
**Intercohort cannibalism and parturition-associated
behaviour of captive-bred swordtail,
Xiphophorus helleri (Pisces: Poeciliidae)**

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**by
Clifford Louis Wilshire Jones
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ABSTRACT

Adult fish that belong to the family Poeciliidae cannibalise juveniles, both in the wild and under captive conditions, but this behaviour has only been partly investigated in the Poeciliidae in some of the commercially valuable species. The objective of the research is to develop an understanding of intercohort cannibalism and parturition-associated behaviour in captive-bred swordtail (*Xiphophorus helleri*), with applications to industry and future research of other poeciliids.

Experiments investigating the effect of adult stocking density and sex ratio on the production of juveniles were used to determine if cannibalism occurs under culture conditions. The average rate of intercohort cannibalism ranged from (5.5 to 53.9%), and was positively density dependent and independent of sex ratio, indicating that males and females were probably equally cannibalistic. The highest number of juveniles (1725.7 ± 141.4) produced per tank over 70 days was obtained from two males and eight females. To develop a better understanding of adult and juvenile behaviour during parturition, fish were observed under controlled laboratory conditions using video and behaviours such as attack (burst of speed by an adult in the direction of a juvenile), escape (avoidance of cannibalism after attack) and cannibalism (predation of a live juvenile by an adult), for example, were identified. Under laboratory conditions most young escaped in downward direction after attack (49%) and most utilised the refuge made that was made available. Furthermore, most attacks (62-65%) and cannibalism (57-84%) occurred at the bottom. Since the presence of refuge significantly increased the rate of juvenile survival under culture conditions, it was hypothesised that the rate of cannibalism could be reduced under farming conditions if juveniles were protected when they escaped downwards. This hypothesis was accepted as it was found that refuge at the bottom of the water column or the inclusion of a false-bottom reduced the rate of cannibalism by 49% and 72%, respectively. Similarly, the hypothesis that the rate of cannibalism could be reduced if juveniles were given protection when escaping sideways (32% of juveniles escaped sideways in the laboratory) was also accepted when tested under farm-scale conditions because a false-side reduced the rate of cannibalism by an average of 45%. Since males and females were equally responsible for cannibalising juveniles in the laboratory, it was hypothesised that the rate of cannibalism would decrease proportionately with the removal of males (i.e. 20% of the cannibals) from the population; the removal of males under farming conditions resulted in a 19.5% reduction in the rate of cannibalism. Since older juveniles were better able to escape cannibalism

than neonates and since adults habituate to stimuli that previously resulted in attack behaviour, it was hypothesised that the rate of cannibalism would remain unaffected by the length of time that juveniles were exposed to adults in the breeding tanks. This hypothesis was also accepted when tested under farm conditions. However, some hypotheses based on laboratory observations were not accepted. For example, a constant low light intensity did not appear to decrease the rate of cannibalism under farm conditions; also, the occurrence of dead and deformed juveniles went unnoticed in the laboratory, and under farm conditions, where adults did not have access to the bottom of the tank, 10% of the harvest consisted of dead and deformed juveniles. It is concluded that technologies, such as bottom-refuge or a false-side, that increase the size of the live-harvest and allow for the removal of potentially less viable offspring are recommended for the commercial production of poeciliids.

The overall similarity of *X. helleri* behaviour between the laboratory experiments and the farm-scale trials suggests that the *post-partum* behaviour of *X. helleri* remains consistent under these different conditions; thus, behaviour under one set of conditions may be used to predict behaviour under other conditions. The application and significance of extrapolations to industry and future research of *X. helleri* and possibly other poeciliids were discussed and the most applicable laboratory observations with the highest extrapolation capacity were proposed. Furthermore, techniques were developed to aid industry and future researchers in making predictions relating to behaviour of *X. helleri* under different conditions based on laboratory observations. The results were used to develop a model indicating that selection pressures against cannibalism are not likely to exist at the rate of cannibalism observed here since the potential genetic gain through kin survival and inclusive fitness was shown to be greater than any potential genetic loss experienced by a victim of cannibalism. The model was successfully tested under a range of social conditions. Other possible explanations for cannibalism in poeciliids, such as parental manipulation, nutritional advantages, opportunistic predation and the recovery of energy are discussed. It is suggested that the most likely proximate cause of cannibalism under captive conditions is opportunistic predation. The theory that cannibalism ensures that only viable genes of the victim are expressed, through inclusive fitness, is a possible ultimate cause of cannibalism, which may have been inherited from feral ancestors of captive-bred *X. helleri*.

CHAPTER 1

Introduction

Cannibalism is the act of killing and consuming an individual or the major part of an individual of the same species (Smith and Reay, 1991). It is also referred to as intraspecific predation (Fox, 1975; Polis, 1981). Cannibalism in fishes can be divided into two broad categories: intracohort cannibalism is cannibalism within an age class, which incorporates the term sibling cannibalism, and intercohort cannibalism is cannibalism between age classes, which includes cannibalism of juveniles by adult fish (Smith and Reay, 1991).

