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ECOLOGY AND CONSERVATION OF THE AGILE FROG
(*RANA DALMATINA*) IN JERSEY

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ABSTRACT

On the island of Jersey, the agile frog (*Rana dalmatina*) has declined to a single small population that is displaying erratic breeding success. Survival of eggs from hatching to metamorphosis can vary from 0% to 17% per year. This gives rise to pulses in recruitment, which are reflected in the age structures of adult frogs. Variations in recruitment - and the resulting population fluctuations (the number of males was estimated to vary between 14 and 42 individuals) – were recorded over a three-year study period and seemed to be linked to variations in the level of predation pressures on the tadpole phase by newts and macroinvertebrates, which in turn are related to hydroperiod. Genetic variation in the Jersey population was compared to populations elsewhere in the species' range using microsatellite DNA analysis. The Jersey population emerged as the least variable population, possibly due to drift and inbreeding. Population Viability Analysis (PVA) models – based on field data - predicted the extinction of the population over 50 years, unless productivity is enhanced (i.e. by protecting the spawn). The *Rana dalmatina* distribution range should be expanded, to reduce the risk of its disappearance from the wild. Although field observations of *Bufo bufo* tadpoles indicated a problem with water quality at one potential introduction site (Cannes de Squez), a laboratory bioassay indicated that tadpoles developed normally in water collected from this site the following year. At Ouaisné, enhancing connectivity within the site may facilitate the creation of a metapopulation of frogs. Garden ponds in proximity of the frogs' breeding habitat represent possible recipients for translocations. PVA models predicted that new *Rana dalmatina* populations created by translocations of relatively low numbers (20 - 120) of captive-bred individuals could be viable in the medium term providing predation of eggs and larvae are controlled.

ACKNOWLEDGEMENTS

Finally, I would like to thank Dominic Currie, with all my heart, for accompanying me during this amazing journey, without even complaining when we ended up catching toads, during a storm, on the night of last year's St Valentine's Day.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GENERAL INTRODUCTION

1.1. GLOBAL AMPHIBIAN DECLINES

Amphibians are diverse in colour, form, behaviour and natural history. They are found in many different ecosystems and habitats, including deserts, grasslands and forests, from sea level to high mountain tops. Most abundant in the tropics, they are also common in temperate zones and even inhabit higher latitudes, such as Alaska and northern Canada (Blaustein & Wake, 1995). As a part of the overall "biodiversity crisis" (Blaustein *et al.*, 1994a), recent reports indicate that numerous amphibian species, anurans in particular, are undergoing population declines, range reduction and even extinction (e.g. Alford *et al.*, 2001; Blaustein & Wake, 1990; Blaustein & Wake, 1995; Young *et al.*, 2001), usually as a result of human activities. Even more alarmingly, widespread amphibian declines have been recorded in "pristine" habitats (Blaustein *et al.*, 1994, Griffiths & Beebee, 1992; Sjögren, 1991). Since the First World Congress of Herpetology, held in Canterbury (UK) in 1989, scientists have started to consider amphibian declines caused by human influences as a global phenomenon (Wake, 1998). The seriousness with which the scientific community have taken this issue was reflected in the organisation of a National Research Council (NRC) sponsored workshop in the USA in 1990 and in the establishment of a special task force on declining amphibian populations (DAPTF), allied with the Species Survival Commission of the IUCN, in 1991 (Gardner, 2001). Reports of declining amphibian populations persisted at the Third World Congress of Herpetology, in 1997 (Collins & Storfer, 2003). Famous examples of species that experienced dramatic declines are the golden toad (*Bufo periglenes*) and the harlequin frog (*Atelopus varius*) (Pounds & Crump, 1994), the cascades frog (*Rana cascadae*) (Fellers & Drost, 1993), the yellow and red-legged frogs (*R. muscosa* and *R. aurora*) (Blaustein & Wake, 1990), and the midwife toad (*Alytes obstetricans*) in the Pyrenees (Márquez *et al.*, 1995). Moreover, Houlahan *et al.* (2000) analysed 936 populations of 157 species from six continents to assess large-scale temporal and spatial variations in population trends. They found that, while the large-scale trends show considerable geographical and temporal variability, amphibian populations have in fact been declining for several decades.

Habitat destruction and/or degradation have probably caused the extermination of more amphibian species than any other factors (Blaustein *et al.*, 1994a; Sala *et al.*, 2000). Construction of new roads, houses and factories has been a continuing cause of amphibian terrestrial and aquatic habitat loss for many decades (Beebee, 1997; Beebee & Corbett, 1998; Fahrig *et al.*, 1995; Green, 2003; Joly *et al.*, 2003; Vos & Chardon, 1998). Furthermore, forest management practices, which modify prevailing temperature, humidity or soil moisture regimes, may be responsible for local population extinctions (Petranka *et al.*, 1993). Other modifications of habitats, produced for example by introductions of predators (Beebee, 1997; Gillespie, 2001; Kats & Ferrer, 2003),

overstocking of ponds with ducks and other wildfowl, which can not only predate on eggs and larvae, but may cause gross eutrophication of water bodies (Beebee & Corbett, 1998), or overdeepening of the ponds, and the consequently increasing the abundances of invertebrate predators of eggs and larval stages (Loman, 2002a) are also factors that negatively influence amphibian populations. However, as mentioned above, not all recorded amphibian declines have happened in areas subjected to heavy human impacts. In fact, dramatic decreases in population sizes of amphibian species inhabiting "pristine" or otherwise legally protected or isolated habitats have been reported on several occasions (e.g. Blaustein *et al.*, 1994a; Griffiths & Beebee, 1992; Sjögren, 1991). Various other, less obvious, agents were consequently identified. (1) The increase in ultraviolet B (UV-B) radiation at the Earth's surface, caused by the depletion of the stratospheric ozone layer, causes decreases in hatching success, higher embryo and larval mortality and may also negatively affect embryo and larval development (e.g. Blaustein *et al.*, 1994a; Blaustein *et al.*, 1995; Blaustein *et al.*, 2003). (2) Pollutants, such as factory emissions or pesticides, herbicides and fertilisers, have a deleterious impact on survival rates of amphibians, as these have absorbent skins and in most cases aquatic larvae (e.g. Gunther & Plotner, 1986; Hecnar, 1995; Mahaney, 1994; Marco *et al.*, 1999). Moreover, contaminants such as pesticides and fertilisers may interact with UV-B radiation synergistically, enhancing their detrimental effects on developing amphibians (Blaustein *et al.*, 2003). Furthermore, increased acidity of ground and pond water, due to increased acidity of rainfall, is suspected to have both lethal and sub-lethal effects on amphibian populations through a number of factors, including enhanced embryo and larval mortality, reduced reproductive output, reduced adult size and alterations in geographic distribution (Gardner, 2001). (3) Recently, in a rising number of cases, spreading of diseases within amphibian populations have been reported (Berger *et al.*, 1998; Blaustein *et al.*, 2001; Bosch *et al.*, 2001; Carey *et al.*, 1999; Daszak *et al.*, 1999; Kiesecker *et al.*, 2001; Lips, 1998; Muths *et al.*, 2003; Rollins-Smith *et al.*, 2002). Most pathogens, such as *Saprolegnia* water moulds, Ranaviruses (genus *Ranavirus*, family Irodoviridae) and trematode parasites (*Ribeiroia* spp.) are currently associated with amphibian mortalities (i.e. die-offs) (Daszak *et al.*, 1999; Kiesecker *et al.*, 2001) but not necessarily with population declines. On the contrary, chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* have been isolated as the main cause of the decline of several amphibian species (Daszak *et al.*, 2003). (4) Amphibian disappearances have been in some cases correlated with climate changes (Kiesecker *et al.*, 2001; Laurance, 1996; Lips, 1998). Gradual changes in the global climate due to human activities have been influencing the population dynamics of many amphibian species in more ways than one. For instance, reduction or changes in rainfall patterns have been proved to reduce amphibian reproduction and recruitment (Lips, 1998; Pounds & Crump, 1994). Moreover,

3

amphibian reproductive cycles are sensitive to climate warming (Carey & Alexander, 2003). Climate change may also play an indirect role in facilitating epidemics and infectious disease (Kiesecker et al., 2001). However, further research is needed to demonstrate the existence of direct causal relationships between declines of amphibian populations and climate changes (Carey & Alexander, 2003).

Finally, not a single mechanism, but the interaction and synergistic effects between different agents may often be behind amphibian declines (Blaustein et al., 2003; Collins & Storfer, 2003; Gardner, 2001). For example, various stresses (man-made factors, such as toxicants in the water) make amphibians more susceptible to diseases, by altering their immune responses to pathogens (Blaustein et al., 1994b; Carey et al., 1999; Daszak et al., 2003).

1.2. REASONS FOR CONSERVING AMPHIBIANS

Many are the reasons that justify all efforts made to try to preserve amphibian populations and species. First of all, loss of amphibian populations has an important effect on the ecosystem, as they often are the most abundant vertebrates in their habitats. Adults are carnivores, whereas the larval stages are mostly herbivorous, thus, as predator and prey, they play an essential role in the trophic dynamics of ecological communities to whom they belong (Blaustein & Wake, 1995).

Moreover, amphibians are generally considered to be important indicators of global environmental health and resilience (Collins & Storfer, 2003; Diamond, 1996; Storfer, 2003). They inhabit both aquatic and terrestrial habitats and therefore are exposed to aquatic and terrestrial pollutants, to which they are particularly sensitive due to their highly permeable skins (Duellman & Trueb, 1986). They are also good monitors because they usually spend most of their lives in fairly confined regions. Ultimately, what causes declines of amphibian populations could harm our species as well (Blaustein & Wake, 1995).

Furthermore, amphibians may greatly contribute to the creation of new pharmaceutical products. Some compounds extracted from secretions of amphibians' skins have already been used, for example, as painkillers, and many more are being investigated for their antibacterial and antiviral properties (Blaustein & Wake, 1995).

Finally, as Wilson (2000) states "…each imperilled species is a masterpiece of evolution, potentially immortal except for rare chance of human choice, and its loss is a disaster".

1.3. *RANA DALMATINA* AND ITS DECLINE IN JERSEY

The agile frog, *Rana dalmatina* (Fitzinger in Bonaparte, 1838) (Fig. 1.1), is a brown frog that can be distinguished from other similar species, such as *R. temporaria* and *R. arvalis*, because of its long hind legs, its flattened snout and the closeness of its eardrums to the eyes (for a detailed review of the species key characteristics, see: Herrmann, 1986).

Figure 1.1. *Rana dalmatina* (Fitzinger in Bonaparte, 1839)

Although the agile frogs' habitat preferences can vary depending on which part of their range they inhabit, they generally live in light deciduous forest, often choosing damp shaded spots at the edge of the woods. They breed in stagnant water or in slow running brooks and ditches, with an optimal depth between 30 and 80 cm (Grossenbacher, 1997). The adults return to the water at the beginning of the reproductive season, but go back to their terrestrial habitat as soon as the breeding period ends. The males only call from underwater and their vocalisations are not very audible (Arnold & Burton, 1985). Egg clumps are attached separately to stable vertical structures (e.g. reed stalks) (Grossenbacher, 1997) and are therefore easily discernible from, for example, the spawn of *Rana temporaria*. As shown in Fig. 1.2, the agile frog has a widespread distribution in Europe, even though its abundances vary widely among locations. In most areas of southern Europe the species is very abundant. On the other hand, in central and northern Europe, its rareness and scattered distribution, together with reported cases of local extinctions, suggest it may be endangered. In most cases, fragmentation and/or loss of habitat seem to be the causes of decline (Grossenbacher, 1997).

Figure 1.2. *Rana dalmatina* distribution range (from: Grossenbacher, 1997)

The Jersey population of agile frogs, once described as "common in the west, getting rarer as one goes east" (Sinel, 1908), has been declining in both range and numbers since the 1940s (Tonge, 1986). In the late 1970s, Le Sueur (1976) reported that only seven populations were still breeding on the island and by the late 1980s only two sites (Ouaisné Common and Noirmont) supported frogs. Finally, in 1987, the population breeding in Noirmont pond was lost, following a spill of agricultural pesticide (Gibson & Freeman, 1997). Hence, at present, only one *Rana dalmatina* population breeds in the wild, at Ouaisné. Loss of breeding sites seems to be the main factor responsible for the disappearance of agile frog populations from most of the past breeding locations (Baker & Gibson, 1995), obviously with the exception of Noirmont. However, other agents, such as introductions of predators and poor water quality due to intensive agriculture, may have played a role in the dramatic decline of *Rana dalmatina* on the island (Baker & Gibson, 1995; Gibson & Freeman, 1997).

1.4. AIMS AND OBJECTIVES

A *Rana dalmatina* captive breeding, reintroduction and habitat management programme, involving various organisations [the Jersey Agile Frog Group, the Herpetology Department at the Durrell Wildlife Conservation Trust, the States of Jersey Planning and Environment Committee, Environmental Services Unit (ESU) and the Société Jersiaise)] started in Jersey in the late 1980s (Gibson & Freeman, 1997; Partridge, 1995). All parties involved recently realised that, in order to

plan effective conservation measures for the species, a clearer understanding of its ecology and of the processes driving the frogs' population dynamics in Jersey was needed. An Action Plan for the conservation of *Rana dalmatina* on the island was consequently published in 2001 (Agile Frog Group, 2001) and the present study initiated the same year. The main objectives of the present research were the following.

(1) Collect as many data as possible about the demography and the processes regulating the dynamics of the *Rana dalmatina* population currently breeding at Ouaisné, through intensive surveying of the breeding site during three consecutive reproductive seasons and by applying standardised methodologies (Chapters 2, 3 and 4).

(2) Analyse the influence of hydroperiod, predation and competition on the development of the frogs' aquatic stages, and their impact on the recruitment of metamorphs (Chapter 3 and 4).

(3) Investigate the effectiveness of placing mesh bags around the frog spawn as a technique for enhancing the frogs' reproductive success (Chapters 3 and 5).

(4) Assess population sizes and reproductive success of the common toads (*Bufo bufo*) – potential competitors to the agile frogs- at Ouaisné and at *Rana dalmatina* introduction and reintroduction sites (Chapters 3 and 7).

(5) Assess the long-term viability of the agile frog population breeding at Ouaisné, by using field data collected for (1) to create models able to predict the population's extinction risk (Chapter 5).

(6) Use a DNA-based technique, and comparisons with other European agile frog populations, to try to ascertain the level of genetic variation of the *Rana dalmatina* population breeding at Ouaisné (Chapter 6).

(7) Finally, outline practical and realistic strategies for the conservation of *Rana dalmatina* in Jersey, by analysing the results obtained during the study and by assessing the success of past management practices (Chapters 7 and 8).

7

CHAPTER 2

STUDY SITES AND GENERAL MATERIALS AND METHODS

2.1. STUDY SITES

2.1.1. INTRODUCTION

The largest of the Channel Islands, Jersey (117 km^2), situated in the Bay of Mont St Michel, is little more than 20 km from the northwest coast of Normandy (France) and 128 km from the English coast (Fig. 2.1). The island has its own government, called the States of Jersey, which comprises 53 elected members. Jersey also has its own system of local administration, fiscal and legal systems, and courts of law. Jersey is neither part of the United Kingdom nor a colony and it is not one of the members of the European Union. It is not represented in the United Kingdom Parliament, whose Acts extend to Jersey only if the island expressly agrees that they should do so. The island has allegiance to the British Crown. Jersey is highly populated, with approximately 88,000 residents and around 600,000 visitors per year, and intensively cultivated, as 54% of its area is farmland. The urban area covers 20% of the total and the rest 26% of the land is represented by semi-natural habitats (States of Jersey, 2003). The underlying geology is largely granite and shale. The overlying soils vary from areas of clay, sandy loess and alluvium with acid soils, particularly over the granite. The climate is milder than that of the British Isles with mean temperatures of 7°C in January and 18°C in August. Summers are generally warm and dry, yet with the occasional drought. Winters are usually mild but with frosts in some years. The island slopes from a height of 153 m on the north coast to 60 m above mean sea level in the south (Jones *et al.*, 1990). The level of biodiversity on the island is quite high and Jersey's geographical position partly explains the large number (33) of UK Red Data Book species supported (States of Jersey, 2003). *Lacerta bilineata* (green lizard), *Podarcis muralis* (wall lizard), *Natrix natrix* (grass snake) and *Anguis fragilis* (slow-worm) are reptiles that can be found in Jersey, whereas the only amphibian species are *Bufo bufo* (common toad), *Triturus helveticus* (palmate newt) and, finally, the agile frog, *Rana dalmatina*. At present, the only site on the island that supports a natural wild agile frog population is a nature reserve called Ouaisné Common (Agile Frog Group, 2001). Frogs are also present in Noirmont pond, a natural breeding site for all the amphibian species present on the island until the late 1980s, where they have been reintroduced since 2000. Finally, in the mid 1990s, tadpoles and froglets were introduced in Grosnez pond, that is situated within Les Landes, a designated Site of Special Interest and historic breeding area for *Rana dalmatina* (Sinel 1908).

All the fieldwork undertaken to collect data for the present study was carried out at the three sites mentioned above, and shown in Fig. 2.2, and the main characteristics of these sites will be described. Finally, as the Jersey *Rana dalmatina* captive rearing programme was also regularly

monitored as part of this project, details about the main captive sites, which are two enclosures, one situated in the Jersey Zoo and the other in a private garden, will be given.
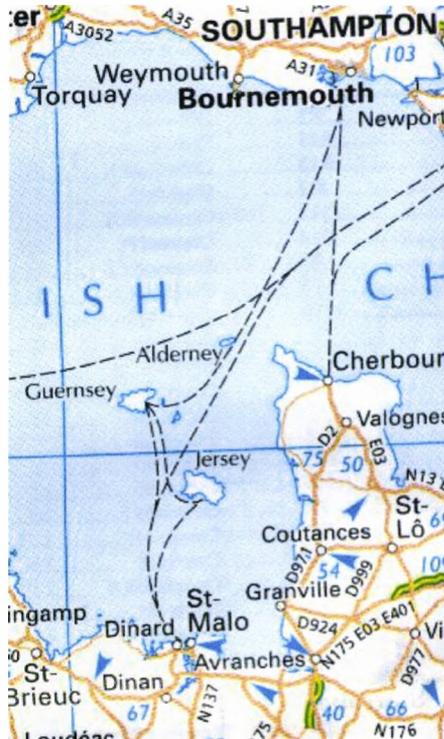
Figure 2.1. Position of Jersey in the English Channel.
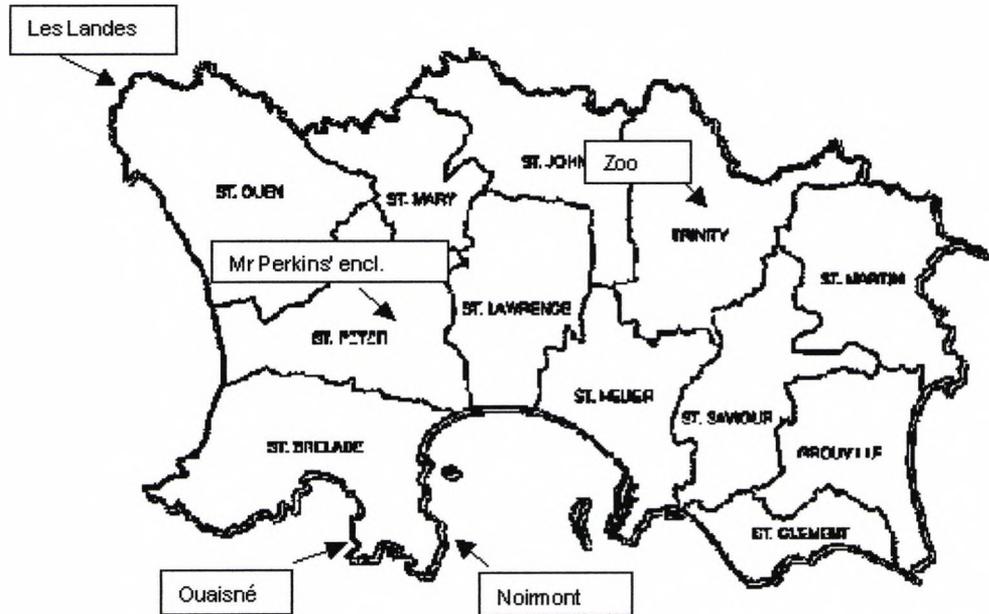
Figure 2.2. Location of the study sites on the island of Jersey.

2.1.2. AMPHIBIAN BREEDING SITES

OUAISNÉ COMMON

Ouaisné Common, situated on the southwest coast, is, despite its relatively small size (10 hectares), one of Jersey's richest and most diverse nature reserves. It presents a variety of habitats, such as wetland areas, blocks of gorse (*Ulex* spp.), dwarf shrub heath, grassland and areas of open sand. The Common is now separated from the beach by a coastal protection wall, built by the Germans when they occupied Jersey during the Second World War. The presence of the wall has played a major role in stabilising the dunes by interrupting the flow of sand being blown from the beach onto the Common and such stabilisation encourages scrub to grow, overshadowing lower growing species. The ESU, department of the States of Jersey, is therefore currently managing the Ouaisné area by controlling the growth of scrub and other invasive species. From time to time, surveys are also carried out at the site and a recent study showed that rabbits (*Oryctolagus cuniculus*) are the most common mammals. Lesser white toothed shrews (*Crocidura suaveolens*), common shrews (*Sorex araneus*), bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*) have also been recorded at the site. Birds that breed in the area are Dartford warblers (*Sylvia undata*), stonechats (*Saxicola torquata*), serins (*Serinus serinus*) and whitethroats (*Sylvia communis*). On the southern edge of the site, a large pond and reed bed hosts a large number of waterfowl, and moorhens (*Gallinula chloropus*) have been seen nesting in the middle of the reed bed. Other birds observed at Ouaisné are snipe (*Gallinago gallinago*), woodcock (*Skolopax rusticola*), skylarks (*Alauda arvensis*), meadow pipits (*Anthus pratensis*) and herons (*Ardea cinerea* and *A. purpurea*) (Le Sueur, 1976; States of Jersey, 2003). All the amphibian and reptile species that can be found in Jersey live and breed at the site. The agile frogs, which are absent from the main pond mentioned above, breed in dune slacks situated in the centre of the Common (Fig. 2.3) and their two main breeding ponds are called North and South Slack (Fig. 2.4).

11
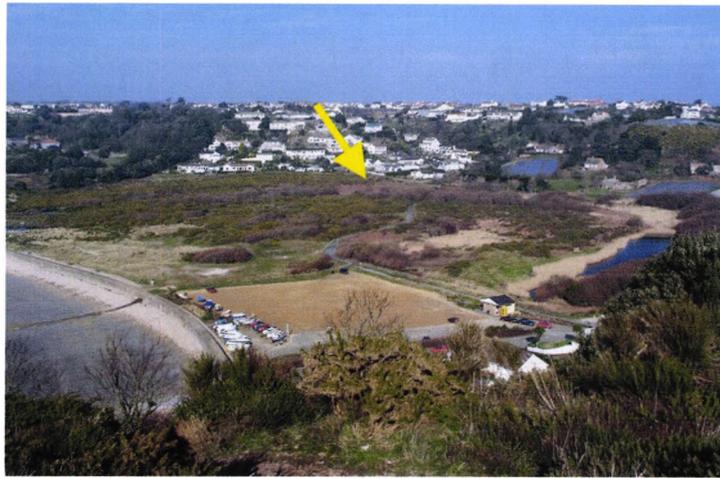
Figure 2.3. The yellow arrow indicates the approximate location of North and South Slack

Figure 2.4. North Slack (above) and South Slack (below).

The vegetation around the ponds mainly consists of willow trees (*Salix* spp.) and gorse (*Ulex* spp.). Marsh pennywort (*Hydrocotyle vulgaris*) and water mint (*Mentha aquatica*) are the most abundant aquatic plants in both water bodies. Many species of insects live in and outside the ponds, the most noticeable being many species of crickets, damselflies, dragonflies, leaf and grasshoppers and plenty of different species of butterflies. Palmate newts and - in some years - common toads also breed in the slacks. Grass snakes, green lizards and slow-worms have been observed in the area surrounding the water bodies, together with many species of birds. The size and depth of North and South Slack vary with the seasons, as they reach their largest size at the end of the winter, gradually desiccating during the spring and are reduced to big puddles by the end of the summer. Both water bodies dry completely during exceptionally dry years. However, this has not happened before the emergence of amphibian metamorphs since the ponds were deepened by the ESU, several years ago (Partridge, 1995). During particularly wet winters, the area around the slacks is flooded and two more ponds are clearly discernible, one, called Small Temporary Pond, just behind the south edge of the South Slack and often connected to it by a narrow corridor of water. The other, the Sump, is on the west side of North Slack, separated from it by thick bushes and gorse. The former usually dries completely by the end of March, whereas the latter holds water approximately until the end of May, depending on the amount of rainfall during the spring. Finally, in September 2001, the ESU team started clearing three more areas of Ouaisné Common, in order to create new habitats for the amphibian and reptile species present at the site. In total, five new slacks have been dug so far, and some more work, aiming to deepen all these new water bodies has been planned and will be completed before the beginning of the 2004 amphibian breeding season.

At Ouaisné, North Slack, South Slack and the area around them were intensely surveyed during the three years of fieldwork undertaken for the present study. However, the rest of the Common was also regularly visited.

NOIRMONT

Noirmont covers an area of approximately 35 hectares and is situated on the southwest coast of Jersey. The site, managed and administered by the Public Services Committee, is characterised by a similar variety of habitats, including a large pond, and those species described for Ouaisné Common, that is located nearby, as shown in Fig. 2.2 (States of Jersey, 2003).

Figure 2.5. Noirmont pond.

The pond (Fig. 2.5), which is a historically important breeding site for *Rana dalmatina*, *Bufo bufo* and *Triturus helveticus*, was heavily polluted in 1986 when a significant quantity of aldicarb, an agricultural pesticide, spilled from a field situated on the boundary of the water body, killing all animals living in it (Gibson & Freeman, 1997). Later on, in 2000, four toad egg strings were introduced by the ESU, their growth monitored and the emergence of metamorphs noted (Racca, 2000). At the same time, monitoring of the water quality showed that there was no residual pollution in the pond and therefore the Agile Frog Group considered the site suitable for the reintroduction of *Rana dalmatina*. A total of 239 tadpoles and 5 froglets, all reared in captive sites, were therefore released in the pond in 2000 (Racca, 2000). The reintroduction programme continued in 2001, when 64 tadpoles and 66 frog metamorphs were released, and in 2003, with the release of 60 tadpoles and 110 froglets. In all cases, the *Rana dalmatina* individuals were taken from the captive populations. In 2002, no spawning occurred in the captive populations, so no tadpoles or froglets were introduced in the pond.

In order to monitor the effects of such reintroductions, Noirmont pond was chosen as one of the study sites and was therefore intensively surveyed from 2001 to 2003.

LES LANDES

Les Landes was designated a Site of Special Interest (SSI) in 1996 as Jersey's largest expanse of maritime heathland (States of Jersey, 2003). Located on the northwest coast, it covers an area of 160 hectares, bounded by 3 km of rugged granite cliffs. Over 200 species of heathland plants, such as heath pearlwort (*Sagina subulata*), spotted rock-rose (*Tuberaria guttata*), allseed (*Radiola linoides*), sand crocus (*Romulea columnae*), dodder (*Cuscuta epithymum*), crossed-leaved

heath (*Erica tetralix*) and lesser skullcap (*Scutellaria minor*) grow in the area. Ravens (*Corvus corax*), peregrine falcons (*Falco peregrinus*), stonechats (*Saxicola torquata*) and Dartford warblers (*Sylvia undata*) are birds that have been recorded at this site. Green lizards and slow-worms inhabit Les Landes. Common toads and palmate newts breed in its wetland, an area called Cannes de Squez, and in a pond at Grosnez (Fig. 2.6), an area situated in the western part of Les Landes. An important site for grazing by sheep and cattle in the past, it is now managed by the ESU. Their activities include bracken control, gorse coppicing (to maintain bird habitats), species monitoring and site patrolling (States of Jersey, 2003). In 1994, the pond situated at Grosnez was chosen by the Agile Frog Group as *Rana dalmatina* introduction site (Gibson & Freeman, 1997). Although it had never supported agile frog populations before, it was known to be a successful breeding site for toads and newts. Moreover, water quality monitoring indicated that there was little, if any, pollution at the site. In 1994, a chestnut paling fence was erected around the pond (Fig. 2.6), to restrict access to people and their pets and a sign informed visitors to the area about the purpose of the fence. Then, 200 frog tadpoles, derived from *Rana dalmatina* captive rearing sites, were released in the pond. In spring 1996, a single clump of spawn was found at the site, and, since it was being heavily predated by *Triturus helveticus*, it was moved to the Zoo for hatching and initial rearing. Later that year, 100 large tadpoles and 20 froglets, developed from the clump found *in situ*, were released in the pond (Gibson & Freeman, 1997). In 1998 and 1999, breeding occurred again and one clump of eggs was laid each year. They were both enclosed in protective mesh boxes to prevent newt predation. Unfortunately, the presence of the boxes seemed to harm the eggs and the spawn did not develop. In 1998, 16 captive reared frogs, from the Zoo enclosure (Fig. 2.7), were released in the pond. The year after, another 25 froglets and 11 large tadpoles, again from the Zoo's spawn, were introduced (Agile Frog Group, 2001). Finally, in 2000, two clumps of frog eggs were found in Grosnez pond. They were immediately moved to the Zoo, where they were successfully reared in an outdoor tank. Later that year, 116 large tadpoles were returned to the pond.

As part of the fieldwork undertaken for the present research, Grosnez pond was intensively surveyed in 2001 and 2002, with the aim of verifying the success of such introductions of *Rana dalmatina*. The wetland at Cannes de Squez was also regularly visited. In 2003, since no frog activity had been recorded in the two previous years, the visits to both sites were only sporadic.

Figure 2.6. Grosnez pond (left) and main water body at Cannes de Squez (right).

2.1.3. CAPTIVE FROG POPULATIONS

ZOO ENCLOSURE

In 1993, an enclosure was built for agile frogs at the Durrell Wildlife Conservation Trust (Jersey Zoo), with funds provided by the Jersey Ecology Trust Fund (Gibson & Freeman, 1997). Within the existing tortoise paddocks, beside the Herpetology Department, an area measuring approximately 20 m² was enclosed with a 1 m high glass wall which had a 15 cm horizontal lip on the side of the enclosure to prevent escapes. The area inside was landscaped with low growing plants, grasses, logs and roof tiles and a 2 m² pond with a maximum depth of 50 cm was dug. The pond was filled to about half its depth with hard core and top soil and a variety of emergent and non-emergent plants were added. The entire enclosure was covered with nylon mesh, to prevent predation by gulls and other birds. Individuals of *Rana dalmatina* were introduced for the first time in 1993 and then again over the next few years (Partridge, 1995). The first spawn was found in 1995 (Gibson & Freeman, 1997) and since then breeding has occurred nearly every year. In 2001, the enclosure was cleaned and completely renovated (Fig. 2.7), but its main characteristics were maintained.

Figure 2.7. *Rana dalmatina* enclosure at Jersey Zoo.

MR PERKINS' ENCLOSURE

In 1994, following a decision taken by the Agile Frog Group (Gibson & Freeman, 1997), a little pond, with an area of approximately $1m^2$ and a 50 cm maximum depth, was dug in the garden in front of the house of Mr Perkins, one of the Agile Frog Group members. An enclosure, with a 2 m high timber framework, covered with netlon, and with a 50 cm high corrugated plastic panels at the bottom, was constructed around the pond. This way, predation by cats, birds and other animals was prevented and, at the same time, the frogs could not escape. Logs and flat stones were placed into the enclosure and aquatic vegetation was added to the pond. Young and adult frogs were introduced in 1994 and in successive years, and soon breeding started to occur quite regularly (Partridge, 1995).

In February 2002, Mr Perkins reported that rats had breached the enclosure, and poison was used to eliminate them. He succeeded in the removal of the rats and, on 15th May, the rangers from the ESU replaced the old enclosure with a new one, more effective in keeping rats and other unwanted animals away from the frogs (Fig. 2.8). The number of animals living in the old enclosure was not known. When it was dismantled, five male and two female frogs were counted (see sections 2.2.2. and 3.2.2.).

Figure 2.8. New enclosure for agile frogs in Mr Perkins' garden.

OTHER CAPTIVE BREEDING SITES

One more pond where frog breeding usually occurs every year is located in another private garden. The owner, Mr Bob Burrow, who is a former member of the Agile Frog Group, reported that various clumps of eggs were laid in 2001 and 2003 and that the resulting metamorphs were later released in Noirmont pond.

The enclosure at the ESU's Frances Le Sueur Centre, where in 2000 an agile frog introduction started (Racca, 2000), was completely under water in 2001, because the compound was heavily flooded during the winter. No frog activity was recorded during any of the three years' fieldwork.

The following places, that have been captive breeding sites in past years, were visited at least once during each year fieldwork period:

- Samarès Manor (two ponds in two enclosures)
- Grainville School (one pond)
- Grosnez House (three garden ponds)
- Mrs Norma Hind's garden (two ponds in two enclosures)

None of these sites appeared to support agile frogs anymore.

2.2. MATERIALS AND METHODS

2.2.1. AMPHIBIAN BREEDING SITES

CAPTURE OF ADULTS

Every year, night surveys were carried out at Ouaisné, in order to catch *Rana dalmatina* and *Bufo bufo* adults. In 2001 and 2002, the water body at Grosnez was also occasionally visited at night, whereas in 2003, due to unavailability of surveyors, this did not happen.

During all three years, surveys were carried out every night for the duration of the anurans' breeding season, from 22.00 hrs to 24.00 hrs. During each survey, the surveyor (who was usually me or one of the ESU rangers) completed a survey form (see Appendix I), with the help of a map of the site. On each occasion, air temperature, weather conditions (Tab. 2.1), maximum, minimum, current water temperature and soil temperature were measured. Each pond was circuited once, in a clockwise direction, walking slowly and shining a torch (B&Q Multifunction Compact Lantern 4.8 V, model JM L8856) around the edges and inside the water. The presence of any amphibians was noted. The location of any vocalisations of frogs and toads was recorded. Attempts to catch all the frogs and toads observed were always made, at Ouaisné, when I was the surveyor, with the help of a dip-net when they were in the water. At Grosnez, toads occurred in very high numbers on the road that leads to the pond and in the pond itself. These animals were counted and as many individuals as possible were captured. Once frogs or toads were caught, their sex was identified. Then, the mass of the frogs was measured, to the nearest 0.25 g, using a 30 g Pesola spring balance, whereas the toads were weighed to the nearest 1 g, using a 100 g scale. The body length (from snout to urostyle) of all anurans was also measured, to the nearest 0.1 mm, using a 30 cm ruler. When in amplexus, both animals were marked and the total mass was measured.

Code	Sky	Wind
0	clear or a few clouds	
1	party cloudy or variable	smoke rises vertically
2	cloudy (broken) or overcast	wind direction shown by smoke drift
3	fog	wind felt on face; leaves rustle
4	drizzle	leaves or small twigs in constant motion; wind extends light flag
5	showers	raises dust and loose paper; small branches are moved

Table 2.1. Codes used to record the weather condition (from: Heyer *et al.*, 1994).

Trovan PIT tags (Heyer *et al.*, 1994) were used to mark all the adult frogs caught during each survey. In 2002 and 2003, each animal was also toe-clipped (Heyer *et al.*, 1994) and the toes

individually preserved in methylated spirit. When marked individuals were recaptured, their measurements were retaken and recorded. Then, all the animals were released at the same place where they had been found. All adult toads caught were marked using the Panjet dye-marking technique, as described by Wisniewski *et al.* (1979), and a different code was chosen every year. When in pairs, both animals were marked and the total mass was measured. Once the anurans started to spawn, the location of each new clump or string of eggs was recorded. The morning after, the number of eggs in each clump was estimated (see "Before hatching" section, below), as well as the percentage of dead eggs and occurrence of fungal infection. The number of new toad egg strings, the overall percentage of dead eggs and the presence of fungal infection were also noted. No night surveys were carried out at Noirmont pond, because no surveyors were available.

MONITORING OF EGGS AND CAPTURE OF TADPOLES AND METAMORPHS

Once the anurans started to lay eggs, day surveys were carried out every 2 – 3 days at all sites in 2001 and 2002, and at Ouaisné in 2003. During each survey, the following variables were recorded:

- air temperature at time of visit
- weather conditions (Tab. 2.1)
- maximum, minimum and current water temperature at about 20 cm depth
- soil temperature at 20 cm depth
- water level

The thermometers in the water were reset every week, in order to record max and min temperature of the week.

The other activities during the surveys varied, depending on the phase of development of the spawn. The same methods were applied to follow the growth of frog and toad spawn, unless otherwise stated.

Before hatching

Every time a new clump or string of eggs was found, its location was recorded. Depth of the water where each frog spawn clump was laid and depth of the clump from the surface were noted. Then, approximately one tenth of the eggs in each clump were counted, and the total number of eggs was estimated by multiplying by ten. This operation, that did not require moving the clumps from where they had been laid, was carried out by at least two independent observers. When there was disagreement, the counting was repeated until agreement was reached. The percentage of live eggs was also recorded. In order to avoid predation by ducks, which were often seen in the area, all the frog clumps were protected by constructing corrals around them using willow twigs, or by placing a willow sapling over them. In 2002 and 2003, all the frog eggs were

enclosed in "bags" made with 1 mm gauge mesh that prevented newt predation (Fig. 2.9). Then, during each survey, frog and toad eggs were examined with a magnifying lens and the stage of the embryos recorded, using a staging table (taken from Gosner, 1960). It was also checked if the percentage of live eggs varied during each stage of the development.

Figure 2.9. Clump of frog eggs in its protective mesh bag.

Hatching

For each clump or string, the date the hatching started, when it finished and the percentage of dead eggs before and after hatching were recorded. The stage at which the embryos started hatching was also noted.

Tadpoles

Netting sessions, using a dip-net (mesh: 1 mm, bag: 0.5 m, handle: 1.48 m), were performed every two weeks to catch frog and toad tadpoles. The shallower areas of each slack were swept in segments (Fig. 2.10), moving around each pond in a clockwise direction. Each segment was swept once, then the contents of the dip net were checked and, when present, the tadpoles placed into buckets filled with pond water. This was repeated for all the segments. All the tadpoles caught were then counted and their total length, body length and tail length were measured to the nearest 0.02 mm using 15 cm long steel dial callipers. Their stage of development was also determined, using a staging table (Gosner, 1960). They were then released. In 2002 and 2003, once the tadpoles had their hind legs well developed, netting sessions were performed for three consecutive days in North and South Slack, at Ouaisné, and mark – recapture of tadpoles was carried out. During the first day, all the tadpoles were anaesthetised with MS222 (Heyer *et al.*, 1994), then measured, staged and marked by cutting the tip of their tail.
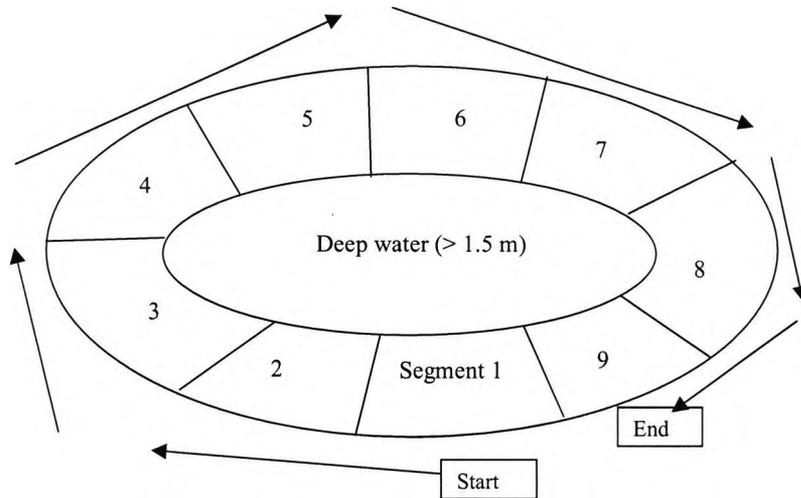
Figure 2.10. Diagram illustrating the areas (segments) of the pond swept during the tadpole netting sessions.

In 2001, the tips were kept and individually placed in small plastic tubes containing absolute ethanol. On the second day, all the tadpoles caught were checked and the ones that still had their tail tip were anaesthetised, measured, staged and marked, while the ones that had already been marked were counted and then released. Finally, during the third day, the number of recaptured and unmarked tadpoles was recorded.

Every time invertebrates were found in the net, they were identified and recorded. The number of newts, adult and larvae, caught during the netting was also recorded.

Metamorphs

At Ouaisné, drift fences and pitfall traps (Heyer *et al.*, 1994) were used to try to catch frog and toad metamorphs as soon as they started emerging from the water. The drift fences were made with 1.5 m high plastic sheets supported by wooden poles. On the side facing the edges of the ponds, pitfall traps were placed, approximately one every 2 metres. They were 25 cm high plastic shrub tubs, positioned in holes dug in the ground, covered by lids which had a circular opening ($\emptyset$= 6 cm), in order to stop the froglets from jumping out. In 2001, the fences encircled the ponds only partially, whereas in the two next years both water bodies were completely enclosed by them (Fig. 2.11). The pitfall traps were checked every morning. They were completely closed when not in use (Chapter 3). In 2001, all the froglets caught in the traps or found walking along the fences were marked with waterproof ink scratched with the needle of a microfine syringe (1 mL, 0.33 x 13 mm) on the skin of one of their back legs or of their throat. In 2002 and 2003 marking was not necessary, as all the metamorphs captured in the traps and along the side of the fence facing the

22

water were released on the other side of the fence, and therefore never caught again. Before being released, both froglets and toadlets had their body length (from snout to urostyle) measured to the nearest 0.1 mm, using a 30 cm rule. They were then weighed to the nearest 0.1 g, using a 5 g Pesola balance. Their colour and the location where they had been found (the number of the trap where they had been captured or the numbers of the traps either side of where they had been captured, if found along the fences) were also recorded. At Grosnez, Cannes de Squez and Noirmont ponds, no standardised methods were used to catch the metamorphs. These sites were regularly visited in 2001 and 2002 and any sightings of froglets and toadlets recorded. In 2003, such surveys were carried out only at Noirmont.

