EDIBLE FROG HARVESTING IN INDONESIA: EVALUATING ITS IMPACT AND ECOLOGICAL CONTEXT

Thesis submitted by

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ABSTRACT

Frogs are harvested in Indonesia for both domestic consumption and export. Concerns have been expressed about the extent of this harvest, but there have been no detailed studies on the edible frog trade in Indonesia, or on the status or population dynamics of the harvested species. To investigate the possible impact of this practise, I collected data on harvesting and trading of frog legs in Java, Indonesia. I also investigated the ecology and population dynamics of the three species that are most heavily harvested: *Fejervarya limnocharis-iskandari* complex, *F. cancrivora* and *Limnonectes macrodon*.

The first step of the study quantified the extent of the Indonesian edible frog leg trade for export and domestic purposes. Harvesting is an unskilled job and serves as livelihood for many people. There is no regulation of this harvest, and species taken and size limits are governed by market demand. The most harvested species in Java are *F. cancrivora* and *L. macrodon*. Harvests occur all year long. The number of frogs harvested fluctuates and is controlled by natural forces such as dry/wet seasons, moon phase, and planting seasons. Records of the international trade in Indonesian frog legs are available from 1969 to 2002; the mass of frozen frog legs exported has increased over the years. I used data on the relative weights of frogs and their legs to estimate how many frogs were represented by the export records. In 1999-2002 an average of 3,830,601 kg of frozen frog legs were exported annually; this represents an export harvest of approximately 28 to 142 million frogs, all with masses greater than 80 g, the minimum size of animals that have legs considered suitable for export. I used data collected by following frog hunters in the field to assess their capture rates and the numbers and sizes of frogs captured. I found that only about one-eighth of frogs

captured are of sizes acceptable for export, and therefore estimate that the domestic market is approximately 7 times as large as the export market.

In the second step of the study, I used field data on *F. limnocharis-iskandari* complex*, F. cancrivora* and *L. macrodon* to gain greater understanding of the population biology and dynamics of the harvested species. Populations of *F. limnocharis-iskandari complex* and *F. cancrivora* were studied in six rice fields in West Java during the planting seasons of 2002 and 2003. Both *F. limnocharis-iskandari* complex and *F. cancrivora* were abundant in the rice fields, with estimated overall mean densities of 193.71 and 39.76 per hectare. The mean density of *F. cancrivora* was very similar to that of unharvested populations in Malaysia (8.6 – 91.2 per hectare) (Jaafar, 1994) and was much higher than the mean density (0.7 frogs/ha) found for *Rana tigerina*, the larger species of edible Indian paddy field frog investigated by Dash and Mahanta (1993). These comparisons suggest that densities of *F. cancrivora* in Javan rice fields are relatively high, despite ongoing harvesting pressure. *F. limnocharis-iskandari* complex and *F. cancrivora* were able to breed continuously all year without an apparent season. Both species showed sexual size dimorphism, with females larger than males. Both appear to be short lived.

The population dynamics of *L. macrocodon* were observed at two stream sites West Java: Cilember and Ciapus Leutik. The habitat at Ciapus Leutik is relatively heavily modified bu human activity, while Cilember is in a nature reserve and ls less disturbed. A mark recapture study was conducted once a month between June 2002 and May 2003 (a continuous 12 month sampling period), and in January, April and July 2004. Skeletochronological analysis of phalanges removed during toe clipping suggests that this species lives longer than *F. cancrivora* or *F. limnocharis-iskandari* complex. Recapture rates of *L. macrodon* in both locations were low and more frogs were observed during periods of higher rainfall. In this study, more *L. macrodon* were found at Cilember than at Ciapus Leutik stream. It is unclear whether the low number of frogs at Ciapus Leutik was caused by over-harvesting or by other factors. There is a need for further monitoring to obtain a greater understanding of the population dynamics of this species.

In conjunction with my field work, I measured a number of parameters to determine the type and extent of pesticide residues present in rice fields, and to attempt to determine whether those and another potential stressor, drought, might be affecting the morphology, body condition, or developmental stability of frogs. I also surveyed populations of the rice field frogs and *L. macrodon* for the emerging disease chytridiomycosis, which could strongly affect the population dynamics of the frogs. Two types of pesticide residues were detected in water and soil: organochlorine (lindane, aldrin, heptachlor, dieldrin and endosulfan) and organophospate (chlorpyrifos and diazinon). Six organochlorines type (BHC, lindane, aldrin, heptachlor, dieldrin and endosulfan) and three organophosphates (propenofos, chlorpyrifos and diazinon) were detected in the livers and leg muscles of both frog species. Almost all pesticide residues were low compared to the Maximum Residue Limit set by WHO and the Government of Indonesia although a few individuals showed higher pesticide residues contents. Pesticide residue levels did not appear to be related to any measure of frog condition or stress. Both species of rice field frogs exhibited relatively low percentages of abnormalities, probably within the normal range. Only *F. limnocharis-iskandari* complex consistently exhibited levels of fluctuating asymmetry in excess of measurement error. Levels of asymmetry differed between characters. Higher limb asymmetries were elevated in 2002 compared to 2003, whilst body condition was lower in 2003. It is possible that the lower body condition in 2003 was caused by stress from an environmental factor, in this case the drought in that year. It is clear that for both *F. limnocharis-iskandari* complex and *F. cancrivora*, fluctuating asymmetry is not a powerful indicator of stress. There was no sign of chytrid infection in any samples of the three species.

To assess the impact of harvesting, I used two approaches. I developed a model of the population dynamics of *F. cancrivora* and ran simulations for ranges of parameters including harvesting rate. The simulation indicated that current levels of harvest may be near the maximum level sustainable by the population. My second approach was to compare data on the population biology and distribution of both *Fejervarya* species to criteria for assessing the conservation status of the species, i.e. IUCN Red Categories and the CITES Res. Conf. 9.24 on the Criteria for Amendment of Appendices I and II.

My assessment using listing criteria showed that neither *F. limnocharis-iskandari* complex or *F. cancrivora* are qualified for inclusion into any IUCN Red List and CITES Appendices. On the other hand, more consideration needs to be given to *L. macrodon.* This frog is not qualified for inclusion in CITES Appendix I. At present, it is not possible to determine whether this species could be listed as vulnerable or in CITES Appendix II due to a lack of data, such as survivorship among stages, and habitat size, on the population biology of this species.

Recommendations for management of the harvest include: 1) regular monitoring of this trade especially in other islands such as Sumatra by the scientific authority of CITES (The Indonesian Institute of Science or Lembaga Ilmu Pengetahuan Indonesia, LIPI), 2) regular monitoring of the numbers of export companies and their middleman, 3) developing a simple identification key and distributing it to the middlemen to ensure correct identifications, 4) assessing the possibility of breeding local frogs for this trade to replace the farming of the exotic frog *Rana catesbeiana*, and 5) ensuring that harvest is limited to species that are not adversely impacted.

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Harvest from the wild

Throughout their existence, humans have hunted animals and gathered plants for food or for protection (e.g. animal skins for clothes or blankets; big leaves for roofs). As human societies have become more complex, so have the uses of wildlife, from simple consumption to a wide variety of uses in many economic and cultural contexts (Ponting, 1991).

Wildlife may be used commercially in non-consumptive ways, for example for tourism, or in consumptive ways, for example as food, medicines and souvenirs (Freese, 1998). Although concern has been raised about the exploitation of wildlife for non-consumptive use, for example, the possibility that human interactions may alter animal behaviours (Orams, 2002), it is consumptive use that has aroused the greatest concern. As human societies evolved from hunter-gatherers into modern societies, the development of agricultural and industrial activities led to the domestication of various plants and animals (Boyden, 1992). This domestication has not eased human pressure on wildlife. Wildlife harvests still occur, both for subsistence and as mass harvests for trading in commerce. Diverse ranges of organisms are removed from the wild for commercial use. These harvests include corals for ornaments, aquaria and food (Pfister and Bradbury, 1996; Bruckner, 2000), edible swiftlet nests and pegasid fishes for medicinal purposes (Lau and Melville, 1994; Vincent, 1997), non-human primates for biomedical research (Achmad and Sulaiman, 2000) and native plants and flowers for decoration (Lamont et al., 2001; Wolf and Konings, 2001).

The impacts of wildlife harvesting in Indonesia are an area of growing concern. Indonesia is an archipelago consisting of more than 17,000 islands, situated between Asia and Australia. The unique biodiversity of Indonesia, in which the western parts are related to the Asia region while the eastern parts are related to Australia, was noted in the nineteenth century by Alfred Russel Wallace (1869) in his celebrated book, "The Malay Archipelago". This high biodiversity means that almost all parts of the archipelago are considered as hotspots for biodiversity conservation (Myers et al., 2000; Wikramanayake et al., 2002).

The harvest of wildlife in Indonesia has played an integral part in the lives of much of its human population, particularly for people that live near forests (Soehartono and Mardiastuti, 1995). Harvested wildlife, such as bush-meat (Clayton et al., 1997; Lee, 2000; Millner-Gulland and Clayton, 2002), reptiles (Shine et al., 1998), and edible swiftlet nests (Lau and Melville, 1994), was originally consumed locally, however it is now traded widely in both domestic and international markets. The international wildlife trade from Indonesia has become a lucrative business, including many species of fishes, birds, reptiles, amphibians, mammals, corals, and insects, which are traded as curios, food, pets, and zoo attractions, and for research, ornamental and medicinal purposes (Soehartono and Mardiastuti, 1995; 2002).

The harvest of Indonesian wildlife, especially for international markets, has been widely scrutinised. Many reports on the harvest and trade in specific Indonesian species have been published in journals and bulletins, particularly in TRAFFIC (Trade Records Analysis of Flora and Fauna in Commerce), and in books (Mulliken et al.,1992; Jenkins, 1995; Shine et al., 1998a, 1998b; Erdelen, 1999; Clayton and Milner-Gulland, 2000; Lee, 2000; Keogh et al., 2001; Robinson and Bennett, 2001; Soehartono and Newton, 2001; Milner-Gulland and Clayton, 2002; Soehartono and Mardiastuti, 2002). The reported impacts of these harvests have varied with the extent of exploitation and the resilience of the harvested species, with the most severe effect being extinction. Many of these reports have raised concerns that trade may endanger populations of the harvested species and have suggested that there is a need for increased control and monitoring of harvests (Primack, 2002).

1.2 Frogs for human use, with the emphasize on Indonesian frogs and threats to their existence

Humans use frogs in various ways including as pets (Spellerberg, 1976; Gorzula, 1986) and for biomedical research (Tyler, 1994, 1999; Pough et al, 2004). However, the greatest volume of trade is in frogs exploited for their legs as human food. People consume frogs in most regions of the world, including Europe, the United States, Asia, and Australia (Jenning and Hayes, 1985; Patel, 1993; Martin, 2000; Schmuck, 2000a; Truong, 2000; Vredenburg et al., 2000; Paltridge and Nanno, 2001; Szilard and Csengele, 2001). Frog legs have become an international commodity, and many countries export and import frozen frog legs. In particular, many countries in Europe and America import large quantities of frog legs, mostly from Asia (Martin, 2000). The demand for frog legs has led to the decline of populations of edible frog species in several countries, including India and the United States of America (Abdulali, 1985; Jennings and Hayes, 1985).

Approximately 450 species of anurans have been recorded in Indonesia, which represents about 11% of the total world anuran species (Iskandar, 1998). Of this number, approximately 14 species are known to be exploited for frog legs (Schmuck, 2000b), although most studies have found that only four species are widely traded in markets: *Fejervarya cancrivora, Fejervarya limnocharis, Limnonectes macrodon*, and

Rana catesbeiana, the latter having been introduced from North America in 1983 for frog farming (Church, 1960; Berry, 1975; Iskandar, 1998; Arie, 1999).

Recently, it has become apparent that many amphibian species worldwide have suffered population declines (Barinaga, 1990; Blaustein and Wake, 1990; Anonymous, 1991; Pechman, 1991; Tyler, 1991; Blaustein, 1994; Blaustein et al., 1994; Pechman and Wilbur, 1994; Blaustein and Wake, 1995;). Many possible reasons have been suggested for declines, including habitat degradation, pollution, ozone destruction, introduced species and epidemic diseases (Blaustein and Wake, 1990, 1995; Anonymous, 1991; Carey, 1993; Carey and Bryant, 1995; Gibbons, et al. 2000, Hofrichter 2000, Mattoon, 2000). Several studies have pointed out an additional possibility: reduction as a result of harvesting for consumption (Alford and Richards, 1999; Cloudsley-Thompson, 1999; Schmuck, 2000b). This threat could be important for the exploited species of Indonesian frogs.

Indonesia is one of the major exporters of frog legs (Niekisch, 1986; Martens, 1991; Schmuck, 2000b). Although the extent of this trade has been criticised (Barfield, 1986; Patel, 1993; Schmuck, 2000a), it has not been studied in detail. The biology and status of the amphibian fauna of Indonesia are poorly known in general. Little is known even for species that are commonly traded, such as *F. cancrivora* and *L. macrodon*. For instance, the only refereed article on *F. cancrivora* in Indonesia dates from 1960 by Church (1960), while the only study on *L. macrodon*, another commonly harvested species, is an unpublished PhD thesis in the Indonesian language, *Bahasa Indonesia*, from 1979 (Sugiri, 1979). Therefore, there are insufficient data available on the status of harvested Indonesian amphibian populations to evaluate possible impacts of the harvest.

Since Indonesian frogs have been harvested in relatively large numbers for many years, harvesting may have affected their status or distribution. However, in evaluating the status of edible frog populations in Indonesia, it is necessary to consider other factors that could also affect them, such as the area of available habitat, possible effects of environmental stressors such as pesticides, and the occurrence of introduced species and disease.

Because of their life history, most frogs are associated with water. However, they occupy a variety of landscapes, from ponds and streams inside forested areas to savannah and even desert areas (Duellman and Trueb, 1994). One possible cause of amphibian population declines is the loss of habitat (Alford and Richards, 1999). Dodd and Smith (2003) summarise the effects of habitat change on amphibians, mostly focusing on negative impacts. As a country with a population of more than 200 million (BPS, 2002), a huge proportion of Indonesian natural habitats have been substantially modified either for habitation or for agriculture, for instance large areas have been modified to serve as rice fields. There are no documented reports on the impacts of these habitat changes on amphibian populations in Indonesia. These impacts may not always be adverse; for example Shine et al. (1999) suggested that reticulated pythons in Sumatra are much more abundant in palm-oil plantations than in natural forests. Positive effects of habitat modification also seem likely to occur in populations of edible frogs in the rice fields. It has often been suggested that rice fields provide highly suitable habitat for frogs such as *F. limnocharis* and *F. cancrivora* (Alexander et al., 1979; Dash and Mahanta, 1993; Jaafar, 1994; Whitten et al., 1999), both species that are harvested in Indonesia (Church, 1960; Premo; 1985).

Even if the results of this study confirms the high populations of the rice fields frogs, concern regarding the impact of the frog harvest is not only related to the viability of frog populations, but also to the fact that frogs provide ecosystem and even economic services, in particular pest control. This may mean that maintenance of elevated populations is desirable in agricultural systems. Duellman and Trueb (1994) noted that most of frogs' diets are insects; therefore frogs are likely to play a role in controlling insect pests in rice fields. A study by Atmowidjoyo and Boeadi (1988) on several species of frogs in Javan rice fields showed that they consumed several species of insects that are considered to be agricultural pests. Abdulali (1985) and Pandian and Marian (1985) reached similar conclusions in their study on the diets of rice field frogs in India.

Many authors have suggested that pesticides may contribute to frog declines and disappearances (Alford and Richards 1999). It has been suggested (Abdulali, 1985; Barfield, 1985; Pandian and Marian, 1985; Oza, 1990; Jaques, 1999) that frog harvesting and pesticide use may interact synergistically. Decreases in frog populations caused by harvesting may allow pest populations to increase, leading to increased use of pesticides, which may then have greater negative effects on frogs.

In 1983, to meet the demand for frog legs, the Indonesian government, through its aquaculture research centre in Sukabumi, introduced *Rana catesbeiana*, the North American bullfrog. The introduction was considered a success and this species has now been distributed to other areas for culturing (Arie, 1999). Several studies in the USA have suggested that feral populations of *Rana catesbeiana* outside the natural range of this species have caused declines of native frog populations (Moyle, 1973; Emlen, 1977; Jennings and Hayes, 1985; Hayes and Jennings, 1986; Lanoo, et al., 1994; Lawler et al., 1999; Adams, 1999, 2000). There are no studies on the impact of the introduction of *Rana catesbiana* on Indonesian native frogs. If feral populations of *Rana catesbeiana* become established in Indonesia, they are likely to become a threat to native species, as forewarned by Iskandar (1998).

Introduced frogs can be detrimental to native frog species as competitors or predators, but also as vectors of disease (Carey et al., 2003). Emerging diseases like chytridiomycosis and ranaviral disease are linked to frog population declines (Berger, et al., 1998; Daszak, et al., 1999), and diseases caused by parasites are also linked to frog deformities (Kaiser, 1999; Leong, 2001). The effects of competition, predation, pesticides, and disease can interact, since environmental stresses due to any of these factors can increase frogs' susceptibility to diseases (Carey and Bryant, 1995; Thieman, 2000).

1.3 The sustainability of wildlife harvests

Sustainability is an important concept in wildlife management (Caughley, 1977; Fitter, 1986; WCED, 1987). The World Commission on Environment and Development, in their report "Our Common Future" (1987), referred to a sustainable use as one that "meets the needs of the present without compromising the ability of future generations to meet their own needs". Thus, a simple definition of sustainability in harvesting natural resources is that the harvest of a certain species for a given time must not surpass the ability of the species to produce offspring, ensuring that the next harvest will not be lower (Caughley, 1977; Fitter, 1986). The maximum rate of harvesting that permits this is known as the maximum sustainable yield (MSY; Mace, 2001).

In terrestrial animals, concerns regarding the effects of harvesting have primarily been raised in the context of animals hunted as game (Fa et al., 1995; Fitzgibbon et al., 1995; Forsyth, 1999; Lee, 2000; Peres, 2000; Beissinger, 2001; Fa and Peres, 2001; Milner-Gulland and Clayton, 2002; Hurtado-Gonzales and Bodmer, 2004). Compared to amphibians, game mammals and birds may be more vulnerable to negative effects of harvesting because of their life history characters, such as slower growth rates, late maturation, high adult survival rates and low number of offspring, as shown by the decline and extinction of bison (*Bison bison*), the passenger pigeon (*Ectopistes migratorius*) and the Labrador duck (*Camptoryhncus labradorius*) in the United Sates of America and many other species (Bolen and Robinson, 1999). On the other hand, there are reports that harvested species from the tropics are able to withstand intensive commercial exploitation, mostly due to their life history traits and ability to tolerate habitat change (e.g., Shine et al., 1999 regarding the reticulated python harvest in Indonesia, and Webb, 2001 on the Hawksbill turtle harvest in Cuba). The harvest of frogs may be more analogous to fisheries; many species of frogs and fishes can potentially have very high rates of increase, and therefore may be able to recover rapidly from over harvesting. Despite this potential, however, some of the world's fisheries have been severely depleted, for instance in the case of North Sea herring, the Atlantic Cod, Hokkaido herring and the Californian and Japanese sardines (Beverton, 1998; Reynolds et al., 2001; Hutchings, 2001, Jackson et al., 2001). Determining suitable levels of frog harvest may therefore present a unique challenge that has not been fully appreciated.

Although not without criticisms, the concept of sustainable harvesting is integral to fisheries management approaches around the world (Mace, 2001). Some fisheries species have long been managed for sustainable harvesting such as the Pacific halibut (McCaugran, 1997) and the Alaska's sockeye salmon (Schmidt et al., 1997). The concept has been successful in other regions, for example in Australian crocodiles and kangaroos (Cairns and Kingsford, 1995; Callister and Williams, 1995). Researchers have proposed conserving other species through sustainable harvesting (see Barbier et al., 1990 for African elephants and Beissinger and Bucher, 1992 for parrots).

To develop a sustainable harvest policy the population ecology and exploitation rates of the harvested species must be known in detail (Rosenberg, et al., 1993). There is a particular need to understand the extent of natural fluctuations caused by environmental factors, food availability, and the abundance of predators (Bolen and Robinson, 1999).

Concern about the extent of the international wildlife trade first appeared in the 1960's at the 7th General Assembly of the IUCN (International Union for the Conservation of Nature and Natural Resources) (Soehartono and Mardiastuti, 2002). It resulted in the birth of CITES or the Convention on International Trade in Endangered Species. The main goal of CITES is to prevent species extinction due to trade by putting bans or restrictions/quotas on trade in vulnerable species (Freese, 1998; Hutton and Dickson, 2000). Indonesia joined the Convention in 1979 (Soehartono and Mardiastuti, 2002). Members of CITES regularly gather in the Conference of Parties to discuss the status and quotas of traded species. Species traded can be put under the lists of Appendices I, II, and III, depending on the degree of protection necessary (Hutton and Dickson, 2000).

Frogs were one of the many species of concern in this Convention. For example, extensive harvesting of frog legs for export in India led to the inclusion of the harvested species in Appendix II of CITES (Pandian and Marian, 1983). Many elements of the fauna of Indonesia have been proposed for inclusion in the CITES appendices (Soehartono and Mardiastuti, 2002). Some proposals have been successful, however some have failed because of lack of data. During the early 1990's several Indonesian *Rana* species were proposed for listing in CITES Appendix II (Martens, 1991). This proposal was rejected because of heavy pressure from Indonesia and support by other CITES members (Favre, 1989; Schmuck, 2000a). Favre (1989) mentions that it is quite difficult to impose a ban on frog exports because there is not enough data on their population status and the extent of the trade to justify a CITES listing.

1.4 Research Focus

Gaps in our knowledge of the trade in edible Indonesian frogs make it difficult to evaluate the impact of this trade and its conservation implications. Presently available data are inadequate to evaluate concerns that have been raised about potential reductions in frog populations due to harvesting. The primary purposes of this study are therefore to provide sufficient empirical data to determine the extent of the Indonesian frog leg trade by collecting and analysing detailed observations on harvesting and trading, to determine the population status and dynamics of the traded species through the collection of field data, and to combine this knowledge to evaluate the impact of harvesting on Indonesian edible frogs. Empirically evaluating the impact of this trade will increase the likelihood of effectively conserving the edible species while maintaining sustainable use. The study is the first research that has attempted to quantify the total extent of the frog harvest in Indonesia. Increased knowledge of the economic and ecological significance of this trade will contribute to the management of the affected species.

1.5 Research Aims

The aims of the research are to:

1. Quantify the extent of the Indonesian edible frog leg trade including both export and domestic consumption.
2. Investigate the population status of the harvested species in enough detail to permit modelling species rates of increase.

3. Investigate the possibility that environmental factors such as pesticides and diseases affect the abundance or condition of the edible species
4. Incorporate the information on population biology and harvesting into a model of the dynamics of harvested populations of one of the most harvested species, the rice field frog *Fejervarya cancrivora*.
5. Investigate whether species are being sustainably harvested and the conservation status of the species in relation to IUCN categories and the CITES convention.

1.6 Thesis outline

The thesis includes nine chapters. **Chapter 1** gives the general introduction and background of the study. **Chapters 2 and 3** examine the extent of the frog leg trade in international and domestic markets, including information on market structure and harvest effort. **Chapter 4** presents detailed information on the species and sites studied, including species' distributions in Indonesia and detailed descriptions of study sites. The population biology of three species of edible frogs, two that live in rice fields (*Fejervarya limnocharis-iskandari* complex and *F. cancrivora*), and one stream frog (*Limnonectes macrodon*), is presented in detail in **Chapters 5 and 6**. Other environmental stressors that might affect the population of edible frogs, especially frogs that live in rice fields, are examined in **Chapter 7**. Conservation status assessment of *F. cancrivora* and *L. macrodon*, through population modelling and conservation criteria based on the IUCN Red List and CITES Appendices is presented in **Chapter 8**. All results are then integrated in **Chapter 9** where I discuss specific conclusions and their implications for the conservation of Indonesian edible frogs.

CHAPTER 2

INTERNATIONAL TRADE IN INDONESIAN FROG LEGS

2.1 Introduction

Frog legs are widely considered to be delicacies and are harvested in many countries such as in Vietnam, the United States of America, and Romania (Jennings and Hayes, 1985; Truong, 2000; Szilard and Csengele, 2001; Torok, 2003). The numbers of frogs taken are sometimes not enough for domestic consumption, so some countries import large quantities of frog legs, mostly from Asia (Martin, 2000). Indonesia is one of the primary exporters of frog legs (Niekisch, 1986; Martens, 1991; Schmuck, 2000b). The extent of the Indonesian frog leg trade has not been studied in detail.

Little information is available on the volume of Indonesian frog leg exports, and data readily available only for certain years (Barfield, 1986; Niekisch, 1986; Martens, 1991; Schmuck, 2000b). Gaps in our knowledge of the trade in edible Indonesian frogs make it difficult to evaluate the impact of this trade and its conservation implications. Although concerns have been raised about the possibility of reductions in frog populations caused by frog hunting (Barfield, 1986; Patel, 1993; Schmuck, 2000a), there has been no means of evaluating the true impact of this activity on Indonesian frog populations, and it might not be as great as people fear.

To understand the impact of this trade in relation to ecological sustainability, it is necessary to assess the edible species trade and harvest by examining both trade information and the distributions and population status of edible frog species. In this chapter and chapter 3 I will examine the trade in Indonesian frog legs for international and domestic markets. In this chapter, I review frog leg exports based on recorded data obtained from the Indonesian Statistical Bureau for the period from 1969 until October 2003 and on interviews with frog exporters.

2.2 Methods

Frog leg export data are usually recorded by the authorities at the point of export, predominantly at seaports or airports. The data are compiled to produce annual trade data, which are published in the *Foreign Trade Statistical Bulletin* by the Indonesian Statistical Bureau. To determine the scale of the trade in frog legs, I examined trade statistics reported in this bulletin. I looked for statistical records for frog leg exports commencing in 1960, and found that 1969 is the first year that explicitly included frog legs in the reported data. Based on these data, I assembled an overall history of the frog leg trade, including information on which countries are major importers, the value of the trade, net weight exported/year, and the locations of major frog sources.

2.2.1 Harvest estimation

To estimate the actual take of frogs for export purposes, I measured snout-vent lengths, whole wet masses, and skinless leg masses of *L. macrodon* (36 females and 27 males) and *F. cancrivora* (32 females and 9 males) to obtain the ratio of wet mass to skinless leg mass for each species. This ratio differed significantly between *F. cancrivora* and *L. macrodon* ($F_{1,102}$ =75.509, P <0.001). However, since data on exports are not presented separately by species, I used the combined estimates of mean mass per pair of legs and mean total mass of frog per mass of legs to estimates the numbers of frogs and total mass of frogs removed from the wild.

The equation to estimate harvest is as follows:

Total number of frogs harvested = annual mean mass of exported frog legs (grams) / mean weight of pair of legs

Table 2-1. The proportion of whole body mass accounted for by leg mass in *F. cancrivora* and *L. macrodon*.

	N	Mean	Std. Deviation	Range
F. cancrivora	42	0.3208	.03143	0.25 – 0.41
L. macrodon	63	0.4160	.06751	0.21 – 0.65
Total	105	0.3772	.07272	0.21 – 0.65

From Table 2-1, on average a frog's legs account for 37.7% of its total weight. The correlation between total weight and length was estimated based on regressions taken from mature *F. cancrivora* and *L. macrodon* captured by harvesters (Figure 2-1). The equation is:

$$Log\ Weight = 2.724*logSVL-3.456\ (F_{1,794}= 5028.869;\ P= 0.000)$$

Estimates were made in two ways:

1) Using harvest data from harvesters (Chapter 3, table 3-2) for frogs with body masses of at least 80 grams, I assumed that the SVL of exported edible frogs is between 89 – 162 mm with mean of 101.43 mm. Since data were taken from harvesters that mostly cater for domestic consumption, the SVL assigned here might be biased to smaller frogs. Predicted total weight: range 71.41 – 365.36 grams, mean = 100.87 grams. Predicted weight of pair of legs (total weight x 37.7%): range 26.96 – 137.81 grams, mean = 38.05 grams.
2) Presuming that frogs taken for export might be larger, I assumed that the SVL of edible frogs is 100 – 150 mm with mean of 125 mm. Predicted total weight: range 98.17 – 296.26 grams, mean = 180.29 grams; Predicted weight of pair of legs: range 36.32 – 109.62 g, mean = 50.56 grams.

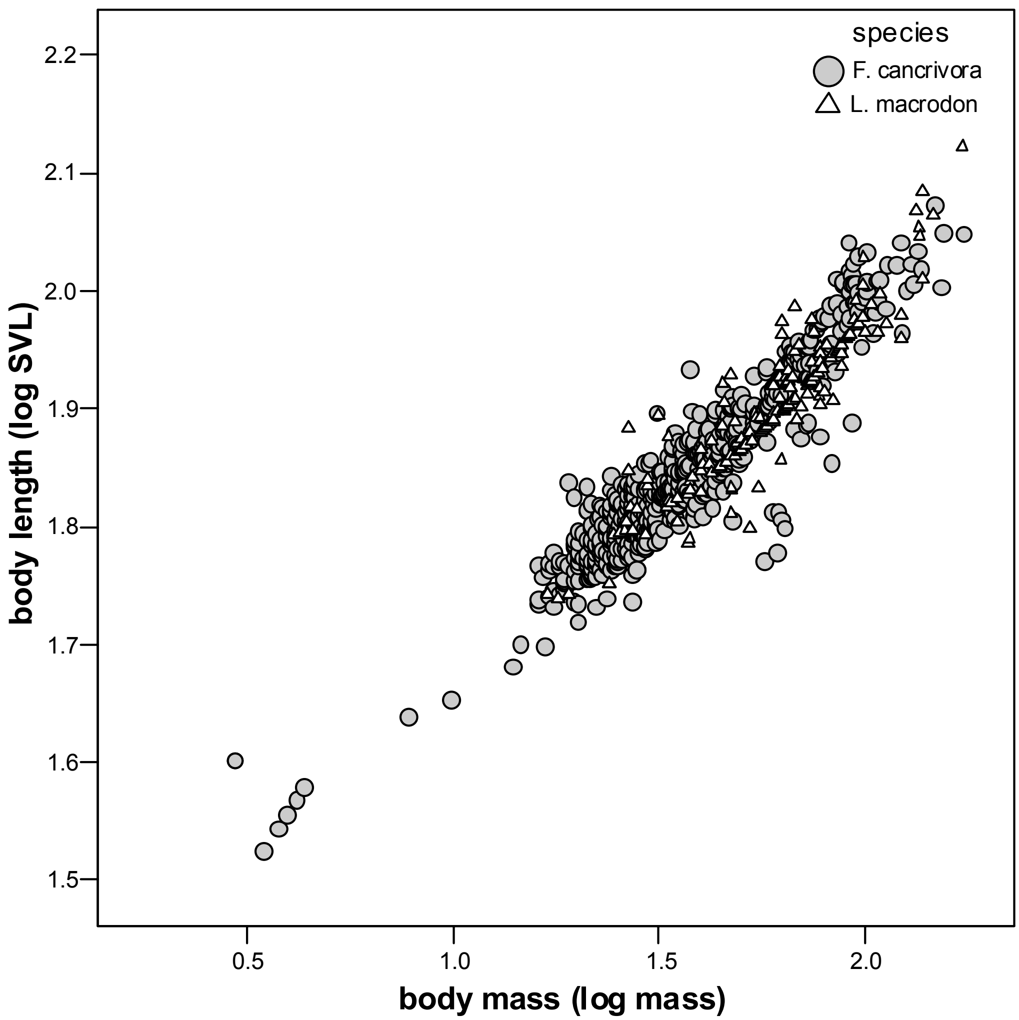

Figure 2-1. The correlation between body length (SVL, mm) and body mass (g) of *F. cancrivora* and *L. macrodon* based on captured frogs from harvester.s

2.2.2 Exporter Profiles

To gather more insight on exports, I interviewed three exporters. Two exporters were interviewed in Surabaya (East Java) during August 2003, and one exporter who resides in Cirebon (West Java) answered the interview questions through email. I defined exporters as persons who manage and operate export Companies, and who buy frogs from middlemen or local traders (see Chapter 3 for information), processed the frogs in their factories and sold them overseas. Questions to exporters consisted of enquires to determine species sold, the seasonality of frog captures, sources of frogs, countries to which exports were destined, and revenue and problems related to frog export.

2.3 Results

The earliest data available on frog leg exports are from 1969 as mentioned by Susanto (1989). Frog legs were listed under fisheries products; from 1969 - 1974 they were listed simply as frog meat. From 1975 – 1980 they were further categorized as frog legs, fresh, and chilled/frozen. The number of categories increased further in later years. The most recent classification of frog legs in the statistical publication is based on systems first developed in the 1989 Indonesian Tariff Classification, and updated in 1991, 1993 and 1996. Meat from frog sources is now registered under five categories which are 1: meat and edible meat of frog legs, fresh or chilled, 2: meat and edible meat of frog legs frozen, 3: meat and edible meat of frogs (excluding legs) fresh or chilled, 4: meat and edible meat of frogs (excluding legs) frozen, and 5: other meat of frogs. However, there is no mention of the species from which the meat or legs are taken.

2.3.1 Species exported

Since species taken for export are not recorded in the statistical data, information on species taken was gathered from interviews with exporters. Interviews revealed that all companies received frogs from suppliers as skinless frog legs. They usually want big frogs and accept *F. cancrivora, L. macrodon* and *Rana catesbeiana*. One company actually only accepts *L. macrodon*, however the manager admitted that since supplies come in the form of skinless frog legs, as long they are of acceptable size, it is possible that they include other species such as *F. cancrivora.*

2.3.2 The volume and value of Indonesian Frog Leg Exports

In this account, all forms of "edible frog meat" are included together. Although since 1991 there are records of frog meat categorized under "edible frog meats excluding leg", the quantity is very small (0.34% of total); I have thus included this quantity with the remainder of the data. Figure 2-2 summarizes export volumes, and makes it apparent that exports of Indonesian frog legs increased between most years from 1969 to 1992, especially during the period 1985 – 1992. Exports of frog legs decreased in 1993, and remained relatively constant thereafter, with a slight increase in 1999. To detect trends, I examined the data in four periods, 1969 - 1978, 1979 - 1988, 1989 - 1998 and 1999 - 2002. The average annual volume increased two to three fold between the early period (1969-1978) and the post-1980 years (Table 2-2). The highest export of frog legs was in 1992, with a total mass of 5,630,434 kg, valued at $US 23,596,841.

Table 2-2. Annual mean volume of frog legs exported and predicted number of frogs taken for export. A is for SVL between 89 – 162 mm, mean 101.43 mm and B is for SVL between 100 – 150 mm, mean 125 mm

Periods	Annual mean mass of frog legs (kg)	Annual range number of frogs taken		Annual mean number of frog taken	
		A	B	A	B
1969-1978	1,402,395	10,176,063 – 52,018,796	12,793,696 – 38,607,229	36,857,617	21,022,568
1979-1988	2,878,087	20,883,983 – 106,756,384	26,256,063 – 79,232,287	75,461,618	43,143,892
1989-1998	4,301,680	31,213,863 – 159,561,473	39,243,143 – 118,423,087	113,056,359	64,484,227
1999-2002	3,830,601	27,795,618 – 142,087,821	34,945,608 – 105,454,519	100,675,504	57,422,529

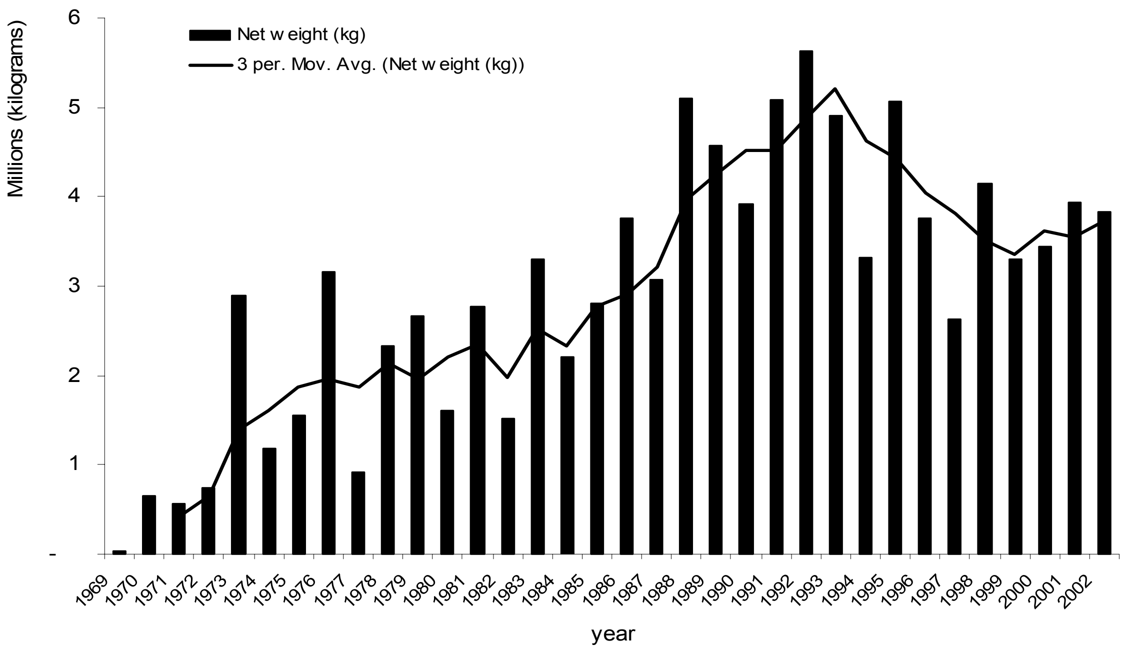

Figure 2-2. Volume of frog legs exported by Indonesia from 1969 – 2002. 3 Moving average: A sequence of averages that are computed from the every three data series to smoothes the fluctuations in data, thus showing the pattern or trend more clearly.

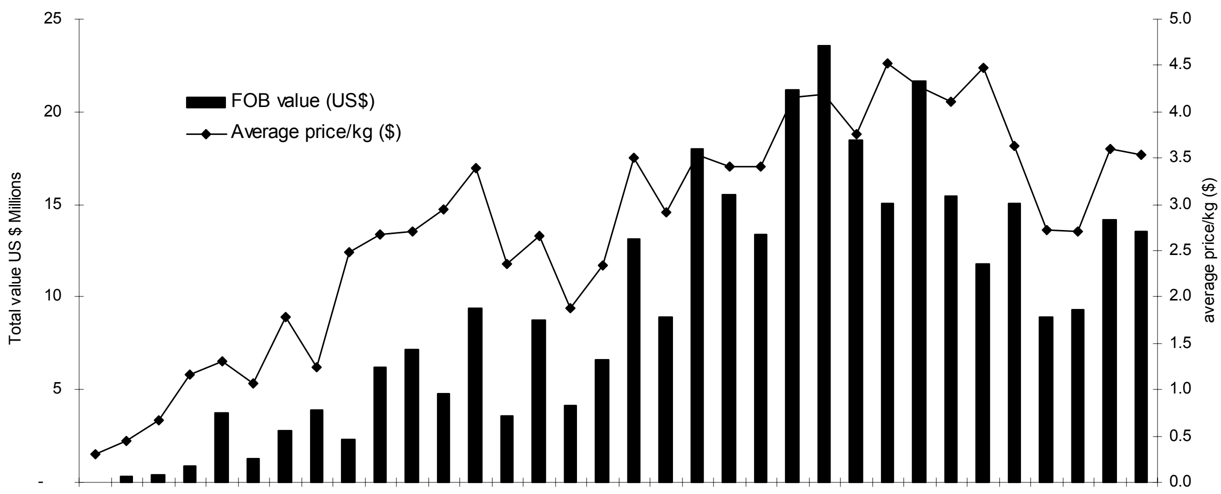

Figure 2-3. Value of frog legs exported by Indonesia from 1969 – 2002. FOB value: value of export which still included shipping costs and the insurance costs) from the point of manufacture to a specified destination.

As would be expected, the value of frog leg exports has risen with the increase in volume (Figure 2-3). The average price of frog legs in US$ increased over the period for which figures were available, with some fluctuations. It was US$ 0.31/kg free on board (FOB)[1] in 1969, and by 2003 it was US$ 3.53/kg .

2.3.3 Destinations of exported frog legs

In total, there are 36 ports of destination for Indonesian frog leg exports. Ten of these are in countries in Asia (Singapore, Malaysia, Hong Kong, Vietnam, Korea, China, Japan, Taiwan, Pakistan and East Timor); two are in Middle East countries (Egypt and Bahrain); 14 are in European countries (Austria, Belgium and Luxembourg, Bulgaria, Czechoslovakia, Denmark, Finland, France, Germany, Italy, Netherlands, Spain, Sweden, Switzerland, and United Kingdom; four are in Latin America (Ecuador, Mexico, Bahamas and Brazil); two are in North America (the USA and Canada) and four are in Pacific countries (Australia, Papua New Guinea, New Caledonia and other countries in Oceania). Although Belgium and Luxembourg are different countries, in the statistical reports they are combined as one entity.

The major importer of Indonesian frog legs is Europe (83.2% of total exports). Of the European total, Belgium and Luxembourg take the greatest proportion (47.6%), followed by France (27.6%) and the Netherlands (21%). Eleven other countries in Europe import frog legs, none taking more than 1% of the European total. In the earliest period (1969-1980), the main European importer of Indonesian frog legs was the Netherlands, however, after the 1980s Belgium and Luxembourg dominated the imports. Exports to Europe tended to fluctuate but increased dramatically after 1985,

[1] FOB. A shipping term that indicates that the supplier pays the shipping costs (and usually also the insurance costs) from the point of manufacture to a specified destination, at which point the buyer takes responsibility.

while exports to Asia tended to increase slightly after 1985 and stabilized around 500 tons annually.

The second largest export destination of Indonesian frog legs is Asia (around 12%). Ten countries in Asia import Indonesian frog legs; the leading importers are Singapore (50.5% of the Asian total), Hong Kong (22.9%) and Malaysia (18.3%). The only Asian country that has imported Indonesian frog legs in every year since 1969 is Singapore. Malaysia has imported continuously since 1981. Two countries in North America (USA and Canada) currently import small volumes of frog legs from Indonesia. These countries and apparently did not import from Indonesia during the 1980s.

The results reported above corresponded with those from my interviews. All exporters reported that most exports go to Europe (Belgium, France, Holland, and Switzerland) and a small number to the United States and Singapore. They also reported that demand for frog legs from overseas has been increasing. One manager said that large increases in demand occurred during the 1980s and since then the market has been relatively constant, increasing at slow rates compared to the 1980s. Exporters also reported that they sometimes could not keep up with demand because they are limited by rates of supply. During February to August (the dry season) supply is usually lower than during September – January (the wet season).

2.3.4 Frog Exporter Profiles

Companies that export frogs usually also export other fisheries products such as prawns and fish. In the Ministry of Fisheries report for 2000 there were 22 frog leg exporting companies listed, employing a total of 3596 people. Almost two thirds (14) of these companies were based in Java and the rest in Sumatra. This number fluctuates from year to year. After trying for two years to track down some of the companies, I found that some of them had closed down and there are more companies that are not listed at all. I interviewed managers from two exporting companies that were not listed in the Ministry of Fisheries Report (2000). One company started in 1972 and the other started in 1999. Both companies export other seafood products, mainly prawns and lobster. Exported frogs mostly came from East Java (Kediri, Madiun, Nganjuk, Bojonegoro, Pasuruan, Jombang, Madura), but also from other parts of Java such as West Java (Tasikmalaya and Cirebon), Central Java (Solo, Cilacap), and outside Java such as Lampung (in the south of Sumatra), Bali and South Kalimantan (Banjarmasin). Both managers declared that they rely mostly on supply from adjacent areas, however supplies from other islands serve as additional capacity, especially during periods of low supply. Most frogs were removed from natural habitat, and only a small number came from frog farming. One of the suppliers of one company in Lampung was a frog farm supplying *Rana catesbeiana*.

Exporting companies usually have cold storage, processing and packaging facilities that work at least 6 days a week, and employ 30-50 full time workers (Figure 2-4). The number of workers can be higher if there is a high demand. One company indicated that sometimes they hire more than 100 people to work part-time. Frogs were processed in compliance with international quality standards (Ministry of Agriculture, 1993). Batches of frogs exported were usually tested beforehand by an appointed laboratory and given certified clearance that the batches were free from bacteria, parasites and other pathogens such as salmonella.

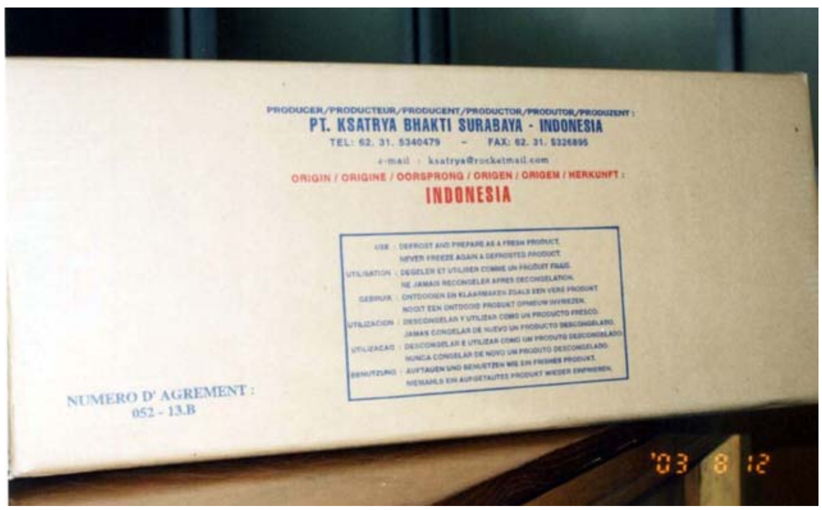

Figure 2-4. Processing frog legs for export (top) and box of skinless frog leg ready to be shipped (centre and below)

2.3.5 The sources of exported frog legs

Export records showed that there are twenty-three ports of exportation. The ports are situated on five main islands: Java (seven ports), Sumatra (11 ports), Kalimantan (three ports), Sulawesi (one port) and Bali (one port). Based on the interviews with exporters, although some frogs originate from other islands, most come from the island on which the company operates. Since it is difficult to differentiate the number taken from each province, I assume that frogs originated on the islands on which exporters are based. On this basis, it is likely that most exported frogs came from Java and Sumatra (see Figure 2-5). Based on interview data, the majority of frog legs from Java come from three provinces, Jakarta, Eastern Java and Central Java, and most frog legs from Sumatra originated in North Sumatra and South Sumatra provinces. The contribution of frog legs from Sumatra to total exports increased during each period as the contribution from Java decreased until the period between 1994-1998, however during 1999 – 2003 the contribution from Java rose again (Figure 2-5).

There are no data regarding whether exported frogs originated from culture or were removed from natural habitats. The frog culture industry in Indonesia began in 1983 with the introduction of bullfrogs (*Rana catesbeiana*) as part of an Indonesian government program to increase frog leg exports (Susanto, 1989; Arie, 1999). In 1999, the government, through the Ministry of Fisheries, launched a program to increase fisheries production for export (PROTEKAN) by developing various aspects of fisheries commodities including freshwater aquaculture by the year 2003. The freshwater aquaculture industry is primarily directed at three commodities: *Tilapia*, frogs and freshwater turtles. Directorate General of Fisheries (DGF, 2001) estimated that frog culture might produce 1,650 tons per year by the year of 2003. Based on this amount, it appears that most exported frogs have been, and will continue to be, removed from natural habitats.

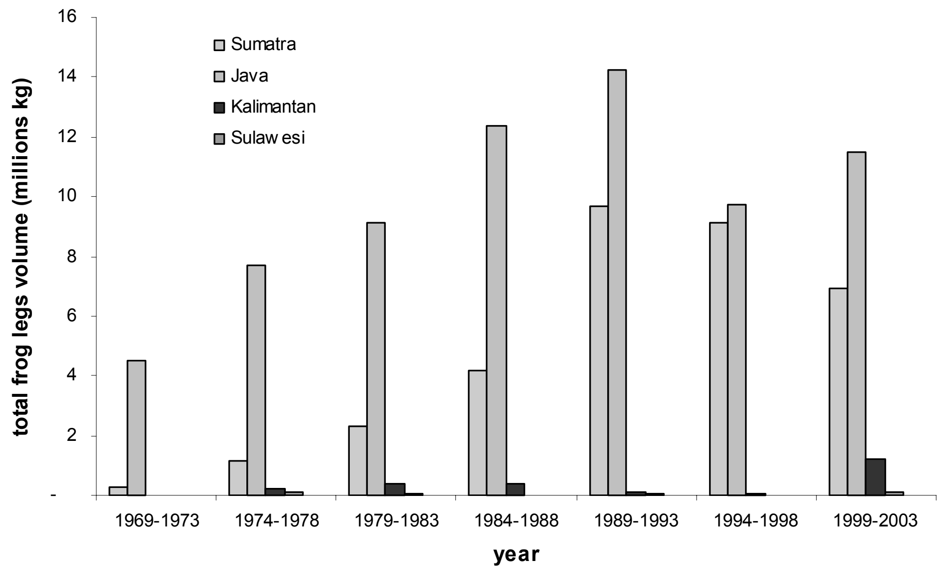

Figure 2-5. The volume of frog legs exported out of ports in four main islands in Indonesia

A discussion with staff from Balai Budidaya Air Tawar (the Centre for Freshwater Aquaculture) in Sukabumi, West Java on October 2001 also revealed that many farmers had stopped culturing *Rana catesbeiana* because of high maintenance costs and disease. This was confirmed by an interview with one of the frog farmers in East Java. This indicates that frog culture is unlikely to contribute substantially to future exports.

2.3.6 Number harvested for export

The mean number of frogs exported annually during 1989-1998 is estimated in the range of 31 – 160 millions frogs per year, depending on the length of frogs used in the equations (Table 2-2). However, the actual number of frogs taken was probably higher than recorded. Exporters will only take frog legs that have no bruises or other imperfections. Because frogs are usually captured using a net on a long pole, some are inevitably bruised, and the number of frogs rejected by exporters varied from 2 - 5% of the total frogs caught, although one middlemen remarked that he had once rejected as much as 30% (Chapter 3). Rejected frogs are sometimes thrown away because some domestic markets prefer live frogs (Chapter 3).

2.4 Discussion

Previous authors have reported that the common edible frogs of Indonesia consist of *Fejervarya cancrivora, F. limnocharis, L. macrodon,* and the introduced species *Rana catesbeiana* (Church, 1960; Berry, 1975; Iskandar, 1998; Susanto, 1989). The problem of identifying the species exported lies in the fact that frogs are usually exported as skinless frog legs. Even if species were identified, the documentation in export papers could be incorrect. For example, Veith et al. (2000) performed DNA analyses to compare imported frog legs from Java and Sumatra with frozen tissues of Indonesian Ranid species. Their results showed that samples of tissue that had been documented as belonging to the species *L. macrodon, F. cancrivora, F. limnocharis,* and *R. catesbiana,* were all in fact *F. cancrivora.*

Exported *Fejervarya cancrivora* and *L. macrodon* are usually taken from the wild. Previous studies have suggested that *Fejervarya cancrivora* is the most common species in rice fields, a habitat mostly associated with humans (Church; 1960; Alcala, 1962; Berry, 1975; Iskandar, 1998). *Limnonectes macrodon* is thought to live mostly near rivers or in small streams (Iskandar, 1998) but in my fieldwork this species was also found in ponds and rice fields (see Chapter 4). Both are large species and are found in areas accessible to harvesters, therefore they are probably the most caught for export purposes, especially in Java.

It is not clear from the statistical records what species are taken for the frog leg trade and in what numbers they are taken; these need to be assessed for management purposes. The species that are harvested are likely to differ among locations. As an archipelago, Indonesia consists of several main islands, and each island has its own unique faunal assemblage. It is likely that outside Java, species other than *F. cancrivora* and *L. macrodon* are taken. Exporters that use supplies from other islands usually record only the names of locally available species (e.g. kodok lembu, the local name for *Rana catesbeiana* or kodok batu, the local name for *Limnonectes macrodon*). Since the frogs taken must be of similar size to *L. macrodon*, it is possible that species captured from other islands may be other members of the genus *Limnonectes*.

Data show that the numbers of frogs caught for export purposes increased significantly between the first period (1969 - 1979) and the second period (1979 - 1988) for which data were available. Before 1985, India and Bangladesh were the main global sources of exported frog legs. However, in 1985 their two edible frog species (*Euphyctis hexadactylus* and *Holobatrachus tigerinus)* were included in Appendix II of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), because of declines in their populations (Abdulali, 1985; Pandian and Marian, 1986; Dash and Mahanta, 1993). A comparison of Indonesian, Indian, and Bangladeshi frog leg export data (Schmuck 2000a) suggested that Indonesian exports rose markedly as exports from Bangladesh and India decreased.

Although some exported frogs were probably transported between islands, it is likely that almost all exported frogs originated on the island where each exporting company resides. Even if we assume that up to 20% of frogs from Java could have originated from other islands, my analyses still show that the harvest from Java is always greater than that from Sumatra. The greater number of frogs taken from Java is likely to be correlated with the fact that Java has the largest area of rice fields, which are the most suitable habitat for edible frogs and the areas most accessible to frog hunters (see Chapter 3). Figure 2-6 shows the total areas of rice fields in the main islands of Indonesia.

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Figure 2-6. Total rice field area in several main islands in Indonesia 1983 to 1999. Data taken from Indonesian Statistical Bureau.

Although the majority of frogs in Java come from three provinces, it is likely that other provinces in Java (such as West Java and Yokyakarta) also contribute to frog leg exports. It is also possible that frog legs exported from Jakarta originate in West Java since Jakarta, the capital city of Indonesia, does not have any habitat suitable for edible frogs, and also Jakarta is the nearest major port to West Java. A small fraction of frog legs from West Java might also go through Central Java.

The major importer of Indonesian frog legs in Europe is Belgium and Luxembourg (47.6%), followed by France (27.6%) and the Netherlands (21%). This contradicts the idea that France is the major importer of Indonesian frog legs (Patel, 1993). However, Belgium and Luxembourg could also serve as gateways to Europe; from these ports of entry frog legs may be re-exported to other European countries.

My data suggested that the number of frogs taken for export purposes is high (see Table 2-2). Data from India suggested that the number taken for export before the ban was around 60 million/year (Pandian and Marian, 1986). Although my estimated number of Indonesian frog harvested is larger than this number, this does not necessarily mean that the Indonesian species are threatened by harvesting. They differ in breeding regimes, environmental condition, and available area of habitat.

Besides export, the domestic sector also utilises frogs. Although most Indonesians are Moslems, and thus are forbidden to eat frogs, ethnic Chinese and persons of other religions may consume large numbers of frog legs. There are no data available on the volume of frog legs used for domestic consumption. The volume of frogs harvested for domestic consumption is analysed more thoroughly in Chapter 3.

CHAPTER 3

EDIBLE FROG HARVESTING AND THE STRUCTURE OF THE DOMESTIC MARKET

3.1 Introduction

Issues regarding the sustainability of game harvests in various tropical forests have been assessed thoroughly by Robinson and Bennett (2000). From this book it is clear that to conserve harvested species, researchers cannot focus only on the ecology and biology of those but also need to examine the human aspects of harvests. In the case of the Indonesian frog harvest the questions that must be addressed are: What is the importance of this harvest? Where does the harvest go? What methods do hunters use to catch the frogs? Will the methods used to catch frogs be detrimental to non-target species? By addressing the human aspects of the harvest we can also get a clearer picture of harvest rates and other issues related to the survival of the harvested species, such as whether the frog harvest is seasonal and whether it is size selective.

Unfortunately, most authors who have dealt with the issue of frog harvesting have neglected the human dimension. Pandian and Marian (1986) briefly mentioned that the ban on Indian frog leg exports resulted in the collapse of this industry and the loss of jobs for many people. This chapter examines these human dimension issues with data on the harvesting and marketing of edible frog legs in Jakarta, West Java and East Java with an emphasis on domestic frog supply.

3.2 Methods

I defined three occupational categories related to frog harvesting. Frog hunters or harvesters are defined as people whose main activity is the actual harvesting of frogs.

Middle men are people who receive frogs directly from frog hunters or harvesters. Local traders sell frogs in local markets; middle men also sometimes serve as local traders. I interviewed frog hunters and frog traders, using the assistance of five field assistants to assess the trade networks in three provinces: Jakarta, West Java and East Java, between September 2001 and October 2003. I selected provinces based on their accessibility and recorded high rates of frog exports (Chapter 2). Middlemen and harvesters were identified and located using information from various traders in the market. Observations in West Java were mainly carried out in the Bogor and Sukabumi districts; activities in East Java were monitored in the Surabaya, Madiun, Mojokerto and Pasuruan districts.

The numbers of hunters and traders interviewed and observed varied among locations due to varying difficulty in accessing the local harvesters. The number of people in each occupational category interviewed in each region is presented in Table 3-1 below.

Table 3-1. Number of people interviewed in each occupational category and region during 2001-2003

	East Java				West Java		Jakarta
	Surabaya	Madiun	Mojokerto	Pasuruan	Bogor	Sukabumi	
Harvester		6	3	3	9	6	
Middle men		2	2	3	3	3	
Local trader		1*			1*		8

* Also act as middle men

Most of the interviews were done in an informal manner, therefore no standard format of questionnaire was distributed to respondents. Questions to harvesters and traders consisted of enquires to determine the characteristics of stakeholders (age, ethnics, job duration), species sold, the seasonality of frog captures, and also to identify revenue distribution among stakeholders in the market chain of the traded species. Since there are no previous data on sizes of populations of edible frogs, to obtain information on the fluctuations of the harvest we also asked harvesters whether the harvest has been lower in recent years. To assess the size classes preferred by harvesters and traders, frogs caught by harvesters (usually harvesters who had been followed by my team) were measured for their snout vent length and mass. The results are presented in a descriptive manner.

In addition, in 2001 I also interviewed a researcher from Balai Budidaya Air Tawar or BBAT (Fresh Water Aquaculture Research) in Sukabumi, an institution that was responsible for culturing the bullfrog (*Rana catesbeiana*) the first time. A bullfrog farmer in East Java was also interviewed during August 2003 to gain information on the problems associated with, and extent of development of, frog farms.

Local markets in each area were also surveyed to record the species and numbers of frogs sold. The market surveys were carried out discreetly or openly depending on the situation. A discreet survey was carried out if it would be likely to upset the traders; in Indonesia that means attracting a large crowd which would make working very difficult. The data were either recorded directly into a small hidden tape recorder or memorized by the investigators and put onto record sheets a short distance away from the market stall.

To calculate harvesting rates in local areas, data were collected by following 10 frog hunters across all of the local area (West Java and East Java) for an overall total of 21 night works. Routes were recorded with GPS to obtain information such as working hours per days and the size of search areas. From this data I estimated catch per hunter-night of effort for each area.

By obtaining data on actual harvesting rates, I could estimate the proportions of frogs taken for domestic consumption and for export. I calculated the proportion of frogs suitable for export as the proportion that were of sizes suitable for export (mass > 80 grams); frogs below this size must be destined for domestic consumption.

3.3 Results

3.3.1. Species harvested

a. Species in trade

Four species of frogs were sold in local markets: *Fejervarya cancrivora, Fejervarya limnocharis-iskandari* complex*, Limnonectes macrodon* and *Rana catesbeiana*. Of the local species, the larger species (*F. cancrivora* and *L. macrodon*) were sold the most. Surveys in East Java only found two species being traded: *F. cancrivora* and *F. limnocharis-iskandari* complex. However, East Java traders did say that they sometimes sold stream frogs matching the description of *L. macrodon*. *Fejervarya limnocharis-iskandari* complex were not only sold as for human consumption but also as fish bait. We rarely saw *Rana catesbeiana* in local markets, however a few supermarkets in Jakarta such as Ranch 99 sold them. Interviews with local traders in all markets revealed that most local consumers dislike the taste of *R. catesbeiana* and prefer local frogs.

In West Java and Jakarta's local markets *F. cancrivora* and *L. macrodon* were usually sold by number rather than by weight, whereas in East Java *F. cancrivora* were sold by weight (whole) (Figure 3-1). Local consumers selected frogs whilst they were still alive. The trader either delivered them to the consumer alive or cut and skinned the legs before delivering them.

Small frogs such as *F. limnocharis-iskandari* complex are either sold alive by number for fish bait or are sold by weight as skinless frog legs. Local markets usually demand live frogs for sale; as such, the outward appearance of frogs is important.

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Figure 3-1. Frogs sold in the markets, either alive (centre) or as skinless legs (top). Below, a costumer buying frog legs in Madiun, East Java *b. Frog sizes*

A total of 736 frogs of three species (*Fejervarya cancrivora*, *F. limnocharis-iskandari complex* and *L. macrodon*) captured by harvesters were measured. Most of the frogs captured were *F. cancrivora* (75.4%), while only a small percentage were *F. limnocharis-iskandari complex* (5.8%) and the rest were *L. macrodon*. Frogs captured were usually mature, except for *F. cancrivora* where a small number of captured individuals were of sizes considered to be juveniles (see Chapter 5 for the description of sex differentiation for *F. cancrivora* and *F. limnocharis-iskandari* complex). The mean SVL of *F. cancrivora* captured was 71.50 mm and mean mass was 41.88 grams. The means for *F. limnocharis-iskandari* complex were 45.29 mm and 10.67 grams, respectively, and those for L. *macrodon* were 80.47 mm and 66.41 grams (Table 3-2).

Table 3-2. Size and Mass of Frogs captured by harvesters; F = female, M = male and J = juvenile

Species	Sex	N	SVL (mm)			Mass (gram)		
			Mean	Range	Std Dev	Mean	Range	Std Dev
Fejervarya cancrivora	F	367	75.10	40.00 -162.00	16.89	48.55	2.94 - 152.40	26.48
	J	5	35.90	33.50 - 38.00	1.75	3.93	3.45 - 4.35	0.35
	M	183	65.54	50.20 - 86.20	6.01	29.55	14.50 - 57.40	8.09
	Total	555	71.60	33.50 - 162.00	15.23	41.88	2.94 - 152.40	24.03
Fejervarya limnocharis-iskandari complex	F	16	43.69	30.00 - 78.00	16.22	10.34	1.87 - 32.00	12.9
	M	27	46.24	32.57 - 70.20	13.75	10.87	2.60 - 41.00	10.04
	Total	43	45.29	30.00 - 78.00	14.58	10.67	1.87 - 41.00	11.04
Limnonectes macrodon		138	80.47	54.74 -138.10	12.78	66.41	17.00 - 146.05	26.49

3.3.2 The process of harvesting frogs and the characteristics of harvesters

a. Profiles of harvesters, middle men and traders

Frog harvesting is a nocturnal job, therefore it is not surprising that all frog harvesters are males. Harvesters' ages are variable, ranging from 25 to 60 years old. The maximum length of time of employment in this work was around 30 years. More than one third of the harvesters (37%) had worked for more than 20 years, nine people (33 %) had worked between 10-20 years, six people had worked (22%) between 5-10 years, and only two people (7%) had worked for less than five years. More than half (56%) worked part-time as frog hunters. During the day they worked as farmers, farm labourers, general labourers, brick makers, public transportation drivers or as middle men. All harvesters were local people, meaning that they live near the place where they capture and sell frogs. East Javan harvesters were either *Maduranese* (Madura is a nearby small island in the north coast of East Java) or East Javanese. All West Javan harvesters were *Sundanese* (Sunda is an ethnic group from West Java). Some of the harvesters were related (brothers or father and sons) to other harvesters or middle men. Full time harvesters usually work nearly every night, taking time off at least once a week and during the full moon. Part-time harvesters usually work only during the high season.

Three middle men were female and the rest were males. Half of the middle men had worked more than five years, 29% had worked for 10-20 years whilst 21% had worked more than 20 years. Four male middle men started their careers as harvesters. One of the middle men from Sukabumi worked as a harvester from the 1950s to the 1970s. He could not remember the exact dates, and used certain historical events as his time marks. In this case he started working as a harvester during the Darul Islamiyah/Tentara Islam Indonesia Rebellion (around 1955) and finished working as a harvester not long after the Communist Party was disbanded (the late 1960s).

Harvesters usually “belong” to (work exclusively with) one of the middle men. They are given upfront money by middle men, usually for transportation, and have to give all of their harvest to their middle man. Middle men also loan equipment, such as carbide lamps, bags and capture devices, to harvesters. They also sometimes provide food or a cup of coffee to harvesters before and after work. However, the loyalties of harvesters usually depend on the price of frogs given by the middle men. The number of harvesters working for one middle man varied; usually between 5 – 40 people each day depending on the season. In the high season the number of harvesters working was usually higher than in the low season. The low season usually occurs during the full moon and in the dry season.

Some of the middle men not only sent their frogs to other distributors or traders but also act as traders in local markets. Most traders had worked more than five years and some "inherited" the work from relatives.

b. Harvesting Techniques

Harvesters use lights powered by carbide or batteries to help them capture frogs. This has led to their occupation being called *pengobor* (West Java) or *penyuluh* (East Java), which literally means men who use torches. To capture frogs, most harvesters walk in or along areas of frog habitat such as rice fields, small fish ponds, ponds inside forested areas, and irrigation ditches near sugar cane or riversides. Only one harvester from Sukabumi indicated he used a dinghy when cruising the river Cikarang to find frogs. The total distance traversed during searches was up to 10 km/night. Working time usually started around 7.00 p.m. and finished around 1.00 a.m. The route taken was rarely far from their home or from their middle man's area; this minimizes travel costs.

Harvesters used active hunting techniques in which frogs were captured using special equipment: a long bamboo pole with a net at the end (West Java) or a long bamboo pole with a three-headed spear (East Java) (Figure 3-2). This long pole helps harvesters to capture frogs that are a bit far from where the harvester is standing. Sometimes frogs hide in crevices; this is where the three-headed spear becomes useful to capture hidden frogs. Harvesters usually tried to avoid spearing frogs in their legs because the resulting bruises would cause their rejection, especially for export purposes. However, sometimes it is difficult to avoid such bruising, especially when frogs are hiding (Figure 3-3). Captured frogs are put in special bags. West Javan harvesters avoided frog mortality during capture because frogs were usually sold live, whereas it did not matter to East Javan harvesters whether frogs were alive or dead, since as soon as their middle men weighed their catch, the frogs' legs were removed and skinned.