In their review of cannibalism in teleost fishes, Smith and Reay (1991) classify cannibalism according to three criteria, namely the age relationship between cannibal and prey, their genetic relatedness and the developmental stage of the prey. For example, the cannibalism of juvenile poeciliids by the parent fish would be described as filial intercohort cannibalism of juveniles. As this work deals with cannibalism of juveniles by both parents and unrelated adults, and not with any form of intracohort cannibalism, the only criterion necessary to differentiate between the types of cannibalism observed here was the genetic relationship between the cannibal and the prey. For the purpose of this thesis, *filial cannibalism* is defined as cannibalism of juveniles by a parent fish, and *non-kin cannibalism* defines cannibalism of juveniles by unrelated adults.

Cannibalism in aquaculture

Cannibalism of fish in larviculture has been reviewed by Hecht and Pienaar (1993) and recently by Baras and Jobling (2002). These reviews focus primarily on intracohort cannibalism, while intercohort cannibalism of eggs, larvae and juveniles in aquaculture have not received equal attention – probably because procedures employed in the intensive aquaculture of most species involve separating age classes (Baras and Jobling,

2002). However, the separation of age classes in some fishes, such as the mouth brooders, guarders and live-bearers, is not always practical or possible. Thus, it is unknown whether or not intercohort cannibalism may affect aquaculture of these species also.

The following background discussion refers to both intercohort and intracohort cannibalism; relatively little research has focused on intercohort cannibalism in aquaculture (Baras and Jobling, 2002) and similarities exist between the two forms. However, the mechanisms driving intracohort cannibalism (sibling cannibalism) may not always be the same as those driving intercohort cannibalism (filial and non-kin cannibalism). Therefore, a clear distinction is drawn between the two forms of cannibalism throughout the work.

Hecht and Pienaar (1993) reported that cannibalism is positively density dependent in all species in which it had been studied to date. This appears true for sibling cannibals (Degani and Levanon, 1983; Hecht and Appelbaum, 1988; Smith and Reay, 1991; Baras and Jobling, 2002) and filial cannibals (Rose, 1959; Warren, 1973; Thibault, 1974; Fox, 1975; Meffe, 1984; Smith and Reay, 1991; FitzGerald, 1991), with the black spotted stickleback, *Gasterosteus wheatlandi*, as the only known exception (FitzGerald, 1991). Cannibalism in that species was reported as density independent, which was attributed to the solitary nature of its behaviour (FitzGerald, 1991).

Intracohort cannibalism among larval African catfish (*Clarias gariepinus*) was suppressed significantly with increased availability of refuge sites (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992; Hecht and Pienaar, 1993). Similarly, filial cannibalism in livebearing mosquitofish (*Gambusia affinis*) and care-giving threespine stickleback (*Gasterosteus*

aculeatus) decreased significantly with an increase in the availability of shelter (Foster *et al.*, 1988; Benoit *et al.*, 2000). However, the rate of predation on juvenile *G. aculeatus* by other predators, such as insects, increased with increasing refuge density although the overall payoff for the decrease in cannibalism with increased vegetation cover was greater than the loss incurred by piscivorous insects (Foster *et al.*, 1988).

Food availability has been found to be negatively correlated to the rate of intracohort cannibalism in larval walleye (*Stizostedion vitreum*), African catfish (*C. gariepinus*), vundu (*Heterobranchus longifilis*), sea bass (*Dicentrarchus labrax*), cod (*Gadus morhua*), koi-carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) under intensive culture conditions (Loadman *et al.*, 1986; Hecht and Appelbaum, 1988; Katavic *et al.*, 1989; Folkvord, 1991; Hecht and Pienaar, 1993; Baras, 1999; Baras and Jobling, 2002). Furthermore, the availability of live food (*Daphnia* spp.) significantly suppressed aggression and cannibalism in juvenile *C. gariepinus* and *O. mykiss* (Hecht and Pienaar, 1993). The effect of food availability on some intercohort cannibals appeared to differ from that of intracohort cannibals. For example, filial cannibalism of juvenile *G. aculeatus* remained unaffected by the amount of food available to the adult fish (FitzGerald, 1992a) and starvation stress in the poeciliid *Poeciliopsis monacha* also had no effect on the number of offspring cannibalised by adult fish (Thibault, 1974). However, this finding was not consistent in at least one poeciliid practising intercohort cannibalism as low food levels increased filial cannibalism of juvenile *G. affinis* (Dionne, 1985).

The effect of light intensity on cannibalistic behaviour appears to vary considerably between fishes with different modes of cannibalism. Loadman, Moodie and Mathias (1986) working with *S. vitreum* observed a decrease in cannibalism at night, which was similar to the reduced rate of sibling cannibalism of juvenile *C. gariepinus* at low-light

intensity (Pienaar, 1990; Britz and Pienaar, 1992; Hecht and Pienaar, 1993). Care-giving Midas cichlids (*Cichlasoma citrinellum*) cannibalised their offspring irrespective of photo-conditions (McKaye and Barlow, 1976), whereas cannibalism in care-giving paradise fish (*Macropodus opercularis*) increased at low-light intensities since parental care is inhibited in the dark, at which time the eggs and juveniles are exposed to cannibalistic conspecifics (Smith and Reay, 1991). The suggestion that cannibalism in species that rely on visual orientation is usually lower under reduced light intensity (Baras and Jobling, 2002) largely concurs with reduced cannibalism at low light intensities for intracohort cannibals (Loadman *et al.*, 1986; Pienaar, 1990; Britz and Pienaar, 1992; Hecht and Pienaar, 1993). This, compared with the example of care-giving *M. opercularis*, further illustrates that there are probably different mechanisms at work on intracohort cannibalism compared with intercohort cannibalism in fishes.