Figure 2.11. Drift fence along the south edge of North Slack in 2001 (above) and encircling the pond completely in 2002 and 2003 (below).

AQUATIC PREDATORS OF **RANA DALMATINA** *AT OUAISNÉ*

During a preliminary survey at Ouaisné (Racca, 2000), the potential aquatic predators of *Rana dalmatina* tadpoles were identified as being palmate newts (*Triturus helveticus*) and various species of macroinvertebrates, such as water beetles, backswimmers, Odonata nymphs and water spiders. Therefore, during all three years, data on the presence and relative abundance of these predators were collected in both slacks at Ouaisné.

Palmate newts

Adult and larval palmate newts were caught in the water during the spring using funnel traps (Griffiths & Langton, 1998). Each trap is made from a plastic drink bottle. The bottle is cut into two sections, so that the top half forms the funnel and the bottom half the receptacle. The top half is inverted and fitted into the bottom half to form the trap (Fig. 2.12). During each trapping session, the traps were submerged around the edge of North and South Slack, one every two metres, in the evening, and were removed early the next morning. Once the newts were caught, their snout-vent length (SVL) and tail length were measured to the nearest 0.1 mm, using a 30 cm rule, as well as their mass, to the nearest 0.1 g, using a 10 g Pesola balance. Prior to release, they were toe–clipped (Heyer *et al.*, 1994), using the same code for all individuals, but different at each marking session. The toes were preserved in individual plastic tubes filled with 70% alcohol.
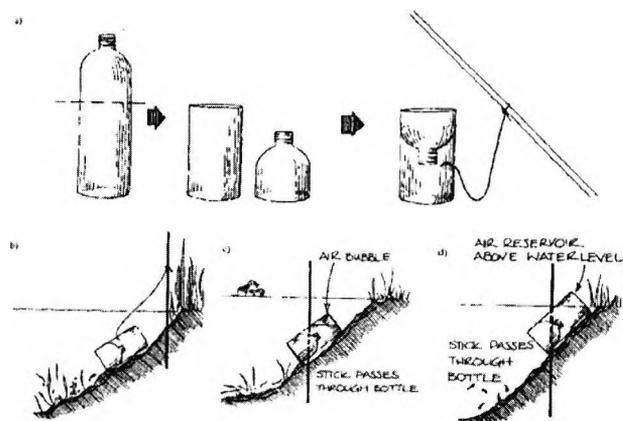
Figure 2.12. Funnel trap (from: Griffiths & Langton, 1998).

In 2002 and 2003, newt metamorphs were caught in the pitfall traps used to catch froglets and toadlets, when emerging from the ponds to disperse in the surrounding area. They were counted and the location where they were found noted. Finally, they were released on the opposite side of the fences.

Aquatic macroinvertebrates

Monthly standardised netting sessions, using a dip-net (mesh: 1 mm, bag: 0.5 m, handle: 1.48 m), were performed, in order to catch and identify the macroinvertebrates present in North and South Slack. In each pond, areas of different depth were identified and measured. During each session, in each of these areas a standard volume of water ("sweeping volume unit") was swept once (see section 3.2.). All macroinvertebrates caught during the netting sessions were put in jars containing 70% ethanol for later identification. Identification of the different taxa followed standard guidebooks (Clegg, 1980; Croft, 1986; Manuel & Shields, 1992).

TERRESTRIAL PREDATORS OF WILD RANA DALMATINA AT OUAISNÉ

Grass snakes (*Natrix natrix*) live and breed at Ouaisné and are probably the main terrestrial predators of agile frogs there. Therefore, they were always recorded if seen. Cats, dogs and birds can also predate on metamorphs, as well as adult frogs, hence all sightings of these animals in the area surrounding the breeding sites were recorded during the three years of fieldwork.

2.2.2. CAPTIVE FROG POPULATIONS

JERSEY ZOO ENCLOSURE

In 2001 and 2003, breeding occurred in the pond inside the *Rana dalmatina* enclosure, situated opposite the Zoo's Herpetology Department. The egg clumps were immediately moved to an outdoor aquarium tank (155 x 38 x 48 cm) containing aged tap water and some aquatic vegetation (Fig. 2.13), to prevent them being eaten by the numerous palmate newts present in the pond. Then, the growth of the eggs was monitored looking at them with a magnifying lens and recording the stage of development of the embryos, using Gosner's table (1960). It was also checked if the percentage of live eggs varied during each stage of the development. Dates of hatching and the percentage of dead eggs before and after hatching were recorded. The stage of development of the hatchlings was also determined. Every two or three weeks, samples of 10 tadpoles were taken from the tank and they were staged. Their total length, tail and body length were also measured to the nearest 0.02 mm, using 15 cm long steel dial callipers. During each survey, air temperature and weather conditions were recorded, as described above. A thermometer was left in the tank for the duration of the fieldwork and current, maximum and minimum water temperature were monitored each week. Once all the tadpoles had completed metamorphosis, they were taken to Noirmont and released in the pond.

Figure 2.13. Aquarium tank (155 x 38 x 48 cm) placed outside of the Herpetology Department at the Jersey Zoo, where the frog egg clumps found in the Zoo enclosure in 2001 and 2003 were reared.

MR KEN PERKINS' ENCLOSURE

In 2001 and 2003, breeding occurred in the pond inside the enclosure situated in Mr Ken Perkins' garden pond. The clumps of frog eggs were moved to an indoor tank in 2001 and to an outdoor aquarium in 2003, where they remained until they were close to the metamorphosis. The development of the spawn was regularly monitored until froglets started to appear. They were then moved to Noirmont pond.

In 2001, following a decision made by the Agile Frog Group, young agile frogs were swapped between the Zoo and Ken Perkins, in order to try to maintain genetic diversity in both captive frog populations. Five immatures were caught in Mr Ken Perkins' enclosure and taken to the enclosure situated at the Zoo. Then, five one-year old frogs, from year 2000's spawn, reared in an indoor tank in the Herpetology Department, were marked with the Panjet technique and moved to Ken Perkins' enclosure.

In February 2002, during the dismantling of the old enclosure, all the frogs found were measured, weighed, PIT-tagged and toe-clipped, before being released in the new enclosure.

CHAPTER 3

POPULATION SURVEYS

The last remaining wild population of agile frogs on Jersey was intensively surveyed from 2001 to 2003, and demographic and ecological data were collected and analysed. Relatively low rates of embryonic and tadpole mortality were reflected by relatively high recruitment rates (survival of eggs from hatching to metamorphosis varied from 2.4% to 17.1% per year). Predation pressures on the terrestrial phase appeared to be quite low. Since recruitment failures have been recorded at Ouaisné in the past, and most of the frogs caught at the site over the three-year period were 1 or 2 years old, it appears that years of high reproductive success are interspersed with years of poor reproductive success. Mark-recapture analysis indicated that adult population size varied between years, but was male-biased with less than 50 males and less than 20 females. Individual frogs recaptured between years increased in mass and length but not in body condition. Surveys carried out at two agile frog reintroduction and introduction sites - Noirmont and Grosnez ponds - highlighted the suitability of the former, but not of the latter. Finally, the agile frog captive breeding programme appeared to be fairly successful, as both captive sites experienced relatively high breeding success two years out of three. In conclusion, the risk of the agile frogs disappearing from the wild as a result, for example, of an environmental accident at Ouaisné, makes the creation of new breeding sites where tadpoles and froglets can be introduced a high priority conservation measure to be undertaken to try to assure a long-term presence of these amphibians on the island.

3.1. INTRODUCTION

The main aim of the present research was to shed light on the dynamics that regulate the last remaining wild population of agile frogs in Jersey. An understanding of population dynamics is necessary when trying to assess, for example, how environmental changes affect the population, or to predict when the population is threatened or endangered with extinction. More generally, such knowledge is essential in creating any long-term plan for the management of a habitat and the conservation of the species living in it (Caughley & Gunn, 1996). In many cases, detailed studies investigating the ecology of a population have highlighted its endangered status, often also identifying possible causes for it, and provided the basis for recovery plans (see, for example: Berglind, 2000; Gittins, 1983; Lips, 1998; Ritcher *et al.*, 2003). The ecology of the natterjack toad (*Bufo calamita*), for instance, an endangered species in Britain since 1975 (Denton *et al.*, 1997), was studied for 25 years. The results of such extensive research provided the foundations for the intensive recovery plan, based on habitat management and reintroductions in restored sites, that has prevented the species from disappearing (Denton *et al.*, 1997). The local case of the red squirrel (*Sciurus vulgaris*), inhabiting the small, fragmented and often urban woodlands in Jersey, is another example. In 1994, the States of Jersey commissioned a study into the ecology and dynamics of the local population of red squirrels, aiming to plan its future conservation, as this species is considered vulnerable in Jersey (Environmental Services Unit, 2003). Consequently, the major threats to the squirrels' long-term viability were identified as fragmentation and loss of habitat, as well as mortality from cats and cars. The importance of supplementary food was also highlighted. The ESU subsequently put into practice the recommendations given once the study was completed. Twenty thousand hedgerow trees were planted, feeding devices were developed and distributed and, to try to reduce the road mortality, warning signs and rope bridges were placed in various locations across the island (Environmental Services Unit, 2003). Many other case studies have been reported in the literature (e.g. Coulson *et al.*, 1999; Regan *et al.*, 2003; Walpole *et al.*, 2001), some internationally renowned for their success, like the recovery of the Mauritius kestrel, *Falcus punctatus* (Cade & Jones, 1993) and the Mallorcan midwife toad, *Alytes muletensis* (Buley & Garcia, 1997).

In light of this, broadening the knowledge of the ecology and dynamics of the only *Rana dalmatina* population breeding in the wild in Jersey appears to be of great relevance. Knowing the rate of growth of the frog population breeding at Ouaisné and its structure, together with the factors affecting it, is probably the primary tool that can allow an assessment of the health of the population. This, in turn, can assist in planning the management of Ouaisné Common, as well as any translocation strategy to reduce the risk of the frogs disappearing from the island as a result of,

28

for example, an environmental accident at Ouaisné. The surveys undertaken over a three-year period, particularly those at Ouaisné Common, were therefore planned and carried out so that as many aspects of the ecology of *Rana dalmatina* as possible could be investigated. As most of the information available at the beginning of the study was fragmented, sometimes anecdotal, data that would allow the determination of the size and age structure of the population of frogs, their reproductive success, the level of intra- and inter-specific competition and predation, as well as their effects on the annual recruitment of juveniles, were collected. At the same time, the degree of success of past introductions and reintroductions of agile frogs at a new site (Grosnez), and at historical breeding pond (Noirmont), was also measured. Finally, an analysis of the data was performed and the results obtained for each year compared, so that a clearer picture of the frog population dynamics could emerge.

3.2. MATERIALS AND METHODS

3.2.1. CAPTURE OF ADULTS

At Ouaisné, the night surveys started on 3rd, 13th and 18th February in 2001, 2002 and 2003 respectively. These dates corresponded to the times when both slacks had completely thawed (2001 and 2003) or filled up with water (2002). Surveys ended after no anurans were observed at the site for at least six consecutive days (25th March 2001, 30th March in 2002 and 20th March in 2003). No standardised night surveys were undertaken at Cannes de Squez, whereas Grosnez pond was visited every other night from 4th February to 8th March in 2001 and on four occasions in 2002 (6th and 26th February and 3rd and 10th March). In 2002 and 2003, all adult frogs caught during the surveys had the second toe of their hind right leg clipped. Later on, the toes, preserved in 70% ethanol, were used for ageing all *Rana dalmatina* individuals with the skeletochronology technique, as described further on in this Chapter (see next section). Finally, *Bufo bufo* adults were marked with the Panjet dye technique, using code 8 in 2001 and code 4 in 2002 (Wisniewski *et al.*, 1979), at both Ouaisné and Grosnez.

3.2.2. AGE DETERMINATION OF RANA DALMATINA ADULTS BY SKELETOCHRONOLOGY

Skeletochronology is a technique based on the observation that skeletal growth is often an annual cyclical phenomenon, laying down lines of arrested growth (LAGs) in the bone tissue that allows the estimation of individual age (Castanet & Smirina, 1990; Castanet *et al.*, 1996; Measey, 2001). At the present time, it is considered to be one of the most appropriate methods for determining individual age in amphibians (i.e. Guarino *et al.*, 1995; Halliday & Verrell, 1988; Measey, 2001). In October and November 2002, and again in September 2003, in the laboratories of the "Dipartimento di Biologia Animale e dell'Uomo" at the University of Turin (Italy),

29

skeletochronology was employed to age Jersey individuals of *Rana dalmatina*, using the clipped toes of wild adult frogs caught during night surveys performed in 2002 and 2003, and those of captive frogs caught in Mr Perkins' enclosure (see Chapter 2).

In 2002, a total of 52 adult frogs (45 in the wild and 7 in captivity) were caught and had the first two phalanges of one of their toes clipped. The second phalange of each clipped toe was used for the skeletochronology. For one of the wild frogs (code 0006212E6F) only the short first phalange was available, because a too small portion of the toe was cut, therefore, that year, the determination of its age was not possible. Luckily, the same frog was recaptured the next year and it was toe clipped again, therefore, in 2003, its age could be determined. In 2003, a total of eight new frogs were toe clipped. Moreover, when a female frog was found dead in Noirmont pond, her complete right hind leg was preserved in alcohol, so that not only the second phalange of one of the toes, but also the femur, could be analysed with the skeletochronology, to confirm that age determination using phalanges corresponds to those obtained from long bones. Each bone used in this study was cleaned, decalcified in 3% nitric acid for 10 minutes and cross-sectioned using a cabinet cryostat. The 16 μ sections were stained with Erlich's haematoxylin for at least 15 minutes and then placed on polysinate slides. Coverslips were mounted using Aquamount. The histological sections were finally analysed under a microscope and the lines of arrested growth (LAGs) counted. For the wild frogs, it was always assumed that a LAG was present along the outer border of each section, even when it was not visible, because, as the animals were all caught at the beginning or during the breeding season, they had already lived for at least one winter. The reason why the most peripheral line was not usually visible is that the bone had just started growing again after the period spent in hibernation, therefore the portion of new periosteal bone was still absent or very small. This assumption was not made for the captive frogs, because they were toe-clipped in May, so their bones had surely grown since the end of the winter and a zone of periosteal bone had already been formed between the last LAG and the outer border of the sections. Finally, as the sections tended to discolour quite quickly (after 2 or 3 months since they were stained), they were all digitally photographed.

3.2.3. MONITORING OF EGGS AND CAPTURE OF TADPOLES AND METAMORPHS

At Ouaisné, in 2001, day surveys were carried out three times a week from 3rd February to 31st May and every day from 1st June to 8th July and from 16th to 22nd August. In 2002, the site was visited three times a week from 18th February to 13th June, every day from 14th June to 12th July and every two days from 31st July to 20th August. Finally, in 2003, surveys were carried out from 13th February to 30th April and from 28th May to 9th June every two or three days and every day from 10th June to 8th August.

Mark-recapture sessions were performed on frog tadpoles in 2002 on 16th, 17th, 18th May in North Slack and 22nd, 23rd, 24th May in South Slack. The next year, they were carried out on 28th, 29th, 30th May in North Slack and 29th, 30th May and 2nd June in South Slack.

Finally, during all three years of fieldwork, drift fences and pitfall traps were used to collect metamorphs. In 2001, two drift fences, constructed as described in Chapter 2, were put along the northern and the southern edge of North Slack. The first was 3.5 m long and the latter 10 m long. Two drift fences were also built around the northern and western sides of South Slack, and they were respectively 3.5 m and 4.5 m long. Along them, 14 pitfall traps were placed, one every two metres, facing the edges of the water bodies. In 2002 and 2003, two separate systems of fences, one completely encircling the North Slack, the second the South Slack, were erected. Along them, on the side facing the water, 11 pitfall traps were placed around the North Slack and 16 around the South Slack, approximately one every two metres and more or less in the same places from one year to the next. Fences and traps were left at the site from 1st June to 22nd August in 2001, from 14th June to 20th August in 2002 and from 10th June to 8th August the next year.

As described in Chapter 2, day surveys were also undertaken during all three years at Noirmont and just in 2001 and 2002 at Grosnez and Cannes de Squez. The first site was visited from mid February to the end of June, once a week in 2001 and 2002, and once every fortnight in 2003. The wetland at Cannes de Squez and Grosnez pond were surveyed twice a week, from the end of January to the end of June. Finally, all three sites were sporadically inspected during the rest of the summer, to try to assess the emergence of metamorphs from the ponds.

3.2.4. SURVEYS AT CAPTIVE BREEDING SITES

During all three years of fieldwork, weekly surveys were performed at the Jersey Zoo enclosure until the end of March, in order to find any frog clumps as soon as they were laid. In 2002, no spawn was found, therefore no other surveys were carried out. In 2001 and 2003, frog spawn was found in the pond and immediately moved to an outdoor tank (as described in Chapter 2) where its growth was monitored weekly until 28th June 2001 and 26th August 2003 respectively. On 28th June 2001, all tadpoles were released into Noirmont pond, after health screening at Jersey Zoo's veterinary laboratory. In 2003, all the animals were released in Noirmont pond after metamorphosis.

As in the Zoo enclosure, in Mr Perkins' pond frog breeding occurred in 2001 and 2003, but not in 2002. The development of the eggs and, later on, of the tadpoles, was followed during weekly visits to the site, until 15th July in 2001 and 26th August in 2003. Then, the tadpoles, in 2001, and the froglets, in 2003, were finally released, together with those from the Zoo's clump, into Noirmont pond.

3.3. RESULTS

3.3.1. CAPTURE OF ADULTS

Rana dalmatina

During all three years of surveys, the frog breeding season at Ouaisné Common started in February and ended in March, lasting between 27 and 41 days, depending on the year (Tab. 3.1). The spawning period varied between 12 days in 2001 and 25 in 2003, as reported in Tab 3.1.

Year	First frog	Last frog	First clump	Last clump	No days for spawning	Min No days presence of frogs
2001	10 Feb	18 Mar	23 Feb	7 Mar	12	36
2002	11 Feb	24 Mar	25 Feb	12 Mar	15	41
2003	21 Feb	12 Mar	23 Feb	20 Mar	25	27

Table 3.1. Duration of adult frogs' presence at the breeding site and of spawning period during 2001, 2002 and 2003 at Ouaisné.

At the beginning of each year's surveys, many frogs were seen, but not caught (Fig. 3.1). This was due to the fact that the frogs are very fast swimmers and were difficult to catch. A higher number of individuals were captured, by the end of the surveys, as a result of the practical experience gained. However, some frogs still managed to escape, and this was mainly because they were often seen in small groups, therefore the capture of one individual almost always made the other ones swim away.
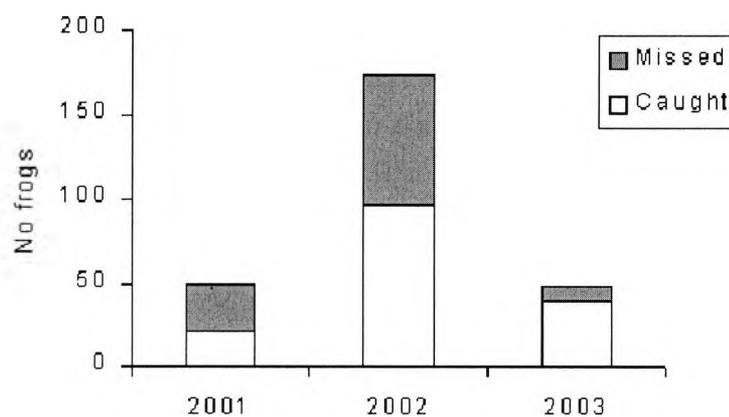
Figure 3.1. Number of frogs caught and missed during the night surveys.

Frog vocalisations were heard on only a few occasions. Three frogs were heard calling in 2001 and just one in both 2002 and 2003. The calls always came from underwater, their volume was very low and they stopped as soon as the surveyor started walking in their direction.

The number of individuals observed during each night survey in 2001, 2002 and 2003, together with the average between maximum and minimum daily air temperature (Fig. 3.2), and total daily rainfall (Fig. 3.3) are reported in the graphs below.
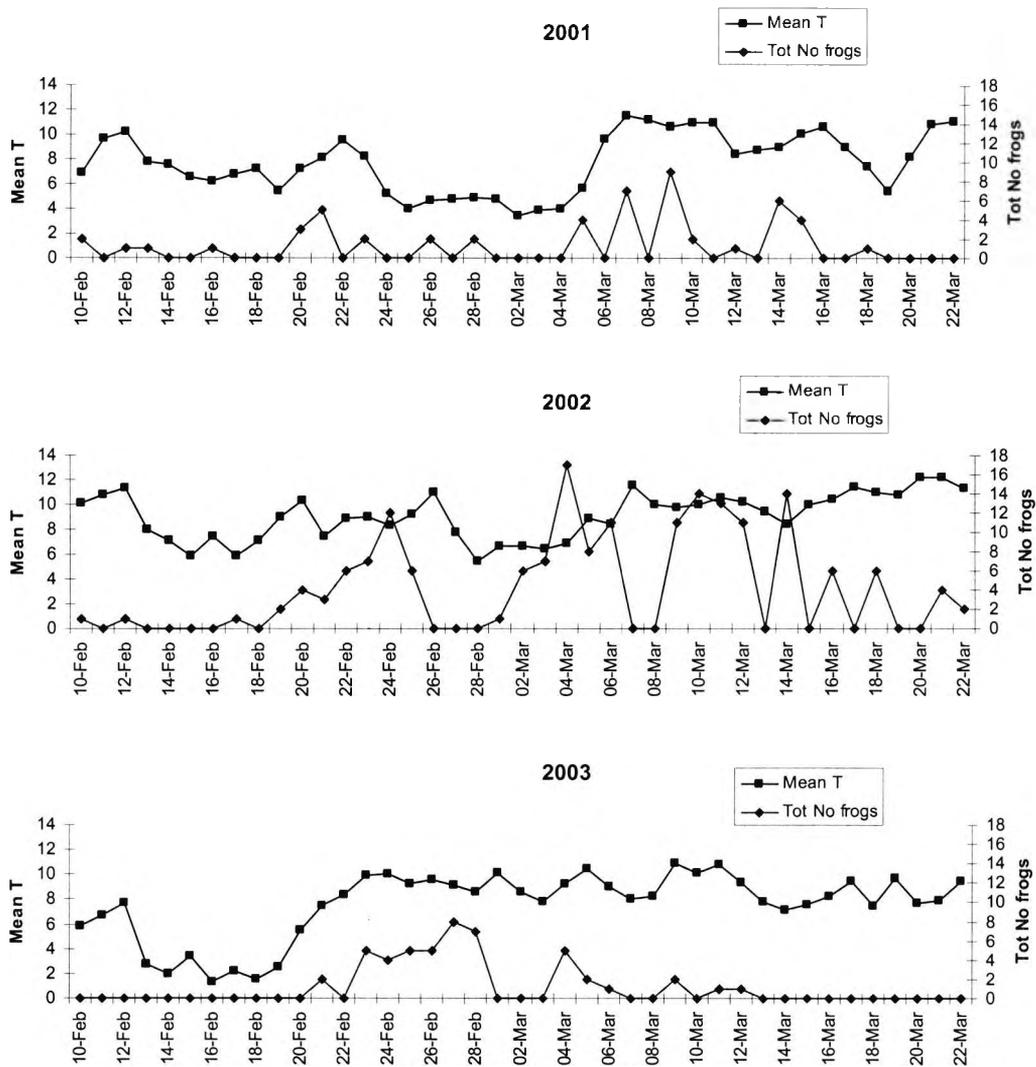
Figure 3.2. Number of adult agile frogs observed during each night survey in 2001, 2002 and 2003. Daily mean temperatures (°C) are also reported (data provided by the Jersey Meteorological Office).
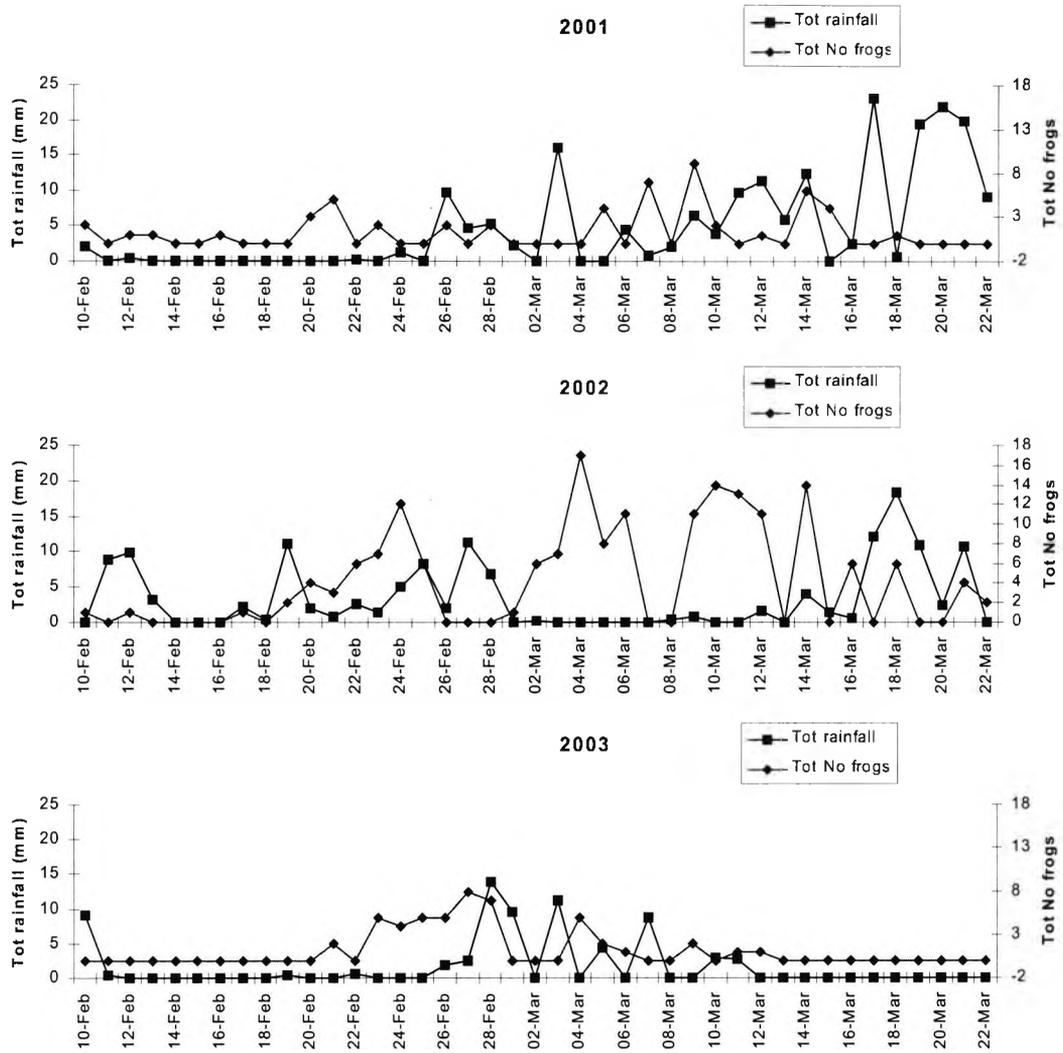
Figure 3.3. Number of adult agile frogs observed during each night survey in 2001, 2002 and 2003. Daily total rainfall (mm) is also shown (data provided by the Jersey Meteorological Office).

The codes of all the frogs marked at Ouaisné are reported in Tab. 3.2. An asterisk next to their code indicates the frogs that were recaptured the year after they had been marked.

Code	Sex	Year marked	Code	Sex	Year marked
00060606F8T*	M	2001	0006039D19	M	2002
0006062BBCT*	M	2001	000603913D	M	2002
0006062D83T	M	2001	00060379AE	M	2002
00060637DCT	M	2001	0006038BB1	M	2002
000607A316T	M	2001	0006204412	M	2002
000605DDD6T	M	2001	00062019E9	M	2002
00060631B5T	M	2001	00062024CE	M	2002
000605CB03T	M	2001	00062015D2	F	2002
00060623CET	M	2001	0006203733	M	2002
0006061BOCT	M	2001	0006201CF7	M	2002
0006030624*	M	2002	0006201F61	M	2002
000603733A*	M	2002	0006204170	M	2002
0006036CAO*	M	2002	000620017C	M	2002
00061FA41F*	M	2002	00062003DA	M	2002
000620169C*	M	2002	0006203736	M	2002
00062041B8*	M	2002	0006205761	M	2002
00061F8754*	M	2002	000620404B	M	2002
00061F8C25*	M	2002	0006200209	M	2002
0006212E6F*	M	2002	00061FA51D	M	2002
00061FBB04*	M	2002	0006202AC7	M	2002
0006036C59	M	2002	0006219D88	M	2002
0006060A61	M	2002	00061FFA17	M	2002
0006062195	M	2002	0006219BEE	M	2003
00060379FF	M	2002	00061F0B03	M	2003
0006036907	M	2002	000620187C	M	2003
000603898A	M	2002	0006200393	M	2003
0006037B97	M	2002	0006153761	M	2003
00060398B9	M	2002	0006385F89	M	2003
00060376D2	M	2002	000638BF2C	M	2003
0006037640	M	2002	00063869F3	F	2003
0006060E45	M	2002	0006446B43	F	2003

Table 3.2. Codes and sex of all the wild adult frogs PIT-tagged at Ouaisné in 2001, 2002 and 2003. An asterisk next to the code indicates a frog that was recaptured the year after being marked.

In total, 10 frogs, all males, were marked in 2001. Two of them were recaptured twice, while 4 of them were recaptured once (Fig. 3.4). No females were PIT-tagged, because they were never caught. Even though spawning occurred, since 7 clumps were found in the ponds, it was never actually observed. Of the frogs marked in 2001, 2 were recaptured the next year (Fig. 3.5). In 2002, 1 female and 42 males were PIT tagged. The female was caught only once, whereas, of the males, 15 were recaptured once, 7 were recaptured twice, 3 were caught 3 times and 1 frog was recaptured 4 times (Fig. 3.4). Ten of the males marked in 2002 were recaptured in 2003 (Fig. 3.5). Finally, 2 females and 7 males were marked in 2003. Of all male frogs caught in 2003 (new and

recaptures from 2002), 7 were recaptured once, 7 twice, 1 was caught 3 times and 4 were captured 4 times (Fig. 3.4).
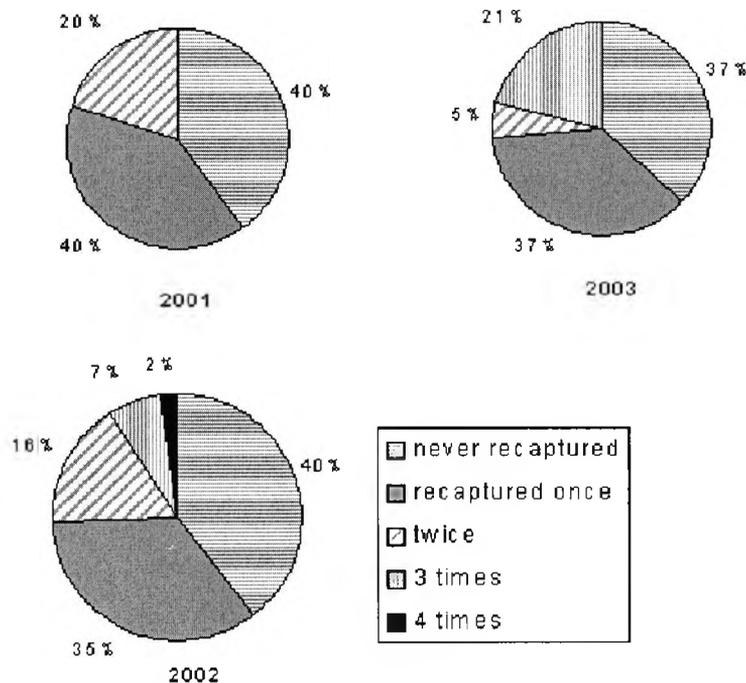
Figure 3.4. Percentage of *Rana dalmatina* individuals never recaptured, recaptured once, twice, three and four times during each year's surveys. No females were ever caught more than once.

Estimates of the size ($\check{N} \pm$ Standard Error) of the adult male populations present at the site during the breeding season in 2001, 2002 and 2003, were calculated by applying the "Weighted Mean Method" (Begon, 1979), using mark – recapture data. As one of the model's assumptions is that, during the sampling period, there are no immigrations or emigrations, only the data collected during the few days identified as each year's peak breeding time (05th March - 15th March 2001, 06th March – 16th March 2002 and 23rd February – 04th March 2003, see Fig. 3.2) were used. The following estimates were obtained: $\check{N} \pm$ SE= 14 $\pm$ 5 in 2001, $\check{N} \pm$ SE= 42 $\pm$ 9 in 2002 and $\check{N} \pm$ SE= 18 $\pm$ 5 in 2003. Despite the relatively low number of males present at the breeding ponds in 2003, especially when compared with those in 2002, that year the highest number of egg clumps was laid. This was probably a consequence of the fact that, overall, the percentage of older, and therefore sexually active, males was higher in 2003 than in 2002 (see Fig. 3.15 in section 3.3.2.).
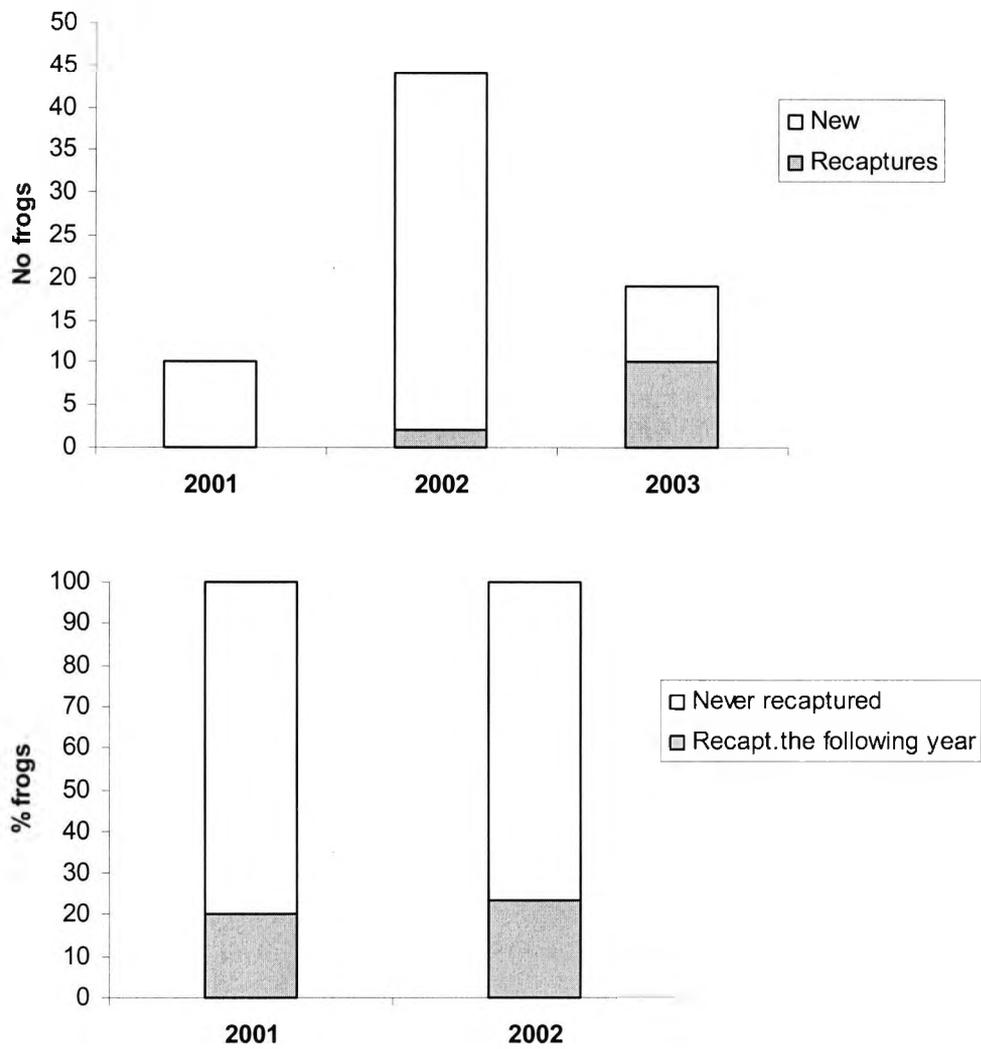
Figure 3.5. Number of new and recaptured frogs caught in 2001, 2002 and 2003 (above) and percentages of individuals that were never recaptured and of those recaptured the year after being marked (below).

The number of days spent at the breeding ponds (that is the number of days between their first capture and their last recapture) by the recaptured males varied between 3 and 11 in 2001, 2 and 25 in 2002 and 2 and 14 in 2003 (Fig. 3.6).
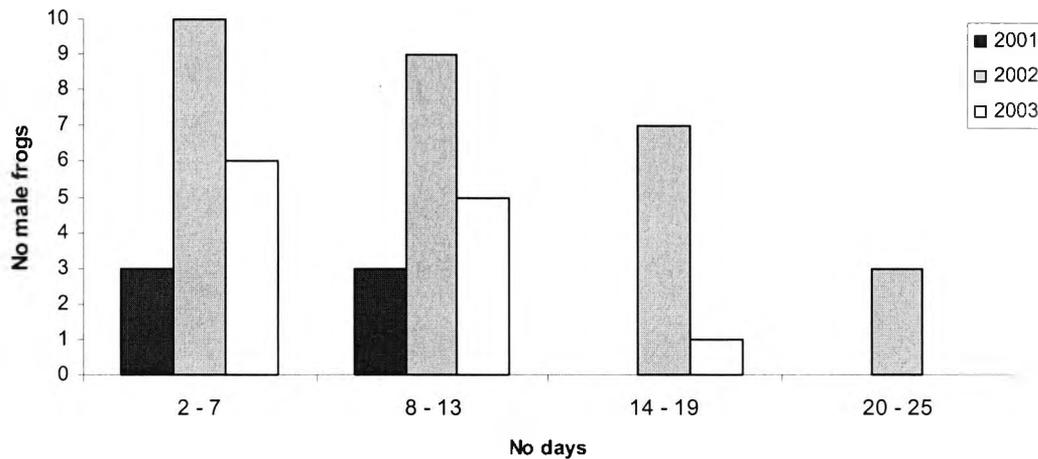
Figure 3.6. Time that male frogs spent at their breeding site in 2001, 2002 and 2003 (on the x axis: 2 - 7= between 2 and 7 days, 8 – 13= between 8 and 13 days, etc.).

The graphs in Fig. 3.7 show mass, body length and body condition (calculated as mass/body length^3 x 10^3) of all males caught in 2001, 2002 and 2003. In case of recaptures, within each year, the means of measurements, taken every time each animal was caught, were considered. A one-way ANOVA test was performed to compare mean mass, body length and body condition of male adult frogs caught in 2001, 2002 and 2003, to verify if the differences between years were significant (Fig. 3.8). The results indicated that there was significant variation between years in body mass and length (F= 4.59, df= 2, 69, p= 0.013 and F= 5.03, df= 2, 69, p= 0.009 respectively). Post-hoc tests (Fisher's LSD test) showed that frogs captured in 2003 were significantly larger in terms of both mass and length than those captured in previous years. However, no significant differences between years were noted in their mean body condition.