Most harvesters work alone. They occasionally worked in pairs when one was new at the work. As soon as a new harvester had learned the techniques, he would work alone. One harvester from Sukabumi worked with a team of 2-4 people during the times when he used a dinghy in the Cikarang River; at other times he worked alone.

c. Harvesting effort

Before going to work, harvesters usually assembled in their middle men's houses to prepare their equipment and determine the route to be taken that day. They usually started to work after 7 p.m. by going to the starting point of their selected route either by foot or public transportation. The mean total time spent by harvesters in traversing their routes was 17948.52 seconds or 4 hours 59 minutes and 8.52 seconds. The starting point was taken as the point at which harvesters began searching for frogs. Along the way, they sometimes rested for snacks or coffee for between 10-15 minutes and one hour. The number of frogs caught ranged from six to 110 frogs, with total wet masses from 508.8 – 5000 grams. Mean catch per unit effort was 8.74 frogs/hour (range 1.79 – 20.50 frogs/hour) or 351.32 grams/hour (range 94.65 – 931.68 grams/hour). Table 3-3 shows the number and mass of frogs captured for each trip for each harvester.

Based upon interviews, during the full moon or the dry season, yields were usually between 1 - 2 kg/harvester/night whereas during the wet season the yield can be between 2 – 5 kg/harvester/night. Several harvesters in West Java counted the yield not by mass but by number. Based on interviews the numbers of frogs caught per day were usually between 30-40 frogs during the low season and 70 – 100 frogs during the high season.

Table 3-3. The average trip length and catch/effort per hour and per km of harvester

	East Java (n=6 days)	West Java (n = 15 days)	Total (n= 21 days)
Average length of Trip (km)	10.80	9.76	10.06
Range	9.46 -12.18	5.07 - 16.00	5.07 - 16.00
SD	1.12	3.29	2.85
Average of Total times (seconds)	19742.17	17231.07	17948.52
Range	17168 - 24691	12073 - 22860	12073 - 24691
SD	2764.35	3347.17	3332.28
Average of Catch/effort (g/hour)	395.28	332.38	351.32
Range	325.71 - 505.13	94.65 - 931.68	94.65 - 931.68
SD	67.71	239.55	203.32
Average of Catch/effort (n/hour)	11.37	7.42	8.74
Range	8.23 - 15.67	1.79 - 20.50	1.79 - 20.50
SD	2.53	5.73	5.17
Average of Catch/effort (g/km)	203.41	165.15	176.63
Range	156.01 - 289.19	50.00 - 349.65	50.00 - 349.65
SD	54.63	105.10	93.10
Average of Catch/effort (n/km)	5.86	3.86	4.53
Range	3.94 - 8.96	0.83 - 9.40	0.83 - 9.40
SD	1.83	2.51	2.45

3.3.3. Market structure

The market structure for wildlife trades can be either competitive or monopolistic (Clayton and Millner-Gulland, 2000). The local frog leg trade follows the classic chain of marketing which includes three to four main stakeholders: harvesters, middle men, suppliers and traders (Figure 3-4). In competitive markets, each stakeholder can enter or leave the market freely while in monopolistic markets, access is controlled and denied by various means such as ownership or law enforcement (Clayton and Millner-Gulland, 2000).

Figure 3-2. Equipment used to capture frogs include lamps, bags and bamboo pole with net in West Java (left) or three headed spears in East Java (right)

Figure 3-3. Rejected frog legs with bruised marks

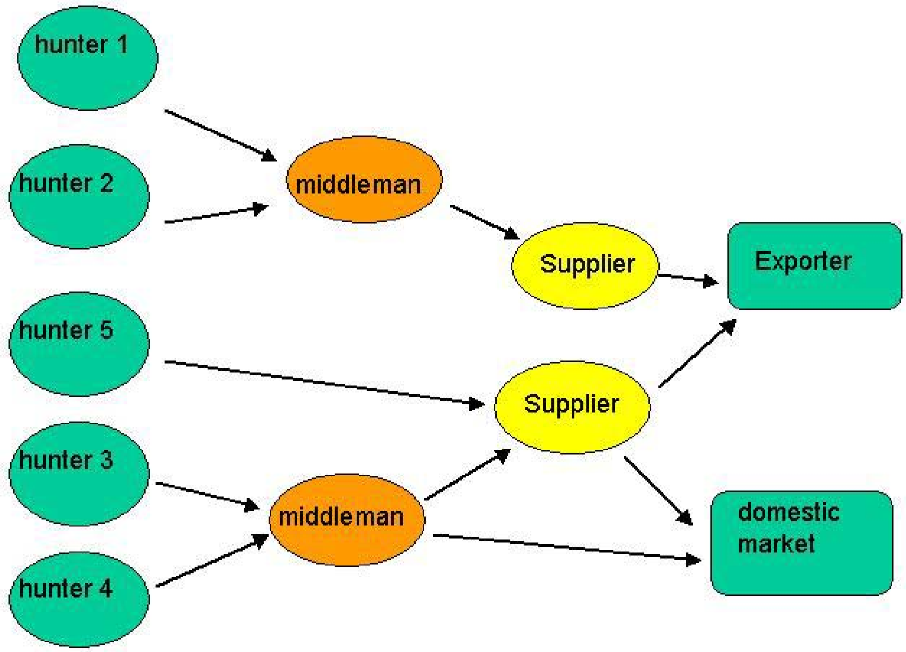

Figure 3-4. The web of frog leg supply from harvesters to consumers usually consist of hunters, middle men, supplier and then the costumer itself either an export company that will distributed the legs to overseas or local consumers in the market.

The structure of the frog leg trade is competitive. Access is not controlled, since the frogs traded are not protected species. Paddy fields are considered as open areas; there is no need to ask permission from paddy field owners to capture frogs as long as harvesters capture frogs without damaging rice plants. Harvesters from other areas usually bring identification and sometimes ask permission from heads of villages. New players enter the market freely, with numbers of harvesters and middle men fluctuating.

a. Price

Frogs were sold either by number (when live) or by mass (either overall frog or as skinless legs). Price varied according to species, size, location, stakeholders, and season (Table 3-4 and 3-5). The prices stated in this result were in Indonesian rupiah

(Rp) for the years 2002 and 2003. The price of *Limnonectes macrodon* was usually higher than that for rice field frogs. In West Java and Jakarta it is usually sold live by number or by weight. Harvesters sold this frog for Rp. 750/frog or Rp. 5000 – 14000/kg to middle men. The cheapest *L. macrodon* sold to consumers was in Bogor (Rp. 1250/frog) compared to Jakarta, where prices ranged from Rp. 1500 (small) to Rp. 3000 (large)/frog, or around Rp. 25000/kg.

The prices of rice field frogs varied according to size and location. There were no standard categories, however traders usually categorized frogs into large, medium or small. Some traders categorized frogs using an alphabetical system: A, B and C. Categories A and B are large and medium frogs, with 9-12 frogs/kg (A), or 13- 25 frogs/kg (B) whereas more than 25 frogs/kg were categorized as C or small. The price per individual and per kilogram was lower for smaller frogs. The price of frogs usually increased 2-3 times from harvester to local consumer (Table 3-4). Frog legs exported are usually from large frogs (category A or B) with prices in year 2003 around US$ 3-4/kg of skinless frog legs or around Rp. 25,200 – 33,600/kg with a currency exchange rate of 1 US$ = Rp. 8,400.

b. Levels of Income and Profit

Harvester income depends on the number or mass of frogs caught, route taken and skills. To minimize costs of transportation, harvesters rarely go far from the place where they sell the catch, preferring to walk whenever possible. Other than transportation, additional costs for each trip were for snacks, coffee and cigarettes. The maximum cost of transportation and snacks (including coffee and cigarettes) along the way that harvesters were willing to spend was Rp 1,000/day during high season. If we assume that transportation cost is nil and the price of frogs is constant at Rp. 6000/kg (mixed), harvesters could make around Rp. 5,000-11,000/ day (low season) and Rp.

11,000 to 29,000/day (high season) (Table 3-7). A full time harvester working for 25 days/month would receive a monthly income of Rp. 245,000 to 635,000. This estimate is probably lower than actual earnings, because harvesters tend to capture bigger frogs (thus increasing their income) and prices fluctuate, and are higher in the low season because of lower supply. Based on interviews, the lowest income of a full time harvester was Rp. 450,000/month whilst the highest was 900,000/month.

For middle men, the gross revenue for each type of frog varied (see Table 3-4 and 3-5). Middle men and traders must also spend substantial amounts of money for fixed costs such as equipment, carbide or batteries, transportation to carry frogs to traders or local markets, and hiring people to cut and skin frogs. Middle men who had connections with exporters usually did not need to cover the costs of transportation since exporters usually come to the middle man. However, interviews revealed that only some middle men distributed their frog legs to exporters. Exporters usually have higher quality control. Interviews with exporters revealed that at least 2-5% of frog legs coming from middle men were usually rejected for various reasons such as the occurrence of bruises or parasite infection. One of the middle men interviewed remarked that he had lost a substantial amount of money because more than 30% of the last batch of frog legs he sent was rejected. This caused him to leave the export market and concentrate on the local market, which he said was much more flexible. Other middle men used other approaches, catering for both local and export markets. Frogs that are too small for export are sold in local markets. Net revenue for middle men and traders varies, ranging from Rp.100,000/day to Rp. 600,000/day.

Table 3-4. Price of frogs (in Indonesian Rupiah) in Jakarta and West Java according to buyers

Stake holder	Sold to	Location	Province	Price of Rice fields frog (*F. cancrivora* and *F. limnocharis-iskandari* complex)				Price of Stream frogs (*L. macrodon*)		
				Small	medium	Big	Mixed	Small	Big	Mixed
Harvester	Middle man	Caringin	West Java	150/frogs		600/frogs				750/frogs
Harvester	Middle man	Cibatok	West Java				6000/kg			10000/kg
Harvester	Middle man	Kelapanunggal	West Java			10000/kg			14000/kg	
Harvester	Middle man	Karangtengah	West Java	5000/kg	5500/kg	6000/kg				
Harvester	Middle man	Pasar Pelita	West Java			13000/kg				
Harvester	Middle man	Ciracap	West Java				5000/kg			6000/kg
Middle man	Consumer	Caringin	West Java			750/frogs			1250/frogs	
Middle man	Distributor	Cibatok	West Java	7000/kg						13000/kg
Middle man	Consumer	Karangtengah	West Java	12000/kg (Bogor)	20000/kg (Jkt)					22000/kg (B*) or 25000/kg (J*)
Middle man	Consumer	Pasar Pelita	West Java	15000 – 17000/kg						18000 – 20000 /kg
Middle man	Distributor	Ciracap	West Java	8000/kg		12000/kg (A & B)				18000/kg
Middle man	Trader	Glodok	Jakarta	6000-8000/kg		12000-15000/kg		1000/frog	2500/frog	20000/kg
Middle man	Trader	Senen	Jakarta		20000/kg (*) or 1500/frogs	35000/kg (*) or 2000/frogs			2500/frog	
Middle man	Trader	Senen	Jakarta	7.000/kg		17000/kg	8000/kg			20000/kg
Middle man	Trader	Petak sembilan	Jakarta			15000/kg				20000/kg
Trader	Consumer	Glodok	Jakarta	15000/kg (*) or 500 - 700/frog		25-30000/kg (*) or 20000/kg		1500/frog	3000/frog	
Trader	Consumer	Senen	Jakarta	15000-25000/kg (*) or 12000-20000 /kg		25000/kg			35000/kg	
Trader	Consumer	Petak sembilan	Jakarta		1000/frog	25000/kg or 2000/frog			30000/kg or 3000 - 4500/frog	

Note:

* means kinless legs otherwise price for live frogs, B* is Bogor, J* is Jakarta

Table 3-5. Price of skinless frog legs in East Java (in Rupiah) unless stated as other form

Stake holder	Sold to	Location	Province	Rice fields frog (*F. cancrivora* and *F. limnocharis-iskandari* complex)			
				Small	medium	Big	Mixed/skinless
Harvester	Middle man	Madiun	East Java				13000/kg
Harvester	Middle man	Madiun	East Java			9000/kg	
Harvester	Middle man	Madiun/Magetan	East Java			10000/kg	
Harvester	Middle man	Mojokerto	East Java				8000/kg (high season) 12000/kg (low season) Live
Harvester	Middle man	Pasuruan	East Java	6000/kg		10000/kg	
Harvester	Middle man	Sidoarjo	East Java			10000/kg (live)	
Middle man	Consumer	Madiun	East Java				10000/kg
Middle man	Consumer	Madiun	East Java	10000/kg	15000/kg	25000/kg	
Middle man	Consumer	Madiun	East Java	10000/kg		25000/kg	
Middle man	Distributor/Exp	Madiun	East Java	7000/kg	15000/kg	20000/kg	
Middle man	Consumer	Madiun/Magetan	East Java	12000 /kg		22500/kg	
Middle man	Consumer	Mojokerto	East Java			25000/kg	
Middle man	Consumer	Pasuruan	East Java			15000/kg (live)	
Middle man	Consumer	Pasuruan	East Java			25000/kg	
Middle man	Consumer	Sidoarjo	East Java	1500/each	2500/each	3000/each	
Exporter	Consumer						25200 – 33600/kg ($US 3 – 4, 1 US$ = Rp. 8400)

Table 3-6. Comparative prices of frogs sold from harvester to local consumer in Indonesian Rupiah, based on the maximum price available for each stakeholder.

Species	Condition	Category	Harvester	Consumer	Gross revenue
L. macrodon	By number		750	3,000	2,250
	By weight/kg		10,000	35,000	25,000
F. cancrivora & *F. limnocharis-iskandari* complex	By number	Small	150	700	550
	By weight/kg	Small	5,000	17,000	12,000
	By weight/kg skinless	Small	6,000	12,000	6,000
	By weight/kg	Medium	5,500	20000	14,500
	By number	Big	600	2,000	1,400
	By weight/kg	Big	5,000	20,000	15,000
	By weight/kg skinless	Big	10,000	30,000	20,000
	By weight/kg	Mixed	6,000	8,000	2,000

Table 3-7. Harvester daily wages assuming that transportation is nil, food and price of frogs were constant

	Snack	Minimum Yield	Maximum Yield	Minimum Wages	Maximum Wages
low season	1000	1	2	5,000	11,000
high season	1000	2	5	11,000	29,000
5 days low season in a month	5000	5	10	25,000	55,000
20 days high season in a month	20000	40	100	220,000	580,000
Monthly wages				245,000	635,000

Note:

- Price of frogs were based on maximum price of mixed frogs (Rp. 6000/kg)
- Wages per day is yield times price of frog minus food
  Monthly wages is wages during low season plus wages during high season

c. Sustainability

About 75% of harvesters in East Java and 61.5 % of harvesters in West Java remarked that yield per year has tended to decrease. Almost all (90%) middle men, in East and West Java remarked the same thing. However, traders and exporters did not share this perception; they only remarked that sometimes supplies were low depending on the season. Harvesters and middle men believe that the declines they have noticed are caused by: 1) increasing numbers of harvesters, 2) increasing numbers of middle men, allowing harvesters to go to other middle men, and 3) habitat change, as more rice fields have been developed for other uses. Very few respondents suggested that the decreased numbers of frogs captured were caused by decreases in frog populations. An account by one of the middle men from Sukabumi who had worked as a harvester in the 1950s through –1970s suggested that some frog populations may have decreased. In West Java more stream frogs than rice field frogs were captured in the 1960s. During the 1970s the number of stream frogs captured decreased and the harvester shifted his capture efforts to rice field frogs. During the 1970s, a one-day yield for one middle man could consist of a maximum of 200-500 kilograms of frogs. Nowadays, with almost the same number of harvesters he can only obtain a maximum 100 to 200 kilograms for 3 days work or about 33 – 66 kg/day, which is a substantial decrease.

One approach to reducing the impact of harvesting on natural frog populations is to farm frogs. Although bullfrogs (*Rana catesbeiana*) were introduced as early as 1983 for frog farming purposes (Susanto, 1989; Iskandar, 1998; Arie, 1999), so far, the frog farming industry in Indonesia has not developed as expected. An interview with a research aquaculturist from BBAT revealed that attempts at frog farming have usually been short lived. Many frog farmers have abandoned their attempts due to the expense of food and maintenance. These factors were also mentioned by an ex- frog farmer interviewed in Surabaya (Figure 3-5).

d. Number of frogs sold in local market

In contrast to frog leg exports (Chapter 2), no data are available on the local trade in frog legs. Because the majority of the Indonesian population are Muslims (85% based on the 2002 census), who are forbidden to eat frogs, frog legs are not a major food for Indonesians. However, if most of the remaining 15% of the 234 million people in the Indonesian population (BPS, 2002) do eat frogs, the domestic market may still be substantial, with up to 35 million consumers.

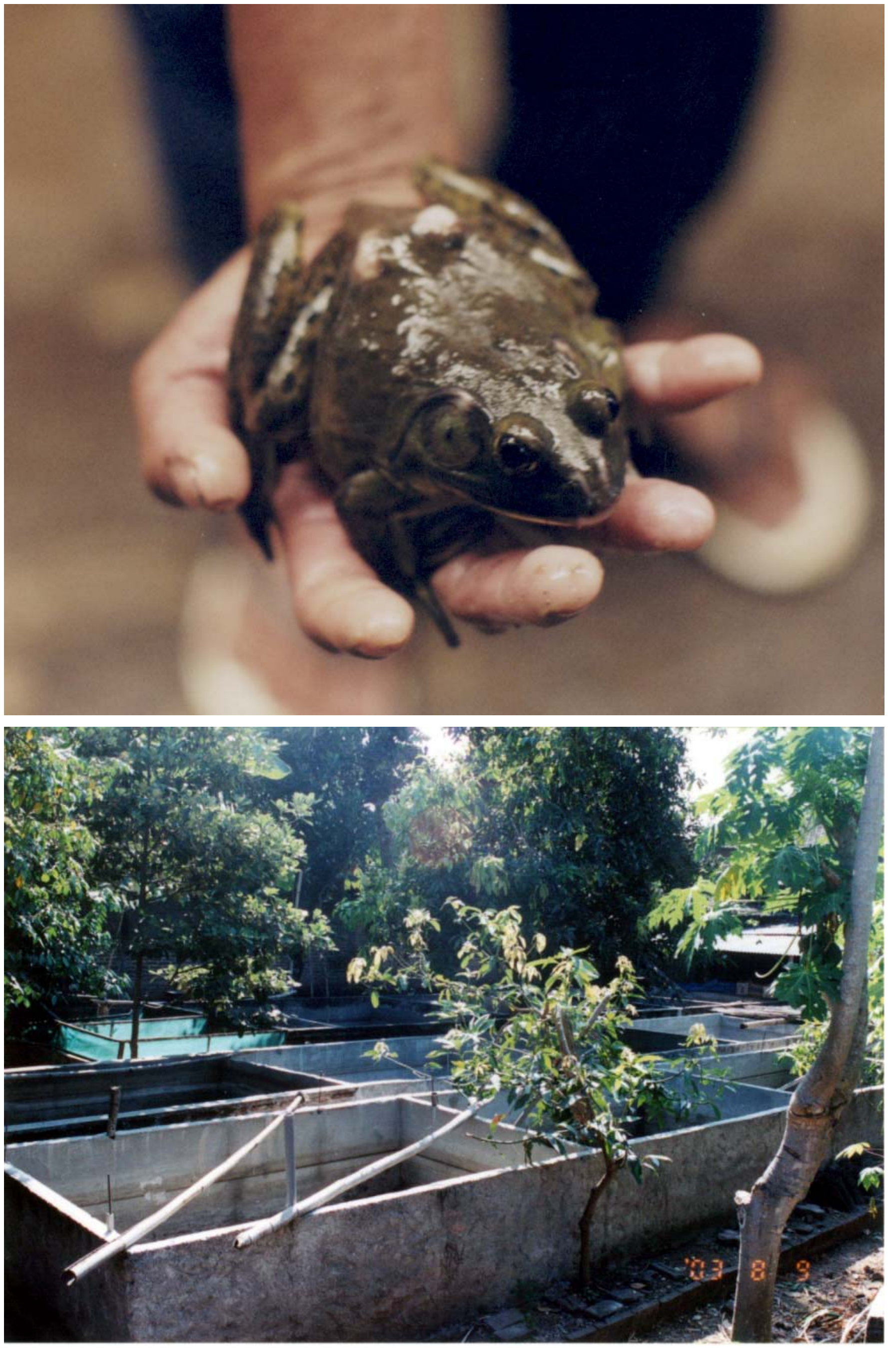

Figure 3-5. *Rana catesbeiana* (top) and abandoned frog farm in Malang, East Java (below)

Determining the total extent of the frog leg trade in local markets is very difficult, because the number of markets is not known; nor is the number of sellers. For example, my survey found at least 15 local markets in Jakarta selling frog legs, excluding supermarkets. Since the export market uses large frogs (Category A, maximum 12 frogs/kg wet weight, each frog ~ 80 gram) almost exclusively, the proportion of captured frogs that enter local markets can be estimated as the proportion of those captured that do not fall in this category, or approximately 88% of frogs (Table 3-8). This suggests that the local market is at least 7 times as large as the export market. This is a minimum figure, since some Category A frogs are not exported.

Table 3-8. Percentage of frogs captured in category A (mass>80 grams), number in brackets refers to actual number

Species	Mass <80 grams	Mass >80 grams
Limnonectes macrodon	71.7 (99)	28.3 (39)
Fejervarya cancrivora	90.8 (504)	9.2 (51)
Fejervarya limnocharis-iskandari complex	100 (43)	
Total	87.8 (646)	12.2 (90)

3.4. Discussion

The species of frogs I found to be sold in the market for human consumption corresponds with those reported by previous authors (Church, 1960; Berry, 1975; Susanto, 1989; Iskandar, 1998). The mean weight of frogs captured varied among species; however it is clear that harvesters select frogs based on their size. I found that the most commonly harvested edible species is *F. cancrivora,* which is abundant in rice fields. Based on its size, *L. macrodon* is also an important component of the harvest, since the price of this frog is usually higher than that of other local species, especially in West Java. Although East Javan harvesters mentioned the occurrence of stream frogs that match the description of *L. macrodon*, my East Java surveys were done during dry periods in which most streams were at low levels and harvesters considered those times as unsuitable for harvesting stream frogs. Thus, this species was not sighted in markets or in the catches of frog hunters in East Java. Analysis of specimens in the Museum Zoology Bogor in Bogor revealed no specimens of this species from East Java. Iskandar (1998) mentioned that this species is endemic to Java and also occurs in southern Sumatra. There are several possible explanations for this species' absence from museum and frog-hunter collections from East Java: this species may occur in East Java but simply not have been encountered in my surveys or by museum collectors, or, the stream frog species traded in East Java could be other species from the same family.

It is not clear how many species of Indonesian frogs are routinely collected and traded for human consumption. A report by Schmuck (2000) indicated that 14 Indonesian species are used in the frog leg trade, plus an additional 10 species of South East Asian origin that may possibly come from Indonesia (Table 3-9). However, I am unconvinced with the accuracy of his report. For instance, the taxonomic name and status of frogs are uncertain (See comments on Table 3-9). Some of the frogs mentioned in this table are probably not used for export because of their small size. Interviews with local traders revealed that species such as *Rana hosii*, which has glands that smell, and *L. kuhlii*, which has wrinkled skin, are avoided. Size-dependent capture influences the sex of frogs caught, especially for *F. cancrivora*. This species is sexually dimorphic; females are larger than males (Church, 1960), and are therefore more likely to be targeted for capture by harvesters. Similar selectivity of harvest was also observed by Jennings and Hayes (1986) who remarked that most California red-legged frogs (*Rana aurora draytonii*) harvested in California were females. The opposite is true in *L. macrodon,* in which the males are larger than the females (Inger, 1966; Emerson and Berrigan, 1993). Size is most important for exports, so frogs taken for export were usually large mature individuals. However, for local consumers, large size is not important. Frogs taken for local consumers can therefore also include young mature frogs. The snout vent length at maturation of *F. cancrivora* is around 45 mm for females and 50 mm for males (Jaafar, 1994).

Based on interviews and market surveys, it is clear that more than 90% of frogs sold in local market or export came from natural habitats. Although more than 20 years has passed since *Rana catesbeiana* were introduced to Indonesia for frog farming, there is no indication that bullfrog farming has succeeded. Problems with bullfrog farms include the high costs of food and medicine, and taste. *Rana catesbeiana* are considered unappetizing in taste and appearance by local consumers. Based on interviews, some frog farms have collapsed due to high mortality of frogs caused by an unknown disease or diseases. The symptoms exhibited by frogs included a warty appearance of the skin.

From an ecological point of view, the introduction of this species to Indonesia was not a wise decision. *Rana catesbeiana* is a predator and is known to prey on local frogs where it has been translocated in the United States, and is implicated as a cause of declines of populations of some frog species (Moyle, 1973; Hayes and Jennings, 1986; Lanoo, et al.,1994; Lawler et al., 1999).

There is no published report indicating that *Rana catesbeiana* has become established in the wild in Indonesia, although Iskandar (1998) mentioned that this could happen. I did not find this species during my fieldwork. However, lack of control of farmers makes it possible that this frog may spread into natural habitats. Two harvesters from Sukabumi indicated that they had found bullfrogs in paddy fields at least once. It is also possible that this frog may spread diseases to wild frogs. Mazzoni et al. (2003) found mass mortality caused by chytridiomycosis in farmed *Rana catesbeiana* in South America.

Table 3-9. Indonesia's edible frog species and their conservation status (adapted from Schmuck, 2002). Name of species is written according to original table by Schmuck, 2002 as well as note on local consumption, exported to and population status. Comments are not original from the table.

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* Iskandar, *pers. comm.*, 2005

The techniques (nets and spears) used by Indonesian frog harvesters are species selective and will not disturb the habitat or non-target species. However the use of spears in East Java is inefficient, causing unnecessary wound marks in the body parts. Frogs that have bruises or wound marks in the thigh are rejected for export purposes.

The timing of the frog harvest is closely related to weather or season and in the case of rice field frogs is also related to the stage of rice planting. The dry season and full moon periods are considered as low season, and most harvesters do not work during these periods. The low season is reflected in the availability of frogs in the local market. Each month, during the full moon, most local traders have low supplies of frogs or do not sell any at all, causing the price of frog legs to increase. This finding is the same as reported by Church (1960) in which, during full moon periods, he found low numbers of frogs in the market. There is no clear reason why there is a relation between the abundance of frogs and the occurrence of full moon. Church (1960) suggested that during this time of the month frogs probably remained concealed in thicker areas of rice plantings and were more difficult to catch. In traditional fisheries, catches are also affected by lunar phase; low catches usually coincide with the full moon. Research by CSRI Marine Science has also revealed that low catches of prawns occur during the full moon, probably because prawns avoid light to avoid predators (Salini, et al., 2001).

Periods after rice harvest were usually considered as one of the best times to hunt frogs since rice plants are mostly cut, cover is sparse, and harvesters can freely move within rice field blocks. Because access to paddy fields is limited at other times, harvesting pressure is less intense. This may give frog populations an opportunity to recover.

It is clear that harvesting frogs is economically important. The trade has been well established for several decades and provides employment and a livelihood for local people. Despite the economic importance of this trade, it seems that it has escaped government attention. There are no formal reports on the local frog trade nor is it regulated. None of the frogs harvested are protected under current Indonesian wildlife laws and there is no regulation or size limit on frogs captured as there is in other countries such as Romania (Török, 2003). In fact, no Indonesian frog species are protected at all under current law, which is based mainly on the Conservation of Natural Resources and Ecosystems Act (number 5) of 1990 (Republik Indonesia 1990).

Frog harvesting requires certain skills, but they can be learned quickly, and harvesters do not require a large capital expenditure to start working. Areas searched are usually accessible, mostly in rice fields and streams in nearby villages. For full time harvesters, the monthly income of ca. Rp. 450,000 – Rp. 900,000 is substantial. As developing country, the GNI per capita in 2002 is US$ 710 (Rp. 5,964,000 with 1 US = Rp. 8,400), well below the mean GNI per capita for South East Asian and Pacific countries of US$ 950 (World Bank Report, 2003). The net revenue for middle men or local traders in the market is also high if compared with white collar income. For instance, a new graduate with a bachelor's degree working as a government employee will only receive around Rp. 700,000 per month, similar to the amount earned by a full time frog harvester.

Since the end of the rule of president Suharto, in 1997, Indonesian economic stability has decreased. In less than 6 months in 1997 the currency exchange rate of 1 US$ increased from around Rp. 2,000 to Rp. 15,000. The economy slowed to a standstill, and in 1998, Indonesia experienced negative growth of – 12% (Akita and Alisjahbana, 2002). Banks and companies were closed, the price of food and other consumer goods have rocketed and unemployment has steadily increased. Frog harvesting has become more attractive to new players, with more people working as harvesters and becoming middle men. There is always a demand for frog legs for export purposes or for local consumption. Although selling frogs in local markets tends to gain lower net revenue per kilogram, the local market is not as highly selective as the export market. There is also a higher demand for frog legs for the local market compared to the export market, making the local market more favourable to new entrants.

With a high influx of new harvesters and traders, there is obviously increased competition among harvesters and middle men. This is likely to lead to declines of frog populations if it continues. However, it is unclear whether edible frog populations in Indonesia, especially in Java, have declined. Interviews did indicate that lower numbers of frogs were caught by each harvester and traded by each middle man, however this could reflect stable frog populations and stable total harvesting rates spread across an increased number of harvesters and traders. Chapter 5 and 6 will provide more direct data on populations of edible frogs in paddy fields and streams.

CHAPTER 4

STUDY SPECIES AND SITES

4.1 Introduction

In this chapter I first describe the common edible frogs of Indonesia and present a brief review of their ecology based on published and unpublished literature, including Master's and Ph.D. theses. I then introduce the sites including a full description of their habitat types and the importance of habitat to frog species. Sites were selected based on their relevance to the objectives of the study, and accessibility.

4.2 Description and distribution of the common edible frogs

Three species were selected for detailed population studies: *Fejervarya limnocharis-iskandari* complex*, Fejervarya cancrivora*, and *Limnonectes macrodon*. These species were selected because they were the most common frogs sold in markets during a preliminary survey in 2001. All species mentioned in this section have also been known by other names, since their taxonomic status has been revised several times. Taxonomy throughout this thesis follows Iskandar and Colijn (2000). Morphological descriptions follow Iskandar (1998), unless otherwise cited.

The distribution maps of edible frogs in Indonesia that I present were made by geo-referencing specimens stored at the Museum Zoology Bogor (MZB) of the Institute for Indonesian Science in Bogor, Indonesia, supplemented by locality records for specimens found in additional surveys. The locations of the three species (*F. limnocharis-iskandari* complex*, F. cancrivora*, and *L. macrodon*) and additional *Limnonectes* species were plotted on to the Indonesian map at the scale of 1:20,000,000 using ArcView 3.1. Almost all specimens in the MZB did not have exact locations, and were therefore assigned the locations of their nearest *Kabupaten* (Residency, a small administrative unit similar to a shire in Australia). Because the distribution records are based on specimens, they may be biased by sampling effort, and reflect in part the areas most surveyed by researchers.

Almost all specimens were determined by the MZB herpetology curators such as S. Kadarsan, Boeadi, Mumpuni and D.T. Iskandar. Identities of all specimens were reconfirmed prior to their inclusion in range maps. Specimens, originally identified as *L. macrodon,* found in three locations in Sumatra were re-identified as *L. blythi*. Some of the species might be split off to other species or re-determined; therefore the distribution maps could differ in the future as new taxonomic information is added. A brief note on the frogs was also added based on additional surveys in eight rice fields, seven streams and one lake in West Java.

4.2.1 The Paddy field frog, <u>Fejervarya limnocharis-iskandari</u> complex

Fejervarya limnocharis Boie, 1835 is also known as *Rana limnocharis* and *Limnonectes limnocharis*. Sexual dimorphism is apparent in this species, with females larger than males. Mature males are characterized by a black M-shaped patch on their throat, nuptial pads on the first finger, and vocal sac.

Iskandar (1998) revised the taxonomy of *Fejervarya limnocharis.* In 1998 he suggested that populations from high elevations should be split off as a separate species. In 2001, Veith et al., revised the *F. limnocharis* systematic and described the new species from Java as *F. iskandari*. This new species could not easily distinguished morphologically and almost identical to *F. limnocharis* but is smaller in size (male SVL up to 40 mm, female SVL up to 50 mm) (Iskandar, 1998). It could be distinguished through molecular identifications (Veith et al., 2001). Iskandar (pers. comm., 2005) indicated that *F. iskandari* is not restricted to highland but well spread all over in Java. It has been found in Cianjur to Sukabumi, Bogor, Malimping and Banjaran (near Bandung) (Iskandar, pers. comm., 2005). Due to its hidden nature, it is uncertain whether specimens stored in MZB or found in my surveys were *F. limnocharis* or the new species mentioned by Iskandar (1998) and Veith et al., (2001). For this reason a single name of *F. limnocharis-iskandari* complex is cited in the whole result of my thesis. Therefore, reports concerning the occurrence or the biology of *F. limnocharis* from the region where the species occurs should also be referred as *F. limnocharis-iskandari* complex.

Fejervarya limnocharis can be found in China (Taiwan, Sichuan, and south of Chuanche (=Yangtze) River and north to Shandong), Nepal, Pakistan, India, Sri Lanka, southern Japan, Philippines, Greater Sunda Islands, and the Lesser Sundas as Far East as Flores (Frost, 2004). In Indonesia this species is distributed from Sumatra, Kalimantan, Sulawesi, Java, Bali, Lombok, and Sumbawa to Flores (Figure 4-2).

Although mature *F. limnocharis* (male snout vent length up to 50 mm; female SVL up to 60 mm) differ in size from *F. cancrivora* (male SVL up to 100 mm; female SVL up to 120 mm), based on my experience, because of similarities in appearance *F. limnocharis* can be mistakenly identified as young *F. cancrivora*. Table 4-1 shown the spread of SVL size of *F. limnocharis-iskandari* complex *and F. cancrivora* in main island groups in Indonesia, whilst Table 4-2 showed the size differences between *F. limnocharis-iskandari* complex *and F. cancrivora*. All measurements were based on samples of specimens stored in MZB.

Webbing between the toes can be used to distinguish the species. Figure 4-1 shows the differences in webbing among *F. limnocharis-iskandari complex, F. cancrivora* and *L. macrodon.*

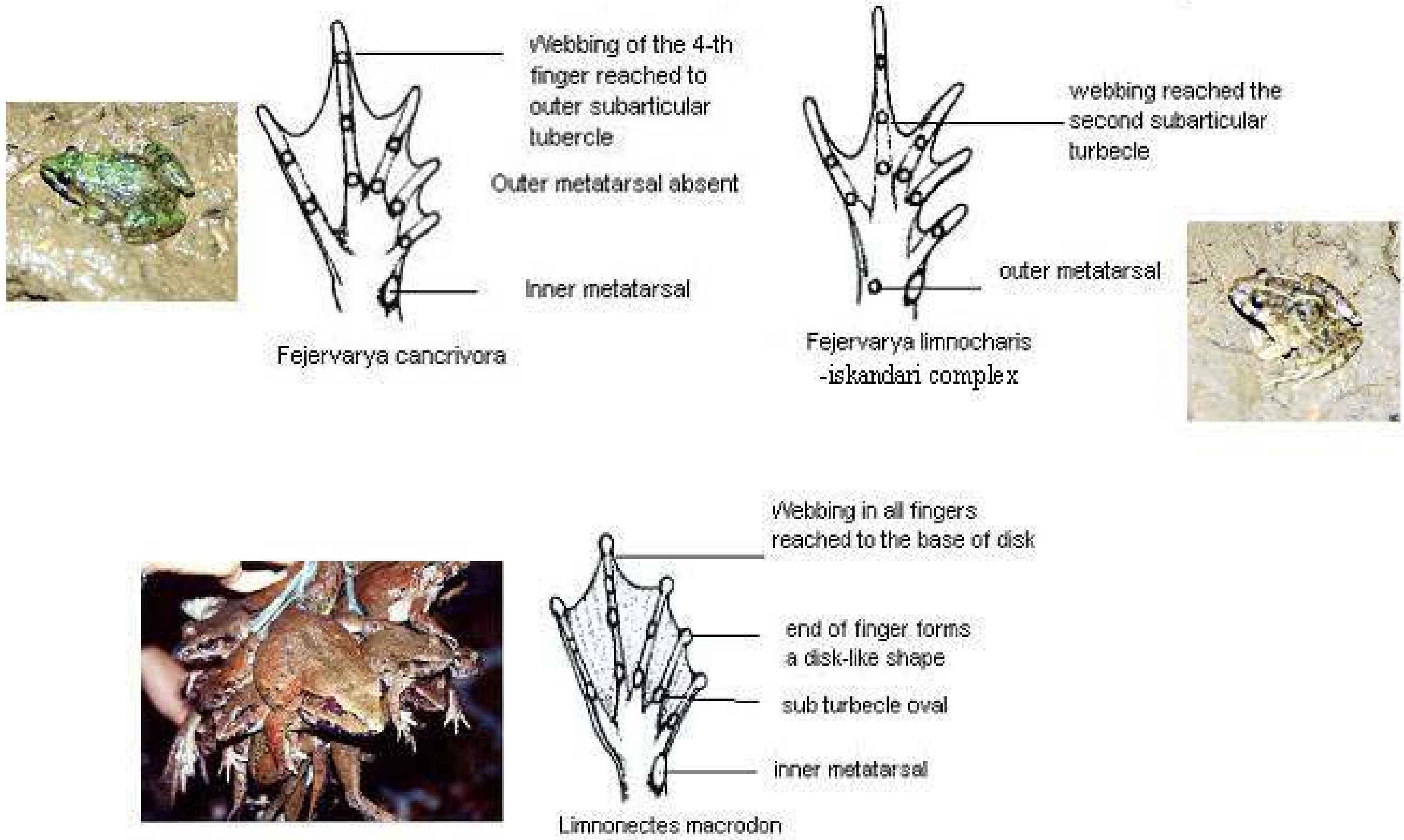

Figure 4-1. Differences in webbing between the toes of *F. limnocharis-iskandari* complex, *F. cancrivora* and L. *macrodon*

Adult *F. limnocharis-iskandari* complex are relatively small with short limbs and pointed fingertips. The head is longer than broad and the snout is pointed. The tympanum is visible. Irregular skins folds are present on the back. The webbing between the toes is not expansive, and at least one phalanx of each toe is free. Both inner and outer metatarsal tubercles are present.

Table 4-1. Mean sizes of specimens (SVL) stored in the Museum of Zoology, Bogor based on island groups. Numbers in brackets refer to range and number of specimens measured.

Species	Java	Bali	Sumatra	Kalimantan	Nusa Tenggara	Sulawesi	Irian Jaya
F. limnocharis-iskandari complex	34.30 ± 8.99 (10.38-75.19; 762)	36.73±7.94 (23.25-47.72; 12)	39.43±10.06 (12.51-72.46;135)	40.43±10.38 (18.16-58.48; 41)	32.75±11.43 (16.82-54.02; 25)	38.09±18.15 (11.10-65.29, 22)	None
F. cancrivora	59.77±18.67 (17.19-98.45; 178)	59.19±15.64 (36.05-70.51; 4)	52.98±17.62 (14.89-85.70;74)	55.25±12.07 (34.42-78.58; 34)	49.62±13.29 (22.38-76.80; 46)	55.55±16.39 (14.46-94.89; 141)	56.80±27.42 (15.88-85.16; 7)

Table 4-2. Mean sizes of specimens (SVL) stored in the Museum of Zoology, Bogor. Numbers in brackets refer to range and number of specimens measured.

Species	Female Mean ± SD mm	Male Mean ± SD mm	Juvenile Mean ± SD mm
F. limnocharis-iskandari complex	38.34 ± 8.61 (24.42-75.19, 535)	38.59±4.18 (20.69-62.59, 297)	20.23±4.71 (10.38-27.66, 169)
F. cancrivora	62.30 ± 14.23 (40.79 – 98.45, 257)	61.07 ± 9.32 (34.42 – 86.49, 142)	28.45 ± 7.30 (14.46 – 39.63, 82)

In life the colour pattern of *F. limnocharis-iskandari* complex resembles mud, with dark green and red-brownish patches. A vertebral stripe is sometimes present, and can be in a range of colours: green, orange, yellow, or brown. The underside is creamy and immaculate, except for the male's dark throat patch. There are usually three dark brown vertical bars on the upper and lower lips, and the dorsal surfaces of the limbs have dark crossbars (Liem, 1971).

Most research on this species has centred on its breeding biology. Alexander et al. (1963, 1979) gave an extensive account of the reproductive biology of *F.*

limnocharis found in Taiwan, including information on sex ratios, body size and age distributions, growth rates, and reproductive biology. Jaafar (1994) observed the reproductive ecology of this species in Malaysia, while Premo (1985) presented the only study on the reproductive biology of this species in West Java. *Fejervarya limnocharis* reproduces throughout the year (Alexander et al., 1963; Berry, 1964; Alexander et al., 1979; Premo, 1985; Jaafar, et al., 1999) although in Taiwan the proportion of females in reproductive condition peaks during spring (Alexander et al, 1979). Jaafar (1994) found that the reproductive pattern of this species in paddy fields in Malaysia is correlated with water levels.

Wide ranges of clutch sizes of *F. limnocharis* have been reported. Premo (1985) reported that the average clutch size of West Java *F. limnocharis* was 1750, while Jaafar et al. (1999) reported that the average clutch for Malayan *F. limnocharis* was 405.5 eggs (sd = $\pm$ 92.45, range 233 - 657). Genetic variation among East Asian populations of *F. limnocharis* is thoroughly reviewed by Toda (1999).

Only two works have examined populations of *F. limnocharis* in rice fields. Dash et al., (1993) gave the average density of *F. limnocharis* in irrigated paddy fields in India as 39.12 frogs/ha, while Jaafar (1994) found adult densities ranging from 6.2 to 14.0 frogs/ha.

Fejervarya limnocharis is common in habitats associated with humans, such as paddy fields and ponds (Berry, 1975; Premo, 1985; Iskandar, 1998). It is often sympatric with *F. cancrivora* (Berry, 1964; Liem, 1971, Berry, 1975; Premo, 1985, Jaafar, 1994; Iskandar, 1998).

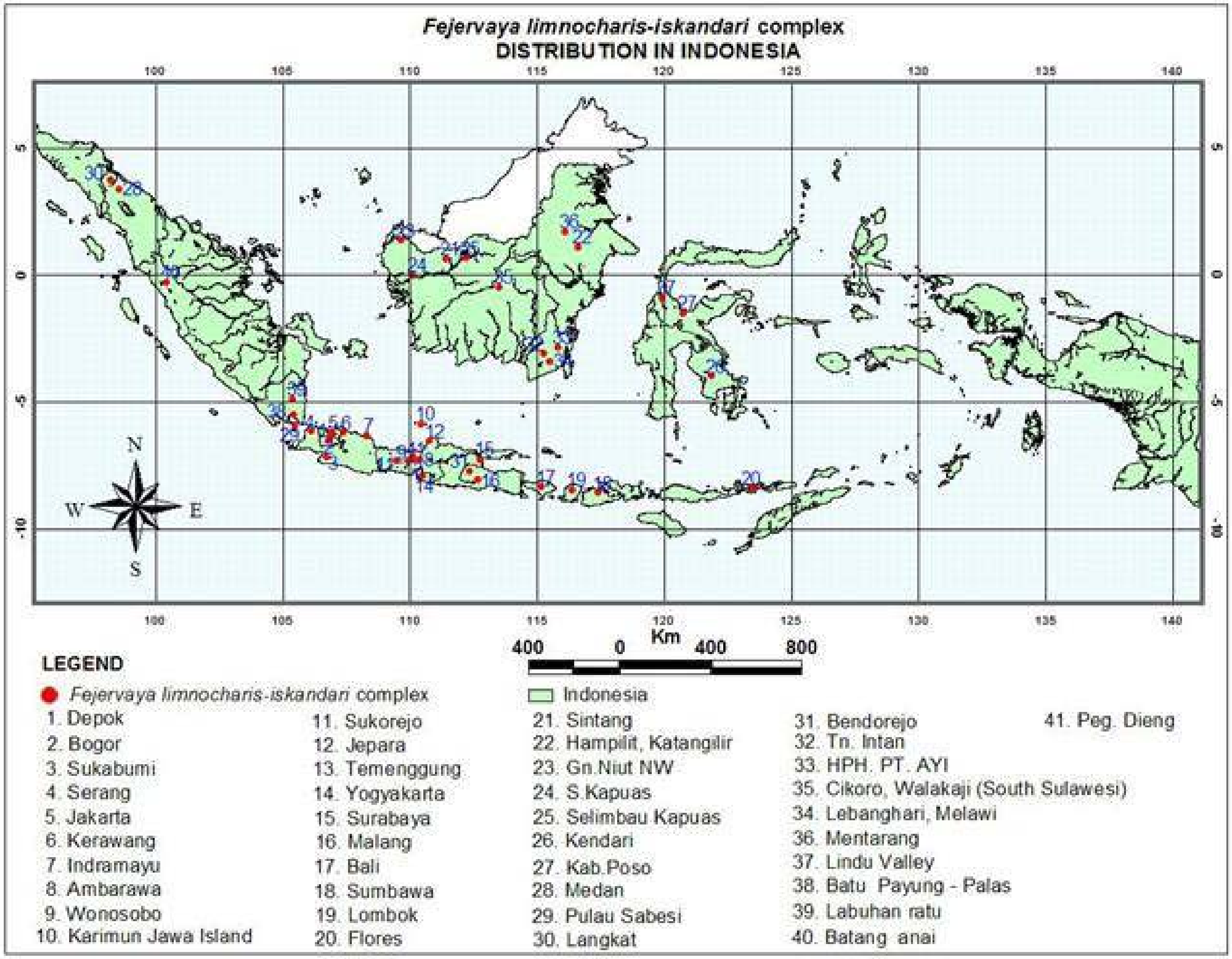

Fig. 4-2. The distribution of *F. limnocharis-iskandari* complex in Indonesia. Points were made based on specimens stored at MZB and additional survey

My survey also found these frogs in streams near paddy field areas, as was also recorded for MZB specimens. My surveys in West Java showed that *F. limnocharis-iskandari* complex were in high abundance at both low and high elevations. At lower elevations (less than 400 m asl) the abundance of this species is similar to that of *F. cancrivora*, however, at upper elevations this species (or the new species mentioned above) dominates the rice fields.

Iskandar mentioned that *F. limnocharis* is mostly found in lowlands and rarely found more than 700 m above sea level (asl), except in areas where rice fields occur. I found the species at higher elevations; up to 900 m asl (Cikaniki village, July 2004). Similarly high elevations were noted for specimens in the collection of the MZB, as noted by Alexander et al. (1979), who mentioned that the species can be found up to 1000 m asl.

4.2.2 The crab eating frog, <u>Fejervarya cancrivora</u>

Fejervarya cancrivora is a sturdy and large frog commonly found in rice fields. Females are larger than males; mature males can be distinguished by the presence of vocal sacs and black patches at the corner of the throat, and nuptial pads on their first fingers. *Fejervarya cancrivora* was also known as *Limnonectes cancrivorous* or *Rana cancrivora* Gravenhorst, 1829. Its common name is "crab-eating frog", because its diet includes large numbers of crabs. The species differs from *F. limnocharis-iskandari* complex by its more extensive webbing between the toes and the absence of the outer metatarsal tubercle. The colour in life is muddy brown or grey, with dark green or brown patches in its skin. It has irregular longitudinal skin folds on its back. As with *F. limnocharis-iskandari* complex, a dorsolateral stripe is sometimes present.

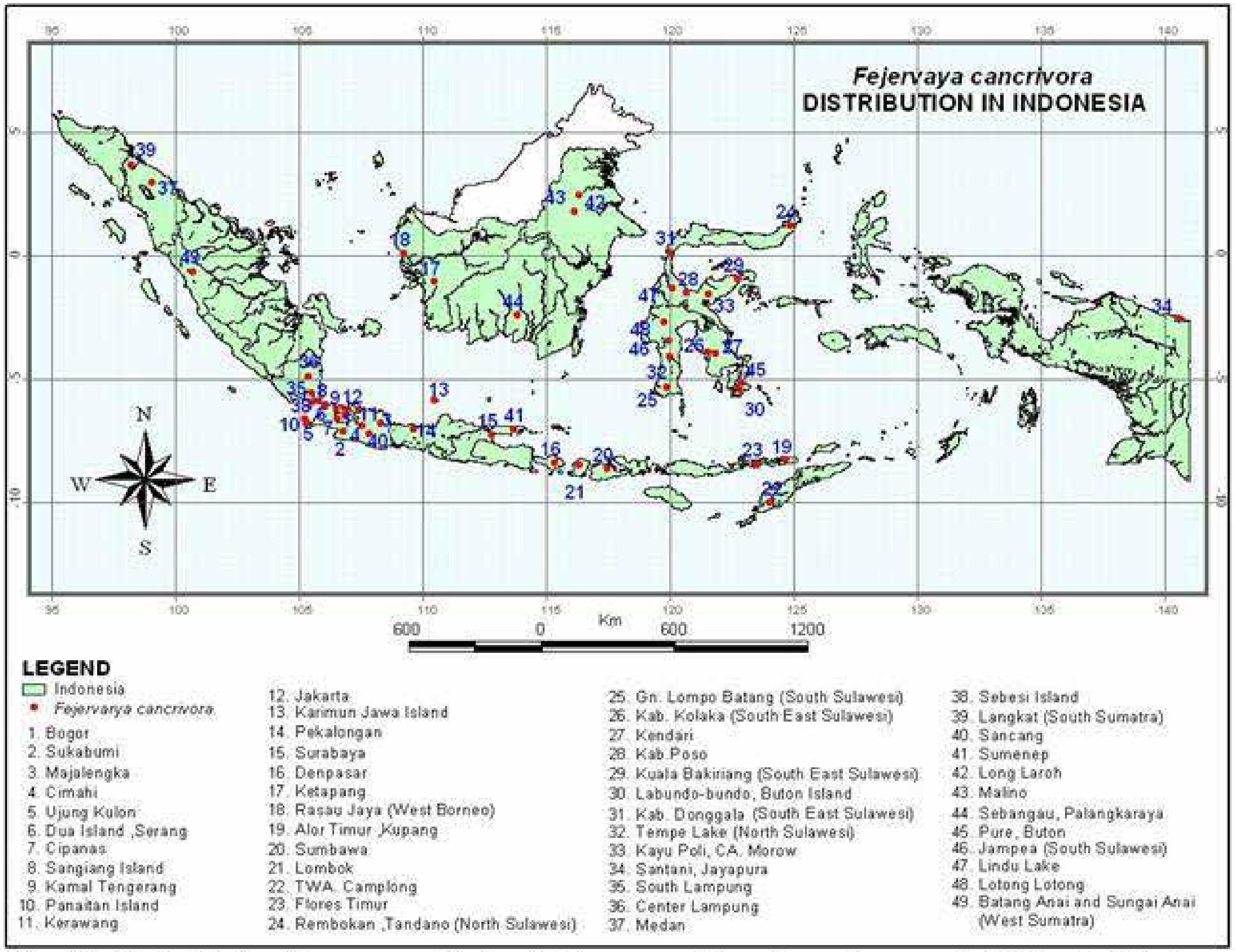

Figure 4-3. The distribution of *F. cancrivora* in Indonesia. Points were made based on specimens stored at MZB and additional survey

Church (1960) and Premo (1985) gave detailed accounts of *F. cancrivora* breeding ecology in western Java, based on weekly examinations of frogs captured by professional harvesters. Church (1960) noted that the species is shy and always ready to jump when approached by humans. Apparently breeding is nearly aseasonal, and eggs can be found at any time of the year, but according to Church (1960), the breeding peaks from August through October.

There are conflicting reports regarding the clutch size of this species. Iskandar (1998) and Jaafer et al. (1999) reported that clutches contain from 500 to more than 1000 eggs. In contrast, Premo (1985) stated that *F. cancrivora* clutches could contain more than 10,000 eggs. Spawning can occur in two or even three batches and may tend to peak during the dark phase of the moon (Church, 1960).

Although *F. cancrivora* is considered common, there are anecdotal accounts suggesting that this species has disappeared from some rice field habitats in which it was formerly abundant (e.g. Pratomo, 1998; Schmuck, 2000; Veith et al., 2001). There is only one detailed population study on this species, carried out in Malaysia by Jaafar (1994). Schmuck (2000) placed *F. cancrivora* on “at risk” status without actual data, most probably because of its importance for human consumption.

Although it is most often encountered in rice fields, *F. cancrivora* is also found in streams, ponds, ditches, and swamps in non-forested areas, mostly in habitats associated with humans (Church; 1960; Alcala, 1962; Berry, 1975; Iskandar, 1998). Both adult and larval *F. cancrivora* can be found in brackish water (Alcala, 1962; Dunson, 1977). An experiment by Dunson (1977) revealed that tadpoles of this species could survive high temperatures and salinities. Field notes accompanying specimens stored at the MZB confirm that this species was mostly found in rice fields, although a few (especially specimens from islands other than Java) were found in streams, lakes and marshes. Unfortunately, some specimens did not have detailed habitat descriptions.

Iskandar (1998) mentioned that *F. cancrivora* could be found at elevations up to 1200 m asl, however a survey I carried out in 14 paddy fields in West Java found that *F. cancrivora* was most abundant at lower elevations. Only one *F. cancrivora* was found at an elevation of more than 600 m, which was in the Citalahab rice fields (an enclave within Mount Halimun National Park) during a survey in July 2004. My surveys also confirmed findings by other researchers that most individuals of this species were found around rice fields (inside the rice fields or in associated irrigation ditches). Only one individual was found in a different habitat; the Cisaketi stream, a forested stream about one kilometre from the nearest human habitation.

This species is distributed from the Malay Peninsula to the Philippines and the Lesser Sundas as far as Flores; Guangxi and northeastern coast of Hainan Island, China; Vietnam (Frost, 2004). In Indonesia, this species is found on almost all major islands from Sumatra to Irian Jaya (Indonesian part of New Guinea Island). Figure 4-3 shows the distribution of *F. cancrivora* in Indonesia.

4.2.3 The Giant Javanese Frog, Limnonectes macrodon

Limnonectes macrodon Duméril and Bibron, 1841 is a large frog. Measurements of 148 specimens stored in the Museum Zoology, Bogor showed that the species could reach a length of at least 168.30 mm. Its taxonomy has undergone several revisions and it is also known as *Rana macrodon.* This species has a large head and smooth skin. The toes are entirely webbed to the tips of the digits, and the tips of the toes are distinctly expanded (Figure 4-1). Skin colour in life is reddish brown to blackish brown.

This frog is part of the ranid fanged frogs, in which males do not have the usual secondary sexual characteristics such as nuptial pads and voice (Inger, 1969). There is some confusion about the identity of this species; most specimens reported as *L. macrodon* from outside Java are not really this species but belong to one of several other species, such as *Limnonectes blythii, L. malesianus, L. leporinus, L. ingeri* and *L. kadarsani* (Iskandar, 1998).

There is no published information on the ecology of this species. A study by Sugiri in 1979 provided information on the basic biology of a species of frog in West Java which at the time was known as a member of the *Rana blythii* complex but is now known as *Limnonectes macrodon* (Iskandar, *pers. comm.*) Almost all fanged frogs have reverse sexual size dimorphism, in which mature males are larger than females (Inger, 1966; Emerson et al., 1993). To differentiate sex in this genus, researchers usually look for the occurrence of a fang-like bone in the jaw (an extension of the lower mandible), which is usually larger in males (Sugiri, 1979, Emerson and Inger, 1992; Tsuji, 2004). As with *F. limnocharis* and *F. cancrivora*, the species breeds all year long, with a peak in the rainy season in September to March (Sugiri, 1979). The number of eggs in one clutch of *L. macrodon* is variable. Sugiri (1979) recorded that this frog can have multiple clutches in one breeding period with a range of 551 – 3,892 eggs in a single clutch. Iskandar (1998) refers to around 1000 eggs in one clutch, which are placed in small ponds beside the river.

The frog is usually found along rivers or small streams with clear water (Iskandar, 1998). My survey found this species at streams, and also near natural ponds inside forested areas (Mount Walat), fish-ponds (Panguyangan) and even in drainage near rice fields (Ciptarasa). It was found from low elevation at about 60 m asl (Cilabruk) up to about 800 m asl (Panguyangan and Ciptarasa).

Figure 4-4. Various type of habitat for *Limnonectes macrodon*: A – stream with fast flowing water and boulder in Caringin (Bogor), B – stream flowing across rice fields in Cilengkong (Sukabumi) , C - Small stream in Cilabruk (Sukabumi) with closed canopy, D – irrigation tributaries in Situ Gunung (Sukabumi)

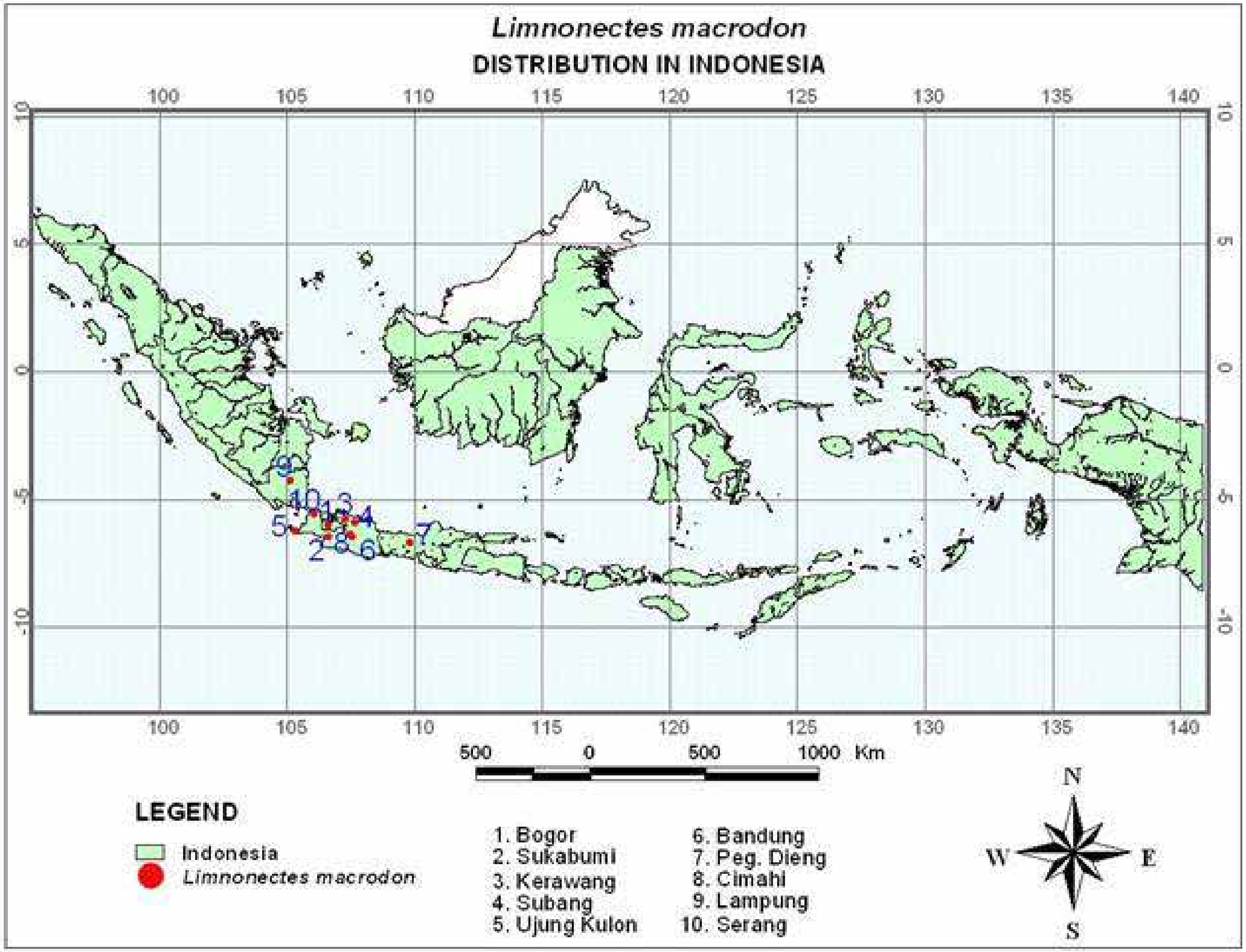

Figure 4-5. The distribution of *L. macrodon* in Indonesia. Points were made based on specimens stored at MZB and additional survey

Figure 4-4 shows typical habitat for this species. *Limnonectes macrodon* occurs from Burma to Malaya, Thailand and Malaysia; Java and Sumatra (Indonesia), and the Rioux Archipelago (Frost, 2004). In Indonesia this species is considered endemic to Java, but is also found in Lampung, South Sumatra (Iskandar, 1998). In Java, it is mostly found in the western part of the island, with only one specimen known from central Java (Figure 4-5).

4.3 The locations

Java island, situated between Sumatra to the west, Borneo to the north, and Bali to the east, is part of the Republic of Indonesia. Indonesia is the fifth most populous country in the world with a population of more than 200 million in 2002 (202,707,418 excluding the populations of the provinces of Moluccas, Irian Jaya and Aceh (BPS, 2002). Although the island of Java comprises less than 10% of the total area of Indonesia, it contains sixty percent of the population. Java thus has an extremely high population density, because of historical influences and fertile soils (Whitten et al., 1997). Table 4-3 shows the essential statistics of Java.

Table 4-3. Essential statistics of the six administrative areas of Java (BPS, 2002)

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Java is an important area for rice production in Indonesia, both historically and currently (van Setten van den Meer, 1979). In 1999 the total area of rice fields in Java was 3,375,381 ha or around 40% of the total Indonesian rice field area. A third of the total area of rice fields in Java is situated in West Java (BPS, 2000).

My research sites are located in three provinces in Java: West Java, DKI Jakarta and East Java. All population ecology work was conducted in rice fields and streams in West Java. A description of each field site is given below (4.3.1 and 4.3.2). Research in Jakarta and East Java involved surveys of traders, harvesters and markets, since the markets of Jakarta and Surabaya (East Java) are the main centres for harvested frogs (see Chapter 3).

Jakarta is the capital city of Indonesia. It is a metropolitan city with a population of 8,379,069 in 2002 (BPS, 2002). West Java was the second largest province in Java after East Java up to 2001, but since late 2001 the province has been split into two provinces, with the western area becoming the province of Banten. West Java is known as one of the wettest regions in Java, with the wettest months from December to April. Based on an agro-climatic map (Whitten et al., 1997) the area to the east and west of Bogor (Bogor and Sukabumi residencies) is considered to be a permanently wet zone where rainfall is more than 200 mm/month for 12 months. That is why the Bogor region is also known as the "rain city".

Six locations were chosen for population studies of *F. limnocharis-iskandari complex* and *F. cancrivora* in the rice fields, whilst two locations were chosen for population studies of *L. macrodon*.

4.3.1 Rice fields

a. Locations

Intensive studies of the population dynamics of *F. limnocharis-iskandari* complex and *F. cancrivora* and of pesticide residues were conducted in six rice fields in the *Kabupaten* (Residencies) of Bogor, Karawang and Subang, from May 2002 until October 2003.

Two locations were sampled in the Bogor area; Caringin and Darmaga village. The *sawah* or rice fields in this area are small and situated between home-gardens (*kebun*) and villages. The area sampled in Darmaga was a research plot owned by the nearby Bogor Agricultural University. The Caringin and the Darmaga rice fields are subject to high annual rainfall. During my study, the wettest month occurred in February 2003 (571 mm in Caringin and 556 mm in Darmaga) and the driest month in July 2003 (43 mm in Caringin and 25 mm in Darmaga). Figure 4-6 shows the monthly rainfall during 2002 and 2003.

Karawang and Subang are situated in the northern coastal lowland part of West Java, with less annual rainfall than Bogor and Sukabumi, and have distinct dry seasons. The countryside is flat, most trees are concentrated within the villages, and rice fields dominate the terrain. The rice fields sampled in Karawang were located at the village of Cikuntul, whilst in Subang they were located at the village of Sukahaji (around 40 km east of Cikuntul).

Samples were taken from four rice fields in these areas:, two in Karawang and two in Subang. In each area I sampled one pesticide rice field (code P) and one non-pesticide rice field (code NP). The fields sampled were situated in huge arrays of *sawah* and the distances between the pesticide and non-pesticide locations were approximately 500 m and 2000 m for Karawang and Subang respectively.

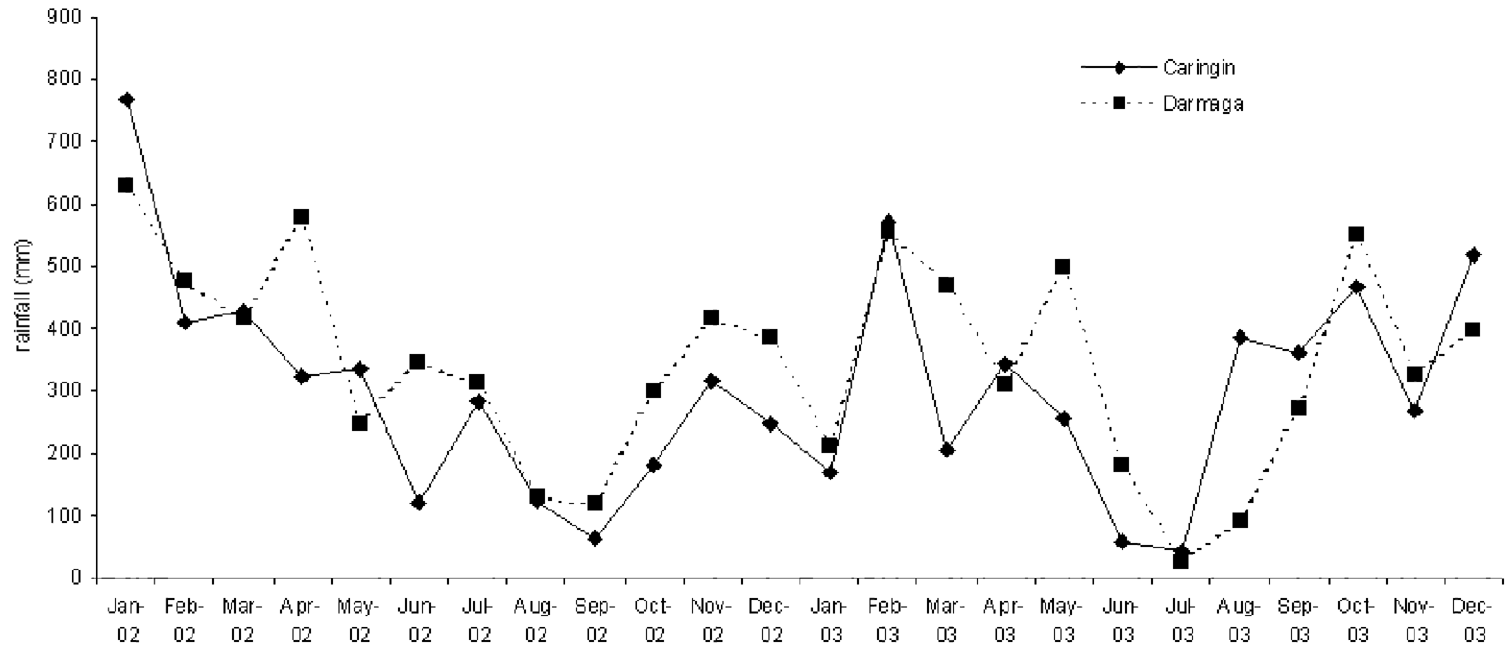


Figure 4-6. Monthly rainfall in Caringin and Darmaga from January 2002 to December 2003. Data from Caringin is taken from Ciawi Station, whilst data for Darmaga is from Darmaga Bogor station. Data source: Stasiun Klimatologi Darmaga Bogor, Badan Meteorologi dan Geofisika Balai Wilayah II).