Culture conditions may induce cannibalism. Intensive aquaculture environments are designed to maximise production per unit volume or area and fish are therefore subject to conditions, such as increased stocking density, lack of alternative food, or reduced opportunity to escape predation, which may elicit cannibalistic behaviour (Smith and Reay, 1991; Baras and Jobling, 2002). Much of the research mentioned above was designed to address this problem and is well summarised by Hecht and Pienaar (1993). Smith and Reay (1991) suggested that cannibalism is of some economic importance in aquaculture but that its impact can be reduced quite easily. Inter- and intracohort cannibalism account for losses under culture conditions of up to more than 90% (Berrios-Herandez and Snow, 1983; Hecht and Pienaar, 1993; Baras *et al.*, 2000a). While Baras and Jobling (2002) agree that the mitigation of cannibalism in aquaculture is largely achievable, they point out that before mitigating measures can be implemented it is necessary to have a complete understanding of the animal's cannibalistic behaviour. This

includes, for example, knowledge of the animal's age at which cannibalism emerges, growth advantages to the cannibal, environmental and social factors that trigger the behaviour, senses used in prey capture and "quality" or viability of the prey (Baras and Jobling, 2002). Therefore, the suggestion by Smith and Reay (1991) that cannibalism in aquaculture can be reduced "relatively easily," requires review.

Summary of cannibalism in the Poeciliidae

A number of poeciliids are known to cannibalise offspring in the wild; this group includes *Gambusia holbrooki*, *Gambusia affinis*, *Poeciliopsis monacha* and *Belonesox belizanus* (Thibault, 1974; Meffe and Snelson, 1989; Nesbit and Meffe, 1993), and these fish have all been reported to cannibalise under captive conditions also. There are further reports that *Poeciliopsis occidentalis*, *Poecilia reticulata*, *Poecilia latipinna*, *Heterandria formosa*, *Xiphophorus variatus* and *X. helleri* also cannibalise juveniles in captivity (Rose, 1959; Meffe, 1984; Meffe and Snelson, 1989; Hubbs, 1991; Jones *et al.*, 1998a; Jones *et al.*, 1998b; Benoit *et al.*, 2000; Kruger *et al.*, 2001a).

A full understanding of the processes involved in cannibalism of poeciliids (and most other fish families) is largely lacking (Meffe and Snelson, 1989). The following paragraph will summarise what is understood regarding intercohort cannibalism of poeciliids. Cannibalism is under genetic control in at least one poeciliid genus, *Poeciliopsis* (Thibault, 1974). Thibault (1974) demonstrated that the behaviour was polygenetically inherited when cannibalistic *P. monacha* were hybridised with non-cannibalistic *P. lucida*. While starvation stress had no impact on cannibalism in *P. monacha* (Thibault, 1974), low food availability affected cannibalism positively in the *G. affinis* (Dionne, 1985). Furthermore, cannibalism in *G. affinis* had a positive effect on somatic growth and reproductive development (Meffe and Crump, 1987). Stocking density positively affected the rate of

cannibalism in *P. reticulata* (Rose, 1959), *X. helleri* (Jones *et al.*, 1998a) and *P. monacha* (Thibault, 1974), while the availability of refuge negatively affected cannibalism in *G. affinis* (Benoit *et al.*, 2000) and *X. helleri* (Jones *et al.*, 1998a) and female poeciliids are reported to be more cannibalistic than males (Warren, 1973; Hubbs, 1991; Nesbit and Meffe, 1993) supposedly because to the female's larger size relative to males (Kruger, 1995). As very little work has been carried out on cannibalism in these fishes, and as their life history is probably conducive to this behaviour (Stenseth, 1985), cannibalism is likely to be more prevalent in the Poeciliidae than previously reported. However, such speculation is only testable once the behaviour of more species in the family has been studied and a more complete understanding of cannibalism in poeciliids becomes available.

An understanding of cannibalism in *X. helleri* under captive conditions, or otherwise, is lacking. For example, the effect of adult stocking density, refuge availability and light intensity on cannibalism in *X. helleri* is unknown, and while there have been reports of sexual differences in cannibalistic behaviour for some poeciliids (Warren, 1973; Hubbs, 1991), it is not known if behaviour differs between male and female *X. helleri* or if behaviour of adults and juveniles changes with time or age. Similarly, while cannibalism may be genetically inherited in the genus *Poeciliopsis* (Thibault, 1974) and while there have been reports of learned behaviour in poeciliid species such as the goldbelly top minnow (*Girardinus falcatus*) (Cantalupo *et al.*, 1995; Bisazza *et al.*, 1998) and the guppy (*P. reticulata*) (Goodey and Liley, 1986), the mechanisms driving cannibalism in *X. helleri* are not yet appreciated. Thus, it would appear that almost nothing is definitively known about cannibalism in *X. helleri*.