Mass

Body length
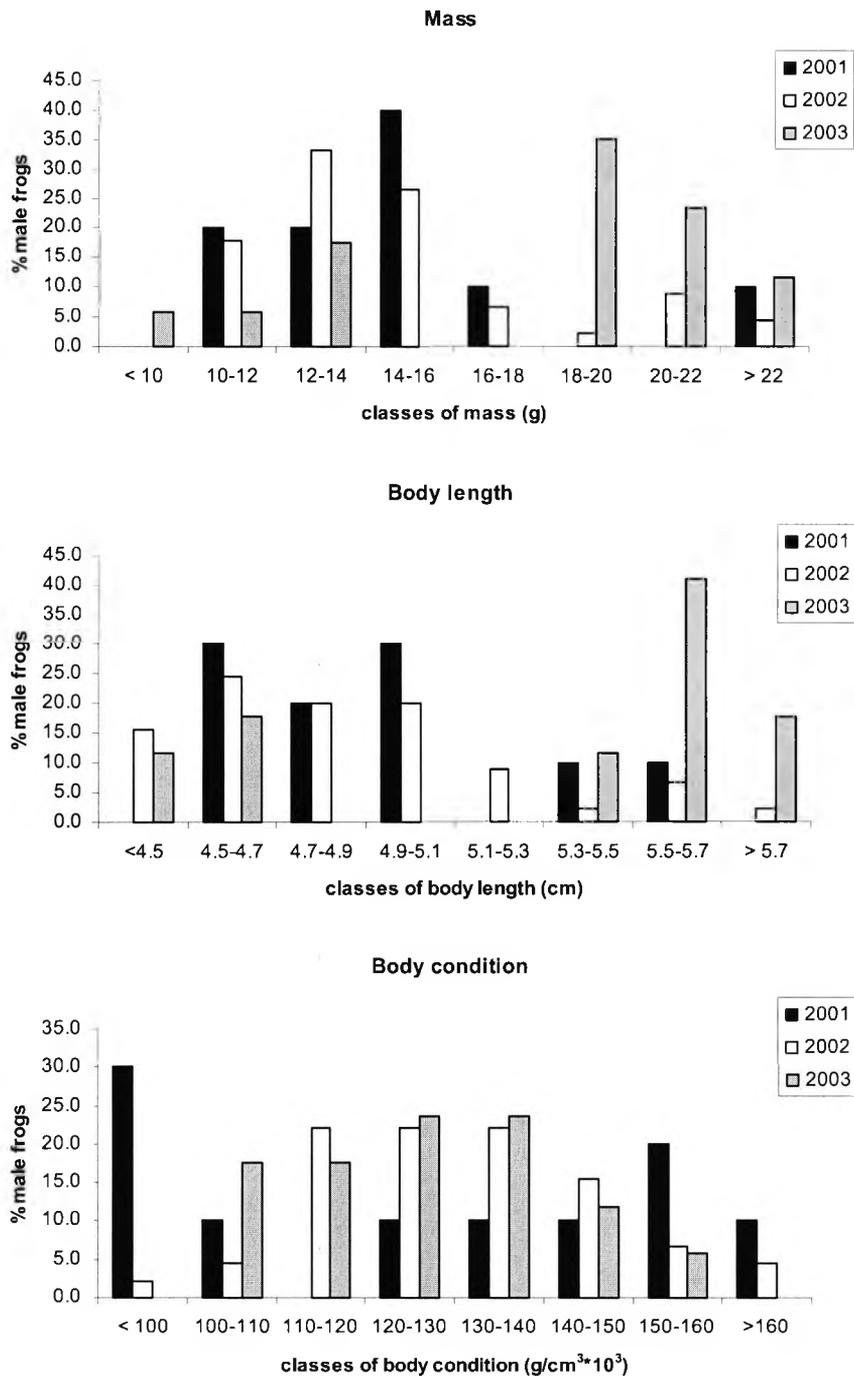
Body condition

Figure 3.7. Mass, body length and body condition (calculated as mass/body length^3 x 10^3) of all males caught in 2001, 2002 and 2003 at Ouaisné.

Mass

Body length
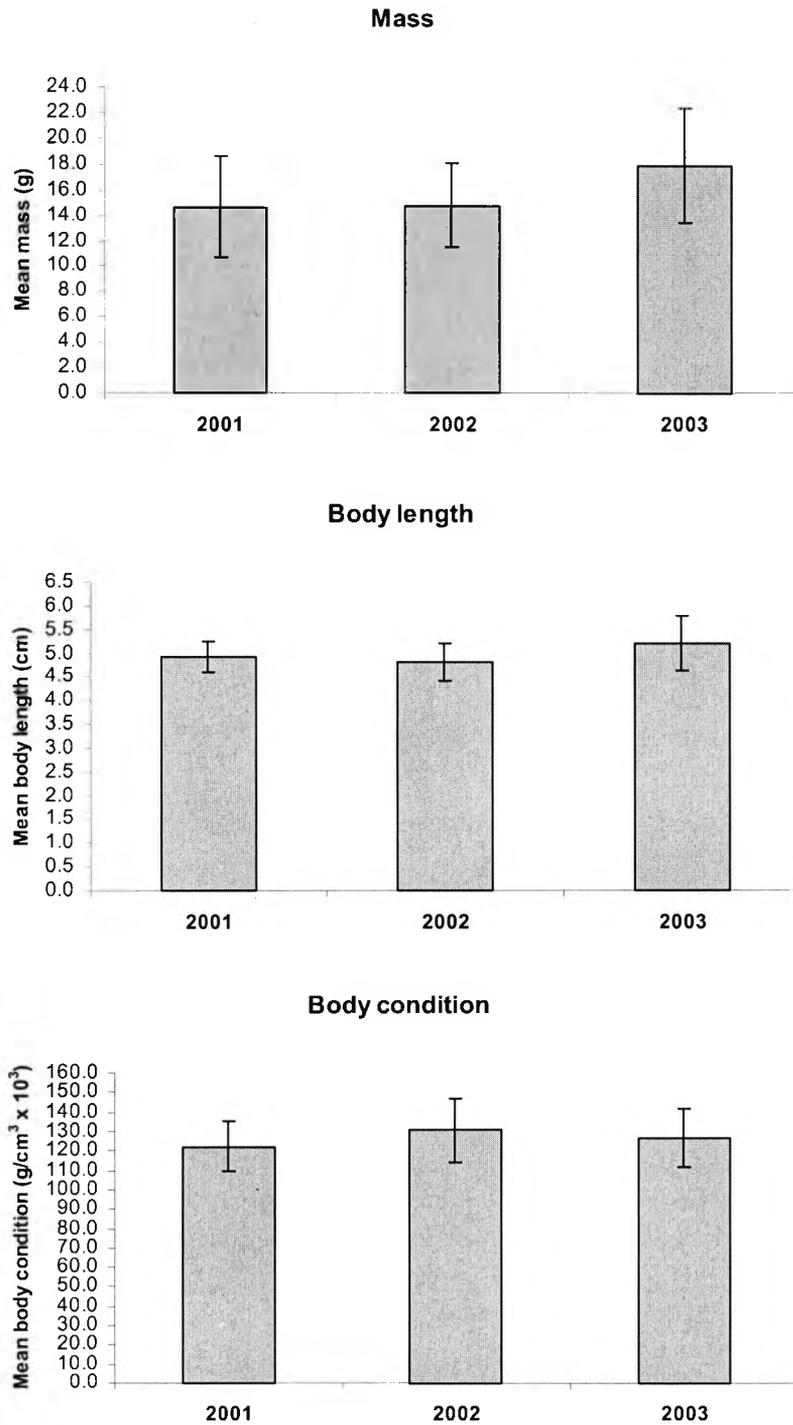
Body condition

Figure 3.8. Mean mass (g), body length (cm) and body condition (g/cm^3 x 10^3), with standard deviations, of male frogs caught in 2001, 2002 and 2003.

Finally, one-way ANOVA tests - performed after log-transforming all variables - showed that the individual frogs marked in 2002 had significantly higher mean mass (F= 3.78, df= 2, 69, p= 0.028) and body length (F= 4.45, df= 2, 69, p= 0.015) when recaptured in 2003, but their body condition did not significantly vary from one year to the next (F= 1.28, df= 2, 69, p< 0.285).

Bufo bufo

In 2001, common toads were seen at night at Ouaisné for less than a month and no more than 11 individuals were present at the site in any one night. They were spotted for the first time on 21st January and never again after 16th February. On 6th February, a pair of individuals was observed spawning and theirs was the only string of eggs laid that year at Ouaisné. The animals marked in 2001 were never recaptured in successive years. In 2002, common toads were seen at night in very low numbers (never more than 7 during a survey) from 11th to 24th February. Only two females were caught and they were not in the slacks, but on the road that leads to Ouaisné Common. No egg strings were found in either North or South Slack, and the same happened in 2003, when no adult toads were seen during the night surveys.

The situation was different at Grosnez (Fig. 3.9), where the toads were numerous, i.e. in the order of hundreds, in both 2001 and 2002 - the two years when night surveys were carried out at the site.
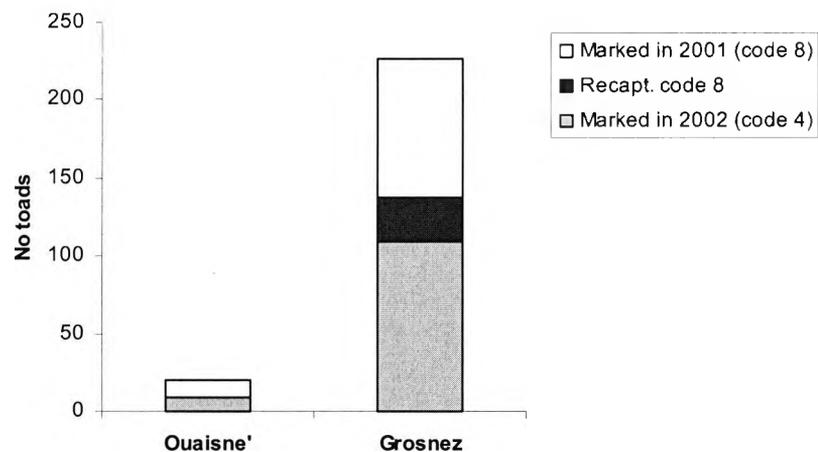
Figure 3.9. Total number of common toads, new and recaptures, caught at Ouaisné and Grosnez in 2001 and 2002.

Mean mass, body length and body condition of male common toads caught in 2001 and 2002 at Ouaisné and Grosnez were compared with ANOVA tests, to verify if there were differences between years and ponds. As shown in Fig 3.10 and 3.11, where an asterisk indicates p≤ 0.05, mass and body length of the animals found at Ouaisné were significantly higher in 2002

41

than in 2001, and in 2002 the toads that bred in Grosnez pond had significantly lower mass and body condition than those present at Ouaisné.
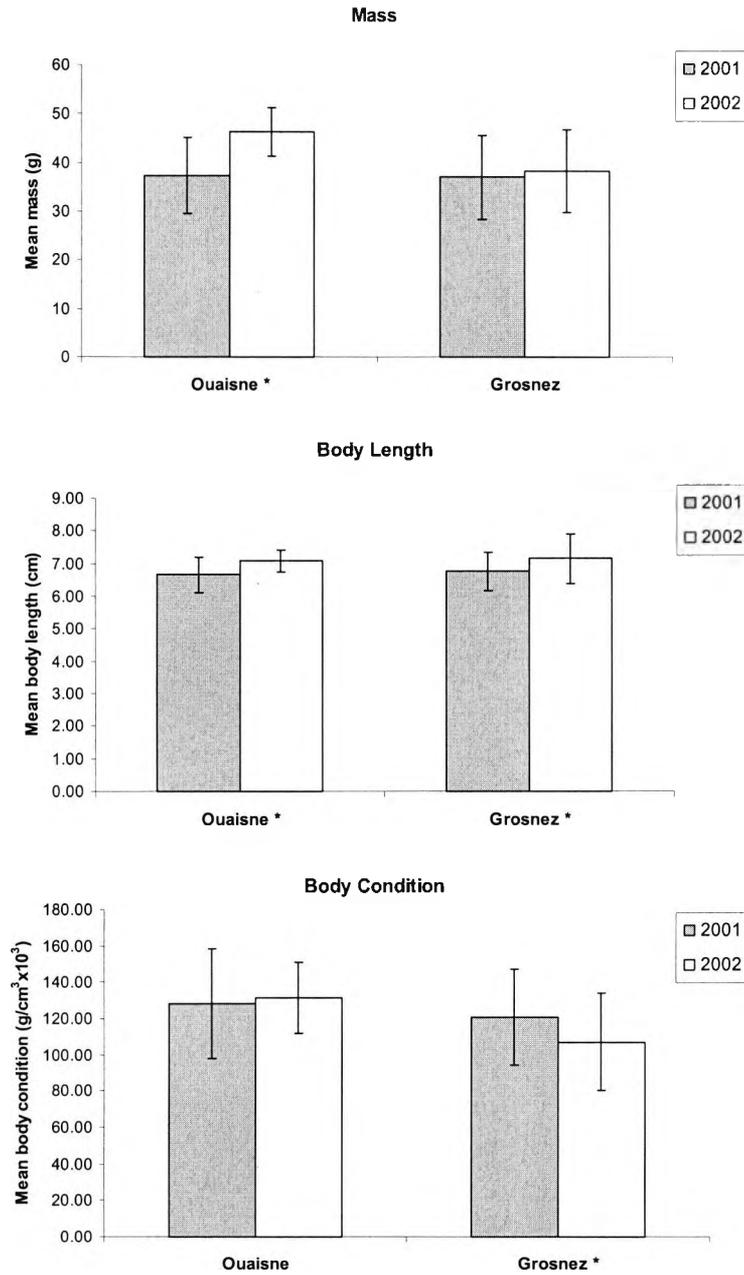
Figure 3.10. Mean mass (g), body length (cm) and body condition (g/cm^3 x 10^3), with standard deviations, of male toads caught in 2001 and 2002 at Ouaisné and Grosnez. The asterisks indicate significant (p≤ 0.05) differences between years.

Mass

Body length
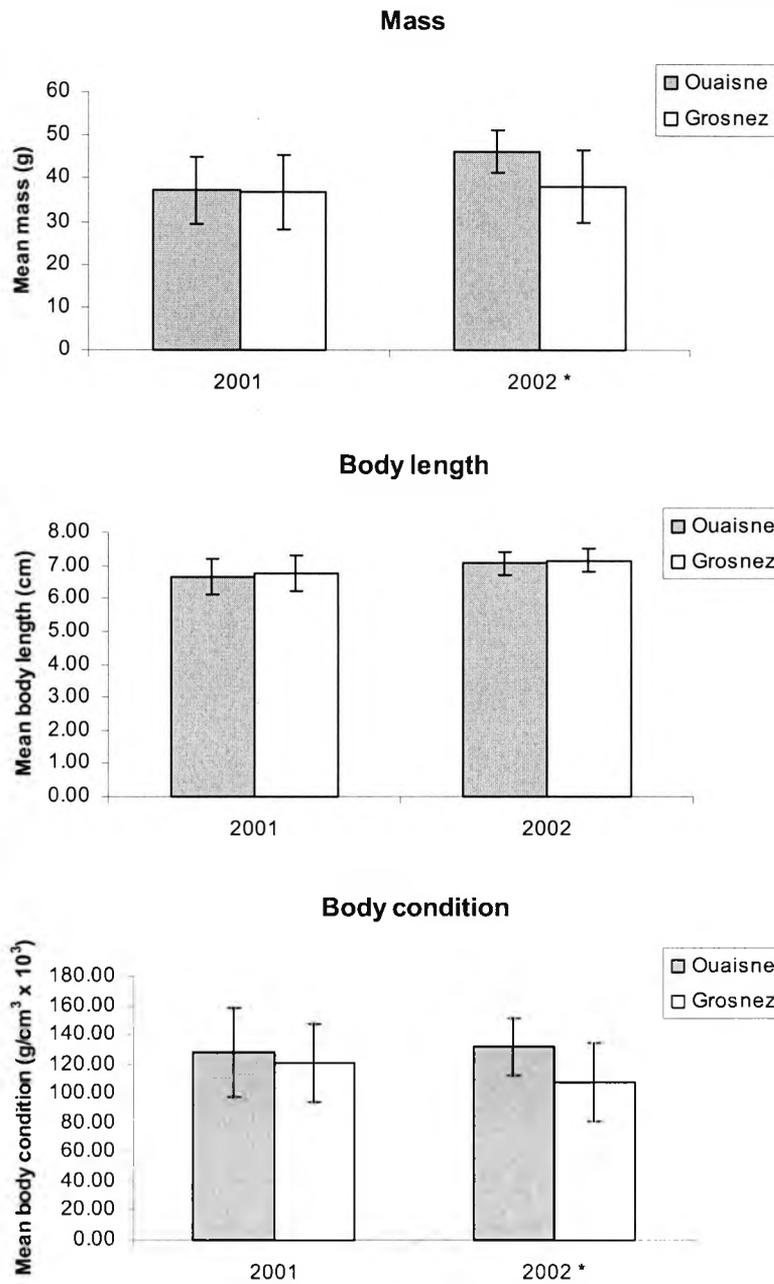
Body condition

Figure 3.11. Mean mass (g), body length (cm) and body condition (g/cm^3 x 10^3), with standard deviations, of male toads caught in 2001 and 2002 at Ouaisné and Grosnez. The asterisks indicate significant ($p \leq 0.05$) differences between ponds.
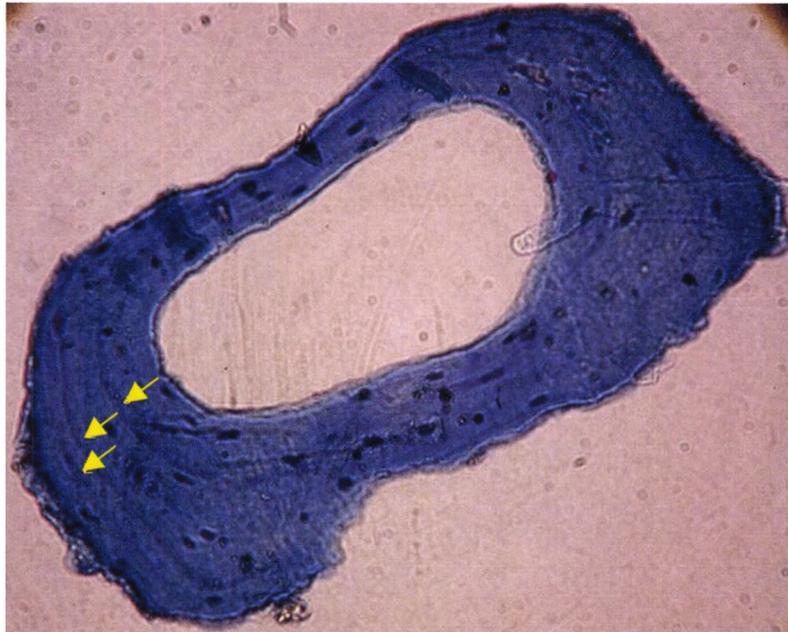
Figure 3.13. Cross-section of the second phalange of a wild agile frog's toe (code 00062024CE). The animal was 4 years old, as three LAGs are visible (where the arrows are) and the fourth, not visible, is on the outer border of the section.
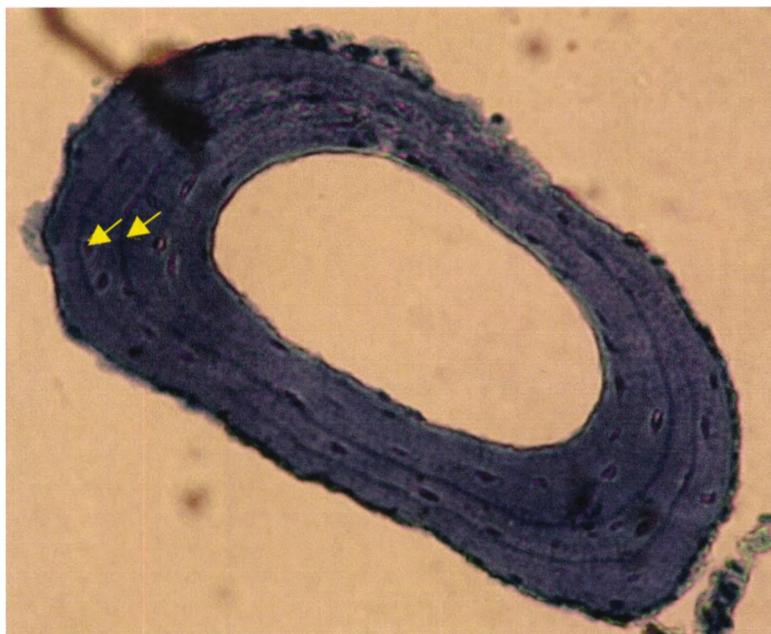
Figure 3.14. Cross-section of the second phalange of a captive agile frog's toe (code KEN 7), with two LAGs.

3.3.2. AGE DETERMINATION OF RANA DALMATINA ADULTS BY SKELETOCHRONOLOGY

There is a certain risk of underestimating the age of amphibians, when using the skeletochronology on phalanges, which are small and often damaged, instead of long bones (Castanet & Smirina, 1990). However, this risk has proved minimal on various occasions (e.g. Kumbar & Pancharatna, 2001; Measey, 2001). Also in the present study, when both a short and a long bone were used to age the same frog, no differences were noted. The animal (a female found dead in Noirmont pond in 2003) was 3 years old. In most individuals, the LAGs were well defined and easy to count (Fig. 3.12 and 3.13). In the wild, most frogs caught in 2002 were one or two years old, while the captive animals were either in their second or third year of life (Fig. 3.14). The next year, when many of the frogs caught during the night surveys were recaptures from the year before, the new frogs, that is those marked in 2003, were mainly 1-year old males. Both females were 3 years old. As shown in Fig. 3.15, in 2002, 75.5% of the wild frogs were 1-year old, 22.2% 2 years old and just 1 individual (2.2%) was 4 years old. The only female caught this year was amongst the 2 year old frogs, as were the 2 males marked in 2001 and recaptured in 2002. In captivity, 4 animals (57.1%, 3 males and 1 female) were 2 years old and 3 frogs (42.9%, 2 males and 1 female) were 3 years old. In 2003, 22.2% of the males were 1-year old, 61.1% 2 years old and 16.7% 3 years old. All females were 3 years old.

Figure 3.12. Cross-section of the second phalange of a wild agile frog's toe (code 00060606F8). One LAG, indicated by the arrow, is clearly visible.

Wild frogs 2002

Captive frogs 2002
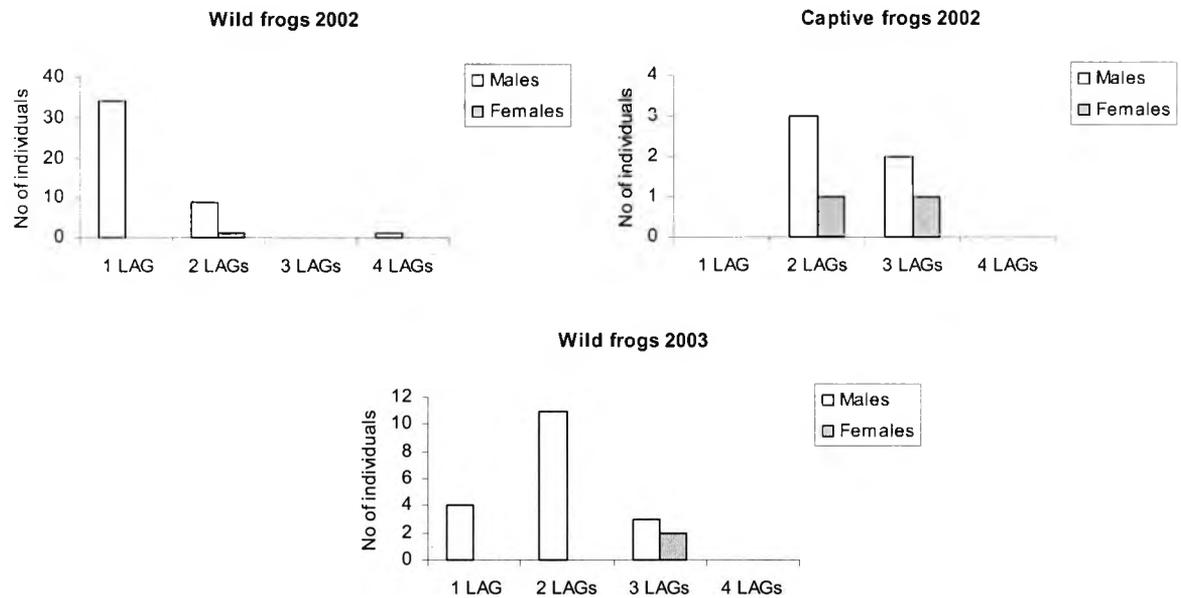
Wild frogs 2003

Figure 3.15. Wild and captive frogs caught in 2002 (above), and wild frogs caught in 2003 (below), which were found to be one, two, three and four years old, by counting the LAGs present on the sections of their second phalanges.

3.3.3. MONITORING OF EGGS AND CAPTURE OF TADPOLES AND METAMORPHS

Rana dalmatina

In 2001, a total of 7 egg clumps were found at Ouaisné, 4 in North Slack, 2 in South Slack and 1 in the Sump. In 2002, 9 clutches, 6 in North Slack and 3 in South Slack, were laid. Finally, in 2003, the number of clumps increased to 19, of which 12 were in North Slack, 3 in South Slack, 3 in the Sump and 1 in Small Temporary Pond. The latter was moved to South Slack as soon as it was found, because otherwise the eggs would have not been able to develop, as the pond usually dries around the end of March. In Noirmont pond, one clump was laid in 2002. The next year, a female frog was found dead in the water, on the top of a clutch of eggs. She had probably been predated while in amplexus and her eggs were not fertilised by the male, who hopefully managed to escape alive. The clump was taken to the Zoo and placed in an outdoor tank filled with aged tap water, but, as expected, none of the eggs developed.

All the clumps found in the wild were attached to aquatic plants or dead twigs. The depth of the water where each clump was laid and the depth of the clumps are shown in Tab 3.3.

Year	Clump (Pond)	Date laid	Depth of water (cm)	Depth of clump (cm)
2001	1(NS)	23-Feb	29	surface
2001	2(NS)	23-Feb	26	bottom
2001	3(NS)	28-Feb	33	10
2001	4(NS)	05-Mar	31	14
2001	5(SS)	24-Feb	50	26
2001	6(SS)	07-Mar	31	23
2001	7(SU)	07-Mar	38	14
2002	1(NS)	04-Mar	42	36
2002	2(NS)	04-Mar	44	30
2002	3(NS)	07-Mar	28	surface
2002	4(NS)	07-Mar	53	24
2002	5(NS)	07-Mar	32	surface
2002	6(NS)	07-Mar	23	19
2002	7(SS)	25-Feb	56	46
2002	8(SS)	07-Mar	23	surface
2002	9(SS)	12-Mar	47	35
2002	10(NO)	15-Mar	29	17
2003	1(NS)	23-Feb	33	10
2003	2(NS)	23-Feb	39	32
2003	3(NS)	25-Feb	36	12
2003	4(NS)	26-Feb	26	26
2003	5(NS)	03-Mar	30	15
2003	6(NS)	04-Mar	44	44
2003	7(NS)	04-Mar	23	23
2003	8(NS)	04-Mar	23	23
2003	9(NS)	04-Mar	23	23
2003	10(NS)	06-Mar	28	15
2003	11(SS)	26-Feb	40	28
2003	12(SS)	03-Mar	31	20
2003	13(SS)	06-Mar	39	15
2003	14(SU)	06-Mar	53	10
2003	15(SU)	06-Mar	50	22
2003	16(NS)	08-Mar	24	15
2003	17(NS)	12-Mar	23	23
2003	18(SS)	20-Mar	38	0
2003	19(SU)	13-Mar	30	17

Table 3.3. Dates when frog egg clutches were found (and pond in which they were laid) in 2001, 2002 and 2003. Depth of the water where each clump was laid and depth of the clumps are also reported (NS= North Slack, SS= South Slack, SU= Sump, NO= Noirmont).

Year	Clump (Pond)	Start hatching (=d after eggs were laid)	Days for hatching
2001	1(NS)	24	12
2001	2(NS)	/	/
2001	3(NS)	24	17
2001	4(NS)	21	15
2001	5(SS)	22	13
2001	6(SS)	22	5
2001	7(SU)	19	9
2002	1(NS)	24	15
2002	2(NS)	24	20
2002	3(NS)	21	11
2002	4(NS)	21	21
2002	5(NS)	19	7
2002	6(NS)	21	8
2002	7(SS)	23	8
2002	8(SS)	19	10
2002	9(SS)	16	11
2002	10(NO)	13	18
2003	1(NS)	30	16
2003	2(NS)	30	16
2003	3(NS)	28	22
2003	4(NS)	27	16
2003	5(NS)	22	22
2003	6(NS)	21	22
2003	7(NS)	23	4
2003	8(NS)	23	4
2003	9(NS)	21	6
2003	10(NS)	27	4
2003	11(SS)	22	6
2003	12(SS)	19	16
2003	13(SS)	21	6
2003	14(SU)	21	5
2003	15(SU)	21	14
2003	16(NS)	25	20
2003	17(NS)	15	20
2003	18(SS)	19	8
2003	19(SU)	14	14
2003	20(NO)	/	/

Table 3.4. Number of days passed from when each clump was laid to the start of the hatching phase and duration of the hatching phase (NS= North Slack, SS= South Slack, SU= Sump, NO= Noirmont).

Hatching invariably started 13-30 days after deposition (Tab. 3.4) when the embryos were at stage 20 and 21 of Gosner's table (Gosner, 1960). In 2003, just when most of the clumps were hatching, or about to start hatching, a spill of oil from a nearby buried heating oil tank occurred in the slacks and surrounding area. All the clutches that were not yet hatching (8 out of 19), were therefore moved to outdoor tanks that had the characteristics of the one located at the Zoo (Chapter

2), placed outside the ESU's Frances Le Sueur Centre. Every clump was separated from the others. Within two or three days, all the clumps in the tanks started to hatch. Water analyses, carried out in both slacks after the incident, soon showed that the level of pollution present in the ponds was below critical values (Pinel, J., pers. comm.), therefore, within a week of the spill, all the newly hatched tadpoles were released in the wild. Before taking them back, the number of tadpoles hatched from each clump was noted and compared to the estimate of the number of eggs present in each clutch made as soon as the spawn was found. In all cases, the discrepancy between the two numbers was low, never higher than 5% of the total (Tab. 3.5), therefore the first estimates were considered correct for all clumps.

Code clump	No eggs (estimated)	No hatchlings (counted)
7 (NS)	450	428
8 (NS)	450	457
9 (NS)	450	472
10 (NS)	600	584
16 (NS)	450	429
17 (NS)	450	431
13 (SS)	650	683
18 (SS)	650	616

Table 3.5. Number of eggs, estimated in the field as soon as each clump was found, and number of hatchlings, counted at the end of the hatching phase, when the clumps were in tanks.

The duration of the hatching phase varied between 5 and 17 days in 2001, 7 and 20 days in 2002 and 4 and 22 days in 2003 (Tab. 3.4). Just one clump (NS2, in 2001), failed to develop at Ouaisné. Overall, each year the percentage of eggs that died during the embryonic development was low, as shown in Fig. 3.16.
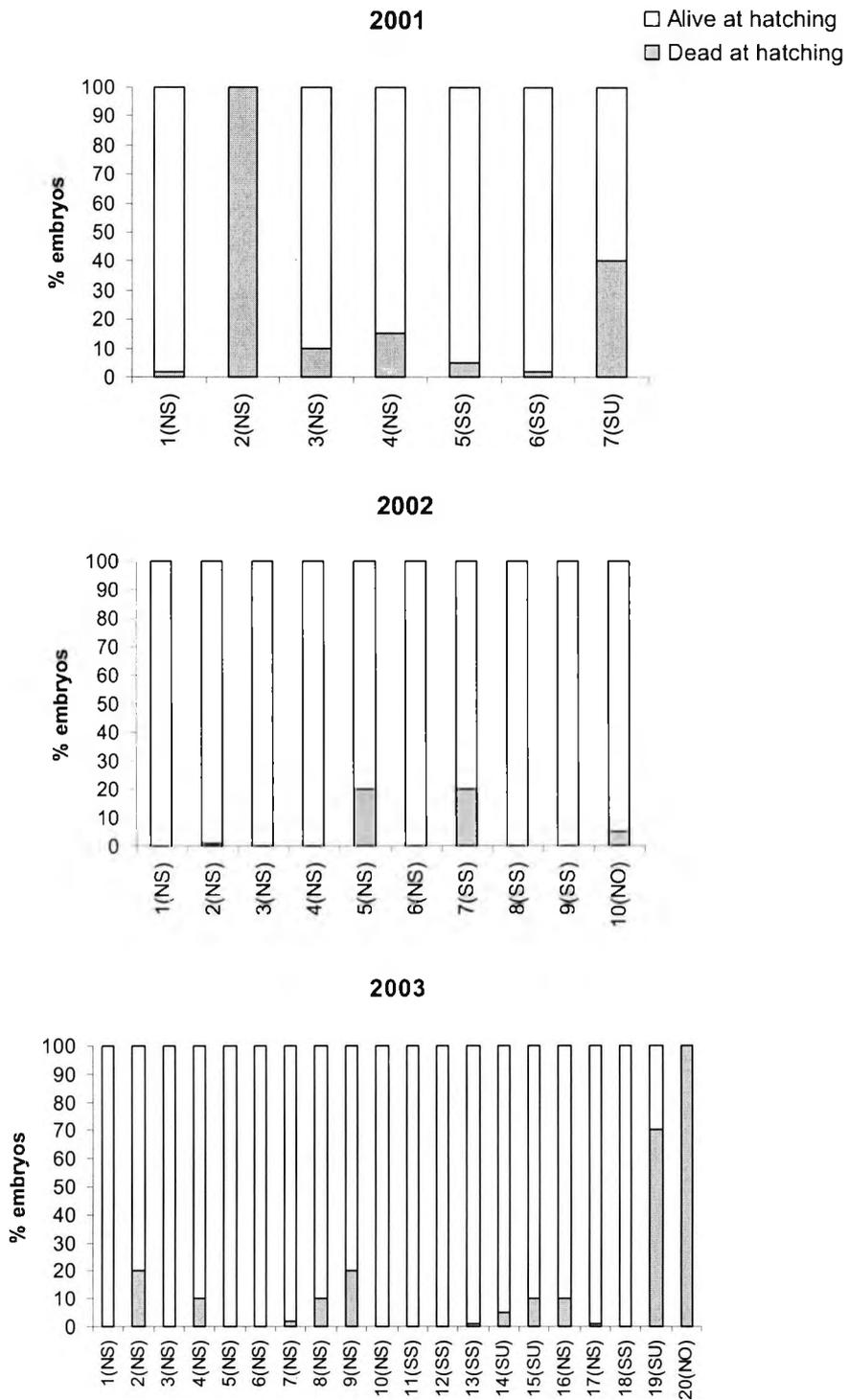
Figure 3.16. Embryonic survival rate in 2001, 2002 and 2003.

No data about the development of tadpoles in the Sump, nor in Noirmont pond, are reported, because too few tadpoles (never more than 3) were caught during the netting sessions performed there. In the graph in Fig. 3.17, the mean total length of tadpoles at stages from 26 to 40 is shown. Only these stages are considered because too few tadpoles (less than 3 individuals in any one year) representing the other stages of development were caught at Ouaisné during the netting sessions. The two-way ANOVA test, which was used to compare the development of tadpoles in the two slacks, showed that there was no significant difference either between ponds or years.
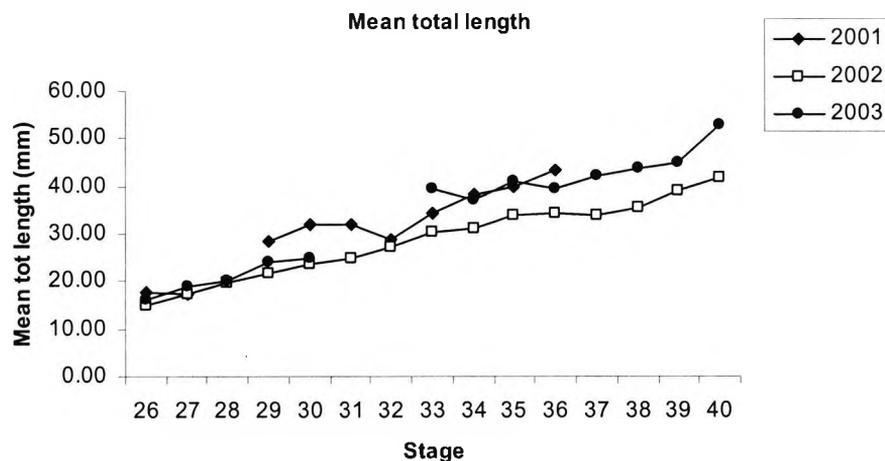
Figure 3.17. Mean total length (mm) of tadpoles at stages from 26 to 40 caught in the slacks at Ouaisné during the three years of surveys.

Fig. 3.18 shows the number of new and recaptured tadpoles caught during the capture - mark – recapture sessions. The estimated tadpole population sizes ($\check{N}$ ± Standard Error), at the time when these sessions were performed, calculated by applying the "Weighted Mean Method" (Begon, 1979), were: $\check{N}$ ± SE= 582 ± 40 in the North Slack and $\check{N}$ ± SE= 543 ± 44 in the South Slack for 2002 and $\check{N}$ ± SE= 1325 ± 271 in the North Slack and $\check{N}$ ± SE= 584 ± 29 in the South Slack for 2003. These estimates reflected reasonably well the actual number of froglets caught using drift fences and pitfall traps in 2002 (553 in North Slack and 427 in South Slack), but were an overestimate in 2003, when 484 and 195 metamorphs were caught respectively in North and South Slack (Fig. 3.19). Fewer froglets were caught around the slacks in 2001, when the drift fences did not encircle the water bodies completely, and therefore some of the emerging animals were certainly missed. In the same year, no froglets were observed around or in the Sump, whereas in 2003, when the pond dried up completely by mid May, causing the death of most of the tadpoles present in it, 17 metamorphs were seen in the area in early June.
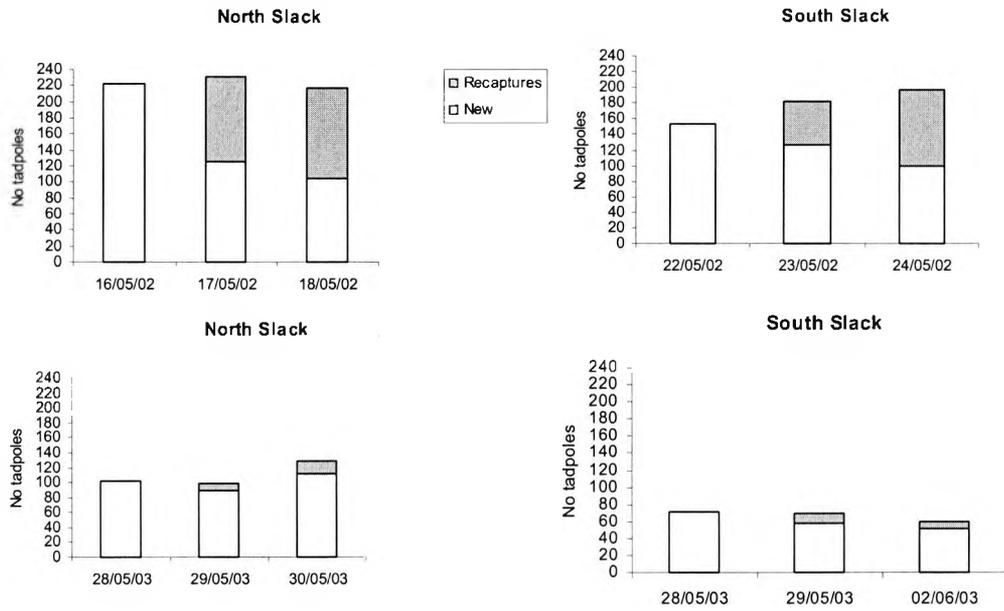
Figure 3.18. Number of tadpoles caught during the capture-mark-recapture sessions performed at Ouaisné in 2002 (above) and 2003 (below).
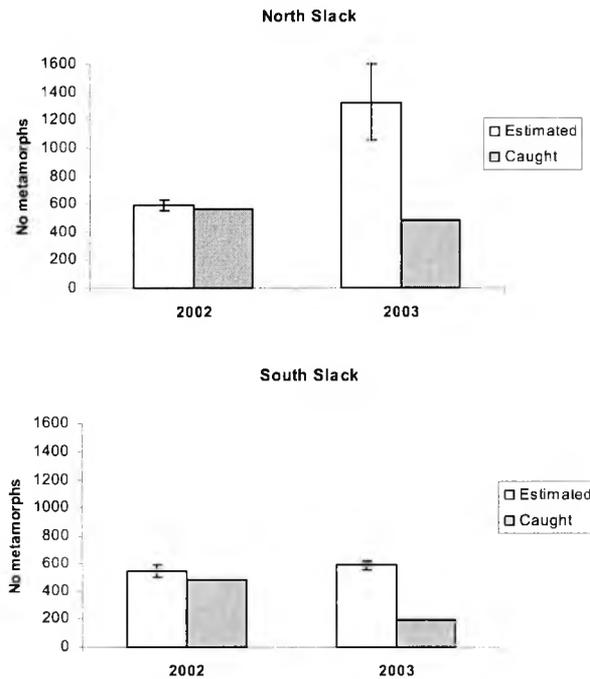
Figure 3.19. Estimated tadpole population size and number of metamorphs caught in North and South Slack in 2002 and 2003.

In Noirmont pond, where one clump of eggs was laid in 2002, a total of 7 metamorphs were observed, outside of the water, on two occasions (8th and 9th July).

At Ouaisné, during all three years, most metamorphs were found in the traps and a small percentage along the fences (Fig. 3.20).
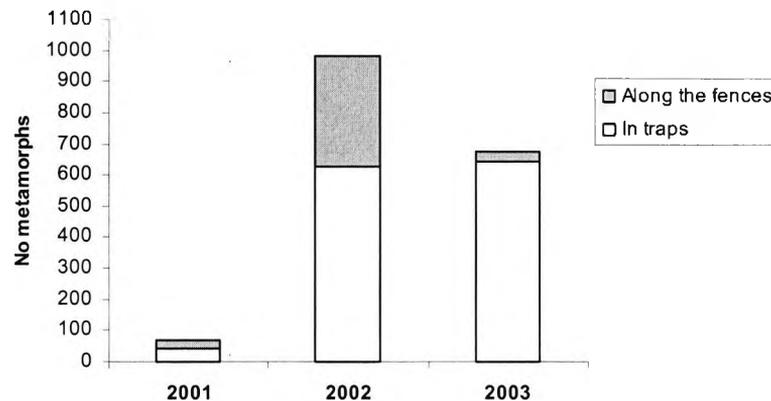
Figure 3.20. Number of metamorphs caught in the pitfall traps and along the fences in 2001, 2002 and 2003.