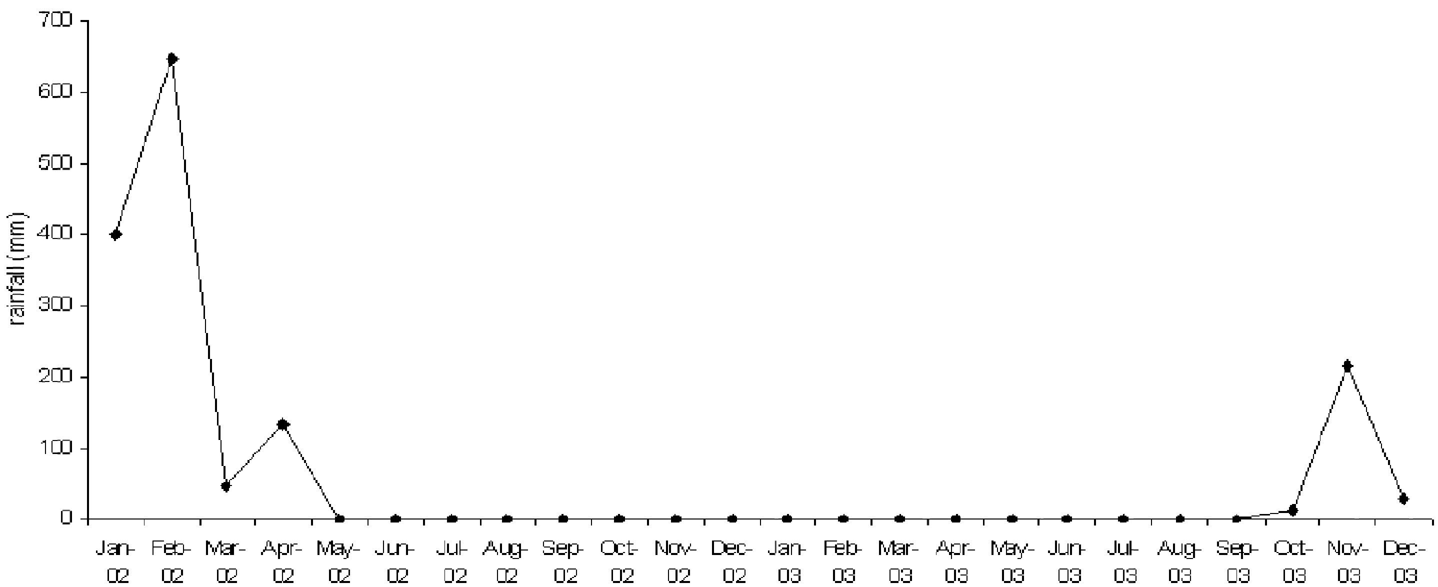


Figure 4-7. Monthly rainfall in Karawang and Subang from January 2002 to December 2003. Data is the same for both locations, since it was only served by Cilamaya karawang station. Data source: Stasiun Klimatologi Darmaga Bogor, Badan Meteorologi dan Geofisika Balai Wilayah II.

The non-pesticide rice fields in Karawang were owned by a single farmer who has not used pesticide since 1990. The non-pesticide rice fields in Subang were owned by a group of farmers who decided to turn to integrated pest management in 2000. Thus the total area of non-pesticide rice fields in Subang is 10 times bigger than the total area of non-pesticide rice fields in Karawang. The relatively short times since pesticide use ceased means that residual pesticides might still be present, particularly the more persistent types such as organochlorines. Unfortunately, there were no fields with less likelihood of pesticide residues available for sampling.

Figure 4-7 shows the monthly rainfall in the area during 2002 and 2003. Throughout the whole period of sampling (May 2002 to September 2003), the meteorological bureau offices in Karawang and Subang area recorded no rain. 2003 was a dry year compared to 2002, with total annual rainfall of 257 mm compared with 1227 mm in 2002. Table 4-4 shows air and water temperatures and relative humidity at the six sites during the study.

Surveys to check the occurrence of the target species were also undertaken in eight other locations: Situ Gede (Bogor), Cisaat (the buffer zone of Mount Gede-Pangrango National Park), Panguyangan and Ciptarasa (both enclaves within Mount Halimun National Park), Citalahab (also an enclave in Mount Halimun NP), Ciracap and Lengkong. Figure 4-8 shows the location of rice field surveys.

The first four locations were surveyed during August-September 2001, Citalahab was surveyed in July 2004, and the last two during October 2003. In March 2004, Situ Gede rice fields were used for a 6-days movement study of *F. cancrivora* (Chapter 5). Full descriptions and locations of these sites appear in Table 4.5.

Table 4-4. Microclimate in the paddy fields of Bogor, Karawang and Subang. Data were recorded at 21.00 – 24.00 hours.

Location	Microclimate	Range	Mean $\pm$ SD
Caringin	Air temp (^oC)	20.0 - 23.5	21.3 $\pm$ 1.0
	Water temp (^oC)	21.0 - 24.5	23.1 $\pm$ 1.1
	Relative Humidity (%)	84 - 96	90.6 $\pm$ 4.1
Darmaga	Air temp (^oC)	21.8 - 26.2	23.7 $\pm$ 1.3
	Water temp (^oC)	24.2 - 26.0	25.2 $\pm$ 0.9
	Relative Humidity (%)	85 - 97	94.1 $\pm$ 3.6
Karawang NP	Air temp (^oC)	22.4 - 27.4	24.9 $\pm$ 1.5
	Water temp (^oC)	24.9 - 28.1	26.3 $\pm$ 1.3
	Relative Humidity (%)	86 - 96	92.1 $\pm$ 3.7
Karawang P	Air temp (^oC)	22.4 - 27.4	25.0 $\pm$ 1.5
	Water temp (^oC)	24.9 - 28.1	26.3 $\pm$ 1.2
	Relative Humidity (%)	86 - 96	92.4 $\pm$ 3.9
Subang NP	Air temp (^oC)	22.0 - 26.0	23.7 $\pm$ 1.5
	Water temp (^oC)	23.2 - 27.0	25.1 $\pm$ 1.4
	Relative Humidity (%)	81 - 98	93.9 $\pm$ 5.0
Subang P	Air temp (^oC)	22.0 - 26.0	23.8 $\pm$ 1.4
	Water temp (^oC)	24.0 - 27.0	25.3 $\pm$ 1.6
	Relative Humidity (%)	81 - 99	93.0 $\pm$ 4.2

Five *sawah* were surveyed in Sukabumi. Except for Ciracap, which was situated in low land, the others were situated in the sloping terrain within an enclave of a National Park (Ciptarasa and Panguyangan, Citalahab, all village enclaves inside Mt. Halimun National Park) or on the border of a National Park (Cisaat and Situ Gunung, bordering Mt. Gede-Pangrango National Park). Although focusing more on the occurrence of *F. limnocharis-iskandari complex* and *F. cancrivora*, I also noted other species present. Figure 4-8 shows the locations of rice fields surveyed during the study.

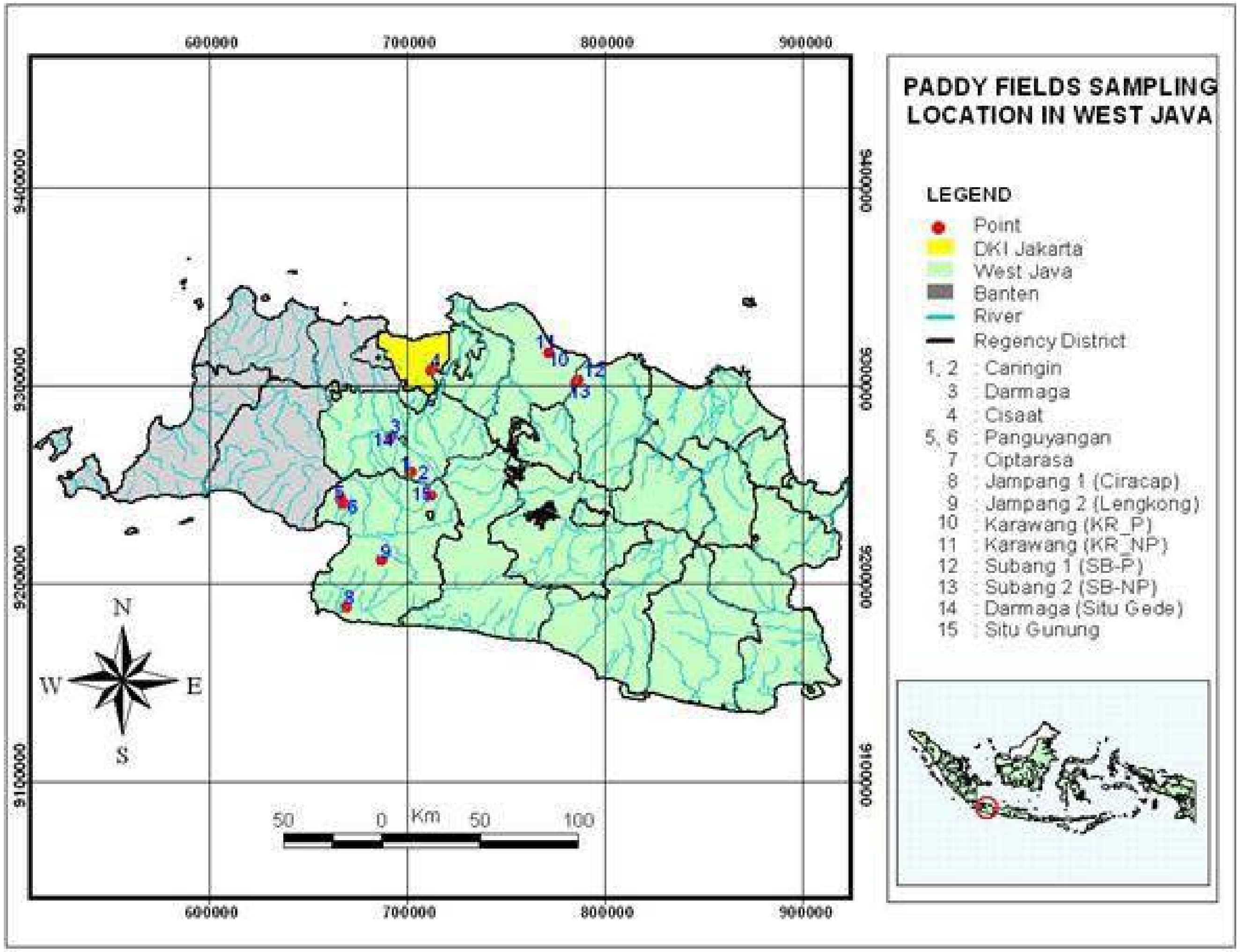


Figure 4-8. Sampling sites in paddy fields in West Java, Indonesia

b. Rice planting methods and management

Rice growing in Indonesia is mostly done using wet-field cultivation, in which rice grows in water and is dependent on rainwater run-off or artificial irrigation (van Setten van den Meer, 1979). Rice fields or *sawah* are an important agriculture landscape in Indonesia. They are the main source of Indonesia's staple food, and are also an important habitat for wildlife, including fish, birds and frogs (Whitten, et. al. 1997). Edible frogs, such as *Fejervarya limnocharis-iskandari complex* and *F. cancrivora*, occur abundantly in rice fields (Chapter 5), thus the habitat is important in sustaining the edible frog trade.

Rice, *Oryza sativa*, is planted either in flat areas or in terrace systems in upland areas. Land is usually separated into blocks of *sawah*, divided by small borders and flooded just before sowing. The borders serve both as property boundaries and as water regulation systems. Preparation begins with the farmer raking and ploughing the rice field with water buffaloes when water fills the field. Rice seeds are planted in small plots, germination takes place 7–15 days later, and rice plants emerge from the water approximately 30 days after sowing. Seedlings are separated and planted in the rice fields. Once seedlings are rooted, water levels (20–30 cm) are periodically reduced (5–10 cm) several times during the season for herbicide and pesticide spraying or fertilization. Rice fields are drained some days before harvesting. Rice is harvested about four months after planting. In intensive areas like Subang and Karawang, the fallow period is short and fields are prepared as soon as possible for another growing season. In other areas, usually those dependent on water from run-off, farmers sometimes grow cash crops such as beans or other vegetables intermittently with the rice-planting season or leave the fields to "wild" aquatic plants. Figure 4-9 shows various stage of rice planting.

Table 4-5. Rice field sites surveyed for *F. limnocharis-iskandari complex* and *F. cancrivora* in West Java, Indonesia

Site name and Location	Eleva-tion (m)	Type of *sawah*	Pesticide use	Water systems	Fallow Time (months)	Harvests/ year
BOGOR						
Caringin: S 06°43.373′- .542′ E 106°49.380′ -.578′	478	Flat	high	Rain dependant	1-2	2
Darmaga S 06°33′50.8″ E 106°44′10.2″	202	Flat	High	Rain dependant	1-2	2
Situ Gede S 06°33'50.8" E 106°44'10.2"	202	Flat	High	Rain dependant	1-2	2
SUKABUMI*						
Cisaat S 06°51′34.9″ E 106° 55′ 06.4″	800	Terrace	High	Rain dependant	1-2	2
Panguyangan S 06°52.219′- .283′ E 106°31.074′ - .253′	760 -800	Terrace	Low	Rain dependant	1-2	2
Ciptarasa: S 06°51.121′ E 106°30.382′	810	terrace	none	Rain dependant	4-5	1
Citalahab (Mount Halimun National park) S 6º44.349´ E 106º31.826´	1091	terrace	none	Rain dependant	4-5	1
Ciracap S 7°20.781′ E 106°31.973′	68	Flat	High	Rain Dependant	1	2
Lengkong S 7°07.969′ E 106°41.679′	594	Flat	High	Rain Dependant	1	2
Situgunung S 6º50.857´ E 106º55.586´	936	terrace	High	Rain Dependant	1	2
KARAWANG						
Karawang P S 06°10.601′ E 107°27.514′	23	Flat	High	Irrigation	2 -4 weeks	2
Karawang NP S 06°10.511′ E 107°27.549′	23	Flat	None for 10 yrs	Irrigation	2 -4 weeks	2
SUBANG						
Subang P S 06°18.094′ E 107°35.054′	29	Flat	High	Irrigation	2 -4 weeks	2
Subang NP S 06°18.626′ E 107°35.074′	28	Flat	None for 3 yrs	Irrigation	2 -4 weeks	2

Note: all locations surveyed in Sukabumi were for the preliminary survey purposes.

The wet cultivation method applied in West Java dictates the structure and use of irrigation systems in each area. The most intensive area, which is called "the rice bowl" such as the rice fields in Karawang and Subang, has intensive irrigation systems developed by the government who manage the flow of water from the nearby reservoirs. Since the area sometimes suffers drought, the planting season in this area depends on the flow of water from irrigation. *Sawah* in this area are assigned to four coded areas and water flows to each coded area in turn. Each farmer in the same coded area will plant rice at the same time. There is usually 2 weeks difference between the timing of water release into areas with adjacent codes.

In most areas in Bogor and Sukabumi, where rain falls almost every month, water supply is not a problem. Thus most *sawah* in these areas depended on surface water run off. The irrigation system here depends on canals that bring water from nearby streams, and farmers can plant their rice any time of the year. This leads to variable timing of planting among farmers, even in adjacent fields.

Most farmers cultivate rice using high yielding varieties developed by the International Rice Research Institute and encouraged by the government. However, in Ciptarasa village in the vicinity of Mount Halimun National Park buffer zone and in other indigenous communities such as Badui (in the province of Banten) only local varieties are grown. Indigenous communities in this area have a strict *adat* rule that allows no modern techniques in their daily lives and have strong ritual habits in preparing the land.

Figure 4-9. Various stages of rice fields. **A** and **B** are rice fields in Caringin during second and sixth weeks of planting. Fallow periods in Karawang (**C**) and Sukabumi (**D**). Rice plants ready to be harvested in Subang (**E**) and terrace rice fields during sowing period in Mount Halimun National Park enclave (**F**)

The need for greater rice productivity has resulted in higher levels of pesticide and fertilizer input to rice fields (Settle, et al., 1996). Moreover, the development of more efficient water irrigation systems has shifted rice planting methods from traditional methods which use low input and fewer planting seasons in a year (one or two times) into high input and more planting seasons in a year (2 or 3 times a year), thus reducing the fallow times. Excess use of pesticide is known to affect the biodiversity of rice field communities in Indonesia (Settle et al., 1996) and pesticides have been implicated as one of the culprits in amphibian declines around the world (Ouelett, et al., 1997; Taylor, et. al, 1999; Bridges and Semlitsch, 2000)

Sawah in Karawang and Subang are usually farmed intensively, with two planting seasons yearly. Although, the government of Indonesia tries to encourage lower use of pesticides, their use by Indonesian farmers is still high. Investigations by Ardiwinata et al. (1997) into pesticide levels in samples of soil, water and rice collected from West Javan areas including Karawang, Subang, and Sukabumi revealed high levels of chlorpirifos, BHC, aldrin, endosulfan and carbofuran in the soil samples. The high cost of pesticides and better understanding of pesticide risk has turned some farmers to integrated pest management system (IPM), using no pesticides and relying on biological pest control. The number of farmers using IPM in Subang and Karawang is still small, and the total area of rice fields under IPM is relatively small. Most blocks of *sawah* under IPM are situated in the middle of a huge array of pesticide-use rice fields and therefore the benefit to them of using IPM is not really known. In contrast to these systems, the indigenous communities in Mt. Halimun National Park area in Sukabumi residency have maintained their traditional agricultural systems. The methods include a long fallow period, low number of planting seasons per year and no use of pesticides. A summary of *sawah* conditions in each location appears in Table 4-5.

4.3.2 Streams

The population dynamics of *L. macrodon* were studied in two locations: Cilember and Ciapus Leutik, from June 2002 to May 2003. Both locations were near forested land, although the location at Ciapus Leutik was nearer to human habitat, in this case Ciapus village.

Additional surveys to find *L. macrodon* were conducted in eight other locations in West Java. Three streams (Cinagara, Cikalang, and Mount Walat streams) were surveyed twice during 2002-2003, and five locations were surveyed once (Cisaketi Stream; Cilabruk Stream, Cilengkong Stream, tributaries around Situ Gunung lake and Situ Gunung stream).

The streams and the lake surveyed differed in various characteristics. Some were located near villages and sometimes crossed through rice fields (Caringin, Cikalang, Mount Walat and Ciapus, Situ Gunung stream, Cilabruk and Cilengkong), others were located in forested areas (Cisaketi and Situ Gunung Lake). Table 4-6 gives the characteristics of the streams and full descriptions of study sites, whilst Figure 4-10 shows the locations of these surveys. Details of the results of the surveys conducted in rice fields and streams appear in chapters 5, 6, and 7.

Table 4-6. Streams and ponds surveyed for *L. macrodon*.

Site name and Location	Elevation (m)	Flow status	Adjacent landscape
BOGOR			
Cinagara – Caringin: S 06°43.373′- .542′ E 106°49.380′ -.578′	479 – 491	Permanent streams	Village and rice fields
Cikalang S 06°43.216′ E 106°49.775′	458	Permanent streams	Village and rice fields
Ciapus Leutik* S 06°39.800′ - .901′ E 106°44.711′ - .715′	709 - 717	Permanent streams	Village and Plantation forest
Cilember* S 06°39.612′ - .631′ E 106°56.701′ - 57.002′	662 – 840 m	Permanent streams	Plantation Forest and Recreational area
SUKABUMI			
Mount Walat S 06°54.879′ E 106°49.445′	595	Permanent streams	Village and rice fields
Cisaketi S 06°44.471′ - .559′ E 106°38.453′ - .882′	951 – 1039	Permanent streams	Forested area, inside Unocal Gas Mining
Cilabruk S 07°20.263′ - .423′ E 106°31.981′ - 32.169′	69 - 101	Permanent streams	Rice field
Cilengkong S 07°07.852′ - .967′ E 106°41.682′ - .725′	578 - 591		Village and Rice fields and crops
Situ Gunung S 06°50.017′ - .077′ E 106°55.399′ - .417′	1045	Permanent streams	Plantation Forest and Recreational area, rice fields
Situ Gunung Lake S 6º50.239´ - .992’ E 106º55.564´ -.652’	921 – 1071	Permanent lake	Plantation Forest and Recreational area, rice fields

* Population study sites. Other sites were preliminary survey

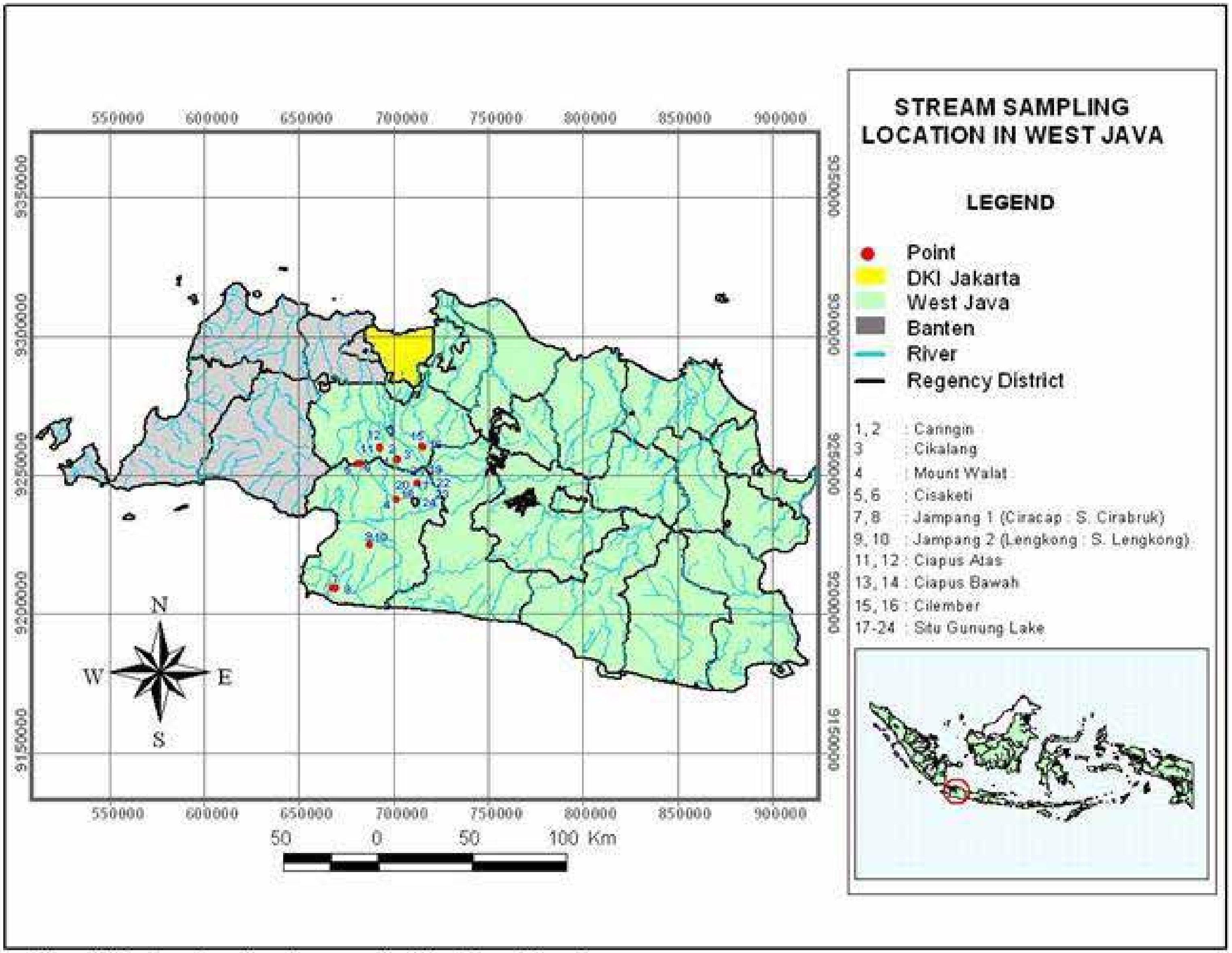


Figure 4-10. Sampling sites in streams in West Java, Indonesia

CHAPTER 5

POPULATION DYNAMICS OF *F. LIMNOCHARIS-ISKANDARI* COMPLEX AND *F. CANCRIVORA*

5.1 Introduction

As a country, Indonesia relies heavily on its agricultural sector. Because rice is the staple food for most of the nearly 200 million inhabitants of Indonesia, it is not surprising that its agricultural land is predominantly paddy fields. Paddy fields are an important habitat for wildlife such as birds, reptiles and amphibians (Whitten et al., 1997) and some 3.5 million hectares of paddy fields are available as habitat for amphibians in the island of Java alone. Compared with other habitats, the number of frog species in agricultural land is usually low (Dash and Mahandata, 1998; Jaafar et al., 2002), however species adapted to the conditions encountered in this system are usually abundant. Some frogs, such as *Fejervarya limnocharis* and *F. cancrivora,* thrive in paddy fields (Church, 1960; Alexander et al., 1963 and 1979, Jaafar, 1994) despite the physiological stress that might occur because of human activity.

Although some studies on paddy field frog species have been done in Indonesia (Church, 1960, Atmowidjojo and Boeadi, 1988; Premo, 1989), these studies have usually focused on aspects of the biology of one species, such as clutch size, fecundity and food habits, by analysing specimens obtained from professional harvesters. Thus there is little detailed information on the population sizes and dynamics of the most common frogs in Indonesian paddy fields: *F. limnocharis-iskandari* complex and *F. cancrivora*. Both species are heavily exploited for human consumption (chapter 2 and 3) and to evaluate the sustainability of current harvesting practices there is a need to have a clear understanding of the population dynamics of these species.

To estimate populations, a number of mathematical models have been developed. To apply these models, parameters must be estimated, usually using data collected from mark-release-recapture studies. Mark recapture techniques have been widely used for estimating population size and other parameters such as mortality, emigration rates, birth and immigration rates for a range of animal groups, such as frogs (Friedl and Klump, 1997; Ramirez et al., 1998; Wood et al., 1998, Peters, 2001), lizards (Bennett, 2000), dolphins (Baird et al., 2001), or insects (Zun'iga et al, 2001).

These techniques can be used for closed or open populations. For the purposes of mark-recapture analysis, closed populations are those in which the effects of birth, death, immigration, and emigration can be assumed to be zero during the periods over which data are collected. Open populations are those that may be affected by these processes during data collection (Krebs, 1999).

Populations of frogs are influenced by many factors, such as climatic conditions (Lips, 1998), food availability (Lima et. al, 1995; Pope and Matthews, 2002), competition and predation (Brönmark and Edenham, 1994; Gillespie, 2001) and territoriality (Heying, 2001). The study area lies in West Java, which is a tropical region with high annual rainfall, and the rice field habitat undergoes repeated harsh physical conditions such as flooding and draining, depending on the stage of the harvest cycle (see Chapter 4 for detailed account). In Malaysia, the reproductive pattern of *F. limnocharis* and *F. cancrivora* in the paddy fields is correlated to the water regime in the rice fields (Jaafar, 1994).

The dynamics of populations can be affected by patterns of individual movements. Because environmental conditions in rice fields are dynamic, it is likely that frogs move between them to avoid the harsher extremes. This leads to questions such as: What becomes of animals when the habitat dries? Do they stay and aestivate or do they move elsewhere? What are the frequency and extent of short-term movements, and how will they affect mark-recapture population estimates? To date, no studies have been conducted on detailed microhabitat use and movement patterns of rice field frogs. In fact, there is little detailed information on movement patterns of most anurans (Beebee, 1996). Animal movements are likely to be affected by the availability of resources and avoidance of predators (Lemckert and Brassill, 2000; Schwarzkopf and Alford 2002, Pough et al., 2004). *F. limnocharis* and *F. cancrivora* are widespread in most of Asia (Frost, 1985) and are found mostly in agricultural land. Information on the dynamics of these populations would give insight into the success of species that inhabit rice fields.

This chapter presents the population biology of F *limnocharis-iskandari* complex and *F. cancrivora* in several paddy fields in West Java, and includes the results of a detailed study on individual movements of *F. cancrivora*.

5.2 Methods

5.2.1 Population study

Populations of both species were studied at six sites: Caringin, Darmaga, Subang 1 (Non Pesticide) and Subang 2 (Pesticide), Karawang 1 (Non Pesticide) and Karawang 2 (Pesticide) over four discrete times which included two planting seasons, one in 2002 and the other in 2003 (table 5-1). All rice field locations are harvested twice a year. Chapter 4 gives detailed descriptions of each site. Field parties consisted of 4-6 persons using battery-powered torches, who surveyed paddy fields between 20.00 hrs and 24.00 hrs. Frogs were collected manually.

In total, twelve days of sampling were carried out at each location, divided into four periods. One plot in a paddy field at each site was sampled for three days in a row, twice in one planting season: first during preparation time or early rice plant growth and later near harvest time or after harvesting. A total of 680 man-hours 45 minutes of sampling were performed, with a mean effort per location per day of sampling of 9 man-hours 27 minutes (range 240 – 1170 minutes, SD = 200.41).

Table 5-1. The location and size of sampling area

Location	Adjacent landscape	N. of blocks	Size (m^2)
Caringin	Vegetable patch and village	4	9016.0
Darmaga/2002	Vegetable patch and village	8	10440.0
Darmaga/2003	Vegetable patch and village	2	1473.5
Kawarang Non Pesticide (NP)	Rice fields	2	3529.7
Karawang Pesticide (P)	Rice fields	2	6691.3
Subang Non Pesticide (NP)	Vegetable patch and village	4	6600.0
Subang Pesticide (P)	Rice fields	2	2860.0

The total area sampled differed among locations due to accessibility and changes in the landscape. During 2003, I had to move the Darmaga sampling area to adjacent rice fields because the area sampled in 2002 had been converted to a vegetable patch; this reduced the area sampled (Table 5-1). Frogs in each site were captured by hand and then marked uniquely based on day of capture by clipping their toes. After marking, frogs were released. In the second and third surveys at each site, marked frogs were noted and unmarked frogs were marked.

For every frog captured, I recorded capture date, plot number, time, location on the plot, sex, body size (measured as snout-vent length, SVL), mass, clip code and also environmental data such as water level, water temperature, substrate temperature, percentage of cloud cover and relative humidity.

Toe clipping is the least costly option for marking amphibians for population study (Heyer et al 1994) and although some scientists have suggested that this technique may reduce survival rates (Clarke, 1972; Golay and Durrer, 1994), measures such as using sterilized equipment to treat the toe with antibacterial ointment helps prevent infection that could lead to mortality (Reaser and Dexter, 1996). A recent study found that the adverse impact of toe clipping could increase with the number of toes removed (McCarthy and Parris, 2004). To minimize the impact of toe clipping, I only clipped one to two toes of each individual captured. When two toes were removed, I clipped one from a forelimb and one from a hindlimb. I also avoided clipping the first digit on the forefeet (the "thumb") because of its potential role in amplexus in males.

5.2.2 Determination of sex

The sexes of *F. limnocharis-iskandari* complex and *F. cancrivora* were differentiated based on secondary sexual characteristics. *Fejervarya limnocharis-iskandari* complex males have pigmentation on their throats, which in adults is distinctly M shaped, whilst in juveniles only a faint trace is visible. Males also have a pair of vocal sacs and during breeding possess nuptial pads. Male *F. cancrivora* also have a black patch on their throats, although this is not as large as in *F. limnocharis-iskandari* complex.

The smallest frogs exhibiting the black patch and vocal sacs were 17.46 mm in SVL weighing 0.6 gram (*F. limnocharis-iskandari* complex) and 25.0 mm in SVL weighing 1.15 gram (*F. cancrivora*). However, only frogs judged to be mature based on body size (based on research by Church (1960), Alexander (1978), Premo (1989), and Jaafar (1994)) were assigned to adult sex categories. Therefore, *F. limnocharis-iskandari* complex designated as males were frogs who exhibited secondary sexual characteristics and were 25 mm or more in length, and females of this species were frogs lacking male secondary sex characteristics and with lengths of 30 mm or more. *F. cancrivora* were assigned similarly, with minimum lengths of 35mm for males and

40mm for females. Individuals with lengths below the cutoffs for their species were classified as juveniles.

5.2.3 Skeletochronology

Selected phalanges taken from toe clips of *F. limnocharis-iskandari* complex and *F. cancrivora* were examined to determine age using skeletochronology. Skeletochronology has been used successfully to estimate age in temperate frogs (Halliday and Verrel, 1988; Ryser, 1988; Reaser, 2000). In this study skeletochronology was carried out on 103 *F. limnocharis-iskandari* complex and 35 *F. cancrivora* from samples taken in preliminary surveys during 2001 in several paddy fields in West Java at Caringin, Darmaga, Ciptarasa, Panguyanan and Ciptarasa.

Clipped toes removed from frogs during surveys were stored in 4% formalin solution for processing. Each toe was wrapped in sponge and placed individually in a cassette, then decalcified overnight in 10% formic acid solution. Toes were then embedded in wax in a vacuum infiltration processor and cut into 10µm thick sections transversely using a rotary microtome. Sections were put on slides and kept in a warming oven at least overnight. They were then placed into two consecutive baths of xylene for two minutes each, and then placed into three consecutive baths of ethanol for 20 dips each before being rinsed in water and stained with Mayer's Haematoxylin and Young's Eosin for 25 minutes. Slides were then rinsed again in water and put into Scott's tap water substitute for two minutes. After a final rinse in water, the slides were put in Young's Eosin stain for five dips and then exposed to a series of 10 dips in ethanol and a final 10 dips in xylene. Cover slips were then mounted with DPX glue and slides were placed on trays in a 60^{o}C oven overnight. The best and clearest sections were chosen for observations and were examined using an Olympus BH dual-head microscope to view marks of skeletal growth.

5.2.4 Sampling bias

To check for sampling bias in paddy field surveys (since frogs were caught mostly when they were 0.5 m or less from the field's perimeter), on at least one occasion at each site, frogs were sampled simultaneously not only from within 50 cm of the perimeter but also inside the plot. To avoid damage to rice plants, this sampling was done after harvesting. Frogs were treated the same as in the first sampling, by marking and releasing them afterward for three consecutive nights. In calculating densities, the sampled area was assumed to be 150 cm wide, except at Darmaga, which was assumed to be 100 cm wide. The width of the border in Darmaga is narrower than other locations (maximum width 15 cm, compared to other locations with maximum width 50 cm). These perimeter widths include the width of pathways that act as borders separating blocks of rice fields.

5.2.5 Movement of F. cancrivora

Additional sampling to assess population densities and movements of *F. cancrivora* was conducted in Darmaga during March 2004. The sampled paddy field had been harvested and half of the blocks in it had been prepared for tilling. Heavy rain occurred during the sampling period and most of the areas were temporarily flooded with water; this flooding usually subsided after a few hours. The new site was approximately 2 km from the site used in the original population study. A party of 11-13 persons surveyed 4,592.83 m^2 of paddy fields (composed of nine blocks) for six days in a row with total effort of 274 man-hours 45 minutes or mean daily effort of 45 man-hours 47 minutes and 30 seconds. Since sampling was focused on the occurrence of *F. cancrivora* other frogs were only noted and released immediately. Each *F. cancrivora* captured was assigned a unique code by clipping its toes based on Hero's (1989) numbering system but omitting codes requiring clipping the "thumb". The snout-vent length and mass of each frog were measured before its release at its point of capture. A flag with the individual's ID number was put at each location where a frog was captured. A path on one side and an irrigation channel perpendicular to the path bordered the blocks of rice fields; we used these as X and Y axes in mapping the positions of frogs. When frogs were recaptured, we noted their position only and released them at the point of capture.

5.3. Data analysis

All data sets were analysed using Microsoft Excel and SPSS (version 12.0.1, 2003). The mass and snout vent length of both species were analysed using descriptive statistics. Mean SVLs of males and females within sites were compared using independent samples t-tests for equal variance. Correlation analyses between log-transformed mass and log-transformed snout vent length were undertaken on both species.

The relationship between SVL and number of lines of arrested growth (LAG) was examined for both species using simple scatter graphs and by analyzing the differences of mean SVL between groups that had LAGs and no LAGs. Size-frequency histograms were produced for both sexes of both species at each of the six study sites for each discrete sampling time. Data on snout-vent length were pooled into 1 cm size class categories. I used log-linear analysis to examine relationships between three categorical variables: size class, location and sampling time.

Analysis of variance (ANOVA) was used to determine whether mean snout vent length (SVL), mean density of captured frogs and mean estimates of density differed between locations, regions (Bogor, Karawang and Subang), category of water level and stage of rice planting.

Frogs densities are given as the number of frogs captured per hectare. Water levels were categorized into four classes (0 = dry, 1= mostly dry, 2 = half of the area wet, 3 = entire area wet or flooded) while stage of rice planting was put into five categories (1 = rice plants aged up to one month, 2 = rice plants aged around 1-2 months, 3 = rice plants aged 3 months to near harvest, 4 = rice plants already harvested and land fallow, 5 = land in preparation for tilling).

Populations were considered to be closed over the short time periods (three or six days) over which each sampling session was conducted. Therefore, for each sampling session, the population sizes for both species in each plot were estimated using the Schnabel (Schnabel, 1938) method for closed populations. The formula is as follows:

$$\hat{N} = \frac{\Sigma_t (C_t M_t)}{\Sigma_t R_t},$$ Where:

$\hat{N}$ = estimated population size

Ct = number of frogs caught on day *t*

Mt = marked frogs at day *t*

Rt = number of frogs recapture on day *t*

Assumptions of the Schnabel method are:

1. The population is constant without recruitment or losses
2. All individuals have the same probability of capture in any given sample
3. Marks persist during the study

4. Marking does not affect catchability

The recapture rates of both species for each sampling time were determined by dividing the number of frogs recaptured from the day before by the total numbers of frogs captured. For each sampling time, recapture rates were determined for days 2 and 3.

In the movement study, movement distances of individual *F. cancrivora* were obtained by calculating distance between mapped capture points. Frogs' movements were analysed separately for each sex using three parameters:

1. Distance moved per day
2. Displacement per day
3. The straightness of the movement trail.

Straightness is the ratio of total cumulative distance moved between times the animal was captured to final displacement from the site of initial capture (Schwarzkopf and Alford, 2002). It indicates what type of movement pattern frogs are following. A value of near 1 means that there is a nearly straight path between start and end observations, whilst 0 indicates that there is little net movement.

5.4 Results

5.4.1 Traits of *F. limnocharis-iskandari* complex *and* *F. cancrivora*

In total, 3671 frogs of eight species were sampled during 2002 and 2003. The most abundant species was *F. limnocharis-iskandari* complex (86.4% of individuals) followed by *F. cancrivora* (8.9 %) and *Occidozyga lima* (2.8 %). *Bufo melanostictus, B. biporcatus*, *B. asper Polypedates leucomystax*, and *R. chalconota* were also occasionally encountered.

Except for the first sampling at Subang 2, *F. limnocharis-iskandari* complex were always more abundant than *F. cancrivora*. Overall, I measured and recorded 685 males, 877 females and 1506 juveniles of *F. limnocharis-iskandari* complex, and 51 males, 95 females and 178 juveniles of F. *cancrivora*. The mean SVL for female *F. limnocharis-iskandari* complex was 36.78 mm (SD = 5.98, range 30.00 - 74.11 mm) while for males it was 37.96 mm (SD = 5.02, ranges 25.00 - 52.50 mm). Mean SVL for female *F. cancrivora* was 53.33 mm (SD = 11.54, ranges 40.00 – 87.86 mm) and for males was 53.37 mm (SD = 10.47, ranges 35.00 – 75.90 mm). Although mean snout vent length of females is slightly smaller than that of males, the difference is not significant, except for *F. limnocharis-iskandari* complex in Caringin (Table 5-2). The maximum snout vent lengths of females of both species were greater than the maximums for males. Figure 5-1 summarizes the SVLs of both species.

Table 5-2. T-tests for sexual dimorphism in snout vent length

Location	*F. limnocharis-iskandari* complex		*F. cancrivora*	
	t-statistic	P	t-statistic	P
Caringin	t_{862}=-5.59	0.000	t_{27}=0.549	0.588
Darmaga	t_{363}=0.420	0.675	t_{9}= 0.130	0.160
Karawang-1	t_{56}=2.13	0.038	t_{18}=1.14	0.270
Karawang-2	t_{110}=1.29	0.199	t_{25}=0.341	0.736
Subang-1	t_{160}=1.94	0.055	t_{16}=-0.551	0.590
Subang-2	t_{62}=-0.341	0.734	t_{37}=-0.918	0.364

The mean mass of female *F. limnocharis-iskandari* complex is 4.40 grams (SD = 2.54, range 1.19 – 17.40 grams) while for males it is 4.78 grams (SD = 1.59, range 1.10 – 12.30 grams). Mean mass for females of *F. cancrivora* was 19.47 grams (SD = 14.84, range 2.10 – 105.00 mm) and for males was 17.22 grams (SD = 8.86, range 3.20 – 43.10 grams). All *F. limnocharis-iskandari* complex and 95.5% of *F. cancrivora* captured weighed less than 40 grams; 3.4% of *F. cancrivora* weighed between 40-80 grams and 1.1% weighed more than 80 grams. Almost all of the largest *F. cancrivora* (with weights > 40 grams) were females. Figure 5-2 summaries the body mass distributions of both species. Traits of *F. limnocharis-iskandari* complex and *F. cancrivora* in each location are shown in Tables 5-3 and 5-4.

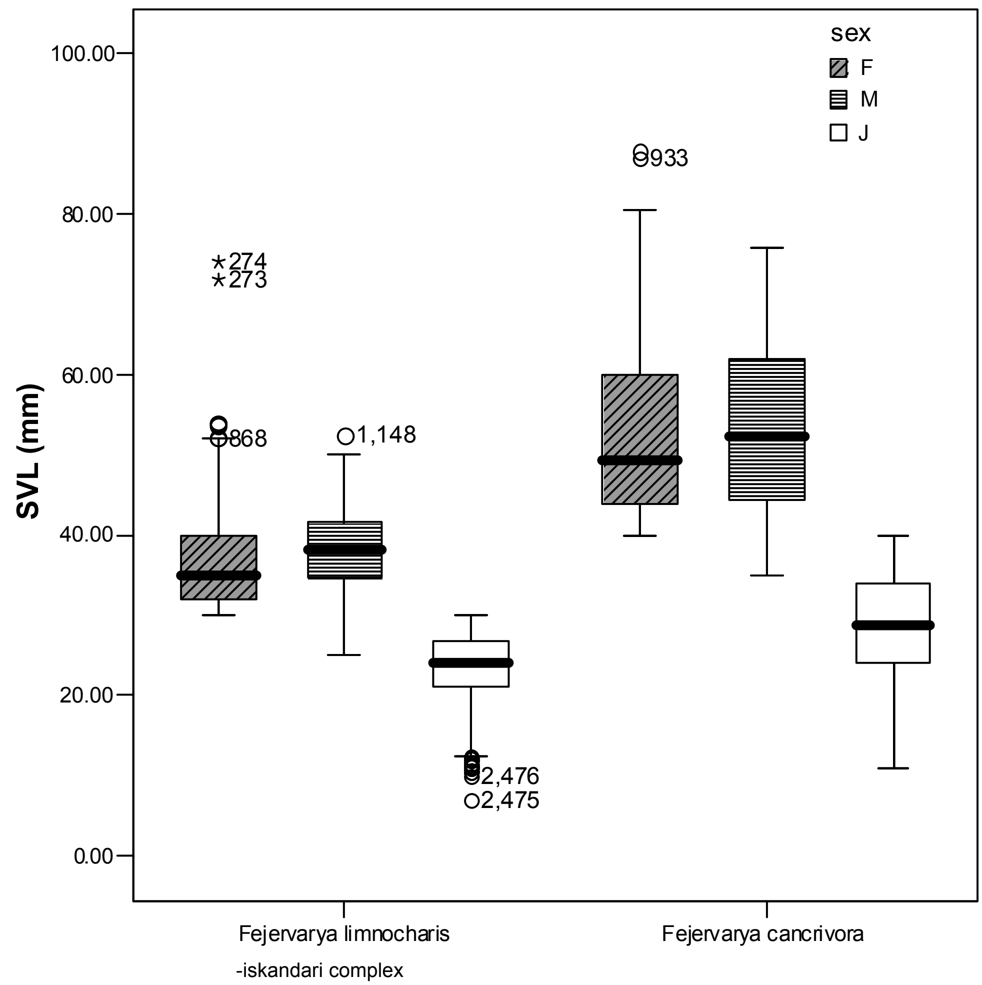


Figure 5-1. Snout vent length of *F. limnocharis-iskandari* complex and *F. cancrivora*: F = female, M = male and J = juvenile

In *F. limnocharis-iskandari* complex there were positive relationships between the log of snout vent lengths and the log weights of females ($r = 0.865$, $N = 863$, $P < 0.05$) and of males ($r = 0.831$, $N = 730$, $P < 0.05$). Similar relationships occurred in *F.*

cancrivora (females: r =0.921, N = 90, P < 0.05, males: r =0.908 N = 49, P < 0.05). These relationships are shown in Figure 5-3.

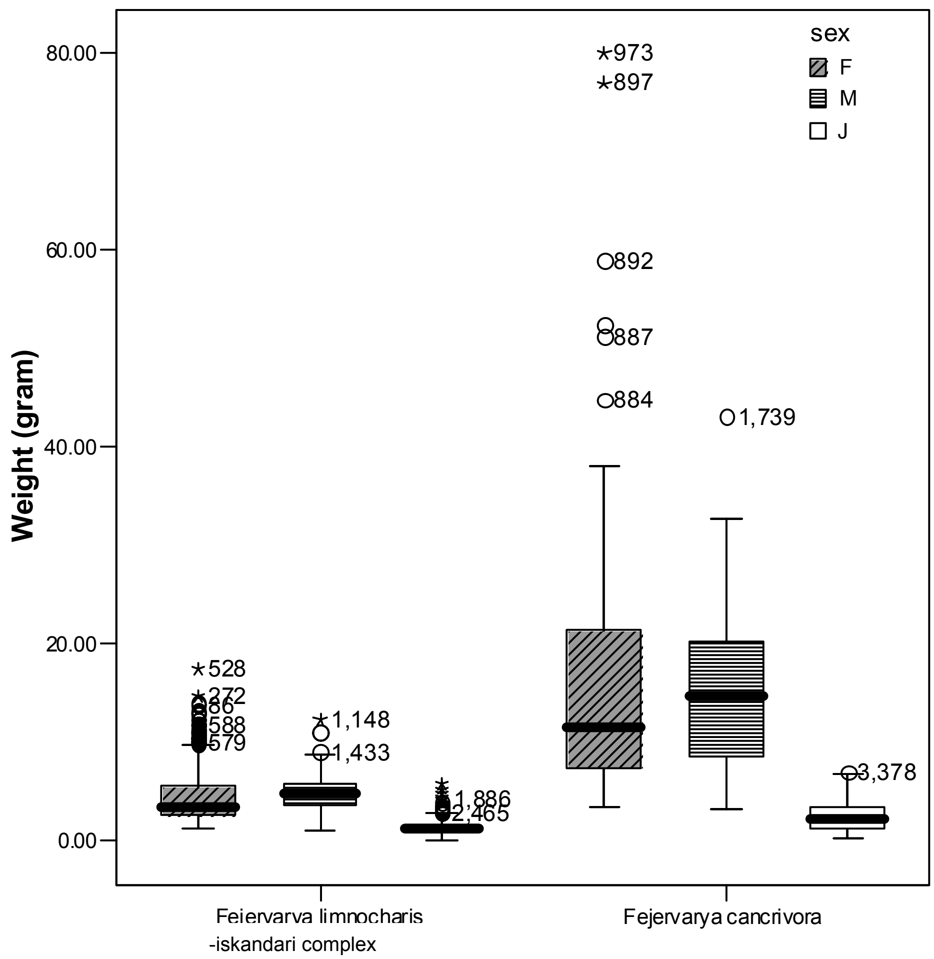


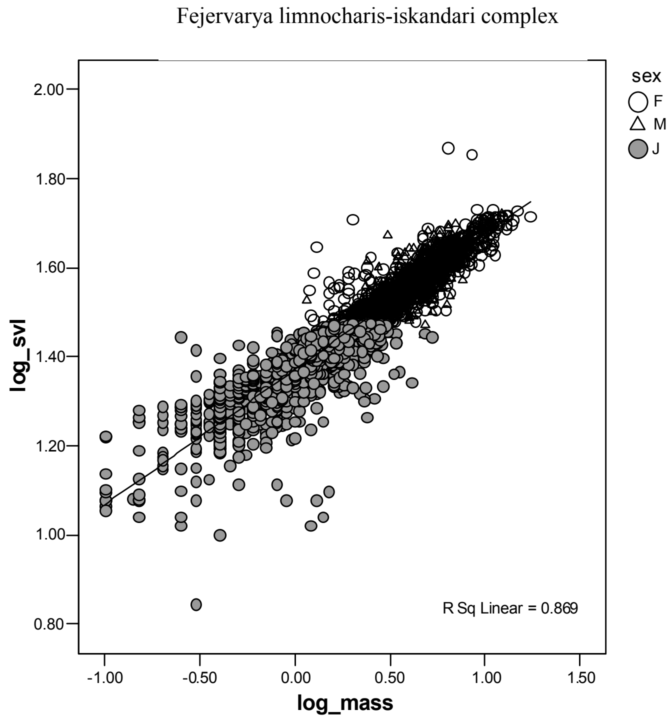
Figure 5-2. Body mass of *F. limnocharis-iskandari* complex and *F. cancrivora*: F = female, M = male and J = juvenile

Table 5-3. Traits of *F. limnocharis-iskandari* complex by site (2002 and 2003)

Location	Sex	N	SVL (mm)				Mass (gr)			
			Min	Max	Mean	SD	Min	Max	Mean	SD
Caringin	Female	498	30.00	74.11	36.39	5.85	1.19	14.70	4.13	2.34
	Male	366	25.00	52.50	38.47	4.68	1.10	12.30	4.75	1.43
	Juvenile	683	12.05	29.96	24.93	3.16	0.14	4.80	1.41	0.59
Darmaga	Female	137	30.00	54.00	39.65	6.73	2.20	17.40	6.08	3.01
	Male	228	25.32	50.00	39.40	4.60	1.40	11.00	5.53	1.41
	Juvenile	101	7.00	29.98	23.95	4.43	0.10	5.70	1.62	0.95
Subang Non Pesticide	Female	86	30.00	50.30	36.03	5.11	1.70	10.50	4.02	2.08
	Male	12	26.58	42.36	35.95	5.48	1.35	8.00	3.60	1.81
	Juvenile	385	11.00	29.78	21.54	3.48	0.10	3.85	0.80	0.47
Subang Pesticide	Female	52	30.00	52.10	35.40	4.95	1.50	13.20	3.33	1.87
	Male	13	18.00	42.36	33.76	7.93	0.80	4.65	2.70	1.53
	Juvenile	118	10.50	29.92	22.98	5.25	0.10	2.50	1.12	0.56
Kerawang Non pesticide	Female	46	30.00	53.90	35.89	6.07	1.80	11.00	4.10	2.39
	Male	12	25.94	44.00	31.83	5.09	1.35	7.50	3.01	1.64
	Juvenile	71	17.84	29.90	24.75	3.34	0.35	2.60	1.38	0.54
Kerawang Pesticide	Female	58	30.12	49.98	36.41	5.27	1.20	13.00	4.42	2.49
	Male	54	25.28	44.50	35.26	4.03	1.30	8.40	3.97	1.50
	Juvenile	148	10.52	29.86	22.95	4.12	0.25	4.40	1.24	0.63

Table 5-4. Traits of *F. cancrivora* by site (2002 and 2003)

Location	sex	N	SVL (mm)				Mass (gr)			
			Min	Max	Mean	SD	Min	Max	Mean	SD
Caringin	Female	15	48.00	80.45	62.32	9.28	9.80	59.00	24.73	15.38
	Male	15	44.40	75.90	60.36	9.95	5.75	43.10	21.30	10.01
	Juvenile	4	30.40	33.61	31.42	1.51	1.70	3.66	2.63	0.91
Darmaga	Female	6	42.00	80.00	60.42	15.52	3.40	77.00	34.10	28.10
	Male	5	39.00	65.68	47.94	10.35	5.00	25.00	10.80	8.07
	Juvenile	20	26.65	39.00	32.86	3.76	1.00	5.40	3.18	1.48
Subang Non Pesticide	Female	10	40.38	65.17	47.89	8.74	4.80	29.00	12.44	9.09
	Male	9	39.78	68.15	50.37	10.35	6.80	27.30	14.71	7.48
	Juvenile	23	14.38	36.81	30.79	6.39	0.30	6.70	3.06	1.81
Subang Pesticide	Female	30	40.00	87.86	47.97	9.43	4.25	80.00	11.15	13.77
	Male	9	39.00	67.58	51.22	8.92	4.50	30.00	11.70	7.91
	Juvenile	67	11.02	39.46	26.04	7.69	0.20	6.50	1.88	1.46
Kerawang Non pesticide	Female	13	41.57	70.10	54.21	10.30	6.40	35.50	17.32	9.59
	Male	7	35.00	62.92	48.62	10.82	3.20	22.20	12.10	7.54
	Juvenile	24	21.31	38.97	30.48	5.33	0.90	5.20	2.75	1.33
Kerawang Pesticide	Female	21	40.21	86.96	55.02	11.95	5.00	38.00	16.15	9.55
	Male	6	41.94	66.22	53.24	7.97	9.85	24.00	15.61	5.32
	Juvenile	40	19.58	39.88	28.07	4.72	0.45	7.00	2.29	1.33



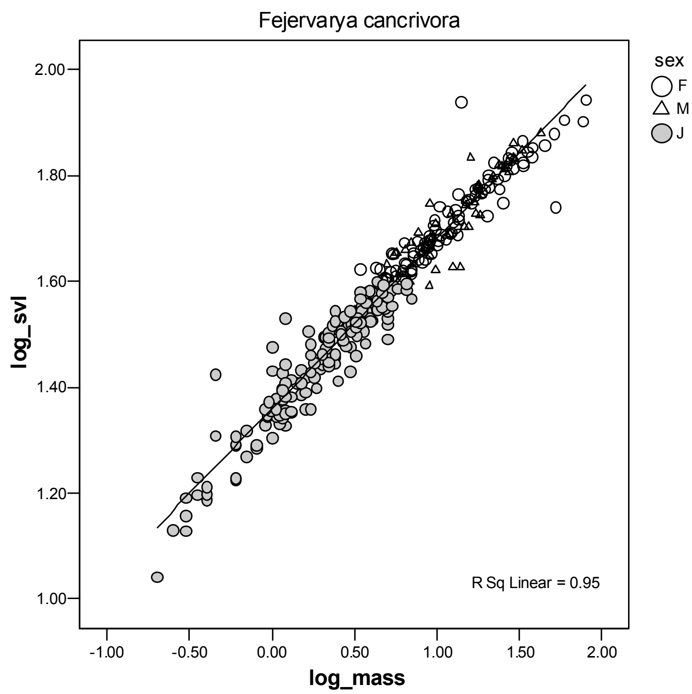


Figure 5-3. Relationship between snout vent length (mm) and mass (gram) of *F. limnocharis-iskandari* complex and *F. cancrivora*. F = female, M = male and J = juvenile

5.4.2 Demographic analysis

Histological analysis of toe clips of *F. limnocharis-iskandari* complex and *F. cancrivora* from several locations in West Java showed that the frog have faint rings in their phalanges, suggesting that they have lines of arrested growth (LAGs) or annual growth rings (Figure 5-4 A, B and C). I was unable to analyse sections of seven samples of *F. limnocharis-iskandari* complex and five samples of *F. cancrivora* due to staining problems (too dark or/and curled section). The maximum numbers of LAGs found in samples from *F. limnocharis-iskandari* complex and *F. cancrivora* were one and two respectively (Figure 5-5). Seventy-three percent of *F. limnocharis-iskandari* complex and 63% of *F. cancrivora* showed no LAGs, suggesting an age of less than one year. There were no significant differences in mean body size between groups that had LAGs and no LAGs, both in *F. limnocharis-iskandari* complex (t_{93} = 0.358, P = 0.723) and in *F. cancrivora* (t_{28} = 0.369, P = 0.723). This suggests that lines of arrested growth may not reflect age in these species. Demographic analyses of both frogs were therefore done based on snout vent length. Length-frequency distributions for each sex of each species during four discrete time periods of sampling in 2002 and 2003 are shown in Figures 5-6 to 5-11. Hierarchical log linear model analysis shows that the number of frogs in each SVL category captured for both species depended on sampling date and location (*F. limnocharis-iskandari* complex: chi-square = 910.5, P <0.0001; *F. cancrivora*: chi-square = 159.799, P = 0.009).

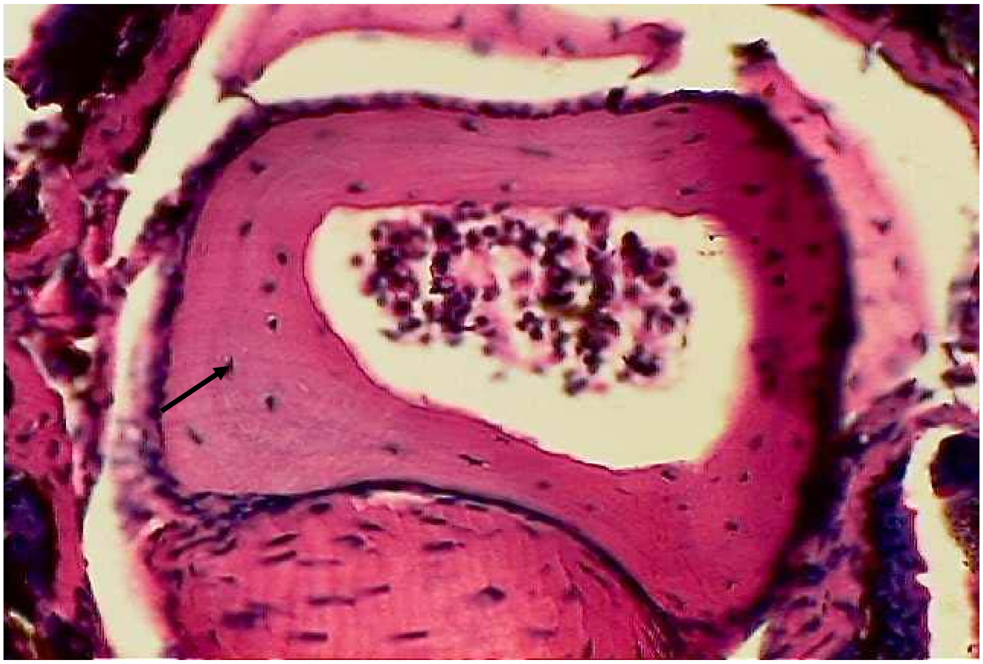


Figure 5-4A. Female *F. limnocharis-iskandari* complex (SVL = 41.5 mm, mass = 5 g), 1 LAG (arrow): scale 40 µm

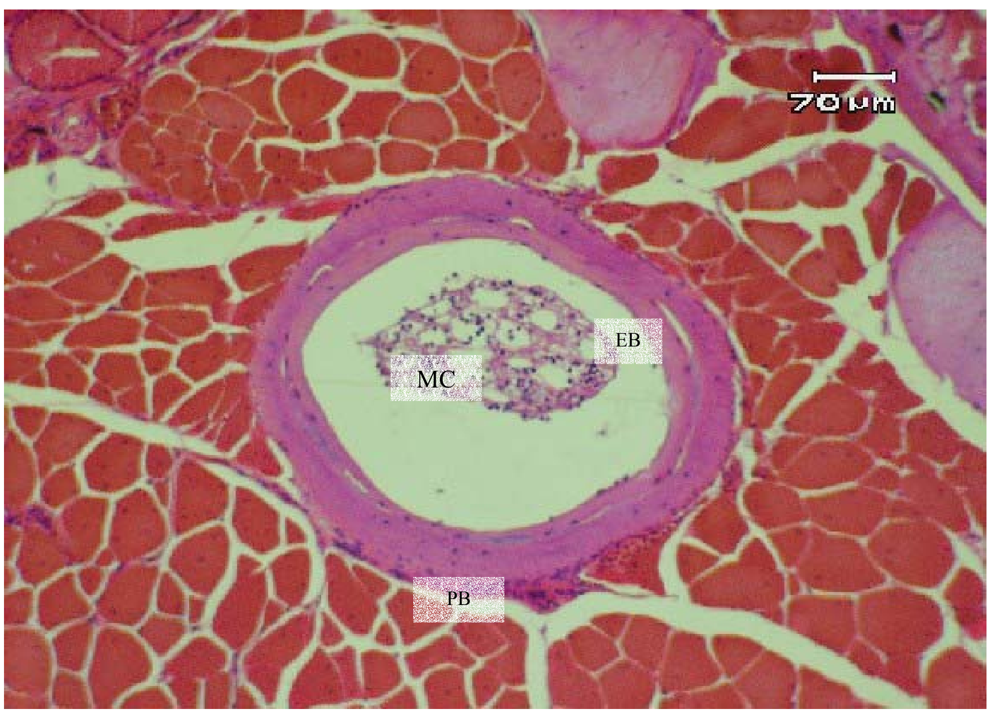
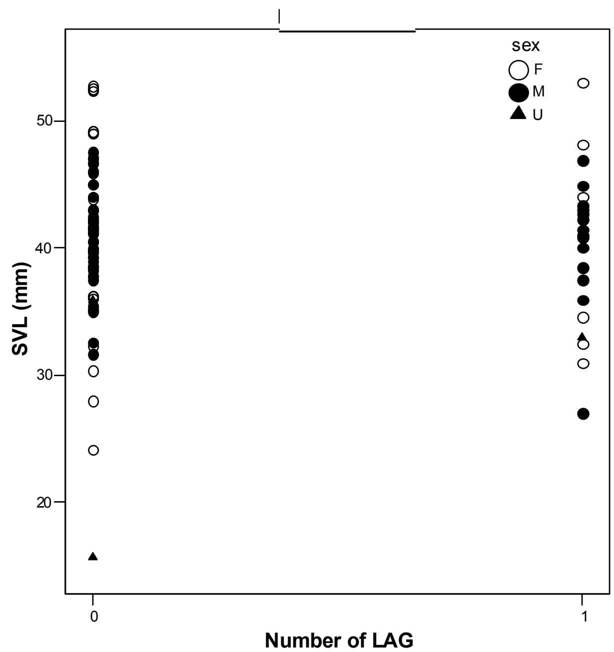


Figure 5-4B. Male *F. limnocharis-iskandari* complex, no LAGs, SVL = 43 mm, mass = 6.4 g. (MC = Marrow cavity; EB = Endosteal Bone; PM = Periosteal Bone)

Figure 5-4C. Female *F. cancrivora* (SVL = 42 mm, mass = 4.6 g); LAG (arrow) = 1

Fejervarya limnocharis-iskandari complex



F. cancrivora

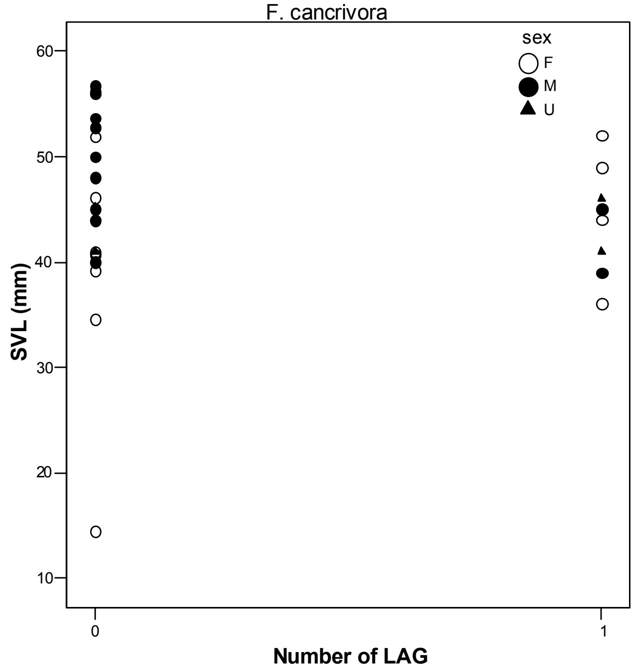


Figure 5-5. Relationships between number of lines of arrested growth and snout vent length for *F. limnocharis-iskandari* complex and *F. cancrivora*. Samples were taken from West Java during September 2001: F = female, M = male and J = juvenile

A. CARINGIN B. DARMAGA

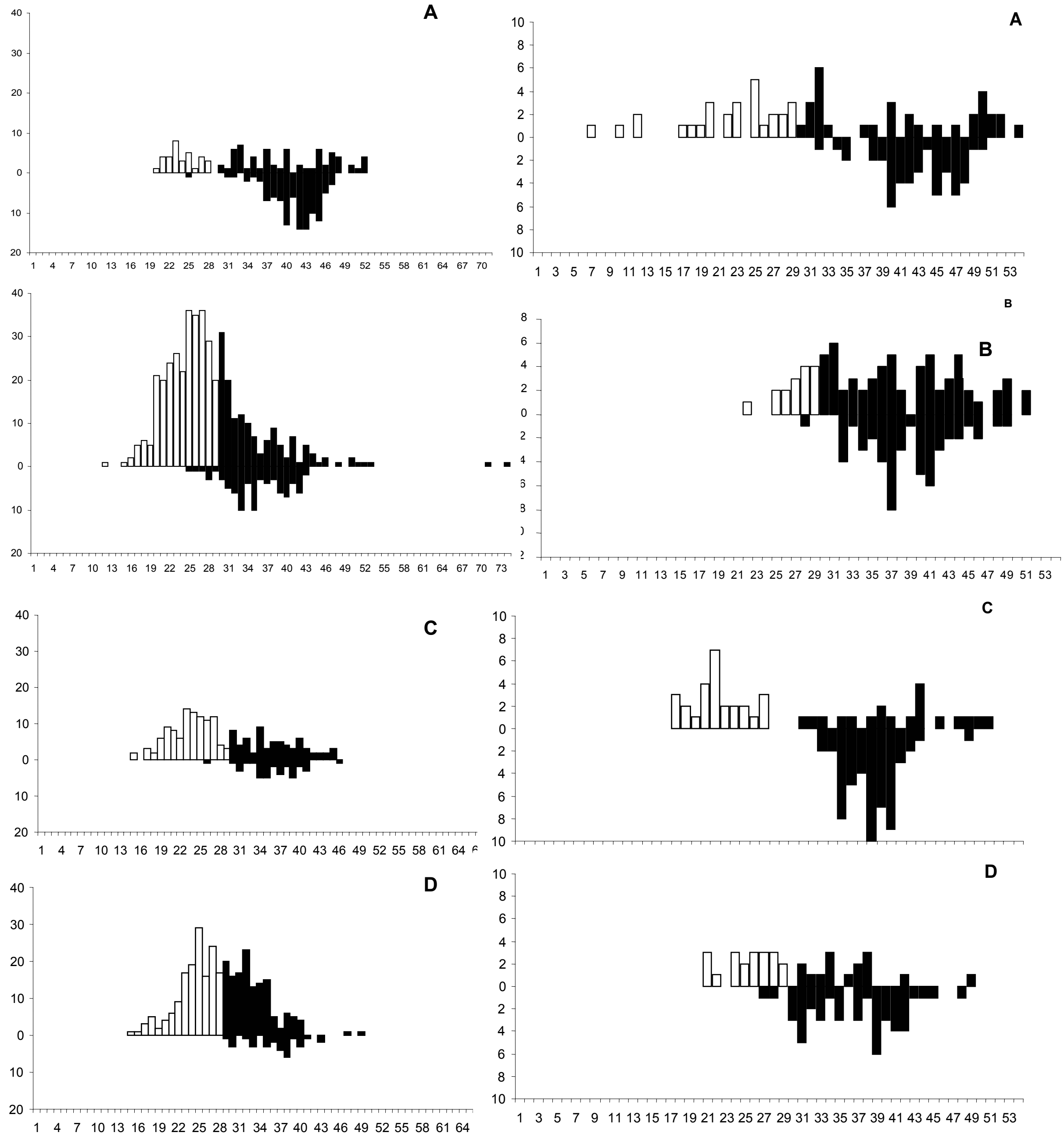


Figure 5-6. Population structure of *F. limnocharis-iskandari* complex in Caringin and Darmaga for four sampling times (2002 and 2003) based on snout vent length (mm) of individuals at first capture in a sampling episode. A to D represent the four sampling episodes (1 to 4, respectively) at each site. The x-axis represents SVL (mm) and the y-axis represents frequency. White bars represent juveniles, black bars above 0 on the y-axis represent females, and black bars below 0 on the y-axis represent males.

