-April to the end of June 2016 during daytime. Due to the large number of locations to be investigated, the visits were replicated spatially instead of temporally (as it was the case in 2003/2004). Therefore, every stream was only visited once but three sections of it were used as spatial replicates. The stream sections to be sampled were chosen to be as close as possible to those examined in 2003/2004. Each section had a length of 25 m and its coordinates were saved in a GPS device (Garmin Oregon 650t). I conducted surveys in an upstream direction to maintain water clarity. Every section of the stream was observed for 15 minutes by walking up the stream slowly and as often as possible ashore instead of in the water to avoid disturbing the larvae. Larvae were actively searched in leaf litter and under stones in stream pools. I only included larvae in my fieldwork as they are spatially restricted to streams (Schmidt et al., 2005), which allows for much higher detection probabilities (Baumgartner et al., 1999). After each location, I disinfected my boots with Virkon S (Antec International – A DuPont Company) to prevent the spread of pathogens (Schmidt et al., 2009).

Observation and site covariates

Presence/absence data from the three visits of the years 2003/2004 and 2016 each were used for analyses of the observation and site covariates. I tested the date and observer as observation covariates to capture observation error. A total of 15 variables were measured to reveal their significance in terms of observation error and larvae occurrence. Observation and

site covariate data were used as a reference for both time periods, until 2003 and from 2003/2004 to 2016.

- **Date:** The date was defined as the number of days passed after the 31th of March of the corresponding year (2003/2004 or 2016). I assumed that the date could be correlated with detection probability because the larvae are more likely to be spotted with increasing size. Additionally, it is expected that the skills of detecting fire salamander larvae improve after an experimenter has performed fieldwork for some time.

- **pH-value:** The pH-value was noted once per section using a universal indicator paper from the brand "Merck". Owing to a change in colour, pH-values could be determined.

- **Water temperature and conductivity:** The conductivity [µS/cm] measures the salinity of the water (Egea-Serrano et al., 2012). The water temperature [°C] and conductivity were measured right below the surface three times per section each (at the beginning, middle and end of the sections) with the ProfiLine Cond 3110 conductivity meter. For the locations that were surveyed by the intern, conductivity measurements are missing. For the statistical analyses, the means of the values were calculated.

- **Presence of fish:** Baumgartner et al. (1999) demonstrated that the density of fire salamander larvae was reduced by the presence of some fish species. I noted whether any fish were present in the stream sections or not (fish detected/fish not detected).

- **Substrate type and size:** As wooden structures represent effective shelters in the stream (Baumgartner et al., 1999), I hypothesized that grain size and substrate structure could also be significant in terms of hiding spots and influence the occurrence of larvae. I visually categorized the substrate type as forest floor, artificial, gravel or stones and the soil size as small, middle-sized, large, very large or other (e.g. for concrete floor).

- **Mean and maximum water depth:** Fire salamander larvae commonly in quiet pools with low flow velocity (Baumgartner et al., 1999), why I assume that water depth could play a role for fire salamander occurrence. With a yardstick, I measured the depth [m] of the three deepest parts in each section, calculated the mean and also noted the maximum water depth.

- **Periphyton:** Periphyton is defined as the total amount of substances – such as bacteria or algae - that attach on aquatic surfaces (Gray, 2013) and are a vital food resource for many stream organisms (Murphy et al., 1981). Three times per section, an area of about 20 cm^2 within the stream was chosen and visually categorized as being covered with an estimated proportion of periphyton (0-20%, 21-40%, 41-60%, 61-80% and 81-100%). The areas were chosen as where the highest amount of periphyton was observed.

- **Stream assessment/naturalness:** The stream assessment was carried out once per section up to a distance of 5 m from the stream. The area was classified with the help of the leaflet «Bioindikation Biologische Bestimmung der Gewässergüte» by the Naturama Aargau (2006) according to ten different characteristics (stream channel, breadth, shore diversity, fastening and vegetation, water depth and flow, ground diversity, fish migration, use). For every characteristic, one to three points were given while higher points describe more unnatural conditions. The average of the sum of these points then gives a number (1-3) which determines the quality of the stream. Higher values describe unnatural conditions and thus a lower quality.

- **Tree and forest type:** I defined the tree type as the entirety of trees in visual distance of the stream and classified it in the groups deciduous, mixed, coniferous or other type of trees. The type of forest was categorized for every section as forest, bushes, no forest or mixed types in the immediate surrounding of the stream.

- **Deadwood and woodpiles:** Deadwood, which could be a consequence of forestry management could, on one hand, induce stress (Price et al., 2006; Bolger, 2014) but on the other hand, it serves together with leaf litter as energy source for many stream organisms (Murphy et al., 1981) that are preyed on by *S. salamandra* (Davic & Welsh, 2004). The amount of deadwood was measured twice per section by defining a ten meter transect line vertical to the stream and then counting the dead trunks and branches with a diameter of 7-14 cm or ≥ 15 cm and noting the presence of woodpiles. Transects were situated ten steps away from the beginning of the sections at each side of the stream.

I collected the data for the following site covariates with the use of ArcGIS (version 10.4.1). I used the "road network", "hydrological network" and "primary surfaces" vector data from the Swiss map "Vector 25" (resolution 1:25'000) from the Federal Office of Topography (provided by karch). All the variables below were measured within a buffer zone around the coordinate that was used as reference for the location investigated in 2003/2004. Because Ficetola et al. (2012) stated that more than 90% of the fire salamander individuals disperse less than 200 m, I used a buffer of 250 m to make sure that I encompassed the area representing the dispersal distance of most individuals.

- **Road network:** The study of Marsh et al. (2016) found that road networks can harm amphibian populations through direct road kill. I took the length [m] of the road classes 1-5 as well as the highways into account. The roads were classified by their width or construction (class five = forest or bicycle paths, class four 1.8-2.7 m, class three 2.8-3.9 m, class two 4-5.9 m, class one ≥ 6 m wide) ("Zeichenerklärung Landeskarten der Schweiz," 2016).
- **Hydrological network:** I measured the length [m] of streams and rivers within the buffer.