Most of the metamorphs emerged from the ponds between mid June and mid July, the period of time considered in Fig. 3.21, where each year's peak of froglets' emergence is shown. In 2002, most of the metamorphs left the water slightly later than in the two previous years. The same graphs also report mean temperatures and total rainfall recorded during the three-day periods taken into consideration.
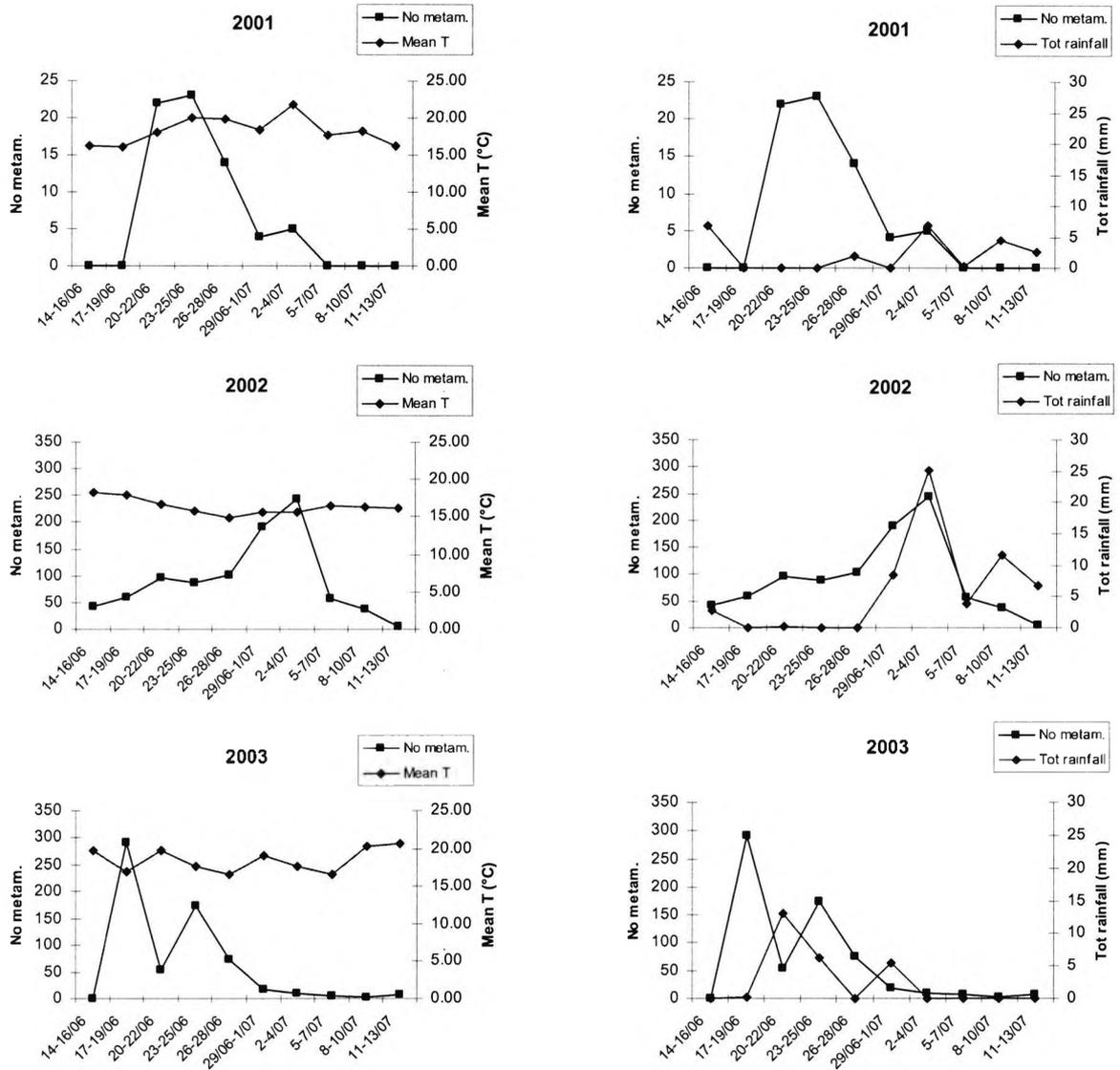
Figure 3.21. Peaks of metamorphs' emergence at Ouaisné. For each year, three-day periods are considered, from 14th June to 13th July. Mean temperature (°C) and total rainfall (mm) recorded during each of these three-day periods are also shown (data provided by the Jersey Meteorological Office).

In Jersey, two varieties of frog skin colour can be distinguished, a reddish one and a green-greyish one (Partridge, 1995). The graph in Fig. 3.22 reports the percentage of metamorphs, caught at Ouaisné, for each of the two varieties. The reddish variety, most abundant in 2001 and 2002, was slightly less represented than the green-greyish one in 2003.
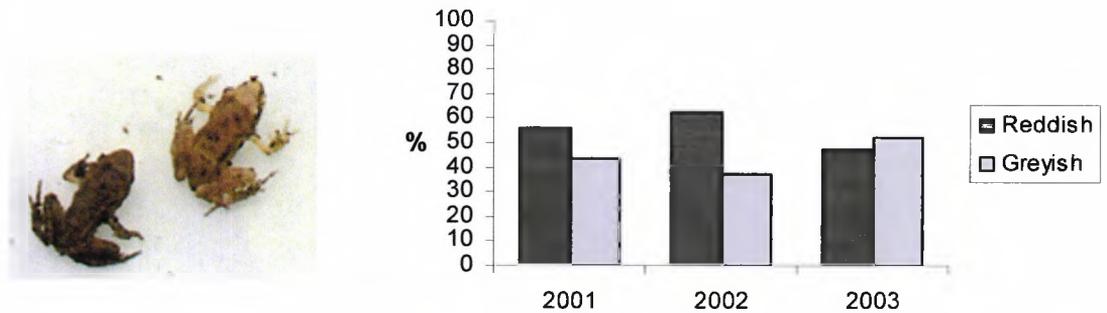
Figure 3.22. The two varieties of skin colour (left). Percentage of reddish and green-greyish (respectively "Reddish" and "Greyish" in the graph) metamorphs caught at Ouaisné in 2001, 2002 and 2003 (right).

The mean body condition of all metamorphs emerged from the slacks during each of the years of surveys, calculated as mass/body length^3 x 10^3, is shown in Fig. 3.23. The results of a one-way ANOVA test showed significant differences in body condition between years (F= 987.67, df= 2, 1369, p< 0.001). Post-hoc tests (Fisher's LSD test) indicated that in 2003 the froglets had significantly higher body condition than in both previous years and that in 2001 the metamorphs' body condition was significantly better than in 2002.
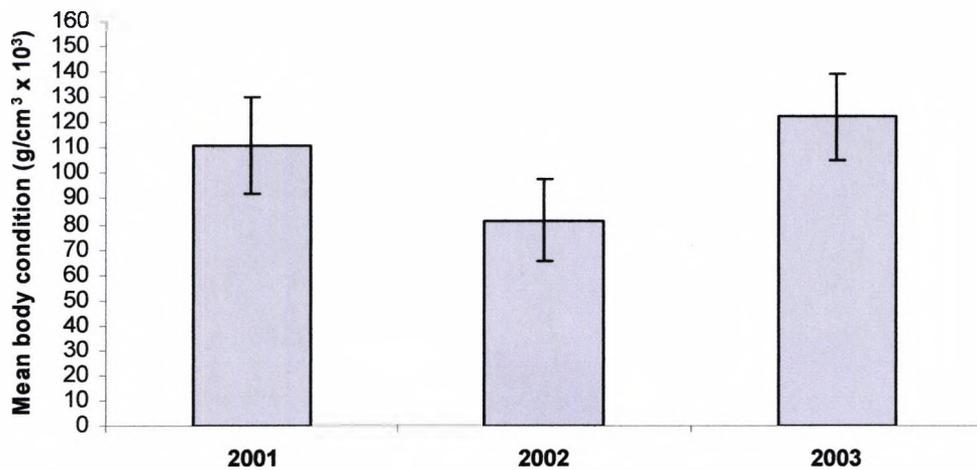
Figure 3.23. Mean body condition (mass/body length^3 x 10^3), and standard deviations, of all metamorphs caught during the trapping sessions performed at Ouaisné in 2001, 2002 and 2003.

One-way ANOVA tests were also used to assess, within each year of study, if metamorphs had different mass, body length and/or body condition depending on their colour. It emerged that in both 2002 and 2003 the green-greyish froglets had significantly shorter body length (F= 6.17, df= 1, 709, p= 0.013 and F= 6.47, df= 1, 589, p= 0.011 respectively) than the reddish ones. Moreover, in 2003 the latter had significantly better body condition (F= 7.20, df= 1, 589, p= 0.007) than the green-greyish metamorphs (Fig. 3.24).
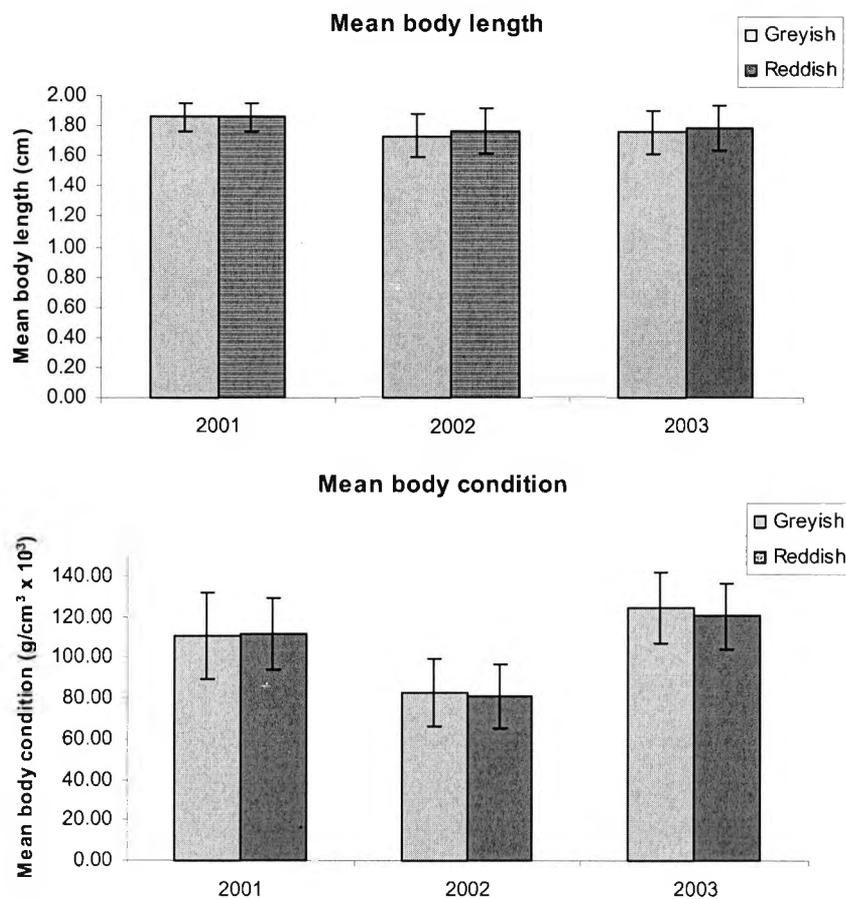
Figure 3.24. Mean body length (cm) and mean body condition (g/cm^3 x 10^3), with standard deviations, of green-greyish and reddish metamorphs caught during the trapping sessions performed at Ouaisné in 2002 and 2003.

A one-way ANOVA test was used to compare the mean body condition of metamorphs from North and South Slack in 2002 and 2003 (Fig. 3.25), but no significant differences emerged. The data for 2001 were not considered in this analysis because, since the drift fences did not

encircled the slacks completely, it was not possible to be certain about the provenance of each froglet.
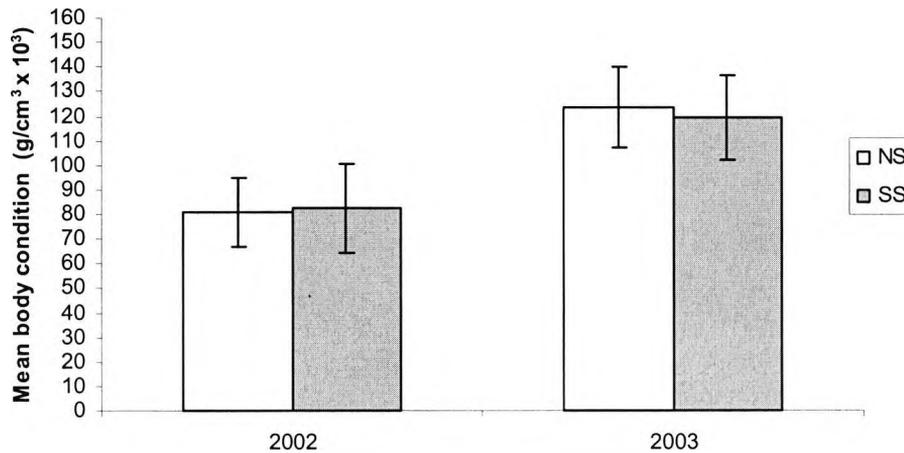
Figure 3.25. Mean body condition (g/cm^3 x 10^3), and standard deviations, of metamorphs caught in North Slack (NS) and South Slack (SS) during the trapping sessions performed at Ouaisné in 2002 and 2003.

In order to verify if the metamorphs in 2002 and 2003 left the ponds in a preferential direction, a two-way ANOVA test was performed on the average number of froglets found every three days in 5 and 8 different zones respectively around North and South Slack. Every zone included a set number of pitfall traps and areas along the fences between consecutive traps, the same each year. In the North Slack, five zones, of which three comprised 2 traps and 2 areas along the fences, and one 2 traps and 3 areas along the fences, were identified, whereas in the South Slack, 8 zones included 2 traps and 2 areas along the fences. The results showed that in 2002, while in the North Slack the froglets did not choose any direction significantly more than the others, in the South Slack they did (F= 8.74, df= 7, 63, p< 0.001). A post-hoc test (Fisher's LSD test) indicated that one of the zones was significantly preferred to the others. The fact that it was the closest zone to the wettest part of the site might have influenced the metamorphs' choice. However, no preferential directions of emergence were detected in 2003.

Finally, Tab. 3.6 summarises the data on the frog spawn clump development in the wild in 2001, 2002 and 2003. The minimum embryonic developmental time (i.e. the number of days from when the last clump of frog eggs was laid to the end of the hatching phase) did not vary considerably between sites or between years. Similarly, the minimum number of days needed by the frog tadpoles to reach metamorphosis, slightly higher in 2001 than in both 2002 and 2003, did

North Slack	2001	2002	2003
Number of clutches	4	6	12
Estimated number of live eggs	1750	4120	6500
First clump laid	23/02/01	04/03/02	23/02/03
Last clump laid	05/03/01	07/03/02	12/03/03
First hatching	23/03/01	26/03/02	25/03/03
End of hatching	10/04/01	18/04/02	16/04/03
Minimum embryonic developmental time (d)	36	42	35
First transformation	15/06/01	13/06/02	10/06/03
Minimum tadpole developmental time (d)	66	56	55
Total number of froglets caught	42	553	484
Min % survival from egg to metamorphosis	2.4	13.4	7.4

South Slack	2001	2002	2003
Number of clutches	2	3	4
Estimated number of live eggs	750	1600	2500
First clump laid	24/02/01	25/02/02	26/02/03
Last clump laid	07/03/01	12/03/02	20/03/03
First hatching	21/03/01	20/03/02	25/03/03
End of hatching	03/04/01	15/04/02	16/04/03
Minimum embryonic developmental time (d)	27	34	27
First transformation	20/06/01	13/06/02	13/06/03
Minimum tadpole developmental time (d)	78	59	58
Total number of froglets caught	26	427	195
Min % survival from egg to metamorphosis	3.5	26.7	7.8

Sump	2001	2002	2003
Number of clutches	1	0	3
Estimated number of live eggs	450	-	2000
First clump laid	07/03/01	-	06/03/03
Last clump laid	07/03/01	-	13/03/03
First hatching	26/03/01	-	27/03/03
End of hatching	04/04/01	-	10/04/03
Minimum embryonic developmental time (d)	28	-	28

Noirmont	2002	2003
Number of clutches	1	1
Estimated number of live eggs	400	50
Date when clump was laid	15/03/02	11/03/03
Start hatching	28/03/02	-
End of hatching	15/04/02	-
Minimum embryonic developmental time (d)	31	-

Table 3.6. Summary of data about the frog wild spawn clump development in 2001, 2002 and 2003.

not present relevant differences between North and South Slack, the only two sites where it was possible to record it (Chapter 2). However, the number of froglets emerged from the water bodies at Ouaisné varied considerably between years. In 2001, at least 2.4% and 3.5% of tadpoles survived to metamorphosis respectively in North and South Slack, whereas much higher survival rates were recorded in the two following years (in 2002, 13.4% and 26.7% respectively in North and South Slack and, in 2003, 7.4% and 7.8%).

Bufo bufo

Tab. 3.7 summarises the data collected in 2001 and 2002 in North Slack, Cannes de Squez, Grosnez pond and Noirmont pond. At Grosnez, despite the abundance of egg strings and then tadpoles, the number of toadlets seen near the pond was not very high in 2001, definitely lower than in the previous year (Racca, 2000). No toadlets were seen in, or around, Cannes de Squez in 2001, when the tadpole development was slower than the average (see Chapter 7), and just 5 in 2002. Near Noirmont pond, only one toadlet was seen in 2001, but this may be due to the fact that the area is very big and difficult to search. In 2002, the situation at Grosnez and Noirmont did not seem to differ much from that of the previous year. In all ponds, between 30% and 50% of the eggs died because of fungal infections in the first year of surveys and around 10% − 30% in 2002. At Ouaisné, no toad egg strings were laid in 2002 or 2003.

2001	Ouaisné	Grosnez	Cannes de Squez	Noirmont
N_o strings	1	≥ 100	≥ 50	6
Start spawning	06-Feb	30-Jan	19-Jan	before 19/02
Start hatching	08-Mar	14-Mar	28-Feb	02-Mar
First transformation	08-Jun	10-Jun	/	03-Jun
N_o of toadlets seen near the pond	10	40	none	1

2002	Grosnez	Cannes de Squez	Noirmont
N_o strings	≥ 100	≥ 70	7
Start spawning	01-Feb	05-Mar	End of February
Start hatching	12-Mar	12-Mar	10-Mar
First transformation	11-Jul	11-Jul	21-Jun
N_o of toadlets seen near the pond	30	5	15

Table 3.7. Summary of data about the toad spawn development in 2001 and 2002.

3.3.4. SURVEYS AT CAPTIVE BREEDING SITES

When the enclosure in Mr Perkins' garden was rebuilt, in May 2002 (Chapter 2), seven frogs, one of which was dead, were found in the compound. In Tab. 3.8, the sex, colour and measurements of all the frogs are shown. Using the skeletochronology technique (see section 3.3.2.), it was found that, in 2002, four animals were 2 years old and three were 3 years old.

No	Sex	Mass (g)	BL (cm)	BC (g/cm^3)x10^3	Colour	Age (years)	Comments
1	F	12.5	4.7	120.40	R	2	
2	F	10.25	4.5	112.48	R	3	
3	M	13.25	5.4	84.15	G	3	
4	M	10	4.3	125.78	G	2	
5	M	10	4.3	125.78	R	3	
6	M	9	4	140.63	R	2	
7	M	9.25	4.5	101.51	R	2	dead

Table 3.8. Agile frogs found inside Mr Perkins' enclosure (BL: body length, BC= body condition, G= green-greyish and R= reddish).

Tab. 3.9 summarises the data about the development of frog egg clumps laid in captivity in 2001 and 2003. In 2002, no spawning occurred in the Zoo enclosure or in Mr Perkins' pond. In 2001, one clump was found in the enclosure at the Zoo and one in the enclosure in Mr Perkins' garden. In the latter, around 40% of the eggs were dead, as a consequence of fungal infection. All clutches were quite small, as their estimated number of eggs did not exceed 150. The exact date the spawn reared in captivity started hatching was missed. All the eggs had hatched by 22nd March at Mr Perkins' site and by 4th April at the Zoo. Virtually no mortality was observed in the Zoo's clump during the embryonic development, while only 50% of the embryos of the clutch found in Mr Perkins' enclosure successfully hatched. Once their development was nearly completed, and they all were at advanced stages of growth, they were released into Noirmont pond. In 2003, one clump was found in the pond located in the Zoo enclosure and two in Mr Perkins' garden pond. By mid April they had all hatched and by the end of August, 40 metamorphs from the Zoo and 123 from Mr Perkins' site were released in the area surrounding Noirmont pond, which at that time of year was completely dry.

Zoo's enclosure	2001	2003
Number of clutches	1	1
Estimated number of live eggs	150	148
Date when clump was laid	14/03/01	12/03/03
Start hatching	-	01/04/03
End of hatching	04/04/01	15/04/03
Number of dead eggs after hatching	0	0
Embryonic developmental time (d)	21	34
Final number of tadpoles/froglets	116	40
% survival from egg	77.3	27.0

Mr Perkins' enclosure	2001	2003
Number of clutches	1	2
Estimated number of live eggs	100	160
Date when clumps were laid	08/03/01	26/03/03
First hatching	-	09/04/03
End of hatching	22/03/01	16/04/03
Number of dead eggs after hatching	30	0
Embryonic developmental time (d)	14	21
Final number of tadpoles/froglets	14	123
% survival from egg	14.0	76.9

Table 3.9. Summary data about the captive frog spawn development in 2001 and 2003. No clumps were laid in captivity in 2002.

3.4. DISCUSSION

For the first time in Jersey, data about the only wild population of agile frogs breeding on the island are now available, thanks to the intensive fieldwork undertaken during three consecutive years, from 2001 to 2003. The results obtained from the analysis of such data allow (1) a comparison with information found in the literature about populations of agile frogs living elsewhere in Europe; (2) an analysis of the main characteristics of the habitat at Ouaisné and of the structure of the *Rana dalmatina* population currently breeding at the site; (3) the isolation of the main factors responsible for the frogs' reproductive success in the wild and, finally, (4) an analysis of the causes of the failure and success respectively of past introductions and reintroductions of *Rana dalmatina* in Jersey. Each of these four points is discussed in more detail below.

(1) At Ouaisné, during the last three years *Rana dalmatina* started breeding from the beginning of February, and in this they appeared to be more similar to frogs inhabiting areas characterised by warm climates, such as Greece (Sofianidou & Kyriakopoulou-Sklavounou, 1983) than to those present in northern countries. For example, Riis (1991) reports that in Denmark breeding does not usually start before April. Also the duration of the spawning period, that in

Jersey varied between 12 and 25 days, was more comparable to that of the Greek frogs than to the much shorter one, between 3 and 6 days, characterising the Danish ones. As temperature, rather than rainfall (Lehman, 1977), influences the beginning of the frogs spawning phase, the fact that the climate in Jersey is usually considerably milder than in other regions comprised in the northern distribution range of *Rana dalmatina* may explain these findings. The egg clumps found at Ouaisné during the last three years were in some cases smaller than those reported in the literature (e.g. Angel, 1947; Terentev & Chernov, 1949; Dottrens, 1963; Riis, 1991). However, in most cases the *Rana dalmatina* clutches laid at Ouaisné contained between 400 and 1000 eggs, so their sizes were within those reported, for example, for populations of Greek agile frogs (on average, between 445 and 1761 eggs per clump) (Sofianidou & Kyriakopoulou-Sklavounou 1983). Also, clumps laid by frogs breeding in the former USSR had, on average, between 600 and 1400 eggs (Bannikov *et al.*, 1977, cited in: Sofianidou & Kyriakopoulou-Sklavounou 1983). The data collected for the present study cannot therefore confirm the locally accepted belief (Partridge, 1985) that the agile frogs lay fewer eggs in Jersey than elsewhere in Europe. However, when compared, for example, with Danish frogs measured by Riis (1991) in the wild, the male adult frogs caught in Jersey appeared to have lower mean body length and mass. Most of them were young (Fig. 3.15), which may explain why they were smaller. A further characteristic that is reported as being peculiar to the Jersey frogs (Partridge, 1995) is the fact that the skin of both adults and metamorphs is either reddish or green-greyish. No information about the presence of the latter variety in European *Rana dalmatina* populations was found in the literature. Although both varieties of skin coloration were observed and recorded during the present research, the results of the analysis of these data (Fig. 3.22) cannot confirm, nor reject, the theory expressed by Partridge (1995) that the red coloration is a cryptic device to allow concealment in the dead leaves of woodlands, typical habitat of *Rana dalmatina*, and that the grey coloration is the result of the Jersey frogs no longer inhabiting woodlands. More data need to be collected and experimental studies have to be performed to shed light on this intriguing matter.

(2) On the whole, a picture of Ouaisné as an adequate (Tab. 3.6) *Rana dalmatina* breeding site emerges from the results of the analyses of the data collected in 2001, 2002 and 2003. First of all, it appears to be a good habitat for the frogs during their terrestrial phase. For instance, quite a high percentage of the animals marked in 2002 returned the next year to the breeding ponds (Fig. 3.5), having therefore survived during their hibernation period. Also, they were considerably bigger in size and this reflects adequate food availability in their winter habitat. Moreover, the aquatic environment offered by the slacks is compatible with the needs of the agile frogs, as low rates of embryonic and tadpole mortality were recorded during all three years of surveys. In 2001, this

resulted in at least 2.4% of the tadpoles completing their development successfully and leaving the water bodies as froglets (Tab. 3.6). This is a good percentage, when compared to the expected 1% – 2% (e.g. Semlitsch, 2002) for wild anuran populations. In 2002 and 2003 the situation improved even further, with an increased number of clumps laid, reflecting higher numbers of females breeding at Ouaisné, and an exceptionally high – respectively 17.1% and 7.5% - recruitment of froglets (Tab. 3.6). The high level of recruitment in 2002 and 2003 may well have been associated with the fact that hatching success was improved in these years by protecting the spawn clumps from predators in nylon mesh bags (see Chapter 2). Finally, once the metamorphs – the most vulnerable phase of a frog' life - emerged from the water and dispersed in the surrounding area, they most likely did not suffer from heavy predation, since grass snakes, their most dangerous predators, presently populate Ouaisné Common in extremely low numbers (McMillan, A, pers. comm.). However, in the past, the situation has not always been so successful at Ouaisné, as data collected during the ten years preceding the start of the present research show. Even though standardised surveys were never undertaken until 2000 (Racca, 2000), the number of clumps laid in the slacks was annually recorded (Partridge, 1995), and is reported, together with the data for 2000 (from: Racca, 2000), 2001, 2002 and 2003, in Fig. 3.26. Moreover, the results of the skeletochronology analysis showed that most of the frogs currently breeding at Ouaisné are young individuals, rarely older than 2 or 3 years old (Fig. 3.15).

These results therefore suggest erratic recruitment of metamorphs at Ouaisné. Years of high reproductive success alternate with years characterised by poor reproductive success. For instance, in 2001 a relatively high number of metamorphs was produced at Ouaisné. As a consequence, in 2002 and 2003 many more animals were present at the site at night (Fig. 3.2) and
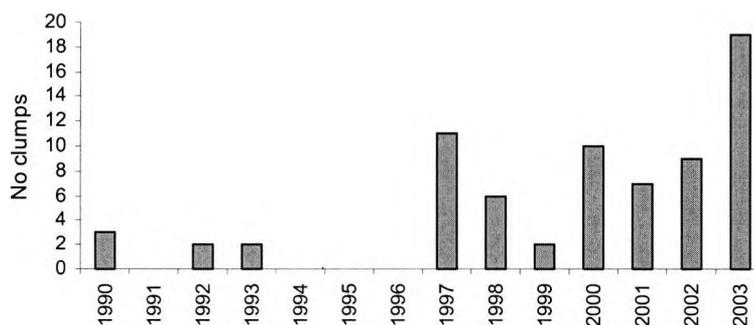
Figure 3.26. Number of *Rana dalmatina* egg clumps found at Ouaisné from 1990 to 2003 (data for 1990 – 1995 from: Partridge, 1995; data for 1996 - 2000 from: Racca, 2000).

two even more successful breeding seasons followed. The age of most adult males showed that they had lived their first winter in 2001/2002, and therefore confirmed the successful outcome of 2001's breeding activity.

(3) From the point of view of the long-term conservation of *Rana dalmatina* in Jersey, it is important to isolate the main factors responsible for the frogs' reproductive success, so that the slacks and the area surrounding them can be properly managed. For example, a much higher proportion of tadpoles successfully completed their development in 2002 and 2003 than in 2001, even though no apparent changes seemed to have occurred at the breeding site. The cause of such a difference may be found by analysing the mechanisms that regulate the relationships between the various components of the ecosystem to which the agile frogs belong. From all the observations made during the last three years, combined with those recorded in the past, the main factors influencing the frog aquatic phase are 1) hydroperiod, 2) water quality, 3) disturbance at the site, 4) competition and 5) predation. First of all, the ponds have to hold water from February at least until the emergence of metamorphs, usually starting at the beginning of June, which lasts several weeks. Since they were deepened by the ESU, in 1997, North and South Slack never dried before the end of July. Secondly, good water quality is required for the anurans' aquatic phase to be successful. So far, the results of water tests regularly carried out by the ESU show that this has never been a problem, so far, at Ouaisné. The level of disturbance at the site is the third factor that can influence the degree of breeding success, and this did not vary during the three years of study, as the area surrounding the slacks was usually very wet at least until April and did not attract any visitors. Then, the level of competition the tadpoles are subjected to may greatly affect their development. In the slacks, the effects of intra-specific competition can be considered to be low, as abundant resources are available to the tadpoles, compared to their density. The only other potential competitors are the common toads, *Bufo bufo*. Very numerous in the past (Le Sueur, 1976), they were present in the slacks in very low numbers in 2001 and 2002 and absent in 2003 (Tab. 3.7), thus it is reasonable to assume that no inter-specific competition influenced the tadpole development. The fifth factor, the predation pressures upon the tadpoles, is therefore likely to be responsible for the differences in recruitment observed between 2001 and the two following years. For this reason, the relationships between frog tadpoles and their aquatic predators were studied in depth, as reported in the next Chapter. Finally, other factors that influenced each year's *Rana dalmatina* reproductive success might have not been isolated during the present research, as it was not possible to carry out any extensive experimental study on the population.

(4) The surveys carried out for the present research also shed light on the status of *Rana dalmatina* reintroduction and introduction programmes, respectively in Noirmont and Grosnez

64

ponds. The occurrence of frog breeding observed in Noirmont pond in both 2002 and 2003, together with the presence of metamorphs in the area surrounding the water body in 2002 give hope for the success of the reintroduction of agile frogs in this site, historically supporting a well established wild population. The female found during 2003's breeding season was 3 years old, meaning that she was one of the individuals taken from one of the captive sites and released in the pond in 2000, the year when the programme started. The site therefore appears to be suitable for the frogs. The knowledge that common toads have now been breeding regularly at the site for the last few years and that the results of the water analyses annually carried out by the ESU did not show signs of water pollution reinforce this conclusion. However, only after several years of breeding or, more importantly, after second generation adults are found breeding at the site, the reintroduction may be considered a success (Semlitsch, 2002). On the other hand, Grosnez pond was not found to be a suitable breeding site for *Rana dalmatina*, as no frog spawning has occurred since 2000, and no adults were ever seen in, or around, the water body during the three years of surveys. Heavy newt predation on frog eggs (e.g. Racca, 2000), a high level of competition with the very numerous common toads – adults (Fig. 3.9) and tadpoles (Tab. 3.7) - present at the site and the fact that the pond tends to dry up early in the year, probably due to a geological fault, are all factors that hindered the establishment of a wild population of frogs at this site.

In conclusion, presently in Jersey Ouaisné Common represents the only truly successful wild breeding *Rana dalmatina* site, as factors such as abundance of resources, low levels of competition and relatively low predation pressures on the anurans (see Chapter 4) make it very favourable to the frogs. Noirmont pond, historically a *Rana dalmatina* breeding location, at the present time appears to be a promising reintroduction site for the species, but needs to be monitored further, so that its long-term suitability for the agile frogs can be assessed. On the other hand, the pond where an introduction of *Rana dalmatina* was attempted, Grosnez pond, did not prove to be suitable for the species. The factors responsible of such a result have now been identified and this acquired knowledge will prove essential when new introductions are planned. In fact, the risk of the agile frogs disappearing from the wild as a result, for example, of an environmental accident at Ouaisné, makes the creation of new breeding sites, where tadpoles and froglets can be introduced, from a good source such as the captive sites, a high priority conservation measure to be undertaken to try to assure a long-term presence of these amphibians on the island.

CHAPTER 4

AQUATIC PREDATORS OF *RANA DALMATINA* IN JERSEY

The main potential aquatic predators of agile frog eggs and tadpoles were palmate newts (Triturus helveticus*) and macroinvertebrates such as damselfly and dragonfly nymphs (Odonata),* *backswimmers* (Notonecta *spp.), water beetles (Ditiscidae,* Dytiscus marginalis *included) and* *water spiders* (Argyroneta aquatica*). The differences in recruitment observed between 2001 and the* *two following years (Chapter 3) may reflect changes in the types and numbers of tadpole predators* *present in the water bodies, and might also have been influenced by the presence (in 2002 and* *2003) or absence (in 2001) of protective mesh bags around the egg clumps. Despite the* Triturus helveticus *population being smaller in 2001 than in 2002 and 2003, the frog eggs were subjected to* *newt predation only in 2001. The same year, dragonfly nymphs (one of the two most voracious* *species of tadpole predators) and backswimmers were significantly more abundant than in 2002* *and 2003. On the other hand, only the damselfly nymphs proved to be significantly less abundant in* *2001 than in 2002, whereas the number of water beetles and aquatic spiders never significantly* *varied from one year to the next. The complexity of the relationships between the different* *components of an aquatic community, and the mechanisms controlling the dynamics of the food* *webs existing in it, cannot be exhaustively uncovered in a short-term study like the present one.* *Nevertheless, at Ouaisné, each year's hydroperiod seems to have had a prominent role in* *regulating the composition of the predator populations. For instance, due to an exceptionally dry* *winter, neither* Rana dalmatina *breeding ponds held any water from October 2001 to January* *2002, and this was certainly the cause of the significant decline in abundance of dragonfly nymphs,* *as their aquatic phase lasts at least one year. In conclusion, the results of the analysis of the data* *collected in the field from 2001 to 2003 suggest that at Ouaisné the size and structure of the wild* *population of agile frogs may be regulated by the level of predation pressures upon the aquatic* *phase of the life cycle. This, in turn, may vary from year to year, because of changes in* *hydroperiod and, therefore, in relative and absolute abundance of predator and prey within the* *aquatic ecosystem.*

4.1. INTRODUCTION

Despite the fact that it was not possible to estimate precisely how many froglets left the ponds, because of doubts about the reliability of the marking technique used (see Chapter 2), the year 2001 seemed to be relatively successful for the wild frogs at Ouaisné (Tab. 3.6). Nevertheless, as only 68 metamorphs were caught, corresponding to 2.7% of the number of eggs laid that year in the two slacks, it is reasonable to assume that the survival rate from egg to metamorphosis was much lower than in 2002 and 2003, when 17.1% and 7.5% of the eggs respectively completed their development successfully. Whilst data were being collected in the field, changes were noted in the composition of the population of tadpole predators, namely palmate newts and various species of aquatic macroinvertebrates, in North and South Slack between 2001 and the two following years. Considering that many studies have already highlighted the importance of the presence - and abundance - of certain predators on the final survival rate of anuran tadpoles (e.g. Babbit & Tanner, 1998; Clegg, 1974; Cooke, 1974; Gascon, 1992; Van Buskirk, 1988), it seems reasonable to assume that the level of predation pressures acting on the aquatic phase of *Rana dalmatina*'s life cycle may greatly influence the recruitment of metamorphs at Ouaisné.

The methods used to sample the most abundant aquatic predators of agile frog tadpoles in North and South Slack are described in the first section of this Chapter. The results of the analyses of the data collected are then discussed, to try to clarify the mechanisms regulating some of the aspects of the dynamics of the wild *Rana dalmatina* population in Jersey.

4.2. MATERIALS AND METHODS

4.2.1. PALMATE NEWTS

Once a month, in March, April and June 2001 funnel traps (Griffiths & Langton, 1998) were placed in North and South Slack, in the evening, between 07.00 hrs and 08.00 hrs, in order to catch palmate newts (*Triturus helveticus*). Twenty-two traps were used in North Slack and 25 in South Slack, placed at 2 metre intervals around the edge of the pond. After they were measured and weighed, newts were toe–clipped (Heyer *et al.*, 1994), using a date code for all individuals (i.e. second toe of their right front leg in March and second toe of their left front leg in April). In May, the code used in April was utilised again, not as a marking technique, but just to collect toes for possible future skeletochronology work. All the clipped toes were put in small plastic containers and preserved in 10% formalin. In 2002 and 2003, instead of placing traps in the ponds once a month, a single intensive trapping session was performed at peak season each year. Again, funnel traps were positioned one every 2 metres in North and South Slack, for 3 consecutive nights (2002: 14th, 15th and 16th April in North Slack and 17th, 18th and 19th April in South Slack; 2003: 9th, 10th

and 11th April in North Slack and 15th, 16th and 17th April in South Slack), in the evening between 19.00 hrs and 20.00 hrs. Twenty traps were used in North Slack in 2002 and 22 in 2003, whereas in South Slack 25 traps were used in both years. Each newt caught had the second toe of its right front leg clipped and the clippings were preserved in small individual plastic tubes filled with methylated spirit.

Finally, in 2002 and 2003, adult and juvenile palmate newts were caught, as they fell in the pitfall traps used to catch froglets and toadlets or walked along the fences (see Chapter 2), when emerging from the ponds to disperse in the surrounding area. They were counted and the location where they were found noted. Finally, they were released on the opposite side of the fences.

4.2.2. AQUATIC MACROINVERTEBRATES

Standardised netting sessions were performed in North Slack and South Slack, in order to catch, identify and calculate abundance and relative abundance of predatory macroinvertebrates inhabiting the ponds. In general, the methods used did not vary between years (Chapter 2), but the area of water sampled each year varied, depending on the size of each pond. In fact, in 2002 the size of the water bodies was smaller than in the two other years, whereas in 2003 the slacks appeared to be more or less the same size as in 2001. During 2001, the sweeping sessions were carried out on 17th April, 15th May and 20th June; in 2002 on 29th April, 29th May and 28th June; and in 2003 on 16th April, 28th May and 30th June. In 2001 and 2003, North Slack had, when measured a few days before the first sweeping session, an approximate total volume of 65.5 m^3, whereas South Slack had approximately 181 m^3. Both ponds were divided into three zones with different depths (Fig. 4.1A), low (from 0 to 60 cm), medium (60 – 120 cm) and high (120 - 180 cm). The sweeping volume unit was 8 m^3, therefore, during each session, in each water body, the area swept was smaller in the deepest zone than in the medium and shallow ones (Tab. 4.1). In 2002, due to an exceptionally dry winter (Fig. 4.8), both North and South Slack were smaller than in 2001 and 2003, with a volume of approximately 7.4 m^3 and 26.5 m^3 respectively, therefore the sweeping volume unit was reduced to 1 m^3. Both slacks were divided into three zones with different depths, low (from 0 to 30 cm), medium (30 - 60 cm) and high (60 – 120 cm) (Fig. 4.1A). Between the first and the two following sessions, the water level in both ponds lowered, and their total volume decreased. They were therefore divided into just two zones with different depths (Fig. 4.1B), low (0 – 30 cm) and medium (30 – 60 cm), but the sweeping volume unit remained unchanged.
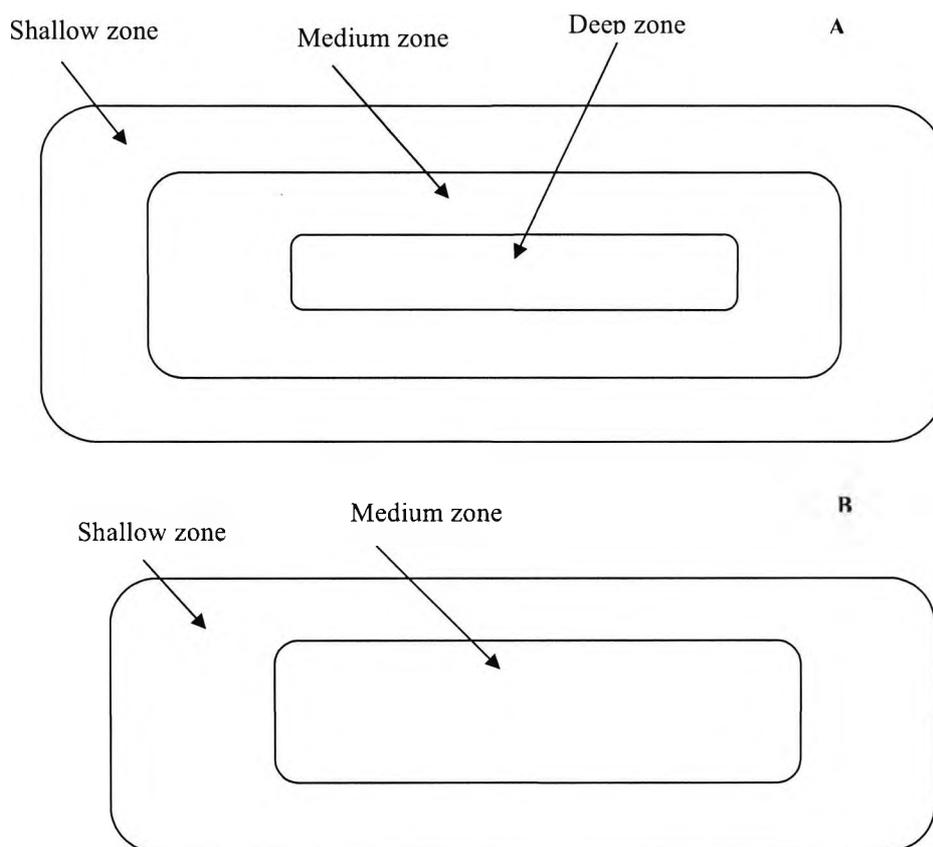
Figure 4.1. In 2001, 2003 and for the first session in 2002, three zones with different depths were identified in North and South Slack (A). During the second and third sweeping sessions of 2002, only two zones (shallow and medium) remained, as both ponds had partially dried (B).