A. KARAWANG NP B. KARAWANG P

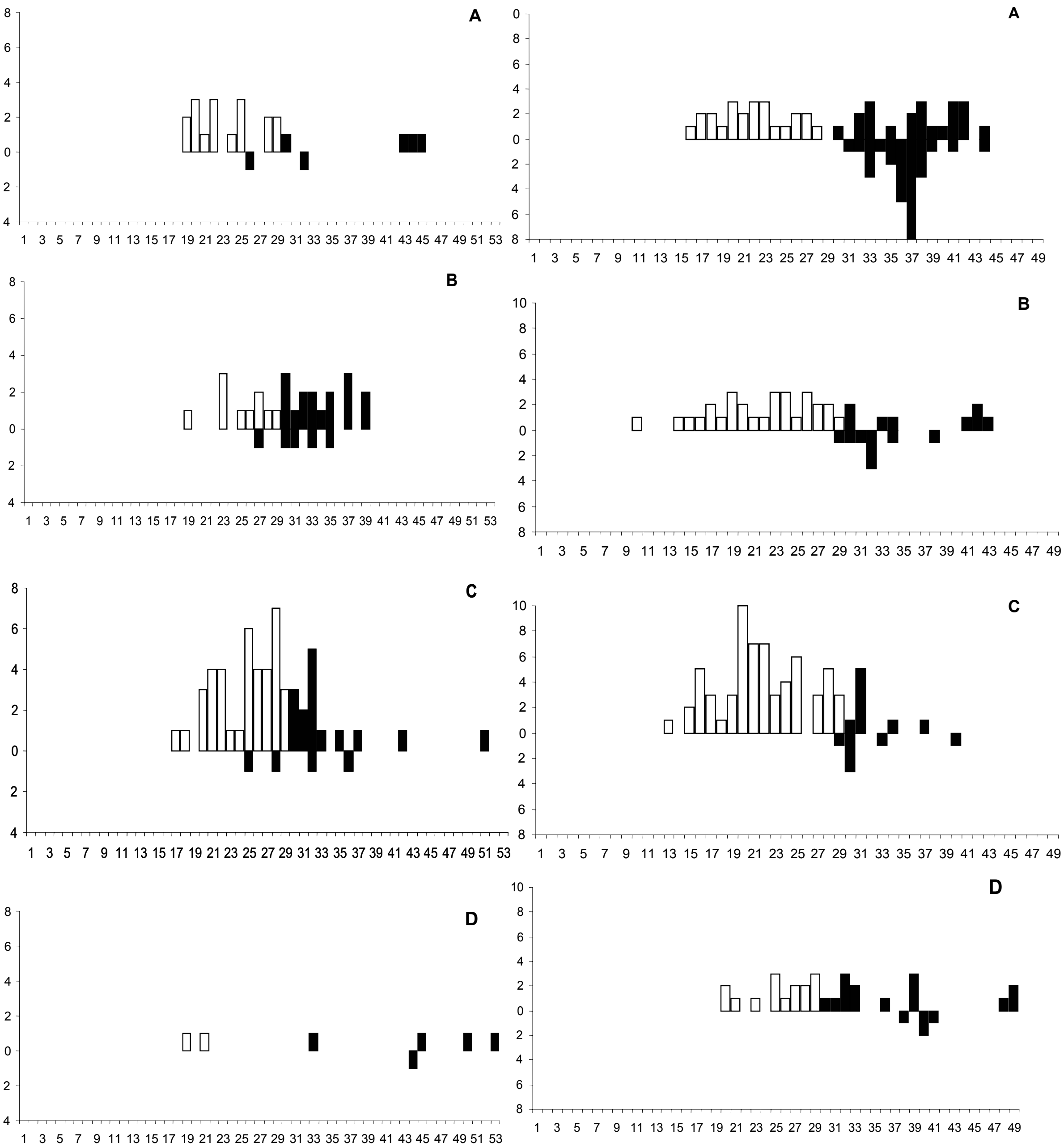


Figure 5-7. Population structure of *F. limnocharis-iskandari* complex in Karawang NP and Karawang P for four sampling times (2002 and 2003) based on snout vent length (mm) of individuals at first capture in a sampling episode. A to D represent the four sampling episodes (1 to 4, respectively) at each site. The x-axis represents SVL (mm) and the y-axis represents frequency. White bars represent juveniles, black bars above 0 on the y-axis represent females, and black bars below 0 on the y-axis represent males.

A. SUBANGNP B. SUBANG P

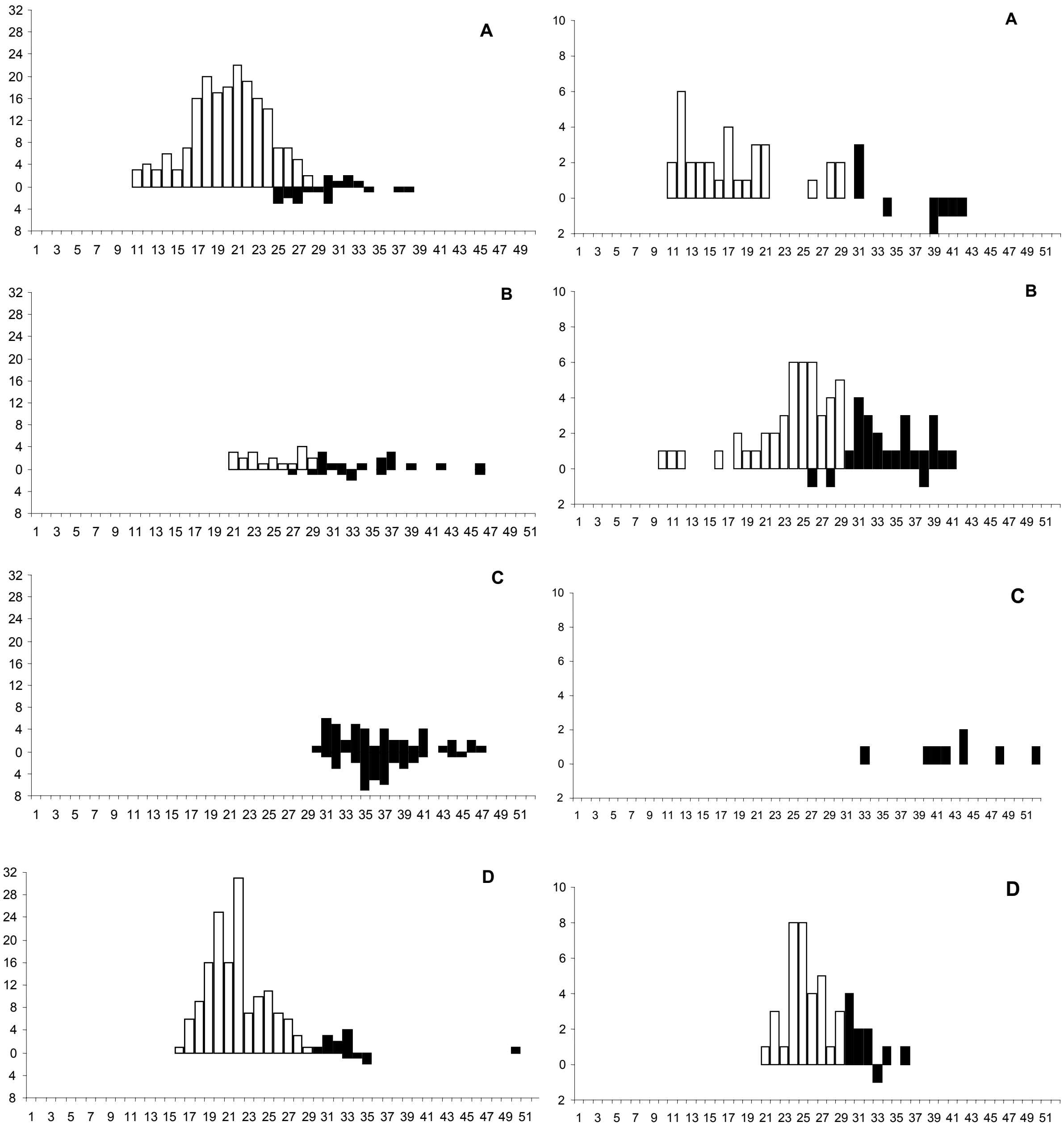


Figure 5-8. Population structure of *F. limnocharis-iskandari* complex in Subang NPand Subang P for four sampling times (2002 and 2003) based on snout vent length (mm) of individuals at first capture in a sampling episode. A to D represent the four sampling episodes (1 to 4, respectively) at each site. The x-axis represents SVL (mm) and the y-axis represents frequency. White bars represent juveniles, black bars above 0 on the y-axis represent females, and black bars below 0 on the y-axis represent males.

A. CARINGIN B. DARMAGA

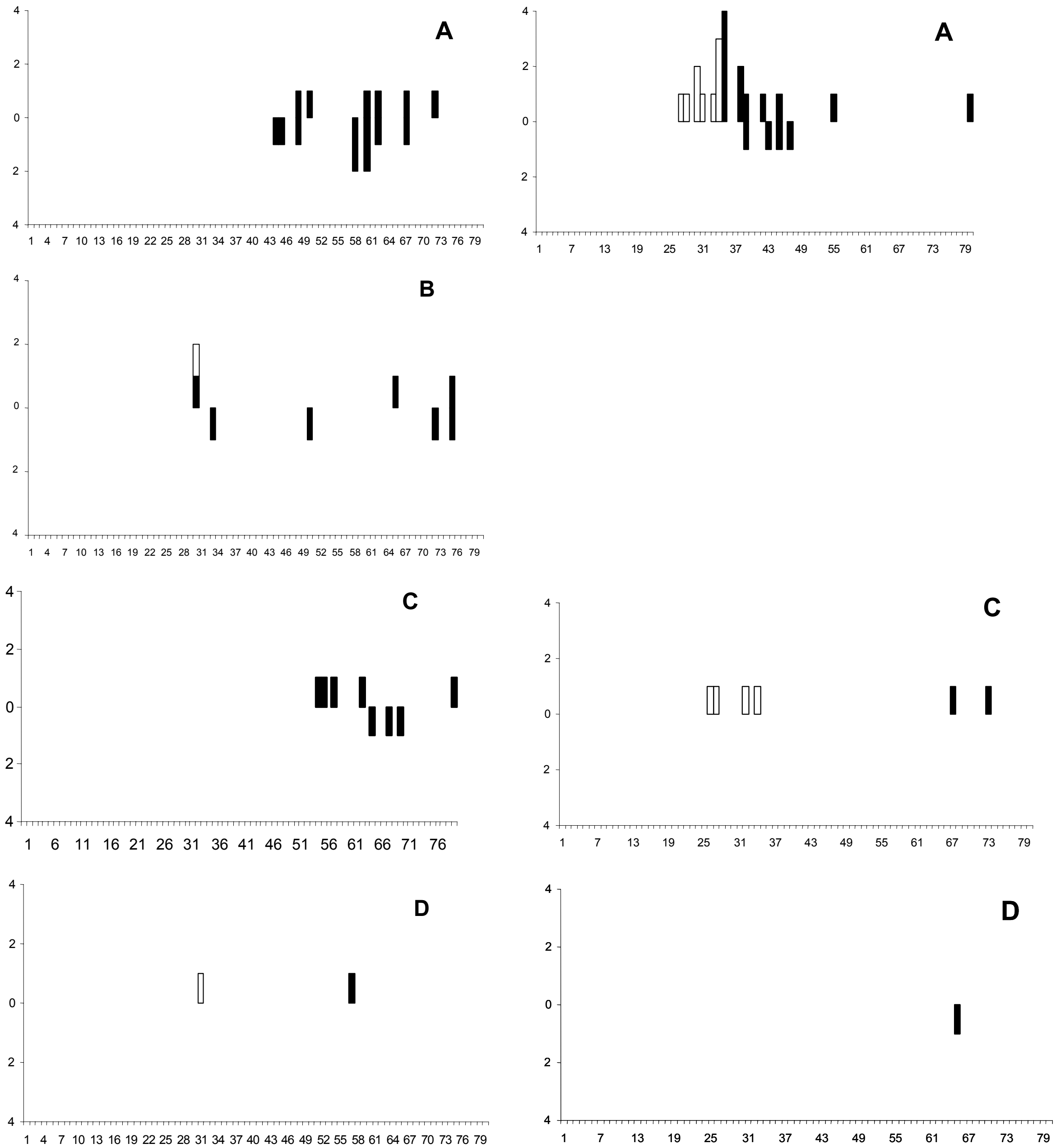


Figure 5-9. Population structure of *F. cancrivora* in Caringin and Darmaga for four sampling times (2002 and 2003) based on snout vent length (mm) of individuals at first capture in a sampling episode. A to D represent the four sampling episodes (1 to 4, respectively) at each site. The x-axis represents SVL (mm) and the y-axis represents frequency. White bars represent juveniles, black bars above 0 on the y-axis represent females, and black bars below 0 on the y-axis represent males.

A. KARAWANG NP B. KARAWANG P

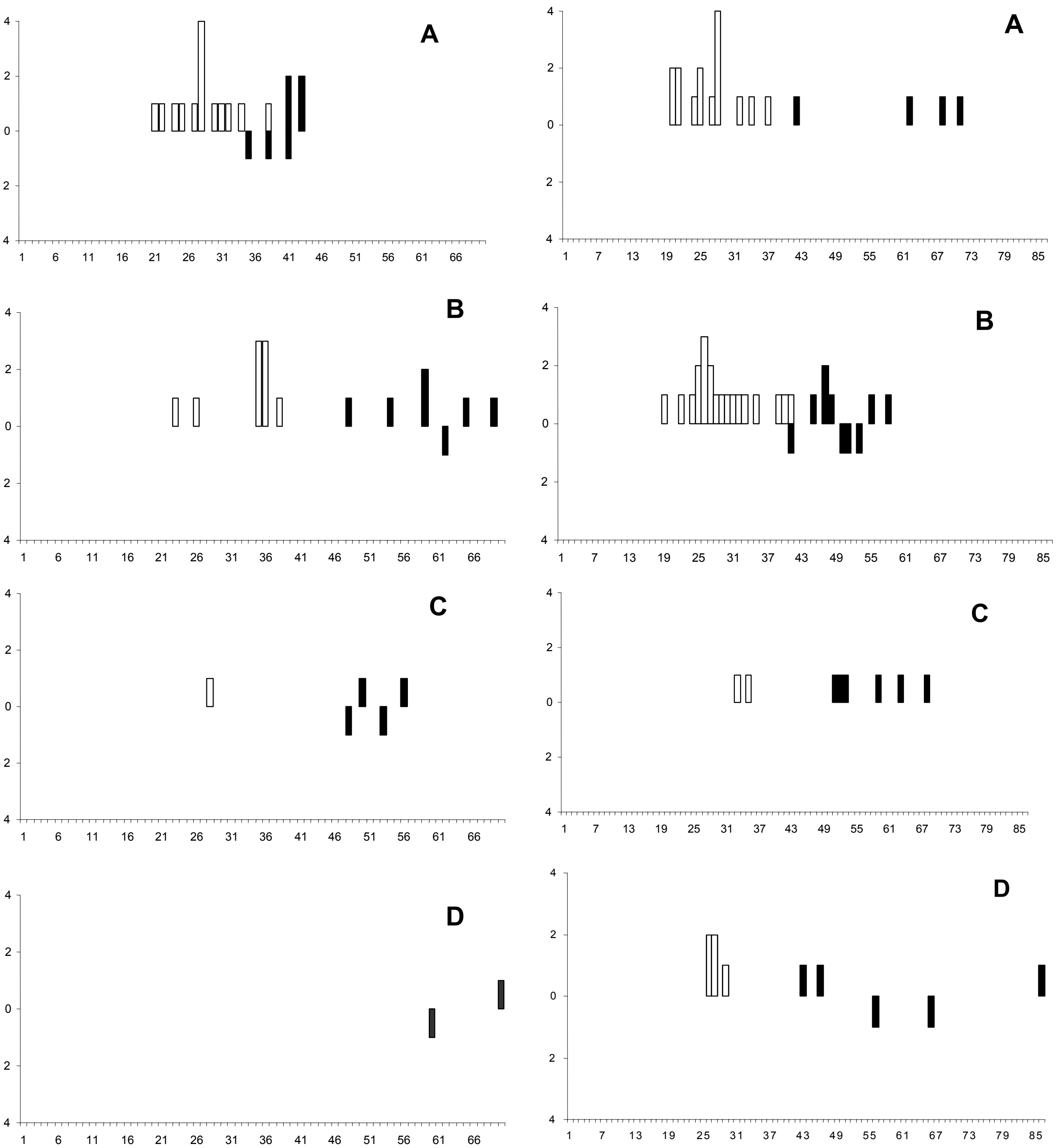


Figure 5-10. Population structure of *F. cancrivora* in Karawang NP and Karawang P for four sampling times (2002 and 2003) based on snout vent length (mm) of individuals at first capture in a sampling episode. A to D represent the four sampling episodes (1 to 4, respectively) at each site. The x-axis represents SVL (mm) and the y-axis represents frequency. White bars represent juveniles, black bars above 0 on the y-axis represent females, and black bars below 0 on the y-axis represent males.

A. SUBANG NP B. SUBANG P

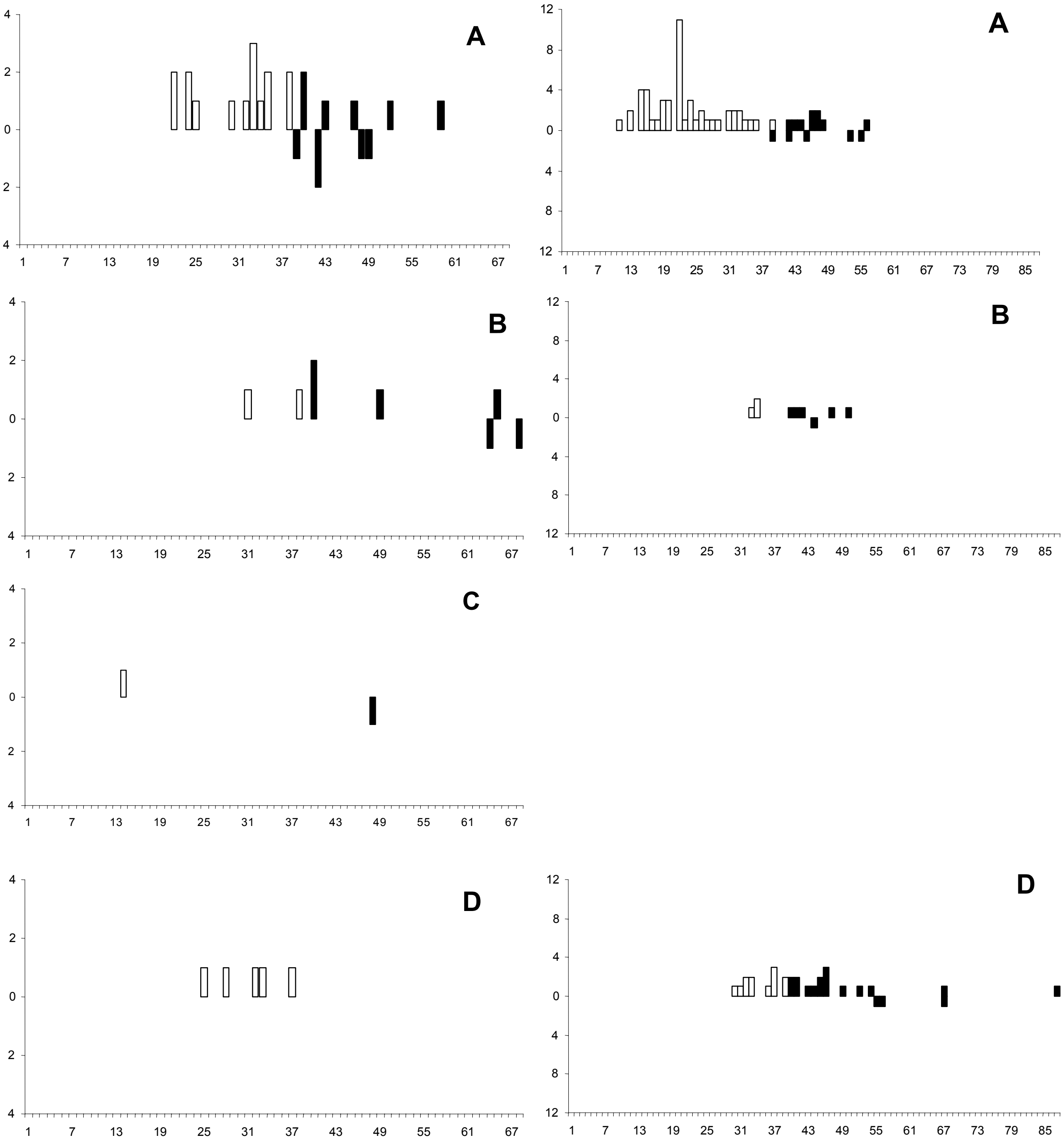


Figure 5-11. Population structure of *F. cancrivora* in Subang NP and Subang P for four sampling times (2002 and 2003) based on snout vent length (mm) of individuals at first capture in a sampling episode. A to D represent the four sampling episodes (1 to 4, respectively) at each site. The x-axis represents SVL (mm) and the y-axis represents frequency. White bars represent juveniles, black bars above 0 on the y-axis represent females, and black bars below 0 on the y-axis represent males.

Linear regressions between total number captured and population estimates calculated using the Schnabel method were carried out for both species. There is a strong correlation between total number captured and estimated population, with r = 0.861 ($F_{1,16}$ = 45.94, P <0.001) for *F. limnocharis-iskandari* complex and r = 0.848 ($F_{1,9}$ = 23.12, P =0.001) for *F. cancrivora*. Therefore, I used the mean density of captured frogs to illustrate and examine differences in density between locations.

The size compositions of frog populations varied among rice fields sampled at different stages of planting and harvest, and may have been affected by water level (Figure 5-12). The mean SVLs of *F. limnocharis-iskandari* complex and F. *cancrivora* differed among stages of rice fields (ANOVA, *F. limnocharis-iskandari* complex: $F_{4,3125}$ = 64.73, P < 0.001; *F. cancrivora*: $F_{4,315}$= 4.80, P <0.005) and water levels (ANOVA, *F. limnocharis-iskandari* complex: $F_{3,3126}$ = 108.16, P <0.01; *F. cancrivora*: $F_{3,316}$ = 4.13, P <0.01). Lower numbers of juveniles were found during the dryer periods that coincide with harvests and fallow periods.

The mean density of *F. limnocharis-iskandari* complex was 242.5 individuals/ha (range = 19.83 – 868.68, SD = 234.19). The mean density of *F. limnocharis-iskandari* complex captured did not differ among locations ($F_{5,18}$ = 2.63, P=0.59).

However, if sites are grouped into regions, with Bogor (Caringin and Darmaga) considered separately from Karawang (Karawang NP and Karawang P) and Subang (Subang NP and Subang P), there is a significant difference in mean density of captured *F. limnocharis-iskandari* complex among these regions ($F_{2,21}$ = 7.52, P < 0.01).

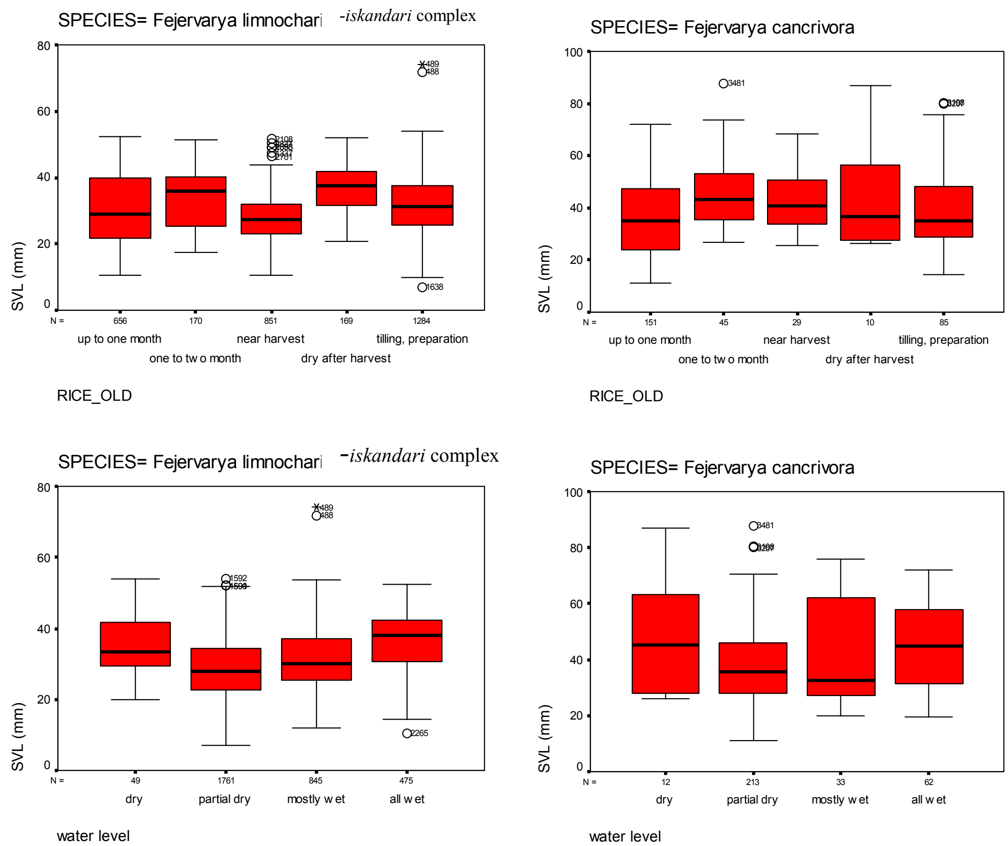
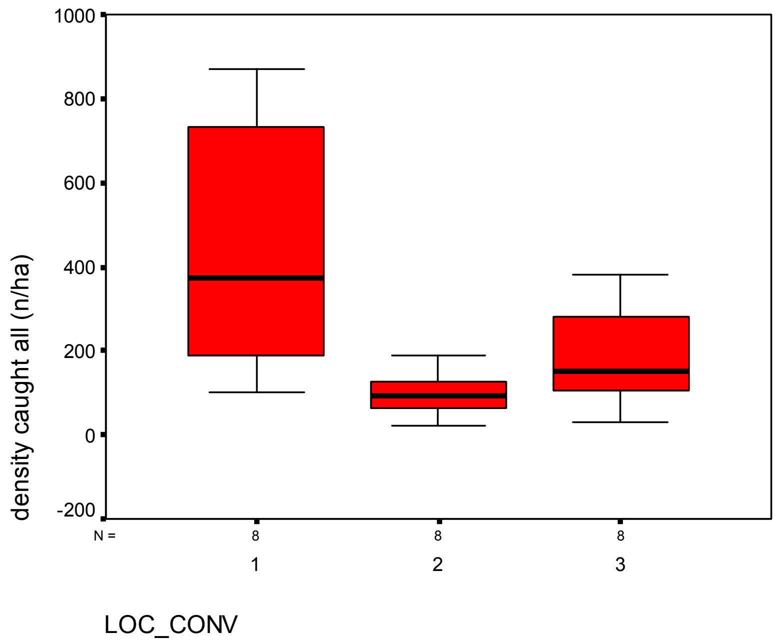


Figure 5-12. Mean size of *F. limnocharis-iskandari* complex and *F. cancrivora* in different stages of planting (top) and water level (bottom)

The mean density of *F. cancrivora* captured was 32.2 individuals/ha (range: 0 – 230.77, SD: 48.94). The mean density of captured *F. cancrivora* (n/ha) did not differ among locations ($F_{5,18}$ = 1.85, P=0.15) or among regions ($F_{2,21}$ = 1.52, P=0.24).

The mean density of *F. limnocharis-iskandari* complex differed among rice fields at different stages ($F_{4,13}$ = 6.75, P <0.005) however it did not differ among fields at different water levels ($F_{3,20}$ = 1.201, P = 0.404). The mean density of *F. cancrivora* did not depend on stage of the rice field ($F_{4,19}$ = 1.17, P=0.35) or on water level ($F_{3,20}$ = 0.623, P = 0.609). Figure 5-13 and 5-14 shows the mean density of both species.



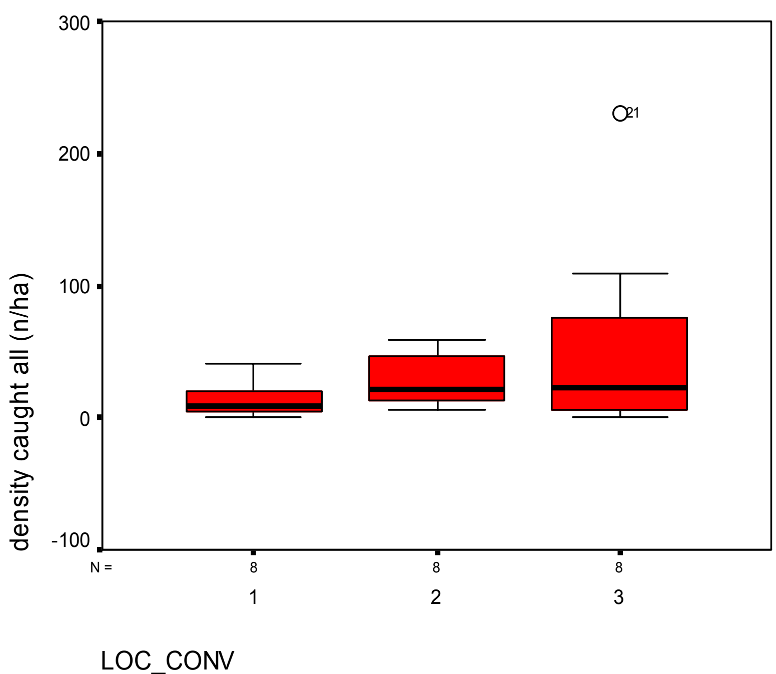


Figure 5-13. Density of *F. limnocharis-iskandari* complex (top) and *F. cancrivora* (bottom) captured in three regions (1 = Bogor, 2 = Karawang; 3 = Subang).

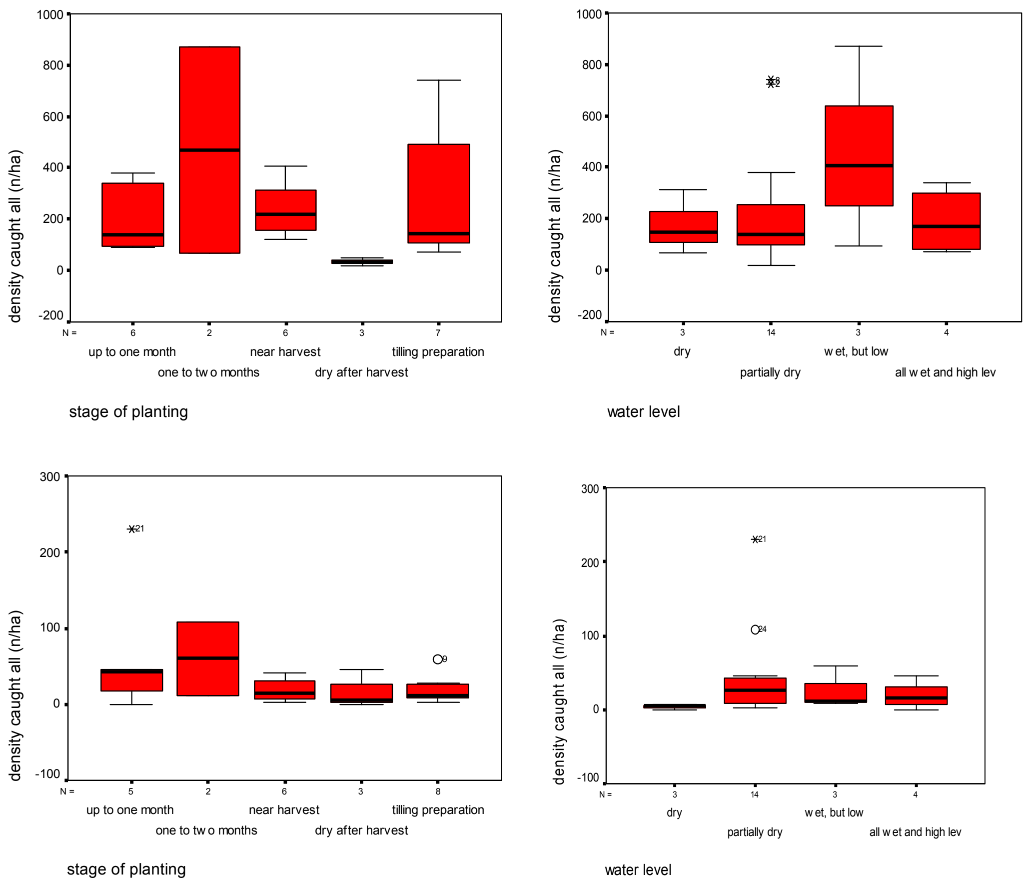


Figure 5-14. Density of *F. limnocharis-iskandari* complex (top) and *F. cancrivora* (bottom) captured in various stages of planting and water level during 2002 and 2003.

5.4.3 Sampling effects

The density of frogs differed between the interior and the borders of rice field blocks; more frogs were found on the edges than in the centre areas (Table 5-5). Mean densities of frogs captured on the edges wre 0.1007 frogs/m^2 (SD = 0.0974, range 0.0030 – 0.2263) for *F. limnocharis-iskandari* complex and 0.0057 frogs/m^2 (SD = 0.0069, range 0.0021 - 0.0160) for *F. cancrivora*. Mean densities of *F. limnocharis-iskandari* complex and *F. cancrivora* captured on the edges did not differ among locations (*F. limnocharis-iskandari* complex*:* $F_{4,5}$ = 1.03, P=0.47; *F. cancrivora*: $F_{3,4}$ = 0.77, P=0.57).

Table 5-5. Densities of frogs captured inside paddy field blocks and on their borders. Numbers in brackets indicate the actual number captured.

Location	Edge size (m2)	Central size (m2)	*F. limnocharis-iskandari* complex		*F. cancrivora*	
			Edge (n/m)	Central (n/m)	Edge (n/m)	Central (n/m)
Caringin	933	8083.00	0.1747 (163)	0.0697 (491)	0.0021 (2)	0.0007 (6)
Darmaga/2003	402.1	1071.40	0.2263 (91)	0.0168 (18)	0.0025 (1)	0 (0)
Karawang Pesticide	437.925	6253.38	0.0183 (8)	0.0042 (26)	0.0160 (7)	0.0005 (3)
Subang Non Pesticide	933	5667.00	0.0815 (76)	0.0034 (19)	0.0021 (2)	0 (0)
Subang Pesticide	675	2185.00	0.0030 (2)	0.0032 (7)		

5.4.4 Population estimates

In total 567 males, 747 females and 1425 juveniles of *F. limnocharis-iskandari* complex, and 47 males, 92 females and 159 juveniles of F. *cancrivora* were marked and released at the six study sites during the period from May 2002 to September 2003. Recapture rates for both species were low; especially for *F. cancrivora* in which no frogs were recaptured more than once (table 5-6). All recaptures occurred within the three-day sampling episodes. No frogs were recaptured across different sampling episodes. It was unusual to get any recaptures when the total number of captured frogs at a site was low (less than about 10). Therefore there were no recaptures from the lowest density populations.

I performed a series of Fisher's Exact Tests, one for each site and sampling period, to determine whether the sex of individuals affected the proportions never recaptured and recaptured at least once. The distribution of recaptures in both species mostly does not appear to differ between sexes (P>0.05) except for *F. limnocharis-iskandari* complex in Caringin and Subang NP (P<0.05). I used one-way ANOVAs to test whether recapture rates differed among stages of rice planting and water levels, I found that the recapture rates of *F.limnocharis-iskandari* complex did not appear to depend on stage of rice planting ($F_{4,67} = 0.65$, P = 0.63) or water level ($F_{3,68} = 0.71$, P = 0.55). There were also no significant differences in mean recapture rates of *F.*

cancrivora with stages of rice planting ($F_{4, 54} = 0.11$, $P = 0.98$) or water level ($F_{3, 55} = 0.51$, $P = 0.68$). Figure 5-15 shows the recapture rate of both species at different stages of rice planting and water level.

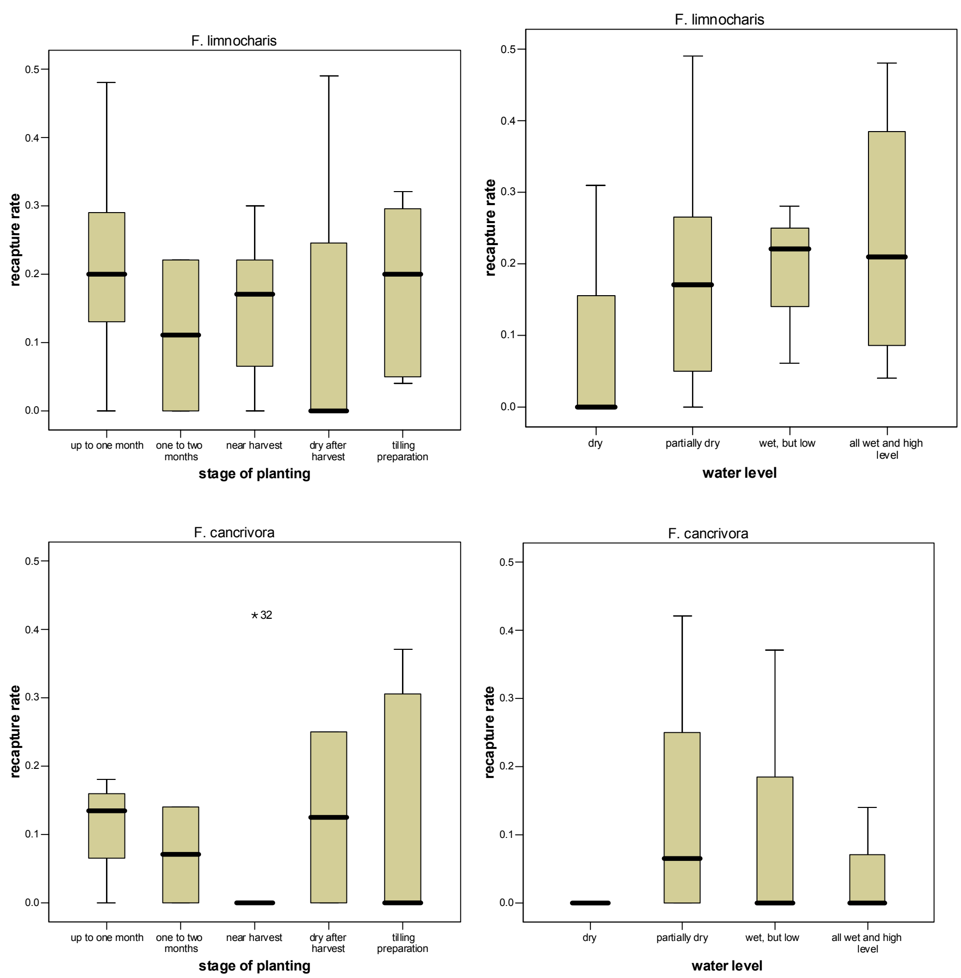


Figure 5-15. Mean rate of recapture for *F. limnocharis-iskandari* complex and *F. cancrivora* in different stage of planting and water level.

The greater effort to maximize capture rates of *F. cancrivora* during the movement sampling carried out in March 2004 yielded recapture rate as high as 0.79 (mean recapture rate: 0.49; range 0.22 – 0.78, SD = 0.25); this is greater than any recapture rates in the earlier population study. The distribution of recaptures of *F. cancivora* in this additional study also did not appear to differ between sexes (P>0.05).

Table 5-6. Range and mean of the two recapture rates (number of recaptures/total frogs captured the day before) estimated for each combination of location and sampling period

Location	Sampling	*F. limnocharis-iskandari* complex		*F. cancrivora*	
		Range	Mean	Range	Mean
Caringin	1	0.44 – 0.52	0.48	0.14 – 0.14	0.14
	2	0.11 – 0.45	0.28	0.00 – 0.00	0.00
	3	0.08 – 0.16	0.12	0.20 – 0.50	0.35
	4	0.12 – 0.23	0.17	0.00 – 0.00	0.00
Darmaga	1	0.25 – 0.30	0.28	0.18 – 0.33	0.26
	2	0.13 – 0.46	0.29	n/a	n/a
	3	0.26 – 0.34	0.30	0.33 – 0.50	0.42
	4	0.00 – 0.62	0.31	0.00 – 0.00	0.00
Karawang NP	1	0.00 – 0.13	0.06	0.08 – 0.67	0.37
	2	0.08 – 0.18	0.13	0.00 – 0.00	0.00
	3	0.17 – 0.21	0.19	0.00 – 0.00	0.00
	4	0.00 – 0.00	0.00	0.00 – 0.00	0.00
Karawang P	1	0.21 – 0.42	0.32	0.00 – 0.00	0.00
	2	0.24 – 0.75	0.49	0.00 – 0.50	0.25
	3	0.22 – 0.23	0.22	0.00 – 0.00	0.00
	4	0.00 – 0.08	0.04	0.00 – 0.00	0.00
Subang NP	1	0.13 – 0.26	0.20	0.12 – 0.14	0.13
	2	0.00 – 0.14	0.07	0.00 – 0.00	0.00
	3	0.19 – 0.30	0.25	0.00 – 0.00	0.00
	4	0.00 – 0.08	0.04	0.00 – 0.00	0.00
Subang P	1	0.00 – 0.13	0.06	0.00 – 0.04	0.18
	2	0.00 – 0.00	0.00	0.00 – 0.00	0.00
	3	0.00 – 0.00	0.00	n/a	n/a
	4	0.00 – 0.00	0.00	0.08 – 0.20	0.14

n/a = not available, no frogs were found during sampling time

The lowest densities of mature *F. limnocharis-iskandari* complex occurred in Karawang NP at sampling times 1 and 4, Subang NP at times 2 and 4, and Subang P at times 3 and 4. The highest density of adults of this species occurred at Darmaga during sampling time 3. The density of females/hectare of *F. limnocharis-iskandari* complex was higher than that of males at most sampling times and sites, except for Caringin time 1 (female: male ratio 0.8:1), Darmaga times 1, 3 and 4 (female: male ratio 0.3:1; 0.3:1 and 0.4:1) and Subang NP time 1 (female: male ratio 0.6:1).

At most sites and sampling times, the density of juvenile *F. limnocharis-iskandari* complex was higher than that of adults, except for Caringin at sampling time

1 (adult: juvenile ratio 2:1), Darmaga at times 2 and 4 (adult: juvenile ratio 11:1 and 2:1) and Karawang at time 2 (no juveniles found). In areas where there were no recaptures (Karawang NP Sampling 2 and 4; Karawang P sampling 1 and 4; Subang NP sampling 2 and 4 and Subang P sampling 3 and 4) juvenile *F. limnocharis-iskandari* complex were always found in low densities (as were adults). Juveniles were found at the highest densities in Darmaga during sampling time 2. Based on mark recapture study, the mean densities of *F. limnocharis-iskandari* complex adults and juveniles were 193.71/ha (range 29.9 – 695.6) and 588.21/ha (range 0 – 16663.7), respectively. Table 5-7 shows the estimated population sizes and densities of *F. limnocharis-iskandari* complex.

The mean densities of *F. cancrivora* adults and juveniles were 39.76/ha (range 0 – 107.14) and 85.27/ha (range 0 - 449.05). At most sites and sampling times, the density of *F. cancrivora* was low, with no recaptures, or this species was absent. Sufficient *F. cancrivora* for population estimates were only found at Caringin at times 1 and 3, Darmaga at times 1 and 3, Karawang NP at time 1, Karawang P at time 2, and Subang P at times 2 and 4.

The highest densities of adult and juvenile *F. cancrivora* were both found in Subang at sampling time 2. The number of juveniles/hectare was higher than the number of adults except for Karawang P at sampling time 2 (adult: juvenile ratio 1.82:1) and Subang P at sampling time 4 (adult: juvenile ratio 1.74:1). Except for Subang P, the density of *F. cancrivora* females/hectare was low compared to that of males. Table 5-8 shows the estimated population sizes and densities of *F. cancrivora.*

Table 5-7. *F. limnocharis-iskandari* complex population estimates based on the Schnabel method.

Site (area m²)	Sampling time	Estimated population size: Male	Female	Juvenile	Adult	F:M ratio	adult density (n/ha)	Juvenile density (n/ha)	Adult: Juvenile ratio
Caringin (9,016)	1	118.13	91.85	99.75	204.52	0.8: 1	226.84	110.64	2.05:1
	2	135.29	321.38	722.18	244.97	2.4:1	271.70	801.00	0.34:1
	3	119.50	161.11	757.20	269.46	1.3:1	298.87	839.84	0.36:1
	4	72.80	263.88	712.88	335.69	3.6:1	372.33	790.68	0.47:1
Darmaga (10,440)	1	74.00	23.38	166.50	108.00	0.3:1	103.45	159.48	0.65:1
	2	66.45	149.25	17.75	206.84	2.2:1	198.12	17.00	11.65:1
Darmaga (1,473.5)	3	92.00	27.17	245.00	102.50	0.3:1	695.62	1662.71	0.42:1
	4	70.88	28.00	39.71	99.00	0.4:1	671.87	269.52	2.49:1
Karawang NP (3,529.7)	1	NA**	NA**	62.00	NA**	NA**	NA**	175.65	NA**
	2	6.00	15.00	NA**	46.00	2.5:1	130.32	NA**	NA**
	3	NA**	17.86	266.00	26.43	NA**	74.87	753.61	0.10:1
	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
Karawang P (6,691.3)	1	44.60	63.00	NA**	101.00	1.4:1	150.94	NA**	NA**
	2	9.29	12.00	48.00	20.00	1.3:1	29.89	71.73	0.42:1
	3	12.00	12.00	162.60	26.67	1.0:1	39.85	243.00	0.16:1
	4	7.00	NA**	NA**	80.00	NA**	119.56	NA**	NA**
Subang NP (6,600)	1	20.20	12.50	516.55	33.00	0.6:1	50.00	782.64	0.06:1
	2	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
	3	40.21	116.86	0.00	106.85	2.9:1	161.89	0.00	NA**
	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
Subang P (2,860)	1	14.00	NA**	317.00	31.00	NA**	108.39	1108.39	0.10:1
	2	3.00	26.77	128.43	30.64	8.9:1	107.14	449.05	0.24:1
	3	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**

Note: NA** stands for not available because there were no recaptures at that site at that time

Table 5-8. *F. cancrivora* population estimates based on the Schnabel method.

Site (area m2)	Sampling time	Population size				F:M ratio	Adult density (n/ha)	Juvenile density (n/ha)	Adult: Juvenile ratio
		Male	Female	Juvenile	Adult				
	1	28.50	8.00	0.00	36.75	0.3: 1	40.76	0.00	NA**
	2	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
Caringin	3	NA**	3.00	NA**	8.00	NA**	8.87	NA**	NA**
(9,016)	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
Darmaga	1	4.00	3.00	42.00	7.50	0.8: 1	7.18	40.23	0.18:1
(10,440)	2	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
Darmaga	3	0.00	1.00	2.00	1.00	NA**	6.79	13.57	0.50:1
(1,473.5)	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
	1	NA**	1.00	52.00	7	NA**	19.83	147.32	0.13:1
Karawang	2	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
NP	3	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
(3,529.7)	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
	1	0.00	0.00	0.00	0.00	NA**	0.00	0.00	NA**
Karawang	2	NA**	21.00	24.67	45.00	NA**	67.25	36.86	1.82:1
P	3	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
(6,691.3)	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
	1	0.00	0.00	0.00	0.00	NA**	0.00	0.00	NA**
Subang	2	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
NP	3	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
(6,600)	4	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
	1	7.00	NA**	NA**	128.00	NA**	NA**	NA**	NA**
	2	3.00	26.77	128.43	30.64	8.9: 1	107.14	449.05	0.24:1
Subang P	3	NA**	NA**	NA**	NA**	NA**	NA**	NA**	NA**
(2,860)	4	0.00	40.00	23.00	40.00	NA**	139.86	80.42	1.74:1
Darmaga (4,592.8)	March 2004	30	44	21.60	67.20	1.3:1	146.32	47.03	3.11:1

Note: NA** means not available since there were no recaptures

5.4.5 F. cancrivora movement

Additional sampling I carried out at Darmaga to examine frog movements resulted in 118 captures of 71 *F. cancrivora,* consisting of 36 females (mean SVL 58.42 mm, range 40.58 – 95.12 mm, SD = 18.94), 16 males (mean SVL 60.79 mm, range 53.57 – 73.82 mm, SD = 5.81) and 19 juveniles (mean SVL 35.81 mm, range 24.52 – 39.61 mm, SD = 3.66. Twenty-nine frogs (42.6%) were captured more than once, with two females recaptured after 5 days, two juveniles recaptured after 4 days, and five frogs recaptured after 3 days (2 females, 2 males and 1 juvenile).

Table 5-9. Summary of capture rates and movement of *F. cancrivora* ($\pm$ SD)

Sex	Capture 1	2	3	4	5	6	Cumulative distance moved (m)	Total dis-placement (m)**	Straightness**
F	18	9	4	0	2	0	5.68 $\pm$ 3.79	4.14 $\pm$ 1.94	0.72 $\pm$ 0.22
M	12	1	3	0	0	0	7.43 $\pm$ 1.59	5.84 $\pm$ 1.52	0.81 $\pm$ 0.11
J	9	7	1	2	0	0	8.63 $\pm$ 11.56	2.11 $\pm$ 1.67	0.67 $\pm$ 0.20

* *Only for frogs captured more than twice; M = male, F = female and J = juvenile

Shapiro-Wilk normality tests indicated that the lengths of daily movements of females and males are not distributed significantly differently from normal (P > 0.1), however, juvenile movement distances were significantly non-normally distributed (P<0.0001). A Kruskal-Wallis test showed that there were no significant differences of cumulative distance moved among classes of individuals (χ^2 = 2.13, P=0.35). A Kruskal-Wallis test also showed that there are no significant differences among classes in the straightness of movement (χ^2 = 0.99, P=0.61).

5.5 Discussion

The mean SVL for females of *F. limnocharis-iskandari* complex in this study was 36.78 mm (range 30 - 74.11 mm) while for males it was 37.96 mm (ranges 25 - 52.50 mm). Premo (1985) reported that the mean snout vent lengths of female *F. limnocharis* from West Java were 52.5 mm (range 41.0-62.5 mm) and of males were 45.6 mm (range 35.5-51.5 mm). In Malaysia, Jaafar (1994) reported mean snout urostyle length of females as 46.8 mm (range 38.0-60.5 mm) and of males as 39.9 mm (range 34.0 – 53.2 mm). Alexander et al. (1979) found that the ranges of snout vent length of mature *F. limnocharis* complex in Taiwan were 30-56 mm (female) and 28-45 mm (male).

Although all authors including Iskandar (1998) reported that the maximum size of female is around 60 mm, my study in Caringin found a female frog with a snout vent lengths of 74.11 mm. During my investigation of the distributions of the study species (chapter 4), the snout-vent lengths of 1001 specimens which was determined by MZB curators as *F. limnocharis* from all over Indonesia stored in the Museum Zoology at Bogor (MZB) were measured; out of which 762 specimens are from Java. The mean snout vent length of female *F. limnocharis* in this Javanese sample was 36.74 mm with a maximum 75.19 mm (specimen ID 3337 captured 9 September 1988 from Cilember, Bogor). The mean snout vent length of male *F. limnocharis* was 37.96 mm with a maximum of 54.52 mm (specimen ID 142 collected in 1930 from Karimun Jawa). On average, females in my samples were smaller than those found in the studies by Premo (1985) and Jaafar (1994), however, my maximum SVLs was greater than the maxima reported by them and also by Alexander et al. (1979). This suggests that the smaller average size of females in my study may reflect poorer growth conditions or higher rates of loss among larger individuals, rather than inherent size differences between the populations in my study and the other studies.

The mean SVL of female *F. cancrivora* in my population study was 53.33 mm (range 40 – 87.86 mm) and for males was 53.37 mm (range 35 – 75.90 mm). Church (1960) gave the range of snout vent length of *F. cancrivora* from Jakarta and Karawang as 64-132 mm (females) and 61-93 mm (males), while Premo gave the mean of male snout vent length as 76.7 mm (range 59.0-87.0 mm) and that of females as 98.6 mm (range 68.0-119.0 mm). According to Jaafaar (1994) the mean snout urostyle length of female *F. cancrivora* in Malaysia was 66.0 mm (range 45.1-104.2) and of males was 61.0 mm (range 40.5-86.2 mm). My measurements of 484 specimens of *F. cancrivora* from all over Indonesia stored in the MZB indicate that the mean snout vent length of females was 62.30 mm (maximum 98.45 mm, specimen ID 2070 captured 6 April 1965 from Bogor Botanical Garden, Bogor) and for males was 61.07 mm (maximum 86.49 mm, specimen ID 477.4 captured 6 December 1954 from Bogor).

The body sizes of frogs depend on several factors such as season, food availability and predation (Duellman and Trueb, 1986). However, in the case of *F. cancrivora*, both Church (1960) and Premo (1985) recorded the size of frogs captured by professional harvesters, and thus their data were probably biased towards larger body sizes, since professional harvesters usually only catch larger frogs. The means and ranges of body size I found in my population samples are substantially smaller than the sizes I found in samples from the catches of professional frog hunters working in Java (Table 3-2); in those samples the mean and range for adult male and female *F. cancrivora* were 65.54 mm (50.20 to 86.20 mm) and 75.10 mm (40.00-162.00 mm), respectively. This differences illustrates the fact that professional hunters concentrate a disproportionate share of harvesting effort on the largest individuals in populations, and also suggests that my population samples may be biased towards smaller, easier-to-catch individuals.

Most previous authors have suggested that both study species exhibit sexual size dimorphism, with females larger than males. In my results for both species, females tended to be slightly smaller than males although the differences in size are not significant. However, the largest individuals of both species found in my study were females. Since all of my sampling areas were subject to extensive hunting by frog harvesters, it is possible that larger frogs, which might mostly be females, were largely absent due to hunting pressures.

There have been no previous efforts to use skeletochronology on Indonesian frogs. Most successful skeletochronological work has been done on temperate species (e.g Halliday and Verrel, 1988; Ryser, 1988; Reaser, 2000). Tropical frogs in North

Queensland (Felton, 1999) and in India (Kumbar and Pancharatna, 2001) also show annual growth patterns in their bones. My skeletochronological results did not indicate that frogs with different numbers of LAGs (only 0 or 1) were different sizes, suggesting that the number of LAGs may not be related to age in these species. The difference between my results and those in other tropical systems may be related to the seasonality of temperature. North Queensland and India have marked temperature differences between dry (winter) and wet (summer) seasons, whereas no marked differences occur in West Java.

The density of frogs differed among locations. Results showed that both *F. limnocharis-iskandari* complex and *F. cancrivora* were found more on the edges than on the central areas of rice blocks. Some of this difference may be caused by sampling bias; it is harder to locate and capture frogs in the central areas of rice fields, particularly during the later stages of the rice planting cycle, when plants are large and dense. This may lead to underestimates of population size, particularly for larger individuals, which are faster and more wary.

Bogor rice fields had the densest frog populations, followed by Subang and Karawang. Most Bogor rice fields depended on surface runoff since rainfall occurs throughout the year. Rice planting can occur as often as three times a year, compared to rice fields in Subang and Karawang which only are planted twice a year. Paddy fields in Subang and Karawang usually rely on irrigation and during fallow periods the area can be very dry. Environmental conditions in Bogor are more suitable for both of the study species and recruitment occurs during most stages of planting as shown in Figure 5-14.

The population structure of both species fluctuated with the stages of rice planting. More smaller frogs were found during tilling and the earlier stages of rice growth, whereas larger frogs were found towards the end of the planting season, especially during harvest and fallow periods which largely coincide with lower water levels and dry periods. It appears that the timing of recruitment coincides with water level, in accordance with Jaafar et al. (1999) findings in Malaysian rice fields.

Although the breeding patterns of *F. limnocharis-iskandari* complex and *F. cancrivora* seemed correlated with the rice planting stage, the lack of distinct annual LAGs in the skeletochronological data suggests that both species grow throughout the year. It is also possible that these frogs do not live long. If paddy field frogs in West Java grow on average according to Jaafar's (1994) equations for growth of F. *limnocharis* and *F. cancrivora* in Malaysia, the ages of F. *limnocharis-iskandari* complex and *F. cancrivora* in this sample would range from 1 week to 21 months (1.9 years) (*F. limnocharis-iskandari* complex) and 1 week to 1 year (*F. cancrivora*). Given the absence of larger *F. cancrivora* in my samples, which suggests that older individuals are absent from my populations, it seems that the populations of *F. cancrivora* I examined tend to be biased towards young mature frogs. Since *F. cancrivora* is more heavily exploited than *F. limnocharis-iskandari* complex the biased population size distribution of *F. cancrivora* suggests that harvesting may be at or near maximum sustainable rates. If almost all older individuals are removed from the populations by harvesting, it is likely that the growth rates of the populations are being negatively affected.

Most previous works on rice field frog populations has focused on their breeding biology (Church, 1960; Alexander et al., 1963 and 1979; Premo, 1985; Jaafar et al., 1999) and has not mentioned the size of the area sampled when reporting the numbers of frogs captured, thus providing no information on the density of frogs. Two studies have provided enough information to allow comparisons with my data. Jaafar (1994) and Dash and Mahanta (1993) both collected frogs for three hours per sampling night with 3-4 persons in their field parties; their daily sampling effort is thus 9 – 12 man-hours, similar to that in my study. Both of these studies also sampled frogs over several months, including more than one season. I can therefore compare my results with those obtained by these authors. Tables 5-10 and 5-11 compares the densities of *F. limnocharis-iskandari* complex and *F. cancrivora* estimated in my study with estimates by Dash and Mahanta (1993) for India and Jaafar (1994) for Malaysia. It is clear that the density of *F. limnocharis-iskandari* complex in Java is higher than the density of *F. limnocharis* in Malaysia and India. However, the mean estimated density of adult *F. cancrivora* in my study is similar to Jaafar's (1994) results. The mean density of *F. cancrivora* (39.76 adult individual/ha) in Java was much higher than the mean density (0.7 frogs/ha) found for *Rana tigerina*, the larger species of Indian paddy field frog investigated by Dash and Mahanta (1993). These comparisons suggest that densities of *F. cancrivora* in Javan rice fields are relatively high, despite ongoing harvesting pressure.

Table 5-10. Comparison of density (frogs/ha) of *F. limnocharis-iskandari* complex in Java estimated in my study using mark-recapture data with estimates from Dash and Mahanta (1993) in India and Jaafar (1994) in Malaysia for *F. limnocharis*

	Java	India	Malaysia
Adult	193.71 (range: 29.9 – 695.6)	n/a	Range 6.2 - 14
Juvenile	588.21 (range: 0 – 1663.7)	n/a	n/a
Overall	n/a	39.1	n/a

Table 5-11. Comparison of density (frogs/ha) of *F. cancrivora* in Java estimated in my study using mark-recapture data with estimates from Dash and Mahanta (1993) in India and Jaafar (1994) in Malaysia

	Java	India (*Rana tigerina*)	Malaysia
Adult	39.76/ha (range 0 – 107.14/ha)	n/a	8.6 to 91.2/ha
Juvenile	85.27/ha (range 0 - 449.05)	n/a	n/a
Overall	n/a	0.7/ha	n/a

Some studies of stream-dwelling frogs have shown that these species have site fidelity (Tessier et al., 1991; Kam and Chen 2000). Other studies have shown that some species migrate seasonally for breeding (Dole and Durant, 1974; Beshkov and Jameson, 1984), and others lack philopatry and move nomadically (Schwarzkopf and Alford, 2002). In my study many individuals were captured only once and I never captured frogs marked from previous sampling occasions, even when these were only one month apart. The environment in rice fields fluctuates frequently in terms of water level and cover (rice plants). Rice in Java is usually planted in large aggregations of rice fields, consisting of hundreds or sometimes thousands of hectares of individual paddy fields owned by different farmers. Each aggregation of rice fields often includes fields in different stages of plant development and water level. Frogs are likely to move between fields to avoid unfavourable environments, such as dry habitats. Most of the lower capture rates in my study occurred during fallow periods, in which the soil in dry fields in areas such as Karawang and Subang was so dry that it cracked. My low recapture rates, even over relatively short time periods with relatively high sampling effort, suggest that both *F. limnocharis-iskandari* complex and *F. cancrivora* may behave similarly to *Bufo marinus* in Australia, which are nomadic (Schwarzkopf and Alford, 2002). They may remain in an area of habitat while conditions are favourable, but move on when they become unfavourable, and not return.

F. cancrivora is the most commonly harvested frog in Java. The low numbers of *F. cancrivora* captured and recaptured in all six paddy fields could suggest that population sizes of this species are low. However, the densities I estimated when mark-recapture calculations were possible were similar to those found by Jaafar (1994) for unharvested Malaysian populations. In my movement study, which involved more intensive sampling effort over a 6-day period, I captured greater numbers of frogs, suggesting that some of the lower counts I obtained were underestimates of density caused by low catchability of frogs. The results of the movement aspect of this study showed that *F. cancrivora* tend to move in relatively straight lines, suggesting that individuals may frequently disperse between rice fields. This must account for the absence of long-term recaptures, which could not be caused entirely by mortality, as that would require that most adults die between each monthly sample, which would lead to rapid extinction of populations. The relatively small sizes of adults I captured, when compared to the same species in other regions and to samples obtained from frog hunters, suggests that harvesting may be removing a large proportion of the larger adults from the populations, which could lead to reduced rates of recruitment. This may also reflect in part lower catchability of larger frogs, which are probably captured more efficiently by frog hunters than by biologists. Although I obtained sufficient data to allow initial evaluations of the status of populations of *F. cancrivora* and *F. limnocharis-iskandari* complex, additional work will be needed to fully understand the dynamics of these species.

CHAPTER 6

THE POPULATION BIOLOGY OF *LIMNONECTES MACRODON*

6.1 Introduction

The Giant Javanese frog, *Limnonectes macrodon,* is commonly harvested for human consumption in Indonesia, especially in West Java (Chapter 3). This frog is a member of the ranid fanged frogs, which inhabit tropical and subtropical Asia, and is unique among anurans in that the males do not have the usual secondary sexual characteristics such as nuptial pads and voice (Inger, 1969). Known previously as *Rana macrodon*, the taxonomic status of this species has undergone several revisions and it has been divided into several other species, such as *Limnonectes blythii, L. malesianus, L. leporinus, L. ingeri* and *L. kadarsani* (Iskandar, 1998). A pioneer study by Sugiri in 1979 provided information on the basic biology of the *Rana blythii* complex in West Java, which is now known as *Limnonectes macrodon* (Iskandar, pers. comm., see Chapter 4). As with most Indonesian frogs, although it is common very little is known of its ecology, life history, or behavioural traits. This makes it difficult to assess the impact of harvesting on this species.

The objective of this study was to assess the population biology of *Limnonectes macrodon* at two streams in West Java: Cilember and Ciapus Leutik. Additional data on the biology of the species was taken from frogs captured by professional hunters.

6.2 Methods

6.2.1 Location

A single 400 m transect was established at both the Cilember (~30 km south of Bogor) and the Ciapus Leutik (~10 km west of Bogor) streams. Maps and detailed site descriptions appear in Chapter 4. The Ciapus Leutik stream is part of the Mount Salak drainage, whereas Cilember is part of the Mount Paseban drainage. Both locations are subject to heavy rainfall and are at the foot of mountainous areas. The Ciapus Leutik stream lies on more gently sloping land than the Cilember stream. The beds of both streams are largely composed of rocks and boulders. Neither stream dries completely, however, during the dry season several parts of the Ciapus Leutik stream dry up to small pools. A summary description of the study sites can be found in Table 6-1 and the appearance of both streams is depicted in Figure 6-1.