Taxonomy of *Xiphophorus helleri*

Xiphophorus helleri is commonly known as the green swordtail. It is a cyprinodontiform of the family Poeciliidae. In a systematic overview of this family, Parenti and Rauchenberger (1989) place the genus *Xiphophorus* in the tribe Poeciliini. The orange-red colouration and the occurrence of black pigmentation in domestic *X. helleri* is not found in feral populations and is a result of the integration of platyfish (*X. maculatus*) genes in captive-bred *X. helleri* (Angus, 1989). However, the hybrids (i.e. orange-red domestic *X. helleri*) were backcrossed to pure *X. helleri* to create a new lineage (Angus, 1989), so that the general characteristics of *X. helleri* appear to be expressed over those of *X. maculatus*. The captive-bred hybrid is now considered by aquarists and scientists as a strain of the green swordtail *X. helleri* (Axelrod and Schultz, 1990). Therefore, throughout this work the study animal is referred to as *X. helleri* (Figure 1.1).

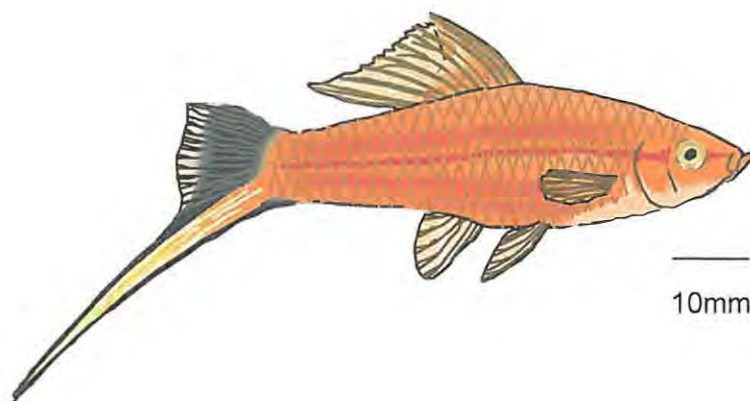


Figure 1.1. A captive-bred adult male swordtail, *Xiphophorus helleri*, similar to those used throughout this work.

The family Poeciliidae is comprised of nearly 200 species (Meffe and Snelson, 1989; Constantz, 1989). However, our general knowledge of its biology and behaviour is limited to only a few genera and to relatively few species within these genera. Generally, scientific investigation has been limited to species with economic value in the ornamental fish trade, use as a biological control or significance to conservation; this includes species from the genera *Gambusia*, *Poecilia*, *Poeciliopsis*, *Heterandria*, *Belonesox* and *Xiphophorus* (Meffe and Snelson, 1989). The following description of the geographic distribution, habitat use and aspects of reproduction of the Poeciliidae must be read with the understanding that our knowledge of the family is strongly influenced by relatively few species whose biology and behaviour may not necessarily be representative of the family as a whole. While the discussion does not focus specifically on *X. helleri*, it is assumed that this species fits the general description of the family, as presently known. *X. helleri* is referred to directly where it is known to differ from the general family description.

Geographic distribution and habitat use of the Poeciliidae

The geographic distribution of the Poeciliidae spans the tropical and subtropical latitudes, with a concentration in Central and South America (Meffe and Snelson, 1989). Poeciliids are typically found in small, shallow water bodies or in the marginal areas of larger lakes and rivers (Meffe and Snelson, 1989). However, their habitat use can vary considerably, not only interspecifically, but also intraspecifically, as there are reports of populations of the same species tolerating vastly different environmental extremes (Krumholz, 1948; Meffe and Snelson, 1989). For example, salinity and temperature-tolerance studies found that many poeciliids are euryhaline and can withstand a broad thermal range (Meffe and Snelson, 1989). However, preference studies showed that poeciliids select salinities typically matching their natural environment, and although minimum and maximum lethal

temperatures may range from 0.5°C to 39°C, respectively, their preferred temperature appears to be in the mid to high 20°C range (Meffe and Snelson, 1989).