	2001 (all sessions)	2002 (1st session)	2002 (2nd and 3rd sessions)	2003 (all sessions)
Shallow zone	27	6.7	6.7	27
Medium zone	7.3	2.2	2.2	7.3
Deep zone	5.3	1	/	5.3

Table 4.10. Area (m^2) swept in the shallow, medium and deep zones of the slacks during the netting sessions performed in 2001, 2002 and 2003 to catch aquatic macroinvertebrates.

All the invertebrates caught during the netting sessions were put in jars containing 70% ethanol and later identified with the help of guidebooks (Chinery, 1993; Clegg, 1980; Croft, 1986; Manuel & Shields, 1992).

69

4.3. RESULTS

4.3.1. PALMATE NEWTS

In 2001, 1 (in South Slack), 2 (1 in each pond) and 7 (6 in South and 1 in North Slack) newts were respectively caught in the first, second and third trapping session at Ouaisné. The following year, a total of 57 individuals (of which 5 were recaptured once) were caught in North Slack and 32 (of which 2 were recaptured once) in South Slack. Finally, in 2003, 12 and 37 individuals were caught respectively in North and South Slack. Just one newt, in South Slack, was recaptured once (Fig. 4.2).
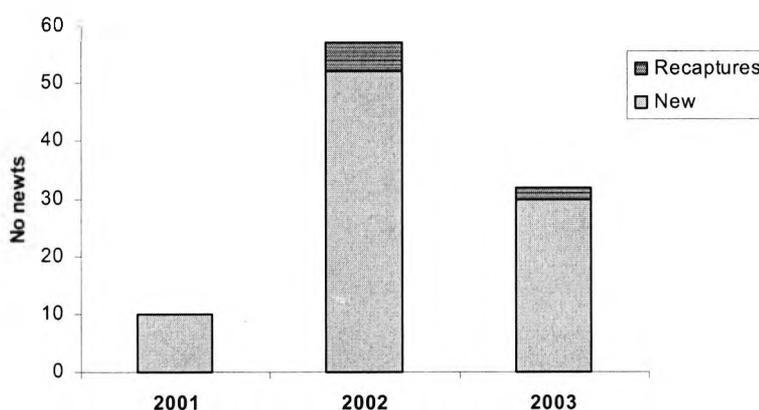
Figure 4.2. Number of new and recaptured newts in 2001, 2002 and 2003 at Ouaisné.

The "Weighted Mean Method" (Begon, 1979) was applied to estimate newt population sizes ($\check{N} \pm$ Standard Error) in 2002 and 2003, when capture - mark – recapture sessions were performed in each slack for three consecutive days. The results are reported in Tab. 4.2.

	$\check{N}_{2002} \pm SE$	$\check{N}_{2003} \pm SE$
North Slack	177 ± 24	47 ± 16
South Slack	182 ± 30	233 ± 38

Table 4.2. Newt population size ($\pm$ Standard Error), estimated with the "Weighted Mean Method", in North and South Slack, in 2002 ($\check{N}_{2002}$) and 2003 ($\check{N}_{2003}$).

A comparison between the average number of newts per trap found in North and South Slack during all trapping sessions in 2001, 2002 and 2003 was performed. The number of traps used every year differed between 2002 and 2001 and 2003, therefore 5 replicate groups of traps were considered for each pond, for each year, and the number of newts found in each replicate was averaged by the number of traps present in it. A two-way ANOVA test was then performed ("year" and "pond" being the two factors). No significant differences between ponds emerged (F= 0.06, df= 1, 24, p= 0.803). However, the results of the analysis showed that the mean number of newts per trap varied significantly between years (F= 22.29, df= 2, 24 and p< 0.001), and a post-hoc test (Fisher's LSD test) showed that it was significantly higher in 2002 than in both 2001 and 2003, as it illustrated in Fig. 4.3.
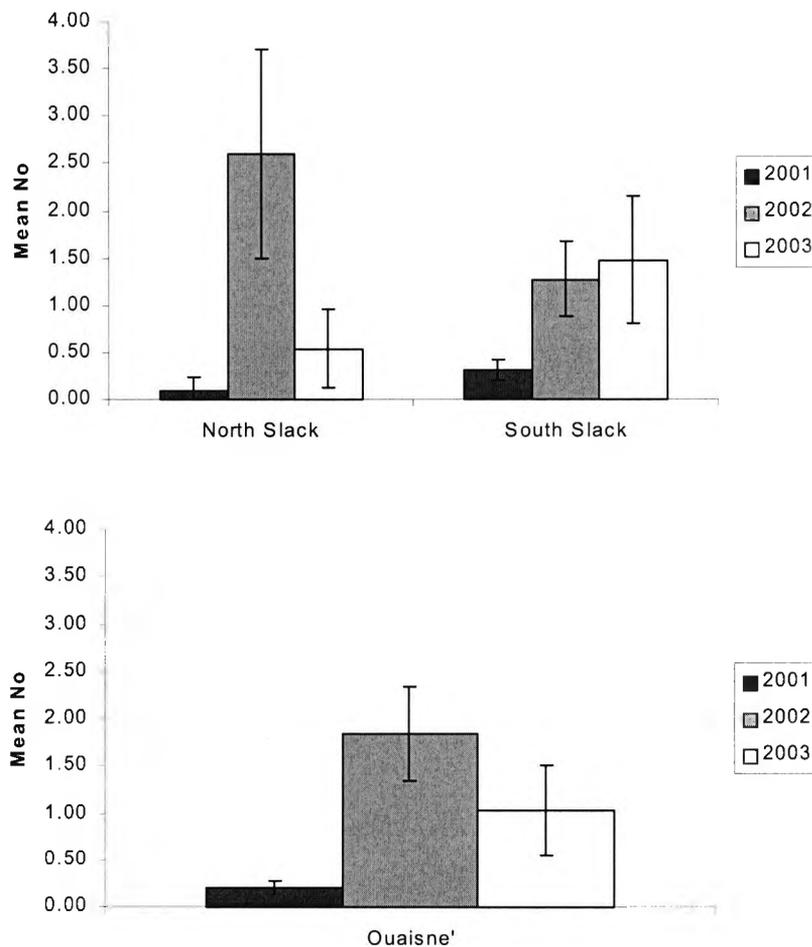
Figure 4.3. Mean number of palmate newts per trap, and standard deviations, in North and South Slack (above) and in both ponds considered together (below), in 2001, 2002 and 2003.

Finally, a one-way ANOVA test was also performed to compare the mean body condition, calculated as {mass (g)/ [SVL (cm)]^3} x 10^3, of the newts caught each year, to verify if the unavailability of frog eggs to the newts in 2002 and 2003 (as they were protected by mesh bags, see Chapter 2) had an impact on the newts' fitness. For this analysis, the data collected in both slacks were considered together, to avoid having too small samples. The result was that while the males appeared to have constant body condition during all three years (F= 0.38, df= 2, 77, p= 0.685), the females did not (F= 4.10, df= 2, 72, p= 0.021), and a post-hoc test (Fisher's LSD test) showed that they had significantly better body condition in 2003 than in 2002 (Fig. 4.4).
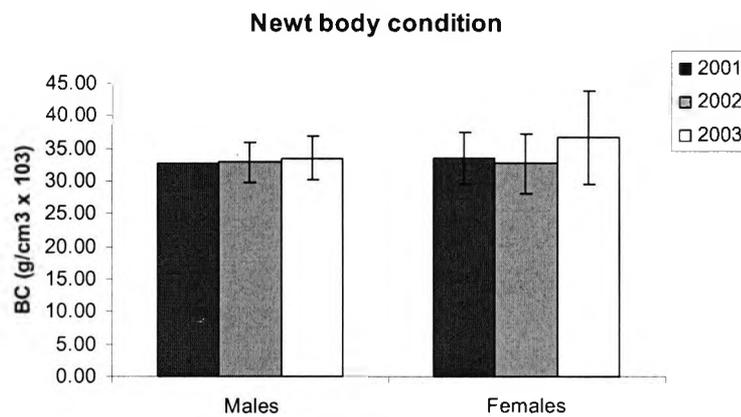
Figure 4.4. Mean body condition (g/cm^3 x 10^3), and standard deviations, of male and female palmate newts at Ouaisné, in both ponds, from 2001 to 2003.
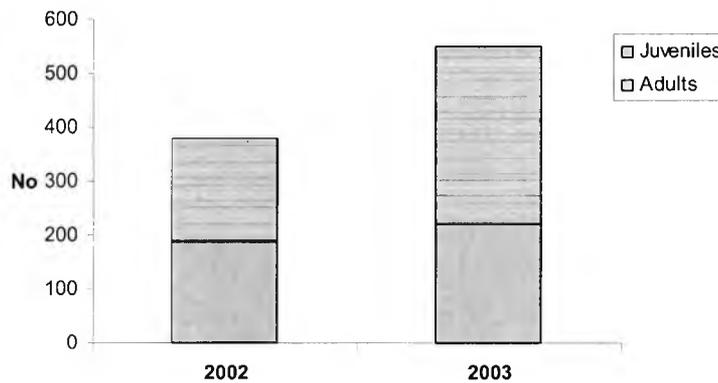
Figure 4.5. Number of adult and juvenile *Triturus helveticus* caught at Ouaisné in 2002 and 2003, in pitfall traps or along drift fences encircling the ponds.

72

Finally, in 2003 a higher number of juvenile newts were caught in pitfall traps or walking along the fences encircling North and South Slack than in the previous year (Fig. 4.5), despite fewer adults being seen at the breeding site in 2003 (Fig. 4.2, Tab. 4.2).

4.3.2. AQUATIC MACROINVERTEBRATES

Among the species of macroinvertebrates found in North and South Slack during the standardised sweeping sessions, damselfly and dragonfly nymphs (Odonata), backswimmers (*Notonecta* spp.), water beetles (Ditiscidae, *Dytiscus marginalis* included) and water spiders (*Argyroneta aquatica*) were identified as possible predators of amphibian tadpoles.

By counting the samples collected, abundance ($n=$ number of individuals of a species found in each pond) and relative abundance (as $p= n/N$, where N is the total number of individuals of all species found in each pond) were calculated. Abundance and relative abundance (the latter transformed in arcsine of its square root), during the three years of surveys, for each species of macroinvertebrates predator of tadpoles, were compared in North and South Slack, performing a multivariate analysis of variance (MANOVA). The months when the sweeping sessions were performed were also considered, as nested factors within the years. The results regarding the abundance values (Fig. 4.6) show significant differences in the invertebrate fauna between months (Wilk's $\lambda= 0.00009$, F= 5.635, df= 30, 18, p<0.0001) and between years (Wilk's $\lambda= 0.01195$, F= 6.518, df= 10, 8, p=0.007), but not between ponds (Wilk's $\lambda= 0.28717$, F= 1.986, df= 5, 4, p=0.263). Looking at the results of the univariate analyses done for each species, and of the post-hoc tests (Fisher's LSD test) performed when the results were significant, abundances of damselfly nymphs and backswimmers varied between months (F= 9.96, df= 6, 8, p= 0.002 and F= 44.4, df= 6, 8, p< 0.001 respectively), being present in the ponds in significantly higher numbers in June than in May and April. Moreover, in 2001 dragonfly nymphs and backswimmers were more abundant than in the two following years (F= 10.13, df= 2, 8, p= 0.006 and F= 33.28, df= 2, 8, p< 0.001 respectively), whereas damselfly nymphs were present in significantly lower numbers (F= 9.19, df= 2, 8, p= 0.008) than in 2002.
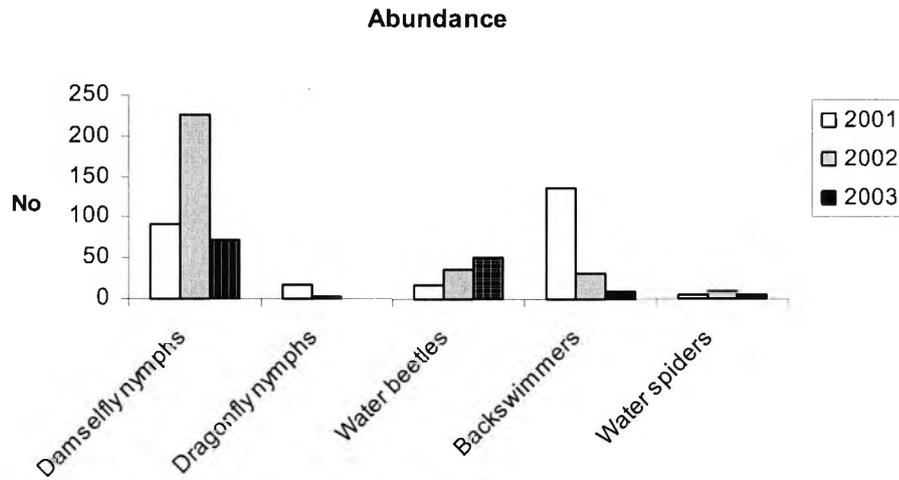
Abundance

Figure 4.6. Abundance of macroinvertebrate predators of tadpole at Ouaisné in 2001, 2002 and 2003.

The results concerning the relative abundance (Fig. 4.7) show a significant difference between years (Wilk's λ= 0.00887, F= 7.696, df=10, 8, p= 0.004), as dragonfly nymphs were proved to be, from the correspondent univariate analyses and post-hoc tests, significantly (F= 8.67, df= 2, 8, p= 0.010) more abundant in 2001 than in the two following years.

Relative abundance
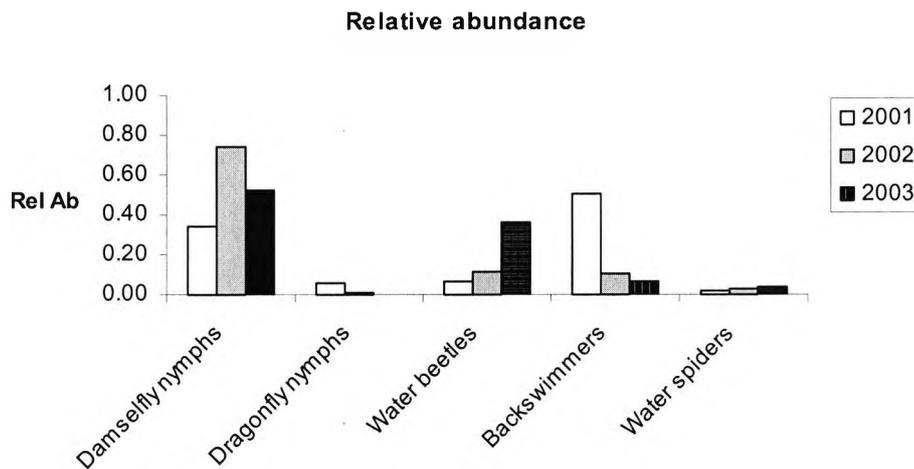
Figure 4.7. Relative abundance of macroinvertebrate predators of tadpole at Ouaisné in 2001, 2002 and 2003.

4.4. DISCUSSION

The level of predation pressures acting upon the aquatic phase of the anuran' life cycle varies between different stages of growth, and is one of the main factors responsible for the annual recruitment of metamorphs (e.g. Babbit & Tanner, 1998). During embryonic development, for instance, lack of mobility and small size make the eggs an easily available food resource to any species of predator inhabiting the pond, from newts, dragonfly nymphs and water beetles – adults and larvae, to backswimmers and damselfly nymphs. However, free-swimming tadpoles are already a much more difficult prey to catch, thanks to their ability to swim fast and hide amongst the vegetation or on the bottom of the pond. As tadpoles grow, the number of species that can predate on them decreases (Calef, 1973; Wilbur, 1980). It is unlikely, for example, given their average dimensions, that damselfly nymphs or water spiders can predate tadpoles after stage 26 (Gosner, 1960), as by then they normally measure at least 20 mm in length (Fig. 3.17). By the time the hind toes start to develop (stage 31), the tadpoles are over 30 mm long, with a body mass of at least 250 mg. This is the threshold size for the predation of *Rana temporaria* tadpoles by smooth newts (*Triturus vulgaris*) (Cooke, 1974). Considering that palmate newts are usually smaller than smooth newts (Arnold & Burton, 1985), it is reasonable to assume that newt predation in Jersey does not affect frog tadpoles once they reach stage 31. However, with their specialised mode of predation (e.g. Clegg, 1974; Gascon, 1992; Skelly & Werner, 1990; Van Buskirk, 1988) dragonfly nymphs and large Ditiscidae can successfully attack tadpoles in their final stages of growth, when they are over 40 mm long (McCormick & Polis, 1982). Thus the differences in recruitment observed between 2001 and the two following years may reflect changes in the types and numbers of tadpole predators present in the water bodies at Ouaisné. For instance, despite the fact that in 2001 the *Triturus helveticus* population was smaller than in 2002 and 2003 (Fig. 4.2), the frog eggs were subjected to newt predation as they were not protected by the mesh bags as they were in the two later years. Interestingly, the use of mesh bags did not have negative effects on the *Triturus helveticus* population size in the slacks (Fig. 4.3 and 4.5), or on the newt body condition (Fig. 4.4). Moreover, the same year, dragonfly nymphs (one of the two most voracious species of tadpole predators) and backswimmers were significantly more abundant than in 2002 and 2003 (Fig. 4.6). On the other hand, only the damselfly nymphs proved to be significantly less abundant in 2001 than in the following year, whereas the number of water beetles and aquatic spiders never significantly varied from one year to the next (Fig. 4.6). The complexity of the relationships between the different components of an aquatic community, and the mechanisms controlling the dynamics of the food webs existing in it, cannot be exhaustively uncovered in a short-term study like the present one. Nevertheless, at Ouaisné, each year's hydroperiod seems to have had a

prominent role in regulating the composition of the predator populations. The fact that, due to an exceptionally dry winter (Fig. 4.8), neither *Rana dalmatina* breeding ponds held any water from October 2001 to the following January was certainly the cause of the significant decline in abundance of dragonfly nymphs, as their aquatic phase lasts at least one year (Chinery, 1993).
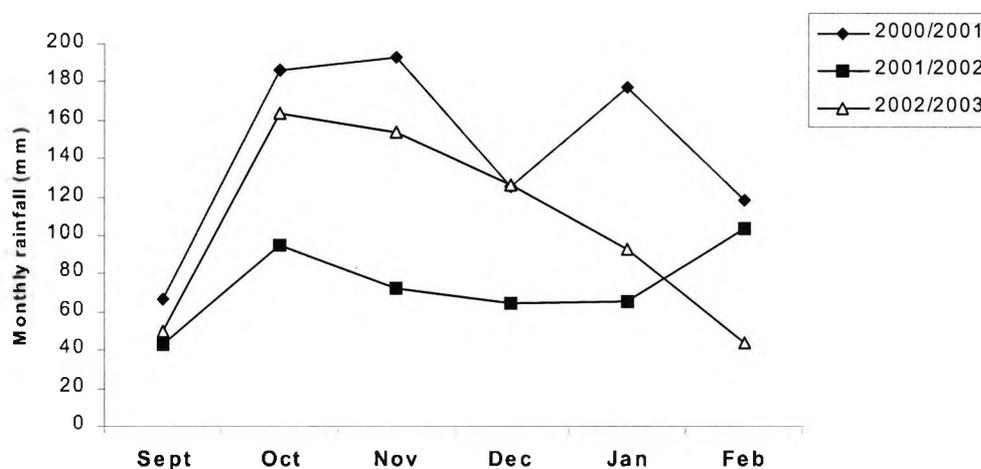
Figure 4.8. Total monthly rainfall (mm) recorded in Jersey from 2000 to 2003, during the autumn and part of the winter (data provided by the Jersey Meteorological Office).

In conclusion, the results of the analysis of the data collected in the field from 2001 to 2003 suggest that at Ouaisné the size and structure of the wild population of agile frogs may be influenced by the level of predation pressures upon the aquatic phase of the life cycle. This, in turn, may vary from year to year, because of changes in hydroperiod and, therefore, in relative and absolute abundance of prey and predators within the aquatic ecosystem.

CHAPTER 5

POPULATION VIABILITY ANALYSIS OF *RANA DALMATINA* IN JERSEY

Using a combination of the demographic data from Ouaisné and data from the literature, Population Viability Analysis (PVA) models were constructed to (1) predict the extinction risk of the Ouaisné frog population over 50 years, assuming no protection and protection of the spawn;(2) assess the feasibility of several agile frog introduction strategies; and (3) assess any variation of extinction risk predicted for the Ouaisné population over 50 years caused by different harvesting and translocation strategies. From the results of the analysis, it emerged that (1) unless protection of the spawn (or any other method enhancing productivity) is undertaken every year, the Jersey Rana dalmatina *population will be extinct in the medium term and very low adult survival appeared to be at least in part responsible for this; (2)* Rana dalmatina *populations, viable at least in the medium term (extinction risk ≤ 0.05), may be established in Jersey by introductions at sites of comparable habitat quality to Ouaisné (assuming protection of the spawn), by introducing relatively low numbers (from 20 to 120, depending on the strategy) of captive-bred metamorphs, and 1-year old frogs when available, over a three-year period. Finally, (3) relatively small numbers of metamorphs (not more than 60 over a three-year period) may be harvested from the Ouaisné frog population, in case they were needed for introduction programmes, as long as protection of the spawn is undertaken. Hence, even in case of scarce availability of animals from the captive populations, planned introductions would not need to be aborted and, even in years when spawning occurs in captivity, the joint use of captive and wild individuals would contribute to increasing genetic variability within the new introduced population. Collection of field data about* Rana dalmatina *ecology and dynamics should be continued in Jersey, as long-term data would allow more precise estimates of the models' parameters. As the uncertainty of the models' predictions decreases, PVA would become an increasingly useful tool that could help to secure the persistence of the agile frogs in Jersey.*

5.1. INTRODUCTION

Endangered species are threatened with extinction from both deterministic and stochastic factors. Examples of deterministic factors are habitat loss, overexploitation and pollution, introduced species and density-dependence (Brook *et al.*, 2002b; Marsh & Trenham, 2001). Stochastic events can be grouped into four categories: demographic, environmental, genetic stochasticity and catastrophes (Caughley, 1994; Simberloff, 1998). If a population is small, it is bound to be particularly susceptible to these forces (Simberloff, 1998). Population Viability Analysis (PVA) is a modelling tool that estimates the future population size and risk of extinction, implicitly dealing with the interacting deterministic and stochastic events affecting the population persistence in the wild (Brook *et al.*, 1997). It works by using life-history or population grow-rate data to parameterise a population model that is then used to project dynamics and estimate future population size and structure (Ludwig, 1999). Various user-friendly software packages (e.g. Brook *et al.*, 1997) are available for the assessment of population viability and this contributes in making PVA the most common form of risk assessment used in conservation biology (Caughley & Gunn, 1996). Several authors (e.g. Coulson *et al.*, 2001; Fieberg & Ellner, 2000; Ludwig, 1999) have expressed concerns about the precision of the predictions of PVA, pointing out that poor data generally cause difficulties in parameter estimation, which in turn leads to unreliable estimates of extinction risks (Ellner *et al.*, 2002). However, the quantitative predictions of many PVA models were tested with field data in various studies (Brook *et al.*, 1997; Brook *et al.*, 2002a; McCarthy & Broome, 2000; McCarthy *et al.*, 2001) and in some cases PVA predictions were found to be "surprisingly accurate" (Brook *et al.*, 2000). Despite its limitations, PVA is therefore generally perceived as one of the most powerful and persuasive tools in conservation biology (Reed *et al.*, 2002). This analysis has already been applied in studies regarding a variety of species (e.g. Hamilton & Moller, 1995; Kendall, 1998: McCarthy & Broome, 2000). For a list of examples and references of studies on many vertebrate, invertebrate and plant species, see: Lindenmayer *et al.*, 1993). In contrast, PVA models have been very rarely applied to amphibian species. In Britain, Halley *et al.* (1996) conducted a PVA to predict the persistence of the common toad (*Bufo bufo*) and of the great crested newt (*Triturus cristatus*) at their breeding sites. The metapopulation dynamics of the great crested newts were further modelled by Griffiths & Williams (2000, 2001) and by Griffiths (in press). Finally, PVA was used as a tool in planning the reintroduction of the pool frog (*Rana lessonae*) in Britain as part of the recovery programme for this species (Williams & Griffiths, 2001).

Surveys were carried out at the only natural breeding site of *Rana dalmatina* in Jersey from 2001 to 2003 (Chapter 2). Much lower metamorph recruitment was observed in 2001 than in the

two following years (Chapter 3). One of the factors likely to be, at least in part, responsible for such a difference was the fact that in 2002 and 2003 all frog egg clumps found in the breeding ponds were protected from predation by enclosing them in mesh bags (Chapter 2). The field data collected during the surveys were therefore used to calculate most of the parameters required for PVA and a model was constructed to compare the extinction risk of the Ouaisné frog population in the medium term, assuming no protection and protection of the spawn. Furthermore, as introductions of *Rana dalmatina* at new sites across Jersey are planned for the near future (Agile Frog Group, 2001), PVA models were used to assess the feasibility of several introduction strategies by predicting the extinction risk, over 50 years, of the population that would be established by each strategy. Finally, the possibility of taking some of the animals needed for such introductions from the frog population currently breeding at Ouaisné was also investigated. Models were therefore designed to assess any variation of extinction risk predicted for the harvested population over 50 years caused by different harvest strategies.

5.2. MATERIAL AND METHODS

5.2.1. COMPUTER PROGRAM

The computer program RAMAS Metapop, Version 4 (Akçakaya & Root, 2002) was used to build stochastic models incorporating the dynamics of the *Rana dalmatina* population currently breeding in Jersey.

5.2.2. OUTLINE OF THE MODELS

Three sets of models were constructed. First of all, models were designed to assess the viability of the *Rana dalmatina* population breeding at Ouaisné over 50 years, assuming a stable environment ("Spawn Management Models", Tab. 5.1). The extinction risk of the population was predicted under two scenarios (1) "No Protection": it was assumed that all clumps of frog eggs laid at the site were not protected with mesh bags, as was the case in the 2001 breeding season (Chapter 2), and (2) "Protection": all clumps were protected with mesh bags, as in 2002 and 2003 (Chapter 2).

The second set of models ("Introduction Models", Tab. 5.1) was designed to investigate the outcome of introductions of *Rana dalmatina* individuals in hypothetical new sites for three consecutive years. Several strategies were tested (Tab. 5.1). Each model predicted the extinction risk of the resulting population over a 50-year period. The first three models (2.1, 2.2 and 2.3) were alternatively tested assuming "No protection" and "Protection" of the eggs. They consisted of introductions of metamorphs only in the first year and of both metamorphs and 1-year old frogs in the second and third years. Number of individuals introduced and carrying capacity of the site

varied amongst models (see Tab. 5.1). The next three strategies (2.4, 2.5, 2.6), were applied only to the "Protection" scenario and differed from the previous ones because the carrying capacity of the introduction site was kept constant, equal to that assumed for Ouaisné (K=200). Models 2.6, 2.7 and 2.8 were specifically designed for the "No protection" scenario, as none of the strategies tested so far were successful unless protection of spawn was assumed. They consisted of introductions of 1-year old frogs during all three years, as they had a higher survival rate than metamorphs (see "Survival" section below). Number of individuals introduced and carrying capacity of the site varied among the models (see Tab. 5.1). Finally, as models 2.3, 2.4, 2.5 and 2.6 predicted extinction risks < 0.05, when assuming protection of the spawn, four new models (2.10 – 2.13) were constructed exclusively for the "Protection" scenario. They had the same N_o and K, respectively, as models 2.3 - 2.6, but only metamorphs were introduced each year (Tab. 5.1).

The third set of models ("Harvest Models", Tab. 5.1) investigated the possibility of taking some individuals from the frog population currently breeding at Ouaisné for introductions programmes such as those simulated by models from 2.1 to 2.6 (Tab. 5.1). The main assumptions of the harvest models were that only metamorphs would be harvested and that all clumps of eggs would always be protected (see Tab. 5.1). Each model predicted the extinction risk of the harvested (i.e. donor) population.

All models were age-structured and assumed a 1:1 sex ratio, as no sure indications of a male- or female-biased sex ratio emerged from the analysis of the field data. Male frogs seemed to be more abundant than females, at Ouaisné, during each year's breeding season (Chapter 3), but the number of females was probably underestimated. In fact, hardly any females were caught in the field, and their abundance had to be derived from the number of egg clumps laid each year in the ponds. This estimate therefore did not include young, non-sexually active individuals. In contrast, immature males were often the majority of the males caught (Fig. 3.15) and this perhaps increased the apparent discrepancy in the sex ratio. All models were replicated 500 times and the average extinction risk was calculated as the proportion of population simulations that declined to extinction over 50 years. Density dependence was incorporated using a simple ceiling model, as no data were available about density effects in post-metamorphic stage frogs (see "Demographic stochasticity" section, below). As true carrying capacities (K) for agile frogs are largely unknown, K was used in a broader sense within the models, mainly as a measure of habitat quality. Rates of recruitment and survival, population age-structure, fluctuations in survival and recruitment with environmental stochasticity were obtained from the data collected in the field, at Ouaisné Common, from 2001 to 2003 (Chapter 3), as described below. All these parameters were used to compile Le-

MODEL SUMMARY

0. ALL MODELS

N_o Replications: 500
Duration: 50 years
Density Dependence: Ceiling model
Stage Matrices and Standard Deviation Matrices: see Tab. II.3 and II.4 (Appendix II)
Sex Structure: Polygynous (3 F/1M)
Catastrophes: None
Dispersal: None

1. SPAWN MANAGEMENT MODELS

Initial abundance: 100
Carrying capacity (K): 200

Model 1.1. "No Protection". Fecundity (= recruitment) rates were based on 2001 data (Chapter 3).
Model 1.2. "Protection". Fecundity (= recruitment) rates were based on 2002 and 2003 data (Chapter 3).

2. INTRODUCTION MODELS

Initial abundance: 0
Characteristics of the models:

	Tot N_o/ Year	K	Scenario	Year 1	Years 2, 3
Model 2.1	40	80	No Protection, Protection	$N_m = N_o$	$N_m = N_{1yr} = 0.5_{No}$
Model 2.2	80	160	No Protection, Protection	$N_m = N_o$	$N_m = N_{1yr} = 0.5_{No}$
Model 2.3	120	240	No Protection, Protection	$N_m = N_o$	$N_m = N_{1yr} = 0.5_{No}$
Model 2.4	20	200	Protection	$N_m = N_o$	$N_m = N_{1yr} = 0.5_{No}$
Model 2.5	40	200	Protection	$N_m = N_o$	$N_m = N_{1yr} = 0.5_{No}$
Model 2.6	80	200	Protection	$N_m = N_o$	$N_m = N_{1yr} = 0.5_{No}$

	Tot N_o/ Year	K	Scenario	Years 1, 2, 3
Model 2.7	80	160	No Protection	$N_{1yr} = N_o$
Model 2.8	100	200	No Protection	$N_{1yr} = N_o$
Model 2.9	120	240	No Protection	$N_{1yr} = N_o$
Model 2.10	120	240	Protection	$N_m = N_o$
Model 2.11	20	200	Protection	$N_m = N_o$
Model 2.12	40	200	Protection	$N_m = N_o$
Model 2.13	80	200	Protection	$N_m = N_o$

[Tot N_o/Year= Total number of individual introduced in any one year; K= Carrying capacity; Year 1, Years 2,3= First, second and third year of introductions; N_m, N_{1yr}= Number of, respectively, metamorphs and 1-year old individuals introduced]

3. HARVEST MODELS

Initial abundance: 100
Carrying capacity (K); 200
Number of individuals harvested each year: Year 1= N_o + 0.5 N_o, Year 2= N_o, Year 3= 0.5 N_o

Model 3.1. N_o= 40
Model 3.2. N_o= 30
Model 3.3. N_o= 20

Table 5.1. Summary of PVA models created using RAMAS Metapop.

slie matrices of survival-fecundity schedules (Akçakaya & Root, 2002) and corresponding standard deviation matrices. All matrices are reported in Appendix II.

5.2.3. PARAMETERS USED IN THE MODELS

AGE STRUCTURE

Female agile frogs generally start breeding when they are 2 years old. Males reach sexual maturity when they are 1 or 2 years old (i.e. Riis, 1991, Sofianidou & Kyriakopoulou-Sklavounou, 1983). Whereas not all 1-year old males return to the ponds to breed, all 2 year olds are capable of breeding. However, some non-breeding 1-year old males may return to the water each year along with breeding individuals. The models therefore used, for each sex, a stable age distribution, based on 4 age classes: 0 yr (= metamorphs), 1yr, 2 yr and $\geq$ 3yr. All 0 yr frogs were not breeding animals, and so were all 1 yr females. Half of the 1 yr males were classified as "breeding" and so were all frogs aged 2, 3 or more.

SEX STRUCTURE

Males and females were included in the same matrix and had identical survival rates. As male agile frogs may mate with several females during the course of a breeding season (e.g. Kneitz, 1999), a polygynous mating system was incorporated with each male mating with up to 3 females.

SURVIVAL

Rates of survival of metamorphs could not be calculated from the data collected in the field. In the literature, no estimates of *Rana dalmatina* metamorph survival from long-term studies were found. However, several authors reported survival rates for other *Rana* species (see, for example: Berven, 1990; Biek *et al.*, 2002; Elmberg, 1990; Gibbons & McCarthy, 1984; Loman, 1984). In the present study, it was assumed that metamorphs had on average 0.25 ± 0.08 probability to survive to age one (value estimated for *Rana temporaria* in: Biek *et al.*, 2002). Adult survival was calculated using data collected in the field (Chapter 3). Two independent methods were used. (1) Jolly-Seber Method (Krebs, 1999), based on capture – mark – recapture of adults. Estimates of annual adult survival were calculated by grouping all data about capture and recapture of males (as hardly any females were ever caught, see Chapter 3) from a single year and treating it as a single sample date, thus giving a total of three samples (one for each year) (see Tab. II.1 in Appendix II). The result was an annual adult survival rate of 0.23. (2) Robson & Chapman Method (Krebs, 1999), based on data on the age composition of the population. Frogs caught at Ouaisné were aged with the skeletochronology technique in 2002 and 2003 (Chapter 3). To estimate annual adult survival, only the age structure of the male population was considered, due to lack of data for the females (see Tab. II.2 in Appendix II). The result was an annual adult survival of 0.32. The Leslie matrices of survival-fecundity schedules used in the RAMAS models therefore used a

survival rate of 0.275 $\pm$ 0.064, calculated as mean $\pm$ standard deviation of the estimates obtained from (1) and (2), for all age classes $\geq$ 1 yr.

FECUNDITY

Estimates of fecundity were based on the number of new individuals (metamorphs) generated by each female within each age class (i.e. recruitment). Fecundities for age classes 0 yr and 1 yr were zero (see "Age Structure" section). Data collected in 2001 were used to calculate fecundities included in the stage matrix "No protection" and data from 2002 and 2003 for the stage matrix "Protection" (Appendix II). As only a few female frogs were caught during the fieldwork (Chapter 3), the total number of females breeding in any one year was considered equal to the number of egg clumps laid in that year. Fecundity (number of metamorphs produced by each female in each age class > 1yr) was assumed not to vary among productive age classes and was calculated by dividing the number of froglets emerged every year by the number of females present at the site the same year. Standard deviation values for fecundity rates were also calculated in order to construct standard deviation matrices, thus incorporating environmental stochasticity into all models. The maximum and minimum numbers of eggs present in any one clump laid at Ouaisné over the three years of study were considered. Mean number of eggs per clump, its standard deviation and what percentage of the mean the standard deviation represented were calculated (Tab. 5.2). This percentage was then applied to the fecundity values previously calculated and the results were included in the standard deviation matrices reported in Appendix II.

Max No eggs/clump	Min No eggs/clump	Mean No eggs/clump	St. Dev.	St. Dev./Mean No
1000	250	558.1	169.9	30.4%

Table 5.2. Maximum, minimum, mean number of eggs per clump, and standard deviation of the mean, laid at Ouaisné over the three-year period of surveys. The percentage of the mean represented by the standard deviation is reported in the last column on the right of the table.

ENVIRONMENTAL STOCHASTICITY

RAMAS Metapop modelled environmental stochasticity assigning to each vital rate, at each time step, a random variate drawn from a lognormal distribution (Akçakaya & Root, 2002). The mean of this distribution was taken from the stage matrices and the standard deviation matrices reported in Appendix II.

DEMOGRAPHIC STOCHASTICITY

Demographic stochasticity was incorporated in all models. RAMAS Metapop modelled it by sampling the number of survivors from a binomial distribution and recruitment from a Poisson distribution (Akçakaya & Root, 2002).

CATASTROPHES

The impacts of extreme environmental events were not incorporated into the models, as none were witnessed during the three-year study.

DISPERSAL

Metamorph and adult dispersal was not incorporated into any of the models. The Ouaisné population and the hypothetical populations established with the introductions were considered closed.

5.2.4. ANALYSES OUTPUT: EXTINCTION RISK AND QUASI-EXTINCTION TIME

RAMAS Metapop calculated extinction risk as the probability that the population abundance declined below a range of abundances at the end of the duration of the simulation (Akçakaya & Root, 2002). The program calculated the 95% confidence intervals for the risk curves by using the Kolmogorov-Smirnov test statistic, D (Akçakaya & Root, 2002).

When the number of individuals declines to a critically small size, the behaviour of a population becomes increasingly difficult to predict due to the impacts of demographic stochasticity and Allee effects; this can result in estimates of the time to total extinction being unreliable (Akçakaya & Root, 2002). The time to quasi-extinction refers to the risk of decline to a threshold level of abundance, below which demographic stochasticity and Allee effects are expected to dominate the behaviour of populations (Akçakaya & Root, 2002). The threshold at which demographic stochasticity and Allee effects were assumed to be important was two individuals, therefore quasi-extinction of a population occurred when the total number of individuals declined to two. RAMAS Metapop calculated the median time to quasi-extinction, the probability that the population declined to the threshold level at a given time step (depicted as a histogram) and the cumulative probability distribution of quasi-extinction probabilities. The cumulative probability curve showed the probability that the population would have declined to the threshold level at or before a given time step; this was estimated by summing the values of the vertical histogram bars up to and including the given year (Akçakaya & Root, 2002).

5.3. RESULTS

5.3.1. SPAWN MANAGEMENT MODELS

The population of agile frogs currently breeding at Ouaisné was found to have a 100% probability of extinction over the next 50 years, with a 10-year median time to quasi-extinction (Fig. 5.1), when it was assumed that the clumps of eggs laid at the site were not protected. Protection of the spawn, however, considerably reduced this risk (Fig. 5.2), effectively making the population viable for at least 50 years (Tab. 5.3).

Model	Scenario	Extinction Risk	95% Confidence Interval	Time to extinction (median)
1.1	No Protection	1	0.9604– 1.000	10
1.2	Protection	0.02	0.000 – 0.060	> 50

Table 5.3. [Models 1.1 and 1.2]. Extinction risk (with 95% confidence interval) and time to extinction (years) for the population of agile frogs breeding at Ouaisné when their eggs were not protected ("No Protection") and when they were ("Protection").
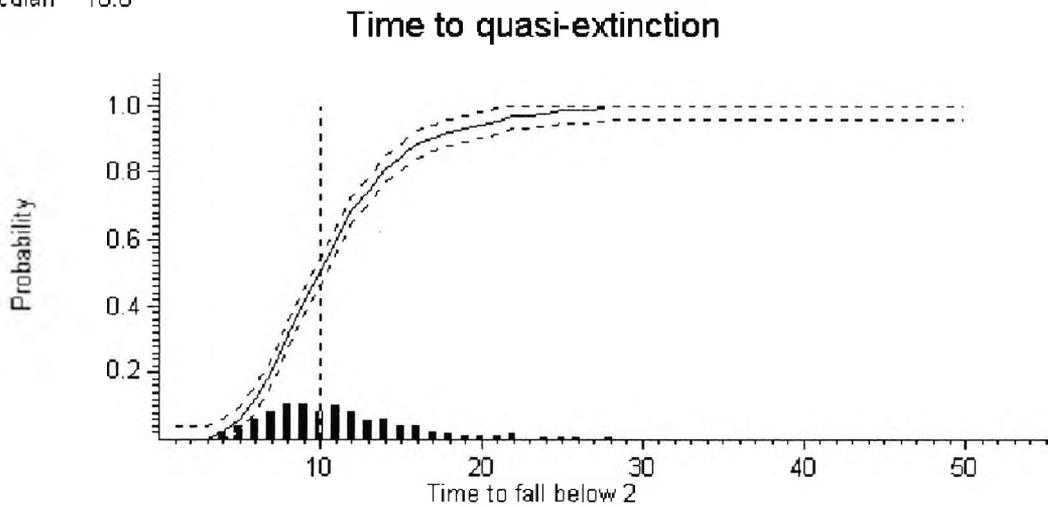
Figure 5.1. [Model 1.1]. Predicted time (in years) to quasi-extinction for the population breeding at Ouaisné in case of no protection of the frog clumps. The median value gives the median number of years to quasi-extinction, indicated by the vertical dotted line. The distribution of quasi-extinction times is illustrated by a histogram: the height of each vertical bar indicates the probability that the population will have declined to 2 individuals at exactly a specific time step. The cumulative probability curve shows the probability that the population will have declined to 2 individuals at or before a given time step. The dashed lines either side of the risk curve indicates the 95% confidence intervals (Akçakaya & Root, 2002).