- **Primary surfaces:** Previous studies found that large habitat availability was anticipated to benefit both adult and juvenile fire salamanders (Schmidt & Zumbach, 2005; Price et al., 2011), why the area [m^2] covered with settlement, lakes and forest was calculated.

Statistical analysis

I used the site occupancy model by MacKenzie et al. (2003) to analyse the data. This model takes imperfect detection into account, can cope with multi-seasonal data and is often used to investigate population distributions (Mackenzie et al., 2003; Kéry & Schmidt, 2008; Fiske & Chandler, 2015). I worked with the package *unmarked* from Fiske and Chandler (2011) and used the function *colext*, which matches the site occupancy model by MacKenzie et al. (2003), to calculate the parameters occupancy (ψ), colonization (γ), extinction (ε) and the detection probability (p) (Fiske & Chandler, 2015). The detection probability refers to the chance of the observer detecting a present population and thus describes the error in observation, while occupancy, colonization probability and extinction probability refer to the process yielding to a change from occupied to unoccupied locations and vice-versa. While with occupancy probability, the percentage of occupied locations after the first time period t is explained, colonization probability and extinction probability describe the change of occupancy for later time periods t+1. In other words: occupancy and extinction probability both account for the same biological processes but relate to a different time period (MacKenzie et al., 2003). Therefore, I could use the reciprocal of the occupancy probability to describe the first extinction event while the second was represented with the extinction probability.

When site occupancy models are used, the following assumptions need to be fulfilled: (1) The population is closed i.e. no migration, births or deaths takes place between the visits of the same year. (2) It is possible to overlook some individuals (= false negative) but it is not possible

to detect individuals that are not there (= false positive). (3) The detection probabilities are independent from one location to the next. Detected populations are noted with the value "1" while non-detection is noted with a "0" (MacKenzie et al., 2002). A study of the Eurasian otter (*Lutra lutra L.*) evaluated the substitution of temporal with spatial replication for population dynamic data analysis as suitable and demonstrated that it yields more reliable detection probability estimates for highly mobile species (Parry et al., 2013). Temporal and spatial replicates yield similar occupancy estimates in surveys of larval salamanders (Keller, 2016). At one location, only one section could be surveyed. In the data from 2003/2004, the presence/absence data is missing at nine locations for at least one section because locations were not always visited thrice.

In a first step, the model was run for both time periods for each parameter - occupancy, colonization, extinction and detection probability – without any covariates and therefore constant parameters ($\psi(\sim 1)$ $\gamma(\sim 1)$ $\epsilon(\sim 1)$ $p(\sim 1)$). After that, I tested the variables for correlations among each other and found that date and water temperature were positively correlated (r = 0.5). To ensure that this correlation would not influence the interpretation of the tested variables, I used the residuals of a regression of temperature against day.

In a next step, I set date and observer as explanatory variables for the detection probability (*p*) to test their relevance in terms of observation error (see Table 1 "Detection probability Model"). After that, I created candidate models for the parameters occupancy and extinction containing each site covariate separately. I left the colonization probability constant (~1) because there were only two cases where I found larvae in previously vacant locations (see Figure 2). The candidate models were then checked for the lowest AIC (Akaike's Information Criterion), which indicates the most parsimonious model (Burnham & Anderson, 2002). For each parameter ψ and ε, I chose the five site covariates with the lowest AIC values, which were then combined in global

models (see Table 1, marked in bold). Then, I tested whether candidate models with only four out of these five site covariates result in better (the ones that are above the corresponding model in bold) or worse (the ones that are below the corresponding model in bold) models than the global model (see Table 1). After that, I put the variables that resulted in worse models when missing in the next model (see Table 2). This allowed discovering the best explanatory variables for each parameter ψ and ε. These variables together with the one for the detection probability then resulted in the best overall model for the data (see Table 2, marked in bold).

All statistical analyses were carried out using the open source program R (Version 3.3.1) (R Development Core Team, 2016). Moreover, all numerical values had been standardized before they were put in the analysis.

Results

I estimated that in 2003/2004, 73.7% (= 84.8 locations, SE = 0.046) of the investigated 115 locations were still occupied by fire salamander larvae. In 2016, a total of 55.8% (= 64.2 locations, SE = 0.047) were occupied. At these locations, the salamanders have either not gone extinct (72.3% = 61.3 locations, SE = 0.049) or they have been recolonized (9.5% = 2.9 locations, SE = 0.055). Moreover, I found that in 2016, at 27.7% (= 23.5 locations, SE = 0.049) out of the 84.8 locations that were occupied in 2003/2004, the fire salamander populations had become locally extinct. To sum up, together with the 90.5% (= 27.3 locations, SE = 0.055) that remained abandoned (= 100%-9.5% from the recolonization), at a total of 44.2% (= 50.8 locations, SE = 0.047) out of the 115 locations fire salamander absence was inferred in 2016 (see Figure 3).

The best model for occupancy probability ψ included two covariates. The effects of class four roads (estimate = 0.492, SE = 0.276, see Figure 4) and the amount of deadwood with a diameter of ≥ 15 cm (estimate = 1.077 SE = 0.369, see Figure 5) were positive. The colonization probability

γ could not be explained by the variables I measured. Extinction probability *ε* was best explained by the variables substrate type (estimate = -0.313, SE = 0.176, see Figure 6), presence of fish (estimate = 1.141, SE = 0.719, see Figure 7), stream naturalness (estimate = 0.922, SE = 0.331, see Figure 8), nearby rivers (estimate = 1.274, SE = 6.755) and the date (estimate = 0.461, SE = 0.328). For the overall model, the nearby rivers were excluded due to the large standard deviation, while the date was excluded because the range of the predicted extinction probabilities was very small (0.9998-1).

The "Red List" observer - which refers to all the volunteers from the year 2003/2004 - showed to have the highest detection probability *p* (estimate = 0.951, SE = 0.014) while lower probabilities were estimated for the intern (estimate = 0.545, SE = 0.100) and myself (estimate = 0.782, SE = 0.034). Altogether, the detection probability p was higher in 2003/2004 (estimate = 0.951, SE = 0.014) than in 2016 (estimate = 0.749, SE = 0.033, see Figure 9). The detection probability model with the observer as explanatory variable had the lowest AIC and was therefore the better of the two tested candidate models (see Table 2).