Table 6-1. Summary descriptions of study sites

	Cilember	Ciapus Leutik
Situation	Varied slope (range 1- 84^o)	Gentle slope (range 1 – 14^o)
Stream width (max)	6.7 m	9.4 m
Flooding regime	Regular flooding, during dry season (3months) only half of the stream bed is inundated	Infrequent flooding, most of the time only half of the stream bed is inundated
Substrata	Rock and sand	Rock, sand and clay
Canopy cover	Mostly closed canopy	Mostly open canopy
Other frog species present	11 species: *Bufo asper* *B. melanostictus* *Megophrys montana* *Huia masonii* *Limnonectes kuhlii* *L. microdiscus* *Rana chalconota* *R. hosii* *Nyctixalus margaritifer* *Philautus aurifasciatus* *Rhacophorus javanus*	10 species: *Bufo asper* *B. melanostictus* *Huia masonii* *Limnonectes kuhlii* *L. microdiscus* *Rana chalconota* *R. hosii* *Rana erythraea* *Leptobrachium* hasseltii *Fejervarya limnocharis-iskandari* complex

Both streams are located within forested areas of the Perum Perhutani Forest Production Unit. However, since the Cilember stream is located within a Natural Recreation Area, the area adjacent to the Cilember stream is more densely forested than that around the Ciapus Leutik stream; the vegetation is mostly composed of natural forest mixed with plantation timber such as *Calliandra calothyrsus, Pinus merkusii* and *Agathis dammara*, and undergrowth such as *Brugmansia suaveolens*, *Ficus sp., Altingia* *excelsa, Achasma megalochilos*, *Selaginella plana*, *Artocarpus heterophyllus*, *Melastoma malabathricum*, *Acacia* sp., *Daemonorops rubra* and bamboo (*Bambusa* sp.).

Figure 6-1. The Ciapus Leutik stream (left) and the Cilember stream (right)

The Cilember stream lies between 662 and 840 m above sea level (asl). It has a steep gradient and is interrupted by seven waterfalls ranging in height from one metre to more than 15 m high. In the Cilember stream transect the first 200 m lies mostly near the lower elevation (662 m a.s.l.), with a shallow gradient. A 15 m high waterfall divides this from the next 100 m. This is followed by a 5 m high waterfall and the final 100 m of the transect. The Cilember stream eventually flows into the Ciliwung River and flows to the city of Bogor.

The elevation of the Ciapus Leutik stream is between 709 and 717 m. As with the Cilember stream, the lower 200 m of the transect is divided from the upper 200 m by a small waterfall, around 3 m high. Half of the area along the transect in the Ciapus Leutik stream is within a forest maintained for harvest by the nearby villages (a community forest). As such, the vegetation is mainly *Pinus merkusii* with undergrowth of a vegetable plant *Buchanaria arborescens.* Other vegetation found in the area includes timber and crops such as *Piper aduncum, Bambusa* sp., *Eupatorium odorata,*, *Tetraria* sp., *Calliandra calothyrsus*, *Amomum* sp., *Selaginella plana*, *Paraserianthes falcataria*, *Mesopsis eminii*, *Ageratum conyzoides*, *Brugmansia suaveolens*, *Vitis* sp., and *Lantana cammara*, planted by inhabitants from the nearby village. As with the Cilember stream, the water from the Ciapus Leutik stream flows to Bogor.

Access to the area inside the Cilember Natural Recreation Unit is restricted to visitors and Perum Perhutani (Perusahaan Umum Perhutanan Indonesia or Indonesian Forest Product Company) officers, whereas access to Ciapus Leutik is open. Figure 6-1 shows the condition of the Cilember and Ciapus Leutik streams.

6.2.2 Sampling

Two days of sampling were conducted at each site during each month from June 2002 to May 2003, inclusive (a continuous 12 month sampling period); additional sampling was carried out in January, April and July 2004. Sampling began during the evening, starting around 19.30, and continued until around 01.00 the next morning. At least 2 people took part in each survey. A thorough search was made for frogs along the entire length of each transect and within 1 m of each side of the streams for a total of 683-man hours for all samples, or around 11-man hours per sampling day. For each frog captured we recorded snout vent length (SVL), weight, microhabitat, position along the transect with reference to markers along the transect, the activity of each individual captured at the time it was first observed, and also measurements of environmental conditions (air and water temperatures, and relative humidity). Each frog was uniquely marked based on the numbering system of Hero (1989) and then released. To minimize the impact of toe clipping (Chapter 5) I excluded clipping the thumb and tried to clip no more than two toes per animal.

Limnonectes macrodon is a shy animal and hard to catch. Because the frog is large and has a distinctive posture that differentiates it from other large stream species (for instance *Bufo asper*), it is possible to identify this species without actually catching it. For each *L. macrodon* observed but not captured, I noted the time and position where it was sighted.

There are almost no morphological differences between the sexes of *L. macrodon*. Unlike most frogs, *L. macrodon* males do not have vocal sacs, nuptial pads, or other obvious secondary sexual characteristics. Based on Sugiri (1979) and Emerson and Inger (1992), male and female fanged frogs can be differentiated by the occurrence of a fang-like bone in the jaw (an extension of the lower mandible). Males have longer protrusions in the mandible, which look like fangs, whilst females have shorter and blunter ones. We used the relative lengths of the "fangs" to determine the sex of *L. macrodon* in the field. To check the reliability of this method, 69 *L. macrodon* were bought from harvesters which included 49 females (SVL range 67.44-118.50 mm) and 20 males (SVL range 56.34-94.42) (see table 6-5). The sexes of the frogs, based on the appearance of the fangs, were noted separately by two research assistants and myself. The frogs were then dissected to definitively determine their sex from their gonads. Unfortunately, this indicated that determining gender using fangs was inaccurate; approximately 36% of the animals assigned to either gender actually belonged to the opposite gender (35.8% of "females" were males and 36.6% of "males" were females). Therefore, data were not analysed separately by sex; all adult frogs were pooled, and analysed separately from juveniles. In addition, I also noted mass, SVL, and mass of fat of each individual dissected, and if female, the reproductive stage based on its mass of eggs. I categorised reproductive stage into three groups: non reproductive (no ovaries found), early gravid stage (ovaries 0.1-9.99 g), gravid (ovaries more than 10 g).

Toe clips from the mark-recapture study were stored in 4% formalin solution for skeletochronological analysis in the lab. Forty-six samples were analysed: seven samples from preliminary surveys in 2001 and 2002 (at Ciptarasa, Panguyangan and Cinagara), 25 samples from Cilember, and 14 samples from Ciapus Leutik. Each toe clip was processed for histological examination using the method presented in detail in Chapter 5. Since each frog was numbered uniquely, phalanges used for skeletochronological analysis were taken from different toes. Due to staining problems (sections too dark or/and curled), which made interpretation difficult, sections from one *L. macrodon* were not included in the analysis. To gain more accuracy in determining the number of lines of arrested growth (LAGs) in the histological sections, I checked each section with two other people.

6.2.3 Data analysis

All data sets were analysed using Microsoft Excel and SPSS (version 12.0.1, 2003). Mass and snout vent length were analysed using descriptive statistics. Analysis of variance (ANOVA) was used to determine whether mean snout vent length (SVL) and mean mass differed between locations. Correlations between mass and snout vent length use log transformed data.

It is not possible to assume that the populations were closed during the study period. I therefore used the Jolly Seber method for open populations to estimate the population number using Program Jolly (version 6.0), which is included in the STAT-

METH program supplement for Krebs (1999). The assumptions of the Jolly Seber method are:

1. Every individual has the same probability of being caught in the t^{th} sample, whether it is marked or unmarked.
2. Every marked individual has the same probability of surviving from the t^{th} to the $(t+1)^{st}$ sample.
3. Marks persist during the study and marks are not overlooked at capture.
4. Sampling is instantaneous and population size does not change during sampling events

Populations were estimated in two ways: separate mark recapture estimates for each month's data and an overall analysis in which I lumped each three consecutive months of data as a single sample. Note that with the Jolly Seber method, initial and final population sizes cannot be estimated, so the results only show population estimates from July 2002 onward, or, for the three-monthly estimates, the second three-monthly period.

Movement distances of individual *L. macrodon* were obtained by calculating the distance between capture points. Observations of frogs' movement were analysed using three parameters:

- Cumulative distance moved per month
- Displacement per month
- Straightness of movement path.

Straightness is the ratio of displacement to cumulative distance moved. A value of near one means that movements were nearly along a straight line, while a value close to zero indicates that movements tend to return to the starting point.

Capture Efficiency (CE) for each sampling occasion was determined by dividing the number of frogs observed by the number of frogs caught. The equation is given below.

$$CE = N_{caught} / N_{total\ (caught\ +\ observed)}$$

6.3. Results

6.3.1 Microclimate

The Cilember and Ciapus Leutik streams were typical of tropical streams, with relative humidity (RH) ranging from 75 to 100 %. The temperature in the Ciapus Leutik stream was approximately 2°C warmer than in the Cilember. The Cilember stream is nearest to Citeko Climate Station while the Ciapus Leutik stream is nearest to Pondok Gedeh Climate Station, both in Bogor (Table 6-2). Total rainfall during June 2002 to May 2003 was 2370 mm and 3025 mm for Cilember and Ciapus Leutik, respectively. The lowest rainfall occurred during September 2002 (23 mm) for Cilember and June 2002 (85 mm) for Ciapus Leutik. The highest rainfall occurred during February 2003 (582 mm) for Cilember and December 2002 (598 mm) for Ciapus Leutik. Figure 6-2 illustrates the mean RH, air and water temperature during the 15 sampling occasions.

Table 6-2. Microclimates at the Cilember and Ciapus Leutik stream sites.

Location	Microclimate	Range	Mean ± SD
Cilember	Air temp (°C)	15.6 - 21.6	18.87 ± 1.24
	Water temp (°C)	16.4 - 21.0	18.87 ± 0.93
	Relative Humidity (%)	75 - 100	95.22 ± 4.86
	Monthly rainfall (mm)*	23 - 582	197.83 ± 145.90
Ciapus Leutik	Air temp (°C)	18.0 – 22.8	20.32 ± 1.18
	Water temp (°C)	19.0 – 24.0	21.63 ± 1.16
	Relative Humidity (%)	83 - 100	94.9 ± 4.66
	Monthly rainfall (mm)*	85 - 598	252.08 ± 156.98

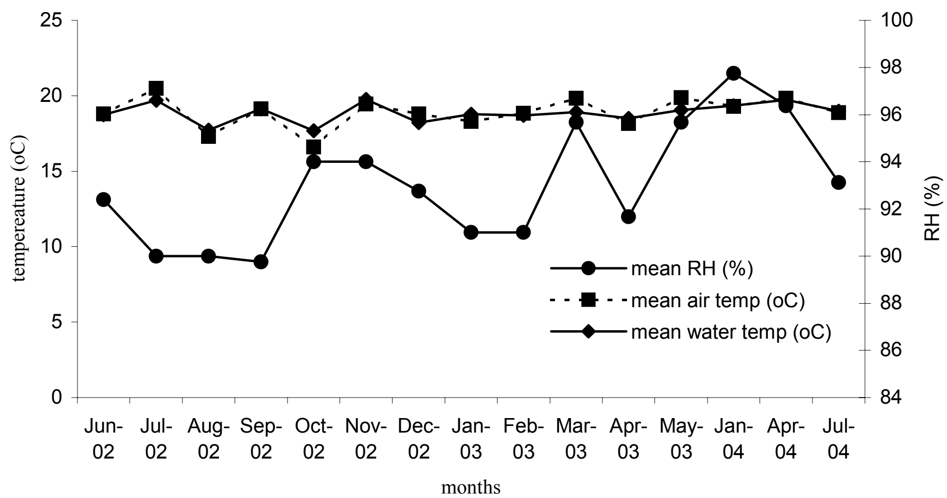
* Data for June 2002-May 2003 were obtained from Climate Stations in Citeko (for Cilember) and Pondok Gedeh (for Ciapus Leutik) from the Badan Meteorologi dan Geofisika, Balai Wilayah III

6.3.2 Captures

A total of 42 individual *L. macrodon* were captured a total of 64 times (including recaptures) at the Cilember stream. Almost three quarters (n = 30) were captured once, six were captured twice, three were captured three times, two were captured four times and one was captured five times (Figure 6-3). Thirty-four frogs were observed but not captured. Mean capture efficiency ± SE in the Cilember stream was 0.71 ± 0.06. The greatest numbers were observed in November 2002 (n = 19), December 2002 (n = 17) and February 2003 (n = 13). The highest density of frogs observed in a 100 m segment of the transect was 7 individuals on 26 December 2002. No frogs were observed during August and October 2002, and July 2004 (Figure 6-4).

Fewer frogs were observed and captured at the Ciapus Leutik stream. A total of 20 individuals were captured, 15 of them once, four twice, and one three times (Figure 6-5), for a total of 26 capture occasions. Eighteen frogs were observed but not captured. Mean capture efficiency ± SE at Ciapus Leutik was 0.59 ± 0.1. The greatest number of frogs were observed in April 2003 (n = 6), February 2003, and July 2004 (both n = 4). Frog density was lower at this stream; the usual density was one or two per 100 m of transect. No frogs were captured during January 2003 and April 2004, however frogs were still observed at those times (Figure 6-6).

A. Cilember



B. Ciapus Leutik

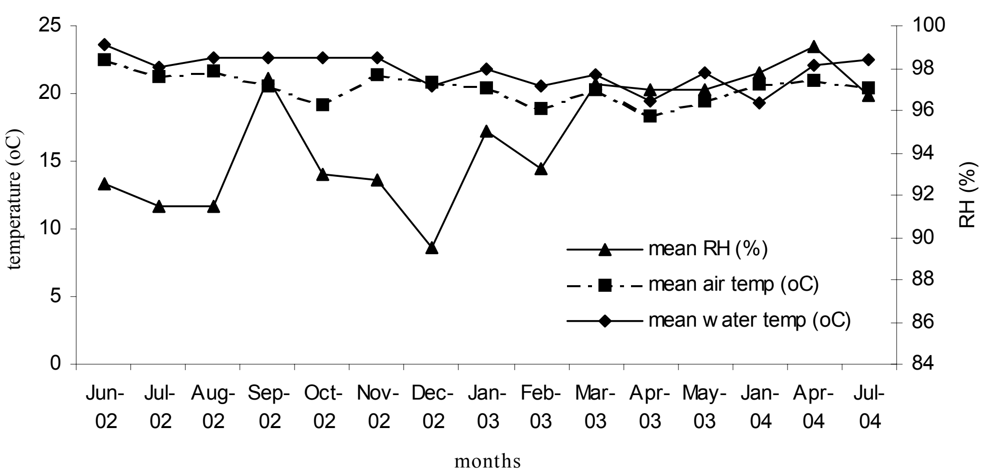


Figure 6-2. Mean relative humidity (%), air and water temperature (^{o}C) in the Cilember and the Ciapus Leutik Stream during sampling.

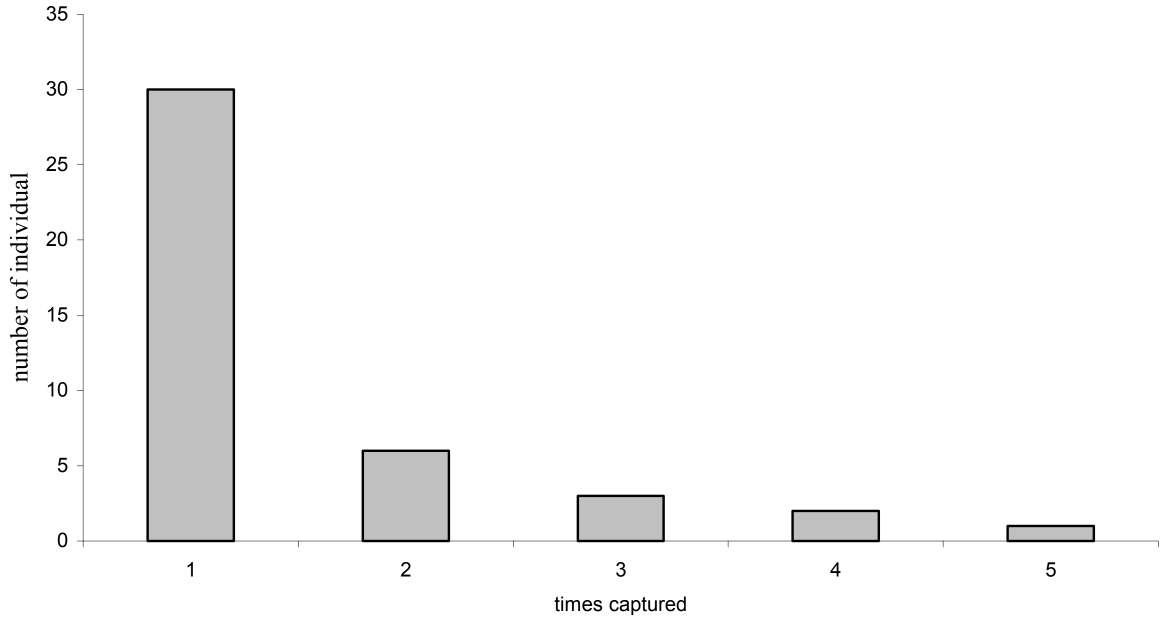


Figure 6-3. Proportion of *L. macrodon* captured in the Cilember stream

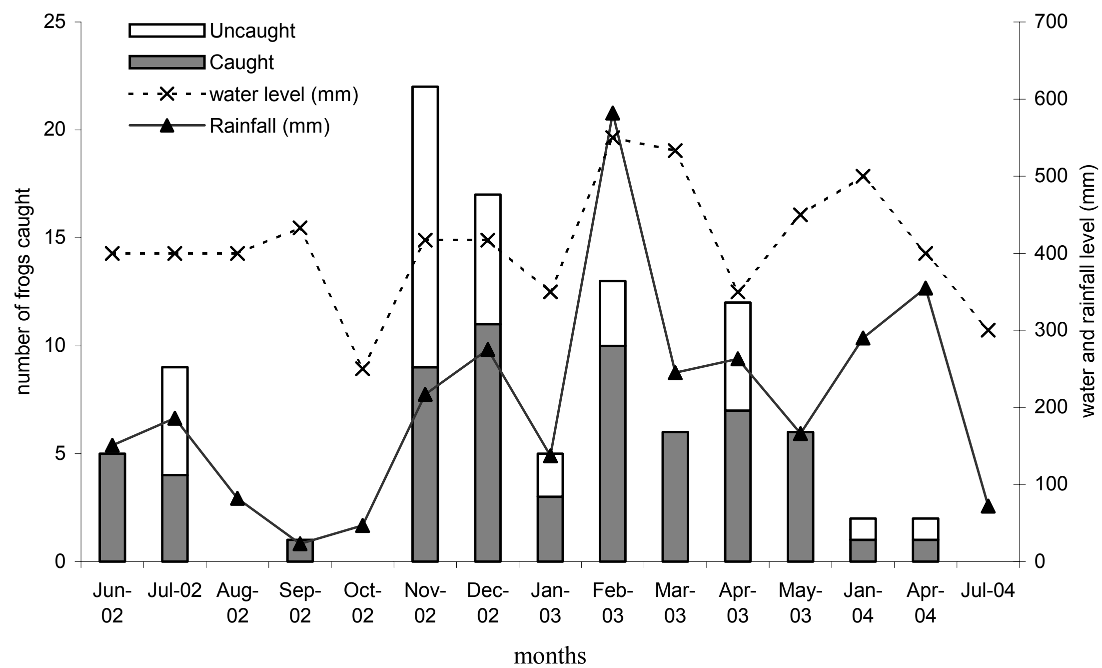


Figure 6-4. Number of *L. macrodon* found in the Cilember stream

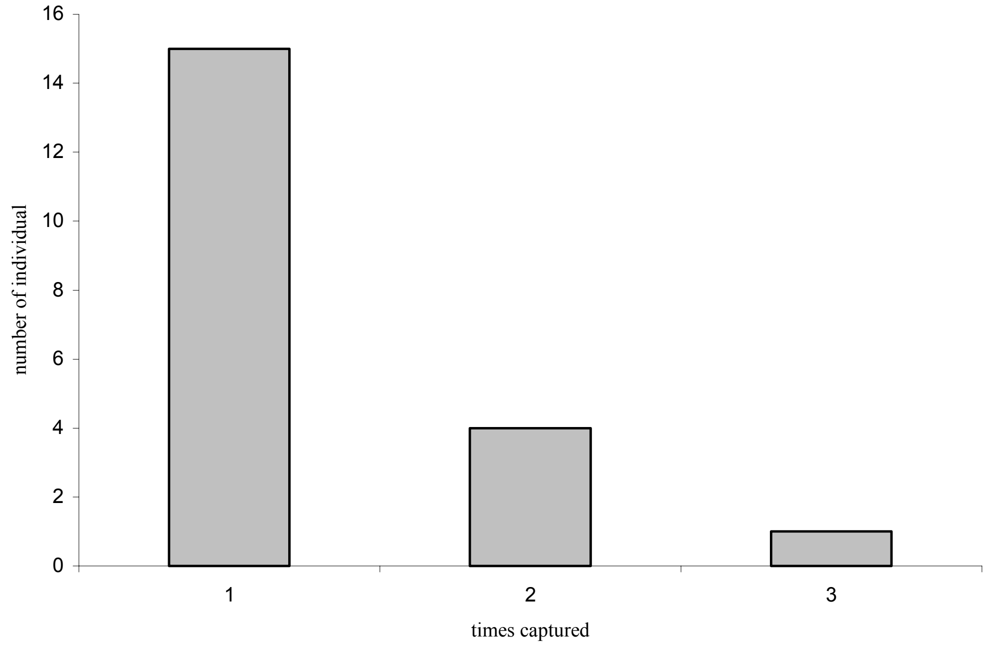


Figure 6-5. Proportion of *L. macrodon* captured in the Ciapus Leutik stream

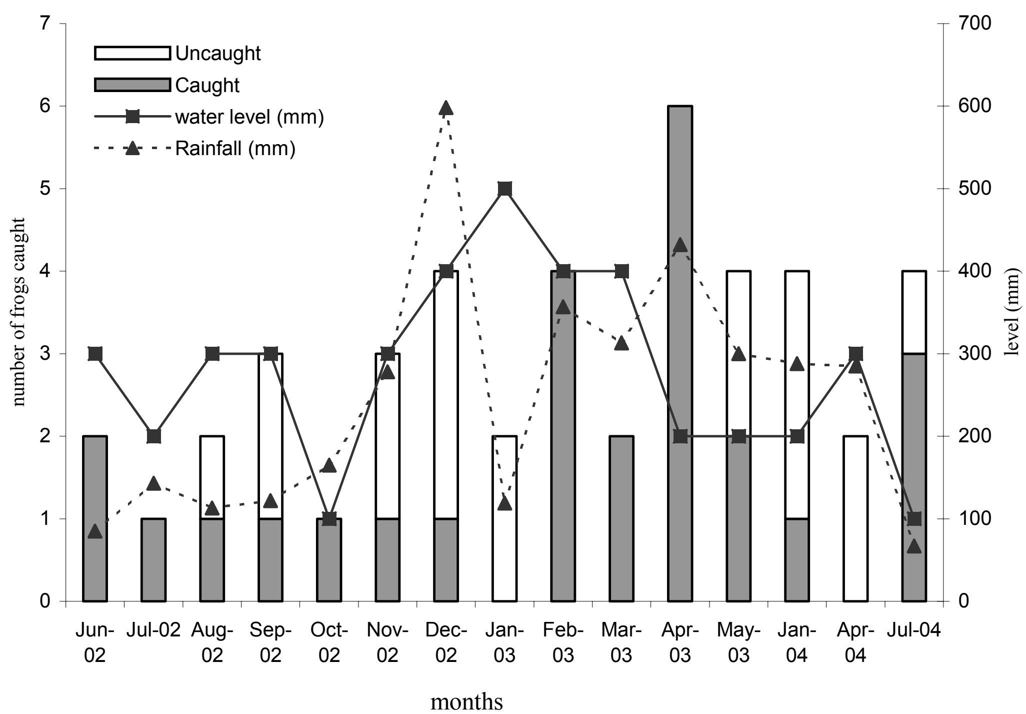


Figure 6-6. Number of *L. macrodon* found in the Ciapus Leutik stream

During the 12-month continuous survey period (June 2002 – May 2003), more frogs were found during the third and fourth 3-month periods (Figure 6-7), which coincided with higher rainfall. There is a positive correlation between numbers of frogs observed and mean monthly rainfall based on these four periods (Cilember r = 0.658, P = 0.020; Ciapus Leutik r = 0.714, P = 0.009).

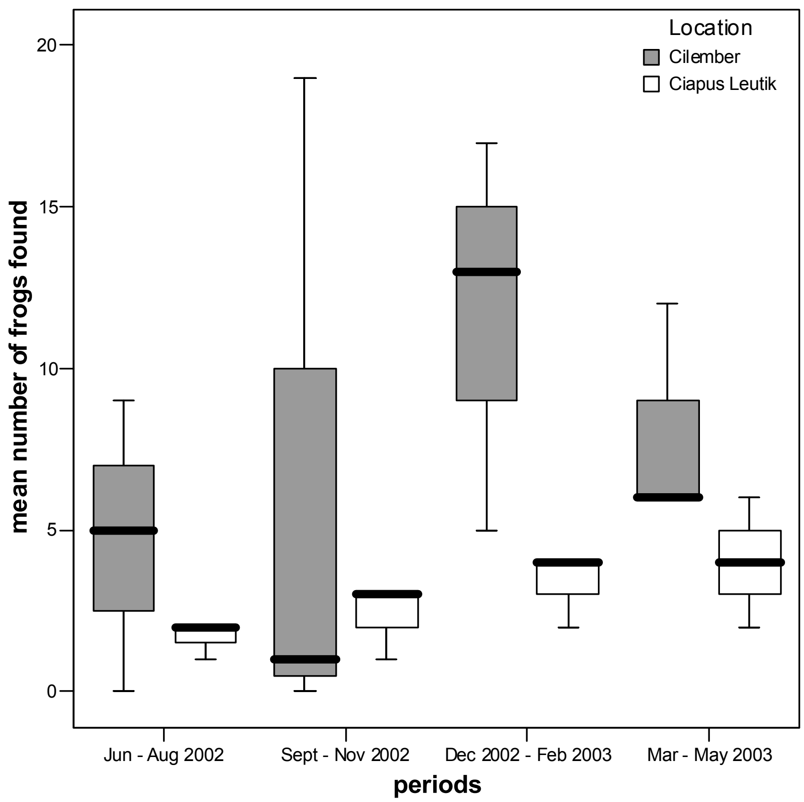


Figure 6-7. Mean frogs found (observed and captured) in the Cilember and the Ciapus Leutik streams during 4-monthly periods in the continuous period between June 2002 and May 2003

6.3.3. Traits of L. macrodon

Including recaptures and frogs encountered but not captured, a total of 142 frogs were encountered across both sites during the study. *L. macrodon* was not the most abundant species at the study sites. *Limnonectes kuhlii* was the most common species at Cilember, while *Rana hosii* was the most common frog at Ciapus Leutik.

The size (snout vent length) of frogs at the time of initial capture was between 47.06 – 105.22 mm, and their mass was from 12 – 178 grams. Figure 6-8 summarizes the size and mass of frogs at the two locations. Table 6-3 details the data for frogs captured at Cilember and Ciapus Leutik.

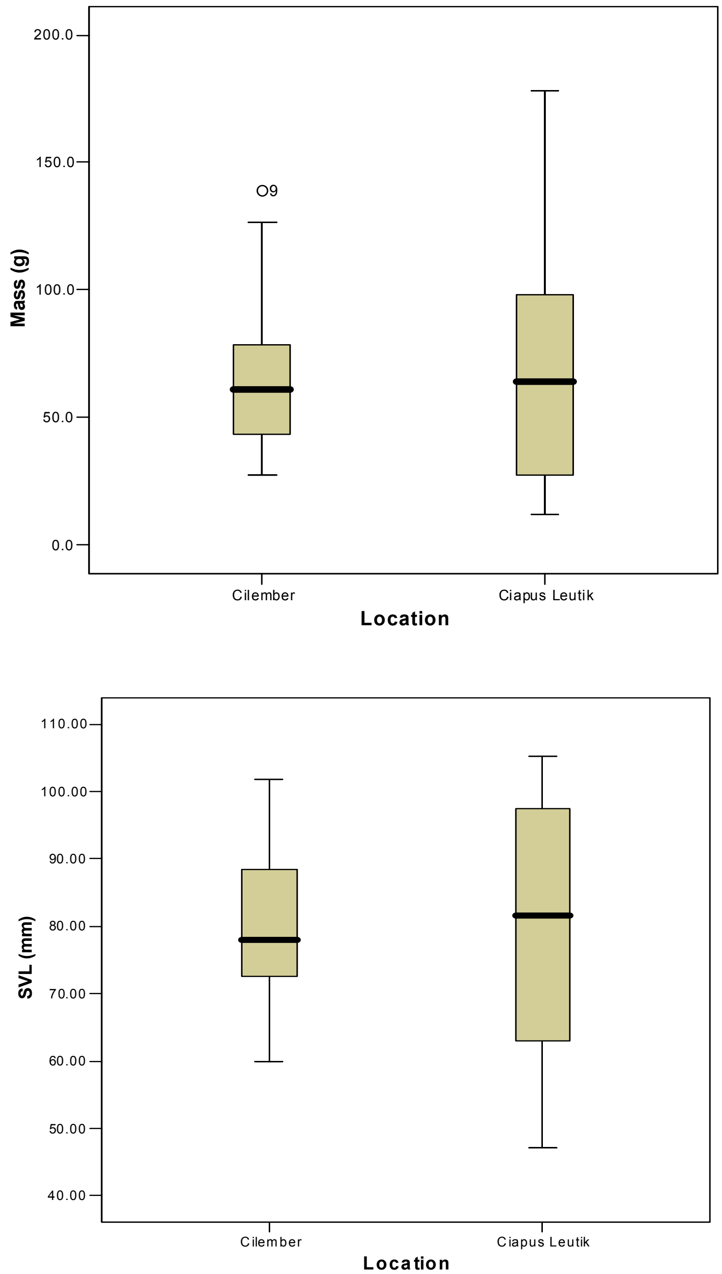


Figure 6-8. Size and body mass of *L. macrodon* in the Cilember and the Ciapus Leutik

Table 6-3. Traits of *L. macrodon* in the Cilember and Ciapus Leutik streams

		SVL (mm)		Mass (grams)	
Location	N	Range	Mean ± SD	Range	Mean ± SD
Cilember	42	60.00 - 101.74	79.67 ± 11.52	27.60 - 136.00	65.35 ± 29.11
Ciapus Leutik	20	47.06 - 105.22	78.25 ± 18.25	12.00 - 178.00	68.41 ± 48.59

There were no significant differences between Cilember and Ciapus Leutik in the mean SVL ($F_{1,60}$ = 0.14, P =0.71) or mass ($F_{1,60}$ = 0.09, P =0.76), of frogs. Log(mass) and log(SVL) were strongly correlated at both sites (Cilember: r = 0.906, N = 42, P < 0.001; Ciapus Leutik: r = 0.949, N = 20, P < 0.001). These relationships are shown in Figure 6-9.

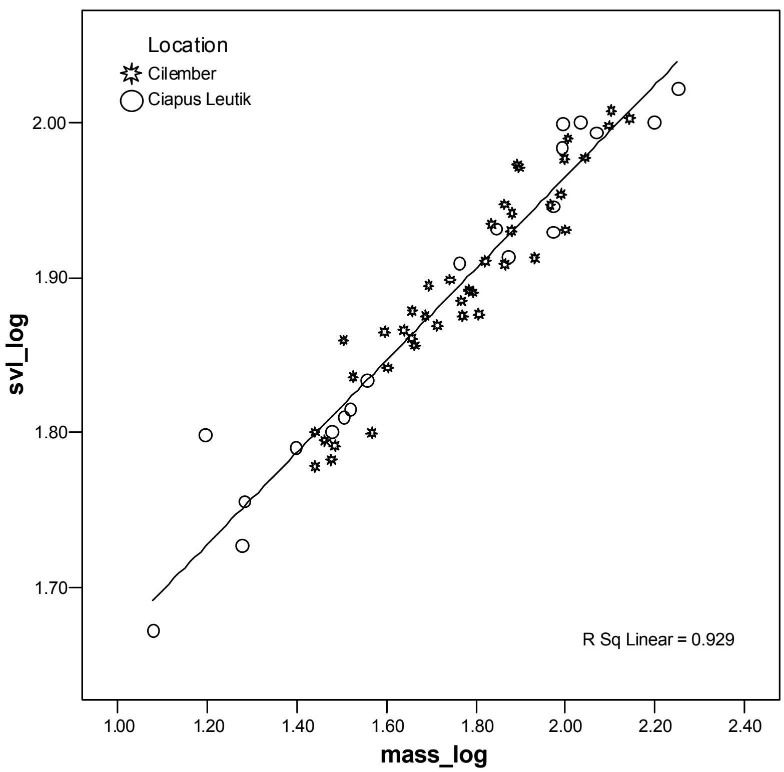


Figure 6-9. Relationship between snout vent length (mm) and mass (gram) of *L. macrodon*

At Cilember a number of frogs were captured repeatedly. Based on these recapture, I determined the monthly growth of SVL and mass of each frog (Table 6-4). Although there was a positive correlation between growth rates of SVL and initial SVL ($r^2 = 0.21$ Figure 6-10) it was not significant ($F_{1,9} = 2.441$, $P = 0.15$). Figure 6-11 shows the gains of the five frogs captured more than two times.

Table 6-4. Snout vent length (mm) and mass (gram) gains of *L. macrodon* at Cilember during June 2002 to May 2003

Code number	Number of LAGs	Initial SVL (mm)	Initial Mass (g)	# re-capture	SVL gain (mm)	Mass gain (g)	Months between	SVL gain /month (mm)	Mass gain /month (g)
SGBER_1	n/a	81.78	85.30	3	5.90	7.90	11	0.54	0.72
SGBER_11	n/a	74.98	48.70	4	18.36	61.50	8	2.30	7.69
SGBER_13	n/a	88.54	73.10	2	3.62	17.40	4	0.90	4.35
SGBER_14	0	81.38	66.20	3	6.30	26.00	9	0.70	2.89
SGBER_18	0	85.10	75.70	1	5.90	6.30	3	1.97	2.10
SGBER_2	n/a	79.16	55.26	1	7.16	22.74	9	0.80	2.53
SGBER_21	0	62.36	29.00	1	27.04	10.50	3	9.01	3.50
SGBER_22	0	93.96	78.10	2	0.26	0.00	1	0.26	0.00
SGBER_33	3	101.74	126.50	2	11.26	23.50	2	5.63	11.75
SGBER_42	3	94.84	110.50	1	0.00	-21.50	8	0.00	-2.69
SGBER_7	n/a	93.50	78.60	1	0.00	0.00	10	0.00	0.00

n/a = not available

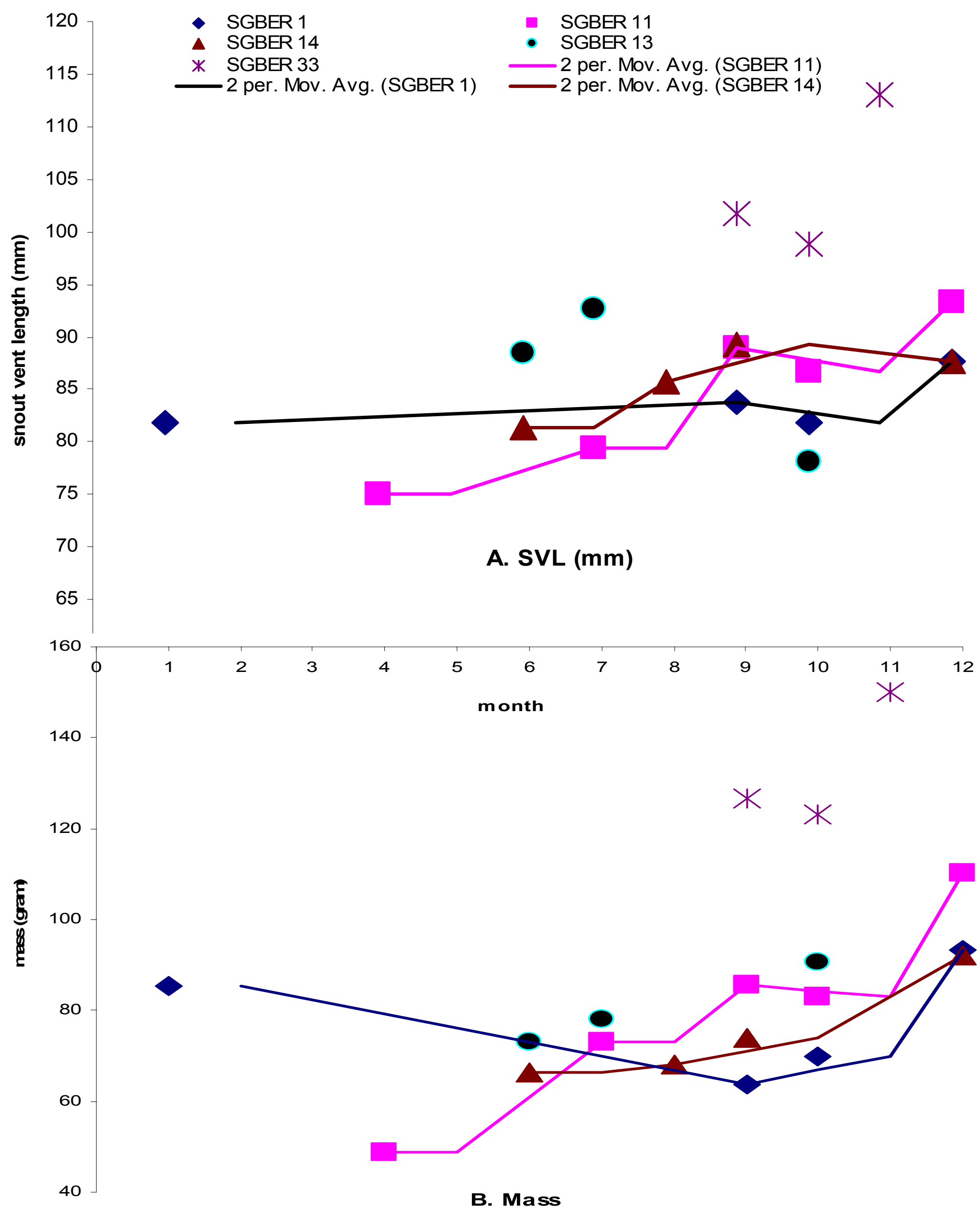


Figure 6-10. SVL and mass gain of five *L. macrodon* in the Cilember stream. Months 1–12 are June 2002 to May 2003 respectively. Trendlines are shown only for frogs recaptured more than three times.

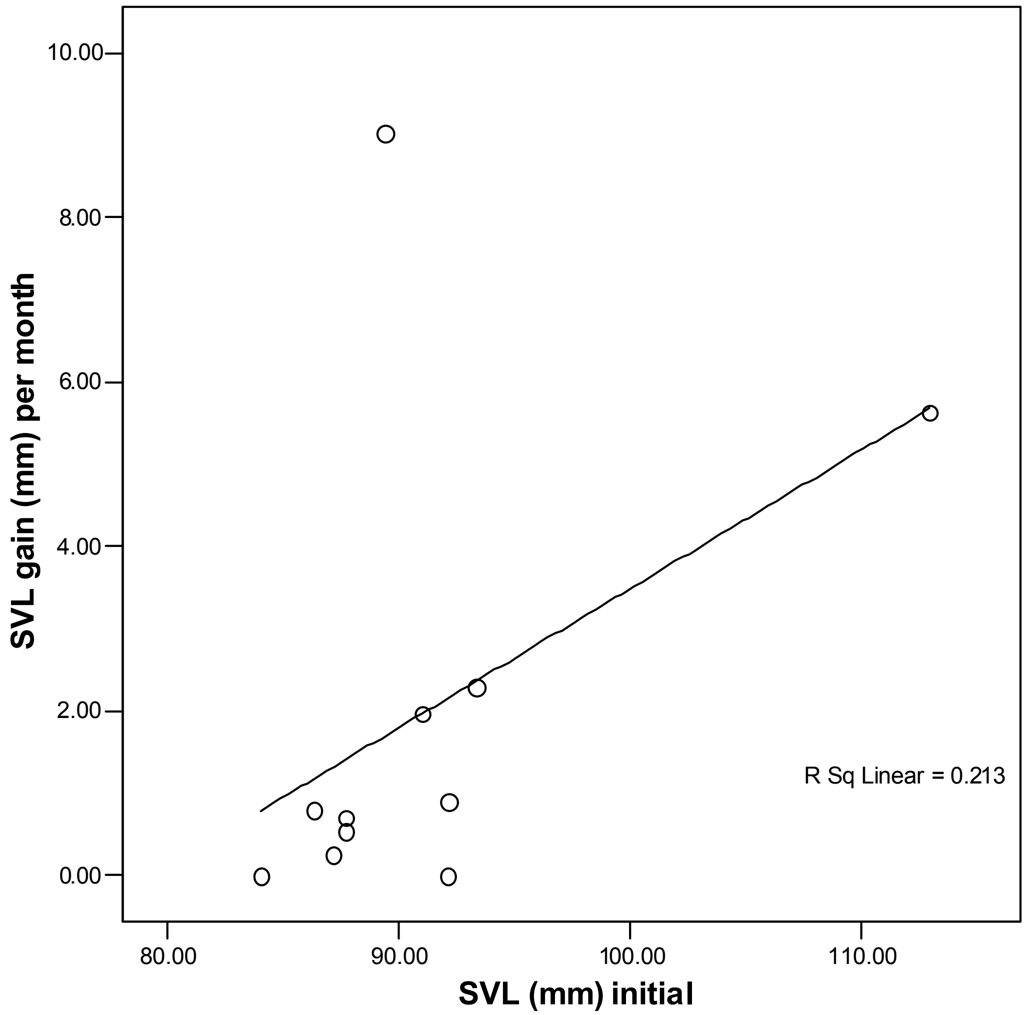


Figure 6-11. Growth rate (mm SVL per month) as a function of snout vent length (SVL) for *L. macrodon* at the Cilember stream

6.3.4. The biology of L. macrodon

Additional data from the *L. macrodon* bought from harvesters in Caringin (West Java) that were used to examine our ability to differentiate sex showed that male and female *L. macrodon* differed significantly in SVL ($F_{1,67}$= 24.72, P<0.001); and mass ($F_{1, 67}$ = 23.88, P < 0.00), with females being larger than males. Table 6-5 presents summary statistics for the SVL and mass of frogs captured by professional harvesters. Most females captured (65.3%) were in reproductive condition; the majority of these (44.9%) were in the early gravid stage (ovaries weight 0.1 – 9.99 grams; Figure 6-12).

Table 6-5. Traits of *L. macrodon* captured by harvester in Caringin (West Java)

		SVL (mm)		Mass (grams)	
Location	N	Range	Mean ± SD	Range	Mean ± SD
Female	49	67.44 - 118.5	92.37 ± 12.18	40 - 172.6	86.02 ± 30.96
Male	20	56.34 - 94.42	77.38 ± 8.96	24 - 94	50.21 ± 16.37

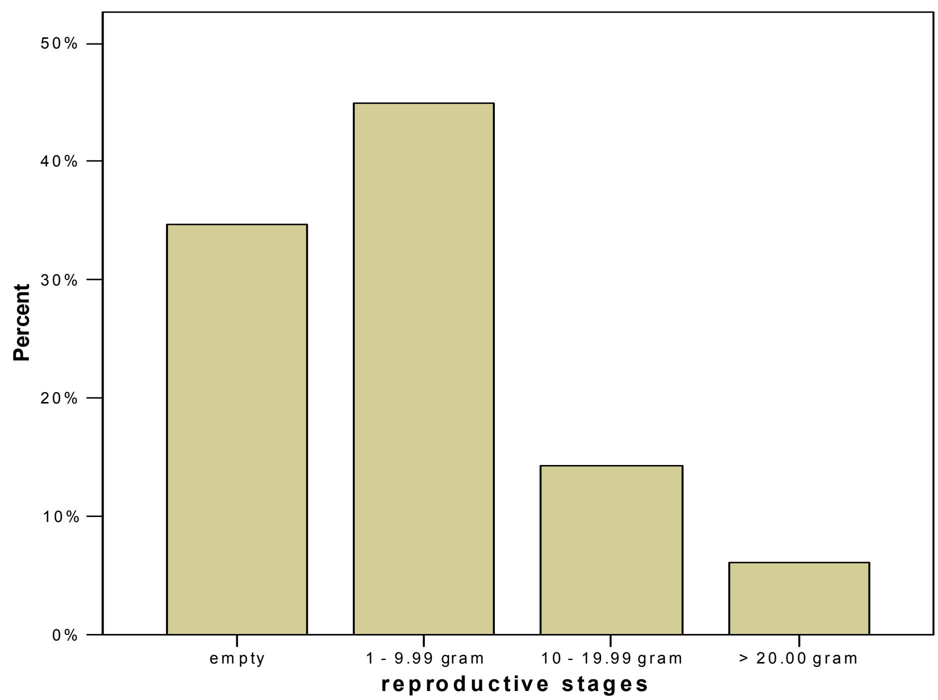


Figure 6-12. Percentages of reproductive female *L. macrodon* harvested in Caringin (n = 49)

There was a significant positive relationship between mass of eggs and body mass ($F_{1,47}$ = 26.69, P <0.001; Figure 6-13). Only two males (10%) had discernable fat bodies, and these were small (both 0.1 grams). Most females had fat bodies, and these were usually larger than those of males. The highest fat mass recorded in a female was 2.5 grams. Most females (95.92%) caught had body fat content less than 1.0 grams. Only 10% of gravid females (ovary mass > 10.00 gram) had fat mass of more than 2.0 g. Thirty percent of gravid females had low fat mass (< 0.4 g).

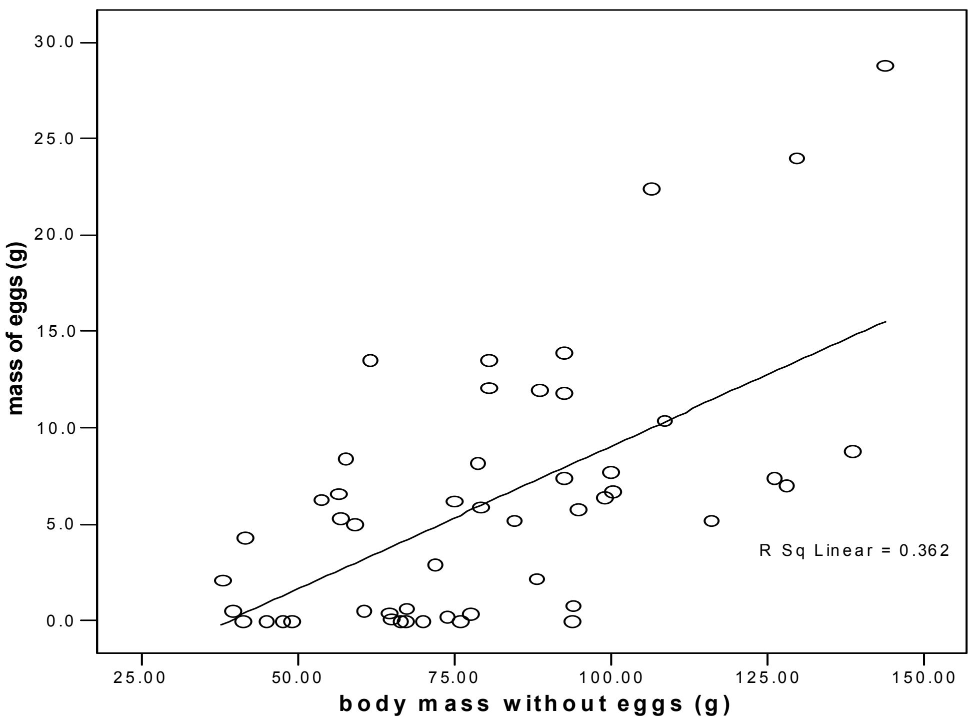


Figure 6-13. Relationship between body mass of *L. macrodon* female versus mass of eggs

6.3.5. Population dynamic and population structure

Because of the low recapture rate at Ciapus Leutik, I was only able to estimate the population of *L. macrodon* at Cilember. More than 60% of recaptures at Cilember occurred from one to three months after first capture. No frogs were recaptured 4 and 5 months after initial capture, but several were recaptured between 6 and 10 months after initial capture. Figure 6-14 shows the timing and the proportions of individual recaptures.

The estimated population sizes of *L. macrodon* at Cilember between July 2002 and March 2003 were between six and 66.7 frogs (Figure 6-15). Lumping the data into three-monthly "samples" produced estimates of 45.4 (September to November 2002) and 36.1 (December 2002 to February 2003; Figure 6-16).

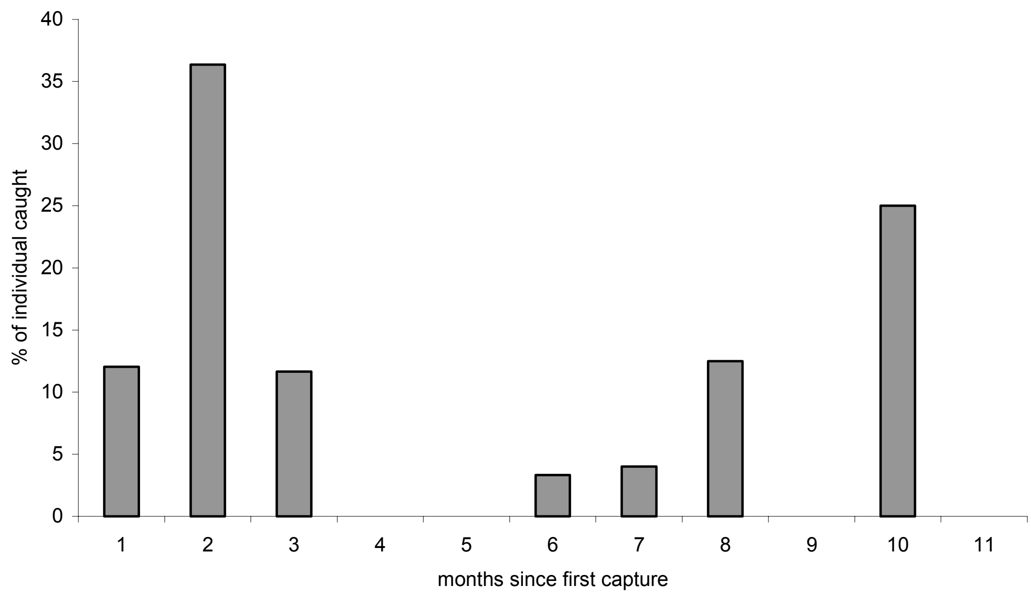


Figure 6-14. The proportion of frogs caught and time in months since first capture at Cilember.

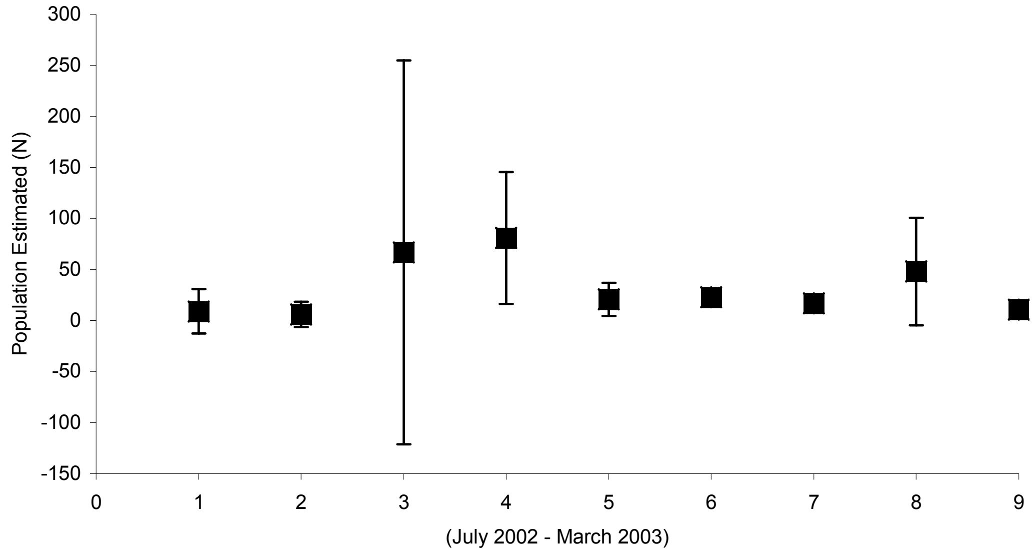


Figure 6-15. Estimated size of the *L. macrodon* population at Cilember (mean ± SE) from July 2002 to March 2003

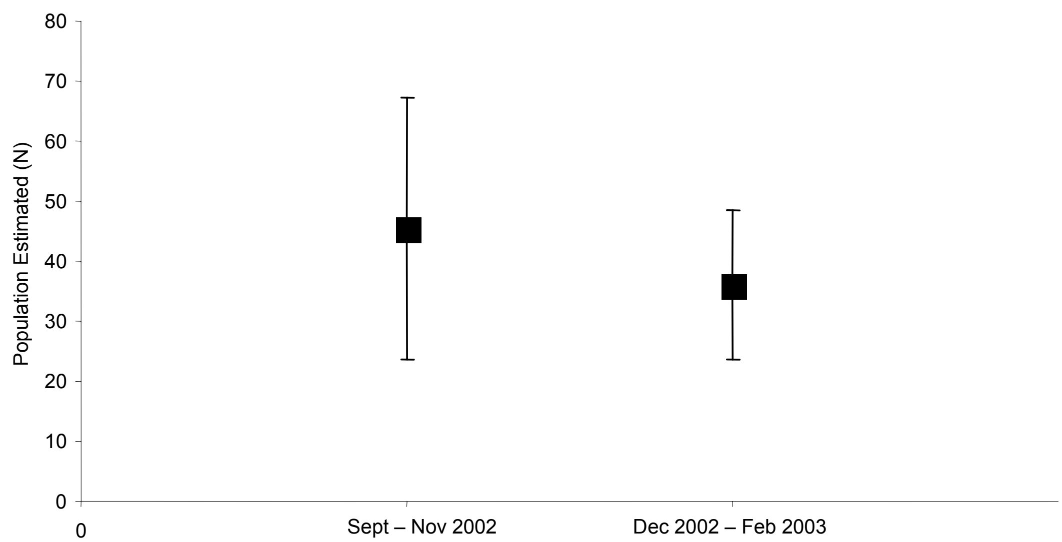


Figure 6-16. Estimated size of the *L. macrodon* population at Cilember (mean ± SE) in the aggregated periods covering September to November 2002 and December 2002 to February 2003

Unlike paddy-field frogs (Chapter 5), histological examination of the phalanges showed the occurrence of darkly stained and distinct lines separated by wider light purple rings, which were interpreted as lines of arrested growth (LAGs, Figure 6-17A and B). I was able to interpret sections taken from of 44 of the 46 individuals captured, and detected up to five LAGs. The predominant "age" class is no LAG (n = 25), next was one LAG (n = 8), two LAGs (N = 4), three LAGs (N = 3) and five LAGs (N = 1). The highest numbers of LAG's found in frogs from Cilember and Ciapus Leutik were five and two respectively.

There was a significant positive relationship between body size and LAG number in *L. macrodon* (Spearman correlation, $r_s = 0.399$, $P = 0.01$); larger *L. macrodon* tend to possess more LAGs than do smaller individuals (Figure 6-18). This suggests that the number of LAGs may reflect age in this species. However, since each LAG class spanned wide and overlapping ranges of snout-vent lengths, it is impossible to predict age from the size of frogs. Therefore I used snout vent length frequency to illustrate population structure.

During June to September 2002, which coincided with dryer periods, the populations of *L. macrodon* in the Cilember and Ciapus Leutik streams consisted mostly of larger frogs (SVL > 80 mm). In the next two three-monthly periods smaller frogs were observed at both sites (Figure 6-19).

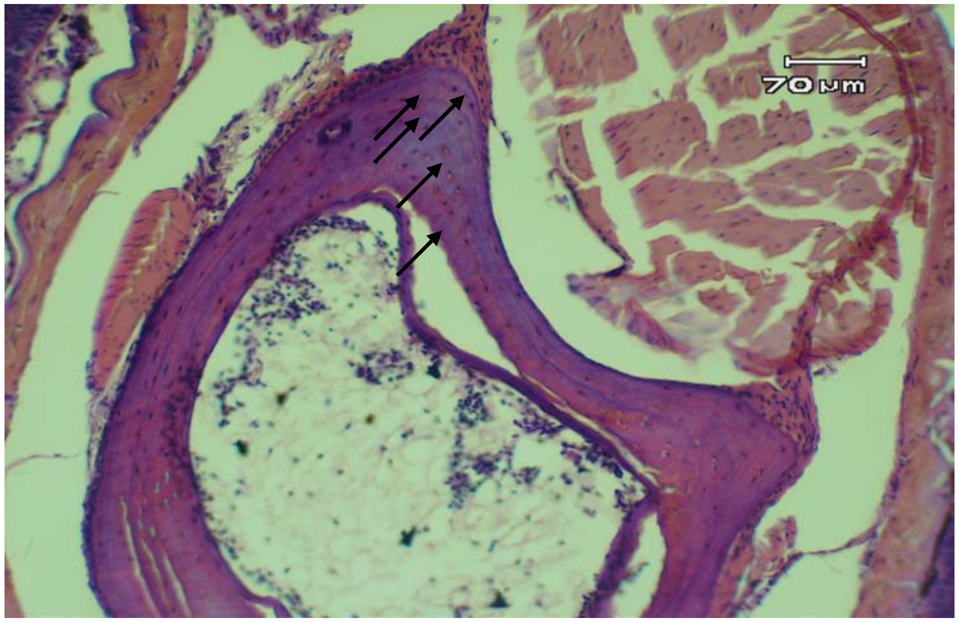


Figure 6-17A. *Limnonectes macrodon* from the Cilember stream with five LAGs; Arrows pointing to LAGs (SVL 77.86 m, mass = 61 g)

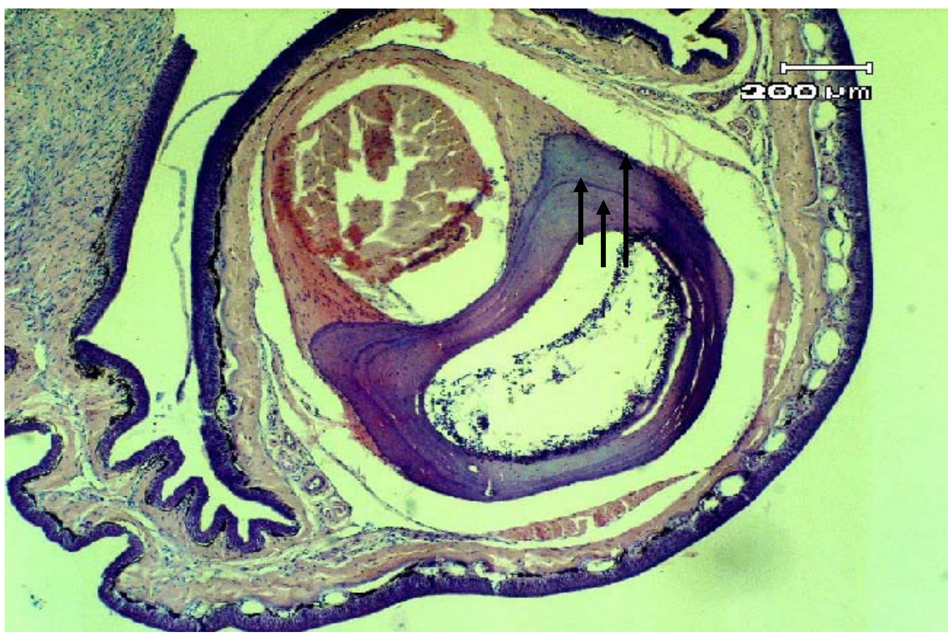


Figure 6-17B. *Limnonectes macrodon* from the Cilember stream with three LAGs; Arrows pointing to LAGs (SVL 101.74 mm, mass = 126.5 g)

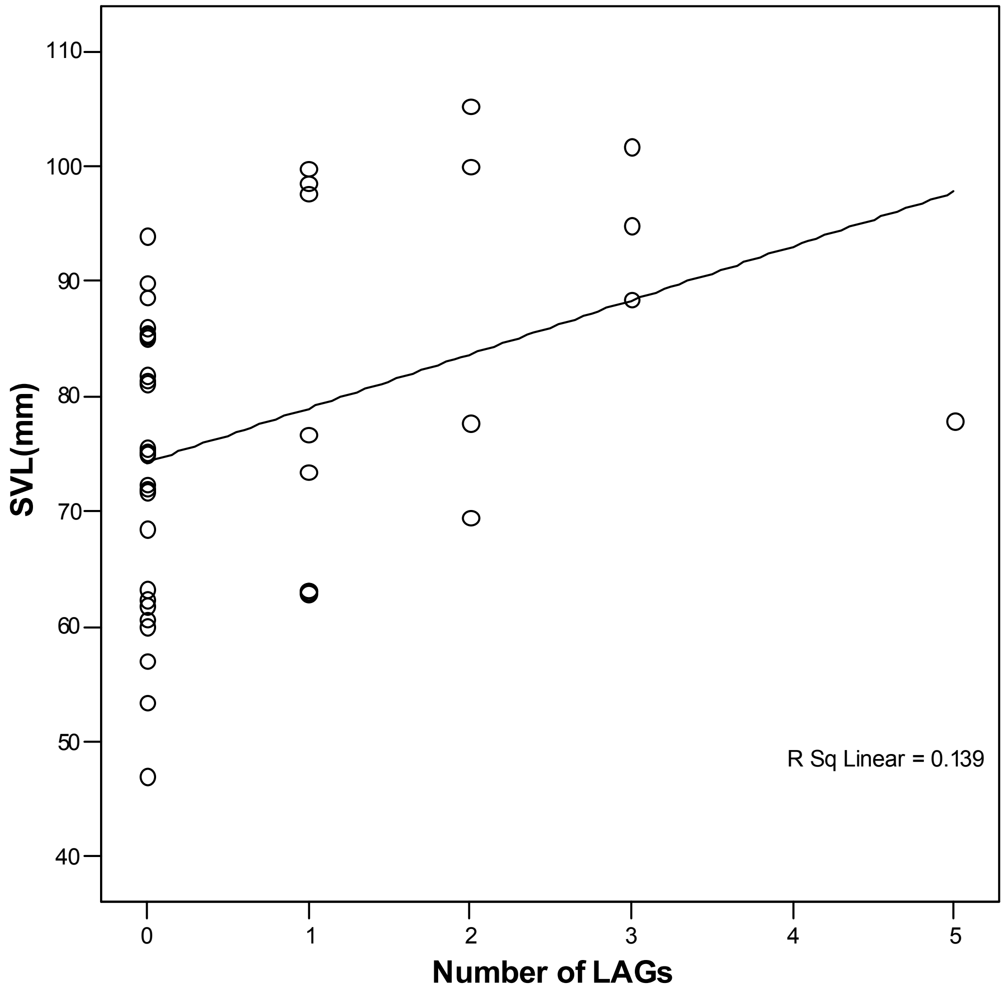
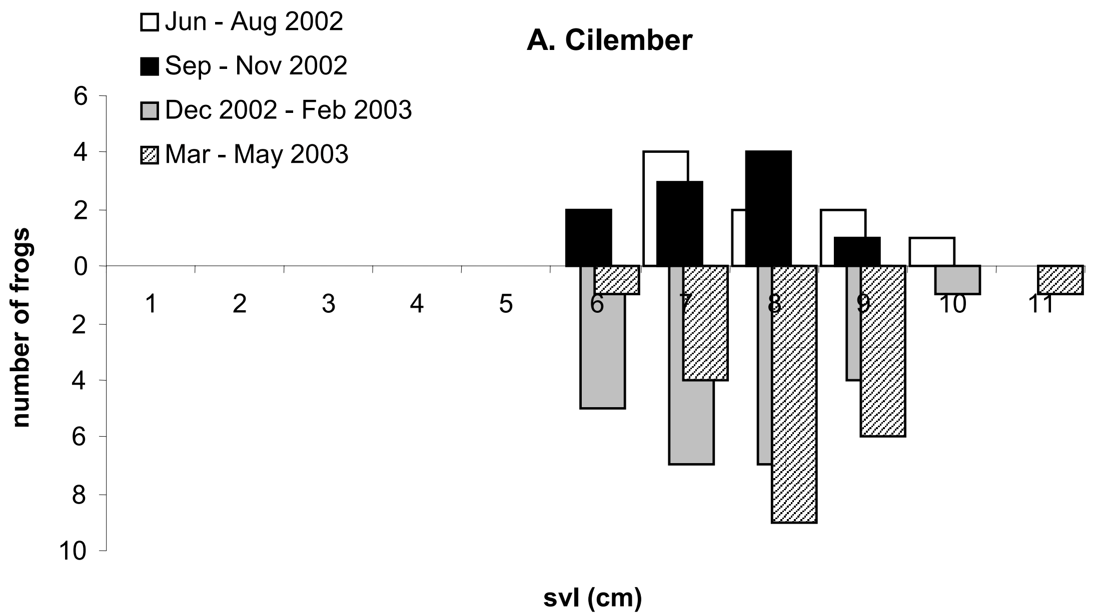


Figure 6-18. Snout vent length (mm) versus number of lines of arrested growth (LAGs) for *Limnonectes macrodon*



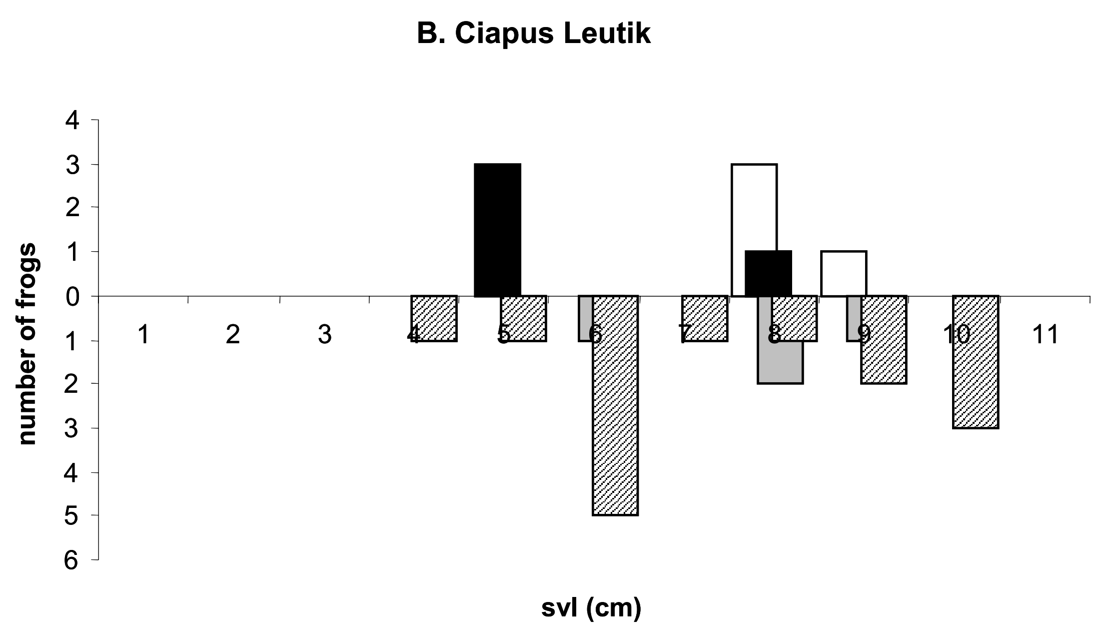


Figure 6-19. Population structure of *L. macrodon* at Cilember (A) and Ciapus Leutik (B) during three-monthly periods (June-August 2002; September- November 2002, December 2002-February 2003 and March-May 2003) based on the frequency of individuals in snout vent length categories

6.3.6 Microhabitat use

At Cilember most *L. macrodon* were found in the lower 200 m of the transect. Although four frogs were captured and marked in the upper 200 m of the transect, all recaptures were from the lower 200 m. *L. macrodon* at Ciapus Leutik were all found in the 0 – 100 m and 200 – 350 m regions of the transect (Figure 6-20).