Extinction risk ~ 0.02

Terminal extinction risk
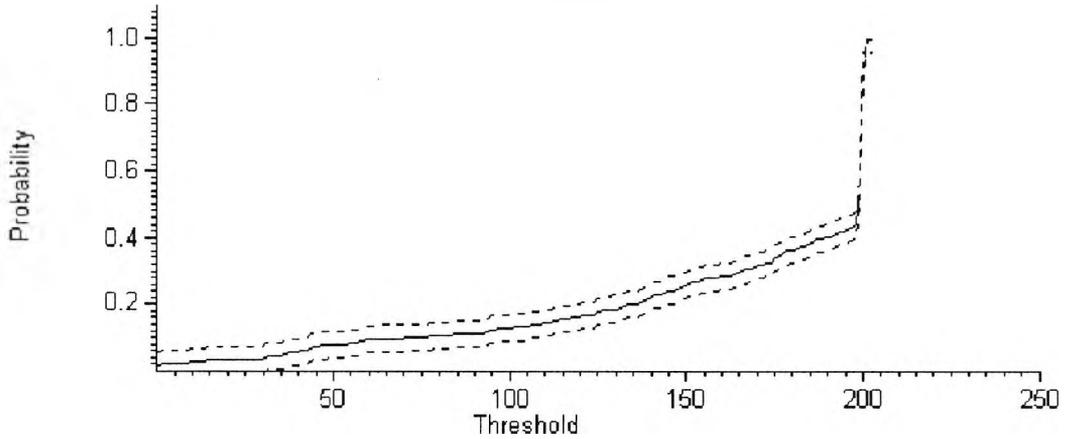
Figure 5.2. [Model 1.2]. Extinction risk for the *Rana dalmatina* population breeding at Ouaisné over 50 years, when the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps. The dashed lines either side of the risk curve indicates the 95% confidence intervals.

The predicted population size over 50 years is shown in Fig. 5.3 and the final stage abundances predicted for the end of the simulation period in Fig. 5.4.

Trajectory summary
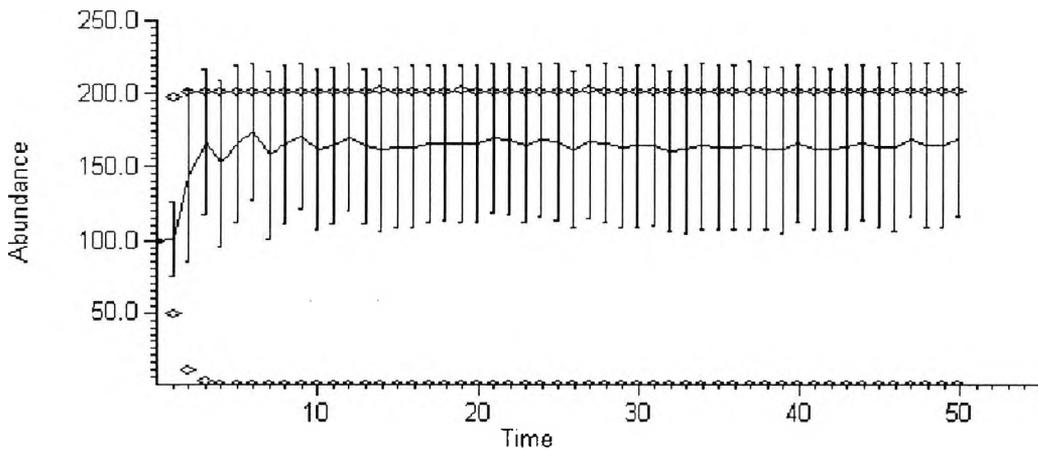
Figure 5.3. [Model 1.2]. Predicted population size over 50 years. A statistical summary of the abundance of the population as it changes through time is shown. The average, ± standard deviation, minimum and maximum abundances are reported.

86

Final stage abundances
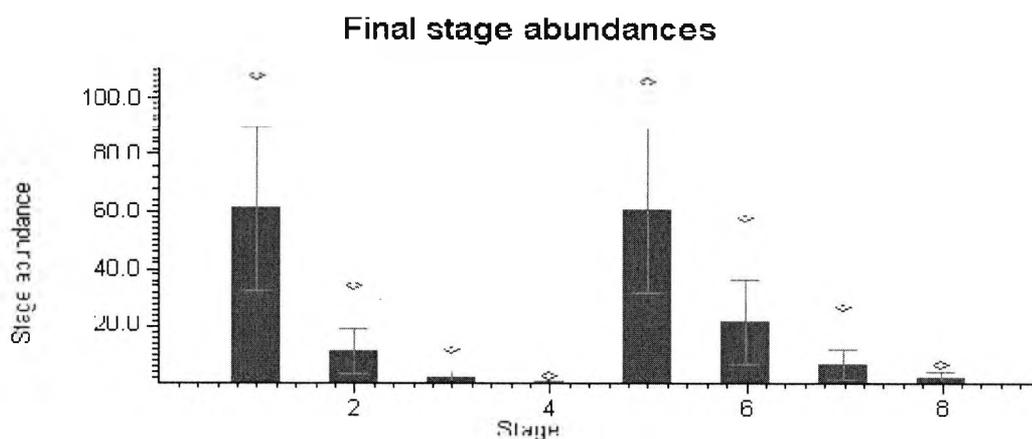
Figure 5.4. [Model 1.2]. Histogram of the distribution of individuals to different stages in the Ouaisné population of frogs at the final step of the simulation (50th year) (Stages 1/5= female/male metamorphs; stages 2/6= female/male 1-year old frogs; stages 3/7= female/male 2 years old; stages 4/8= female/male 3 years old or older individuals).

5.3.2. INTRODUCTION MODELS

None of the introduction strategies simulated assuming no protection of spawn were successful. In all cases the resulting population was extinct before the end of the 50-year period considered in the simulations (Tab. 5.4). Times to quasi-extinction predicted by each model are shown in Appendix II, Fig. II.1 – II.6.

Model	Tot No/Year	K	Extinction Risk	95% Confidence Interval
2.1	40	80	1	0.960 – 1.000
2.2	80	160	1	0.958 – 1.000
2.3	120	240	1	0.958 – 1.000
2.7	80	160	1	0.960 – 1.000
2.8	100	200	1	0.960 – 1.000
2.9	120	240	1	0.956 – 1.000

Table 5.4. Extinction risk (with 95% confidence interval) predicted over a 50-year period, for each of the introduction strategies simulated assuming no protection of spawn.

On the other hand, most of the extinction risks predicted for the introduction models simulated assuming protection of spawn were low and in six cases below 0.05 (Tab. 5.5). Among these, two (2.10 and 2.13) presupposed introductions of just metamorphs for three consecutive years. The time to quasi-extinction curve for model 2.1 and predicted extinction curves for all other models are reported in Appendix II, Fig. II.7 – II.16.

Model	Tot No/Year	K	Extinction Risk	95% Confidence Interval
2.1	40	80	0.76	0.722 – 0.802
2.2	80	160	0.06	0.020 – 0.100
2.3	120	240	0.01	0.000 – 0.046
2.4	20	200	0.03	0.000 – 0.070
2.5	40	200	0.02	0.000 – 0.058
2.6	80	200	0.01	0.000 – 0.052
2.10	120	240	0.01	0.000 – 0.048
2.11	20	200	0.27	0.226 – 0.306
2.12	40	200	0.08	0.044 – 0.124
2.13	80	200	0.02	0.000 – 0.066

Table 5.5. Extinction risk (with 95% confidence interval) predicted over a 50-year period, for each of the introduction strategies simulated, assuming protection of the clumps of eggs laid in the water body each year.

5.3.3. HARVEST MODELS

The best harvest strategy was the one, among the three, that presupposed harvesting the lowest number of individuals from Ouaisné, in total, over the three-year period. However, it caused the extinction risk of the donor population to increase from 0.02 (Tab. 5.3) to 0.06 (Tab. 5.6). In Appendix II, the graphs in Fig. II.17, II.18 and II.19 report extinction risk curves for the donor population for each harvest model.

Model	Year 1	Year 2	Year 3	N_{tot}	Extinction Risk	95% Confidence Interval
3.1	60	40	20	120	0.10	0.060 – 0.140
3.2	46	30	14	90	0.08	0.040 – 0.120
3.3	30	20	10	60	0.06	0.018 – 0.098

Table 5.6. Extinction risks predicted for the donor population for each of the harvest models (N_{tot}= total number of individuals harvested over three years).

5.4. DISCUSSION

The models here presented had several major limitations. The data used to calculate most of the models' parameters reflected the characteristics that the frog population breeding at Ouaisné had for just three years. As amphibian populations can fluctuate greatly over time (Pechmann *et al.*, 1991), it was a too short period of time to allow any generalisations, and therefore precise predictions. Moreover, some of the parameters, essential components of the Leslie survival-fecundity matrices used in all models, could not be drawn from the field data, for example the survival rate of metamorphs and the juvenile survival. A value of metamorph survival was therefore taken from the literature and the survival rate of juvenile frogs was considered equal to that of the adults. It is impossible to establish the accuracy of such assumptions for *Rana dalmatina*

[although the parameters could be tested by performing a sensitivity analysis (e.g. Cross & Beissinger, 2001)]. Furthermore, the lack of knowledge about the response of the species to threats (i.e. density dependence), about the population genetics (i.e. inbreeding coefficient) and the dynamics of adult dispersal added a great deal of uncertainty to the model predictions (Burgman & Possingham, 2000). However, PVA can be used solely for comparison, to evaluate the effectiveness of different management options (Brook *et al.*, 2000). Hence, the predictions of the models designed to assess the viability of the population of frogs in Jersey, and of those built to plan introduction and harvest strategies, may nevertheless be useful, as long as they are interpreted more in a qualitative rather than a quantitative manner. From the results of the analysis, three main conclusions can be drawn. (1) Unless protection of the spawn is undertaken every year to enhance productivity, the *Rana dalmatina* population currently breeding at Ouaisné will be extinct in the medium term (Tab. 5.3, Fig. 5.1). Very low adult survival appeared to be at least in part responsible for this. The adult frogs in Jersey seemed to have just under 28% probability to survive from one breeding season to the next, whereas, on average, various species of frogs were found to have between 38% and 69% probability of adult survival (Biek *et al.*, 2002; Gibbons & McCarthy, 1984). If the clumps of eggs are protected (or productivity is enhanced by some other method, such as predator control), the population will be likely to avoid extinction for at least 50 years (Fig. 5.2). Moreover, at the end of this period the youngest individuals will comprise most of the population (Fig. 5.4). This result was consistent with observations in the field (Chapter 3). It reflected the necessity of constant metamorph recruitment for the population to be viable, as adult survival was low and the population completely isolated. (2) *Rana dalmatina* populations, viable at least in the medium term (extinction risk $\leq$ 0.05), may be established in Jersey by introductions at new sites. Assuming protection of the spawn, metamorphs, and 1-year old frogs when available, should be introduced to sites of comparable habitat quality to Ouaisné. The animals could be taken from the captive populations (Chapter 2). Introductions would generally require relatively low numbers of animals and would last three years in total (Tab. 5.5). As no adults would be needed, low numbers of metamorphs would have to be reared in captivity just until they were 1 year old, and this would not entail major costs or labour. (3) Relatively small numbers of metamorphs (not more than 60 over a three-year period, see Tab. 5.6) may be harvested from the agile frog population that currently breeds at Ouaisné, in case they were needed for introduction programmes, as long as protection of the spawn is undertaken. This means that, even in case of scarce availability of animals from the captive populations, planned introductions would not need to be aborted. Moreover, even in years when spawning occurs in captivity, the joint use of captive and wild individuals would contribute to increasing genetic variability within the new introduced population.

In conclusion, this analysis showed how even a short term study focusing on the dynamics of a population can provide useful information for the construction of models that can help in planning conservation measures. Collection of field data about *Rana dalmatina* ecology and dynamics should therefore be continued in Jersey, as long-term data would allow more precise estimates of the models' parameters. As the uncertainty of the models' predictions decreases, PVA would become a very useful tool, among a variety of other decision-making aids (Ellner *et al.*, 2002), that could help to secure the persistence of the agile frogs in Jersey.

CHAPTER 6

MICROSATELLITE DNA ANALYSIS OF *RANA DALMATINA* IN JERSEY

Microsatellite DNA loci were employed to measure the level of genetic variation within the endangered population of Rana dalmatina *in Jersey and to compare it to three other populations in other parts of the species' range. Since no primers specifically isolated for* R. dalmatina *were available, eleven* R. temporaria *and six* R. latastei *primers were used. It emerged that five* Rana temporaria *and two* R. latastei *loci were also present in DNA extracted from* R. dalmatina. *Four of these loci were polymorphic in at least one of the populations of frogs analysed, thus allowing a comparison of genetic variation between the four populations studied. The Jersey population was the least variable, as only one locus was not monomorphic. Considering the present and past small size of this population, and its total isolation, drift and inbreeding seem likely to have caused such low variation. The most variable population was from Germany, which is approximately at the centre of the range of* Rana dalmatina. *On the other hand, the population from northern Italy, where the Alps represent an important geographical barrier to the frogs' movements, showed little variation, being monomorphic for 2 out of 4 loci. These findings have important practical consequences and open a series of new research opportunities that could ultimately provide knowledge essential for the future management of* Rana dalmatina *in Jersey, its habitat and its captive breeding programme.*

6.1. INTRODUCTION

Small populations tend to be considered under the highest threat of imminent extinction, simply because of their size (Caughley & Gunn, 1996). However, cases are known of very small populations that have persisted for a very long time, like, for example, the Socorro Island hawk *Buteo jamaicenensis socorroensis* population, that comprises just 20 pairs and has probably been this small throughout its entire history (Walter, 1990). Also, introduced species with large population sizes and geographical ranges often originated from very few founders (e.g. Skellam, 1951; Simberloff, 1989). Nevertheless, systematically collected observations of small populations show that, once they are down to what is defined as their "minimum viable size" (see, for example: Lande, 1988; Simberloff, 1988), they have an extremely high probability of extinction. Hence, as theorised in the "Small Population Paradigm" (Caughley, 1994) several forces may weigh disproportionately on small populations, as an effect of their smallness, as opposed to the "Declining Population Paradigm", based on the assumption that if a population is under threat, it means that something external has changed, the current size of the population being of no great relevance (Caughley, 1994). The most important amongst these forces are demographic stochasticity, environmental stochasticity and genetic threats (e.g. Simberloff, 1998). In Chapter 5, the first two are discussed in detail, in relation to the probability of long-term persistence of *Rana dalmatina* in Jersey. Aspects of the genetics of the agile frog population breeding on the island are the subject of the present section. Microsatellite DNA loci, used as molecular markers, were employed to try to measure the level of inbreeding in the Jersey frog population, as this is essential information when carrying out a species recovery plan. Microsatellites (or simple sequence repeats, SSRs) are short tandemly repeated sequence motifs with units of 1 – 6 base pair length, found in both prokaryotic and eucaryotic genomes. They are present in both coding and noncoding regions of the DNA and usually show a high degree of polymorphism (Jehle & Arntzen, 2002). The main characteristic that makes them good molecular markers to use when studying population genetics is their high variability, thus they can be used to obtain information on the allele frequencies in populations (Zane *et al.*, 2002). Microsatellites are amplified with specific PCR (Polymerase Chain Reaction, see for example: Burke *et al.*, 1992) primers and the different alleles separated along an electrophoretic gradient in routine laboratory procedures. The major drawback of microsatellites is that they need to be isolated *de novo* from species that are being examined for the first time, like the agile frogs, and this is an expensive and time consuming process (Parker *et al.*, 1998). However, cross-species amplification is sometimes possible (e.g. Rico *et al.*, 1996). As primers isolated for two species of brown frogs, *Rana temporaria* and *R. latastei,* were available, they were

used in the present study to assess the occurrence of inbreeding in the Jersey *R. dalmatina* population.

6.2. Materials and Methods

6.2.1. Sampling of Rana dalmatina

Samples of *Rana dalmatina* were collected in Jersey, France, Italy and Germany (see Tab. 6.1 for details of sampling locations, number of samples collected and dates).

Population	N_o	Collected by	Date	Location
Jersey	40	L. Racca	2002	Ouaisné Common
France	40	L. Racca	2002	Bois d'Ardennes (Avranches)
Italy	39	Dr. E. Marzona	2003	Airasca (Torino)
Germany	30	Dr. A. Kupfer	2003	Brühl
	10	Dr. T. Bobbe		Dieburg

Table 6.1. Details of *Rana dalmatina* sampling (N_o = number of samples collected at each location).

In Jersey, where the agile frogs are highly endangered because of their low numbers (see Chapter 3), no animals were sacrificed for this study. Grown tadpoles were caught in each of the two main *Rana dalmatina* breeding ponds and their tails were clipped, as described in Chapter 2. In France, 4 hatching tadpoles, still feeding from the jelly, were collected from each of a total of 10 clumps. In Germany and Italy, respectively 40 (from two different sites, see Tab. 6.1) and 39 free swimming tadpoles were sampled when they were at least at stage 31 of Gosner's table (1960). All samples were preserved in Eppendorf tubes filled with pure ethanol.

6.2.2. DNA Extraction

For the first series of tests (see next section), DNA was extracted from Jersey and French samples following a phenol/chloroform extraction protocol (modified from: Sambrook *et al.*, 1989). Small (ca 3 mm) sections of tadpole tail tips were used. To each, 585 µl TES buffer (50 mM Tris-HCl pH 8, 25 mM EDTA and 0.1 M NaCl), 60 µl Tris buffer (1 M Tris-HCl pH 8), 25 µl SDS [10% (w/v) SDS], 5 µl DTT (1 M Dithiothreitol) and 25 µl proteinase K were added. After thorough mixing, the tubes were incubated at 55°C overnight. Then, 0.5 ml phenol/chloroform (an equal volume mixture of water-saturated phenol and chloroform) was added and each tube was mixed thoroughly for about 1 minute and then centrifuged at 5,000 rpm for 5 min. The aqueous layer was removed and transferred to a new Eppendorf tube. The aqueous layer was then once

again extracted with 0.5 ml of phenol/chloroform, as above. Then, 50 μl 3 M sodium acetate was added to the clean aqueous layer, followed by 1 ml pure ethanol. This was mixed thoroughly and left for at least 1 hour at 4°C. The DNA was then pelleted by centrifugation at 13,000 rpm for 10 min and the supernatant was removed and discarded. Then, 1 ml wash buffer (30% 0.1 M Tris-HCl pH 8, 70% ethanol) was added to each tube. This was again centrifuged at 13,000 rpm for 5 min. The supernatant was discarded and the pellet was dried under vacuum until completely desiccated. The final pellet was dissolved in 100 μl sterile distilled water and stored at -20°C.

For the second and third series of tests (see sections 6.2.4. and 6.2.5.), DNA was extracted from samples from all locations using an adaptation of a Chelex-100 protocol (Walsh *et al.*, 1991). Small sections (ca 3 mm) of tadpole tail tips were incubated overnight at 56°C in an Eppendorf tube containing a mixture of 40 μl Chelex suspension and 160 μl sterile distilled water. Each extract was then vortexed, immersed in a boiling water bath for 8 min, vortexed again and centrifuged at 13,000 rpm for 3 min. The supernatant was then removed and kept, as source of DNA, at -20°C.

6.2.3. TESTING FOR RANA TEMPORARIA *AND* R. LATASTEI *MICROSATELLITE LOCI IN* R. DALMATINA *DNA*

Eleven primer pairs for *Rana temporaria* and 6 for *R. latastei* microsatellite loci were tested with DNA extracted from *R. dalmatina* (see Tab. 6.2 for references). PCR reaction mixtures (20 μl in total) contained: 1μl (25 - 100 ng) DNA, 2μl reaction mix from Genpack (150 mM ammonium sulphate, 100 mM Tris-HCl pH 8 and 35 mM $MgCl_2$), 0.2 μl nucleotide mix [10 mM dGTP, tri-Na/1.5 H_2O (5.8 mg/ml), 10 mM dCTP, di-Na 2 H_2O (5.5 mg/ml), 10mM dTTP, tri-Na (5.5 mg/ml) and 10mM dATP, di-Na/2H_2O (6 mg/ml)], 0.04 μl *Taq*EXPRESS DNA polymerase (20U/μl, GenPak), 0.8 μl of 10μM primer pair (0.4 μl Forward and 0.4 μl Reverse) for the first 10 loci of Tab. 6.2, or 0.2 μl (0.1 μl of F and 0.1 μl R) for all the others and, finally, sterile distilled water up to 20μl. PCR reactions were performed using a Techne PHC-3 thermal cycler, at the conditions described in Rowe and Beebee (2001a) and Garner & Tomio (2001), although in some cases annealing temperatures were lowered (see Tab. 6.2). For the first 10 loci listed in Tab. 6.2, the thermal cycler used the following programme: one cycle of 94°C for 4 min followed by four to eight touchdown cycles: 94°C for 1 min, annealing temperature for 1 min (Tab. 6.2), 72°C for 1 min. Touchdown temperatures generally decreased in 2 °C steps (two cycles per touchdown step) until the final annealing temperature was reached, with a total of 35 cycles altogether. For R*temp*μ1, R*temp*μ3 and R*temp*μ5, the final touchdown step was 3 °C.

Locus	Ts (°C)	Ta (°C)	N_o cycles at Ta	Reference	GenBank accession No
R*temp*μ1	66	53	23	Rowe &Beebee, 2001a	AF297972
R*temp*μ2	62	52	27	Rowe &Beebee, 2001a	AF297973
R*temp*μ3	66	55	25	Rowe &Beebee, 2001a	AF297974
R*temp*μ4	64	56	25	Rowe &Beebee, 2001a	AF297975
R*temp*μ5	66	53	23	Rowe &Beebee, 2001a	AF297976
R*temp*μ6	62	48	27	Rowe &Beebee, 2001a	AF297977
R*temp*μ7	64	56	25	Rowe &Beebee, 2001a	AF297978
R*temp*μ8	66	58	27	Rowe &Beebee, 2001a	AF297979
R*temp*μ9	66	58	27	Rowe &Beebee, 2001a	AF297980
R*temp*μ10	56	46	25	Rowe &Beebee, 2001a	AF297981
Rt$_2$Ca9	/	57	35	Garner & Tomio, 2001	AF327358
RlatCa 18	/	48	35	Garner & Tomio, 2001	AF327356
RlatCa27	/	48	35	Garner & Tomio, 2001	AF327357
RlatCa9	/	48	35	Garner & Tomio, 2001	AF327359
RlatCa21	/	48	35	Garner & Tomio, 2001	AF327360
RlatCa17	/	58	35	Garner & Tomio, 2001	AF327361
RlatCa41	/	58	35	Garner & Tomio, 2001	AF327362

Table 6.2. *Rana temporaria* and *R. latastei* microsatellite loci used in the present study. Ts and Ta indicate the starting and final annealing temperature respectively. The number of cycles at the final annealing temperature (N_o cycles at Ta), the GenBank accession codes for each locus and references of the papers in which the loci were first described are also reported.

For Rlat and Rt$_2$ loci, all PCRs were performed at the following conditions: 3 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at annealing temperatures (Tab. 6.2) and 30 sec at 72°C, followed by a final step of 10 min at 72°C. PCR products were then electrophoresed on 2% agarose gels. Ethidium bromide (4 μl) was incorporated in each gel and ten 15 μl samples (4 Jersey, 4 French and 2 control samples containing no DNA), each mixed with 5 μl loading buffer, were loaded. Also 5 μl of a 100 bp ladder were loaded each time. The gels were run for 60 – 70 min at 90 – 100 Volts and finally photographed using an Eagle – Eye videostill camera with an UV transilluminator (Sambrook *et al.*, 1989).

6.2.4. TESTING AMPLIFIED MICROSATELLITE LOCI FOR POLYMORPHISM

To test loci for polymorphism, all primers giving clear products in the initial screening (R*temp*μ1, R*temp*μ4, R*temp*μ5, R*temp*μ9, Rt$_2$Ca9, RlatCa17 and RlatCa41), were tested with 10 individuals from each one of the four sampled populations (see Tab. 6.1). For each sample, the PCR mixture (20 μl in total) contained: 3 μl DNA (25 - 100 ng), 2μl reaction mix from Genpack (150 mM ammonium sulphate, 100 mM Tris-HCl pH 8 and 35 mM MgCl$_2$), 0.2 μl nucleotide mix [10 mM dGTP, tri-Na/1.5 H$_2$0 (15.8 mg/ml), 10 mM dCTP, di-Na 2 H$_2$0 (5.5 mg/ml) and 10mM dTTP, tri-Na (5.5 mg/ml)], 0.04 μl *Taq*EXPRESS DNA polymerase (20U/μl GenPak), 0.024 μl labelled ATP [α-^{33}P] dATP (92 Tbq/mmol), different quantities of unlabelled dATP [1 mM dATP, di-Na/2H$_2$O (1.2 mg/ml)], depending on the locus (0.2 μl for R*temp*μ1, Rt$_2$Ca9 and RlatCa41; 0.12 μl for RlatCa17, R*temp*μ4 and R*temp*μ9; 0.4 μl for R*temp*μ5), different quantities of primers (10μM), depending on the locus (0.8 μl for R*temp*μ1, R*temp*μ4, R*temp*μ5 and R*temp*μ9; 0.4 μl for Rt$_2$Ca9 and RlatCa41; 0.2 μl for RlatCa17), and, finally, sterile distilled water up to 20μl. The PCR conditions were those reported in the previous section. PCR products were analysed by electrophoresis through standard sequencing gels [6% (w/v) polyacrylamide], followed by autoradiography (Rowe *et al.*, 1997). M13 sequence markers were used to size PCR products.

6.2.5. GEL ANALYSIS OF POLYMORPHIC MICROSATELLITE LOCI

For loci that showed polymorphism in at least one of the four populations, the whole process described in the previous section was repeated using all samples from Jersey, France, Germany and Italy. The allele size for each locus was recorded for each individual.

Finally, expected and observed heterozygosities and conformity to Hardy-Weinberg equilibrium were determined using the computer program Genepop version 3.1 (Raymond & Rousset, 1995). For these analyses, the samples from Brühl and Dieburg (see Tab. 6.1, German population) were considered separately.

6.3. RESULTS

When *Rana temporaria* and *R. latastei* microsatellite loci were tested with *R. dalmatina* DNA, respectively 5 and 2 of them amplified (Fig. 6.1). Of these, 4 (3 from *R. temporaria* and 1 for *R. latastei*) were polymorphic in at least 1 of the frog populations considered in the analysis (in bold in Tab. 6.3). As shown in Tab. 6.3, just 1 locus was polymorphic in the Jersey population, 2 in

Figure 6.1. Example of agarose gel photographed using an Eagle – Eye videostill camera with an UV transilluminator. Primers for the microsatellite locus RlatCa41 (right) gave clear products when tested in *Rana dalmatina* DNA (the fifth sample on the right hand-side is the control), whereas those for RlatCa27 did not, because the product of the negative control had the same size as the other samples (primer dimer) (left). The size ladder is visible on the left of the first sample for RlatCa27.

	Jersey (n = 40)	France (n = 40)	Italy (n = 39)	Germany (Brühl, n = 30)	Germany (Dieburg, n = 10)
R*temp*μ4	1 allele	1 allele	1 allele	1 allele	1 allele
Rt₂Ca9	1 allele	1 allele	1 allele	1 allele	1 allele
RlatCa41	1 allele	1 allele	1 allele	1 allele	1 allele
R*temp*μ1	1 allele	2 alleles	1 allele	2 alleles	2 alleles
R*temp*μ5	2 alleles	2 alleles	3 alleles	1 allele	3 alleles
R*temp*μ9	1 allele	1 allele	1 allele	1 allele	2 alleles
RlatCa17	1 allele	3 alleles	2 alleles	3 alleles	2 alleles

Table 6.3. *Rana temporaria* and *R. latastei* microsatellite loci also present in *R. dalmatina* DNA. In bold are the ones that were polymorphic in at least one of the agile frog populations sampled for this study.

the Italian population and 3 in the animals from France. In the German population, 3 loci were polymorphic at Brühl and all 4 at Dieburg. When a locus was polymorphic in more than one population of frogs, the number of alleles varied amongst populations (Tab. 6.3 and 6.5). Tab. 6.4 reports the number of alleles, and their size range, for each of the polymorphic loci in *Rana dalmatina*, as well as the corresponding data for *R. temporaria* and *R. latastei* taken from the literature (for R*temp*μ1, R*temp*μ5 and R*temp*μ9: Rowe & Beebee, 2001a; for RlatCa 17: Garner & Tomio, 2001). The sizes of all *Rana dalmatina* alleles are reported in Tab. 6.5. Observed heterozygosity values were considerably smaller than expected for R*temp*μ1 in the population from Brühl (Germany), for all loci in the other German population and for RlatCa17 in all populations (Tab. 6.6).

Locus	In *Rana dalmatina*		From literature*	
	No alleles	Size range (bp)	No alleles	Size range (bp)
*Rtemp*μ1	2	87 – 89	12	92 – 130
*Rtemp*μ5	5	125 - 139	8	117 – 157
*Rtemp*μ9	2	92 - 105	2	94 – 100
RlatCa17	4	91 - 98	5	113

* see text for references

Table 6.4. Number of alleles, and their size ranges, for all polymorphic microsatellite loci, in *Rana dalmatina* (left) and *R. temporaria* or *R. latastei* (right).

*Rtemp*μ1	Alleles	
	087 bp	089 bp
Jersey	0.000	1.000
France	0.276	0.724
Italy	1.000	0.000
Brühl (Germany)	0.707	0.293
Dieburg (Germany)	0.929	0.071

*Rtemp*μ5	Alleles				
	125 bp	128 bp	130 bp	134 bp	139 bp
Jersey	0.855	0.145	0.000	0.000	0.000
France	0.833	0.167	0.000	0.000	0.000
Italy	0.735	0.206	0.059	0.000	0.000
Brühl (Germany)	1.000	0.000	0.000	0.000	0.000
Dieburg (Germany)	0.500	0.000	0.000	0.250	0.250

*Rtemp*μ9	Alleles	
	92 bp	105 bp
Jersey	1.000	0.000
France	1.000	0.000
Italy	1.000	0.000
Brühl (Germany)	1.000	0.000
Dieburg (Germany)	0.727	0.273

RlatCa17	Alleles			
	91 bp	95 bp	96 bp	98 bp
Jersey	0.000	1.000	0.000	0.000
France	0.050	0.800	0.000	0.150
Italy	0.135	0.000	0.000	0.865
Brühl (Germany)	0.000	0.815	0.037	0.148
Dieburg (Germany)	0.000	0.778	0.000	0.222

Table 6.5. Allelic frequencies, for each locus, in each of the populations studied. For all loci, the size of each allele is also given (bp).

		$R temp\mu1$	$R temp\mu5$	$R temp\mu9$	RlatCa17
Jersey	H_E	/	0.251	/	/
	H_O	/	0.237	/	/
France	H_E	0.405	0.282	/	0.339
	H_O	0.342	0.278	/	0.100
Italy	H_E	/	0.420	/	0.237
	H_O	/	0.471	/	0.000
Brühl (Germany)	H_E	0.422	/	/	0.319
	H_O	0.103	/	/	0.000
Dieburg (Germany)	H_E	0.143	0.682	0.416	0.366
	H_O	0.143	0.167	0.182	0.000

Table 6.6. Expected (H_E) and observed (H_O) heterozygosity values for all loci, in all populations.

All populations were tested at all loci for Hardy-Weinberg equilibrium (probability test) and heterozygosity deficit. The results show that all loci apart from $R temp\mu1$ were out of equilibrium and showed heterozygosity deficits in the German population from Dieburg, and for $R temp\mu1$ in the German population from Brühl. Furthermore, RlatCa17 was out of equilibrium and showed heterozygosity deficits in all populations where polymorphism was observed (Tab. 6.7).

Probability test	Jersey	France	Italy	Brühl (Germany)	Dieburg (Germany)
$R temp\mu1$	/	NS	/	p= 0.001	NS
$R temp\mu5$	NS	NS	NS	/	0.0216
$R temp\mu9$	/	/	/	/	0.0155
RlatCa17	/	p< 0.0001	p< 0.0001	p< 0.0001	0.0118

Heterozygote deficit	Jersey	France	Italy	Brühl (Germany)	Dieburg (Germany)
$R temp\mu1$	/	NS	/	p= 0.001	NS
$R temp\mu5$	NS	NS	NS	/	0.0130
$R temp\mu9$	/	/	/	/	0.0464
RlatCa17	/	p< 0.0001	p< 0.0001	p< 0.0001	0.0118

Table 6.7. Hardy-Weinberg probability test (deviation from equilibrium is considered significant when $p \leq 0.05$; NS= not significant), above, and heterozygote deficit probability values (significant when $p \leq 0.05$), below.

Although heterozygosity deficit may indicate the occurrence of one or more null alleles, the fact that it was significant for all loci but one across the Dieburg sample and for all loci across the Brühl population suggests that the populations' structure was not at Hardy-Weinberg equilibrium. This may not be the case with RlatCa17, for which the existence of null alleles seems likely, as this locus exhibited heterozygote deficiency also in France and in Italy.

6.4. DISCUSSION

In most animals and plants, there is a tendency for more heterozygous individuals to have faster growth rates, lower metabolic demands, shorter developmental times, greater developmental stability and greater disease resistance (for references, see: Endler, 1991). Decline in the number of individuals of a population, especially when lasting several generations, may lead to inbreeding and an increasing probability that any gene will be homozygous (Wright, 1969). This increased homozygosity can cause inbreeding depression, which results in lower fitness, as deleterious recessives normally masked by a different dominant allele, are exposed (Simberloff, 1998). Another threat to small populations is genetic drift, that is the decline in genetic variation caused by loss of alleles by chance. Which alleles become incorporated into gametes and – to some effect – which gametes get to form zygotes are random processes. In a closed population, this loss of variation is opposed only by mutation, as immigration is null (Caughley & Gunn, 1996). However, the smaller the population is, the fewer mutations occur, so the rate of loss of variation to drift is larger. Finally, a third genetic process that may threaten small populations is the fixation of harmful alleles, by drift, before natural selection can eliminate them (Simberloff, 1998). Fixation by drift causes deleterious mutations to accumulate and this can cause the population size to decrease, therefore accelerating the rate of fixation of still more deleterious mutations (Lynch *et al.*, 1995). This process, known as "mutational meltdown", eventually leads to extinction (Gilpin & Soulé, 1986). In light of this, better knowledge and understanding of the level of genetic variation within the endangered population of *Rana dalmatina* in Jersey are undeniably vital for its long-term conservation. Tools available since the advent of molecular ecology make this task achievable.

The present research used microsatellite loci as molecular markers to investigate the genetic variation of the only population of agile frogs breeding in the wild in Jersey. No microsatellite loci have been isolated in *Rana dalmatina* yet and therefore some of those found in two other *Rana* species were tried. Despite the fact that cross-species microsatellite amplification success rates can be quite low in ranid frogs (Primmer & Merila, 2003), the results showed that 5 *Rana temporaria* and 2 *R. latastei* loci were also present in DNA extracted from *R. dalmatina*. Furthermore, 4 of these loci showed polymorphism in at least one of the populations of frogs used in the analysis, thus allowing a comparison of genetic variation between the four populations studied. The Jersey population was the least variable, as only one locus had more than one allele (Tab. 6.3). Considering the small size of this population, the fact that it has probably been even smaller in past years (see Fig. 3.26) and its total isolation, being the only agile frog population in Jersey, drift and inbreeding seem likely to have caused such low variation. However, to be able to confirm this assertion, and to determine if inbreeding has led, or is leading, to a decline in the

fitness of the individuals, a greater number of loci should be employed (Nei, 1978). Also, genetic analyses should be accompanied by experimental tests, investigating the fitness of the frogs, both in their larval and adult phases, since microsatellite diversity and fitness estimates are not always correlated (Rowe & Beebee, 2001b).

Interestingly, in the present research the most variable population was from Germany, which is approximately at the centre of *Rana dalmatina*'s distribution range (Fig. 1.2). On the other hand, the population from northern Italy, where the Alps represent an important geographical barrier to the frogs' movements, showed little variation, being monomorphic for 2 out of the 4 loci that were polymorphic in the German frogs from Dieburg. More analyses, using a higher number of microsatellite loci, need to be done in order to clarify all the questions that this preliminary research has started to raise. Nevertheless, the present study has been important for more than one reason. First, its findings obviously have important practical consequences and open a series of new research opportunities that could ultimately provide knowledge essential for the future management of *Rana dalmatina* in Jersey, its habitat and its captive breeding programme. In particular, at least until primers are designed specifically for the agile frogs, those designed for other *Rana* species, which are numerous, published and available (for references, see: Jehle & Arntzen, 2002) can be tested on *R. dalmatina*. If amplification follows, they can then be used as essential tools in population genetics studies not only in Jersey, but wherever declines in population sizes are observed. Moreover, genetic data would help to address the controversial idea of introducing continental frogs to Jersey, in case the Jersey population hits a catastrophe. They might also eventually justify the need for increased efforts towards maintaining the genetic integrity of the captive population as a "safety-net". Finally, the present study attracted the attention of the local media (various radio and television programmes and articles in the local newspaper were dedicated to the precarious status of the Jersey agile frogs), and also sponsors, from the island's business world. One of the goals of the "Species Action Plan for the agile frog in Jersey" (Agile Frog Group, 2001), that is raising the profile of the frogs' conservation programme, was therefore achieved.

CHAPTER 7

BIOASSAY OF THE WATER QUALITY AT GROSNEZ AND CANNES DE SQUEZ USING *BUFO BUFO* TADPOLES

Aquatic-breeding amphibians are particularly sensitive bioindicators of aquatic toxicity because of their permeable skin, biphasic life and pattern of embryonic development. Abnormalities in the development of Bufo bufo *tadpoles at Cannes de Squez – a potential introduction site for* Rana dalmatina *- were noted in 2001. A bioassay was carried out in 2002 to try to assess the quality of the water at this site. Toad tadpoles from Cannes de Squez and Grosnez ponds were reared under experimental conditions in containers filled with water from Cannes de Squez, Grosnez and - used as a control - Ouaisné. Their development was closely monitored and their growth compared until metamorphosis. Interestingly, during the experiment the water from Cannes de Squez did not have any inhibiting effects on any groups of toad tadpoles and they all reached metamorphosis. Moreover, unlike the previous year, in the summer of 2002 toadlets emerged successfully from the main water body at Cannes de Squez. These results suggest that either unknown factors - rather than water quality - negatively affected the tadpole development in 2001, or the characteristics of the water at Cannes de Squez changed from 2001-2002 due to differences in winter rainfall, a factor that influences the water quality in the pond by diluting the run-off water coming from the fields situated in the area nearby. Therefore, also considering that the adult toad population size at Cannes de Squez is one of the biggest in Jersey, it appears that years when the water quality of the pond is good for the development of the toad tadpoles sometimes alternate with years of very poor water quality. Finally, the tadpoles reared in water taken from Ouaisné were significantly smaller than those living in water from the other two sites. The reason why this happened is unclear, since water quality analyses did not show any abnormal values. Considering that the toad population in the slacks has been drastically declining in the past few years, it would be timely to carry out a detailed investigation into the water quality of the ponds at Ouaisné. Remembering that it is the only wild breeding site for* Rana dalmatina *in Jersey, such a study is not just desirable, but urgently needed.*

7.1. INTRODUCTION

The presence of toxicants in an aquatic system can in many cases be indicated by adverse effects on the animals and plants inhabiting it. At present, bioassays are increasingly used as sensitive indicators of pollutant toxicity, since they are rapid, inexpensive and applicable to a variety of toxicants (Calevro *et al.*, 1999). Aquatic-breeding amphibians are particularly sensitive bioindicators of aquatic toxicity because of their permeable skin, biphasic life and pattern of embryonic development (Wake & Morowitz, 1990). The presence of toxicants in aquatic habitats can also alter amphibian immune responses to pathogens, thus increasing their mortality rates (Carey *et al.*, 1999). Gunther & Plotner (1986), for instance, demonstrated that various pesticides, fertilisers, as well as cleansing, washing and dish-washing products, have strong toxic effects on amphibian eggs and tadpoles. Moreover, under increasing concentrations of nitrite and nitrate ions in the water, tadpoles of various anuran species and *Ambystoma gracile* larvae reduced feeding activity, swam less vigorously, suffered abnormalities and oedema, and eventually died (Marco *et al.*, 1999). Andrén *et al.* (1988) tested larval stages of *Rana dalmatina*, *R. temporaria* and *R. arvalis* in laboratory bioassays in relation to water acidification. In all species, egg mortality and time needed for embryonic development to hatching increased when pH declined. Moreover, a recent study (Hayes *et al.*, 2002) showed how water-borne atrazine, probably the most commonly used herbicide in the world, caused retarded development and hermaphroditism in male leopard frogs (*Rana pipiens*). Finally, amphibians are affected by presence in the water of heavy metals (Calevro *et al.*, 1999), oil (Mahaney, 1994), benzene (Vinnichenko *et al.*, 1998) and various pesticides (Hall & Swineford, 1980).