Discussion

The aim of this study was to show the population trends of the fire salamander in Northern Switzerland in the recent past. I also wanted to reveal potential causes for the declining population trend of *S. salamandra* and found that many features of the habitat are correlated with the change in occupancy of the populations. From the 115 revisited locations that used to be inhabited by fire salamanders before 2003, only 84.8 (SE = 0.046) were occupied in 2003/2004 and 64.2 (SE = 0.047) in 2016, which represents an overall decline of 44.2%. The trend of the decline rates of the two time periods are very similar with 26.3% in the first and 27.7% in the second time period. I found that in these two periods of time, local extinction of fire salamanders

is associated with the amount of deadwood with a diameter of ≥ 15 cm, small roads, stream naturalness, presence of fish and substrate type. These findings are in line with the results from Manenti et al. (2009) who also found that both terrestrial and aquatic variables could affect fire salamander occupancy. My results show that the relative importance of the variables has changed over the years as in the two time periods, different variables played a role for local extinction. Terrestrial variables such as the amount of deadwood with a diameter of ≥ 15 cm and proximity to roads of class four played a role in the first decline period while stream naturalness, substrate type and presence of fish were relevant in the second period.

The change in occupancy in the first decline event, from before 2003 to 2003, was driven by the determinants of occupancy probability. My results indicate that fire salamander occurrence in the first decline event depended fundamentally on the terrestrial adult stage while aquatic variables played a minor role in that period. It seems as if - in terms of deadwood and the road network – a substantial change around the year 2003/2004 managed these variables not being limiting variables anymore. This hypothesis is supported by the fact that in 2003, the Ministerial Conference on the Protection of Forests in Europe (MCPFE) acknowledged deadwood European-wide as an indicator for forest diversity (MCPFE, 2002; Scheidegger et al., 2013). Since then, the average deadwood volume in Swiss forests has increased significantly (Scheidegger et al., 2013). I hypothesize that, as larvae are restricted to aquatic habitats (Baumgartner et al., 1999), the amount of deadwood with a diameter of ≥ 15 cm only has an indirect effect on the larvae but rather matters for adult fire salamanders. The positive impact of the number of thick deadwood on fire salamander occurrence could be explained by the findings of Baumgartner et al. (1999) stating that deadwood might provide possible hiding places for adult individuals. The investigation of the road network within a distance of 250 m of the stream reference point showed that only roads of the class four (width 1.8-2.7 m) have an impact on the occupancy

probability of the fire salamander larvae. Up to the year 2003, about 35'600 km of roads of class four or five (width of 1.8-2.7 m) were built but the exact length of newly built roads of class four remains unclear (FRS, 2008). The length of roads of class four was – in contrast to my expectation - positively associated with larvae occurrence, which could possibly be explained with their ecological lifestyle: Firstly, fire salamander larvae are bound to aquatic habitats (Schmidt et al., 2005). Terrestrial predators of the larvae might cross roads more frequently, which increases their risk of being hit by vehicles (Fahrig & Rytwinski, 2009). This negative association between their predators and the road network may be beneficial for the fire salamander occurrence. Secondly, adult fire salamanders use stone walls close to the roads as hiding places (Rebelo & Leclair, 2003), why I assume a larger road network to positively influence *S. salamandra*.

The change in occupancy in the second decline event was driven by the determinants of extinction probability. In this second decline event, the aquatic variables showed to be better determinants: Substrate type was found to have an impact on the extinction probability of the fire salamanders. Contrary to the expectation that artificial substrate types (such as plastic pipes or concrete walls) would be correlated with reduced larvae occurrence, this type was the one with the lowest extinction rate, followed by forest floor, stones and sand/gravel. Artificial substrate types are often used in order to preserve a stream system when roads or architectural structures are being built over or next to the stream channel (Patt et al., 1998). I hypothesize that this may pose a benefit for the salamanders since their predators might be strongly negatively influenced by the anthropogenic changes within the stream and stay away from that area. This hypothesis is supported by the findings of Gorman et al. (1978) stating that the diversity of fish species depends on the substrate type of the stream and that natural streams with a higher structure diversity usually also maintain a higher fish species diversity. Nevertheless, fish were detected in eleven of the investigated streams and the statistical model positively associated the

presence of fish with the extinction probability of the larvae. This supports the previously mentioned hypothesis that predators could limit larvae occurrence. My results show that fire salamander larvae can cope with artificial substrate types but does indicate tolerance towards general anthropogenic changes: Naturalness of the stream and its environment was tested with a stream assessment and showed that extinction probability is positively correlated with a higher stream assessment value (1-4) and thus a lower stream naturalness. My results are in line with many studies about habitat quality and the harm of anthropogenic influences on amphibians and whole ecosystems (Hamer & McDonnell, 2008; Price et al., 2011). High extinction probabilities or larvae absence could also be a consequence of larvae that have been flushed away due to heavy rainfalls or even early emigration to the terrestrial habitat after metamorphosis (Baumgartner et al., 1999). Such as has been shown in a paper investigating the conservation of some turtle species, low survival rates up to the adult stage do not always significantly contribute to the overall population decline of a species (Heppell, 1998). It would therefore be interesting to look at the reproductive contribution (annual survival rates and fecundity) of the developmental stages of fire salamander populations.

In conclusion, this study indicates that the population of fire salamanders in Northern Switzerland has continued to decline over the past decade. The local extinction rate of the fire salamander larvae is affected by terrestrial, such as deadwood with a diameter of at ≥ 15cm and proximity to roads of class four, as well as aquatic variables, such as stream naturalness, the length of nearby rivers, presence of fish and substrate type. I would therefore highly recommend integrating not only the structure of a stream when planning conservation projects but also the fish population and forestry management. Moreover, what has been shown with fire salamanders – that the relative importance of the tested variables in terms of species occurrence can change through years – could also be the case for various other species. Conservation

projects should thus whenever possible collect long-term data including habitat variables covering all environments inhabited through the life cycle of the species as well as different spatial scales in their data collection to get an overview over the status of the species. Finally, this study shows that anthropogenic influences do not always negatively affect nature but can be beneficial for certain species and therefore should always be considered as part of a big system.