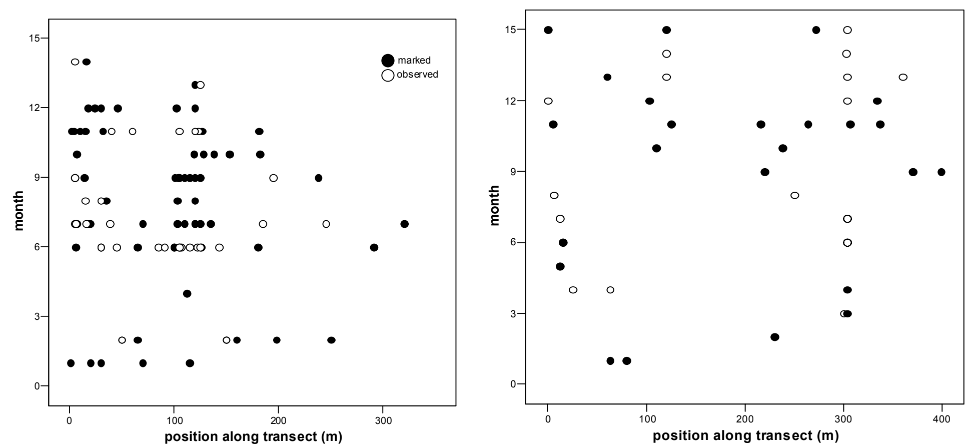


Figure 6-20. The positions of *L. macrodon* along the transects in the Cilember stream (left) and the Ciapus Leutik stream (right). Months 1 – 12 are June 2002 to May 2003. Month 13 = January 2004, month 14 = April 2004, month 15 = July 2004. No *L. macrodon* were found in month 15 in the Cilember stream.

Most frogs were found in relatively protected locations such as holes or rock crevices where they mostly sat quietly (Figure 6-21). Some were found in open areas such as grassy banks, or were swimming in the water. However, most frogs found in open areas were usually quick to jump and swim away and were therefore difficult to catch.

6.3.7 Movement

Movement results are presented only for Cilember. The number of frogs recaptured at Ciapus Leutik was too low for meaningful estimation of movement parameters. The mean cumulative distance moved per month was 11.41 m (Table 6-6).

Of the 21 movements recorded, two-thirds were ten metres or less. The longest movements were two mean monthly movements of 60 m, which happened within the span of one (October to November 2002) and two months (March to May 2003).

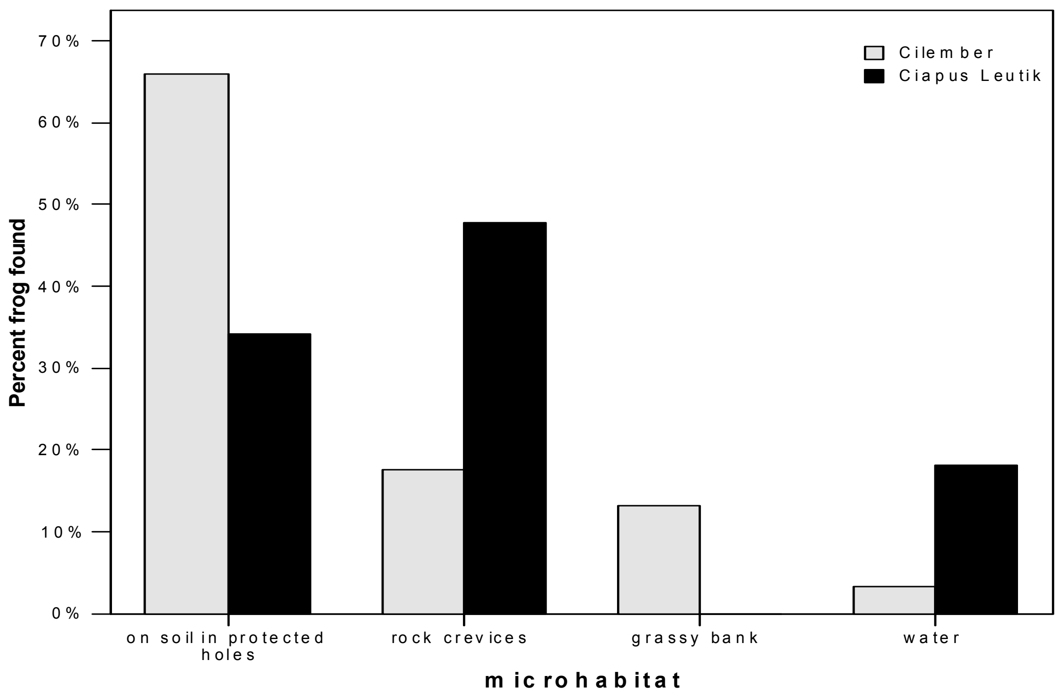


Figure 6-21. The percentage of *L. macrodon* found in four types of microhabitat

Table 6-6. Summary of movement of *L. macrodon* at the Cilember stream

	Distance movement per month (m)	Displacement per month **	Straightness**
Mean $\pm$ SD	11.41 $\pm$ 17.25	5.75 $\pm$ 4.61	0.6 $\pm$ 0.32
Range	0.33 - 60	0.33 – 11.75	0.29 – 1.00

** only for frogs recaptured more than once

6.4 Discussion

Mean SVL for *L. macrodon* in this study was 79.21 mm (range 47.06 – 113 mm) and mean mass was 66.34 grams (range 12 – 178 grams). The largest frogs captured in this study were 113 mm in SVL (mass = 110 grams) and 178 grams in mass (SVL = 105.22 mm). Most frogs captured were less than 80 mm SVL, which is the modal size captured by professional harvesters. The maximum sizes of frogs found in this study are less than the maximum sizes of frogs captured by harvesters in previous years. For instance, based on Sugiri (1979), the biggest *L. macrodon* captured by harvesters in West Java was 209.8 g (male, SVL = 119 mm, captured September 1975) and 214.0 g (female, SVL = 121 mm, captured in May 1976). The latter also had the longest SVL for female, whilst for a male the largest SVL was 144 mm (mass 109.4 g, captured in August 1976).

Recaptures at Cilember showed that most individuals were growing relatively rapidly. Growth in frogs is usually rapid when they are small but slows towards an asymptote when frogs mature (Ramirez et al., 1998). In my data, one individual with an SVL of more than 100 mm was growing more rapidly than some smaller individuals, suggesting that rapid growth can continue past reproductive size in at least some individuals.

Most work on fanged frogs indicates that adult males are larger than females (Inger, 1966; Emerson and Ward, 1998), however this study found the opposite result. The sex of individuals in my field study was very uncertain due to the difficulty of sexing individuals, however additional data from *L. macrodon* bought from harvesters in Caringin (West Java) that was used to differentiate sex indicated that females were on average larger than males. Sugiri (1978) concluded that there were no significant differences in mass or SVL between male and female *L. macrodon* from West Java.

Both Sugiri's (1979) and my study found that professional harvesters captured more females, which is the same trend as that for *Fejervarya cancrivora* (Chapter 3), in which females are larger than males. These results suggest that *L. macrodon* exhibits a pattern of sexual dimorphism different from that of other fanged species. Since both Sugiri's and my data were derived from professional harvesters, they may reflect biases in collection. Large female *L. macrodon* could be easier to catch than large males, possibly because most females captured by professional harvesters were gravid; males may also be more wary. As with other species of frogs, mating of fanged frogs such as *L. blythi* involves an active movement of gravid females to search for suitable adult males (Emerson and Inger, 1992)

Sugiri (1979) found that the smallest female with ovaries in the early gravid stage (ovaries weight ca. 2.1 g) was 72 mm SVL and weighed 50.1 g. In my study the smallest female frog that had ovaries in this stage was 67.44 mm and weighed 40 g. This suggests that the minimum reproductive size for female *L. macrodon* is probably around 60 to 65 mm SVL.

My results show that skeletochronology may be applicable to *L. macrodon.* LAGs found in *L. macrodon* were quite distinct, suggesting that growth was slower during certain times, most probably during the dry season, which only occurs for around three months each year. Compared to the rice field frogs, *L. macrodon* tends to be philopatric, remaining in sites during the dry season. In temperate species, LAGs are usually visible, and are formed during winter (Halliday and Verrel 1988; Ryser 1988; Reaser 2000). Results from this study confirmed the findings of Felton (1999), and Kumbar and Pancharatna (2001) that skeletochronology can be used to determine the ages of tropical frogs.

Because I found a relatively large number of *L. macrodon* individuals with more than a single LAG, but found only zero or one LAGs in *F. limnocharis-iskandari* complex and *F. cancrivora*, it is possible that *L. macrodon* may be longer-lived than either of these species. However, further study is required to validate that LAGs are formed annually in *L. macrodon* and the other species. Although it seems likely that increasing numbers of LAGs indicates increasing age in *L. macrodon*, the exact relationship between LAG number and age is not clear from my results. Number of LAGs and SVL are correlated, but each LAG class spans a wide and overlapping range of snout-vent lengths. Also, because frogs were not recaptured and clipped a second time for reanalysis after one year, there is no direct evidence that LAGs are formed annually. Therefore, there is a need to validate the relationship between LAGs and age, ideally by re-clipping recaptured frogs.

The lower limit of snout vent length for LAG-1 in this study was 62.88 mm, and for the early reproductive stages of females was 67.44 mm. These figures suggest that mature *L. macrodon* have a SVL of at least 60 mm, which may be attained at approximately one year of age. The populations of *L. macrodon* at Cilember and Ciapus Leutik consisted mainly of adult individuals. I did not find any tadpoles during the study, nor did I observe reproductive activity. However, some frogs were found sitting near pool-like areas on the edge of the streams near the banks (Figure 6-22) that would be suitable habitat for breeding not only for *L. macrodon* (Iskandar, 1998) but also for other *Limnonectes* species such as *L. kuhlii* (Tsuji and Lue, 2000) and *L. blythi* (Emerson, 1992).

Figure 6-22. Pool-like area in the Ciapus Leutik stream where *L. macrodon* was sometimes found

Monthly population estimates either could not be calculated using Jolly Seber method, or had relatively high standard errors. The number of individuals captured at Ciapus Leutik was so low that monthly population estimation was impossible, and monthly population estimates at Cilember varied greatly (Figure 6-14). Estimates using the Jolly-Seber technique on data aggregated into three-monthly periods were more reliable, with smaller standard errors. It seems likely that variation in capture probabilities play a factor in the variability of population estimation. It is likely that capture probability is not constant, but is affected by seasonal factors and short-term effects on frog behaviour. For example, it appeared that more frogs were active during higher rainfall periods (Figure 6-7).

Capture efficiency also could affect population estimates; at Cilember and Ciapus Leutik it varied between 0.59 and 0.71, which means for every two to three frogs captured, one frog escaped. *Limnonectes macrodon* were rarely found in open areas, most were found in protected microhabitats, which made them harder to find. The frogs were also very agile and had a tendency to escape during capture and handling. After initial capture, the probability of recapture might decrease. Probability of capture also differs between individuals in the population, which explained why some individuals could only be recaptured more than six months after their initial capture, while most were recaptured between one to three months after initial capture. Frogs also moved upstream and downstream, although movement seemed restricted by natural boundaries, such as the occurrence of waterfalls, most notably at Cilember. The movements of *L. macrodon* at Cilember were probably related to selection of sites needed for specific activities, such as breeding and foraging. Site selection could change over time, which might explain why some frogs move more further than others.

Frogs were found in lower numbers at Ciapus Leutik than at Cilember. These apparent differences in population density could have been caused by differences in factors such as microclimate, food availability and predation rates (Duellman and Trueb, 1994). No data are available regarding food availability at the sites, however it is likely that microclimate and prey abundance in both locations were relatively similar. Risk of predation is presumably higher from human hunting than from natural predators such as snakes. As mentioned before, the survey area at Cilember falls within the boundary of Perum Perhutani recreation area where access is restricted to paid visitors. Because frog harvesters tend to keep costs low by capturing frogs in unrestricted areas (Chapter 3), there is no known professional harvesting at the Cilember stream. However, there is always a possibility that professional or amateur harvesters could catch frogs that moved outside the fenced area. We found no professional harvesters during our surveys at Ciapus Leutik, nevertheless some frog harvesters mentioned this area as one of their regular places to capture frogs. Although there was no significant difference between mean SVL and mass of frog between Cilember and Ciapus Leutik, skeletochronological evidence suggests that the Cilember population includes more older frogs than Ciapus Leutik. This, and the low density of frogs at Ciapus Leutik, could be related to its greater accessibility to frog harvesters. However, further monitoring is needed before it is possible to draw any conclusions regarding whether over-harvesting is the cause of low numbers of frogs at the Ciapus Leutik stream.

CHAPTER 7

EFFECTS OF ENVIRONMENTAL STRESS

7.1 Introduction

The population dynamics of amphibians depend on the quality of the environments they occupy. Stress in the environment caused by natural factors such as climate change or human-induced factors such as chemical contaminants could directly or indirectly affect the dynamics and status of populations. Many scientists believe that amphibians are at high risk from contaminants and other stress effects that could interact with other factors (Alford and Richards, 1999), and which have resulted in several reported amphibian population declines across the world during the past 15 years (Barinaga, 1990; Blaustein and Wake, 1990; Anonymous, 1991; Pechman et al., 1991; Tyler, 1991; Blaustein, 1994; Blaustein et al., 1994; Pechman and Wilbur, 1994; Blaustein and Wake, 1995).

Many scientists have suggested that their environmental sensitivity should make amphibians as good indicators of environmental quality; this idea has been reviewed thoroughly by Sparling et al. (2000). It is clear that the habitats and ecology of amphibians make them susceptible to environmental stressors such as contaminants and diseases. For example, amphibians that live in agricultural fields are at risk from exposure to chemicals in pesticides or fertilizers (Ouellet et al., 1997; Jocelyn, 1999). Because of their life cycles (Duellman and Trueb, 1998) amphibians can be exposed to multiple risks as they pass through different life stages.

Amphibians have developed various adaptations and tolerances to environmental stressors. For instance, to avoid water loss amphibians can alter their behaviour through hibernation or looking for shade, or can reduce the surface area exposed to the air to reduce evaporative water loss (Duellman and Trueb, 1986). Tolerances to environmental stressors have been investigated in *Fejervarya cancrivora*. Tadpoles of this species can tolerate water temperatures up to 40^{o}C and salinity up to 40% of sea water, and survive at rates greater than 50% in 80% sea water (Dunson, 1977). Although adaptations like these can reduce the risk to amphibians from natural environmental stressors, changes in the level of their exposure to these stressors could still produce increased physiological stress, slowing the development and growth rate of juveniles and increasing the vulnerability of frogs to disease (Carey and Bryant, 1995; Taylor et al., 1999).

Concerns about environmental stressors are usually related to human-induced stressors. Pesticides have been applied to agricultural land in developing countries for decades; this has been perceived either as an advantage because it can increase crop production, or as a source of problems in environmental health (Igbedioh, 1991; Ecobichon, 2001). Rice fields or *sawah* are an important agricultural landscape in Indonesia. They have been subjected to intensive pesticide use, mostly subsidised and encouraged by the government. The need to increase rice productivity by combating pests has led to the application of high levels of pesticides to rice fields. Fox (1993), Van der Eng (1994) and Settle et al. (1999) describe the background of pesticide use in Indonesia; highlighting the fact that excess use of insecticides has produced more insecticide-resistant pests.

Pesticides have been implicated as one of the reasons for amphibian problems around the world. The impact of pesticides on frogs has been documented by many authors (Cooke, 1970, 1973, 1977, 1981; Brooks, 1981; Mohanti-Hejmadi and Dutta, 1981; Osborn and French, 1981; Berrill, et al., 1998; Boone and Semlitsch, 2001; and Relyea and Mills, 2001). In addition to their direct toxic and teratogenic effects, stresses caused by environmental toxicants can increase frogs' susceptibility to diseases (Carey and Bryant, 1995; Taylor et al., 1999; Thieman, 2000).

As with other wildlife, frogs are also exposed to various pathogens. Disease in amphibians has recently become a centre of attention, with intensive investigation of emerging diseases like chytridiomycosis and ranaviral disease that are linked to frog population declines (Berger et al., 1998; Daszak et al., 1999). Amphibian chytridiomycosis is a fatal disease caused by the fungus *Batrachochytrium dendrobatidis* (Berger, et al., 1999).

Although amphibian deformities are considered a separate problem to global amphibian declines (Cohen, 2001), alarm has been raised by the finding of increased morphological abnormalities, especially in North America (Ouellet, et al., 1997; Helgen et al, 1998; Meteyer et al., 2000). Pesticides have been suggested as one of the causes of deformities found in frogs around the world (Ouelett, et al., 1997; Helgen et al., 1998) although recent evidence suggests that parasites are probably responsible for many amphibian deformities (Kaiser, 1999; Leong , 2001).

Pesticide residue research has been undertaken for some frogs in agricultural areas in various parts of the world (Gilliland et al., 2001), however there are no reports on levels of pesticide residues in Indonesian frogs. There also are no reports on the occurrence of diseases such as chytridiomycosis in Indonesian frogs.

Developmental Stability Analysis (DSA) is a method that has been used to detect stress in various species from mammals to insects (Soule and Baker, 1968; Pankakoski, 1985; Clarke, 1994; Tsubaki, Y. 1998). The most common methods for measuring and analysing developmental stability involve measurements of fluctuating asymmetry (FA; Palmer and Strobeck, 1986; Alford, et al., 1999). FA is defined only at the population level; it is a measure of the extent and frequency of random departures from perfect bilateral symmetry in normally symmetrical structures. The aim of this study was to investigate the nature of environmental stresses on frogs, focusing on rice field frogs, by 1) documenting the occurrence and frequency of morphological anomalies, 2) analysing levels of pesticide residues in water, soil and in the bodies of *F. limnocharis-iskandari* complex and *F. cancrivora* found in rice fields, 3) measuring levels of fluctuating asymmetry in *F. limnocharis-iskandari* complex and *F. cancrivora* for comparison among populations potentially subjected to different levels of stress, and 4) investigating whether amphibian chytridiomycosis occurs in three species of frogs (*F. limnocharis-iskandari* complex , *F. cancrivora*, and *L. macrodon*) in West Java.

7. 2. Methods

7.2.1. Study area

The research sites are located in West Java province (see Chapter 4 for full site descriptions). Samples for pesticide analyses and frogs measured for DSA were taken from six paddy fields: Caringin, Darmaga, Karawang P (Pesticide), Karawang NP (Non pesticide), Subang P and Subang NP; these are the locations where populations of *F. limnocharis-iskandari* complex and *F. cancrivora* were studied (Chapter 5). Toe-clips taken during the population studies at the paddy field sites and at two stream sites (Cilember and Ciapus Leutik, Chapter 6) were analysed histologically for diagnosis of chytridiomycosis.

7.2.2 Morphological abnormalities

While doing preliminary surveys with harvesters in Caringin, I noted that several individuals captured showed morphological abnormalities. I decided to record any morphological abnormalities found in each individual captured during my population study (Chapter 5). Morphological abnormalities were described and categorised using terms adapted from Tyler (1999), Meteyer et al. (2000), and Johnson et al. (2001). I also present preliminary data taken from harvested frogs captured by frog hunters in Caringin (Bogor) during 2001 and 2002, data from Ciptarasa, Panguyangan and Cisaat taken from 2001 preliminary surveys, and data from specimens located in the Museum Zoology Bogor (MZB) in Cibinong, Bogor, Indonesia.

Site prevalence for each species is calculated as (number of abnormal individuals)/(total number of individuals) X 100. I used chi-squared homogeneity tests for each species to examine possible differences in the proportions of normal and abnormal frogs among sites. I excluded locations in which less than 20 frogs were found from this analysis.

7.2.3. Pesticide analysis in rice fields

a. Sample collection

Water, soil, and frog samples were obtained during the harvest season in September 2002. Water was collected and put into glass bottles and soil samples were taken using a grab and put inside plastic bags. Three samples of soil and water were taken from each location and combined for analysis. Three *Fejervarya limnocharis-iskandari* complex and three *F. cancrivora* were taken from each of the six rice fields using hand collection. No *F. cancrivora* were found in Darmaga during the sampling period, therefore samples of this species were only taken from the five other locations. Snout vent length, mass and sex of each frog were recorded after collection (Table 7-1).

Frogs were brought alive to the Toxicology Laboratory in Bogor. They were then euthanized and liver and leg muscle tissue was removed for analysis. All sampling and laboratory analyses were undertaken according to an Animal Ethics permit (A 748) granted by the James Cook University Animal Ethics Sub-Committee.

Table 7-1. Data on individual frogs analysed for pesticide residues in 6 rice fields

Origin	*F. limnocharis-iskandari* complex			*F. cancrivora*		
	Sex	SVL (mm)	Mass (g)	Sex	SVL (mm)	Mass (g)
Caringin	F	50.40	13.00	F	80.20	37.40
	F	44.50	10.20	F	60.40	20.80
	F	45.50	11.60	M	50.60	9.40
Darmaga	F	51.84	11.3	n/a	n/a	n/a
	M	48.12	9	n/a	n/a	n/a
	M	49.56	8.9	n/a	n/a	n/a
Kawarang NP	F	37.46	4.4	F	65.84	26.4
	F	30.53	2	M	62.92	22.2
	F	35.74	3.55	F	59.55	17
Karawang P	M	51.16	9.85	F	55.98	16.20
	M	39.33	4.3	J	22.88	1.6
	F	28.22	2.6	M	51.16	9.85
Subang NP	F	49.12	n/a	M	49.07	12
	F	41.23	5.5	M	64.02	27.30
	M	46.31	8.4	F	38.37	6.50
Subang P	F	36.72	4.5	F	50.92	9.5
	F	41.22	4.65	F	41.84	5.60
	F	34.72	3.70	M	33.54	3.95

n/a: Not applicable

b. Pesticide residue analysis

Pesticide residue levels in the liver and leg muscles of frogs were analysed at the Toxicology Laboratory, Agriculture Biotechnology and Genetic Research Institute (ABIOGRI), Bogor, West Java, Indonesia using Gas Chromatography (GC) and High Pressure Liquid Chromatography (HPLC). Pesticide residue analyses used the standard methods approved by the Indonesian Pesticide Commission (1997), which include (1) field sampling (2) sample preparation, (3) sample extraction and (4) pesticide residue analysis. Detection limits based on parts per million (ppm) for each residue using this method were as follows: BHC (0.001 ppm), aldrin (0.0068 ppm), heptachlor (0.0006 ppm), dieldrin (0.0006 ppm), endosulfan (0.0020 ppm), chlorpyrifos (0.0005 ppm), profenofos (0.0008 ppm) and diazinon (0.0006 ppm).

Pesticide residue values were checked against the Maximum Residue Limits (MRL) for meat products specified in guidelines from the Indonesian Health Ministry (IHM) and the World Health Organization (in the Codex Alimentarius). Table 7-2 summarizes the maximum residue limits based on IHM and WHO guidelines.

Table 7-2. Maximum Residue Limits Based on the WHO Codex Alimentarius and the Indonesia Ministry of Health Decree 1996 for meat products.

Type of pesticide	Codex Alimentarius (WHO)	Indonesian Ministry of Health Decree, 1996 (on meat)
Aldrin	0.2 mg/kg (meat other than marine mammals)	0.2 mg/kg
Dieldrin	0.2 mg/kg (meat other than marine mammals)	0.2 mg/kg
BHC		0.3 mg/kg
Lindane	2 mg/kg (meat of cattle, pigs & sheep)	0.7 mg/kg
Heptachlor	0.2 mg/kg (meat other than marine mammals)	0.2 mg/kg
Endosulfan	0.1 mg/kg (meat other than marine mammals)	0.2 mg/kg
Chlorpyrifos	0.1 mg/kg (chicken meat)	0.05 mg/kg
Propenofos	0.05 mg/kg (meat other than marine mammals)	0.05 mg/kg
Diazinon	0.02 mg/kg (chicken meat)	0.7 mg/kg

Note: mg/kg is equivalent to ppm

c. Statistical Analysis

In calculating mean residue levels, non-detected values were considered as 0. Non-parametric, multivariate tests (Multi-response Permutation Procedure with Monte Carlo resampling; MRPP/NERM; BLOSSOM version W200.3.2; Cade and Richards, 1999) were used for hypothesis testing to eliminate possible multiple-comparisons problems. Statistical analyses were performed by separately analyzing the data on residue levels in liver and leg muscle and also by considering pesticide level in liver and leg muscle together as mean pesticide level in the body. I performed MRPP/NERM tests to investigate possible differences in: (1) pesticide residues between species and (2) pesticide residues in both *F. limnocharis-iskandari* complex and *F. cancrivora* among locations. Additional tests were undertaken to examine the differences between pairs of locations such as between pesticide and non pesticide sites (Karawang NP vs Karawang P; Subang NP vs. Subang P), between locations within one region (Caringin vs. Darmaga) and between regions (Karawang vs Subang; Karawang vs Bogor; Subang vs Bogor). All tests were performed with a significance level of α=0.05.

7.2.4 Developmental Stability Analysis

A total of 278 *F. limnocharis-iskandari* complex and 85 *F. cancrivora* were captured from six locations during two periods (Sampling-1 in 2002 and Sampling-3 in 2003, see Chapter 5 for more information on the sampling regime) and measured for developmental stability analysis (Table 7-3).

Table 7-3. Numbers of frogs measured for developmental stability analysis

Location	*F. limnocharis-iskandari* complex		*F. cancrivora*	
	Male	Female	Male	Female
Caringin	39	41	6	7
Darmaga	46	27	3	5
Karawang NP	2	27	3	9
Karawang P	5	18	0	12
Subang NP	26	29	0	11
Subang P	5	13	6	23

I measured SVL, the lengths of the radio-ulna bones on the left and right sides of the forelimb, and the lengths of the tibio-fibula bones on each side of the hindlimb to the nearest 0.01 mm using digital callipers (Figure 7-1). Each frog's measurements were taken three times, and individuals were remeasured in random sequence. This measurement protocol was based on that suggested by Alford et al. (1999). The data were then analysed using a program in SAS (version 6.11) that summarises the difference between right to left measurements of each character (R-L) and calculates tests for directional symmetry, anti symmetry, measurement errors and fluctuating asymmetry based on Palmer and Strobeck (2003).

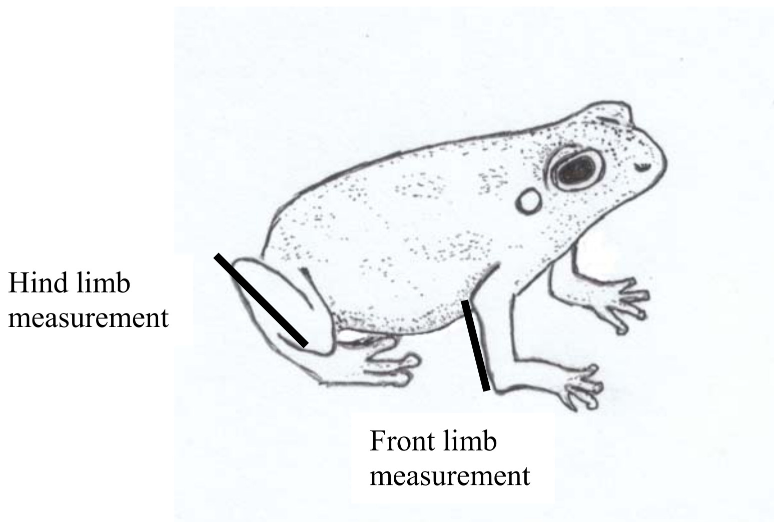


Figure 7-1. Measurement of hind limb and front limb characters of *F. limnocharis-iskandari* complex and *F. cancrivora*

To remove size effects (Palmer and Strobeck, 2003), data for *F. limnocharis-iskandari* complex were transformed by taking their logarithms and reanalysed. Differences between sexes and among sites were tested for using F-tests on levels of unsigned (absolute value) asymmetry measured for individuals. If unequal variance was detected, non-parametric tests were used instead.

To determine whether asymmetry was related to a more conventional measure of fitness, an index of body condition was calculated for each individual. The index used was the residuals from a regression of mass on snout vent length (Dyson et al., 1998). This was tested for correlation with individual values of unsigned asymmetry using Spearman rank correlation, which does not require the assumption that any relationship is linear, only that it is monotonic.

Body condition was also tested for differences between sites and sampling times using data from population study sampling-1 and sampling-2 (Chapter 5) which were taken at the same time as the measurements of fluctuating asymmetry. Whenever possible, these tests were done using ANOVA. When sets of sites or sampling times had unequal variances, tests were performed nonparametrically using Mann–Whitney *U*-tests or Kruskal-Wallis tests.

7.2.5 Chytridiomycosis

Diagnostic histology was undertaken on phalanges taken from toe clips of 103 *F. limnocharis-iskandari* complex*,* 35 *F. cancrivora* and 46 *L. macrodon*. The histological examinations were run simultaneously with the skeletochronological analysis detailed in Chapters 5 and 6, using the same samples. Clipped toes of frogs taken during field surveys were stored in 4% formalin solution. Each toe was wrapped in sponge and placed individually in a cassette, then decalcified overnight in 10% formic acid solution. Toes were then embedded in wax and cut into 10µm sections. Sections were put on slides and kept in a warming oven at least overnight. The slides were then put in water and stained with Erlich's haematoxylin for 20 minutes followed by two washes in water and two minutes in Scott's tap water substitute; they were then mounted with DPX glue. Sections were examined for the presence of amphibian chytrid zoosporangia in the outer keratin layer using standard diagnostic techniques (Berger et al., 2000).

7.3 Results

7.3.1 Morphological abnormalities

I examined a total of 4331 frogs of 23 species from two habitats (paddy fields and streams) and found 20 types of deformities. I divided deformities into two general types: developmental abnormalities and trauma abnormalities (injuries). I distinguished trauma abnormalities based on the appearance of old scars or, if they involved digits, the occurrence of digital re-growth. Developmental abnormalities occurred in limbs (amelia, micromelia, brachymelia, hemimelia, ectromelia, taumelia, cutaneous fusions), digits (ectrodactyly, brachydactyly, syndactyly, polydactyly, clinodactyly), the back-bone (scoliosis), the eyes (anophthalmy) and the skin (a lump-like tumor). Trauma included digit amputation, limb amputation, limb fractured, lip wounds and skin wounds. Table 7-4 shows the types of abnormalities found, whilst Figure 7-2 shows several deformities in frogs.

A preliminary survey in Caringin during 2001 and 2002 found abnormalities in frogs caught by professional hunters. During my first survey in September 2001 I only noted the number of frogs with abnormalities, I did not note types of abnormalities (Table 7-5).

Table 7-4. Types of abnormalities found in frogs of West Java

No	Condition	Description	Found in frogs in
1	Amelia	No evidence of limb	Paddy fields & streams
2	Micromelia	Abnormal smallness of limb/s	Paddy fields
3	Brachymelia	Short limb/s	Paddy fields
4	Hemimelia	Absence of distal portion of limb	Paddy fields
5	Ectromelia	Incomplete limb	Paddy fields & streams
6	Taumelia	Long bone oriented 90^0 out of alignment	Paddy fields
7	Cutaneous fusions	Fusion of limb long bones by skin web	Paddy fields
8	Ectrodactyly	Missing toe/s	Paddy fields & streams
9	Brachydactyly	Short toe/s	Paddy fields & streams
10	Syndactyly	Fused fingers	Paddy fields & streams
11	Bifid terminal phalanx	Presence of extra digit (form of polydactyly)	Paddy fields & streams
12	Clinodactyly	Curved digits	Paddy fields & streams
13	Anophthalmy	Absence of one or both eyes	Stream
14	Trauma – Digit amputation	Presence of scar or wound in digit/s	Paddy fields & streams
15	Scoliosis	Curve spine	Paddy fields
16	Skin	Presence of tumour like lump	Paddy fields
17	Trauma – Limb amputation	Presence of scar or wound in limb/s	Paddy fields & streams
18	Trauma – Lips scarred	Presence of scar or wound around lips	Paddy fields
19	Trauma – skin wound	Presence of scar or wound in skin	Paddy fields & streams
20	Trauma – Limb fractured	Fractured Limb bone	Paddy fields & streams

Table 7-5. Numbers of frogs showing abnormalities captured by professional frog hunters. Number in brackets is the actual number of frogs exhibiting this type of abnormality. Several frogs showed multiple types of abnormalities (*)

Species	N abnormal	N total	Type of abnormalities	Date
F. cancrivora	13	323	No data available	22 September 2001
F. limnocharis-iskandari complex	4	26	Syndactyly (3) Amely (1)	4 October 2001
F. cancrivora	15	90	Brachydactyly (2)* Ectrodactyly (5)* Syndacytyly (10)*	31 March 2002
L. macrodon	3	76	Ectrodactyly (3)	31 March 2002

Figure 7-2. Anophthalmy (no eye) in *Rana chalconota* found in Situ Gunung in 2003 (top); Trauma – limb amputation in *F. cancrivora* (left, bottom) and Developmental abnormality – Amelia in *F. cancrivora* (right, bottom)

In paddy fields, abnormalities were found in four species, *Fejervarya limnocharis-iskandari* complex (143 of 3523, 4.06%), *Fejervarya cancrivora* (22 of 361, 6.09%), *Occidozyga lima* (1 of 117, 0.85%), and *Limnonectes kuhlii* (1 of 1). At least some *F. limnocharis-iskandari* complex exhibited abnormalities on almost every sampling occasion (25 out of 29 sampling occasions), at site-specific frequencies ranging from 1.01 to 11.72%. *F. cancrivora* with abnormalities were found on 11 occasions, with frequencies ranging from 3.00 – 16.13 % (Figure 7-3 and 7-4). The other two species occurred in low numbers and few were found with abnormalities. Although a number of *L. macrodon* captured by professional hunters exhibited morphological abnormalities, no abnormalities were found during field surveys. Chi-squared homogeneity tests indicated that rates of abnormalities differed significantly among locations in *F. limnocharis-iskandari* complex (χ^2 = 44.74 *df* = 24, P = 0.0063) but did not differed significantly for *F. cancrivora* (χ^2 = 9.905 *df* = 6, P = 0.1287).

The oldest specimen of *F. limnocharis-iskandari* complex stored in the MZB that exhibited abnormalities was a juvenile frog captured on 16 November 1921 from Bogor. This specimen was missing one leg (amelia) (Table 7-6). The oldest specimen of *F. cancrivora* stored in the MZB that exhibited abnormalities was a female frog from Bogor captured on 26 July 1954 that had ectrodactyly and a bifid phalanx. Examination of individuals of these species captured by frog hunters revealed low numbers of abnormalities of several types including syndactyly, brachydactyly, ectrodactyly and amelia. No specimen of *L. macrodon* showed abnormalities.

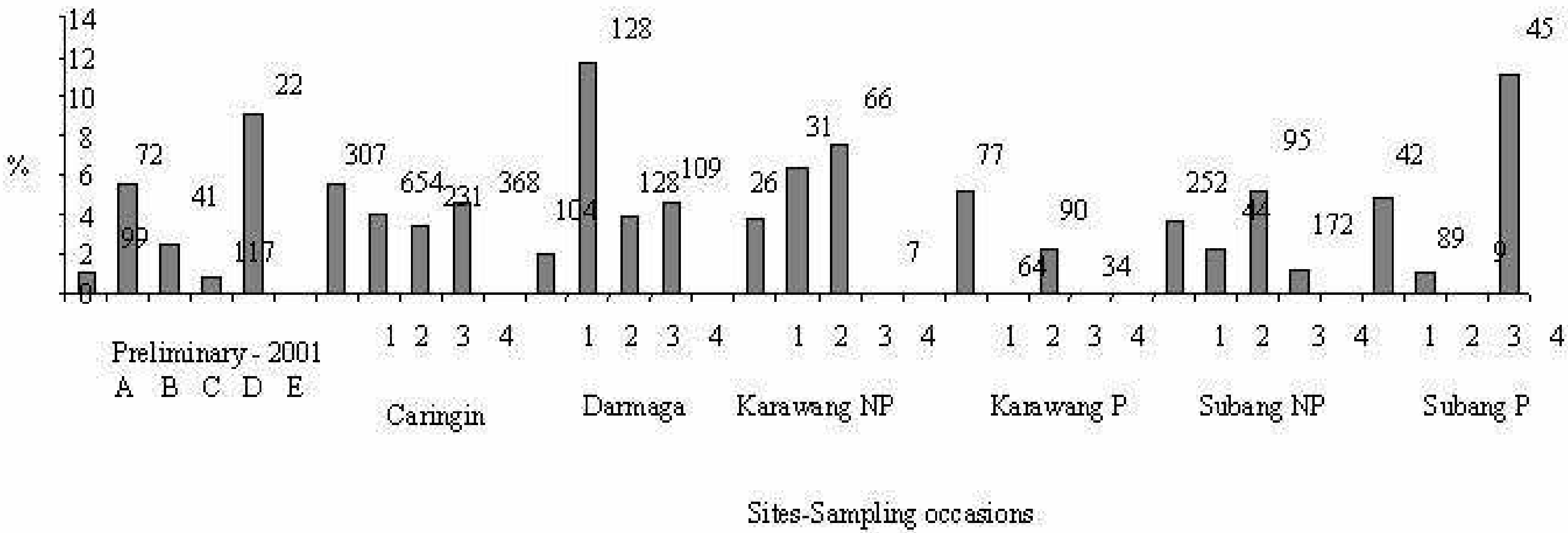


Figure 7-3. Prevalence of *F. limnocharis-iskandari* complex abnormalities per site and sampling occasions. Total sampling occasions: 29. Total sampling occasions with abnormalities = 25. Number on top of each bar refer to actual numbers of *F. limnocharis* found. Preliminary 2001 refers to preliminary surveys undertaken during 2001 with A = Caringin, B = Cisaat, C = Ciptarasa, D = Panguyangan and E = Situ Gede. Other samplings occurred the same time with population study of rice field's frog, number 1 – 4 refers to sampling 1 to 4.

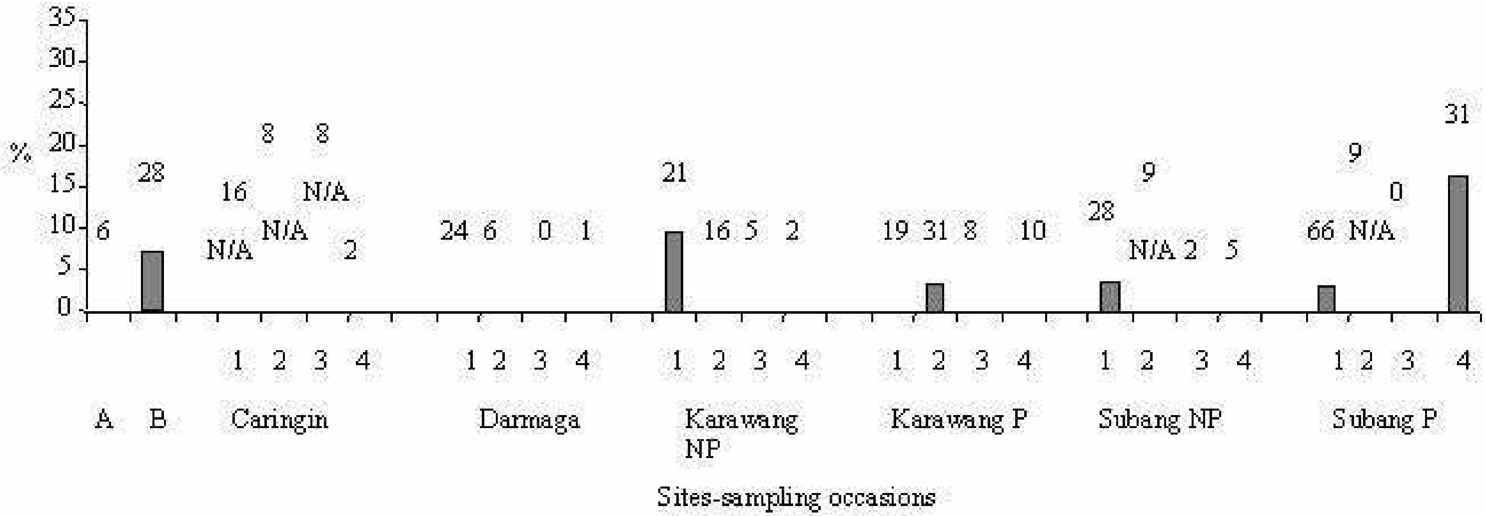


Figure 7-4. Prevalence of *F.cancrivora* abnormalities per site and sampling occasions. Total sampling occasions: 24. Total sampling occasions with abnormalities = 11. Number on top of each bar refers to actual numbers of *F.cancrivora* found. A and B refers to preliminary research in 2001, with A = Caringin, B = Situ Gede. Other samplings occurred the same time with population study of rice field's frog, number 1 – 4 refers to sampling 1 to 4.

Table 7-6. Type of morphological abnormalities found in specimens of *F. limnocharis-iskandari* complex and *F. cancrivora* stored in Museum Zoologi Bogor (MZB).

Collection Number	Collection Date	Sex	SVL (mm)	Location	Type of abnormalities
F. limnocharis-iskandari complex					
057.10	16-Nov-1921	J	10.38	Bogor	amelia
2172.8	1967	M	41.91	Bogor	brachydactyly
2097b	30/03/1971	J	26.13	Indramayu	ectrodactyly
2679.2	July-1984	F	34.06	Bogor	ectrodactyly
3360.3	August-1988	F	41.24	Bogor	ectromelia
3308 (51)	August-Sept 1988	F	39.51	Bogor	ectromelia
3308 (51)	10-Aug-1988	F	40.68	Bogor	ectromelia
5565	Agust-Sept-1997	F	32.17	Bogor	brachydactyly
3427.4	16-Jun-1997	F	46.34	Sukabumi	ectromelia
4644.16	11-Jan-1999	M	45.04	Bogor	ectromelia
5775	14-Jul-2000	M	45.19	Bogor	ectromelia
3409.19	15-Sep-2003	F	43.85	Sukabumi	amelia
F. cancrivora					
341.5	26-Jul-1954	F	88.39	Bogor	ectrodactyly & bifid phalanx
2310.1	16-Aug-1964	F	49.42	Bogor	ectromelia
2107.1	25-Sep-1969	M	75.36	Bogor	brachydactyly
3425.14	15-Jun-1997	M	69.85	Sukabumi	ectrodactyly
6921			75.97	Bogor	amelia

7.3.2 Pesticide levels

Two types of pesticide residues were detected in water and soil: organochlorines (lindane, aldrin, heptachlor, dieldrin and endosulfan) and organophospates (chlorpyrifos and diazinon). Pesticide residue levels varied among locations. Most pesticides were low or not detected at most sites. BHC and lindane (both organochlorines) were never detected in water and soil. Only dieldrin in soil was detected in all locations. Levels of residues in water and soil are presented in Table 7-7.

Table 7-7. Pesticide residue (ppm) in water and soil in 6 locations

Type of pesticide	Caringin		Darmaga		Karawang NP		Karawang P		Subang NP		Subang P	
	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil
Lindane	0.0015	0.0015	ND	ND	0.0800	0.0179	0.0092	0.0173	0.0010	0.0098	0.0109	0.0164
Aldrin	ND	ND	0.0305	ND	0.0401	ND	0.0769	0.0502	ND	0.0828	0.0670	0.0623
Heptachlor	0.0186	0.025	0.0045	ND	0.0097	0.0166	0.0138	ND	0.0015	ND	0.0139	ND
Dieldrin	ND	0.0011	ND	0.0053	ND	0.0015	ND	0.0186	0.0013	0.0014	0.0036	0.0029
Endosulfan	ND	ND	ND	ND	ND	0.0088	ND	0.0179	0.0065	0.0121	ND	ND
Chlorpyrifos	0.0105	0.0058	ND	0.0096	0.0128	0.0643	ND	ND	ND	0.0337	0.0205	0.0298
Diazinon	ND	ND	0.0212	ND	0.0256	ND	ND	0.0324	ND	0.0308	0.0238	0.0310

Note: ND = Not detected

Six organochlorines (BHC, lindane, aldrin, heptachlor, dieldrin and endosulfan) and three organophosphates (propenofos, chlorpyrifos and diazinon) were detected in the livers and leg muscles of both species. Pesticide residues in livers were higher than in leg muscles for both species. Mean levels of organochlorine residues in *F. limnocharis-iskandari* complex and *F. cancrivora* livers and leg muscles were mostly lower than the maximum residue limit set by the WHO or IHM, except for endosulfan residues in the liver of *F. limnocharis-iskandari* complex in Subang NP. However, some individual frogs exceeded maximum pesticide limits set either by the WHO or IHM for aldrin, heptachlor, dieldrin and endosulfan.

Organophospate residue limits were exceeded in some locations. Mean chlorpyrifos residue exceeded limits in *F. limnocharis-iskandari* complex livers from Subang NP, also in the livers of *F. cancrivora* from Karawang NP and Karawang P, and in the leg muscles of *F. cancrivora* from Subang P. One *F. limnocharis-iskandari* complex from Subang P also exceeded the chlorpyrifos residue limit.

At Karawang NP and Karawang P the livers of both *F. limnocharis-iskandari* complex and *F. cancrivora* had mean values of propenofos residues higher than the maximum residue limits set by the WHO and IHM. Mean values of propenofos residue for *F. cancrivora* leg muscles in the Subang P and mean residue for *F. limnocharis-iskandari* complex livers in Darmaga were also higher than the maximum residue level.

Diazinon levels in livers of *F. limnocharis-iskandari* complex from the Caringin, Karawang NP, Karawang P, Subang P sites, and also in leg muscles from Darmaga, were higher than the maximum residue limit set by the WHO. In *F. cancrivora,* mean values for diazinon exceeded the WHO standard only in liver from the Subang NP site. Summary statistics for levels of pesticide residues in both species of frogs are provided in Tables 7-8 to 7-11.

The MRPP tests indicated that levels of pesticide residues in livers and leg muscles or did not differ between the species when they were compared across all locations (P = 0.5012 for differences in mean pesticide residues; P = 0.1534 for differences of pesticide residues in liver and P = 4280 for differences of pesticide residues in muscle). However, pesticide residues in the livers of *F. limnocharis-iskandari* complex differed significantly among locations (P = 0.011), while there are no significant differences in pesticide residues in *F. cancrivora* among all locations.

Tests examining the differences between pairs of locations with and without current pesticide application showed that there were no significant differences in pesticide residues in either livers and leg muscles of *F. limnocharis-iskandari* complex and *F. cancrivora* between the Karawang NP and the Karawang P locations or between the Subang NP and the Subang P locations. There was also no significant difference in pesticide residues in both livers and leg muscles for *F. limnocharis-iskandari* complex between the Darmaga and Caringin sites.

Comparisons between regions showed that there were significant differences in pesticide residues in the livers of *F. cancrivora* (P = 0.0339), and in mean pesticide residues in both tissues of *F. limnocharis-iskandari* complex, between Karawang and Subang (P = 0.0317). However, there were no significant differences of pesticide residues in either species between Karawang and Bogor or Subang and Bogor.

Table 7-8. Mean organochlorine (ppm ± standard deviation) residue in *F. limnochari-iskandari* complex

	BHC		Lindane		Aldrin		Heptachlor		Dieldrin		Endosulfan	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Liver												
Caringin	0.0473+ 0.0497	ND-0.0991	ND	ND	0.143+ 0.1536	0.0309-0.3181*+	0.1286+ 0.2123	ND-0.3736*+	0.0598+ 0.0626	ND-0.1249	0.0393+ 0.0681	ND-0.1179+
Darmaga	ND	ND	0.1126± 0.1549	0.0062-0.2903	0.1822± 0.1612	ND-0.3066*+	0.0818± 0.0808	0.0175-0.1725	0.0879± 0.1522	ND-0.2637*+	ND	ND
Karawang NP	ND	ND	0.2184+ 0.2081	ND-0.4143	0.052+ 0.041	0.0209-0.0985	0.1288+ 0.1384	ND-0.2752*+	0.0379+ 0.0657	ND-0.1138	ND	ND
Karawang P	ND	ND	0.2363+ 0.255	ND-0.5066	0.0307+ 0.0532	ND-0.0921	0.0319+ 0.0552	ND-0.0956	0.0103+ 0.0178	ND-0.0309	ND	ND
Subang NP	0.1316± 0.0517	0.0725-0.1683	0.2018± 0.1197	0.0794-0.3186	0.087± 0.1507	ND-0.2611*+	0.0875± 0.1515	ND-0.2624*+	0.0911± 0.0904	ND-0.1807	0.2232± 0.0839*+	0.1331-0.2993*+
Subang P	0.005+ 0.0053	ND-0.0106	0.0352+ 0.0308	ND-0.0571	0.0057+ 0.0099	ND-0.0171	0.0048+ 0.0054	ND-0.0107	0.005+ 0.0087	ND-0.015	0.0599+ 0.0638	ND-0.127+
Leg muscle												
Caringin	ND	ND	0.0023+ 0.002	ND-0.0039	ND	ND	0.0023+ 0.0022	ND-0.0043	ND	ND	0.0243+ 0.0035	0.0203-0.0264
Darmaga	ND	ND	0.0216+ 0.0373	ND-0.0647	0.0153+ 0.0264	ND-0.0458	0.0189+ 0.0327	ND-0.0567	ND	ND	0.0184+ 0.0319	ND-0.0552
Karawang NP	ND	ND	0.0186± 0.0323	ND-0.0559	0.007± 0.0122	ND-0.0211	0.0199± 0.0188	ND-0.0373	0.0013± 0.0022	ND-0.0038	0.0169± 0.0293	ND-0.0507
Karawang P	ND	ND	ND	ND	0.0086± 0.0149	ND-0.0259	0.0359± 0.0474	ND-0.0896	0.0006± 0.001	ND-0.0018	0.0021± 0.0036	ND-0.0062
Subang NP	0.0093+ 0.0143	ND-0.0258	ND	ND	ND	ND	0.0133+ 0.0159	0.0017-0.0314	ND	ND	0.0337+ 0.0329	0.011-0.0715
Subang P	0.0048± 0.0143	ND-0.0145	0.0143± 0.0248	ND-0.0429	ND	ND	0.0018± 0.00312	ND-0.0054	0.0375± 0.0503	ND-0.0946	0.0964± 0.0451	0.0592-0.1466+

Note : ND= Not detected;* exceeds Indonesian Ministry Health Decree, 1996 based on pesticide residue in meat,
+exceeds WHO codex alimentarius

Table 7-9. Mean Organophosphate residue (ppm ± standard deviation) in *F. limnocharis-iskandari* complex

	Chlorpyrifos		Propenofos		Diazinon	
Liver	Mean	Range	Mean	Range	Mean	Range
Caringin	ND	ND	0.0467+0.0809	ND-0.1402*+	0.0467+0.0809+	ND-0.1401+
Darmaga	ND	ND	0.0628±0.1087*+	ND-0.1883*+	ND	ND
Karawang NP	ND	ND	0.1434+0.1242*+	ND-0.2158*+	0.0328+0.0569+	ND-0.0985+
Karawang P	ND	ND	0.2953+0.3105*+	ND-0.619*	0.0556+0.0962+	ND-0.1667+
Subang NP	0.099±0.0872*	ND-0.1647*	0.1785±0.3091*+	ND-0.5354*+	0.0242±0.042+	ND-0.0727+
Subang P	0.0039+0.0068	ND-0.0118	ND	ND	ND	ND
Leg muscle						
Caringin	0.0018±0.0032	ND-0.0055	ND	ND	ND	ND
Darmaga	0.0014±0.0024	ND-0.0042	ND	ND	0.0278±0.0298+	ND-0.0592+
Karawang NP	0.0059±0.0104	ND-0.01795	0.0019±0.0033	ND-0.0058	ND	ND
Karawang P	0.0036±0.0062	ND-0.0107	0.0016±0.0028	ND-0.0049	0.0106±0.0103	ND-0.0205+
Subang NP	ND	ND	0.0301±0.0521	ND-0.0903*+	ND	ND
Subang P	0.0483±0.054	ND-0.1065*	ND	ND	ND	ND

Note : ND= Not detected; * exceeds Indonesian Ministry Health Decree, 1996 based on pesticide residue in meat;
+ exceeds WHO codex alimentarius

Table 7-10. Mean Organochlorine pesticide residue (ppm ± standard deviation) in *F. cancrivora*

	BHC		Lindane		Aldrin		Heptachlor		Dieldrin		Endosulfan	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Liver												
Caringin	0.0045 ± 0.0039	ND-0.007	0.0132 ± 0.004	0.0096-0.0175	ND	ND	0.0426 ± 0.0708	ND-0.1243	0.019 ± 0.0271	ND-0.05	0.0047 ± 0.0081	ND-0.0141
Karawang NP	ND	ND	0.3204 + 0.504	ND-0.9014*	ND	ND	ND	ND	0.0641 + 0.1111	ND-0.1924	ND	ND
Karawang P	ND	ND	0.1105 ± 0.1043	ND-0.2072	0.0342 ± 0.0593	ND-0.2072*	0.0675 ± 0.0663	ND-0.1326	0.0247 ± 0.0427	ND-0.074	0.1513 ± 0.2621	ND - 0.454 *
Subang NP	0.0381 ± 0.0353	ND-0.0696	0.2421 ± 0.3415	ND-0.6328	0.0162 ± 0.028	ND-0.0485	0.0226 ± 0.0391	ND-0.0677	0.00927 ± 0.016	ND-0.0278	0.0809 ± 0.093	0.0158-0.1877
Subang P	0.0167 + 0.0289	ND-0.0501	0.0266 + 0.0217	0.0096-0.0511	ND	ND	0.0012 + 0.002	ND-0.0035	0.0304 + 0.0365	0.007-0.0725	0.0047 + 0.0081	ND-0.0141
Leg muscle												
Caringin	0.0009 ± 0.0017	ND-0.0029	0.0017 ± 0.0016	ND-0.0031	ND	ND	0.0039 ± 0.0031	0.0008-0.007	ND	ND	0.0055 ± 0.0088	ND-0.0157
Karawang NP	ND	ND	0.0108 + 0.0039	0.0067-0.0145	0.004 + 0.0069	ND-0.012	0.004 + 0.0026	0.0016-0.0067	0.0046 + 0.0051	ND-0.0101	0.0129 + 0.0205	ND-0.0365
Karawang P	ND	ND	0.0112 ± 0.0131	ND-0.0256	0.0106 ± 0.0184	ND-0.0319	0.0029 ± 0.0025	ND-0.0043	ND	ND	0.0042 ± 0.0045	ND-0.0089
Subang NP	ND	ND	0.0037 ± 0.0065	ND-0.0112	ND	ND	0.0049 ± 0.0084	ND-0.0146	ND	ND	0.0154 ± 0.0248	ND-0.044
Subang P	ND	ND	0.0128+0.0113	ND-0.0213	0.1449 + 0.1493	ND-0.2982*	0.0197 + 0.0275	ND-0.0511	0.0831 + 0.1168	ND-0.2167*	0.0092 + 0.0159	ND-0.0276

ND= Not detected; * exceeds Indonesian Ministry Health Decree, 1996 based on pesticide residue in meat; + exceeds WHO codex alimentarius

Table 7-11. Mean organophosphate pesticide residue (ppm ± standard deviation) in *F. cancrivora*

Type of pesticide	Chlorpyrifos		Propenofos		Diazinon	
Liver	Mean	Range	Mean	Range	Mean	Range
Caringin	ND	ND	0.0364±0.063	ND-0.1092*ˑ	0.0188±0.0276	ND-0.0505
Karawang NP	0.4897±0.4899*ˑ	ND-0.9798*ˑ	0.1855±0.1897*ˑ	ND-0.3791*ˑ	ND	ND
Karawang P	0.1171±0.2028*ˑ	ND-0.3514*ˑ	0.2129±0.3687*ˑ	ND-0.6387*ˑ	ND	ND
Subang NP	0.0059±0.0103	ND-0.0178	0.0119±0.0206	ND-0.0357	0.0245±0.0217ˑ	ND-0.0413ˑ
Subang P	ND	ND	0.0058±0.01	ND-0.0174	0.0125±0.0094	0.006-0.0233ˑ
Leg muscle						
Caringin	0.0044±0.0076	ND-0.0131	0.0025±0.0043	ND-0.0074	ND	ND
Karawang NP	0.01857±0.0322	ND-0.0557*	0.0005±0.0009	ND-0.0016	ND	ND
Karawang P	0.0075±0.0065	ND-0.0119	ND	ND	0.0104±0.0181	ND-0.0313
Subang NP	0.0147±0.013	ND-0.0244	0.0083±0.0143	ND-0.0248	ND	ND
Subang P	0.102±0.1379*ˑ	ND-0.2588*ˑ	0.0695±0.0747*ˑ	ND-0.1486*ˑ	ND	ND

Note: ND= Not detected;* exceeds Indonesian Ministry Health Decree, 1996 based on pesticide residue in meat;
ˑ exceeds WHO codex alimentarius

Tables 7-12 and 7-13 summarise the results of comparisons of levels of pesticide residues for both species among locations, and Figures 7-5 to 7-10 show the mean pesticide residues in water, soil and frogs in six locations.

Table 7-12. Differences of pesticide residues between pair of location

Questions	Karawang NP vs. Karawang P	Subang NP vs. Subang P	Caringin vs. Darmaga
Is there a significant difference of pesticide residue content in liver and leg muscle in *F. limnocharis-iskandari* complex?	NO	NO	YES
Is there a significant difference of pesticide residue content in liver and leg muscle in *F. cancrivora?*	NO	NO	N/A

N/A = not available, there is no sample for pesticide content in *F. cancrivora* in Darmaga

Table 7-13. Differences of pesticide residues between pair of region

Questions	Karawang vs. Subang	Karawang vs. Bogor	Subang vs. Bogor
Is there a significant difference of pesticide residue content in liver in *F. limnocharis-iskandari* complex?	NO	NO	NO
Is there a significant difference of pesticide residue content in leg muscle in *F. limnocharis-iskandari* complex?	NO	NO	NO
Is there a significant difference of mean pesticide residue content in *F. limnocharis-iskandari* complex?	YES	NO	NO
Is there a significant difference of pesticide residue content in liver in *F. cancrivora?*	YES	NO	NO
Is there a significant difference of pesticide residue content in leg muscle in *F. cancrivora?*	NO	NO	NO
Is there a significant difference of mean pesticide residue content in *F. cancrivora?*	NO	NO	NO

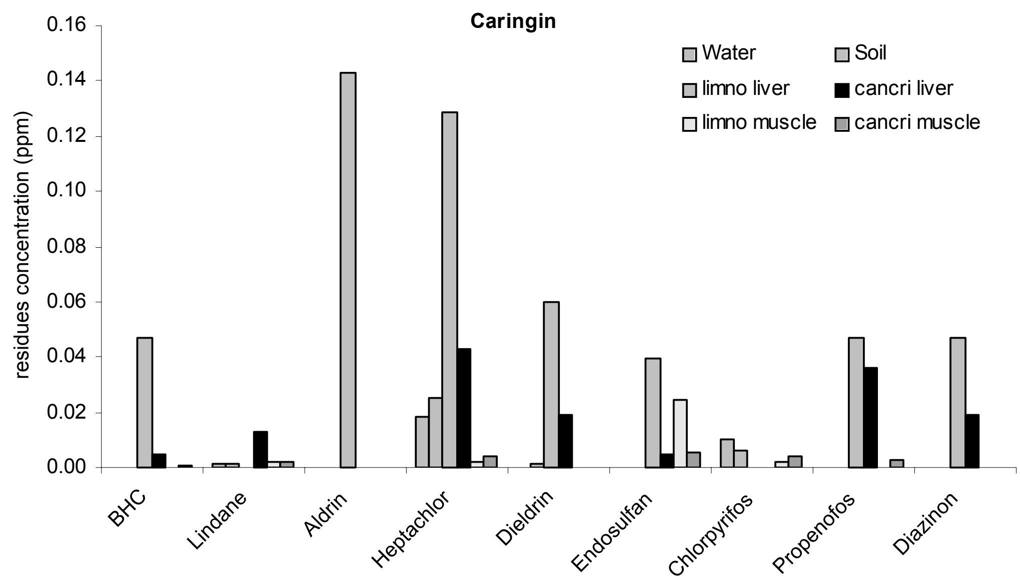


Figure 7-5. Mean pesticide residues (ppm) in water, soil, livers and leg muscles of *F. limnocharis-iskandari* complex and *F. cancrivora* in Caringin

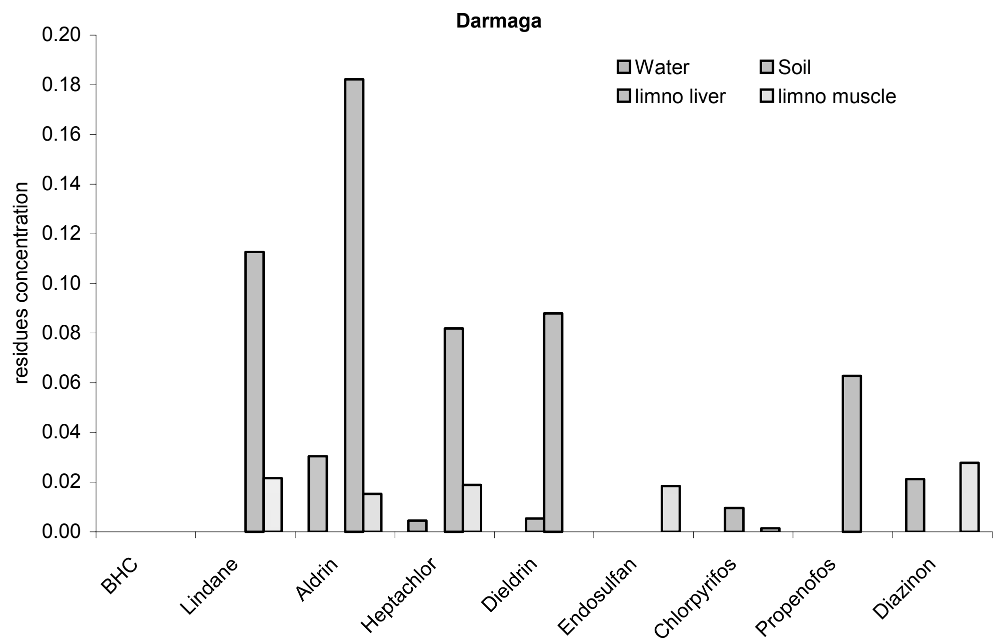


Figure 7-6. Mean pesticide residues (ppm) in water, soil, livers and leg muscles of *F. limnocharis-iskandari* complex and *F. cancrivora* in Darmaga

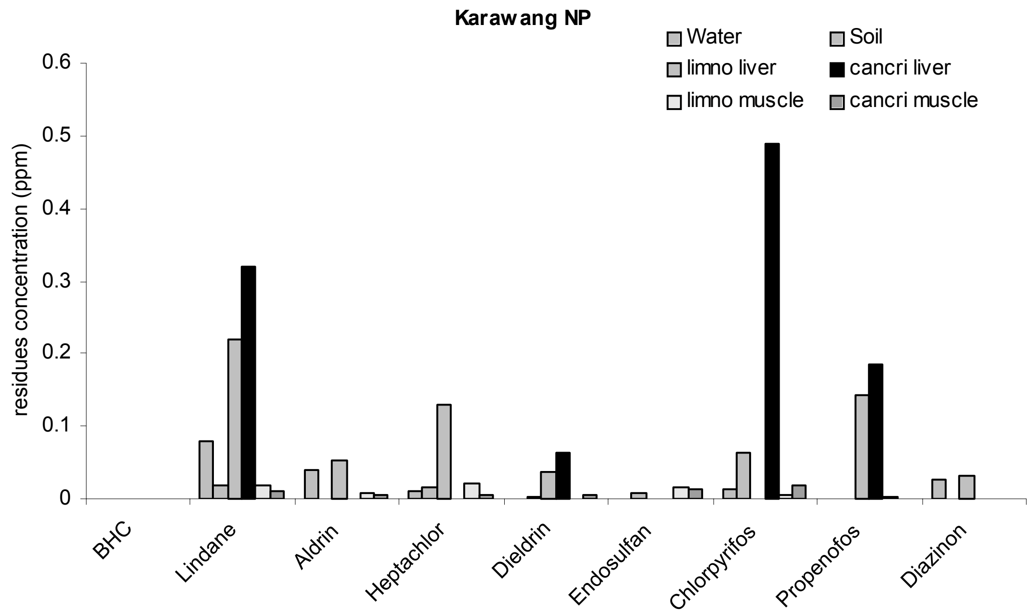


Figure 7-7. Mean pesticide residues (ppm) in water, soil, livers and leg muscles of *F. limnocharis-iskandari* complex and *F. cancrivora* in Karawang NP

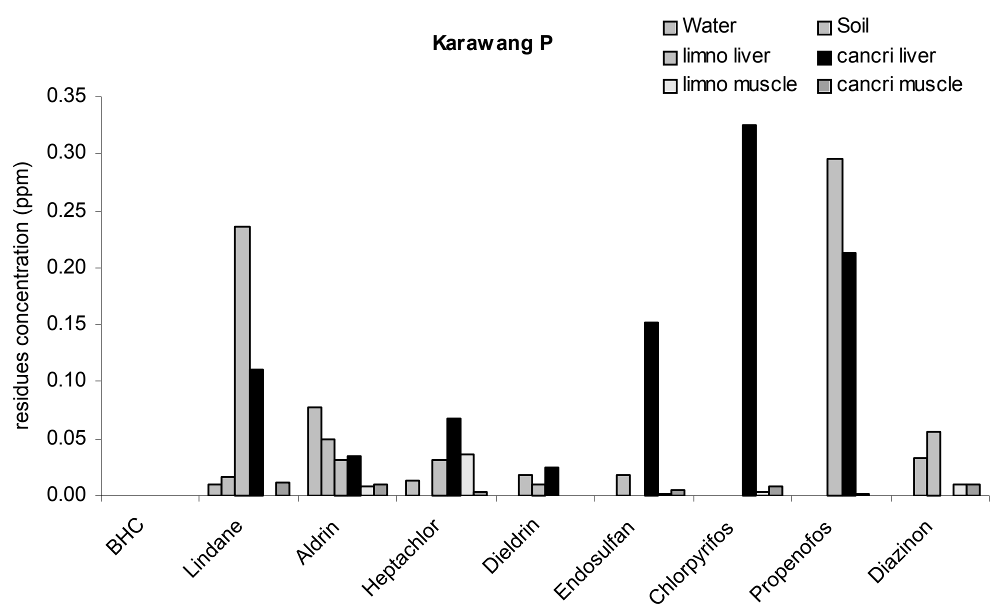


Figure 7-8. Mean pesticide residues (ppm) in water, soil, livers and leg muscles of *F. limnocharis-iskandari* complex and *F. cancrivora* in Karawang P

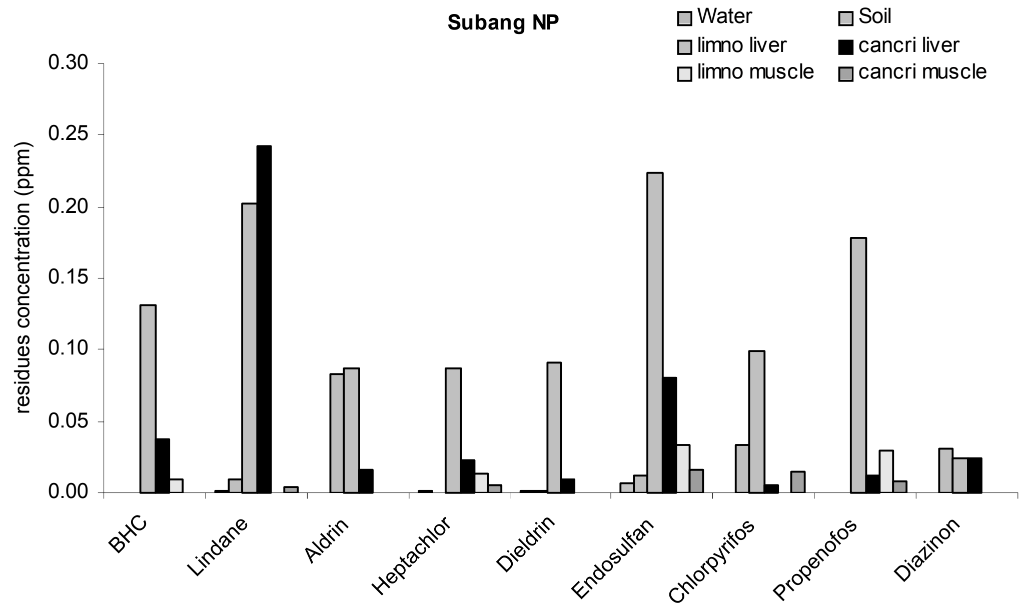


Figure 7-9. Mean pesticide residues (ppm) in water, soil, livers and leg muscles of *F. limnocharis-iskandari* complex and *F. cancrivora* in Subang NP

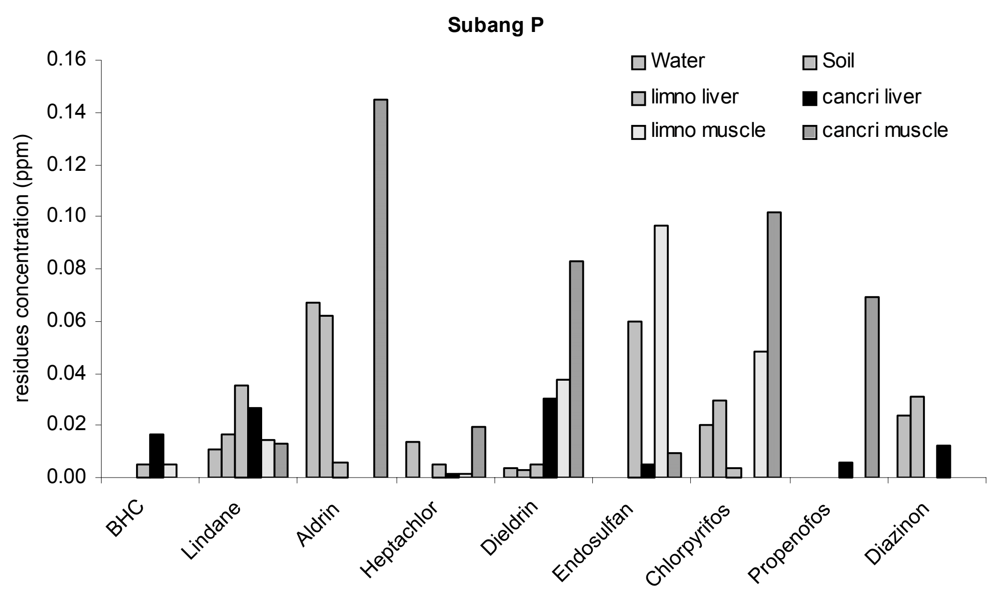


Figure 7-10. Mean pesticide residues (ppm) in water, soil, livers and leg muscles of *F. limnocharis-iskandari* complex and *F. cancrivora* in Subang P

7.3.3 Fluctuating Asymmetry

Overall results for FA appear in tables 7-14 to 7-17. Analyses of log transformed data showed that there is no directional asymmetry present in either sex of *F. limnocharis-iskandari* complex in 2002 and 2003. FA plus antisymmetry formed a significant proportion of the measured departures from symmetry in *F. limnocharis-iskandari* complex but not in *F. cancrivora*. I therefore excluded *F. cancrivora* from further analyses.