Many poeciliids inhabit geologically unstable and ecologically harsh habitats (Meffe and Snelson, 1989). Their tolerance of extreme environmental conditions contradicts the general theory that K-selected species inhabit more stable environments (Bruton, 1989). Furthermore, when poeciliids are exposed to increasingly unpredictable environmental conditions they tend to become increasingly more K-selected. For example, Weeks and Gaggiotti (1993) attributed an increase in offspring size at birth in the *P. monacha*-*P. lucida* species complex to increased environmental stress, such as food deprivation and increased predation. Similarly, when food was less available, when and under conditions of low salinity, sailfin molley (*P. latipinna*) produced larger eggs (Trexler, 1997). These findings further contrast the general theory that species become more r-selected under increased environmental instability (Bruton, 1989). Nonetheless, their tolerance of extreme conditions explains why many poeciliid species inhabit geologically unstable and ecologically harsh habitats (Meffe and Snelson, 1989). For example, populations of *G. affinis* were reported to occur in ice-covered lakes (Krumholz, 1948; Meffe and Snelson, 1989) while other populations were reported to inhabit hot-springs that sometimes reached temperatures in excess of 40°C for short periods (Meffe and Snelson, 1989). However, not all poeciliid species occur in such varied habitats. For example, some species from the *G. nobilis* species group are endemic to springheads in the Chihuahuan Desert of Texas and Mexico and occur in no other habitat types (Meffe and Snelson, 1989).

Reproductive biology in the Poeciliidae

All poeciliids reproduce using internal fertilization (Wourms, 1981; Meffe and Snelson, 1989; Parenti and Rauchenberger, 1989; Constantz, 1989). The anal fin is sexually dimorphic, with an elongation and thickening of the third, fourth and fifth fin rays to form an intromittent organ in the males (Constantz, 1989). The modification of these organs (gonopodia) varies among the poeciliids. Typically, species with long gonopodia visually monitor the position of the organ during copulation (Constantz, 1989). However, species belonging to the genus *Xiphophorus* have short gonopodia that are positioned out of eye view and their gonopodia have developed structures or hooks that position the intromittent organ during insemination (Constantz, 1989).

Another characteristic common to the family is the females' ability to store sperm in the folds of the ovary and oviduct, enabling fertilisation of successive broods from a single copulation (Constantz, 1989). Furthermore, there is evidence that female *Xiphophorus* species nourish stored sperm with secretions from the corpus luteum (Meffe and Vrijenhoek, 1981). Although new sperm fertilises a greater portion of the subsequent brood, sperm can be stored and used to fertilize eggs for up to eight months (Constantz, 1989). Fertilisation in poeciliids may occur one, two, or seven days after the previous parturition. Members of the genus *Xiphophorus* fall into the second category, with a two-day period between parturition and fertilisation (Constantz, 1989).

Fecundity in *X. helleri* is positively correlated to female length (Milton and Arthington, 1983; Kruger *et al.*, 2001a). Kruger *et al.* (2001a) reported that female *X. helleri* with an average standard length from 61.8 to 63.3mm had an average fecundity of 190 embryos. The females observed by Milton and Arthington (1983) averaged 39.6 ± 0.07 mm in length, with an average fecundity of 60 embryos per female.

All but one species (*Tomeurus gracilis*) belonging to the Poeciliidae retain embryos within the body of the female for the duration of embryonic development (Parenti and Rauchenberger, 1989; Constantz, 1989; Meffe and Snelson, 1989). It is this characteristic that distinguishes the Poeciliidae, in part, from other families of the order Cyprinodontiformes (Parenti and Rauchenberger, 1989). The female has paired ovaries that are fused into a sac-like organ with convoluted peritoneal folds that are highly vascularised (Wourms, 1981); embryo development takes place in this organ. Constantz (1989) recognises two types of livebearing in the poeciliids, lecithotrophy and matrotrophy. Lecithotrophy is the term used to describe development where the embryos are nourished by yolk deposited before fertilization of the egg (Wourms, 1981). These poeciliids produce large eggs with a diameter of approximately 2mm (Constantz, 1989). All species belonging to the genus *Xiphophorus* are lecithotrophs. Matrotrophy involves a process in which embryos are nourished primarily by the female rather than by yolk reserves (Wourms, 1981; Constantz, 1989). Matrotrophy and lecithotrophy would subsume the terms viviparity and ovoviviparity, respectively (Constantz, 1989), by the accepted definition of these terms. However, if viviparity is defined simply as bearing live young (Wourms, 1981) then all poeciliids, with the exception of *T. gracilis*, would be considered viviparous. Thus, the frequent reference in the literature that poeciliids are generally viviparous.

The period of gestation varies among poeciliid species and is thought to be temperature dependent, but typically ranges between 20 and 30 days (Constantz, 1989). There is disparity in the reported interbrood period (i.e. the time between successive parturitions) of *X. helleri*. Siciliano (1972) and Kruger *et al.* (2001a) reported an average interbrood period 35 days for *X. helleri*. Tavalga and Rugh (1947) found *X. helleri* to have an

interbrood period of 29 days. It would appear that the difference lies either in the parturition to fertilization period, or in the length of gestation, or both. However, there is no known explanation available in the literature for these reported differences. Ovulation occurs immediately before parturition, which takes place due to muscular contractions of the ovarian wall (Constantz, 1989). The embryos move from the ovary into the ovarian cavity and out into the environment via the gonoduct (Constantz, 1989).

It is immediately after parturition that the juveniles, which are fully developed at parturition (Constantz, 1989), are thought to become susceptible to cannibalism (Rose, 1959; Thibault, 1974; Jones *et al.*, 1998a; Jones *et al.*, 1998b; Kruger *et al.*, 2001a).