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Tables and Figures

Table 1: Candidate models of the five (or two for the detection probability) best site covariates for occupancy ψ, extinction ε and detection probability p. γ refers to the colonization probability. The global models with all five or two covariates is shown in bold while the other models only contain four out of the five variables. (~1) refers to constant parameters, the missing variable shows which variable from the global model is missing in the candidate model, K = Number of parameters, ΔAIC = Difference between the AIC (Akaike's Information Criterion) and the model with the best AIC, ω = Akaike weight, X15 = deadwood with a diameter of ≥ 15cm, Settl = area covered with settlement, Wald = area covered with forest, Kl2 = roads of the class two, Kl4 = roads of the class four, SA = stream assessment/naturalness, Sotp = substrate type, Dat = date, Riv = length of nearby rivers, F = presence of fish, Obs = observer.

Occupancy probability Model				Missing variable	K	ΔAIC	ω
Ψ(X15+Settl+Wald+Kl4)	γ(~1)	ε(~1)	*p*(~1)	Kl2	8	0.00	0.30
Ψ(X15+Settl+Kl2+Kl4)	γ(~1)	ε(~1)	*p*(~1)	Wald	8	0.0008	0.30
Ψ(X15+Kl2+Wald+Kl4)	γ(~1)	ε(~1)	*p*(~1)	Settl	8	1.08	0.18
Ψ(X15+Settl+Kl2+Wald+Kl4)	**γ(~1)**	**ε(~1)**	***p*(~1)**	**–**	**9**	**1.99**	**0.11**
Ψ(X15+Settl+Kl2+Wald)	γ(~1)	ε(~1)	*p*(~1)	Kl4	8	2.17	0.10
Ψ(Settl+Kl2+Wald+Kl4)	γ(~1)	ε(~1)	*p*(~1)	X15	8	9.73	0.002
Extinction probability Model							
Ψ(~1) γ(~1) ε(~SA+Dat+Riv+F+Sotp)			***p*(~1)**	**–**	**9**	**0.00**	**0.26**
Ψ(~1) γ(~1) ε(~SA+Dat+Riv+Sotp)			*p*(~1)	F	8	0.51	0.22
Ψ(~1) γ(~1) ε(~SA+Riv+F+Sotp)			*p*(~1)	Dat	8	0.77	0.19
Ψ(~1) γ(~1) ε(~SA+Dat+Riv+F)			*p*(~1)	Sotp	8	0.95	0.18
Ψ(~1) γ(~1) ε(~SA+Dat+F+Sotp)			*p*(~1)	Riv	8	1.72	0.12
Ψ(~1) γ(~1) ε(~Dat+Riv+F+Sotp)			*p*(~1)	SA	8	7.29	0.01
Detection probability Model							
Ψ(~1) γ(~1) ε(~1) p(~Obs)				Dat	6	0.00	0.64
Ψ(~1) γ(~1) ε(~1) p(~Dat+Obs)				**–**	**7**	**1.17**	**0.36**
Ψ(~1) γ(~1) ε(~1) p(~Dat)				Obs	5	38.83	0.00

Table 2: Model selection for occupancy ψ, colonization γ, extinction ε and detection probability p separately and combined in the best overall model in bold. (~1) refers to constant parameters, K = Number of parameters, ΔAIC = Difference between the AIC (Akaike's Information Criterion) and the model with the best AIC, ω = Akaike weight, X15 = deadwood with a diameter of ≥ 15cm, Kl4 = roads of the class four, SA = stream assessment/naturalness, Lake = area covered with lakes, Sotp = substrate type, Dat = date, Riv = length of nearby rivers, F = presence of fish, Obs = observer.

Best models combined				K	ΔAIC	ω
Ψ(X15+Kl4)	*γ*(~1)	*ε*(~1)	*p*(~1)	6	48.29	0.00
Ψ(~1)	*γ*(~1)	*ε*(~1)	*p*(~1)	4	61.65	0.00
Ψ(~1)	*γ*(~1)	*ε*(~SA+Dat+Riv+F+Sotp)	*p*(~1)	9	46.97	0.00
Ψ(~1)	*γ*(~1)	*ε*(~1)	*p*(~Obs)	6	23.90	0.00
_Ψ_(X15+Kl4)	***γ*(~1)**	**_ε_(~SA+F+Sotp)**	***p*(~Obs)**	**11**	**0.00**	**1.00**