In Jersey, while surveying anuran breeding ponds across the island (Chapter 2), abnormalities in the development of *Bufo bufo* tadpoles were noted in 2001 at Cannes de Squez. While the development of toad tadpoles in Grosnez pond was normal, and many toadlets were seen around the pond in the summer (see Tab. 3.6), at Cannes de Squez most of the tadpoles seemed to stop growing just after their hind limbs had appeared. A sample of forty tadpoles from Cannes de Squez was therefore moved, on 14th June 2001, to an outdoor tank (120 x 38 x 38 cm), placed outside the Herpetology Department at the Jersey Zoo, filled with aged tap water. There, their further development was closely monitored, by regularly measuring their body length, tail length and total length, to the nearest 0.02 mm, with 15 cm long steel dial callipers. After less than two weeks, they had all metamorphed, while no toadlets were yet present in the wild. The tadpoles originally came from a small water body, where their growth might have been inhibited as an effect of crowding (i.e. Brockelman, 1969; Licht, 1967; Smith, 1987). The fact that their development resumed in a much more crowded environmental, the tank at the Zoo, eliminated this possibility. It

was therefore concluded that characteristics of the aquatic habitat at Cannes de Squez, such as water quality, were likely to be the cause of the poor tadpole growth in the wild. Given the importance of Cannes de Squez as a *Bufo bufo* breeding site on the island, and the fact that it was one of the locations taken into consideration for possible *Rana dalmatina* introductions (Partridge, 1995), a bioassay was carried out in 2002 to try to assess the quality of the water at the site. Toad tadpoles from Cannes de Squez and Grosnez ponds were reared under experimental conditions in containers filled with water from Cannes de Squez, Grosnez and, used as a control, Ouaisné. Their development was then closely monitored and their growth compared. The main assumption of this study was that, if toxicants were present in the water from Cannes de Squez, the tadpoles reared in it, regardless of their origin, would show abnormal development. Although testing *Rana dalmatina* tadpoles would have also been potentially useful, this was not possible, as frog spawning did not occur in 2002 in any of the captive sites and no frog tadpoles were therefore available for the work.

7.2. Materials and Methods

On 21st March, a total of 360 hatching *Bufo bufo* tadpoles, comprising 180 tadpoles from three different sibships in Grosnez and 180 from three different strings from Cannes de Squez, were taken to the ESU's Frances Le Sueur Centre. They were all stage 19 or 20 of Gosner's table (Gosner, 1960). Groups of five sibling tadpoles were then placed in 400 mL plastic containers filled with water taken from Grosnez, Cannes de Squez or Ouaisné (Tab. 7.1). Tadpoles from each of the sibships from each of the sites were therefore raised in water collected from both of the collection sites. In addition, tadpoles from each sibship from each site were also raised in water collected from Ouaisné. This resulted in a 3 x 2 crossed design with 'sibships' as a nested factor within 'sites'. The pH of the water and the concentration of nitrates, nitrites and ammonia were measured in all sites at the beginning and at the end of the experiment. Each experimental unit was replicated 4 times and the resulting 72 containers were randomly placed on a table, situated opposite two large windows, in a corner of the main room at Frances Le Sueur Centre. Every two – three days, all the containers were emptied and refilled with fresh water, collected from the three sites on the same day. Every time the water was changed, the containers were randomly reshuffled. For the duration of the study, the toad tadpoles were fed *ad libitum* with flake fish food. The experiment started on 26th March and ended on 10th June, when all the tadpoles had metamorphosed. However, from 15th May to the end, the design of the experiment changed, because by then some of the tadpoles were close to metamorphosis and the toadlets could climb out of the containers. Therefore, all the data used in the "Results" section refer to the period up to 8th May, assuming that, if there had been any differences in the development of the tadpoles grown in

water taken from different ponds, they would have been observable by then. From 15th May, some of the tadpoles were kept for a further 4 weeks to verify if metamorphosis occurred in all treatments.

Treatment No	Source of tadpoles	Sibship No	Source of water
1	Grosnez	1	Grosnez
2	Grosnez	1	Cannes de Squez
3	Grosnez	1	Ouaisné
4	Grosnez	2	Grosnez
5	Grosnez	2	Cannes de Squez
6	Grosnez	2	Ouaisné
7	Grosnez	3	Grosnez
8	Grosnez	3	Cannes de Squez
9	Grosnez	3	Ouaisné
10	Cannes de Squez	1	Grosnez
11	Cannes de Squez	1	Cannes de Squez
12	Cannes de Squez	1	Ouaisné
13	Cannes de Squez	2	Grosnez
14	Cannes de Squez	2	Cannes de Squez
15	Cannes de Squez	2	Ouaisné
16	Cannes de Squez	3	Grosnez
17	Cannes de Squez	3	Cannes de Squez
18	Cannes de Squez	3	Ouaisné

Table 7.1. Design of the experiment. Each experimental unit ("treatment") represents the contents of one of the plastic containers and was replicated four times.

For this final phase of the study, six plastic aquaria measuring 30 x 15 x 12 cm were used. Space limitations made it impossible to maintain the same experimental design as before, so only the tadpoles from one sibship for each pond (sibship III from Grosnez and sibship II from Cannes de Squez), were used because they had the lowest mortality. Of the six aquaria, two were filled with water from Grosnez, two with water from Cannes de Squez and two with control water (taken, as before, from Ouaisné). The tadpoles from Grosnez and Cannes de Squez were then placed in the aquaria using the same water quality treatments as before. Once a week each tadpole was staged, using Gosner's staging table (1960), and its body length, tail length and total length were measured to the nearest 0.02 mm, using 15 cm long steel dial callipers. Tadpoles found dead were always removed as soon as they were noted. When the tadpoles reached stage 42/43 (see Gosner, 1960), i.e. when the forelimbs started to appear, they were released into the ponds from which they were collected. The experiment ended once all tadpoles were released. Using the data collected up to 8th

May, a week-by-week statistical analysis was carried out, applying ANOVA (General Linear Model) tests. The mean total length of the tadpoles reared in different treatments (Tab. 7.1) was compared, considering population (Grosnez or Cannes de Squez), sibship - nested within the populations - and water (from Grosnez, Cannes de Squez or Ouaisné) as factors that might have caused differential tadpole growths, and the interaction between population and water and between water and sibship. The mean initial size of the tadpoles was also taken into account, as the covariate. Post-hoc tests (Fisher's LSD test) were subsequently performed to compare the pairwise means.

7.3. RESULTS

Of the 180 tadpoles taken from Grosnez pond, 6 tadpoles (two from each sibship) died during the experiment. One of them was in water coming from Grosnez, 2 in water from Cannes de Squez, and 3 were in containers filled with control water. The mortality was slightly higher among the tadpoles taken from Cannes de Squez, with 19 of them found dead during the experiment. Eight came from sibship I, 5 from sibship II and 6 from sibship III. Four of them were reared in Grosnez water, 6 in water from Cannes de Squez and 9 in control water. All tadpoles found dead were immediately removed from the containers and the mean length of the remaining tadpoles was subsequently used in the analyses. All the other tadpoles grew normally and no anomalies were observed.

The results of the water analyses carried out at Grosnez, Cannes de Squez and Ouaisné, at the beginning and at the end of the study, are reported in Tab. 7.2.

Date	Site	pH	Nitrites (mg/L)	Nitrates (mg/L)	Ammonia (ppm)
21st March	Grosnez	7.5	0	1.0	0.25
21st March	Cannes de Squez	6.5	0	0.4	0.25
21st March	Ouaisné	7.0	0	0	0
13th June	Grosnez	7.5	0	10	0.25
13th June	Cannes de Squez	7.4	0	0	0.50
13th June	Ouaisné	7.0	0	0	0

Table 7.2. Results of the water analyses carried out at Grosnez, Cannes de Squez and Ouaisné, at the beginning and at the end of the study.

The results of the statistical analyses (Tab. 7.3, Fig. 7.1 and 7.2) show that every week, apart from week 4, the tadpoles raised in water from Grosnez and Cannes de Squez were significantly larger than those reared in control water. Differences between the mean size of tadpoles taken from the two different sites - and from different strings of eggs - existed from the

beginning of the study. However, when the starting size was controlled for as a covariate, the tadpoles reared in the control water still appeared to be smaller than those in water from Grosnez and Cannes de Squez. As shown in Fig. 7.1 and 7.2, this also emerged when considering each sibship of each population separately.

Week	Population	Sibships within pop	Water	Interaction: population x water	Interaction: water x sibship
1	$F_{1,54}=85.64$	$F_{4,54}=21.86$	$F_{2,54}=4.11$	$F_{2,54}=0.74$	$F_{8,54}=4.51$
	p=0.000	**p=0.000**	**p=0.022**	p=0.481	**p=0.000**
2	$F_{1,53}=2.85$	$F_{4,53}=1.59$	$F_{2,53}=5.36$	$F_{2,53}=1.10$	$F_{8,53}=1.43$
	p=0.97	p=0.192	**p=0.008**	p=0.341	p=0.207
3	$F_{1,53}=0.27$	$F_{4,53}=1.83$	$F_{2,53}=4.59$	$F_{2,53}=0.62$	$F_{8,53}=0.78$
	p=0.606	p=0.137	**p=0.015**	p=0.542	p=0.626
4	$F_{1,53}=0.53$	$F_{4,53}=2.71$	$F_{2,53}=0.56$	$F_{2,53}=4.90$	$F_{8,53}=0.56$
	p=0.469	p=0.105	p=0.694	**p=0.011**	p=0.572
5	$F_{1,53}=4.62$	$F_{4,53}=1.58$	$F_{2,53}=4.37$	$F_{2,53}=0.87$	$F_{8,53}=0.32$
	p=0.036	p=0.193	**p=0.018**	p=0.425	p=0.956
6	$F_{1,53}=5.49$	$F_{4,53}=0.67$	$F_{2,53}=6.34$	$F_{2,53}=2.02$	$F_{8,53}=1.22$
	p=0.023	p=0.613	**p=0.003**	p=0.143	p=0.307
7	$F_{1,53}=4.10$	$F_{4,53}=0.47$	$F_{2,53}=4.45$	$F_{2,53}=1.66$	$F_{8,53}=0.94$
	p=0.048	p=0.756	**p=0.016**	p=0.200	p=0.491

Table 7.3. Week by week ANOVA test on tadpole size. The significant results ($p \leq 0.05$) are in bold (df values are indicated as subscripts to F).

Grosnez, sibship I

Grosnez, sibship II

Grosnez, sibship III
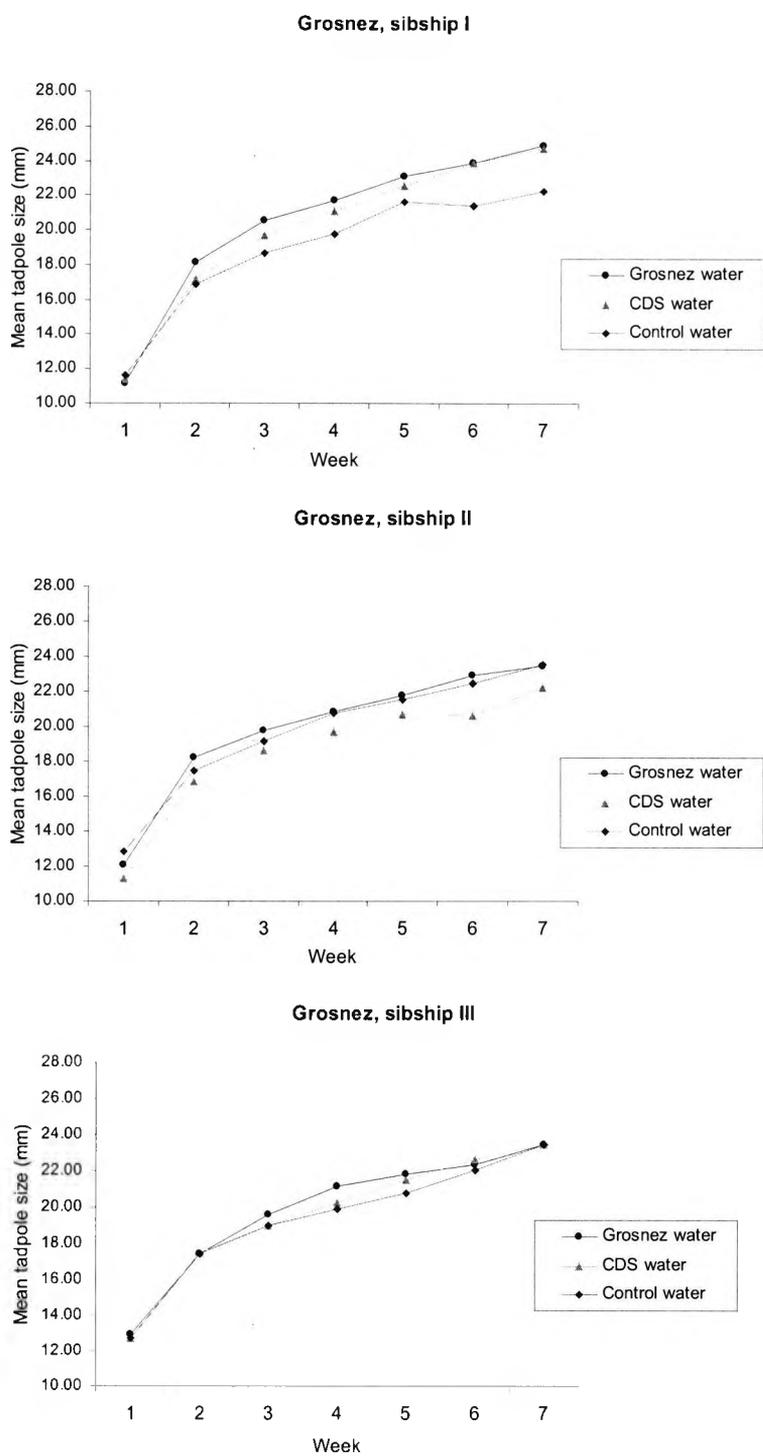
Figure 7.1. Mean size (mm) of toad tadpoles taken from three different strings of eggs (sibship I, II and III), found in Grosnez pond, and reared in experimental conditions in water coming from Grosnez, Cannes de Squez or Ouaisné.
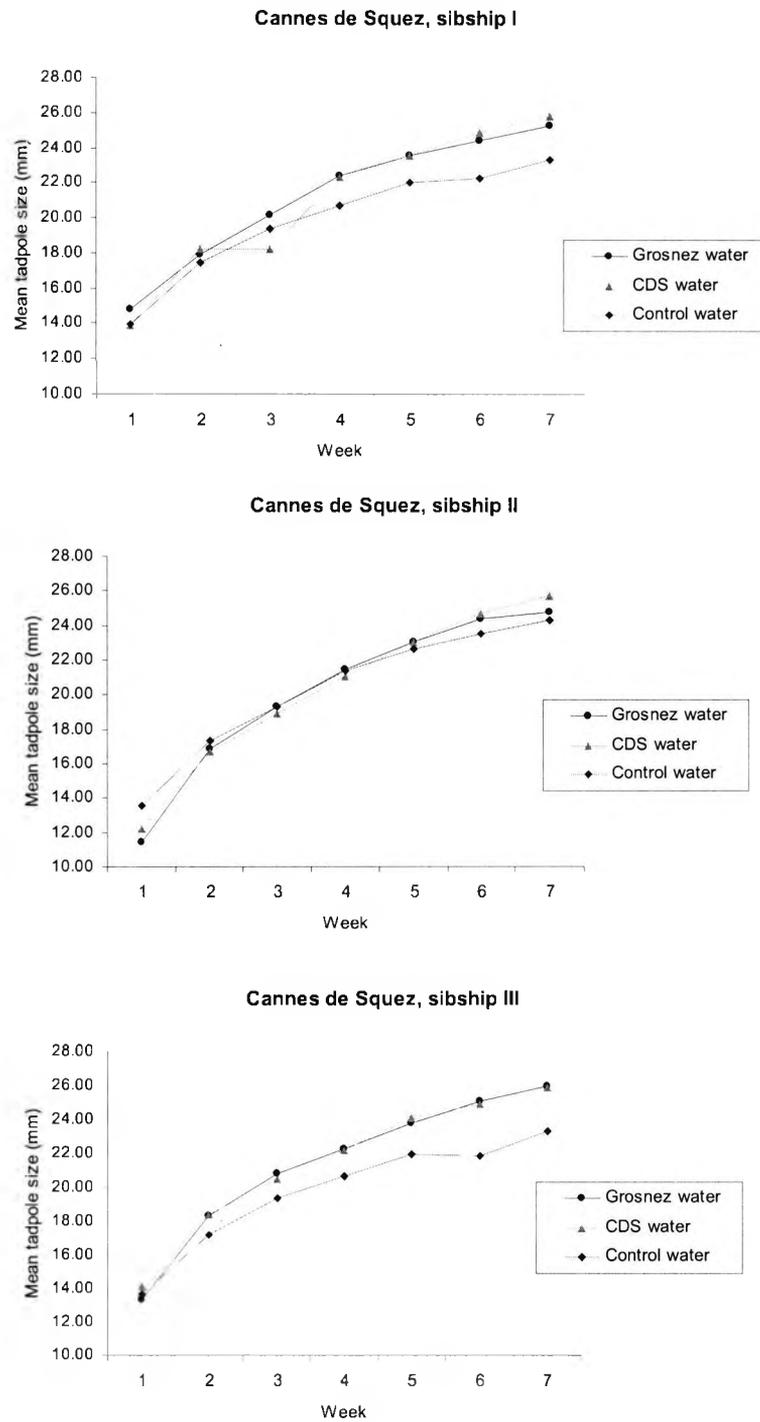
Figure 7.2. Mean size (mm) of toad tadpoles taken from three different strings of eggs (sibship I, II and III), found in Cannes de Squez pond, and reared in experimental conditions in water coming from Grosnez, Cannes de Squez or Ouaisné.

7.4. DISCUSSION

The main aim of the experiment was to attempt to verify if the characteristics of the water present in Grosnez and Cannes de Squez differed, by studying their influence on the growth of tadpoles of *Bufo bufo*. The reason why such a study was undertaken was that the previous year quite dramatic differences in the development of the tadpoles in the two sites were observed in the wild. In 2001, while the tadpoles at Grosnez grew normally and started leaving the water at the beginning of June, the ones at Cannes de Squez seemed to stop developing at the appearance of the hind legs. This resulted in the total absence of metamorphs emerging from the pond. Since the tadpoles taken from this site to an aquarium tank at the Zoo, filled with aged tap water, immediately started developing again and reached metamorphosis, it was assumed that the characteristics of the water at Cannes de Squez inhibited the growth of the toad tadpoles. Interestingly, during the experiment carried out in 2002, the water from Cannes de Squez did not have any inhibiting effects on any groups of toad tadpoles, whether they came from Cannes de Squez or Grosnez, and they all reached metamorphosis. Moreover, unlike the previous year, in the summer of 2002 toadlets emerged successfully from the main water body at Cannes de Squez (see Tab. 3.6). These results suggest that either unknown factors, rather than water quality, negatively affected the tadpole development in 2001, or the characteristics of the water at Cannes de Squez changed from one year to the next. This might have been caused by difference in winter rainfall (winter 2000/2001 was unusually dry, see Fig. 4.8), a factor that influences the water quality in the pond by diluting the run-off water coming from the fields situated in the area nearby. Therefore, also considering that the adult toad population size at Cannes de Squez is one of the biggest in Jersey, it seems reasonable to conclude that years when the water quality of the pond is good enough for the numerous toad tadpoles to develop up to metamorphosis sometimes alternate with years of very poor water quality. However, more research is needed in order to reach more definitive conclusions.

Another intriguing finding was that the tadpoles reared in water taken from the slacks at Ouaisné - considered as control water - had significantly smaller mean sizes than those living in water from the other two sites, Grosnez and Cannes de Squez. The reason why this happened is unclear, since the water tests performed did not show any abnormal values (Tab. 7.2) and more extensive water analyses were not carried out on 2002 at Ouaisné. Considering that the toad population in the slacks has been drastically declining in the past few years (i.e. Partridge, 1995; Racca 2000), it would be timely to carry out a detailed investigation into the water quality of the

ponds at Ouaisné. Remembering that it is the only wild breeding site for the agile frogs in Jersey, such a study is not just desirable, but urgently needed.

CHAPTER 8

CONCLUSIONS & RECOMMENDATIONS

8.1. POPULATION FLUCTUATIONS AND VARIATIONS IN HYDROPERIOD

The size of amphibian populations, especially those of aquatic-breeding species, can fluctuate enormously over time. Even though the extent to which amphibian populations can fluctuate has not yet been exhaustively explored (Pechman *et al.*, 1991), examples reported in the literature show that population size fluctuations can be significant (Berven, 1990; Loman, 2002b; Semlitsch *et al.*, 1996), over three orders of magnitude among years in some cases (Pechman *et al.*, 1991). Schuster (2001) studied an Austrian population of *Rana dalmatina* for a 14-year period and reported that several years of population growth were followed by years of declines. Another population of agile frogs, living on a Danish island, also fluctuated in size over 15 years (Briggs, 1997). This tendency to alternate periods of population growth with times when the number of individuals declines is a characteristic typical of species with high potential for increase ("r-strategists", as opposed to "K-strategists" which are species with fairly constant population densities; Godfray & Crawley, 1998). Many factors determine the magnitude of such fluctuations, each affecting every phase of the amphibians' life cycle to different extents, depending on which processes drive the population dynamics. First of all, little is known about the forces influencing adult and juvenile survival during their terrestrial phase (Pechman *et al.*, 1991). However, body size, population density, and abundances of predators and prey are likely to be the most important (Berven, 1990). Also, rainfall can be correlated to breeding population size, as dry years increase the risk of mortality associated with migrations to the water to breed (Semlitsch, 1983). Hydroperiod, pond temperature, predation, competition, tadpole density and food availability, all influence egg and tadpole development and ultimately determine the rate of each season's reproductive success (i.e. recruitment) (e.g. Alford, 1989; Berven, 1990; Loman, 2002a,b; Morin *et al.*, 1988; Pechman *et al.*, 1991). Moreover, catastrophic events such as fires and droughts are responsible for possibly the most dramatic natural declines in population sizes, by increasing all individuals' mortality and by causing complete recruitment failures (Simberloff, 1998). Finally, human actions that cause, for example, fragmentation or loss of habitats and pollution of water bodies may determine fluctuations of amphibian population size, and so does the presence of roads along amphibian migration routes by increasing adult mortality rates (e.g. Beebee & Corbett, 1998; Blaustein *et al.*, 1994; Lips, 1998; Vos & Chardon, 1998). Separating human impacts from natural fluctuations can sometimes be difficult (Pechman *et al.*, 1991), but long-term studies into the ecology of populations often offer solutions to this problem (Marsh, 2001).

The study carried out in Jersey from 2001 to 2003 focusing on the population dynamics of the only *Rana dalmatina* population breeding in the wild was by no means long enough to allow any general conclusions to be drawn about the species. However, it gave an insight into some of the

processes that drive the dynamics of the population and their relative impact on the various stages of the amphibians' life cycle. As concluded in Chapter 3, a picture of Ouaisné Common as an adequate - sometimes exceptionally successful - *Rana dalmatina* breeding site emerged. However, the frog population size fluctuated even over such a short study-period, with significant differences between 2001 and the two following years. Variations in the level of predation pressures upon the amphibians' aquatic phase was highlighted as one of the possible factors that caused erratic recruitment of metamorphs (Chapter 3), and therefore fluctuations of population sizes over time. The reproductive success of the frogs varied together with the composition of the aquatic community of egg and tadpole predators, especially macroinvertebrate species (Chapter 4). At the same time, differences in absolute and relative abundances of such predators were linked to variations in hydroperiod. As mentioned above, hydroperiod influences aquatic-breeding amphibian population sizes in many ways. If a pond dries before metamorphosis starts, the result is complete recruitment failure. Moreover, by determining the length of the aquatic phase and the level of tadpole density, it can also affect the size of metamorphs, as smaller froglets generally result from faster tadpole development (e.g. Loman, 2002a,b). Juvenile survival was positively correlated to size at metamorphosis and larger juveniles tended to be larger as adults in a long-term study of populations of wood frogs (*Rana sylvatica*) (Berven, 1991). North and South Slack, the breeding ponds for *Rana dalmatina* at Ouaisné, generally hold water until metamorphosis is completed, at least since they were deepened several years ago (Chapter 2). However, both ponds dry completely in case of prolonged scarce rainfall during the autumn and early winter months, as observed in 2001, and can therefore be defined as semi-permanent ponds (Collinson *et al.*, 1995). The correlation between the period of time when a pond holds water and the composition and structure of their community of invertebrate species is well documented in the literature (e.g. Brönmark & Hansson, 1998; Laurila & Kujasalo, 1999; Semlitsch, 2002; Wilcox, 2001). For example, the composition of water beetles assemblages, adults and larvae, differed among 77 ponds characterised by different site-water durations (Eyre *et al.*, 1992). Also, Collinson *et al.* (1995) found that water bodies with shorter hydroperiod had fewer species than ponds with longer water permanence and different assemblages of Odonata, water bugs and water beetles. Loman (2002a) investigated common frog (*Rana temporaria*) tadpole survival and metamorph production in 18 Swedish ponds for several years and his findings were consistent with the results obtained in Jersey (Chapters 3 and 4). He concluded that when ponds dried completely, at least once every few years, they contained relatively small numbers of egg and tadpole predators and so produced more metamorphs than those that never dried. These considerations have to be taken into account, when conservation measures are planned to secure the future of *Rana dalmatina* at Ouaisné in the long

term. As the frog's aquatic phase seems to have a pivotal role in determining the size of the adult population, it is advisable that such measures are focused towards increasing the frequency of years of high metamorph recruitment.

8.2. VIABILITY OF THE AGILE FROG POPULATION CURRENTLY BREEDING AT OUAISNÉ

When predictions for the future of the population of frogs currently breeding at Ouaisné were modelled in a Population Viability Analysis (Chapter 5), its extinction was projected, with 100% probability, within the next 50 years. However, when productivity is enhanced (i.e. by protecting the eggs) the population became viable for the duration of the period of time considered, and its predicted extinction risk was extremely low (Chapter 5). Thus, protection of the spawn is a conservation measure that, as it does not require much effort, should be adopted every year, at Ouaisné, as soon as possible. Moreover, the risk associated with the population inhabiting just a small area and mainly breeding in two ponds next to each other is another aspect to be taken into account when planning conservation actions. A single catastrophic event, such as a spill of pollutants into the water during the reproductive season, could kill most of the population and make the habitat unsuitable for the frogs for a very long time, as was the case at Noirmont, historically an agile frog breeding site (Chapter 2). Moreover, the results of genetic analyses have suggested that the *Rana dalmatina* population living at Ouaisné had relatively low levels of genetic variation, possibly due to drift and inbreeding, when compared to agile frog populations from mainland Europe (Chapter 6). This is hardly surprising, considering that genetic variation is usually correlated with population size (Frankham, 1995). Low genetic variation can also be a consequence of the fact that the population has been living on an island for thousands of years (Chapter 2). It has been indicated, in fact, that within a species genetic variation tends to be lower in island populations than in mainland populations, as the former are prone to suffer from an increase in inbreeding and reduced levels of gene flow caused by isolation (e.g. Berry, 1996; Godfray & Crawley, 1998; Hitchings & Beebee, 1996). For example, Frankham (1998) calculated inbreeding coefficients, from allozyme and microsatellite heterozygosity measures, for 210 island populations and their related mainland populations. All island populations were significantly inbred, whereas mainland populations generally showed high levels of genetic variation. In another study (Eldridge *et al.*, 1999), heterozygosity values, from microsatellite loci analyses, of island and mainland populations of the black-footed rock-wallaby (*Petrogale lateralis*) were compared. As before, the results unequivocally indicated that all island populations had significantly lower genetic diversity than those living on the mainland. The tendency to have low genetic variation, typical of small populations and of those inhabiting islands, however, does not necessarily coincide with inevitable

extinction. In fact, cases of small populations, which, despite very low levels of genetic variation, have persisted for a very long time, are well documented (see Chapter 5. Also: Hitchings & Beebee, 1996; Lacy, 1987; May, 1994; Menzies, 1991). However, although this has not always been proved to be true (e.g. Rowe & Beebee, 2001b), low levels of fitness may be correlated with low levels of genetic variation (Ballou, 1997; Frankham, 1998; Newman & Pilson, 1997; O'Brien *et al.*, 1987; Primmer *et al.*, 2003; Ralls *et al.*, 1988; Saccheri *et al.*, 1998). For example, the highly inbred island populations of the black-footed rock-wallaby mentioned above (Eldridge *et al.*, 1999) also exhibited reduced fitness. In a study on amphibians, the more heterozygous female green frogs (*Hyla cinerea*) were, the higher their reproductive success was (McAlpine, 1993). Furthermore, Hitchings & Beebee (1996) found that low levels of fitness were associated with low levels of genetic variation in populations of common frogs (*Rana temporaria*) breeding in urban areas. As already highlighted in the discussion concluding Chapter 6, more genetic analyses, accompanied by experimental tests, should be carried out in Jersey to measure the fitness of the frogs. Finally, whatever the reason for the low genetic variability detected in the frog population breeding at Ouaisné, either a result of their life history, or a consequence of being on an island, any plans for its conservation have to aim to ultimately increase, or at least maintain, the current level of population genetic variation. The range of the agile frog in Jersey should be expanded, not only locally, but across the whole island, with the use or creation of suitable new sites and introductions of *Rana dalmatina* individuals from the captive sites, as suggested by the strategies described in the next section.

8.3. STRATEGIES FOR THE CONSERVATION OF *RANA DALMATINA* IN JERSEY

8.3.1. CONSERVATION OF THE EXISTING RANA DALMATINA *POPULATION*

First of all, the whole of Ouaisné Common should be carefully managed, as it is important that both aquatic and terrestrial elements of the amphibian habitat are preserved (Bullock *et al.*, 1998; Semlitsch, 2002, Skelly, 2001). Some essential measures of habitat management have already been implemented by the ESU in the last few years and should therefore be continued. Conservation practices required at Ouaisné, most of which suggested by Bullock *et al.* (1998) and Semlitsch (2002), are the following. (1) The level of disturbance caused by people visiting the area or walking their dogs should be kept at the lowest possible level. For example, signs about presence of the species at the site, and about its endangered status, should be spread across the Common. Ideally, access to the area where the breeding ponds are, especially during the amphibians' breeding season, should be forbidden. (2) The growth of vegetation should be controlled through pruning and coppicing and the spread of invasive species prevented, to avoid

excessive shading and eutrophication of the water bodies. (3) Piles of logs or other debris should be left in various locations across the Common, as they provide shelter and hibernation sites. (4) The continued absence of possible barriers (i.e. walls, thick hedges) to the anurans' annual migrations should be assured. (5) The water quality should be monitored, through regular analysis of samples taken from North and South Slack. (6) Surveys of the aquatic breeding site should be carried out from mid-January to the end of the anurans breeding season (usually mid or end of July). By applying the methods described in Chapter 2, ecological and demographic data that could prove essential in a potentially necessary future species recovery plan would be systematically collected. At the same time, frog spawn should be protected (Chapter 2). (7) Populations of frog predators, such as grass snakes, ducks and herons, should be regularly monitored and adequate actions should be undertaken whenever their population sizes increase to dangerous levels.

The persistence of the *Rana dalmatina* population currently breeding in Jersey may be also secured through conservation measures focusing on expanding its range at Ouaisné and on increasing the population size. This may be achieved by augmenting the availability of aquatic habitats. A possible course of action should aim to create a *Rana dalmatina* metapopulation within the Common. A metapopulation is a group of small populations connected by occasional movements of individuals between them (Simberloff, 1998) and may have lower extinction risks than just one larger population (Driscoll, 1998; Laan & Verboom, 1990; Marsh & Trenham, 2000; Semlitsch, 2002; Sjögren, 1991). Single small populations may disappear over time, but usually their habitat patches are reoccupied (Caughley & Gunn, 1996), and dispersal has in some cases been proved to be crucial in compensating for local extinctions of frog communities (Laan & Verboom, 1990). For instance, habitat management aimed at enhancing connectivity, within Ouaisné Common, could facilitate natural colonisation of some of the numerous new slacks recently created by the ESU (Chapter 2). Alternatively, frogs could be introduced to ponds situated in gardens of some of the private properties surrounding the Common. Some of these gardens are quite large, and this would increase their potential for being managed sympathetically with the frogs' habitat requirements. The choice of adequate sites should be made with great caution. Various features make a garden pond suitable for anurans (Fig. 8.1) and should be studied and eventually recreated. Moreover, as the owners of the ponds would obviously be involved, their willingness to cooperate, in the long term, in any conservation practices necessary for the well being of the frogs should also be ascertained.

117
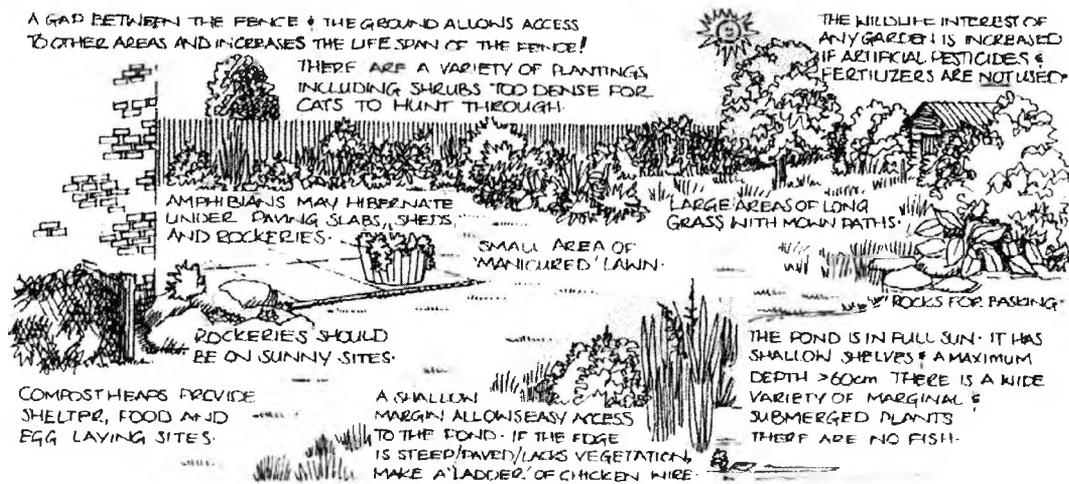
Figure 8.1. Garden showing positive features for amphibians (and reptiles) (from: Bullock *et al.*, 1998).

However, translocation is a complex issue (Trenham & Marsh, 2002), as discussed in the next section, where suggestions on how *Rana dalmatina* introductions should be carried out are also given. Finally, once new populations of frogs are established at Ouaisné, their habitats should be monitored constantly, following the guidelines listed at the beginning of this section. At the same time, as essential sources of individuals for introductions, the *Rana dalmatina* captive breeding sites need to be monitored, by applying the methodology described in Chapter 2. Should the captive agile frog populations grow beyond the carrying capacity of the existing sites, new ones, with characteristics similar to those of Mr Perkins' and the Zoo enclosures (Chapter 2) should be created. Also, it has to be noted that adverse genetic change may occur within populations held in captivity as a result of inbreeding depression, loss of genetic variation and genetic adaptation to captive environments (Sutherland, 1998). Hence, molecular techniques and experimental tests (Chapter 6) will need to be employed to assess the level of genetic variation and fitness of the frogs held in captivity in Jersey.

8.3.2. FOUNDATION OF NEW RANA DALMATINA POPULATIONS

Preserving the agile frog population currently breeding at Ouaisné is, as discussed above, of great importance. However, even assuming a viable metapopulation of frogs was successfully created, it would probably not be enough to secure the long-term persistence of *Rana dalmatina* on Jersey. For instance, should a fire destroy Ouaisné Common, the frog population would also be wiped out and the species would become extinct in the wild. Consequently, attempts to establish agile frog populations in other sites across Jersey should be made, so that extinctions of single populations would not necessarily jeopardise the future of the species on the island. Moreover,

118

fragmentation and loss of habitat were probably the cause of the disappearance of most of the populations of *Rana dalmatina* historically breeding in Jersey (Chapter 1), with the exception of Noirmont pond, where a pollutant spilled in the water killing most of the anurans, adults and spawn, present in it (Chapter 2). Restoring or creating new sites so that they are suitable for the frogs seems to be the best conservation action for the agile frogs. In order to achieve this goal, (1) animals need to be available from the captive breeding sites; (2) locations with suitable habitats have to be found or created; and finally, (3) translocation strategies need to be carefully devised and put into practice. The first point has been discussed in the previous section. A series of features make a habitat suitable for amphibian species such as *Rana dalmatina*. For example, food availability needs to be high in both aquatic and terrestrial habitats. On the other hand, predation pressures should be fairly low and the total absence of fish in all water bodies used by the frogs to breed is required. Moreover, as discussed in section 8.1, it is preferable if the ponds dry periodically, as this will expose sediments to oxidation processes, thereby releasing nutrients, and reduce invertebrate abundances (e.g. Loman, 2002a). Several small breeding ponds are better than just one larger one (Bullock *et al.*, 1998). Additionally, they should be characterised by good water quality, as high levels of fertilisers or pesticides, for example, negatively influence the egg and tadpole development (Chapter 7). Furthermore, terrestrial habitat, undisturbed and continuous with the wetland, extending up to 200 m from the shoreline, must be provided, as it is essential for maintaining juvenile and adult breeding populations (Semlitsch, 2002). More specific features that both aquatic and terrestrial habitats should have to be considered suitable for frog communities (Fig. 8.2), together with technical details about most aspects of their management, are reported, for example, in the Herpetofauna Workers' Manual (Gent & Gibson, 1998). Translocations of individuals to locations that historically were breeding sites for the species seem to have higher probability of success than when amphibians are introduced in areas outside their historical range (Griffith *et al.*, 1989, cited in: Semlitsch, 2002). Naturally, the factors that caused past extinctions in historical sites have to be isolated and eliminated. In Jersey, Noirmont pond was a *Rana dalmatina* breeding site in the past and, since it was considered suitable again, has been a recipient for several anuran introductions during the last few years (Chapter 2). Since the start of the introduction programme, adult frogs have returned to the pond to breed for two consecutive years (Chapter 3), thus the success of the translocations may be defined as "intermediate" (Tab. 8.1). Hence, the pond may be considered as a candidate for further translocations, along with new sites in other parts of the island. Finally, having assessed the availability of suitable recipients and perhaps using, once again, PVA models such as those described in Chapter 5 as predictive tools, translocation strategies should be planned and carefully carried out.
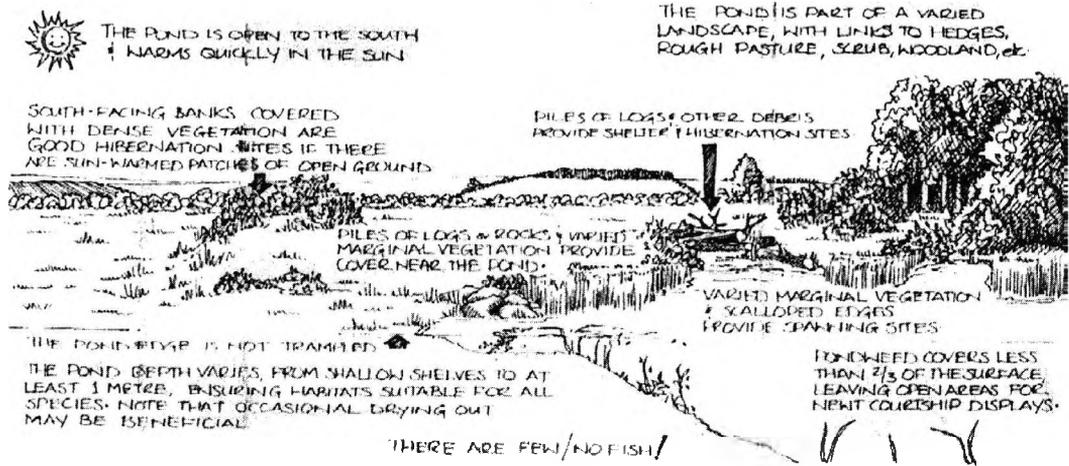
Figure 8.2. Site in countryside showing a series of positive features for amphibian species (from: Bullock *et al.*, 1998).