Objectives and rationale

Cannibalism is a prevalent and naturally occurring behaviour in fishes and is not an abnormal occurrence limited to animals in captivity or otherwise stressed populations (Fox, 1975; Polis, 1981; Smith and Reay, 1991), as was previously thought by ethologists in the school of Lorenz (1966) and Eibl-Eibesfeldt (1961). Polis (1981) encapsulated a discussion of this topic with the suggestion that Dawkins (1976), in his book entitled *The Selfish Gene*, asked the wrong question: "Why is cannibalism relatively rare?" Polis (1981) advocated that a more appropriate question would be, "... why cannibalism is relatively common..."

Cannibalism has been identified in 36 of the 410 teleost families listed by Nelson (1984). Smith and Reay (1991) suggested that the occurrence of this behaviour is probably even more widespread in fishes than proposed in their review. Although it has been associated with an extensive range of taxa and life-history strategies it is particularly well represented in piscivorous species and in precocial species that offer parental care (Smith and Reay,

1991; Lindstrom, 2000). The reason for the latter was explained using a mathematical model developed by Stenseth (1985), from which it was suggested that cannibalism would be expected to evolve in species where the victim of cannibalism had a low reproductive potential and the reproductive output of the cannibal was high.

Furthermore, using this model Stenseth (1985) was able to show that cannibalism was likely to be an evolutionary stable strategy in species where reproductively mature individuals show increased survival and where reproductive output increases with age. As these are typical K-strategies, Stenseth (1985) concluded that cannibalism is likely to evolve in K-selected animals. Species of the family Poeciliidae are typically K-selected as they all reproduce using internal fertilisation, and with the exception of *T. gracilis*, they all bear precocious offspring (Constantz, 1989). Therefore, it is possible that cannibalism occurs as an evolutionary stable strategy in poeciliids. This hypothesis is supported by reports that some poeciliids cannibalise their offspring not only under captive conditions but also in the wild (Thibault, 1974; Meffe and Snelson, 1989; Nesbit and Meffe, 1993). However, in an ecological overview of the Poeciliidae, Meffe and Snelson (1989) concluded that cannibalism in this family is poorly understood.

The overall aim of this study is to develop an understanding of *X. helleri* behaviour during the parturition period, and to explain *post-partum* cannibalism of juveniles, and to develop a better understanding of the mechanisms associated with this behaviour. Another goal is to utilise the data to provide insight into the phenomenon and the consequence of filial and non-kin cannibalism of juvenile poeciliids in general. It is hoped that the findings will be applicable to future research and the commercial production of not only *X. helleri* but also other poeciliid species.

Structure of experimental investigation

The objectives of the first experiments (Chapter 2) were to determine whether or not and to what extent cannibalism takes place under captive conditions and to develop a better understanding of the effect of population size and structure on the behaviour of *X. helleri*. The experiments were designed as a foundation upon which subsequent experiments could be developed.

Once having determined that cannibalism occurs under captive conditions, further experiments were designed to determine when and where cannibalism occurred, under what conditions it took place, how the adults cannibalised the juveniles, which adults were most responsible for cannibalism and how the juveniles behaved during and after parturition. These experiments were carried out under controlled laboratory conditions and constitute Chapters 3, 4 and 5 (Figure 1.2).

The experimental studies described in Chapters 2 to 6 were designed to follow each other chronologically. However, they were also designed to stand-alone as individual studies. Therefore, each of these chapters includes an abstract, definitions, equations and general materials and methods that are common to more than one chapter are repeated to ensure that each study can be understood as an independent study.

Based on the findings presented in Chapter 2 and the observations reported in Chapters 3, 4 and 5, a number of conclusions regarding the behaviour of *X. helleri* during parturition seemed possible. However, several of these conclusions were based on the behaviour of fish kept in small glass aquaria under laboratory conditions; thus, it was not known whether the behaviour of *X. helleri* was truly represented or whether the behaviour could be extrapolated to describe behaviour of the species under different environmental