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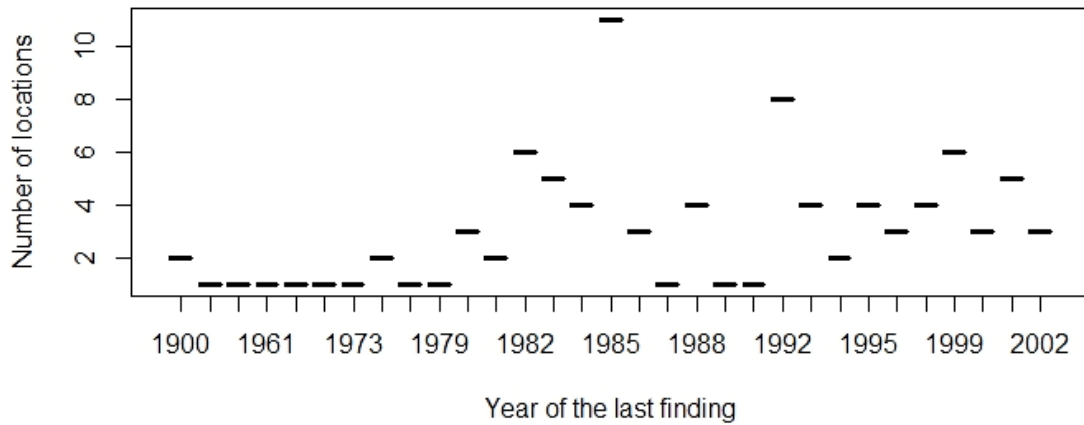
Figure 1: Plot showing the number of locations where the last detection of fire salamander larvae was recorded for each year from 1900 to 2002. The median year of the last finding is 1988.
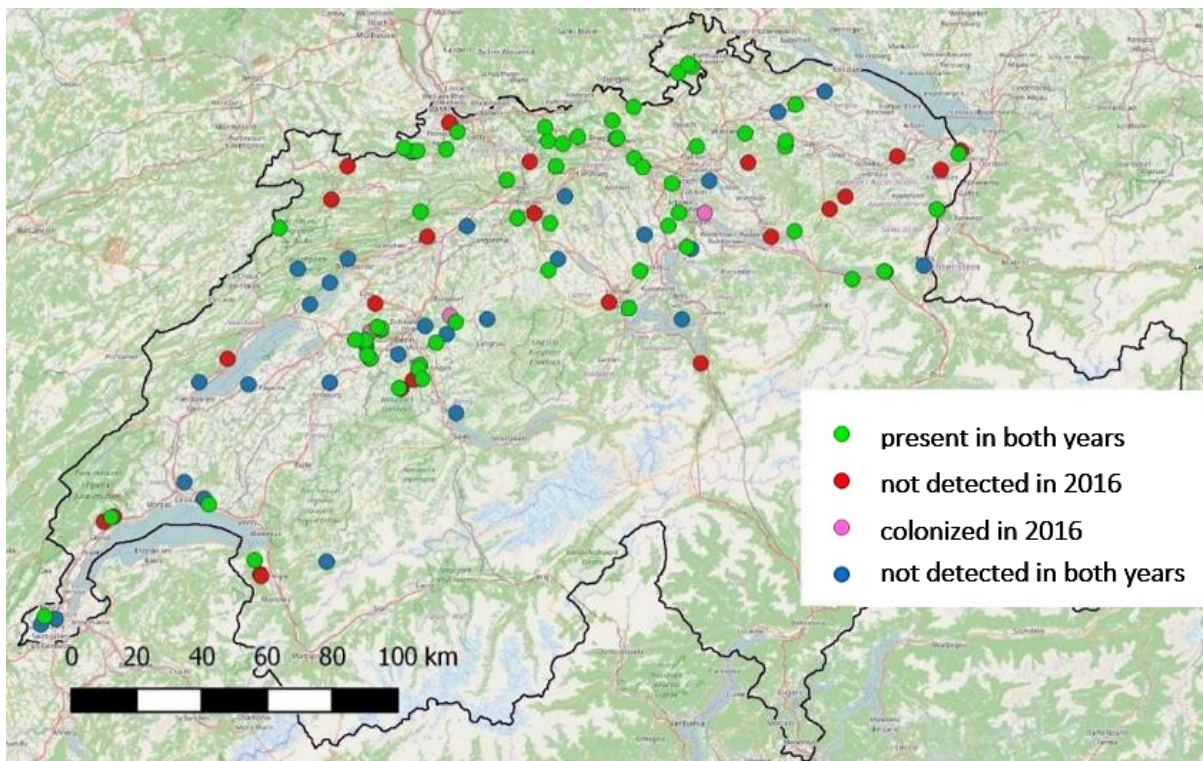
Figure 2: Map of Switzerland showing the reference points of the 115 investigated locations. "Present in both years" refers to locations where fire salamander larvae were detected in 2003/2004 and 2016 while "not detected in both years" refers to locations where no larvae were detected in 2003/2004 and 2016. "Not detected in 2016" shows locations where larvae were only detected in 2003/2004 (and before the year 2003) and "colonized in 2016" refers to locations where larvae were detected in 2016 but not in 2003/2004. Reference: OpenStreetMap of qGIS and dataset provided by karch.
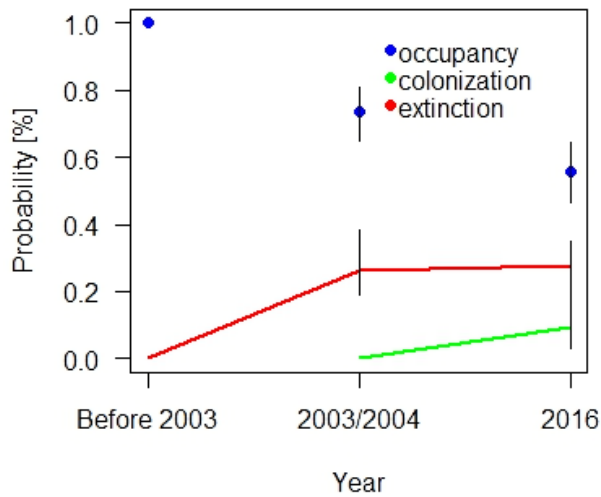
Figure 3: The estimated occupancy (Ψ), colonization (γ) and extinction (ε) probabilities and 95% confidence intervals when covariates – also detection probability (p) - were set as constant (Ψ(~1) γ(~1) ε(~1) p(~1)). Colonization and extinction are shown as linear processes because data between the years are missing. The calculated probabilities refer to the state of the respective previous year.
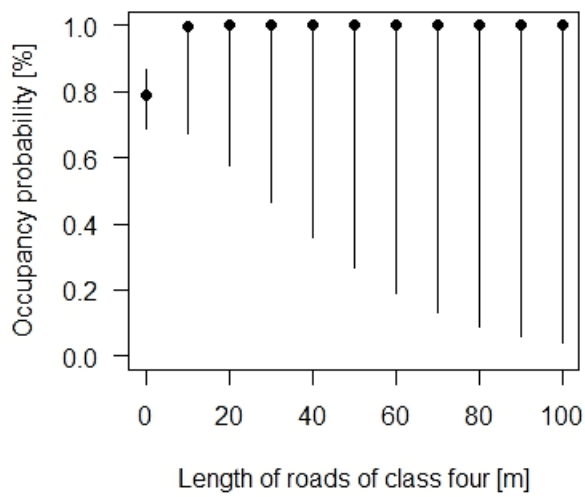
Figure 4: Predicted occupancy probabilities and 95% confidence intervals associated with the length of roads (width 1.8-2.7m) within 250m of the stream.