However, as mentioned above, translocation is a complex issue (Trenham & Marsh, 2002), and translocations potentially have costs in addition to the possible benefits derived from founding new *Rana dalmatina* populations. The most obvious is the risk of disease transmission that translocating pathogens along with the host species would cause (Cunningham, 1996, Seigel & Dodd, 2002). Moreover, translocation of cospecifics can alter natural patterns of genetic variation (Driscoll, 1988). Also, uncertainty about amphibian terrestrial preferences can result in inadequate habitats being chosen for introductions (Pechman *et al.*, 1991). In the past, amphibian translocations have not always had high success rates and in several cases were complete failures (see, for examples: Beck *et al.*, 1994; Dodd, in press, cited in Seigel & Dodd, 2002). Nevertheless, translocation plays a potentially important role in amphibian conservation and in some situations, like that of *Rana dalmatina* in Jersey, is the best strategy to promote regional population persistence (Trenham & Marsh, 2002). Steps to minimise the risk of spreading diseases can be taken (for general guidelines, see: Cunningham, 1996). This, together with careful evaluation and planning of all aspects of translocation projects, may increase probability of success. Cases where translocations were crucial elements of amphibian recovery plans are well documented, like for example for the Mallorcan midwife toad (*Alytes muletensis*) in Mallorca (Buley & Garcia, 1997) and the natterjack toad (*Bufo calamita*) in Britain (Denton *et al.*, 1997). In light of this, and considering the scarcity of alternative valuable options, translocations may greatly enhance the probability of *Rana dalmatina*'s long-term persistence not only at Ouaisné (see previous section), but, more generally, on Jersey. For aquatic-breeding amphibians, the preferred stage for founding new populations is generally the egg or aquatic larval stage (Semlitsch, 2002). Adults are usually only seasonally available, sometimes difficult to catch and often occur in small numbers. Moreover, they are

philopatric, thus the probability that they will emigrate from translocation sites, towards they original ponds, is high (Semlitsch, 2002). Furthermore, if captive sites are sources of animals for translocations, as is mainly the case in Jersey (Chapters 2 and 3), rearing high numbers of individuals to their adult age can be difficult, as it entails availability of space, costs and regular monitoring. To increase the probability of success when eggs are used for introductions, they should be "head-started" (Semlitsch, 2002) in cages placed in the ponds until the free-swimming stage [stage 25 of Gosner's (1960) table]. The mesh bags described in Chapter 2 may be used as an alternative to the cages. Another option, that would increase the larval survival rate without creating prolonged costs and efforts, is to rear the eggs and tadpoles in captivity for the duration of their development and release them in translocation sites as newly metamorphed froglets or juveniles. This would increase the overall probability of success of the introductions, as metamorphs and juveniles have higher survival rates than the aquatic stages [the former averaging between 6% and 26%, the latter between 1% and 5% (Semlitsch, 2002)]. The exact number of individuals needed for amphibian translocations is uncertain because of the scarcity of data with which to evaluate numbers used in past projects (Semlitsch, 2002). However, by using life-history or population growth-rate data, derived from surveys carried on in the field for reasonably long periods [at least 5 or 6 years (Marsh, 2001)], models may be designed and used to predict future size and structure of populations founded by translocation (Ludwig, 1999). As reported in Chapter 5, PVA models, whose assumptions were mainly based on data collected at Ouaisné, were used to test several introduction strategies in new sites with sizes comparable to that of Ouaisné, or smaller. The results of the analysis should be taken just as general guidelines, rather than actual translocation plans (see discussion at the end of Chapter 5). Nevertheless, they show that potentially successful outcomes can be achieved by introducing between 20 and 80 individuals a year, for three consecutive years (combinations of metamorphs and 1 year olds, Tab. 5.5). Alternatively, introductions of just metamorphs would require at least 80 animals per year, for 3 years. In all cases, for the new population to be viable for at least 50 years, protection of the spawn would be indispensable. Moreover, a total of 60 froglets over a three-year period could probably be harvested from the population of frogs currently breeding at Ouaisné, and used for the translocations, without greatly increasing the donor population's extinction risk (Tab. 5.6). In all cases, to maximise genetic diversity, samples of eggs from as many distinct masses as possible should be taken to be reared for introductions (Semlitsch, 2002). Furthermore, monitoring the level of reproductive success of newly established populations, as a way to measure the success of the translocation programmes, should be an essential part of the programmes themselves (Denton *et al.*, 1997). Only after multiple years of breeding, or even after a cohort of second-generation adults

is found breeding, should the translocation be considered a success (Semlitsch, 2002). Criteria such as those outlined by Denton *et al.* (1997), and reported in Tab. 8.1, can be used to determine relevant levels of monitoring and measures of success.

Initial success: emergence of metamorphs from ponds (in case egg/tadpoles were originally introduced)
Intermediate success: return of adults to breed for the first time
Complete success: continuation of breeding for 5 years
Failure: adults fail to return for 5 – 10 years

Table 8.1. Criteria for evaluation of translocation programmes' success (from: Denton *et al.*, 1997).

Long-term management of the habitat of all new founded populations should follow, as well as standardised and continuous surveying of the populations themselves.

In conclusion, conservation actions should be focused on preserving the existing captive and wild *Rana dalmatina* populations. Ecological and genetic research on the species should be continued, by applying methodologies described in this thesis. At the same time, new opportunities for the frog populations to expand should be created, to ultimately enhance the probability that the species will continue to inhabit Jersey in the long term.

APPENDIX I

NIGHT SURVEY FORM

Name			Date			

BEGIN	Time		END	Time	
	Wind code			Wind code	
	Sky code			Sky code	
	Air T				

Water T	max	
	min	
	curr	

	CALLS		Comments	ANURANS IN THE WATER				Comments
Area (*see below)	Frog calls	Toad calls		Male frogs	Female frogs	Male toads	Female toads	

	ANURANS OUTSIDE THE WATER				Comments
Area (*see below)	Male frogs	Female frogs	Male toads	Female toads	

* **Areas** (see map): A, B, C, D, E, F, G, H.

EXAMPLE OF SURVEY SITE MAP
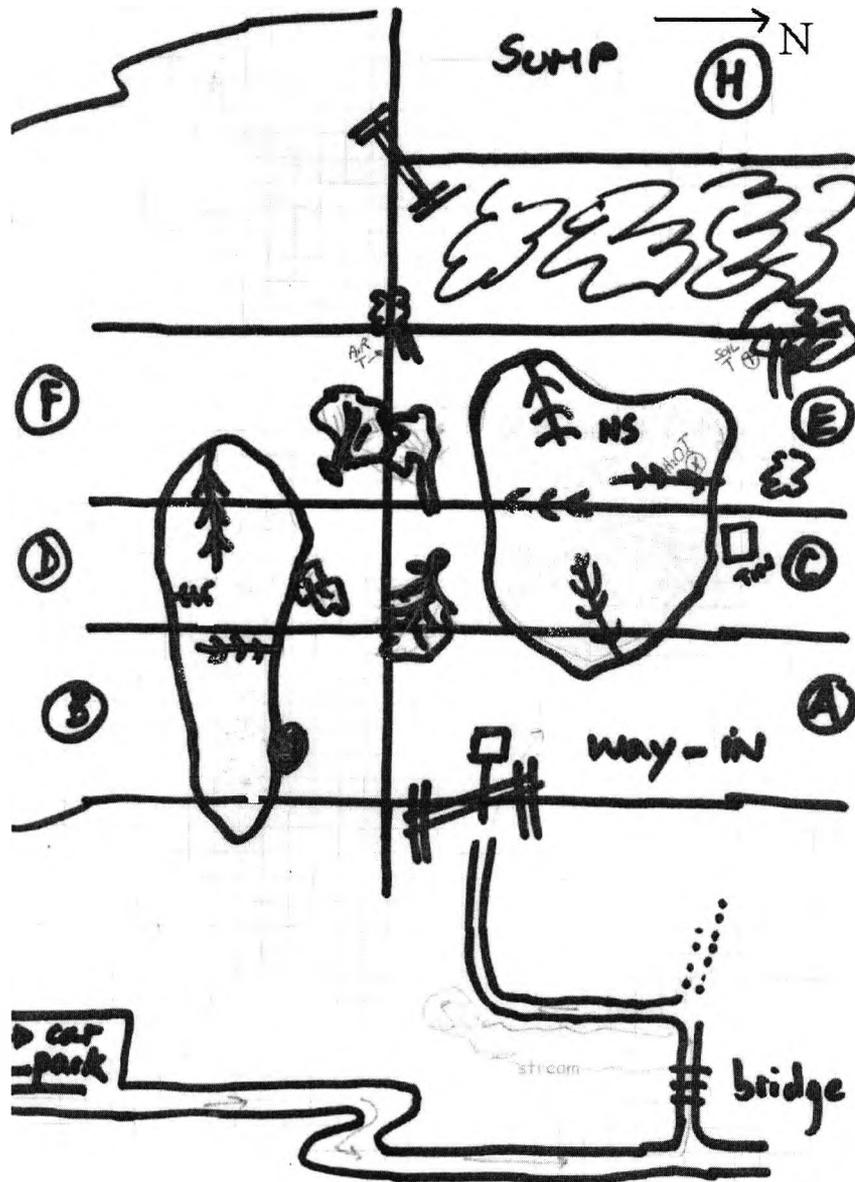
APPENDIX II

ESTIMATES OF ADULT SURVIVAL

JOLLY-SEBER METHOD

Time of last capture (year)	Time of capture (year)		
	2001	2002	2003
2001		2	0
2002			10
Total marked (m_t)	0	2	10
Total unmarked (u_t)	10	42	7
Total caught (n_t)	10	44	17
Total release (s_t)	10	44	17

Table II.1. Mark – recapture data used to estimate *Rana dalmatina* adult survival with the Jolly-Seber Method (see: Krebs, 1999).

ROBSON & CHAPMAN METHOD

1 year old	2 years old	3 years old	> 3 years old
45	21	3	2

Table II.2. Total number of 1, 2, 3 year old and older male frogs caught at Ouaisné in 2001, 2002 and 2003. These data were used to estimate adult survival with the Robson & Chapman Method (Krebs, 1999).

MODEL MATRICES

STAGE MATRICES

No protection

	Female 0 yr	Female 1 yr	Female 2 yr	Female ≥ 3 yr	Male 0 yr	Male 1 yr	Male 2 yr	Male ≥ 3 yr
Female 0 yr	0.000	0.000	5.650	5.650				
Female 1 yr	0.250	0.000	0.000	0.000				
Female 2 yr	0.000	0.275	0.000	0.000				
Female ≥ 3 yr	0.000	0.000	0.275	0.275				
Male 0 yr	0.000	0.000	5.650	5.650	0.000	0.000	0.000	0.000
Male 1 yr	0.250	0.000	0.000	0.000	0.250	0.000	0.000	0.000
Male 2 yr	0.000	0.275	0.000	0.000	0.000	0.275	0.000	0.000
Male ≥ 3 yr	0.000	0.000	0.275	0.275	0.000	0.000	0.275	0.275

Protection

	Female 0 yr	Female 1 yr	Female 2 yr	Female ≥ 3 yr	Male 0 yr	Male 1 yr	Male 2 yr	Male ≥ 3 yr
Female 0 yr	0.000	0.000	33.180	33.180				
Female 1 yr	0.250	0.000	0.000	0.000				
Female 2 yr	0.000	0.275	0.000	0.000				
Female ≥ 3 yr	0.000	0.000	0.275	0.275				
Male 0 yr	0.000	0.000	33.180	33.180	0.000	0.000	0.000	0.000
Male 1 yr	0.250	0.000	0.000	0.000	0.250	0.000	0.000	0.000
Male 2 yr	0.000	0.275	0.000	0.000	0.000	0.275	0.000	0.000
Male ≥ 3 yr	0.000	0.000	0.275	0.275	0.000	0.000	0.275	0.275

Table II.3. The upper left quadrant includes number of daughters per female (as fecundity elements) and the survival rates of females. The lower left quadrant includes the number of sons per female (as fecundity elements) and the lower right quadrant includes the survival rates of males.

STANDARD DEVIATION MATRICES

No protection

	Female 0 yr	Female 1 yr	Female 2 yr	Female ≥ 3 yr	Male 0 yr	Male 1 yr	Male 2 yr	Male ≥ 3 yr
Female 0 yr	0.000	0.000	1.718	1.718				
Female 1 yr	0.030	0.000	0.000	0.000				
Female 2 yr	0.000	0.064	0.000	0.000				
Female ≥ 3 yr	0.000	0.000	0.064	0.064				
Male 0 yr	0.000	0.000	1.718	1.718	0.000	0.000	0.000	0.000
Male 1 yr	0.030	0.000	0.000	0.000	0.030	0.000	0.000	0.000
Male 2 yr	0.000	0.064	0.000	0.000	0.000	0.064	0.000	0.000
Male ≥ 3 yr	0.000	0.000	0.064	0.064	0.000	0.000	0.064	0.064

Protection

	Female 0 yr	Female 1 yr	Female 2 yr	Female ≥ 3 yr	Male 0 yr	Male 1 yr	Male 2 yr	Male ≥ 3 yr
Female 0 yr	0.000	0.000	10.087	10.087				
Female 1 yr	0.030	0.000	0.000	0.000				
Female 2 yr	0.000	0.064	0.000	0.000				
Female ≥ 3 yr	0.000	0.000	0.064	0.064				
Male 0 yr	0.000	0.000	10.087	10.087	0.000	0.000	0.000	0.000
Male 1 yr	0.030	0.000	0.000	0.000	0.030	0.000	0.000	0.000
Male 2 yr	0.000	0.064	0.000	0.000	0.000	0.064	0.000	0.000
Male ≥ 3 yr	0.000	0.000	0.064	0.064	0.000	0.000	0.064	0.064

Table II.4. Standard deviations of the values included in the stage matrices (see: Tab. II.3).

MODEL PREDICTIONS: FIGURES

INTRODUCTION MODELS

Median ~ 12.3
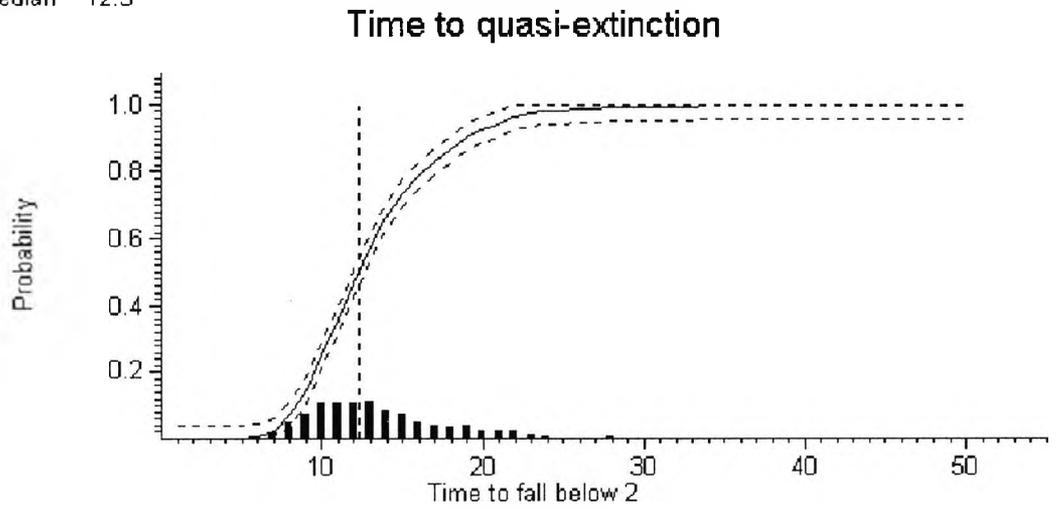
Figure II.1. [Model 2.1, "No Protection"]. Predicted time (in years) to quasi-extinction, when introduction model 2.1 is simulated, assuming the frog clumps were never protected.

Median ~ 15.6
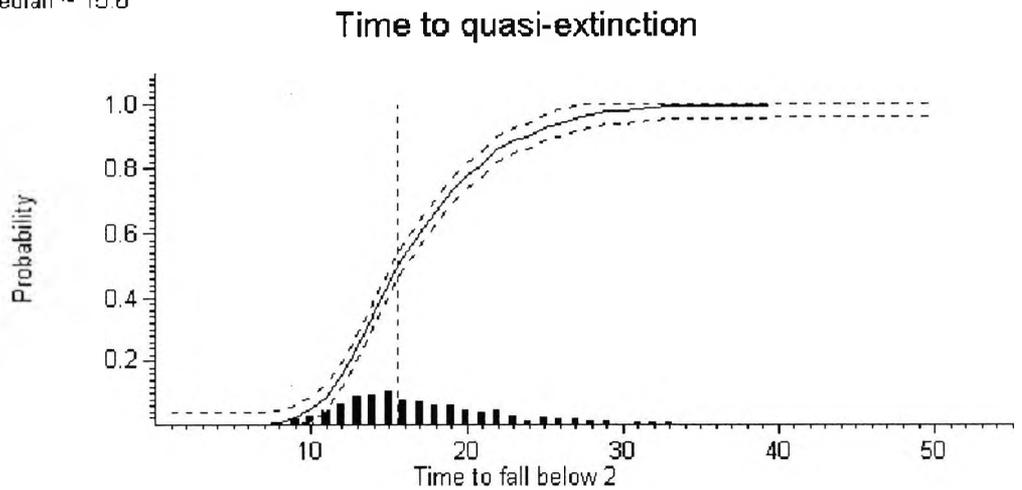
Figure II.2. [Model 2.2, "No Protection"]. Predicted time (in years) to quasi-extinction, when introduction model 2.2 is simulated, assuming the frog clumps were never protected.

127

Median ~ 17.6

Time to quasi-extinction

Figure II.3. [Model 2.3, "No Protection"]. Predicted time (in years) to quasi-extinction, when introduction model 2.3 is simulated, assuming the frog clumps were never protected.

Median ~ 15.4

Time to quasi-extinction
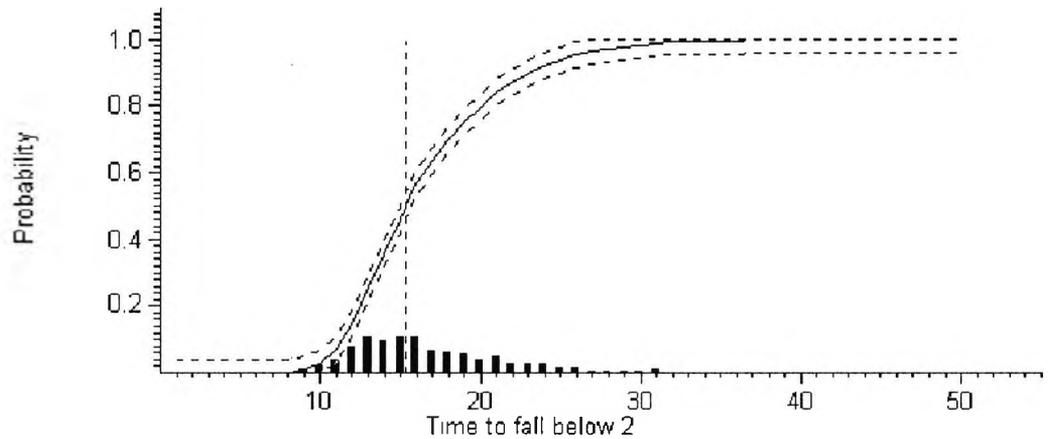
Figure II.4. [Model 2.7, "No Protection"]. Predicted time (in years) to quasi-extinction, when introduction model 2.7 is simulated, assuming the frog clumps were never protected.

Median ~ 16.6

Time to quasi-extinction
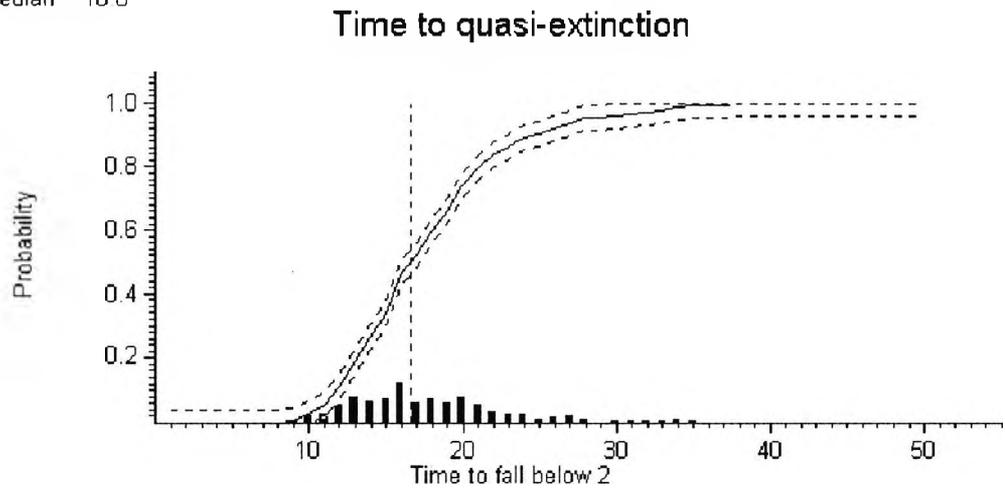
Figure II.5. [Model 2.8, "No Protection"]. Predicted time (in years) to quasi-extinction, when introduction model 2.8 is simulated, assuming the frog clumps were never protected.

Median ~ 17.6

Time to quasi-extinction
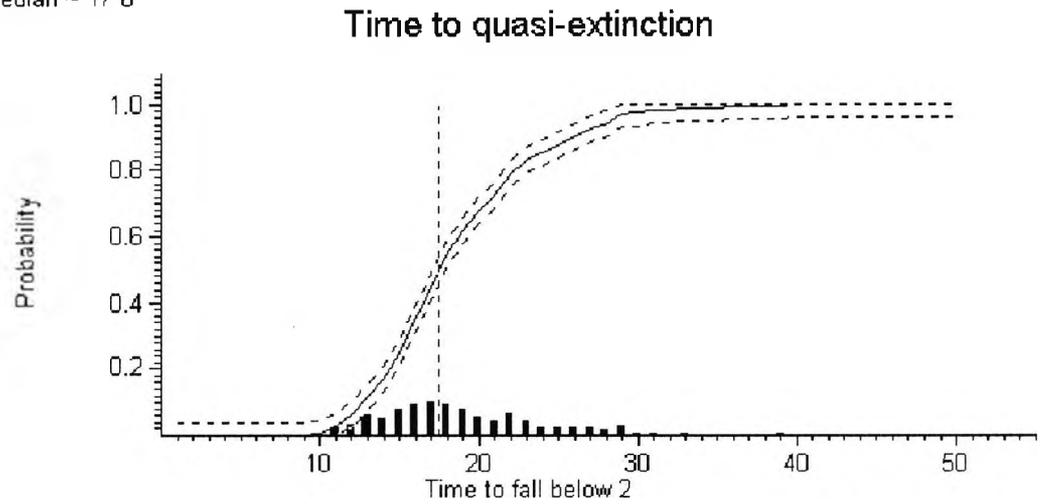
Figure II.6. [Model 2.9, "No Protection"]. Predicted time (in years) to quasi-extinction, when introduction model 2.9 is simulated, assuming the frog clumps were never protected.

Median ~ 24.8

Time to quasi-extinction
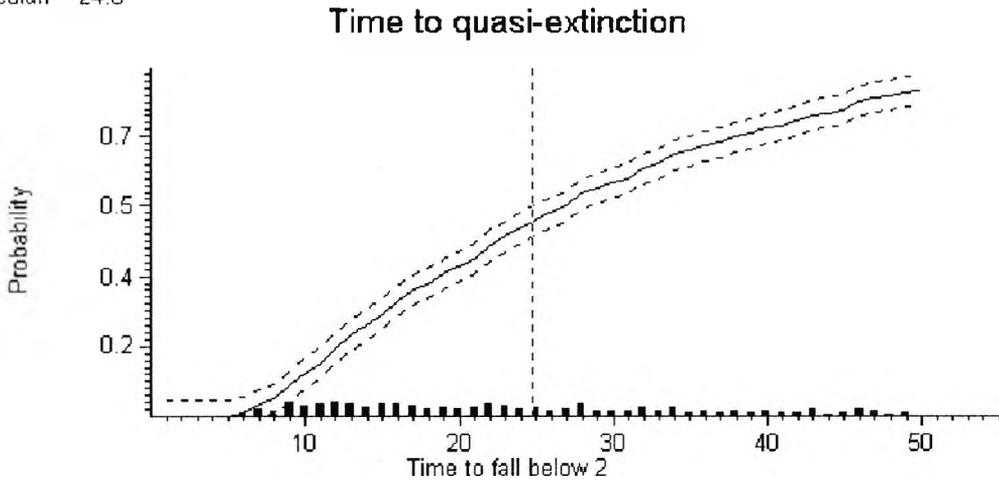
Figure II.7. [Model 2.1, "Protection"]. Predicted time (in years) to quasi-extinction, when introduction model 2.1 is simulated, assuming the frog clumps were protected every year.

Extinction risk ~ 0.06

Terminal extinction risk
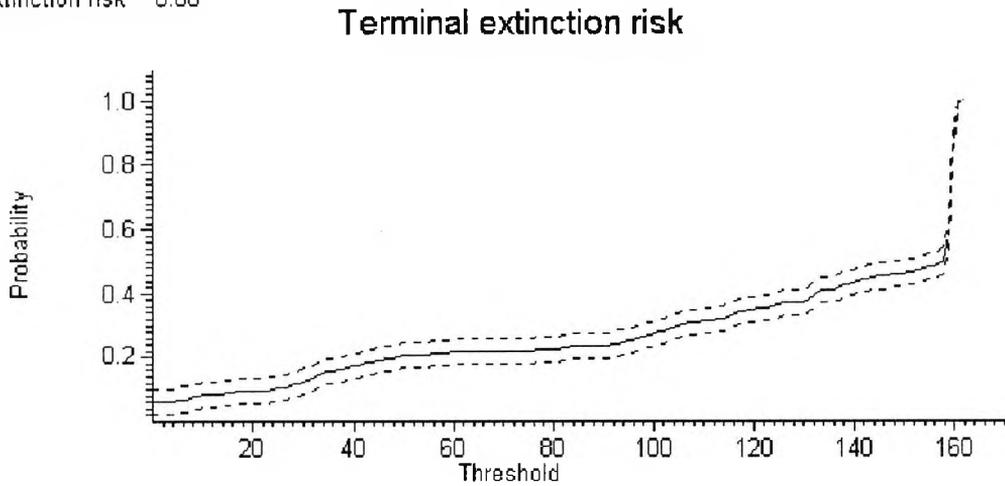
Figure II.8. [Model 2.2, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.2 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.01

Terminal extinction risk
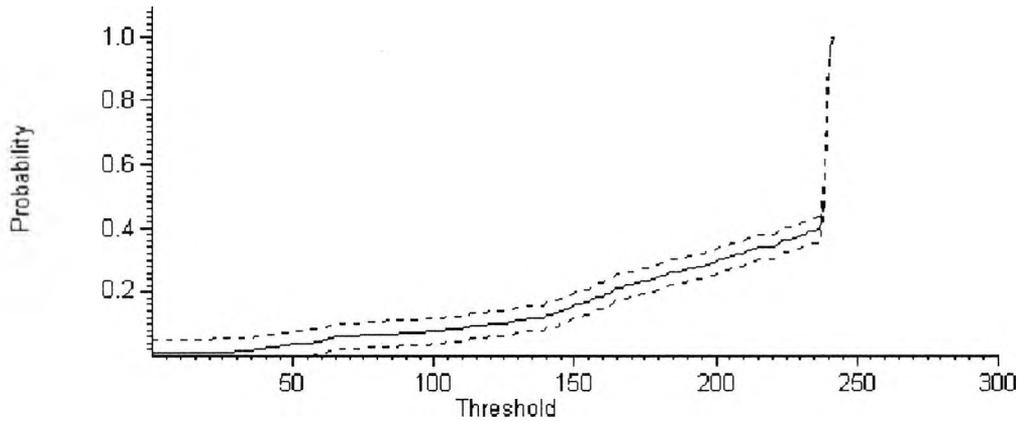
Figure II.9. [Model 2.3, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.3 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.03

Terminal extinction risk
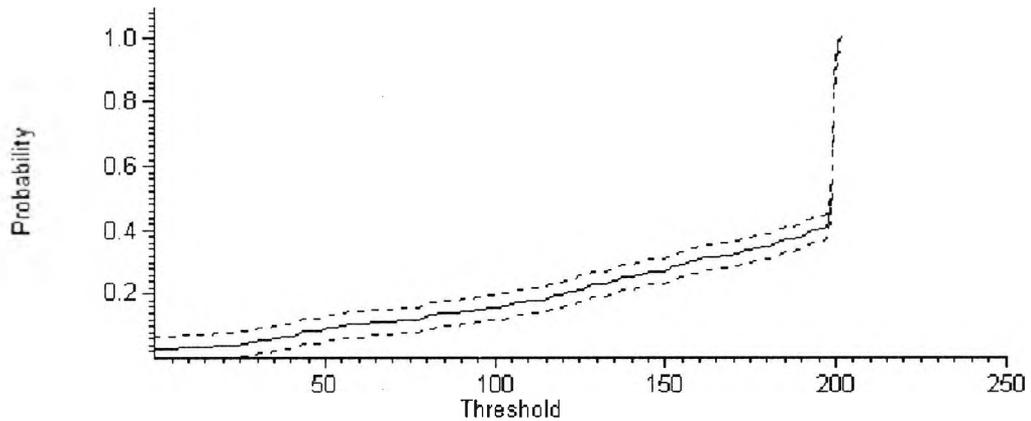
Figure II.10. [Model 2.4, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.4 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.02

Terminal extinction risk
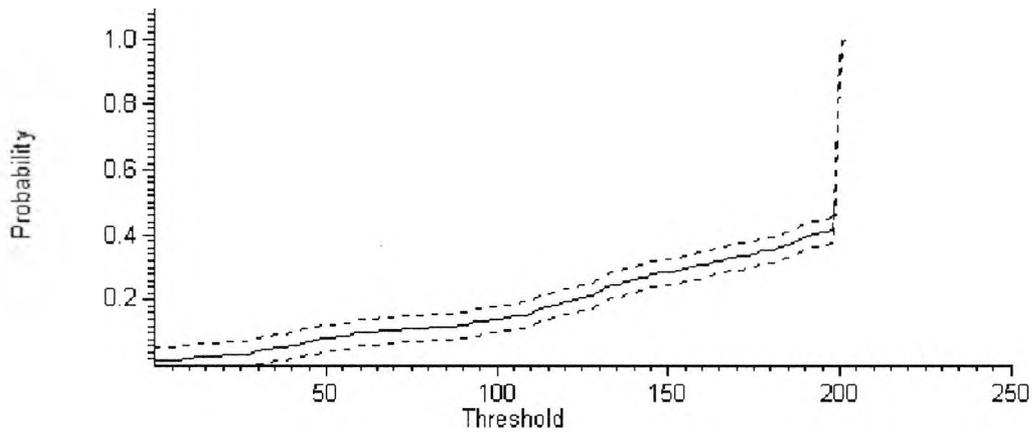
Figure II.11. [Model 2.5, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.5 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.01

Terminal extinction risk
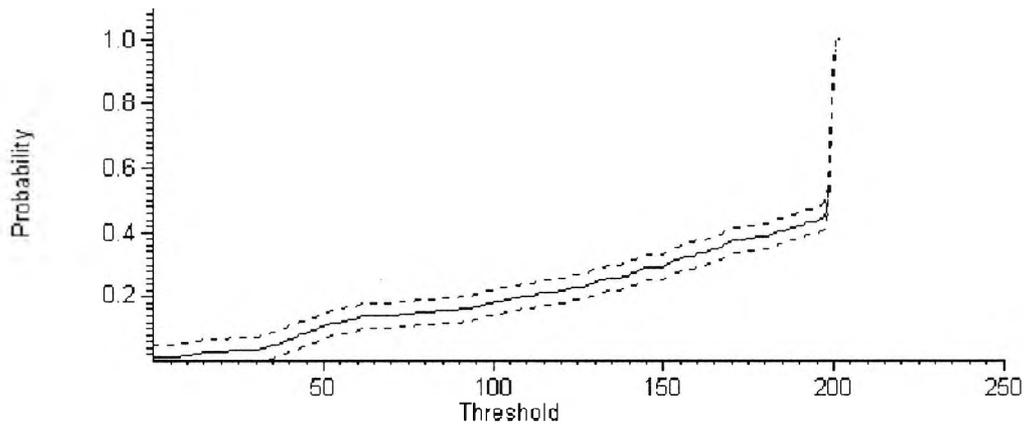
Figure II.12. [Model 2.6, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.6 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.01

Terminal extinction risk
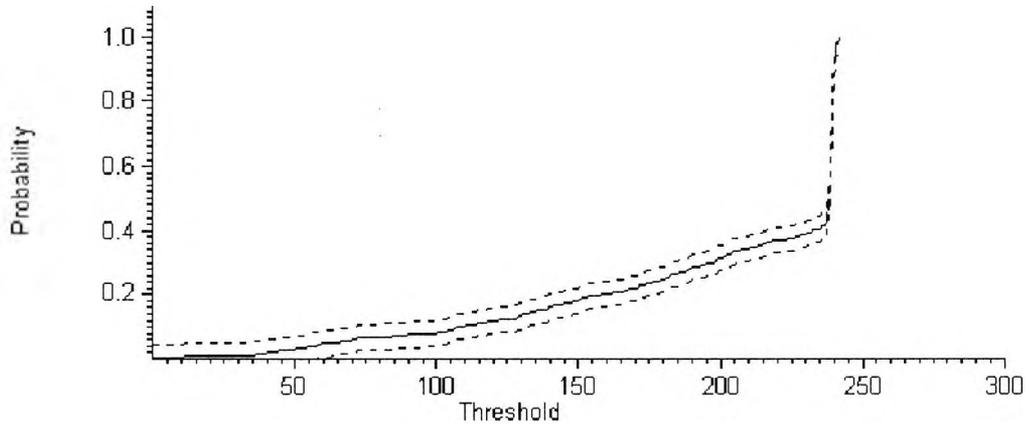
Figure II.13. [Model 2.10, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.10 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.27

Terminal extinction risk
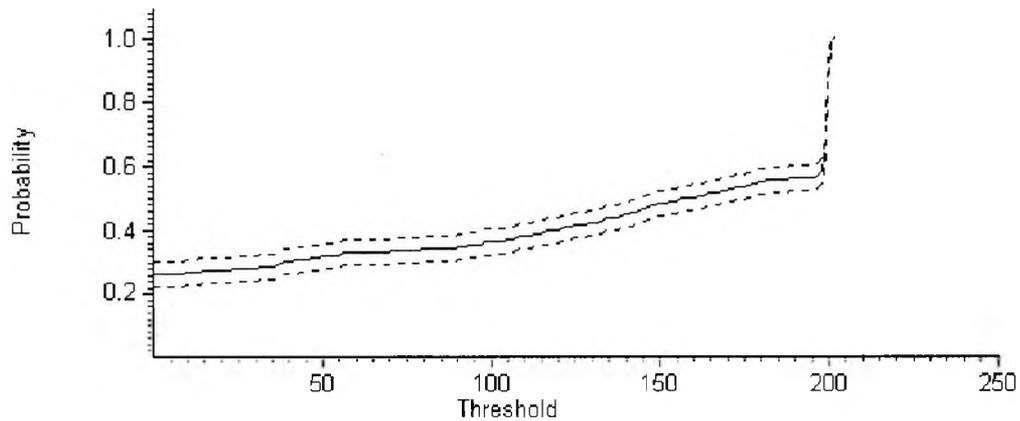
Figure II.14. [Model 2.11, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.11 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.08

Terminal extinction risk
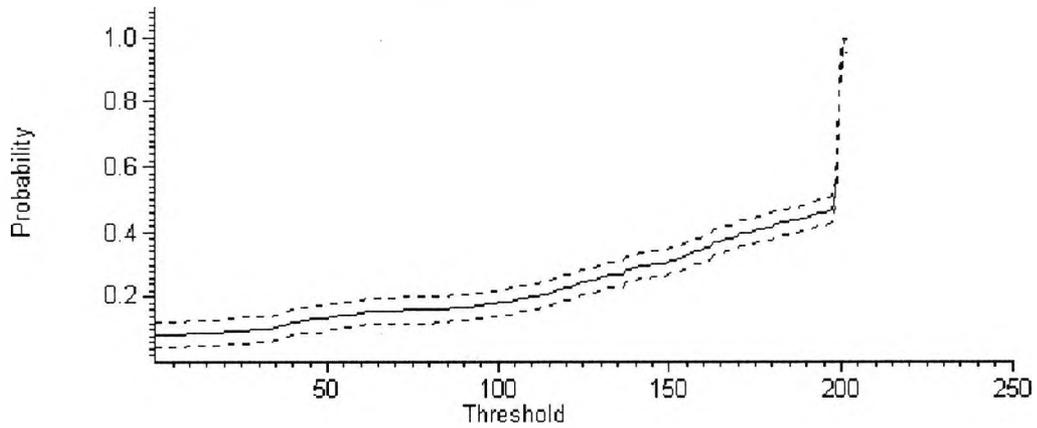
Figure II.15. [Model 2.12, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.12 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0 02

Terminal extinction risk
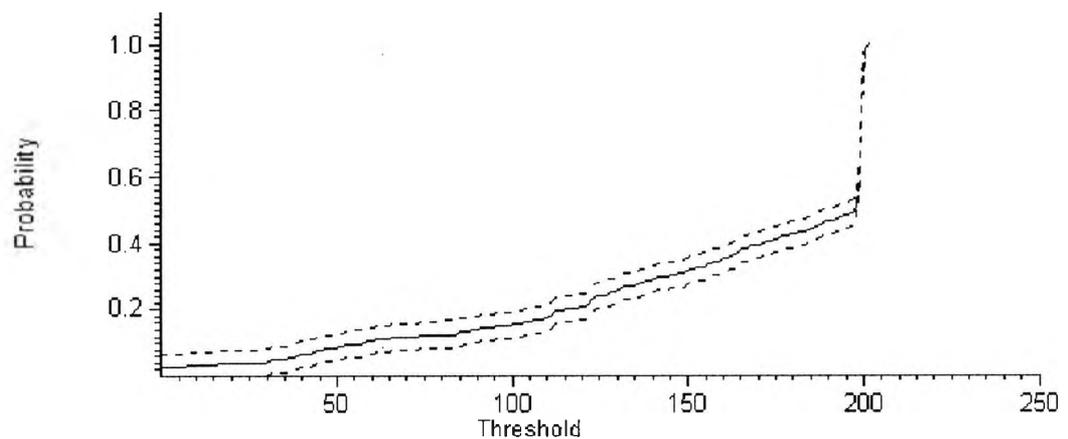
Figure II.16. [Model 2.13, "Protection"]. Predicted extinction risk over a 50 year period, when introduction model 2.13 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

HARVEST MODELS

Extinction risk ~ 0.10

Terminal extinction risk
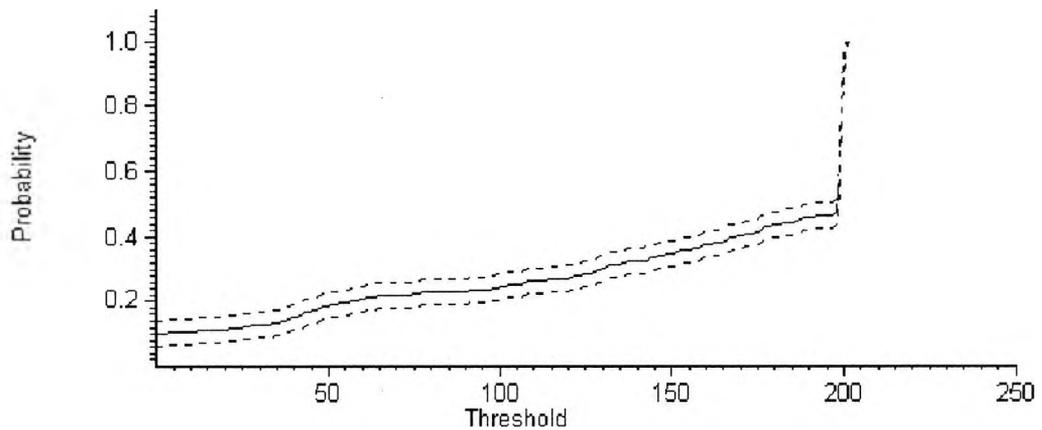
Figure II.17. [Model 3.1]. Predicted extinction risk over 50 years, when harvest model 3.1 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.08

Terminal extinction risk
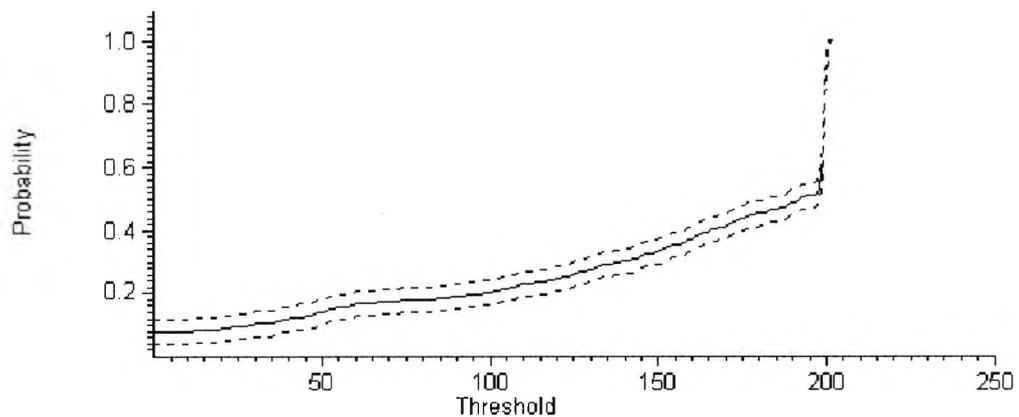
Figure II.18. [Model 3.2]. Predicted extinction risk over 50 years, when harvest model 3.2 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

Extinction risk ~ 0.06

Terminal extinction risk
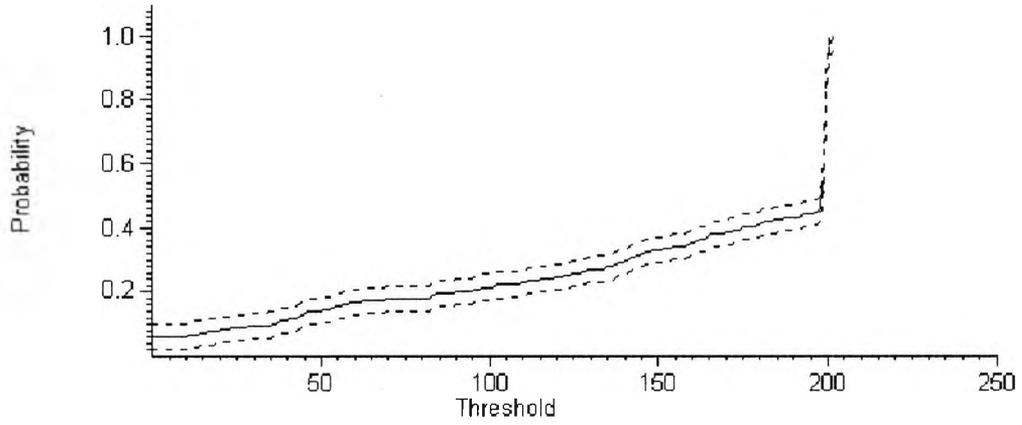
Figure II.19. [Model 3.3]. Predicted extinction risk over 50 years, when harvest model 3.3 is simulated, assuming the frog clumps were protected every year. The graph shows the probability that the population abundance will end up below a range of abundances at the end of the duration time steps.

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