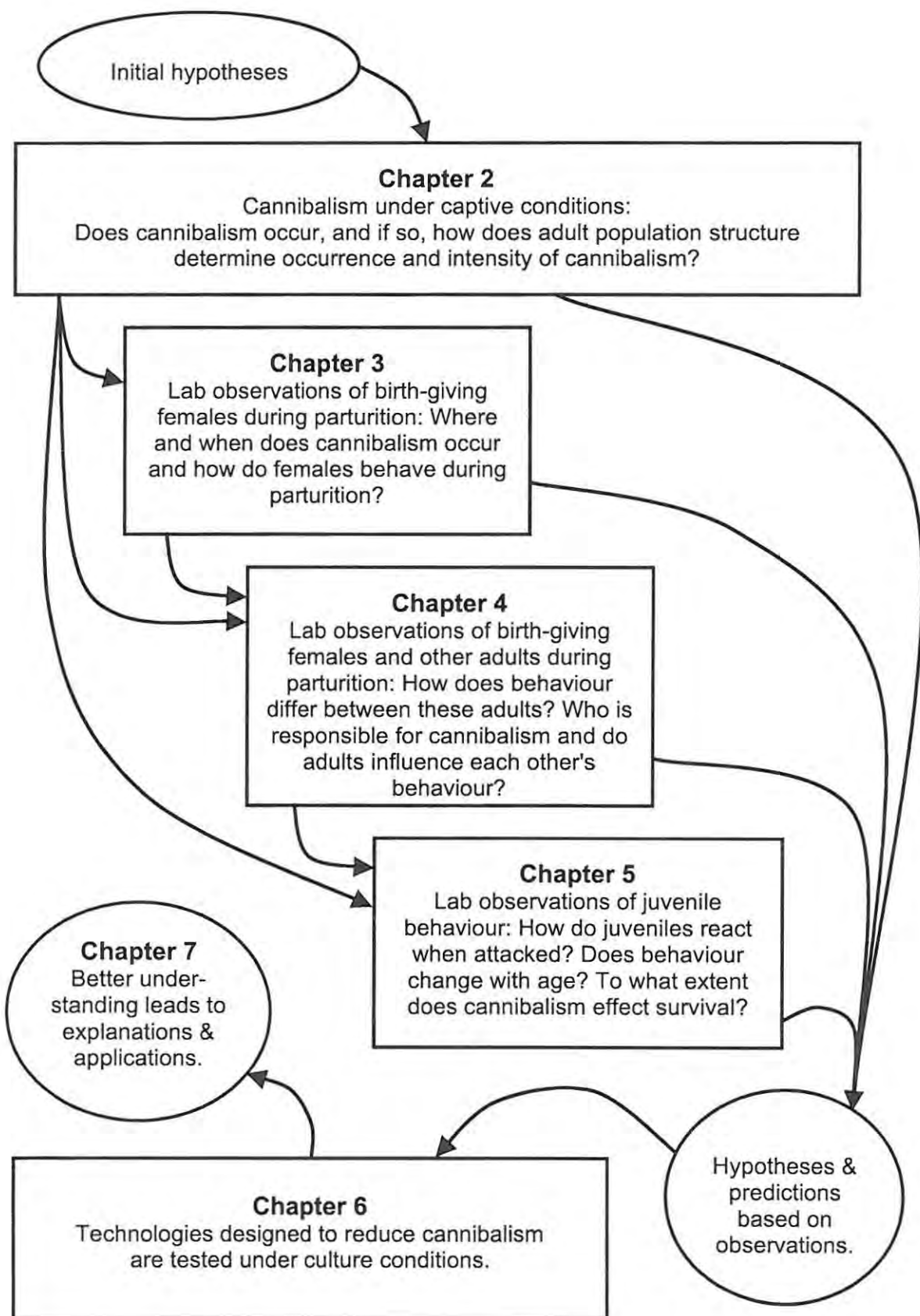


Figure 1.2. Structure of the experimental investigation into the *post-partum* behaviour of *Xiphophorus helleri*.

conditions. Therefore, hypotheses based on the findings presented in Chapters 2 to 5 were tested under different culture-environment conditions as described in Chapter 6 (Figure 1.2).

Finally, the possibility that the findings of laboratory experiments could be used to predict behaviour of *X. helleri* under culture conditions is considered (Chapter 7), and it is shown that the laboratory observations were applicable to different environmental conditions. Applications to industry and future research and possible explanations for cannibalism in *X. helleri* and other poeciliids, such as parental manipulation, nutritional advantages, opportunistic predation, the recovery of energy and the genetic gain or loss experienced by the victims of cannibalism, are also considered in the concluding discussion.

CHAPTER 2

EFFECT OF ADULT SEX RATIO AND BROODSTOCK POPULATION DENSITY ON JUVENILE SURVIVAL UNDER CAPTIVE CONDITIONS

ABSTRACT

Filial cannibalism has been identified as a potential constraint to the intensive culture of *Xiphophorus helleri*. The objective of this study was to verify this assertion, and quantify the effect of refuge availability and quantity, broodstock stocking density, and adult sex ratio on the rate of cannibalism under culture conditions. The availability of refuge had a significant effect on the number of juveniles harvested, while the quantity of shelter did not affect harvest size. The number of juveniles harvested was significantly lower at a stocking density of four adults per tank, but did not increase significantly when stocking density was raised from 10 to 16 adults per tank. Although the additional females contributed to the number of juveniles produced, the increased number of cannibals in the tank counterbalanced this contribution. The rate of cannibalism was density dependent, being significantly lower at four adults per tank ($5.5 \pm 9.6\%$) compared to 16 per tank ($53.9 \pm 4.2\%$), and was independent of sex ratio, indicating that males and females are equally cannibalistic. The highest number of juveniles ($1,725 \pm 141$) per tank over a 70-day period was obtained at a social structure of two males and eight females. These results suggest that a 300L broodstock tank should be stocked with a maximum of two males and between five and eight females to obtain the greatest number of harvestable juveniles per tank. However, it was hypothesised that a better understanding of adult and juvenile behaviour during the parturition period might lead to the development of production procedures or technologies that would mitigate cannibalism and thus increase production under captive conditions.