The level of unsigned asymmetry for each character in *F. limnocharis-iskandari* complex during 2002 and 2003 did not significantly differ between males and females (Table 7-18). In *F. limnocharis-iskandari* complex, forelimbs were significantly more asymmetrical than hindlimbs at all locations during 2002 and 2003 (Table 7-19). Figures 7-11 and 7-12 show the mean asymmetries $\pm$ 95 % confidence intervals in each characters for *F. limnocharis-iskandari* complex.

ANOVAs comparing levels of unsigned asymmetry across years with location disregarded showed that levels of asymmetry were higher in male forelimbs ($F_{1,109}$ = 17.733; P = 0.000) and hindlimbs ($F_{1,109}$ = 5.770; P = 0.018) in 2002 than in 2003; while for females it was higher in forelimbs ($F_{1,154}$ = 10.970; P = 0.001) in 2002 than in 2003 but hindlimbs did not differ significantly ($F_{1,154}$ = 0.003; P = 0.954). Table 7-20 details the outcomes of tests for differences in levels of asymmetry between years in *F. limnocharis-iskandari* complex from each location.

Table 7-14. Palmer and Strobeck (2003) Fluctuating Asymmetry (FA10A), measurement error (ME2), tests for significance of directional asymmetry (DA) and FA + Antisymmetry for each character of *F. limnocharis-iskandari* complex. Measurements not transformed

Year	Sex	N	Hind Limb FA	Hind Limb ME	Hind Limb DA (P value)	Hind Limb FA + Antisym (P value)	Fore Limb FA	Fore Limb ME	Fore Limb DA (P Value)	Fore Limb FA + Antisym (P value)
2002	F	63	0.11001	0.23607	0.11119	0.0004165	0.15193	0.29310	0.96290	0.0000395
2002	M	55	0.31338	0.26968	0.21324	0.0000000	0.20606	0.29310	0.20702	0.0000000
2003	F	88	0.13091	0.20905	0.00690	0.0096385	0.08134	0.17277	0.05781	0.0000042
2003	M	66	0.07880	0.21675	0.04891	0.0000000	0.06268	0.16024	0.72152	0.0098451

Table 7-15. Palmer and Strobeck (2003) Fluctuating Asymmetry (FA10A), measurement error (ME2), tests for significance of directional asymmetry (DA) and FA + Antisymmetry for each character of *F. limnocharis-iskandari* complex. Measurements log transformed

Year	Sex	N	Hind Limb FA	Hind Limb ME	Hind Limb DA (P value)	Hind Limb FA + Antisym (P value)	Fore Limb FA	Fore Limb ME	Fore Limb DA (P Value)	Fore Limb FA + Antisym (P value)
2002	F	63	0.0023418	0.0049654	0.16401	0.000336	0.007481	0.016182	0.64942	0.000398
2002	M	55	0.0073043	0.0057836	0.20424	0.000000	0.010849	0.011505	0.16661	0.000000
2003	F	88	0.0027225	0.0046239	0.16401	0.000000	0.004590	0.008561	0.09996	0.000001
2003	M	66	0.0017115	0.0050032	0.20424	0.016962	0.002785	0.009838	0.76741	0.061062

Table 7-16. Palmer and Strobeck (2003) Fluctuating Asymmetry (FA10A), measurement error (ME2), tests for significance of directional asymmetry (DA) and FA + Antisymmetry for each character of *F. cancrivora*. Measurements not transformed

Year	Sex	N	Hind Limb				Fore Limb			
			FA	ME	DA (P value)	FA + Antisym (P value)	FA	ME	DA (P Value)	FA + Antisym (P value)
2002	F	59	0.00433	0.00567	0.06964	0.00000	0.006369	0.01176	0.02995	0.00004
2002	M	13	0.00274	0.00659	0.15292	0.09601	0.005402	0.01475	0.07867	0.13958
2003	F	6	0.09432	0.20713	0.20250	0.14839	0.025672	0.18652	0.34597	0.42164
2003	M	3	0.19688	0.15238	0.400091	0.01267	-	0.23748	0.91836	0.88294

Table 7-17. Palmer and Strobeck (2003) Fluctuating Asymmetry (FA10A), measurement error (ME2), tests for significance of directional asymmetry (DA) and FA + Antisymmetry for each character of *F. cancrivora*. Measurements log transformed

Year	Sex	N	Hind Limb				Fore Limb			
			FA	ME	DA (P value)	FA + Antisym (P value)	FA	ME	DA (P Value)	FA + Antisym (P value)
2002	F	59	0.21749	0.22759	0.10377	0.00000	0.11201	0.20844	0.14286	0.00004
2002	M	13	0.11341	0.30486	0.16528	0.13374	0.12655	0.29283	0.05099	0.08282
2003	F	6	0.00112	0.00315	0.22273	0.23124	0.00165	0.00649	0.38037	0.32558
2003	M	3	0.00250	0.00196	0.39393	0.01319	-	0.00592	0.78196	0.86225

Table 7-18. Analysis of variances (ANOVA) for differences of unsigned asymmetry between sexes in *F. limnocharis-iskandari* complex

Year	Front limb	Hind limb
2002	$F_{1,120} = 0.212$, P = 0.646	$F_{1,118} = 3.120$, P = 0.080
2003	$F_{1,154} = 1.043$, P = 0.309	$F_{1,154} = 0.916$, P = 0.340

Table 7-19. Results of Kruskal Wallis tests for significance of differences between asymmetry of fore- and hindlimbs in each class of individuals of *F. limnocharis-iskandari* complex in 2002 and 2003.

Sex	Year	F	P
Male	2002	$\lambda^2 = 6.203$, *df* = 1	0.013*
	2003	$\lambda^2 = 13.315$, *df* = 1	0.000**
Female	2002	$\lambda^2 = 25.015$, *df* = 1	0.000**
	2003	$\lambda^2 = 18.476$, *df* = 1	0.000**

* Differences significant at the 0.05 level** Differences significant at the 0.01 level

Table 7-20. Results of one-way test of means differences of mean asymmetry of *F. limnocharis-iskandari* complex between year 2002 and 2003

Sites	Sex	Front limb		Hind limb	
		F	P	F	P
Caringin	Male	$F_{1,32} = 5.708$	0.023*	$F_{1,32} = 0.540$	0.468
	Female	$F_{1,40} = 8.986$	0.0005**	$F_{1,40} = 0.106$	0.747
Darmaga	Male	$F_{1,37} = 2.281$	0.139	$F_{1,37} = 4.176$	0.048*
	Female	$F_{1,25} = 12.053$	0.002**	$F_{1,25} = 0.112$	0.741
Karawang NP	Female	$F_{1,25} = 1.861$	0.185	$F_{1,25} = 0.497$	0.487
Karawang P	Male	$F_{1,4} = 19.978$	0.021*	$F_{1,4} = 35.317$	0.010*
	Female	$F_{1,16} = 0.598$	0.451	$F_{1,16} = 2.111$	0.166
Subang NP	Male	$F_{1,24} = 4.827$	0.038*	$F_{1,24} = 3.315$	0.081
	Female	$F_{1,27} = 0.211$	0.650	$F_{1,27} = 0.063$	0.804
Subang P	Female	$F_{1,11} = 0.917$	0.359	$F_{1,1} = 0.179$	0.680

* Difference significant at the 0.05 level

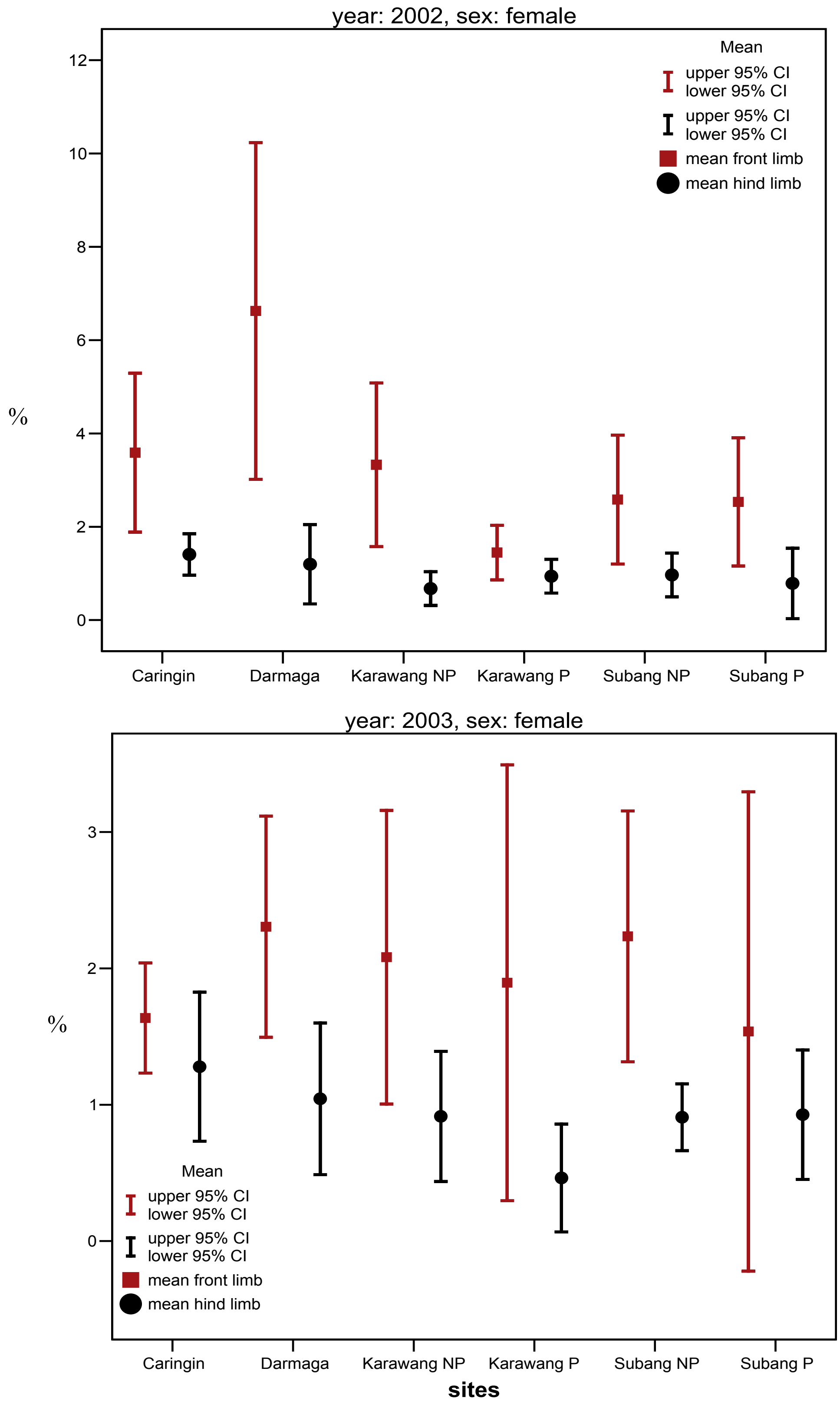


Figure 7-11. Percent asymmetry of female *F. limnocharis-iskandari* complex during 2002 and 2003

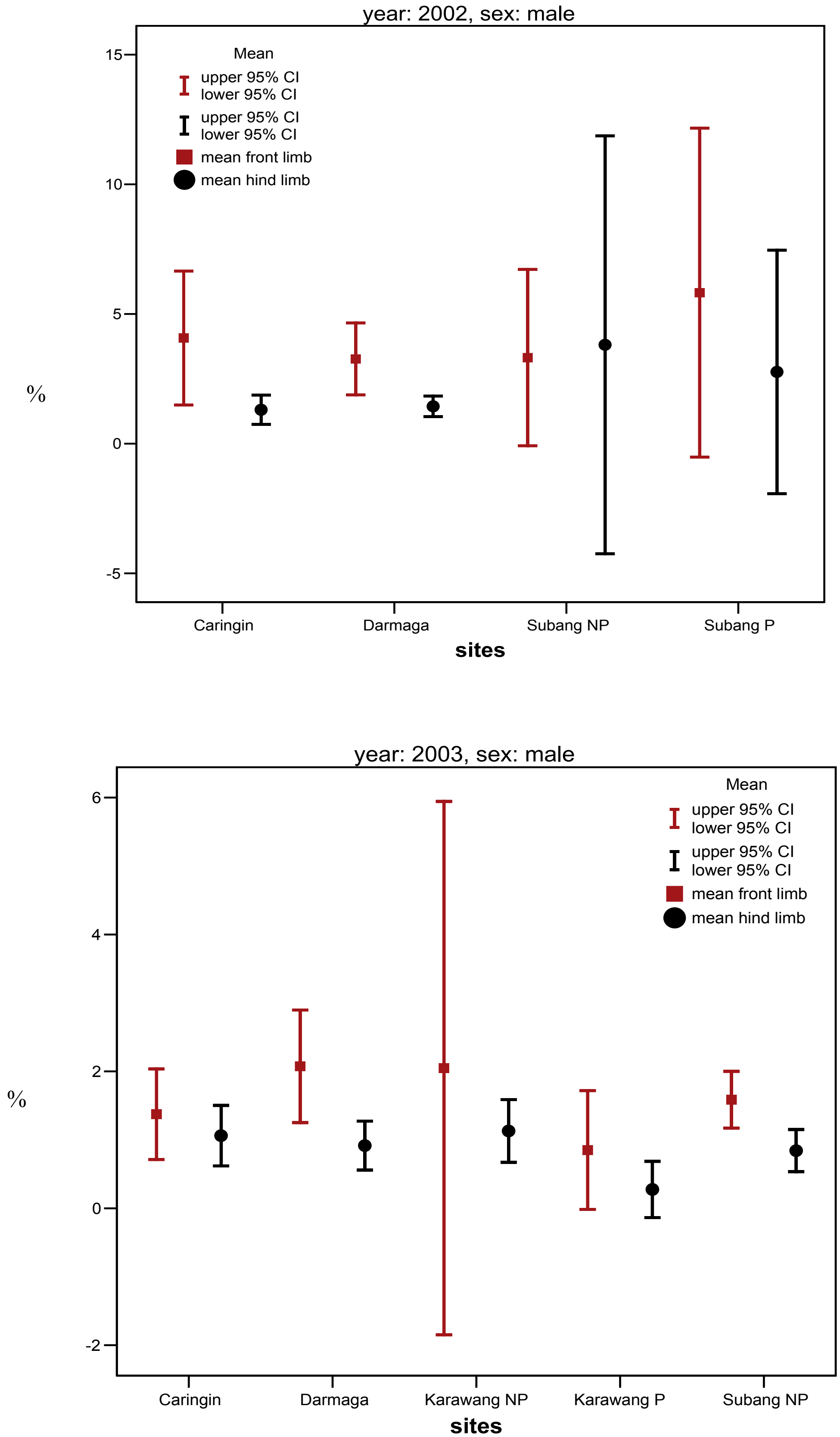


Figure 7-12. Percent asymmetry of male *F. limnocharis-iskandari* complex during 2002 and 2003

For both characters, I tested each sex separately for differences in asymmetry among sites following a stepwise procedure. Initially, I grouped the locations into three regions: Bogor (Caringin and Darmaga), Karawang (Karawang NP and Karawang P) and Subang (Subang NP and Subang P). Pairwise tests for each character in each sex of each species between regions indicated that there were no significant differences in any character or sex between the Karawang and Subang regions; I therefore combined the data for Karawang and Subang into one region (Karawang/Subang) and tested it against Bogor. With this grouping, there were significant differences of mean asymmetry in both sexes of *F. limnocharis-iskandari* complex between the two regions. Table 7-21 summarises these differences.

Table 7-21. Results of tests for differences in mean asymmetry of *F. limnocharis-iskandari* complex between the Bogor region and the combined Karawang/Subang region

Sex	Year	Front limb	Hind limb
Male	2002	$F_{1,44} = 2.470$, $P = 0.123$	$F_{1,44} = 0.509$, $P = 0.479$
	2003	$F_{1,62} = 1.082$, $P = 0.164$	$F_{1,62} = 1.078$, $P = 0.303$
Female	2002	$F_{1,43} = 6.199$, $P = 0.017$*	$F_{1,43} = 8.684$, $P = 0.005$**
	2003	$F_{1,84} = 5.579$, $P = 0.020$*	$F_{1,84} = 0.961$, $P = 0.330$

*Difference significant at the 0.05 level; ** Difference significant at the 0.01 level

Using data from the population study of *F limnocharis-iskandari* complex from sampling-1 (2002) and sampling-3 (2003), I found significant correlations between body condition and snout vent length (r_s= -.169, n = 1103, P < 0.0001). Analysis of variance showed that body condition differed among males, females, and juveniles ($F_{2,1100} = 8.483$, $P < 0.0001$). The condition indices of males and females were similar, while juveniles tended to have higher body condition index values.

A more detailed analysis showed that significant differences in body condition among the classes of individuals of *F. limnocharis-iskandari* complex occurred in Caringin sampling-1, Darmaga sampling-3, Karawang P sampling-1 and 3, and in sampling-1 at Subang P (Table 7-22).

As with asymmetry, body condition differed among sites (Table 7-23) and between sampling times. Overall, there were significant difference in body condition among all classes of individuals (females, males and juveniles) between 2002 and 2003 (t_{275} = 3.251, P = 0.001 for females; t_{308} = 7.029, P < 0.001 for males and t_{514}= 2.614, P = 0.009 for juveniles). The body condition of all classes was lower in 2003.

Table 7-22. Results of Kruskal-Wallis tests for differences in body condition among males, females, and juveniles of *F. limncoharis* in six locations at one or two sampling times.

Location	Sampling	Chi-square	Df	Significant
Caringin	1	9.604	2	0.008**
	3	1.397	2	0.497
Darmaga	1	4.600	2	0.100
	3	7.008	2	0.030*
Karawang NP	1	2.622	2	0.270
	3	3.226	2	0.199
Karawang P	1	6.668	2	0.036*
	3	110.132	2	0.006**
Subang NP	1	1.273	2	0.529
	3	2.796	1	0.095
Subang P	1	6.823	2	0.033*

* Difference significant at the 0.05 level ** Difference significant at the 0.01 level

Table 7-23. Results of Kruskal-Wallis tests for differences of in body condition index of *F. limnocharis-iskandari* complex among sites.

Sex	Sampling	Chi-Square	Df	P
Female	1	6.452	5	0.265
	3	23.155	5	<0.001
Male	1	13.496	5	0.019
	3	63.207	4	<0.000

Spearman correlations examining possible relationships between individual body condition and developmental instability levels, as indexed by unsigned fore- and hindlimb asymmetry, demonstrated no significant relationship for either the forelimbs (r_s = –0.013, n = 262, P = 0.840) or the hindlimbs of F. *limnocharis-iskandari* complex (r_s = 0.026, n = 262, P = 0.674).

7.3.4 Chythridiomycosis

I examined prepared sections of the toes of 184 individuals (103 *F. limnocharis-iskandari* complex*,* 35 *F. cancrivora* and 46 *L. macrodon*. and found no sign of chytrid infection in any of the samples. All samples had thin layers of outer epidermal keratin, which is typical of uninfected frogs.

7.4 Discussion

Results on the frequencies of morphological abnormalities indicated that the frequencies of abnormalities in *F. limnocharis-iskandari* complex and *F. cancrivora* in West Java were relatively low and probably within the normal range (Tyler, 1999 and Oullet, 2000), although in some cases there were elevated percentages of abnormalities (>5%). The other two species (*Occidozyga lima* and *Limnonectes kuhlii*) in which abnormalities were detected had sample sizes too small for meaningful estimates of the frequency of abnormalities. Because my data were collected almost exclusively from late juvenile and adult frogs, I might have found higher frequencies in *F. limnocharis-iskandari* complex and *F. cancrivora* if I had sampled tadpoles or immediately post-metamorphic frogs, reducing or eliminating the probable effects of differential survival.

Residues of many pesticides were present, some at relatively elevated levels. The existing pesticide residues in soil, water and frogs reflect the high level of pesticides used in the recent past, and in some cases at present, by farmers in the habitat. Research by Ginoga in the Sukamandi rice fields in Subang (1999) revealed that all her farmer respondents (n=60) used insecticides, sometimes more than one kind. They were using primarily organophosphates, carbamates and pyrethroids (Ginoga, 1999). Although most organochlorine pesticides are now usually not used around the world because of their persistence and impact on health and the environment (Carson, 1962; Abdullah, 1991) and have been replaced by organophosphates, a small number of farmers in the Subang area (5%) still used organochlorines as of 1998 (Ginoga, 1999). The occurrence of organochlorine residues in my study area could result from current use of this type of pesticide. However, organochlorines have long periods of persistence in the environment (Carson, 1962; Quisey et al., 1995, VanWyk et al., 2001; Smith and Gangolli, 2002). Concentrations of organochlorines detected therefore reflect cumulative patterns of use over an extended time period. Different levels of organochlorine residues might have been found if adipose tissues, in which these lipophilic substances accumulate, had been sampled in addition to muscle and liver (Ayas et al., 1997).

Some individual frogs contained high levels of organochlorine and organophosphate residues. Ginoga's research in Subang (1999) revealed that organochlorine residues were also high in water birds. For instance, Aldrin residues in the livers of *F. limnocharis-iskandari* complex frogs in two areas (Darmaga 0.1822 ppm; Caringin 0. 143 ppm) and *F. cancrivora* leg muscles in Subang P (0.1449 ppm) were almost the same as the mean aldrin residues in livers of black cormorants and little egrets (respectively 0.12156 ppm and 0.15815 ppm) that resided in the Sukamandi paddy fields in Subang (Ginoga, 1999). Residues of other pesticides were generally lower in frogs than in water birds (Table 7-24). In a rice field habitat, water birds occupy a higher trophic level than frogs. The comparison of my results with those of Ginoga (1999) in Table 7-24 suggests that in general, concentrations of pesticide residues increase along the trophic chain in the Subang region (Table 7-24).

Table 7-24. Mean pesticide residues in water and soil, and in the livers of frogs and water birds in Subang. Data for water birds taken from Ginoga's (1999) research in Sukamandi rice fields; Data on water, soil and frogs from the Subang P rice field site.

Type of pesticide	Water	Soil	*F. limnocharis-iskandari* complex	*F. cancrivora*	Black cormorant	Little egret	Pond Heron
BHC	ND	ND	0.0050	0.0167	0.1776	0.0294	0.0308
Lindane	0.0109	0.0164	0.0352	0.0266	-	-	-
Aldrin	0.0670	0.0623	0.0057	ND	0.1216	0.1582	0.0783
Heptachlor	0.0139	ND	0.0048	0.0012	-	-	-
Dieldrin	0.0036	0.0029	0.0050	0.0304	0.0319	ND	0.0392
Endosulfan	ND	ND	0.0599	0.0047	0.0985	0.0789	0.0344
Chlorpyrifos	0.0205	0.0298	0.0039	ND	0.0924	0.2757	0.0535
Propenofos	ND	ND	ND	0.0058	-	-	-
Diazinon	0.0238	0.0310	ND	0.0125	0.1193	ND	0.0999

The reason that the non-pesticide and pesticide rice fields in the Subang and Karawang areas did not differ significantly in pesticide residue levels could be that the total areas of rice fields where pesticides are not presently used were small, and they adjoined other rice fields where pesticides are still in use. They also shared irrigation channels, so that water runoff from pesticide fields could enter non-pesticide fields. In fact, it could be suggested that in terms of pest reduction, non-pesticide rice fields were actually benefiting from the pesticide rice fields because they were getting similar concentrations pesticides without the need to buy them. The lack of differences in concentrations in frog tissues could reflect movements of frogs between fields, as demonstrated in chapter 5. Frogs frequently move between blocks of rice fields, leaving dry blocks for those with higher water levels, and are thus likely to move between fields where pesticides are not applied and those where they are.

In terms of food products, the detection of some residues in the leg muscles of both species of rice field's frog indicates that frog legs originating from rice fields could pose a health risk for human consumption. I suggest further pesticide monitoring in this area to obtain further data on levels of pesticide residues in frogs and the environment. These may suggest that pesticide use should be moderated to minimize potential adverse impacts on human health and the environment.

My results showed that levels of asymmetry differed between characters. The same result was also mentioned by McCoy and Harris (2003) in salamanders. For fluctuating asymmetry in the fields, I suggest measuring multiple characters to gather more information.

Unsigned asymmetry did not differ between the Karawang or Subang pesticide and non-pesticide sites for either the forelimbs or the hindlimbs of either sex of *F. limnocharis-iskandari* complex. These results are in accordance with the finding that there are no differences in pesticide residue levels between the non-pesticide and pesticide sites. Frogs in Indonesian rice fields have been exposed to pesticides for many years (Figure 7-13) and may have developed resistance to any direct negative effects they may have experienced from pesticides. Since there are no data on asymmetry levels in rice field frogs before pesticide use, it is uncertain if the levels of asymmetry I found were elevated at all sites in response to pesticide use.

Figure 7-13. Pesticide and insecticide use (kg/ha)in rice fields in Indonesia. Data from Statistical Bureau (BPS).

Juveniles of *F. limnocharis-iskandari* complex tended to have better body condition than adults. This could reflect greater selection for good body condition acting on survival in juveniles, or could reflect changes in allocation of resources, with adults investing more energy in reproduction.

There were no significant correlations correlation between asymmetry and body condition in *F. limnocharis-iskandari* complex. Interpretation of this relationship is complicated by comparisons among years. Results showed that both asymmetry and the body condition of *F. limnocharis-iskandari* complex were lower in 2003. If higher asymmetry reflects greater stress, which produces lower body condition, then the results would show a lower body condition in all classes of individuals during 2002, not in 2003.

It is clear that in the frogs I studied FA is not a powerful indicator of stress. This is similar to results that have been obtained by other researchers (Bjorksten et al., 2000; McCoy and Harris, 2003). McCoy and Harris (2003) suggest using a range of methods to detect stress. One problem with my results for asymmetry in *F. cancrivora* may have been the relatively low sample sizes I obtained. Accurate estimates of FA, which is a population parameter, require reasonably large sample sizes.

Changes in other environmental factors might explain the differences I found in levels of body conditions between years. Annual rainfall differed substantially among locations and years. Rainfall in Caringin and Darmaga was higher than in Karawang and Subang (Figure 7-14).

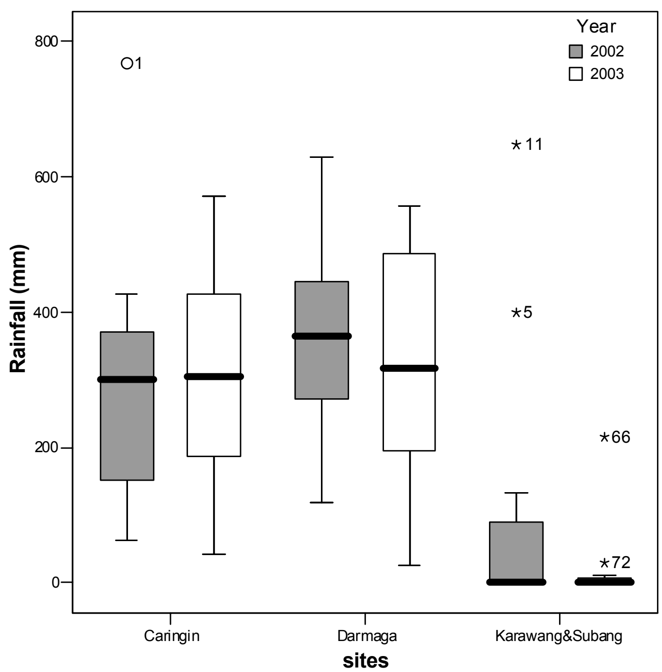


Figure 7-14. Mean annual rainfall in Caringin and Darmaga and Karawang and Subang regions. Data taken from Meteorological Station in Ciawi Station (for Caringin), Darmaga station (for Darmaga) and Cilamaya (for Karawang and Subang). Data for 2002 and 2003 are from monthly rainfall from January to December.

In all locations the total amount of rainfall in 2003 was lower than in 2002 (see Chapter 4 for detailed monthly rainfall figures for the regions). During 2003 in the Subang and Karawang areas no rain occurred for 9 months. My observations in May

2003 showed that most rice field areas failed to produce because irrigation could not supply water because of water shortages in major reservoirs. Lowered body conditions in 2003 could thus have been produced by the short-term effects of water stress, or by food shortages or behavioural changes associated with the relatively low availability of water.

Chytridiomycosis has been found infecting amphibians almost worldwide (Berger et al., 1999). Chytridomycosis had not been looked for in samples from Indonesian frogs before my study, and my results show that at least for three species of frogs occurring in West Java, there is no evidence of infection by the amphibian chytrid. In Australia, chytrid prevalences are higher and frogs are more likely to be negatively affected by the amphibian chytrid at higher elevation, cooler sites with permanent water (Berger et al. 1998, 2004). Most paddy fields in Indonesia occur in lowland areas and the habitats experience periodic wet and dry phases. A dry phase during the fallow period can last for more than one month, potentially eliminating the amphibian chytrid from the environment. Frogs like *L. macrodon* that live at higher elevations in association with more permanent water may be at greater risk, and much additional sampling is needed before Indonesia can be declared to be free of the amphibian chytrid fungus.

My results suggest that environmental stress affected *F. limnocharis-iskandari* complex more than *F. cancrivora*. This is indicated by the occurrence of morphological abnormalities in *F. limnocharis-iskandari* complex on almost all sampling occasions at all locations. One possible source of stress that could have affected *F. limnocharis-iskandari* complex more than *F. cancrivora* is population density. In almost all sampling locations, the densities of *F. limnocharis-iskandari* complex were substantially higher than those of *F. cancrivora* (Chapter 5). However, since both species are abundant in rice fields, it seems that both *F. limnocharis-iskandari* complex and *F. cancrivora* can cope effectively with any environmental stresses peculiar to these habitats.

CHAPTER 8

CONSERVATION STATUS OF THE EDIBLE FROGS

8.1 Introduction

It is known (Chapters 2 and 3) that large numbers of frogs are harvested annually in Indonesia for export and the domestic market. Conservationists have queried whether this threatens the survival of the harvested species (Barfield, 1986; Niekisch, 1986; Martens, 1991, Patel, 1993; Primack, 1999; Schmuck, 2000). Answering this question can be approached by 1) applying a model of the population dynamics of the harvested frogs that includes harvesting rate, and 2) evaluating data on the population biology and distribution of the harvested species in light of criteria for assessing conservation status, i.e. the IUCN Red Categories and the CITES Res. Conf. 9.24 on the Criteria for Amendment of Appendices I and II.

Assessment of sustainable harvesting is a complex task, and requires examining ecological, biological and economic issues. Examining these complex issues requires simplifying real-life systems into models that can be simulated using computer tools (Chi, 2000). Assessments of harvesting for several species, mostly mammals, have used this modelling approach (Slade et al., 1998; Hurtado-Gonzales and Bodmer, 2004). These models attempt to simulate the dynamics of a system, identifying components and processes by which they interact (Odum, 1971; Jeffers, 1978). The system is decomposed into state variables (stocks) and rates of change between stocks (parameters or flows), and a model is assembled connecting them. These models are often characterized by internal feedback mechanisms, such as nonlinearities, delays and uncertainties (Sterman, 1992). These basics (feedback, stocks and flows, time delays, nonlinearities) are essential foundations for effective systems thinking and modeling.

Once a model is assembled and its parameters are well enough known, it can be used to predict whether harvesting is sustainable, and to examine the sensitivity of sustainability to changes or inaccurate estimates of parameters.

In the early development of modeling, simulations usually used complex custom computer programs in relatively low-level languages such as FORTRAN, BASIC, and C. More recently, modeling and simulation development has been simplified by the development of symbolic modeling tools such as Powersim and STELLA (Chi, 2000).

Assessing the conservation status of species is another approach that can be used to evaluate the impact of harvesting. For example, Soehartono and Mardiastuti (2002) performed a series of assessments on Indonesian wildlife species (i.e. *arowana* fish, monkey, swiflets and *ramin* plants) to determine whether they appeared to be threatened by trade.

From my previous chapters, it is clear only two of the three species I studied (*F. cancrivora* and *L. macrodon*) are important in the Indonesian frog leg trade. The small size of *F. limnocharis-iskandari* complex makes it unsuitable for export, and even in the domestic market, only relatively small numbers of this species are sold (Chapter 2 and 3). The populations of *F. limnocharis-iskandari* complex in the study areas were high (Chapter 5) and it is known that this species is distributed widely in Indonesia (Chapter 4). As such, this chapter will focus on the conservation status of *F. cancrivora* and *L. macrodon.*

8.2 Methods

8.2.1 Modelling the impact of frog harvesting

a. General model description

A model of frog population dynamics was developed using Powersim version 2.5. The model included four classes of individuals: eggs, tadpoles, juveniles and adults. The pool of adults was subdivided into males and females.

The population dynamics of the frogs are determined by total rates of recruitment, survival and mortality (Figure 8-1). If recruitment plus survival exceeds total mortality, the population grows, otherwise it declines.

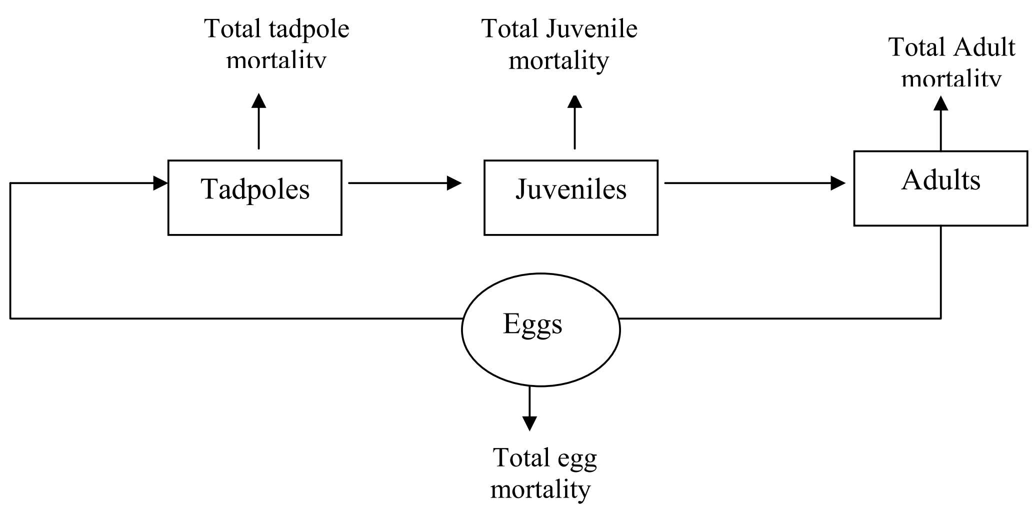


Figure 8-1. Flowchart of frog population dynamics. Population will grow if survival of juveniles is greater than total adult mortality. Population decline occur when total adult mortality exceeds young survival. Total mortality in eggs, tadpoles and juveniles is the sum of mortalities caused by potential food limitation, prolonged dry periods and predation. Total mortality in adult is the sum of mortalities caused by potential food limitation, natural predation and harvest

For the model to be an effective tool in examining population status, data must be available to estimate the values of most of the parameters of the model, such as size of habitat, survival between stages, fecundity, number of reproductive episodes per year, and so on. As more data become available, parameter estimates can be refined and additional parameters can be added, making the model more realistic. For the two species I studied, there were sufficient data in my study and in the literature to estimate enough parameters to model the dynamics of *F. cancrivora.* I therefore used my model to simulate the dynamics of *F. cancrivora* on the island of Java.

b. Harvesting simulation model

A flowchart of my model of frog population dynamics, including most relevant aspects of frog ecology and harvesting, is presented in Figure 8-2. I simplified this model by focusing only on frog population sizes and harvesting, omitting the explicit consideration of natural predator population sizes and food availability. The impacts of predator population size and food availability are still included, since they affect rates of survival.

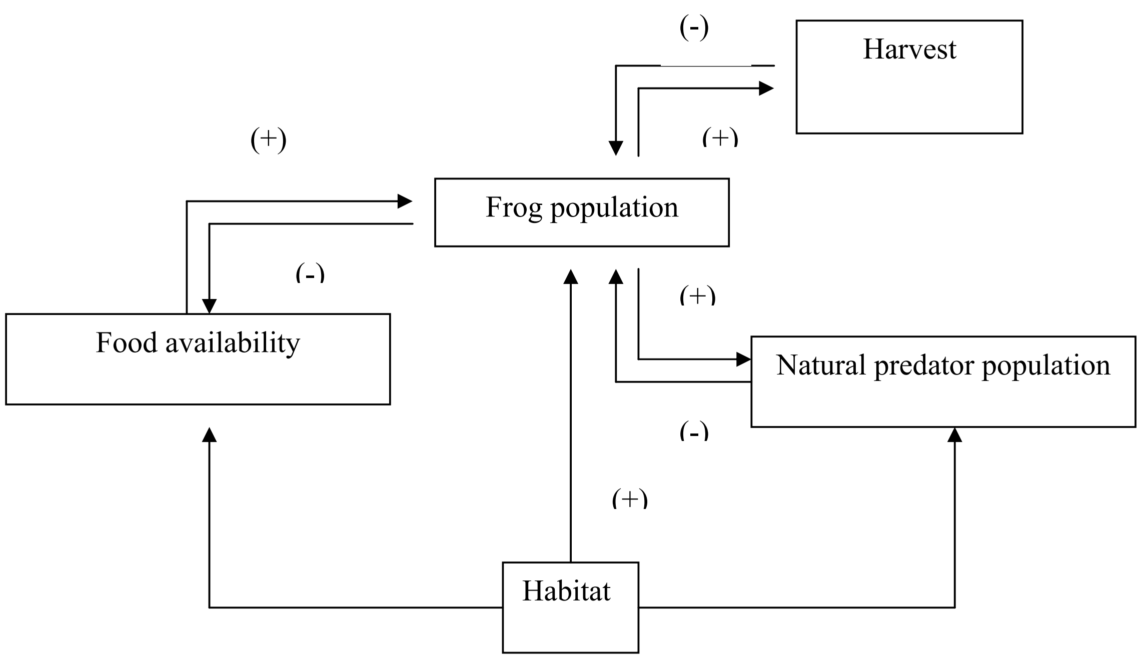


Figure 8-2. Diagram of systems that influence the population of frog. Frog population depends on food availability, habitat, natural predator population and harvest

The frog's preferred habitat will vary among species. *F. cancrivora* is most abundant in wet rice fields or *sawah*, so the area of these is used as the area of habitat available. This area, in hectares, is taken from estimates for Java in 1999, published by the Indonesian Statistical Bureau.

Data on the biology and ecology of *F. cancrivora* from my study and published studies were used to estimate parameters affecting their recruitment and survival. Church (1970), Berry (1975), and Premo (1985) have published very high estimates of fecundity for this species based on dissections, which is up to 10,000 eggs. Iskandar (1998) and Jaafar (1994), who counted eggs in spawned clutches, estimate 1,000 eggs per clutch. This discrepancy suggests that females may lay several clutches in quick succession within a breeding season. All authors agree that *F. cancrivora* breed year round, but that peaks in reproductive activity usually occur twice a year. The timing of breeding is related to the timing of water level in the rice fields (Jaafar, 1994 and this study). Jaafar (1994) concluded that females could produce multiple clutches per season. Rice planting usually occurs two to three times in a year, thus I assume that breeding could occur up to four times a year (two major breeding episodes per reproductive season times two seasons), with between 1,000 and 10,000 eggs deposited per major breeding episode.

Of the eggs spawned in each year, a proportion die before hatching. Eggs that survive enter the tadpole stage. A relatively high proportion of tadpoles die before metamorphosis; those that survive enter the terrestrial juvenile stage. Rates of survival of eggs and tadpoles were obtained from Jaafar (1994), who estimated rates as 0.06 and 0.05 respectively.

Rates of survival of *F. cancrivora* through the terrestrial juvenile stage have not been measured. As an initial estimate, I used the juvenile survival rate of *Rana grylio*, a pond frog harvested in the United States, which is 0.72 (Wood et al., 1998). This estimate is relatively high, and as part of my analysis I examined the implications of other possible patterns of juvenile survival. Adult frogs will breed and eventually leave the system either through death or emigration. The model assumes that there is no immigration and emigration into the system since it simulates the aggregate population of all rice fields in Java.

The model assumes that there are two causes of adult frog mortality: 1) natural mortality, which includes such factors as drying, food limitation, and predation and 2) harvest. I combined natural mortality rates into a single parameter, which I kept separate from harvesting rate. For each run of the model, all rates are constant; they do not depend on density.

From the data on total frog leg exports from 1969 – 2003 (Chapter 2), it appears that the annual total number of frogs exported increased from 1969 to approximately 1993, and has since remained approximately constant, with some fluctuations. The data I collected on domestic harvesting (Chapter 3) indicated that in 2003-2004 the domestic harvest was approximately seven times the export harvest; for the model I have assumed that this ratio remains constant. The total harvest of frogs is estimated based from the highest number of frogs exported from Java (1993) times 0.75 (the proportion of harvested frogs that are *F. cancivora*) times 8, which adds in the sevenfold larger harvest for domestic purposes. Based on estimated harvest using actual data from harvesters (Chapter 3), the range of harvest for 1993 is 200 – 600 million, with a modal estimate of 400 million.

The initial number of mature frogs is based on the mean density of *F. cancrivora* (40 individuals/ha) estimated in my mark-recapture study (Chapter 5), multiplied by the habitat size (3,375,381 ha). The initial number of juveniles is also based on the mean density of juvenile *F. cancrivora* estimated from my study.

Jaafar (1994) estimated that *F. cancrivora* is short-lived (maximum 2-3 years). The female to male adult ratio in the field varied from 0.3:1 to 1.3:1 (Chapter 5). Because females were mostly taken (ratio of female: male harvest was 2: 1, see Chapter 3), I put the fraction of females in the population as 0.3 and males as 0.7. It is possible that many females lived as little as one year after reaching maturity. In order to produce conservative estimates, I set the model to allow each female to breed only four times, assuming assumed that year-to-year survival of adult frogs is zero. All females were assumed able to breed before they were taken. The simulation time is expressed in years.

To examine the sensitivity of this model to my parameter estimates and assumptions, I varied several parameters (juvenile survival rate, times breeding in a year, and harvest rate) while holding the values of other parameters constant. I evaluated the behaviour of the model by running it for 20 years for each set of parameters. Because I did not incorporate density dependence into the model, it is not realistic to expect it to stabilize at a constant population size. I classified the outcome of each run as either positive, if the population was predicted to be increasing at year 20, or negative, if the population was predicted to be decreasing. Table 8-1 shows the equations and numbers used in the simulation.

8.2.2. Conservation status

To assess their conservation status, I examined the properties of both frogs against criteria for IUCN Red List and the CITES Res. Conf. 9.24 on the Criteria for Amendment of Appendices I and II.

Table 8-1. The equations and numbers used in the simulation.

Type of parameter	Note
initial	juveniles = 506307150
flow	juveniles = -dt*juveniles_mortality -dt*juveniles_survival +dt*tadpoles_survival
initial	matures = density*Habitat_size
flow	matures = -dt*mature_mortality +dt*juveniles_survival
initial	tadpoles = 8438452500
flow	tadpoles = +dt*eggs -dt*tadpoles_mortality -dt*tadpoles_survival
auxiliary	eggs = birth_rate*egg_rate_survival
auxiliary	juveniles_mortality = juveniles*(drying+food_scarce+juvenile_predation)
auxiliary	juveniles_survival = juveniles*juv_rate_survival
auxiliary	mature_mortality = mature_natural_death+harvest
auxiliary	tadpoles_mortality = tadpoles* (food_scarcity+habitat_drying+tadpole_predation)
auxiliary	tadpoles_survival = tadpoles*tadpole_rate_survival
auxiliary	birth_rate = females*number_of_eggs_per_clutch*times_breeding_in_a_year
auxiliary	females = matures*frac_female
auxiliary	males = matures*fract_male
auxiliary	mature_natural_death = matures*natural_death
constant	density = 40 frogs/ha
constant	drying = 0.08
constant	egg_rate_survival = 0.04
constant	const food_scarce = 0.1
constant	food_scarcity = 0.24
constant	frac_female = 0.3
constant	fract_male = 0.7
constant	habitat_drying = 0.35
constant	Habitat_size = 3375381 ha
constant	const harvest = 240000000
constant	juv_rate_survival = 0.72
constant	juvenile_predation = 0.1
constant	natural_death = 0.2
constant	number_of_eggs_per_clutch = 1000
constant	tadpole_predation = 0.35
constant	tadpole_rate_survival = 0.06
constant	times_breeding_in_a_year = 4

8.3 Results

8.3.1 Population models including harvesting

a. The simulation

Figure 8-3 shows the population dynamic model for *F. cancrivora*. I used the simulation to explore the implications of harvesting and variation in vital rates for the total Javan population of this species as described in the methods. Populations fluctuated considerably for the first 5-years before either increasing or decreasing steadily in numbers (Figures 8-4, 8-5). These initial fluctuations occur as the population approaches its stable age distribution. Tables 8-2 and 8-3 summarise the impact of variation in harvesting rates, fecundity, number of major reproductive episodes per year, and changes in juvenile survival to reproduction on the sustainability of the Javan population of *F. cancrivora*. The results show under a variety of parameter combinations and harvesting rates, the estimated total harvest (between 100,000,000 and 500,000,000 frogs per year) in Java is sustainable. They also show that it is not sustainable for many parameter combinations. This suggests that the true situation is that frogs are being harvested at rates very near the boundary between sustainability and unsustainability.

b. Model limitations

The limitations of this model are mostly caused by lack of information. More research is needed to improve the estimates of population parameters, for instance the survival of terrestrial juveniles to maturity and the numbers of times that females reproduce. It would also be very useful to incorporate the effects of density dependence, which could lead to lower sensitivity to changes in harvesting rate.

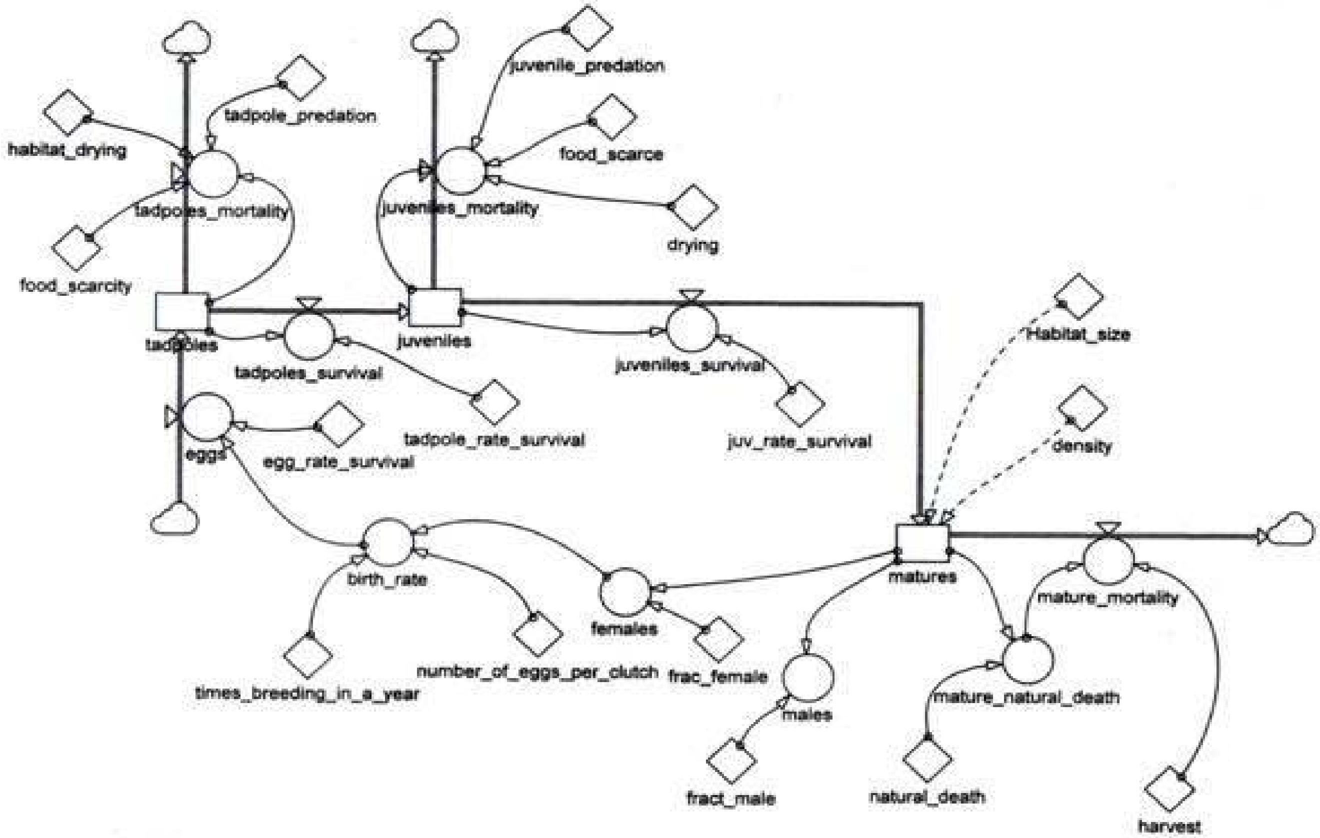


Figure 8-3. Model of population dynamic of *Fejervarya cancrivora* in rice field habitat using program Powersim

Population

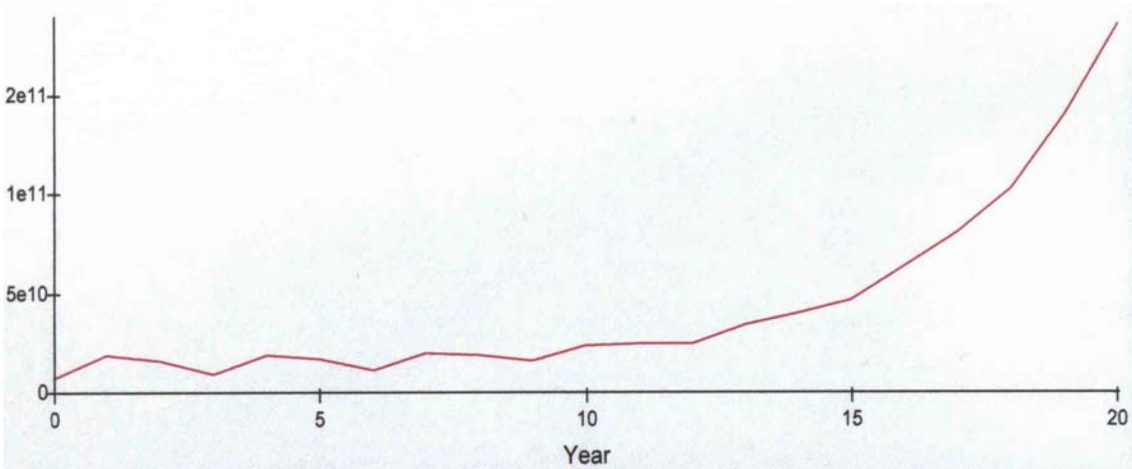


Figure 8-4. Tadpole population dynamics with level of harvesting 200 million, number of reproductive episodes per female = 4, with 1000 eggs per episode, and juvenile survival 0.72

Population

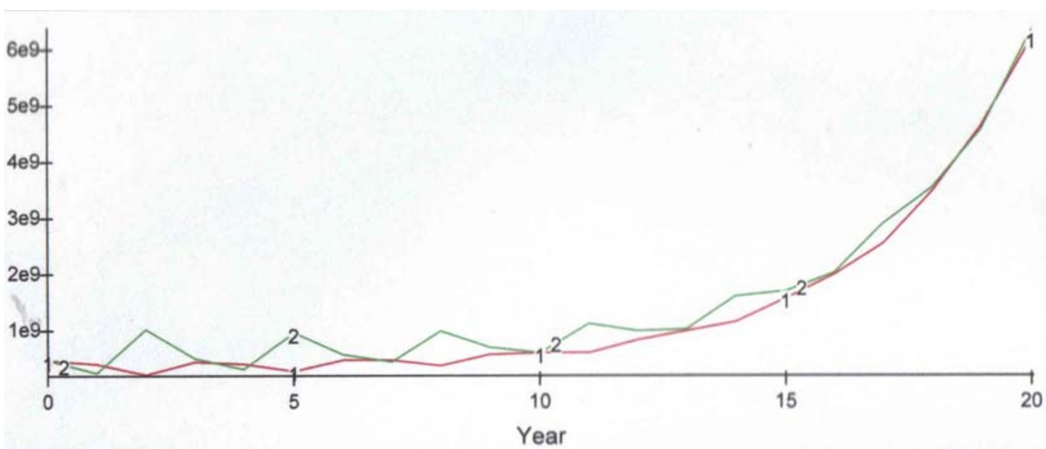


Figure 8-5. Dynamics of juveniles and mature frogs with level of harvesting = 200 million, number of reproductive episodes per female = 4, with 1000 eggs per episode, and juvenile survival 0.72

Table 8-2. Result of 20 year simulations using different combinations of total harvest, number of major reproductive episodes per female, and fecundity per reproductive episode. Juvenile survival to reproduction in this set of simulations is constant at the level (0.72) estimated by Wood et al. (1998) for *Rana grylio.* (+) means population is increasing at year 20, so the given level of harvest should be sustainable; (-) means population is decreasing and the harvest is unsustainable.

Harvest (in millions)	Survival	Number of times breeding	Fecundity (number of eggs) in thousands 1	2	3	4	5	6	7	8	9	10
200	0.72	1	-	+	+	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+	+	+	+
		4	+	+	+	+	+	+	+	+	+	+
300	0.72	1	-	-	-	+	+	+	+	+	+	+
		2	-	+	+	+	+	+	+	+	+	+
		3	-	+	+	+	+	+	+	+	+	+
		4	+	+	+	+	+	+	+	+	+	+
400	0.72	1	-	-	-	-	-	-	-	-	+	+
		2	-	-	-	-	+	+	+	+	+	+
		3	-	-	+	+	+	+	+	+	+	+
		4	-	-	+	+	+	+	+	+	+	+
500	0.72	1	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	+
		3	-	-	-	-	-	-	+	+	+	+
		4	-	-	-	-	+	+	+	+	+	+
600	0.72	1	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-

The model also did not allow for short-term variation in parameters, as might be caused by environmental stresses such as droughts, diseases, fluctuations in the availability of food or the abundance of competitors or predators, or changes in levels of pollution from pesticide use. The impact of long-term environmental stresses such as pesticide levels is, however, integrated into the survival rate constants.

Table 8-3. Result of 20 year simulations using different combinations of total harvest, number of major reproductive episodes per female, and fecundity per reproductive episode. Juvenile survival rate to reproduction in this set of simulations is reduced to 0.5 and 0.3. (+) means population is increasing at year 20, so the given level of harvest should be sustainable; (-) means population is decreasing and the harvest is unsustainable

Harvest (in millions)	Survival	Number of times breeding	Fecundity (number of eggs) in thousands									
			1	2	3	4	5	6	7	8	9	10
200	0.5	1	-	-	+	+	+	+	+	+	+	+
		2	-	+	+	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+	+	+	+
		4	+	+	+	+	+	+	+	+	+	+
300	0.5	1	-	-	-	-	-	-	-	+	+	+
		2	-	-	-	+	+	+	+	+	+	+
		3	-	-	+	+	+	+	+	+	+	+
		4	-	+	+	+	+	+	+	+	+	+
400	0.5	1	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	+	+	+	+
		4	-	-	-	-	-	+	+	+	+	+
500	0.5	1	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-	-	+
		4	-	-	-	-	-	-	+	+	+	+
60	0.5	1	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-
200	0.3	1	-	-	-	-	-	-	+	+	+	+
		2	-	-	-	-	-	+	+	+	+	+
		3	-	-	+	+	+	+	+	+	+	+
		4	+	+	+	+	+	+	+	+	+	+
300	0.3	1	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	+	+	+	+
		4	-	-	-	-	+	+	+	+	+	+
400	0.3	1	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-

The model also assumes that the Javan population can be treated as a single entity, when of course it is actually subdivided into subpopulations which are isolated dynamically to unknown extents. There may, however, be at least some connectivity among all of the populations in the island, as it appears from my data (Chapter 5) that *F. cancrivora* has low site fidelity (Chapter 5) and moves relatively freely, at least on a local scale.

8.3.2 Conservation status

Comparisons of the population parameters of F. *cancrivora* and *L. macrodon* to IUCN Red List and the CITES criteria are given in Tables 8-4 to 8-7. From this assessment *F. cancrivora* is not qualified for listing in any threat category under IUCN Red List criteria nor CITES Appendix I and II. On the other hand, more consideration needs to be given to *L. macrodon.* This frog seems inappropriate to be included in CITES Appendix I, however, it is possible that this species might qualify for listing as vulnerable or in CITES Appendix II if more information was available on its population biology. Based on my market surveys, in which I found that there are often hundreds of frogs of this species sold every day, it is clear that the number of mature *L. macrodon* currently is more than 10,000 individuals (Chapter 3). The distribution is also likely to be more than 20,000 km^2, since the area of West Java alone is 34,597 km^2 (BPS, 2002). The limited distribution I found for this species (Chapter 4) could be due to a lack of surveys. I could not make any informed estimate regarding possible future declines of this species because of lack of data, such as survival rates of this species between stages and detailed knowledge of the actual area of habitat available to it.

Table 8-4. Assessment of biological criteria of *F. cancrivora* and *L. macrodon* against guidelines for inclusion in the IUCN Red List

Criteria	Guidelines	*F. cancrivora*	*L. macrodon*
Extinct	The last individual had died	NO	NO
Extinct in the wild	Only survive in cultivation, in captivity, or as a naturalised population well outside the past range	NO	NO
Critically endangered	Population in the wild has, by observed, estimated, inferred or suspected, declined at least 80% over the last 10 years or three generations whichever is the longer,	NO	NO
	Extent of occurrence estimated to be less than 100 km^2 or area of occupancy estimated to be less than 10 km^2,	NO	NO
	Population estimated to number less than 250 mature individuals and is estimated continuing decline of at least 25%, within 3 years or one generation whichever is the longer	NO	NO
Endangered species	Population has, by observed, estimated, inferred or suspected, declined of at least 50% over the last 10 years or three generations whichever is the longer,	NO	NO
	Extent of occurrence estimated to be less than 5000 km^2 or area of occupancy estimated to be less than 5000 km^2,	NO	NO
	Population estimated to number less than 2500 mature individuals and is estimated continuing decline of at least 20%, within 20 years or 5 generations, whichever is the longer	NO	NO
Vulnerable	Population has, by observed, estimated, inferred or suspected, declined of at least 20% over the last 10 years or three generations whichever is the longer,	NO	NO
	Extent of occurrence estimated to be less than 20,000 km^2 or area of occupancy estimated to be less than 20,000 km^2, population estimated to number less than 10,000 mature individuals and is estimated continuing decline of at least 10%, within 100 years	NO	NO?

Table 8-5. Assessment of biological criteria of *F. cancrivora* and *L. macrodon* against guidelines for inclusion in CITES Appendix I

Biological Criteria		*F. cancrivora*	*L. macrodon*
A	The wild population is small and is characterised by at least one of the following		
1	An observed, inferred or projected, decline in the number of individuals or area and quality of habitat	NO	NO
2	Each population being very small	NO	NO
3	A majority of individuals, during one or more life-history phases, being concentrated in one sub-population	NO	NO
4	Large short-term fluctuations in the number of individuals	NO	NO
5	High vulnerability due to the species biology or behaviour including migration	NO	NO
B	The wild population has a restricted area of distribution and is characterized by at least one of the following		
1	Fragmented or occurrence at very few locations	NO	NO
2	Large fluctuation in the area of distribution or the number of sub-populations	NO	NO
3	High vulnerability due to the species biology or behaviour including migration	NO	NO
4	An observed, inferred or projected decline in area distribution or the number of sub-populations or the number of individuals or area and quality of habitat reproductive potential	NO	NO
C	A decline in the number of individuals in the wild which has been either		
1	Observed as ongoing or having in the past	NO	NO
2	Inferred or projected decrease in area or quality of habitat or levels or pattern of exploitation	NO	NO
3	Threats from extrinsic factors such as pathogens	NO	NO
4	Decreasing reproductive potential	NO	NO
D	If the species is not included in Appendix I, it would satisfy with A, B or C period of five years		
Recommended status for Appendix I		Not qualified	Not qualified

Table 8-6. Assessment of biological criteria of *F. cancrivora* and *L. macrodon* against guidelines for inclusion in CITES Appendix II in accordance with Article II, paragraph 2 (a) of the CITES Convention

	Biological Criteria	*F. cancrivora*	*L. macrodon*
A	The wild population will meet at least either the following criteria in the near future		
1	An observed, inferred or projected decline in the number of individuals or area and quality of habitat	NO	NO
2	Each population being very small	NO	NO
3	A majority of individuals, during one or more life history phases, being concentrated in one sub population	NO	NO
4	High vulnerability due to the species biology or behaviour including migration	NO	NO
B	It is known, inferred or projected that the harvesting of specimens from the wild for international trade has or may have a detrimental impact on the species by either		
1	Exceeding, over an extended period, the level that can be continued in perpetuity	NO	?
2	Reducing it to a population level at which its survival would be threatened by other influences	NO	?
	Recommended status for Appendix II	Not qualified	Need more data

Table 8-7. Assessment of biological criteria of *F. cancrivora* and *L. macrodon* against guidelines for inclusion in CITES Appendix II in accordance with Article II, paragraph 2 (b) of the CITES Convention

	Biological Criteria	*F. cancrivora*	*L. macrodon*
A	The resemble specimens of a species included in Appendix II under the provisions of Article II, paragraph 2 (a), or in Appendix I, such that a non expert, with reasonable effort, is unlikely to able to distinguish between them	NO	NO
B	The species is a member of a taxon of which most of the species are included in Appendix II under the provision of Article II, paragraph 2 (a), or in Appendix I, and the remaining species must be included to bring trade in specimens of the others under effective control	NO	NO
	Recommended status	Not qualified	

8.4 Discussion

From the simulation exercise, it appears likely that although the level of the removal of mature *F. cancrivora* by human harvesting is high, the population can sustain the harvest. However, the simulation results also show that the number of frogs currently harvested is probably near the sustainable rate. Any increase in harvesting, or decrease in the rates of survival and reproduction of frogs due to other causes, could lead to rapid depletion of populations. Thus, it is high time for the local authorities to monitor this trade.