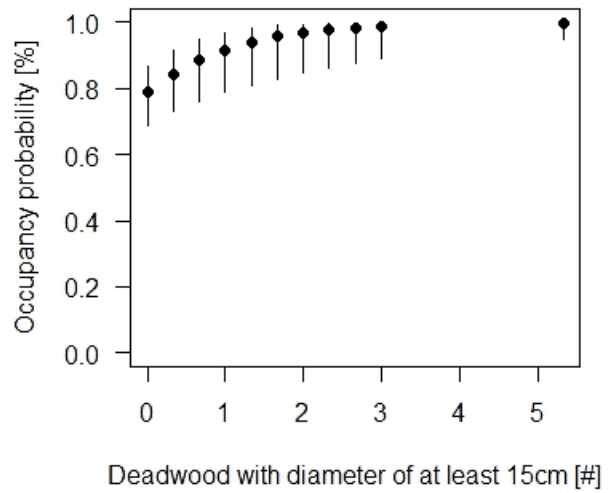
Figure 5: Predicted occupancy probabilities and 95% confidence intervals associated with the amount of deadwood with a diameter of ≥ 15cm.
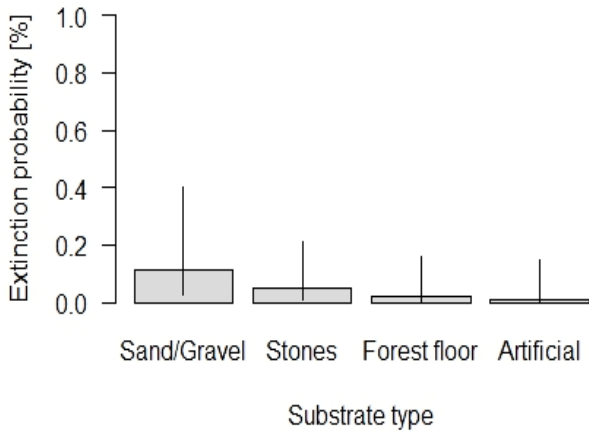
Figure 6: Predicted extinction probabilities and 95% confidence intervals associated with the substrate type.
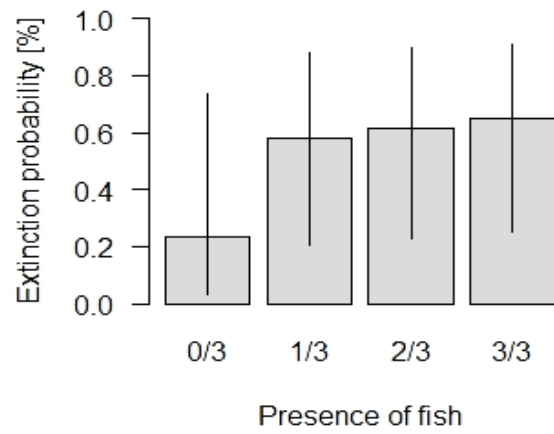
Figure 7: Predicted extinction probabilities and 95% confidence intervals associated with the presence of fish at 0, 1, 2 or all of the three visits (in 2003/2004) or sections (in 2016), respectively.
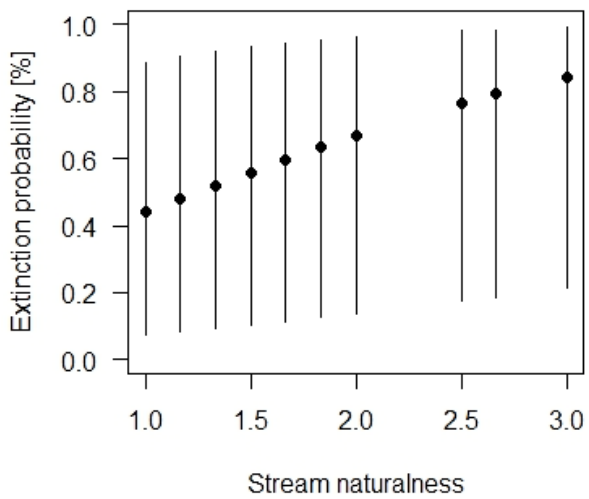
Figure 8: Predicted extinction probabilities and 95% confidence intervals associated with stream naturalness based on the stream assessment. A value of 1 stands for natural streams while a value of 3 stands for unnatural streams.
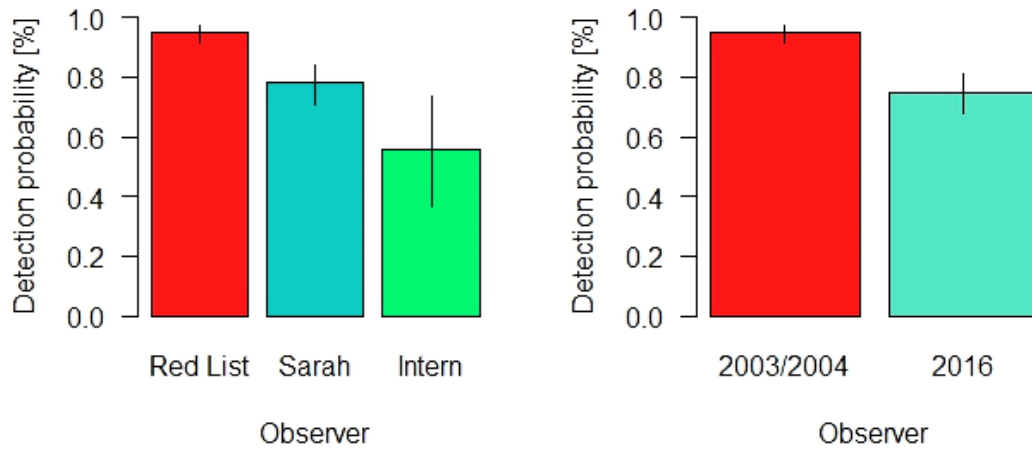
Figure 9: Predicted detection probabilities and 95% confidence intervals associated with the observers. On the left: The observer "Red List" refers to all the volunteers in the years 2003/2004 while "Sarah" refers to myself and "Intern" to the intern helping me in 2016. On the right: To compare the detection probabilities of the years 2003/2004 and 2016, observers "Sarah" and "Intern" were combined to represent the detection probability in the year 2016. The red bar (left: "Red List", right: "2003/2004") shows the same value.