Despite the limitations of this model, it still gives a picture of population trends in relation to harvesting. It is recommended that the model should be developed, with further study allowing more complete parameterisation and more accurate estimates of the parameters I was able to include.

My evaluation of the status of *F. cancrivora* and *L. macrodon* using the criteria for the IUCN Red List and CITES Appendices made it clear that *F. cancrivora* is not qualified for any listing or inclusion in the appendices. The distribution of *F. cancrivora* is from the Malay Peninsula to the Philippines and the Lesser Sundas as far as Flores; Guangxi and north-eastern coast of Hainan Island, China; Vietnam (Frost, 2004). In Indonesia, this species is found in almost all major Indonesian islands including Sumatra, Kalimantan, Java, Bali, Sulawesi and recently Irian Jaya (the Indonesian part of the island of New Guinea). The distribution of *L. macrodon* is also wide, from Burma to Malaya, Thailand and Malaysia; Java and Sumatra (Indonesia), and the Rioux Archipelago (Frost, 2004). However, in Indonesia this species is considered endemic in Java, but is also found in Lampung, South Sumatra (Iskandar, 1998). In Java, it is mostly found in the western part of the island (Figure 4-4).

There are no previous studies of the population status of either species in Indonesia. My study in West Java indicated that populations of *F. cancrivora* were abundant, with a mean density of 40/ha in rice fields. Although concern has been expressed that this species seems to be hard to find in some rice fields (Pratomo, 1997; Schmuck, 2000), it presently occurs in good population densities at a number of sites distributed over West Java. Field experience indicates that *F. cancrivora* can be difficult to catch and moves actively among rice fields, especially during drying periods (Chapter 5). This could account for the previous reports of localised declines in abundance. Wild populations of *L. macrodon* in my study fluctuated over time, with a maximum of seven frogs found per 100 m of stream length (Cilember, December 2002, see Chapter 6). It appears to be common in West Java. The relative scarcity of information on population status of this species suggests a need for long-term monitoring to detect possible future declines.

Both *F. cancrivora* and *L. macrodon* have high reproductive rates and breed all year long (Sugiri, 1979; Premo, 1985; Jaafar, 1994, this study). *F. cancrivora* is found most abundantly in rice fields rather in than natural habitats such as ponds and streams. Although rice fields have been a part of the landscape for a sufficient period that they could be considered a part of the natural environment, their occurrence relies on human interference. It seems that the frogs have immigrated to rice field areas naturally and regard rice fields as suitable habitat compared to other habitat. *F. cancrivora* has the ability to survive the harsh conditions of rice fields and this ability has helped this species thrive in the rice fields along with *F. limnocharis-iskandari* complex.

Surveys of *F. cancrivora* in other wetland areas (ponds, streams and marshes) found no or few individuals (Chapter 4). It appears that by developing large areas of rice fields, humans have probably greatly increased the total abundance of the frog species that inhabit them. In the past few years, the development of rice fields in Indonesia has moved towards outer Java and encompasses areas that traditionally had no rice culture, such as Irian Jaya. The availability of rice fields in other areas has either allowed an increase in the distribution of *F. cancrivora* or caused it to increase in abundance and be detected in new areas; it has only been found in Irian Jaya since the institution of rice culture there (Chapter 4). *L. macrodon* is also not restricted to streams in undisturbed forested areas, and can be found near human habitation (villages or irrigation channels for rice fields).

Biological criteria for the inclusion of *F. cancrivora* and *L. macrodon* in CITES Appendix II in accordance with Article II, paragraph 2 (b) of the CITES Convention state that a non-expert with reasonable effort could distinguish between edible specimens. However, in practicality it is difficult to differentiate both species by their skinless legs. In its live form mature *F. cancrivora* can be distinguished from its sympatric species *F. limnocharis-iskandari* complex or *L. macrodon*. However, non-experts would have difficulty distinguishing *L. macrodon* with some other *Limnonectes* genus such as *L. blythii* and *L. paramacrodon.* To date, no *Limnonectes* are listed under appendices of CITES.

Should any edible frogs be included in any conservation measures in the future, the management authorities should account for the fact that it is difficult to correctly identify (1) species of *Limnonectes* from live specimens and (2) edible species from frozen legs as shown by Veith et al., (2000). The first step in managing the trade is to assure that identification of the frogs is correct. There is a need to develop a simple key-identification for local authorities and traders, and distribute it, so they can identify species correctly.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSION

9.1 Introduction

Understanding the Indonesian frog leg harvest requires cross-disciplinary knowledge, including information on frog biology, population dynamics, and conservation biology, and an understanding of the human aspects of the harvest. The literature concerning the species harvested is surprisingly sparse, and much is relatively old, reaching back more than 20 years. There is more information on the basic biology (such as diet and breeding biology) of harvested species, but it is mostly found in grey literature, e.g. unpublished theses, in a language other than English (Bahasa Indonesia). The results of my study fill gaps in basic understanding of the biology, population ecology, and conservation biology of edible frogs and their interactions with humans, and allow me to make recommendations for the conservation of edible frogs, especially the two most important species: *F. cancrivora* and *L . macrodon.*

9.2 The extent of the Indonesian edible frog leg trade

My study is the first to estimate the total number of frogs harvested in Indonesia, including those harvested for export and for domestic markets. The estimated harvest for export fluctuates annually, and during the period from 1989 – 1998 was higher than those of India before the edible frogs of India were listed under CITES (Pandian and Marian, 1999; Chapter 2). During the last three years for which I had data (1999 – 2002) the level of frog leg exports declined slightly. This decline does not necessarily indicate declining harvests, but may reflect a shift from international to domestic markets, or a reduction in international demand.

My study indicated that the harvest for domestic markets is greater than that for exports (Chapter 3), however, my estimates are subject to uncertainty. They were based on the ratio of frogs captured by harvesters that had suitable mass for export purposes to the frogs that were captured but were not suitable for export because they were too small. Although exporters and middlemen interviewed insisted that frogs exported should have a minimum weight, the number of exporters and middlemen interviewed in this study was limited. It is possible that some of the countries that import frog legs are lowering the criteria, e.g. importing legs from frogs weighing less than 80 grams.

Because of the unregulated nature of this product, the species and sizes of individuals taken are governed by market demand. Hence, if markets accept smaller frogs, they will also be harvested. Although harvests occur throughout the year, the rate of harvesting fluctuates. Peak harvests occur during the wet season and no moon. At a local scale, frog harvesting in rice fields is also limited by the stage of rice plant growth. Although access to rice fields is mostly free, harvesters need to take care that their actions do not damage rice plants; as a result they rarely venture inside rice blocks except during certain times e.g. after rice is harvested. Thus their capture effort is usually limited to areas within a one-meter radius of the block borders, which also serve as pathways. Those limitations may limit the impact of harvesting on frog populations, since the majority of their habitat is protected from harvesting pressure.

At peak season the number of harvesters tends to rise as part-time harvesters enter the workforce. Harvesting is not a highly skilled job and provides a substantial income for unskilled workers. It is evident that frog harvesting in Indonesia, at least in Java where my study was done, has a long history that predates the first recorded exports in 1969. It has a large economic value and well-established trading networks. The number of people involved in this trade is high if we take account full- and part-time harvesters, middlemen, traders, people working in processing plants and export companies, and people working in restaurants or *warung* (street vendors) who serve frog leg delicacies. From my estimates of the total harvest (ca. 400,000,000 frogs per annum) and harvesting rates (ca. 100 frogs per harvester-day) and length of the working year (ca. 200 days), it is possible to estimate that on the order of 20,000 persons are employed in frog harvesting in Java. Any regulation of this trade should take account of the involvement of local people who depend on it for their livelihood.

While my study focused on the trade from Java, the results give an overall picture of the frog leg trade in Indonesia. Frogs are harvested primarily from rice fields or areas near human habitations rather than forested areas since frogs are more abundant in these areas and access is easier. The same trend is probably true for other islands. My study indicated that currently most harvest for export purposes comes from Java. In the future, if increased population leads to a decreased area of rice fields in Java, the harvest for export will probably shift to other islands such as Sumatra, Bali, Kalimantan and Sulawesi. Even though frog farming has been encouraged by the government, it is unlikely that frog farming will develop in Indonesia as long as the cost of frogs harvested in the wild is lower than that of farmed frogs and the species farmed are considered to be unappetizing by local markets.

9.3 The population dynamics and biology of the harvested species

The population status of the three most common species of edible frogs (*F. limnocharis-iskandari* complex, *F. cancrivora* and *L. macrodon*) is addressed in Chapters 5 and 6. My study has provided the first estimates of the densities of *F. limnocharis-iskandari* complex and *F. cancrivora* in Indonesia. My field surveys in West Java revealed that the mean densities of *F. limnocharis-iskandari* complex adults and juveniles were 211.76/ha and 514.68/ha, respectively; whilst for *F. cancrivora* adult and juveniles they were 39.76/ha and 85.27/ha. The densities of both species in my study were similar to, and in fact higher than, their densities in Malaysia (Jaafar, 1994). Given that the locations in my study are subjected to frog harvesting while Jaafar's study sites were not, these results suggest that harvesting has not severely depleted the population of either species.

The population structures of both species differed among rice planting stages; juveniles were found mostly during tilling and the earlier stages of rice plant growth. The timing of recruitment of both species is related to water level, an observation also made by Jaafar et al., (1999) in the rice fields of Malaysia. The ability of the frogs to breed several times a year (Church, 1960; Jaafar, 1994) has allowed the populations of both species to flourish despite continuous harvest. Both species showed sexual size dimorphism, with females larger than males, which causes more females than males to be captured by harvesters.

Similarly to *F. limnocharis-iskandari* complex and *F. cancrivora, L. macrodon* also showed a tendency for sexual size dimorphism. Although most authors state that adult males of fanged frogs are larger than females (Inger, 1966; Emerson, 2001) my study found the opposite: female *L. macrodon* were larger than males, the same pattern displayed by the rice field frogs. My study suggests that the minimum reproductive size for female *L. macrodon is approximately* 60 mm SVL.

My population estimates for *L. macrodon* were mostly too variable to be strongly relied upon, and some mark-recapture parameters could not be estimated at all. This was caused by low numbers of recaptures of this species, especially at Ciapus Leutik. The probability of capture differed between capture periods; more frogs were found during times of higher rainfall than in the dry season. Capture efficiency at

Cilember and Ciapus Leutik varied between 0.59 and 0.71; for every two to three frogs captured, one frog escaped. The density of frogs varied from one per 100m at Ciapus Leutik to seven per 100 m at Cilember. Although lower numbers of frogs were found at Ciapus Leutik there is no evidence that this was caused by over-harvesting in the area.

9.4 Environmental stressors

My results suggest that rice field frogs are subject to environmental stressors. Both *F. limnocharis-iskandari* complex and *F. cancrivora* in my study showed signs of morphological abnormalities, caused both by developmental abnormalities and traumatic injuries. Although the frequencies of abnormalities in my study were relatively low compared with results of some other studies (Ouellet, 2000), the results indicate that stresses may affect the condition of frogs in this habitat.

Pesticide use in agriculture is thought to be a major threat to wildlife (Igbedioh, 1991; Safford and Jones, 1997) including amphibians (Cooke, 1973, 1977 and 1981; Bridges and Semlitsch, 2000; Hayes et al., 2002; Kiesecker, 2002). The existing pesticide residues I found reflect a history of substantial use of pesticides by farmers. Both frog species accumulated pesticides, either through direct contact with the environment or through ingestion of contaminated prey. Pesticides have been used in Indonesia for many years (Fox, 1993; Van der Eng, 1994 and Settle et al., 1996), so both species have been exposed to pesticides for many generations. The relatively high densities I found for both rice-field species suggest that their population sizes are not being negatively affected by pesticide exposure. Despite pesticide use, the insects and other invertebrates the frogs feed upon appear to be abundant in the rice fields (Settle et al., 1996). Most juveniles found in the survey had good body condition, indicating that they were not food-stressed.

There was no sign of chytridiomycosis in the three species I studied. Chytridiomycosis has been found infecting many species of frogs around the world (Berger et al., 1998) and although to date no infection has been found in the species I surveyed, or in any Indonesian frogs, there is a possibility that this fungus could be found in other species of Indonesian frogs more susceptible to this disease, or in other regions or habitats. It is likely that the absence of this disease in Indonesia reflects the lack of surveys, and since it can cause sudden dramatic collapses of frog populations (Berger et al, 1998), efforts should be made to gain a greater understanding of its status in Indonesia.

My analysis of developmental stability determined that in *F. limnocharis-iskandari* complex there is a significant excess of fluctuating asymmetry over measurement error. This indicates that developmental precision was not perfect, which may have been a reflection of environmental stress. However, I was not able to determine whether any particular stress might have been the cause of the observed levels of fluctuating asymmetry. I did not find any significant differences of fluctuating asymmetry between frogs captured in pesticide and non-pesticide locations (Chapter 9), however I also found no major differences in pesticide levels in the environment or in frogs between these sites. More importantly, I found no correlation between body condition and level of unsigned asymmetry in individuals. Body condition, however, was correlated with environmental conditions, being lower in the relative drought experienced in 2003. My results suggest that at least in the species studied, developmental stability analysis is not a powerful tool to detect short-term stress (e.g. the impact of drought).

9.5 The sustainability of harvest and conservation status of the edible frogs

The attitudes revealed in my surveys of people involved in the frog-leg trade indicate that most traders are not overly concerned with the conservation of the edible species. Although some harvesters and middlemen expressed concern at low harvest rates, there is a general belief among the stakeholders that the harvest can sustain trade for an unlimited time. There is also almost no political will to regulate the trade. Working out which government agencies are responsible for the trade is complicated. For instance, in statistical data frog legs were listed as fisheries commodities and thus are under the jurisdiction of the Ministry of Marine Affairs and Fisheries. The Ministry of Marine Affairs and Fisheries itself is a young department, which developed from a directorate general under the Ministry of Agriculture. When my study began, the frog harvest, which is considered to be an inland fishery, was still under the jurisdiction of the Ministry of Agriculture. Later, with reorganization of the government departments, it was moved into the Ministry of Marine Affairs and Fisheries. However, the frog leg trade is not the main focus of this Ministry, compared with other commodities such as prawns, tuna or other marine fisheries. Exporting frog legs also involves the Provincial Office of Industry and Trade.

The export of Indonesian wildlife in particular has resulted in the decline of some species and has been closely monitored by the international public (Soehartono and Mardiastuti, 2002). To answer the problem, the Government of Indonesia developed a conservation strategy to promote the protection of life support systems, preservation of genetic resources and sustainable utilization of living resources. The Government of Indonesia ratified the CITES Convention through Presidential Decree No 43 in 1978 (Soehartono & Maridastuti, 2002) and later on promulgated a ministerial decree from the

Ministry of Forestry regulating the capture, transport and trade of wild flora and fauna (PHPA, 1990).

For the implementation of CITES, the Government of Indonesia designated the Directorate General of Forest Protection and Nature Conservation (Direktorat Jenderal Perlindungan Hutan dan Konservasi Alam, PHKA) as the management authority and The Indonesian Institute of Science (Lembaga Ilmu Pengetahuan Indonesia, LIPI) as the scientific authority. The latter is responsible for advising PKA on the status of species subjected to harvest for international trade, whilst the former establishes the annual harvest quota. As Indonesia has become one of the major exporters of frog legs in the world (Chapter 2), and it is well known that the exported frogs are mostly wild-caught, it is not surprising that the Indonesian frog harvest has also been questioned by international investigations (Barfield, 1986; Niekisch, 1986; Martens, 1991; Patel, 1993; Schmuck, 2000). Although no Indonesian frog species has been included in the CITES convention, the PKA does set a quota for this harvest as part of an attempt to regulate the harvest of wildlife of non protected and non-CITES species. For instance, the annual quota in 2001 is 5,000 *F. cancrivora* for pets and 25,000 *L. macrodon* for skins plus additional 5,000 for pets (PKA, 2000).

The establishment of annual quotas of *F. cancrivora* and *L. macrodon* by the PKA showed the absurdity of the situation. It is clear from my results that millions of these species are caught annually to satisfy the international and domestic demands of the food industry. The PKA quotas did not acknowledge the harvest for food. Also if the number of frogs harvested is reduced to the number established by the PKA, the frog harvesting industry will cease to exist. From the stand point of law, the exclusion of edible species from protected species means that there are no legal issues in harvesting these species, which is probably the reason why the annual quota put by the PKA has no influence whatsoever on the actual harvest of frogs in the wild.

Attempts to regulate the harvest of frog legs will be impossible without acknowledging the already established market for frog legs and the stakeholders involved in this industry. Even if an export quota were set, controlling the domestic market will be complicated and most likely will not succeed. The majority of harvesters are uneducated and poor and will not accept quotas or acquiesce if the practice of harvesting frogs, which has provided livelihoods for many years and is not objected to by most rice field owners, is stopped by the government. Harvesting occurs on almost all Indonesian islands, making law enforcement difficult. Any restrictions on the harvest, as have been attempted by the PKA, require a detailed knowledge of the status and dynamics of populations of the harvested species.

As stated before, the scientific authority responsible for CITES in Indonesia is LIPI. However, LIPI has limited resources for monitoring harvested species (Amir et al, 1998). There is a need for LIPI to develop greater cooperation with other scientific organizations (such as universities) and non-governmental organizations to monitor these species. Collaboration with professional bodies such as IRATA (the Indonesian Reptile and Amphibian Trader Association) to monitor the harvest of several species of reptiles is one of the examples of cooperation already operating (Saputra, 1998). In the case of frog legs, there are no reports of attempts by any professional body to survey the level of the frog harvest. Although the name of IRATA suggests the inclusion of amphibian traders in this professional body, it does not include the frog leg industry; only the live amphibian trade for the pet industry (Saputra, *pers. comm.*). The organization that deals with the frog leg industry is most likely included in the Cold

Storage & Fisheries Commodities Trader Association (Asosiasi Pengusaha Cold Storage dan Produsen Exportir Hasil Perikanan Indonesia; APCI).

Although the estimated numbers of frogs harvested annually in Indonesia are huge (Chapter 2 & 3) there is no evidence that the harvest has depleted the populations of rice field frogs. However, the present level of harvest of *F. cancrivora* may be near the maximum level sustainable by the population, as indicated by the results of my modelling (Chapter 8) and by the fact that the population structure of *F. cancrivora* in the field was mostly biased to young mature frogs (Chapter 5) when compared with unharvested populations that have been studied elsewhere.

Fieldwork showed that *F. limnocharis-iskandari* complex were found in high abundances (Chapter 5). The densities of *F. cancrivora* populations were also high (Chapter 5), and (Chapter 8) it did not meet any criteria for listing as a protected species. Modelling and assessment of *F. cancrivora* using the IUCN Red List and CITES criteria also showed that it does not qualify for any inclusion, as long as the level of harvesting and habitat size remain constant. The conclusion that both Indonesian rice field frogs' populations appear to be able to sustain the relatively high rates of harvest they are experiencing differs from the conclusions reached in research on Indian edible frogs (Abdulali, 1985) that resulted in the inclusion of *Euphyctis hexadactylus* and *Holobatrachus tigerinus* in CITES Appendix 2. My results are similar to those found in a series of studies on the harvest of pythons and monitors in Indonesia during late 1990's (Shine et al., 1996; Boeadi et al., 1998; Shine et al., 1998a, 1998b, 1998c, 1998d, 1998e, and 1998f). These studies showed that the populations of harvested species have been able to withstand continuous harvest pressure due to their life history traits including rapid growth, early maturation, and relatively high reproductive output and also their ability to thrive in disturbed landscapes.

Assessment of L. *macrodon* in the market and fields showed that this species is still considered abundant, especially in the western part of Java. However, there is a need for more studies to assess the status of this species. It occurred at relatively low densities, and I did not collect sufficient data on either harvesting rates or population parameters to allow a full evaluation of the sustainability of its harvest.

9.6 Conclusions and Recommendations

My study suggests that the current levels of harvest of *F. cancrivora* and *F. limnocharis-iskandari* complex are sustainable. The impact of harvesting on *L. macrodon* is unclear. Current levels of harvest appear likely to affect *L. macrodon* populations more than *F. cancrivora*, however, there is a need to do more research on the population biology and ecology of this species.

Although I conclude that there is no justification for including the harvested species in the Red List or in the CITES appendices, the scientific authority (LIPI) should regularly monitor the trade. Monitoring of the frog leg trade should occur not only in Java but also in the other main islands, especially Sumatra. From the export statistics, the centre of exportation in Sumatra is in Medan, thus the monitoring in Sumatra should begin from Medan and surrounding North Sumatran areas. Monitoring should include collection of data on species harvested, size harvested, the spatial and temporal patterns of harvest, and the population ecology of the species harvested.

Because of the potential economic gain from this harvest, especially for export purposes, the government (e.g. the ministry of fisheries and marine affairs) should also monitor this harvest. Monitoring the harvesters itself might prove difficult due to the open nature of this work. One way to trace the number of people involved in this export might be through the export companies. All export companies maintain strong relationship with their middleman, and middleman with their harvesters. The government should monitor the numbers of export companies and their middleman and regularly update it.

Another concern regarding this trade is incorrect species identifications in export documents. With enough effort, species identification is not difficult to do while the frog is still alive. On the other hand, identifying species from frozen leg is likely to require the use of molecular genetics, which is costly. Therefore there is a need for local trade officials to impose correct species identification on the middleman levels. Developing a simple identification key and distributing it to the middlemen thorough exporting companies would be the first step to ensure correct identifications.

There is a need to assess the possibility of farming local frogs for this trade. If it proved successful and cost-efficient, local frogs farming could replace the farming of the exotic species *Rana catesbeiana*, which has become established as an exotic and threatened native fauna in other places where it has been introduced for farming.

In conclusion, the Indonesian frog leg trade employs a large number of people and is an important sector of the economy. There is presently no evidence that harvesting is being conducted at unsustainable levels, and therefore there does not seem to be any conservation need to reduce the rate of harvesting. There is a need to improve the quality and quantity of information available on the industry, and our understanding of the biology of the species harvested, so that developing problems can be averted before they become serious. There is also a need to ensure that harvesting is limited to species that are not adversely impacted.

REFERENCES

Abdullah, A. R. 1991. Persistent organochloride pesticides in the tropical environment. *Wallaceana* 64: 1-6.

Abdulali, H. 1985. On the export of frog legs from India. *J. Bombay Nat. Hist. Soc.* 2: 347-375.

Achmad and F. G. Sulaiman. 2000. *Monyet dan beruk sebagai komoditi ekspor dan peluangnya dalam bisnis di Indonesia.* Prosiding Seminar Primatologi Indonesia, Yokyakarta.. p: 52-66

Akita, T. and A. Alisjahbana. 2002. Regional income inequality in Indonesia and the initial impact of the economic crisis. *Bulletin of Indonesian Economic Studies* 38(2): 201-222.

Alcala, A. C. 1962. Breeding behaviour and early development of frogs of Negros, Philippine islands. *Copeia* 1962 (4): 679 - 726.

Alexander, P. S., C. M. Chang and C. H. Yang. 1963. Reproductive variation in the female rice frog *Rana limnocharis* during the spring season in Taiwan. *Biological Bulletin Tunghai University* 20: 1-4.

Alexander, P. S., A. C. Alcala and D. Y. Wu. 1979. Annual reproductive pattern in the rice frog *Rana l.limnocharis* in Taiwan. *Journal of Asian Ecology* 1979 (1): 68-78.

Alford, R., K. Bradfield and S. Richards. 1999. Measuring and analysing developmental instability as a tool for monitoring frog populations. In: A. Campbell (eds) *Declines and dissapearances of australian frogs*. Canberra, Environment Australia: 34-43 pp.

Alford, R. A. and S. J. Richards. 1999. Global amphibian declines: A problem in applied ecology. *Ann.Rev. Ecol. Syst.* 30: 133-165.

Anonymous. 1991. Declining amphibian populations - a global phenomenon? Findings and recommendation. Workshop on declining amphibian population on 19-20 February 1990. *Alytes* 9 (2): 33-42.

Ardiwinata, A. N., J. Soejitno, E. S. Harsanti, H. Purwanti and S. Y. Jatmiko. 2001. Jenis dan kelimpahan biota di ekosistem tanaman pangan. Jakenan, Pati, Jawa Tengah, Loka Penelitian Tanaman Pangan**:** 13 pp.

Arie, U. 1999. *Pembibitan dan pembesaran bullfrog.* Jakarta, Penebar Swadaya.104 pp.

Atmowidjojo, A. H. and Boeadi. 1988. Food prey on the stomach content of frogs. *Biotrop Spec. Publ.* 32: 77-80.

Ayas, Z., N. E. Barlas and D. Kolankaya. 1997. Determination of organochlorine pesticide residues in various environments and organisms in Giiksu delta, turkey. *Aquatic Toxicology* 39 (1997): 171-181.

Baird, R. W., A. M. Gorgone, A. D. Ligon and S. K. Hooker. 2001. Mark-recapture abundance estimate of bottlenose dolphins (*Tursiops truncatusi*) around Maui and Lana'i, Hawai'i during the winter of 200/2001. Report. La Jolla, Southwest Fisheries Science center, National Marine Fisheries Service USA.

Barbier, E. B., J. C. Burgess, T. M. Swanson and D. W. Pearce. 1990. *Elephants, economics and ivory.* First Edition. London, Earthscan Publication Ltd.154 pp.

Barfield, S. 1986. Indonesia's frog legs. *Journal of Environmental Health* 48 (6): 324.

Barinaga, M. 1990. Where have all the froggies gone? *Science* 247: 1033-1034.

Beebee, T. J. C. 1996. *Ecology and conservation of amphibians.* London, Chapman & Hall.214 pp.

Beissinger, S. R. and E. H. Bucher. 1992. Can parrots be conserved through sustainable harvesting? *Bioscience* 42 (3): 164-174.

Bennett, D. 2000. The density and abundance of juvenile *Varanus exanthematicus* (Sauria: Varanidae) in the coastal plain of Ghana. *Amphibia-Reptilia* 21 (3): 301-306

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Stratcombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Farantelli and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forest of Australia and Central America. *Proc.Natl.Acad.Sci. USA* 95: 9031-9036.

Berger, L., R. Speare and A. Hyatt. 1999. Chytrid fungi and amphibian declines: Overview, implications and future directions.In: A. Campbell (eds) *Declines and disappearances of Australian frogs*. Canberra, Environment Australia: 23-33 pp.

Berger, L., R. Speare, H. Hines, G. Marantelli, A. Hyatt, K. McDonald, L. Skerratt, V. Olsen, J. Clarke, G. Gillespie, M. Mahony, N. Sheppard, C. Williams and M. J. Tyler. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal* 82: 31-36.

Berger, L., R. Speare and A. Kent. 2000. Diagnosis of chytridimicosis in amphibians by histologic examination. *Zoo's Print Journal* XV (1): 184-190.

Berrill, M., D. Coulson, L. Mcgillivray and B. Pauli. 1998. Toxicity of endosulfan to aquatic stages of anuran amphibians. *Environmental Toxicology & Chemistry* 17 (1): 1738-1744.

Berry, P. Y. 1964. The breeding patterns of seven species of Singapore anura. *J. Anim. Ecol.* 33: 227-243.

Berry, P. Y. 1975. *The amphibian fauna of Peninsular Malaysia.* Kuala Lumpur, Tropical Press.127 p.

Beshkov, V. A. and D. L. Jameson. 1980. Movement and abundance of the yellow-bellied toad *Bombina variegata*. *Herpetologica* 36 (4): 365-370.

Beverton, R. 1998. Fish, fact and fantasy: A long view. *Reviews in Fish Biology and Fisheries* 8: 229-249.

Bjorksten, T., P. David, A. Pomiankowski and K. Fowler. 2000. Fluctuating asymmetry of sexual and nonsexual traits in stalk-eyed flies: A poor indicator of developmental stress and genetic quality. *Journal of Evolutionary Biology* 13 (1): 89-97.

Blaustein, A. R. 1994. Chicken little or Nero's fiddle? A perspective on declining amphibian populations. *Herpetologica* 50 (1): 58-97.

Blaustein, A. R. and D. B. Wake. 1990. Declining amphibian population: A global phenomenon? *Trends in Ecology and Evolution* 5 (7): 203-204.

Blaustein, A. R. and D. B. Wake. 1995. The puzzle of declining amphibian population. *Scientific American*: 56-61.

Blaustein, A. R., D. P. Wake and W. P. Sousa. 1994. Amphibian declines: Judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* 8 (1): 60-71.

Boeadi, R. Shine, Sugardjito, M. Amir and M. H. Sinaga. 1998. Biology of the commercially-harvested ratsnake (*Ptyas mucosus*) and cobra (*Naja sputatrix*) in Central Java. *Mertensiella* 9 :99-104.

Boyden, S. 1992. *Biohystory: The interplay between human society and the biosphere - past and present.* Paris, UNESCO.265 pp

Boone, M. D. and R. D. Semlitsch. 2001. Interactions of an insecticide with larval density and predation in experimental amphibian communities. *Conservation Biology* 15 (1): 228-238.

BPS. 1969 – 2003. Statistik Perdagangan luar negeri Indonesia (Indonesian foreign trade statistics) Vol I & II. Biro Pusat Statistik Indonesia, Jakarta.

BPS, 2000. Survey Pertanian : Luas Lahan Menurut Penggunaannya di Jawa *Agricultural Survey Land Area by Utilization in Java*. Biro Pusat Statistik, Jakarta.

BPS. 2002. Statistik Kesejahteraan Rakyat : Survey Sosial Ekonomi Nasional. Biro Pusat Statistik, Jakarta.

BPS. 2002. Statistik Kesejahteraan Rakyat : Survey Sosial Ekonomi Nasional. Biro Pusat Statistik, Jakarta.

Bridges, C. M. and R. D. Semlitsch. 2000. Variation in pesticide tolerance of tadpoles among and within species of Ranidae and patterns of amphibian decline. *Conservation Biology* 14 (5): 1490-1499.

Brönmark, C. and P. Edenham. 1994. Does the presence of fish affect the distribution of tree frogs (*Hyla arborea*)? *Conservation Biology* 8(3): 841-845.

Brooks, J. A. 1981. Otolith abnormalities in *Limnodynastes tasmaniensis* tadpoles after embrionic exposure to the pesticide dieldrin. *Environmental Pollution Series A* 25: 19-25.

Bruckner, A. W. 2000. New threat to coral reefs: Trade in coral organisms. *Issues in Science and Technology*. Fall 2000: 63-68.

Cade, B.S, and J. Richards, 1999, User Manual for Blossom Statistical Software: Fort Collins, CO, U.S. Geological Survey, 107 pp.

Cairns, S. C. and R. T. Kingsford. 1995. Harvesting wildlife: Kangaroos and waterfowl.In: R. A. Bradstock, T. D. Auld, D. A. Keith et al. (eds) *Conserving biodiversity: Threats and solutions*, Surrey Beatty & Sons: 260-272.

Callister, D. J. and L. M. Williams. 1995. Australia's native wildlife trade: Scale, trends and impacts.In: R. A. Bradstock, T. D. Auld, D. A. Keith et al. (eds) *Conserving biodiversity: Threats and solutions.*, Surrey Beatty & Sons.: 273-291.

Carey, C. 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountain of Colorado. *Conservation Biology* 7 (2): 355-362.

Carey, C. and C. J. Bryant. 1995. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. *Environmental Health Perspective* 103 (Suppl 4): 13-17.

Carson, R. 1962. *Silent spring.* New York, Houghton Mifflin Company.297 pp.

Caughley, G. 1977. *Analysis of vertebrate populations.* New York, John Wiley and Sons.234 pp.

Chi, H. 2000. Computer simulation models for sustainability. *International Journal of Sustainability in Higher Education* 1 (2): 154-167.

Church, G. 1960. The effects of seasonal and lunar changes on the breeding pattern of the edible Javanese frog, *Rana cancrivora* Gravenhorst. *Treubia* 25 (2): 215-233.

Clarke, R. D. 1972. The effect of toe clipping on survival in fowler's toad (bufo woodhouse fowleri). *Copeia* 1972 (1): 182-185.

Clarke, G. M. 1994. Developmental stability analysis: An early warning system for biological monitoring of water quality. *Australian Biologist* 7 (2): 94-104.

Clayton, L. and E. J. Milner-Gulland. 2000. The trade in wildlife in north Sulawesi, Indonesia. In: J. G. Robinson and E. L. Bennett (eds) *Hunting for sustainability in tropical forests*. New York, Columbia University Press: 473 - 496.

Clayton, L., M. Keeling and E. J. Milner-Gulland. 1997. Bringing home the bacon: A spatial model of wild pig hunting in Sulawesi, Indonesia. *Ecological Application* 7 (2): 642-652.

Cloudsley-thompson, J. L. 1999. *The diversity of amphibians and reptiles: An introduction.* Berlin, Springer-Verlag.254 pp.

Cohen, M. M. J. 2001. Is Kermit the frog in trouble? *American Journal of Medical Genetics* 104: 99-100.

Cooke, A. S. 1970. The effect of pp'-ddt on tadpoles of the common frog (*Rana temporaria*). *Environmental Pollution* 1: 57-71.

Cooke, A. S. 1973. The effects of DDT, when used as a mosquito larvacide, on tadpoles of the frog *Rana temporaria*. *Environmental Pollution* 5: 259-273.

Cooke, A. S. 1977. Effects of field applications of the herbicides diquat and dichlobenil of amphibians. *Environmental Pollution Series A* 12: 43-50.

Cooke, A. S. 1981. Tadpoles as indicators of harmful levels of pollution in the field. *Environmental Pollution Series A* 25: 123-133.

Dash, M. C. and J. K. Mahanta. 1993. Quantitative analysis of the community structure of tropical amphibian assemblages and its significance to conservation. *J. Biosci.* 18 (1): 121-139.

Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5 (6): 735-748.

Directorate General of Fisheries (DGF). 2001. Program peningkatan eskpor hasil perikanan 2003 . Jakarta, Indonesia.

Duellman, W. E. and L. Trueb. 1994. *Biology of amphibian.* Baltimore, The Johns Hopkins University Press.670 pp.

Dunson, W. A. 1977. Tolerance to high temperature and salinity by tadpoles of the Phillipine frog, *Rana cancrivora*. *Copeia* 1997: 375-378.

Dole, J. W. and P. Durant. 1974. Movements and seasonal activity of A*telopus oxyrhynchus* (anura: Atelopodidae) in a Venezuelan cloud forest. *Copeia* 1974 (1): 230-235.

Dyson, M. L., S. P. Henzi, T. R. Halliday and L. Barrett. 1998. Success breeds success in mating male reed frogs (*Hyperolius marmoratus*). *Proc. Roy. Soc. Lond. B.* 265: 1417–21.

Ecobichon, D. J. 2001. Pesticide use in developing countries. *Toxicology* 160 (2001): 27–33.

Emerson, S. B. 2001. A macroevolutionary study of historical contingency in the fanged frogs of Southeast Asia. *Biological Journal of the Linnean Society* 73: 139–151.

Emerson, S. 1992. Courtship and nest-building behavior of a Bornean frog, *Rana blythi*. *Copeia* 1992(4): 1123-1127.

Emerson, S. B., C. N. Rowsemitt and D. L. Hess. 1993. Androgen levels in a Bornean voiceless frog, *Rana blythi*. *Can. J. Zool.* 71: 196-203.

Emerson, S. B. and R. F. Inger. 1992. The comparative ecology of voiced and voiceless Bornean frogs. *Journal of Herpetology* 26 (4): 482-490.

Emerson, S. B. and R. Ward. 1998. Male secondary sexual characteristics, sexual selection, and molecular divergence in fanged Ranid frogs of Southeast Asia. *Zoological Journal of the Linnean Society* 122: 537–553.

Erdelen, W., Ed. 1998. *Conservation, trade and sustainable use of lizards and snakes in Indonesia*. Mertensiella 7. Rheinbach, Germany.

Favre, D. S. 1985. *International trade in endangered species: A guide to CITES.* The Netherlands, Martinus Nijhoff Pub.415 p.

Felton, A. 1999. Determinants of male mating success in the microhylid frog, *Cophixalus ornatus*. Bachelor of Science Honours Thesis. The Department of Zoology and Tropical Ecology, James Cook University. Townsville. 71 pp.

Fitter, R. 1986. *Wildlife for man: How and why we should conserve our species.* First edition. London, Collins.223 pp.

Fox, J. J. 1993. Ecological policies for sustaining high production in rice: Observations on rice intensification in Indonesia. In: H. Brookfield and Y. Byron (eds) *South-east Asia's environmental future: The search for sustainability*. Kuala Lumpur, Oxford University Press: 211-241 pp.

Freese, C. H. 1998. *Wild species as commodities: Managing market and ecosystems for sustainability.* Washington, Island Press.319 pp.

Friedl, T. W. P. and G. M. Klump. 1997. Some aspects of population biology in the European treefrog, *hyla arborea*. *Herpetologica* 53 (3): 321-330.

Frost, D. R. 1985. *Amphibian species of the world: A taxonomic and geographical reference*. USA, Allen Press.732 pp.

Frost, D.l R. 2004. Amphibian Species of the World: an Online Reference. Version 3.0 (22 August, 2004). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.php. American Museum of Natural History, New York, USA.

Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy and C. Winne. 2000. The global decline of reptiles, deja vu amphibians. 2000. BioScience 50 (8):653-666.

Gillespie, G. R. 2001. The role of introduced trout in the decline of spotted tree frog (*Litoria spenceri*) in south-eastern Australia. *Biological Conservation* 100 (2001): 187-198.

Gillilland, C., C. Summer, M. Gillilland, K. Kannan, D. Villeneuve, K. Coady, P. Muzzall, C. Mehne and J. Giesy. 2001. Organochlorine insecticides, polychlorinated biphenyls, and metals in water, sediment, and green frogs from southwestern michigan. *Chemosphere* 44 (3): 327-339.

Ginoga, L. N. 1999. Pencemaran insektisida pada tiga spesies burung air di areal persawahan Sukamandi, Subang, Jawa Barat. MSc. Thesis. Pascasarjana, Insitut Pertanian Bogor, Bogor. 95 pp.

Golay, N. and H. Durrer. 1994. Inflammation due to toe-clipping in natterjack toads (*Bufo calamita*). *Amphibia-Reptilia* 15: 81-83.

Gorzula, S. 1996. The trade in dendrobatid frogs from 1986 to 1993. *Herpetological Review* 27(3): 116-123.

Halliday, T. R. and P. A. Verrell. 1988. Body size and age in amphibians and reptiles. *Journal of Herpetology* 22 (3): 253-265.

Hayes, T., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. Stuart and A. Vonk. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proc Natl Acad Sci U S A* 99 (8): 5476-80.

Hayes, M. P. and M. R. Jennings. 1986. Decline of ranid frog species in western North America: Are bullfrogs (*Rana catesbiana*) responsible? *Journal of Herpetology* 20: 490 - 509.

Helgen, J., R. G. McKinnell and M. C. Gernes. 1998. Investigation of malformed northern leopard frogs in Minnesota. In: M. J. Lannoo (eds) *Status and conservation of Midwestern amphibians*. Iowa, University of Iowa Press: 288-297..

Hero, J. 1989. A simple code for toe clipping anurans. *Herp Review* 20 (3): 66-67.

Heying, H. E. 2001. Social and reproductive behaviour in the Madagascan poison frog, *Mantella laevigata*, with comparisons to the dendrobatids. *Anim. Behav.* 61: 567-577.

Hofrichter, R. E. 2000. *The encyclopedia of amphibians.* Ontario, Key Porter Books Lim.215 pp.

Hurtado-Gonzales, J. L. and R. E. Bodmer. 2004. Assessing the sustainability of brocket deer hunting in the Tamshiyacu-tahuayo communal reserve, northeastern Peru. *Biological Conservation* 116 (2004): 1-7.

Hutchings, J. A. 2001. Conservation biology of marine fishes: Perceptions and caveats regarding assignment of extinction risk. *Can. J. Fish. Aquat. Sci.* 58: 108–121.

Hutton, J. and B. Dickson. 2000. *Endangered species threatened convention: The past, present and future of CITES.* London, Earthscan Publication Ltd.202 pp.

Igbedioh, S. O. 1991. Effects of agricultural pesticides on humans, animals, and higher plants in developing countries. *Archives of Environmental Health* 46 (4): 218-224.

Inger, R. F. 1966. *The systematics and zoogeography of the amphibia of Borneo.* Chicago, Field Museum of Natural History.402 pp.

Iskandar, D. T. 1998. *Amfibi Jawa dan Bali.* Bogor, Puslitbang Biologi-LIPI.132 p.

Iskandar, D. T. and E. Colijn. 2000. Preliminary checklist of Southeast Asian and New Guinean herpetofauna: I. Amphibians. *Treubia* 31 (3 Supplement): 1-133.

Jaafar, I. H. 1994. The life history, population and feeding biology of two paddy field frogs, *Rana cancrivora* gravenhorst and *R. limnocharis* boie, in Malaysia. Doctor of Philosophy Thesis. Faculty of Science and Environmental Studies, University Pertanian Malaysia, Malaysia. 228 pp.

Jaafar, I. H., A. Ismail and A. Kurais. 1999. Correlations of reproductive parameters of two tropical frogs from Malaysia. *Asiatic Herpetological Research* 8: 48-52.

Jaafar, I., A. Ektella, M. Mansor and S. Shahrudin. 2002. A comparative study of the amphibian community at three sites in Penang state, Malaysia. *FROGLOG* 50.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, J. A. Estes, T. P. Hughes, S. Kidwell, C. B. Lange, H. S. Lenihan, J. M. Pandolfi, C. H. Peterson, R. S. Steneck, M. J. Tegner and R. R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293: 269-638.

Jeffers, J. N. R. 1978. *An introduction to system analysis: With ecological applications.*, University Park Press.

Jenkins, M. D. 1995. *Tortoises and freshwater turtles: The trade in Southeast Asia.* Traffic International.48 pp.

Jennings, M. R. and M. P. Hayes. 1985. Pre-1900 overharvest of California red-legged frogs (*Rana aurora draytonii*): The inducement for bullfrog (*Rana catesbiana*) introduction. *Herpetologica* 41: 94-103.

Jocelyn, K. 1999. Link to parasites grows stronger. *Science* 286 (5449): 2434.

Johnson, P. T. J., K. B. Lunde, E. G. Ritchie, J. K. Reaser and A. E. Launer. 2001. Morphological abnormality patterns in a Californian amphibian community. *Herpetologica* 57 (3): 336-352.

Kaiser, J. 1999. A trematode parasite causes some frog deformities. *Science* 284: 731-732.

Kam, Y. C., T. C. Chen, J. T. Yang, F. C. Yu and K. M. Yu. 1998. Seasonal activity, reproduction, and diet of a riparian frog (*Rana swinhoana*) from a subtropical forest in Taiwan. *Journal of Herpetology* 32 (3): 447-452.

Kellner, J. R. and R. A. Alford. 2003. The ontogeny of fluctuating asymmetry. *The American Naturalist* 161 (6): 931-947.

Kiesecker, J. M. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? *PNAS* 99 (15): 9900–9904.

Krebs, C. J. 1999. *Ecological methodology.* 2nd Edition. California, Addison Weley Longman, Inc.620 pp.

Kumbar, S. M. and K. Pancharatna. 2001. Occurrence of growth marks in the cross section of phalanges and long bones of limbs in tropical anurans. *Herpetological Review* 32 (3): 165-167.

Lau, A. S. M. and D. S. Melville. 1994. International trade in swiftlet nests with special reference to Hong Kong, Traffic International: 35 pp.

Lamont, B. B., R. MArsula, N. J. Enright and E. T. F. Witkoski. 2001. Conservation requirements of an exploited wildflower: Modelling the effects of plant age, growing conditions and harvesting intensity. *Biological Conservation* 99: 157-168.

Lanoo, M. J., K. Lang, T. Waltz and G. S. Phillips. 1994. An altered amphibian assemblage: Dickinson county, Iowa, seventy years after Frank Blanchard's survey. *American Midland Naturalist* 131: 311-319.

Lawler, S. P., D. Dritz, T. Strange and M. Holyoak. 1999. Effects of introduced mosquito fish and bullfrogs on the threatened California red-legged frog. *Conservation Biology* 13 (3): 613-622.

Lee, R. J. 2000. Impact of subsistence hunting in north Sulawesi, Indonesia and conservation options. In: J. G. Robinson and E. L. Bennett (eds) *Hunting for sustainability in tropical forest*. New York, Columbia University Press: 455-472.

Lemckert, F. and T. Brassil. 2000. Movements and habitat use of the endangered giant barred river frog (*Mixophyes iteratus*) and the implications for its conservation in timber production forests. *Biological Conservation* 96 (2000): 177-184.

Leong, T. M. 2001. Parasitic copepods responsible for limb abnormalities. *Froglog* 46 (3).

Liem, D. S. S. 1971. The frogs and toads of Tjibodas national park Mt. Gede, Java, Indonesia. *The Philippine Journal of Science* 100 (2): 131-161.

Lima, A. P., W. E. Magnusson and D. G. Williams. 2000. Differences in diet among frogs and lizards coexisting in subtropical forests of Australia. *Journal of Herpetology* 34 (1): 40-46.

Lips, K. R. 1998. Decline of a tropical montane amphibian fauna. *Conservation Biology* 12 (1): 106-117.

Martens, H. 1991. Trade in frog legs of wild SE Asian *Rana* species: Some facts and considerations. Germany, Scientific Authorities of CITES, Germany.

Martin, R. E. 2000. Other aquatic life of economic significance: Frogs and frog legs.In: R. E. Martin; E.P. Carter; G. J. J. Flick and L. M. Davis (eds) *Marine and freshwater products handbook.* Lancaster, Technomic Pub. Co. Inc.: 279 - 287.

Mace, G. M. and R. Lande. 1995. Assessing extinction threats: Toward a reevaluation of IUCN threatened species categories. In: D. Ehrenfeld (eds) *To preserve biodiversity - an overview*. Cambridge, Blackwell Science, Inc.and The society for conservation biology: 51-60.

Mattoon, A. 2000. Amphibia fading. *World Watch* 13 (4): 12-23.

Mazzoni, R., A. A. Cunningham, P. Daszak, A. Apolo, E. Perdomo and G. Speranza. 2003. Emerging pathogen of wild amphibians in frogs (*rana catesbeiana*) farmed for international trade. *Emerging Infectious Diseases* 9 (8): 995-998.

McCarthy, M. A. and K. M. Parris. 2004. Clarifying the effect of toe clipping on frogs with Bayesian statistics. *Journal of Applied Ecology* 41: 780-786.

McCaughran, D. A. 1997. Seventy-five years of halibut management success.In: D. A. Hancock, D. C. Smith, A. Grant and J. P. Beumer (eds) *Developing and sustaining world fisheries resources: The state of science and management:2nd world fisheries congress proceeding*. Collingwood, Australia, CSIRO Pubising: 680-686 pp.

McCoy, K. A. and R. N. Harris. 2003. Integrating developmental stability analysis and current amphibian monitoring techniques: An experimental evaluation with the salamander *Ambystoma maculatum*. *Herpetologica* 59 (1): 22-36.

McDonald, K. R. and R. A. Alford. 1999. A review of declining frogs in northern queensland.In: A. Campbell (eds) *Declines and disappearances of Australian frogs*. Canberra, Environment Australia: 14-22.

Meteyer, C. U., I. K. Loeffler, J. F. Fallon, K. A. Converse, E. Green, J. C. Helgen, S. Kersten, R. Levey, L. Eaton-Poole and J. G. Burkhart. 2000. Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. *Teratology* 62: 151-171.

Milner-Gulland, E. J. and L. Clayton. 2002. The trade in babirusas and wild pigs in north Sulawesi, Indonesia. *Ecological Economics* 42 2002): 165-183.

Ministry of Agriculture. 1993. Pedoman teknis pemanfaatan dan pengolahan paha kodok beku. Badan Penelitian dan Pengembangan Pertanian Pusat Penelitian dan Pengembangan Perikanan.69 pp.

Ministry of Fisheries. 2000. Profil perusahaan pengolahan dan eksportir hasil perikanan Indonesia. 2000. Jakarta, Departemen Eksplorasi Laut dan Perikanan Direktorat Jenderal Perikanan.

Mohanti-Hejmadi, P. and S. K. Dutta. 1981. Effects of some pesticides on the development of the Indian bullfrog *Rana tigerina*. *Environmental Pollution Series A* 24: 145-161.

Moyle, P. B. 1973. Effects of introduced bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin valley, California. *Copeia* 1973: 18-22.

Mulliken, T. A., S. R. Broad and J. B. Thomsen. 1992. *Perceptions, conservation and management of wild birds in trade.* Traffic international.106 p.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858

Niekisch, M. 1986. The international trade of frogs' legs. *Traffic Bull.* 8: 7-10.

Odum, E. P. 1971. *Fundamentals of ecology.* Third Edition. Philadelphia, WB Saunders Comp.574 pp.

Osborn, D. and M. C. French. 1981. The toxicity of the mothproffing chemical Eulan WA new to frog *Rana temporaria* tadpoles. *Environmental Pollution Series A* 24: 117-123.

Orams, M. B. 2002. Feeding wildlife as a tourism attraction: A review of issues and impacts. *Tourism Management* 23 (2002): 281-293.

Ouellet, M. 2000. Amphibian deformities: Current status of knowledge. In: D. W. Sparling, G. Linder and C. A. Bishop (eds) *Ecotoxicology of amphian and reptiles*. Pensacola, Society of Environmental Toxicology and Chemistry (SETAC): 617-696 pp.

Ouellett, M., J. Bonin, J. Rodrigue, J. DesGranges and S. Lair. 1997. Hindlimb deformities (electromelia, ectrodactyly) in free-living anurans from agricultural habitats. *Journal of Wildlife Disease* 33 (1): 95-104.

Palmer, A. R. and C. Strobeck. 1986. Fluctuating asymmetry: Measurement, analysis, patterns. *Ann.Rev. Ecol. Syst.* 17: 391-421.

Palmer, A. R. and C. Strobeck. 2003. Fluctuating asymmetry analyses revisited. In: M. Polak (eds) *Developmental instability (di): Causes and consequences*, Oxford University Press : 279-319

Paltridge, R. and T. Nano. 2001. Digging for frogs in the Tanami desert. *Australian Geographic*: 25-26.

Pandian, T. J. and M. P. Marian. 1986. Production and utilization of frogs: An ecological view. *Proc. Indian Acad. Sci. (Anim. Sci.)* 95(3): 289 - 301.

Pankakoski, E. 1985. Epigenetic asymmetri as an ecological indicator of muskrat. *J. Mamm.* 66 (1): 52-57.

Patel, T. 1993. French may eat Indonesia out of frogs. *New scientist*. 1868: 7.

Pechman, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Cadwell, L. J. Vitt and W. Gibbons. 1991. Declining amphibian populations: The problem of separating human impacts from natural fluctuations. Science 253: 892-895.

Pechman, J. H. K. and H. M. Wilbur. 1994. Putting declining amphibians populations in perspective: Natural fluctuations and human impacts. *Herpetologica* 50: 65-84.

Peter, A. H. 2001. Survival of adults of the water frog *Rana lessonae* and its hybridogenetic associates *Rana esculenta*. *Can. J. Zool.* 79: 652-661.

Pfister, C. A. and A. Bradbury. 1996. Harvesting red sea urchins: Recent effects and future predictions. *Ecological Application* 6 (1): 298-310.

PKA (Perlindungan Konservasi Alam). 2000. Penetapan jatah penangkapan jenis-jenis satwa dan pengambilan jenis-jenis yang tidak dilindungi undang-undang dan tidak termasuk didalam appendix CITES untuk perdagangan periode 2001. Ministry of Forestry. Bogor. Indonesia.

Ponting, C. 1991. *A green history of the world: The environment and the collapse of great civilizations.* New York, Penguin Books.430 pp.

Pope, K. L. and K. R. Matthews. 2002. Influence of anuran prey on the condition and distribution of *rana muscossa* in the Sierra Nevada. *Herpetologica* 58 (3): 354-363.

Pough, F. H., R. M. Andrews, J. E. Cadle, M. L. Crump, A. H. Savitzky and K. D. Wells. 2004. *Herpetology.* Third Edition. London, Pearson Prentice Hall.726 p.

Pratomo, H. 1997. Keragaman dan ekologi genus Rana (Amphibia: Ranidae) di daerah Bogor, Sukabumi dan Cianjur. Unpublished master thesis. Program Pascasarjana, Institut Pertanian Bogor, 88 pp.

Premo, D. B. 1985. The reproductive ecology of a ranid frog community in pond habitats of West Java, Indonesia. PhD Dissertation. Department of Zoology, 184 pp.

Primack, R. B. 2002. *Essentials of conservation biology.* Third Edition. Sunderland, Massachusetts, Sinauer Associates, Inc.698 pp.

Quisey, P. M., D. C. Donohue and J. T. Ahokas. 1995. Persistence of organochlorines in breast milk of women in Victoria, Australia.. *Fd Chem. Toxic.* 33(I): 49-56.

Ramirez, J., R. C. Vogt and J. Villarreal-Benitez. 1998. Population biology of a neotropical frog (*Rana vaillanti*). *Journal of Herpetology* 32 (3): 338-344.

Reaser, J. K. 2000. Demographic analysis of the Columbia spotted frog (*rana luteiventris*): Case study in spatiotemporal variation. *Can. J. Zool.* 78.

Reaser, J. M. and R. E. Dexter. 1996. Toe clipping effects on *Rana pretiosa* (spotted frogs). *Herpetological Review* 27 (4): 195-196.

Relyea, R. A. and N. Mills. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proc. Natl. Acad. Sci. USA.* 98 (5): 2491-2496.

Reynolds, J. D., S. Jennings and N. K. Dulvy. 2001. Life histories of fishes and population responses to exploitation. In: J. D. Reynolds, G. M. Mace, K. H. Redford and J. G. Robinson (eds) *Conservation of exploited species*. Cambridge, Cambridge University Press: 147-168.

Ryser, J. 1988. Determination of growth and maturation in the common frog, *Rana temporaria* by skeletochronology. *Journal of Zoology* 216: 673-685.

Republik Indonesia 1990. Undang-undang Republik Indonesia No. 5 tahun 1990 tentang Konservasi Sumber Daya Alam Hayati Dan Ekosistemnya.

Richards, S. J., K. R. McDonald and R. A. Alford. 1993. Declines in populations of Australia's endemic tropical rainforest frogs. *Pacific Conservation Biology* 1: 66-77.

Robinson, J. G. and E. L. Bennett, Eds. 2000. *Hunting for sustainability in tropical forest*. Biology and resource management series. New York, Columbia University Press. 1000 pp.

Rosenberg, A. A., M. J. Fogarty, M. P. Sissenwine, J. R. Beddington and J. G. Shepherd. 1993. Achieving sustainable use of renewable resources. *Science* 262(5135): 828-829.

Ryser, J. 1988. Determination of growth and maturation in the common frog, *Rana temporaria* by skeletochronology. *Journal of Zoology* 216: 673-685.

Safford, R. J. and C. G. Jones. 1997. Did organochlorine pesticide use cause declines in Mauritian forest birds? *Biodiversity and Conservation* 6: 1445-1451.

Salini, J., D. Brewer, M. Farmer and P. Jones. 2001. Lunar periodicity of prawns and by-catch in trawls by the Gulf of Carpentaria, northern Australia. *Marine Biology* 138: 975-983.

Saputra, G. 1998. Irata's role in conservation and sustainable use of reptiles. In: W. Erdelen (eds) *Conservation, trade and sustainable use of lizards and snakes in indonesia. Martensiella (9)*: 23-25 pp.

Schmidt, D. C., G. B. Kyle, S. R. Carlson, H. J. Geiger and B. P. C. Finney. 1997. Alaska's sockeye salmon fishery management: Can we learn from success? In: D. A. Hancock, D. C. Smith, A. Grant and J. P. Beumer (eds) *Developing and sustaining world fisheries resources: The state of science and management:2nd world fisheries congress proceeding.* Collingwood, Australia, CSIRO Publishing: 687-695.

Schmuck, J. 2000a. Trade and species conservation. In: R. Hofrichter (ed) *The encyclopedia of amphibians.* Ontario, Key Porter Books Lim.: 228-241.

Schmuck, J. 2000b. Amphibians in human nutrition. In: R. Hofrichter (ed) *The encyclopedia of amphibians.* Ontario, Key Porter Books Lim.: 214-217.

Schnabel, Z. E. 1938. The estimation of total fish population in a lake. *Amer. Math. Monthly* 45: 348-352.

Schwarzkopf, L. and R. A. Alford. 2002. Nomadic movement in tropical toads. *Oikos* 96: 492-506.

Settle, W. H., H. Ariawan, E. A. Astuti, W. Cahyana, A. L. Hakim, D. Hindayana, A. S. Lestari and Pajarningsih. 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. *Ecology* 77 (7): 1975-1988.

Shine, R., P. S. Harlow, J. S. Keogh and Boeadi. 1996. Commercial harvesting of giant lizards: The biology of water monitors, *Varanus salvator*, in southern Sumatra. *Biological Conservation* 77: 125-134.

Shine, R., P. S. Harlow, J. S. Keogh and Boeadi. 1998. The allometry of life-history traits: Insights from a study of giant snakes (*Python reticulatus*). *J. Zool., Lond.* 244: 405-414.

Shine, R., Ambariyanto, P. S. Harlow and Mumpuni. 1998. Ecological traits of commercially harvested water monitors, *Varanus salvator*, in northern Sumatra. *Wildlife Research* 25: 437-447.

Shine, R., Ambariyanto, P. S. Harlow and Mumpuni. 1998. Reticulated pythons in Sumatra: Biology, harvesting and sustainability. *Biological Conservation* 87 (1999): 349-357.

Shine, R., P. Harlow, Ambariyanto, Boeadi, Mumpuni and J. S. Keogh. 1998. Monitoring monitors: A biological perspective on the commercial harvesting of Indonesian reptiles. *Mertensiella* 9: 61-68.

Shine, R., P. S. Harlow, J. S. Keogh and Boeadi. 1998. The influence of sex and body size on food habits of a giant tropical snake, *Python reticulatus*. *Functional Ecology* 12: 248-258.

Shine, R., Ambariyanto, P. S. Harlow and Mumpuni. 1999. Ecological attributes of two commercially-harvested python species in northern Sumatra. *Journal of Herpetology* 33: 249-257.

Slade, N. A., R. Gomulkiewicsz and H. M. Alexander. 1998. Alternatives to Robinson and Redford's method of assessing overharvest from incomplete demographic data. *Conservation Biology* 12 (1): 148-155.

Smith, A. G. and S. D. Gangolli. 2002. Organochlorine chemicals in seafood: Occurrence and health concerns. *Food and Chemical Toxicology* 40 (2002): 767–779.

Soehartono, T. and A. Mardiastuti. 1995. *An overview of wildlife trade in Indonesia*. 2nd Meeting of the Conference of the Parties to the United Nation Convention on Biological Diversity. Jakarta.

Soehartono, T. and A. Mardiastuti. 2002. *CITES implementation in Indonesia.* Indonesia, Nagao Natural Environment Foundation.339 pp.

Soehartono, T. and A. C. Newton. 2001. Conservation and sustainable use of tropical trees in the genus Aquilaria II. The impact of gaharu harvesting in Indonesia. *Biological Conservation* 97(2001): 29-41.

Soule, M. and B. Baker. 1968. Phenetics of natural populations. Iv. The population asymmetry parameter in the butterfly *Coenomympha tullia*. *Heredity* 23: 611-614.

Sparling, D. W., G. Linder and C. A. Bishop. 2000. *Ecotoxicology of amphibians and reptiles.* Columbia, SETAC Technical Publications.877 pp.

Spellerberg, I. F. 1971. The amphibian and reptile trade with particular reference to collecting in europe. *Biological Conservation* 10: 221-232.

Sterman, J. D. 2002. All models are wrong: Reflections on becoming a systems scientist. *System Dynamics Review* 18 (4): 501-531.

Sugiri, N. 1979. Beberapa aspek biologi kodok batu (*Rana blythi*) Boulinger, Ranidae, Anura-Amphibia) di beberapa wilayah indonesia dan kedudukan taksanya. PhD thesis. Fakultas Pasca Sarjana, Institut Pertanian Bogor, Bogor. 188 pp.

Susanto, H. 1989. *Budidaya kodok unggul.* Jakarta, Penebar Swadaya..

Szilard, N. and K. Csengele. 2001. Amphibian harvesting in Romania. *FROGLOG* (44).

Taylor, S. K., E. S. Williams and K. W. Mills. 1999. Effects of malathion on disease susceptibility in Woodhouse's toad. *Journal of Wildlife Disease* 35 (3): 536-541.

Tessier, C., D. Slaven and D. M. Green. 1991. Population density and daily movement patterns of Hochstetter's frogs, *Leiopelma hochstetteri*, in a New Zealand mountain stream. *Journal of Herpetology* 25 (2): 213-214.

Thiemann, G. W. 2000. Patterns and consequences of behavioural responses to predators and parasites in *Rana* tadpoles. *Biological Journal of the Linnean Society* 71: 513–528.

Toda, M. 1999. Historical biogeography of East Asian populations of *Rana limnocharis* (amphibia:Anura): A review. In: H. Ota (eds) *Tropical island herpetofauna: Origin, current diversity, and conservation*. Amsterdam, Elsevier Science B.V.: 299-315.

Török, Z. 2003. Action plan for sustainable exploitation of *Rana ridibunda* stocks from the Danube delta biosphere reserve (Romania). *FROGLOG* (60).

Trenerry, M. P., W. F. Laurance and K. R. McDonald. 1994. Further evidence for the precipitous decline of endemic rainforest frogs in tropical Australia. *Pacific Conservation Biology* 15: 150-153.

Truong, N. Q. 2000. Amphibian uses in Vietnam. *FROGLOG* (38).

Tsubaki, Y. 1998. Fluctuating asymmetry of the oriental fruit fly (*dacus dorsalis*) during the process of its extinction from the Okinawa islands. *Conservation Biology* 12 (4): 926-929.

Tsuji, H. 2004. Reproductive ecology and mating success of male *Limnonectes kuhlii*, a fanged frog from Taiwan. *Herpetologica* 60 (2): 155-167.

Tsuji, H. and L. Kuang-Yang. 2000. The reproductive ecology of female *Rana (Limnonectes) kuhlii*, a fanged frog of Taiwan, with particular emphasis on multiple clutches. *Herpetologica* 56 (2): 153-165.

Tyler, M. J. 1991. Declining amphibian populations - a global phenomenon? An Australian perspective. *Alytes* 9 (2): 43-50.

Tyler, M. J. 1994. Frog cures herpes. *Australian Natural History Magazine*. 24: 10-11.

Tyler, M. J. 1999. *Australian frogs: A natural history.* Sydney, Reed New Holland.192 pp.

van der Eng, P. 1994. Development of seed-fertilizer technology in Indonesia rice agriculture. *Agricultural history* 68 (1): 20-54.

van Setten van der Meer, N. C. 1979. *Sawah cultivation in ancient java: Aspects of development during the indo-javanese period, 5th to 15th century.* Canberra, Faculty of Asian Studies in association with ANU Press.168 p.

van Wyk, E., H. Bouwman, H. Van der Bank, G. H. Verdoorn and D. Hofmann. 2001. Persistent organochlorine pesticides detected in blood and tissue samples of vultures from different localities in south africa. *Comparative Biochemistry and Physiology Part C* 129 (2001): 243-264.

Veith, M., J. Kosuch, R. Feldmann, H. Martens and A. Seitz. 2000. A test for correct species declaration of frog legs imports from Indonesia into the European Union. *Biodiversity and Conservation* 9: 333-341.

Veith, M. J., A. M. Ohler and A Dubois. 2001. Systematics of *Fejervarya limnocharis* (Gravenhorst, 1829) (Amphibia, Anura, Ranidae) and related species. 2. Morphological and molecular variation in frogs in the Greater Sunda Islands (Sumatra, Java, Borneo) with definition of two species. Alytes 2001: 5-28.

Vincent, A. C. J. 1997. Trade in pegasid fishes (sea moths) primarily for traditional chinese medicine. *Oryx* 31 (3): 199-208.

Vredenburg, V., Y. Wang and G. Feller. 2000. Scientific meeting raises awareness of amphibian decline in asia. *FROGLOG* (42).

Wallace, A. R. 1869. *The Malay archipelago.* London, MacMillan.

WCED. 1987. *Our common future.* Oxford, Oxford University Press.393 p.

Wikramanayake, E., E. Dinerstein, C. J. Loucks, D. M. Olson, J. Morrison, J. Lamoreux, M. McKnight and P. Hedao. 2002. *Terrestrial ecoregions of the indo-pacific: A conservation assessment.* Washington, Island Press.643 p.

Whitten, A. J., R. E. Soeriaatmadja and S. A. Afiff. 1996. *The ecology of Java and Bali.* Singapore, Periplus Editions (HK) Ltd.1028 p.

Wolf, J. H. D. and C. J. F. Konings. 2001. Toward the sustainable harvesting of epiphytic bromeliads: A pilot study from the highlands of Chiapas, Mexico. *Biological Conservation* 101: 32-31.

Wood, K. V., J. D. Nichols, H. F. Percival and J. E. Hines. 1998. Size-sex variation in survival rates and abundance of pig frogs, *Rana grylio*, in northern Florida wetlands. *Journal of Herpetology* 32(4): 527-535.

World Bank Report, 2003. Indonesia at a glance. Electronic Source accessible at http://www.worldbank.org/data/countrydata/aag/idn_aag.pdf. Access on 21 July 2004.

Zun´iga, J. A., J. F. Barrera, J. Valle and T. Williams. 2002. Estimating populations of *Idiarthron subquadratum* (orthoptera: Tettigoniidae) using mark-recapture methods in coffee plantations in Chiapas, Mexico. *Environ. Entomol.* 31 (3): 515-522.

Wood, K. V., J. D. Nichols, H. F. Percival and J. E. Hines. 1998. Size-sex variation in survival rates and abundance of pig frogs, *Rana grylio*, in northern Florida wetlands. *Journal of Herpetology* 32 (4): 527-535.