Appendix

Table A1: The investigated 115 locations with the corresponding reference coordinates (X and Y). The number of the locations

(Nr.) refers to the numbering in the dataset of the year 2003/2004. V1, V2 and V3 refer to the three visits (in grey for the year

2003/2004) or the three investigated sections (in white for the year 2016). "1" = larvae found, "0" = no larvae found, "NA" =

no data available. The date of the visits shows when the data were collected (in grey for 2003/2004, in white for 2016).

									Date of the visits			
Nr.	X	Y	V1	V2	V3	V1	V2	V3	V1	V2	V3	V1/2/3
1	584350	223500	0	0	0	0	0	0	30.04.	28.05.	25.06.	04.05.
2	579000	216500	0	0	0	0	0	0	30.04.	28.05.	25.06.	04.05.
3	710200	267450	0	0	0	0	0	0	16.05.	26.05.	17.06.	05.05.
4	541330	152840	0	NA	NA	0	0	0	01.05.	NA	NA	18.05.
5	557900	130180	1	1	1	0	0	0	04.05.	07.06.	27.06.	27.05.
6	558150	129700	1	1	1	0	0	0	04.05.	07.06.	27.06.	27.05.
7	556300	134360	1	1	1	1	1	1	04.05.	07.06.	27.06.	27.05.
8	600075	185175	0	1	1	0	0	0	09.05.	14.05.	26.05.	17.05.
9	700760	261010	0	1	1	1	1	1	11.05.	20.06.	28.06.	18.04.
10	542690	151150	1	1	1	1	0	0	26.05.	13.06.	23.06.	18.05.
11	668350	253300	1	1	1	1	1	1	04.05.	18.05.	08.06.	28.04.
12	661825	264250	1	0	1	1	1	1	26.04.	01.05.	07.06.	21.05.
13	594050	202400	1	NA	NA	0	1	0	25.04.	NA	NA	16.05.
14	594200	203000	1	NA	NA	1	1	1	17.05.	NA	NA	16.05.
15	593040	203370	1	1	0	0	1	0	26.04.	10.05.	29.05.	16.05.
16	648200	242000	NA	NA	NA	0	0	0	01.04.	01.04.	01.04.	03.05.
17	577775	133900	0	0	0	0	0	0	NA	NA	NA	25.05.
18	634250	235500	1	1	1	1	1	1	28.04.	24.05.	07.06.	09.05.
19	763400	256500	1	1	1	1	1	0	11.05.	28.05.	29.06.	11.05.
20	764000	257480	1	1	1	0	0	0	14.05.	24.05.	16.06.	11.05.
21	668000	268300	1	1	1	1	1	1	04.05.	01.06.	08.07.	21.05.
22	723800	273700	0	0	0	0	0	0	01.05.	15.05.	09.06.	05.05.
23	662900	259000	1	1	1	1	1	1	10.05.	31.05.	28.06.	02.05.
24	631050	246625	1	1	1	1	1	1	27.04.	27.05.	22.06.	20.06.
25	605750	237250	1	1	1	0	1	0	04.05.	14.05.	11.06.	27.05.
26	651800	259425	1	1	1	0	1	1	01.05.	24.05.	20.06.	21.05.
27	569600	220600	0	0	0	0	0	0	11.05.	29.05.	15.06.	20.05.
28	496500	117650	0	0	0	0	0	0	30.04.	21.05.	28.06.	24.05.
31	589500	199500	1	1	1	0	0	1	24.05.	09.06.	15.07.	01.06.
32	590850	194000	1	1	1	1	0	0	03.05.	25.05.	14.06.	26.05.
33	589750	197700	0	0	0	0	0	0	26.04.	18.05.	21.06.	01.06.
34	607125	203700	0	0	0	0	0	0	18.04.	24.05.	22.06.	15.06.
35	514400	147900	1	1	NA	0	0	0	01.05.	24.05.	NA	17.05.
36	511300	146400	1	1	NA	0	0	0	26.04.	17.05.	NA	25.05.
37	701800	252500	1	1	1	0	0	0	30.04.	25.05.	30.06.	14.06.

38	678650	233650	1	1	1	1	1	1	03.05.	18.05.	29.05.	18.05.
40	667300	209300	1	1	1	1	1	1	27.04.	11.05.	01.06.	21.06.
41	685480	227000	0	0	0	0	0	0	26.04.	15.05.	04.06.	07.05.
42	663300	259150	1	1	1	1	1	1	01.05.	17.05.	01.06.	02.05.
43	712600	257400	1	1	1	0	1	1	30.04.	22.05.	24.06.	06.05.
44	670850	250750	1	1	1	1	1	1	07.05.	23.05.	18.06.	28.04.
45	590900	194100	1	1	1	1	1	1	27.04.	11.05.	07.06.	26.05.
46	579575	240850	1	1	1	0	0	0	27.04.	15.05.	02.06.	26.05.
47	646200	223500	0	0	0	0	0	0	29.04.	12.05.	01.06.	20.04.
48	712700	259050	1	1	1	1	1	1	23.05.	14.06.	28.06.	06.05.
49	605600	192200	1	0	0	1	1	1	01.05.	20.05.	30.06.	05.06.
50	689300	237650	0	0	0	1	0	1	07.05.	23.05.	04.06.	10.05.
51	605200	191050	1	1	1	0	1	1	25.04.	18.05.	30.05.	05.06.
52	679500	246100	1	1	1	1	1	0	06.05.	23.05.	05.06.	28.04.
53	645500	250750	1	1	1	1	1	1	27.04.	11.05.	08.06.	20.06.
54	613250	255550	1	1	1	1	1	1	22.04.	08.05.	17.06.	01.06.
55	603175	254675	1	1	1	1	1	1	29.04.	12.05.	18.06.	20.05.
56	603550	255000	1	1	1	1	1	1	29.04.	12.05.	18.06.	20.05.
57	604750	255125	1	1	1	1	1	1	25.04.	12.05.	18.06.	20.05.
58	606300	188060	1	1	1	0	1	1	24.04.	15.05.	03.06.	05.06.
60	625500	205750	0	0	0	0	0	0	28.04.	19.05.	07.06.	19.04.
61	607750	229950	1	1	1	0	0	0	18.04.	11.05.	05.06.	27.05.
62	684200	227500	1	1	1	1	1	1	01.05.	16.05.	09.06.	07.05.
63	643000	258100	1	1	1	1	1	1	24.04.	16.05.	16.06.	06.06.
64	733000	218950	1	1	1	1	1	1	04.05.	11.05.	18.05.	04.05.
65	643500	220300	1	1	1	1	1	1	10.05.	25.05.	08.06.	20.04.
66	610400	198750	1	1	1	1	0	0	10.05.	28.05.	04.07.	15.06.
67	584350	250600	1	1	1	0	0	0	26.04.	23.05.	11.06.	26.05.
68	492000	116000	0	0	0	0	0	0	18.05.	24.05.	15.06.	24.05.
69	745350	255250	1	1	1	0	0	0	27.04.	24.05.	24.06.	25.05.
70	573150	210100	0	0	0	0	0	0	03.05.	27.05.	09.06.	20.05.
71	616500	260700	1	1	1	1	1	0	02.05.	18.05.	08.06.	01.06.
72	671650	231000	0	0	0	0	0	0	26.04.	29.05.	23.06.	18.05.
73	603600	187800	1	1	0	0	0	0	24.04.	09.05.	21.05.	05.06.
74	586500	199600	1	1	1	1	1	0	06.05.	18.05.	25.05.	02.06.
75	592400	210300	0	1	NA	0	0	0	23.05.	25.06.	NA	23.06.
76	554800	186600	0	0	0	0	0	0	28.04.	29.05.	02.06.	09.05.
77	642200	262200	1	1	1	1	1	1	16.05.	02.06.	23.06.	06.06.
78	670450	220300	1	1	1	1	1	1	02.05.	02.06.	17.06.	21.04.
79	616400	178000	0	0	0	0	0	0	12.05.	28.05.	11.06.	17.05.
80	619400	233050	0	0	0	0	0	0	25.04.	23.05.	12.06.	27.05.
81	637750	252050	1	1	1	0	0	0	04.05.	31.05.	15.06.	20.06.
82	616200	204850	1	1	1	0	1	0	27.04.	18.05.	08.06.	19.04.
83	661400	211000	1	1	0	0	0	0	29.04.	18.05.	15.06.	21.04.
84	686600	257000	1	1	1	0	1	0	08.05.	28.05.	15.06.	06.05.

85	614100	263300	1	1	1	0	0	0	21.04.	17.05.	22.06.	01.06.
87	708900	230900	1	1	1	0	0	0	30.04.	02.06.	18.06.	29.04.
88	513500	147660	1	1	1	0	0	1	04.05.	25.05.	07.06.	17.05.
89	685025	280200	1	1	1	1	1	1	23.05.	14.05.	08.06.	25.04.
90	680900	278400	1	1	NA	0	1	1	23.04.	20.05.	NA	25.04.
91	647330	257400	1	1	1	1	1	1	03.05.	24.05.	21.06.	06.06.
92	599200	195300	0	0	0	0	0	0	04.05.	15.05.	07.06.	23.06.
93	688750	193300	1	1	1	0	0	0	26.04.	18.05.	14.06.	22.06.
94	754050	223450	0	0	0	0	0	0	23.05.	04.06.	20.06.	25.05.
95	683000	206250	0	0	0	0	NA	NA	05.05.	19.05.	01.06.	22.06.
96	690450	247000	0	0	0	0	0	0	18.05.	31.05.	23.06.	10.05.
97	493200	118400	1	1	1	1	1	1	26.04.	12.05.	04.06.	24.05.
98	681700	237550	1	1	1	1	0	1	02.06.	06.06.	08.06.	18.05.
99	639200	236900	1	1	1	0	0	0	02.05.	12.05.	06.06.	09.05.
100	590000	195100	0	1	1	0	1	1	27.04.	18.05.	09.06.	26.05.
101	715800	232700	1	1	1	0	1	1	27.04.	11.05.	07.06.	29.04.
102	540200	187400	0	0	0	0	0	0	27.04.	18.05.	07.06.	17.05.
103	683750	281000	1	1	1	1	1	1	27.04.	18.05.	08.06.	30.04.
104	548700	194200	1	1	1	0	0	0	01.05.	23.06.	15.06.	20.05.
105	578900	187000	0	0	0	0	0	0	27.04.	11.05.	25.05.	09.05.
106	614500	206700	0	0	0	0	1	0	29.04.	20.05.	18.06.	19.04.
107	599450	185350	1	1	1	0	1	1	11.05.	24.05.	08.06.	17.05.
108	643850	234075	0	1	0	0	0	1	24.04.	22.05.	07.06.	09.05.
110	590950	201900	0	0	0	1	1	1	11.05.	22.05.	01.06.	16.05.
111	535500	157800	0	0	NA	0	0	0	30.04.	07.07.	NA	18.05.
112	600875	255950	1	1	1	1	0	0	02.05.	15.05.	01.06.	20.05.
113	613500	201300	0	0	0	0	0	0	27.04.	17.05.	01.06.	15.06.
114	564250	232550	1	1	1	0	1	0	25.04.	23.05.	10.06.	11.05.
115	742800	221400	1	1	1	1	0	0	26.04.	23.05.	20.06.	04.05.
116	742500	221500	1	1	1	0	1	0	26.04.	23.05.	20.06.	04.05.
117	715350	269700	1	1	1	1	1	1	24.04.	23.05.	17.06.	05.05.
118	725950	239400	1	1	1	0	0	0	07.04.	29.05.	11.06.	10.06.
119	730525	243150	1	1	1	0	0	0	07.04.	29.05.	11.06.	10.06.
120	757425	240100	1	1	1	1	1	1	07.04.	29.05.	11.06.	25.05.
121	758325	251675	1	1	1	0	0	0	07.04.	29.05.	11.06.	11.05.