INTRODUCTION

Cannibalism has been identified as a potential constraint to the commercial culture of many fishes (Folkvord, 1991; Smith and Reay, 1991; Hecht and Pienaar, 1993; Ottera and Folkvord, 1993; Baras and Jobling, 2002), including *Xiphophorus helleri* (Jones *et al.*, 1998a; Kruger *et al.*, 2001a). While different aspects of intercohort cannibalism have been studied in livebearers such as the guppy, *Poecilia reticulata* (Rose, 1959; Warren, 1973; Dahlgren, 1979; Loekle *et al.*, 1982), the eastern mosquitofish, *Gambusia holbrooki* (Nesbit and Meffe, 1993) and some *Poeciliopsis* species (Thibault, 1974; Fox 1975; Meffe 1984; Meffe and Crump, 1987), cannibalistic behaviour of *X. helleri* has not been addressed.

Previous research has shown that the rate of cannibalism among many poeciliid species is related to adult population density (Rose, 1959; Warren, 1973; Thibault, 1974) and the sex of the parent fish (Warren, 1973; Hubbs, 1991; Kruger, 1995). For example, in *P. reticulata* adult population density was inversely related to the number of juveniles produced (Rose, 1959). Fecundity and fertility of *P. reticulata* were also significantly higher at a population density of 0.18 females/L than at 0.7 females/L (Dahlgren, 1979), and increased adult aggression has been observed in this species at a population density of 24 females/L in comparison to six females/L (Warren, 1973). Furthermore, differences in male and female aggression have been reported for some poeciliids, with females being considerably more aggressive than males (Warren, 1973; Hubbs, 1991).

FitzGerald (1991; 1992a and 1992b) explained the adaptive significance of filial cannibalism in fishes in terms of parental investment where the cannibalism of a parent's own offspring is thought to maximise the parent's lifetime reproductive success. It was argued that the loss of eating one's own young was counterbalanced by enhanced

survival and an enhancement of the cannibal's future reproductive success. This is also called *parental manipulation* (Hrdy, 1979; Polis, 1981; FitzGerald and Whoriskey, 1992). The cannibalism of non-kin individuals, called heterocannibalism by Rohwer (1978) and non-kin cannibalism by Smith and Reay (1991), could be adaptive because of the nutritious food item obtained by the cannibal as well as the reduction of conspecific competition for resources (Fox, 1975; Hrdy, 1979; Jones, 1982; FitzGerald, 1991; FitzGerald and Whoriskey, 1992). As filial cannibalism is particularly prevalent in fishes with parental care, FitzGerald (1991 and 1992a) used the behaviour of parental care-giving species to explain the adaptive significance of filial cannibalism in terms of parental manipulation. For example, filial cannibalism in the threespine stickleback (*Gasterosteus aculeatus*) was more common towards the end of the breeding season (Belles-Isles and FitzGerald, 1990) and ovo-cannibalism increased with a decrease in the guarding male's body condition (FitzGerald, 1992a). This was also true for the painted greenling (*Oxylebius pictus*) (DeMartini, 1987) and river bullheads (*Cottus gobio*) (Marconato and Bisazza, 1988). Furthermore, the physical condition of the threespine stickleback was found to deteriorate over the breeding season, and males that were fed conspecific eggs lost weight more slowly than males that did not cannibalise (FitzGerald, 1992a). FitzGerald (1991 and 1992a) suggested that the late season increase in filial cannibalism of the threespine stickleback enabled males to survive the winter period, thus increasing their chances of reproducing again during the following season. Similarly, by looking at the number of eggs found in the stomach of the Cortez damselfish (Pomacentridae: *Stegastes rectifacenum*) it was demonstrated that these largely herbivorous fish cannibalise small numbers of eggs on a continuous basis (Hoelzer, 1988). This was used to support the hypothesis that this species uses cannibalism as a mechanism to satisfy its energy requirements, thus ensuring its own survival and the subsequent survival of future broods.

A number of hypotheses have been drawn from the work carried out on parental care-giving species. For example, it has been hypothesised that in species that exhibit biparental care it is likely that both the male and female will commit filial cannibalism, but the extent of cannibalism should differ according to the relative investment made by each of the parents (FitzGerald, 1992a). It was further hypothesised that when investment in parental care is the same for both parents, the male should be less cannibalistic than the female on the assumption that sperm is less "expensive" to develop than eggs (FitzGerald, 1992a).

The aim of this study was to confirm that filial and/or non-kin cannibalism of juveniles takes place *post partum* in captive-bred *X. helleri*. It was also hypothesised that adult population density is inversely related to juvenile survival and that females are more cannibalistic than males.

MATERIALS AND METHODS

The experimental system

The experimental system consisted of sixteen 300L fibreglass tanks (Figure 2.1), which formed part of a 30,000L closed recirculating system (Figure 2.2). Water was pumped from beneath the gravel bed of the biological filter to the tanks by a 1.1kW pump, via a 60mm delivery pipe. The flow of water into each tank was controlled by a 25mm adjustable valve, which was set to maintain a water flow of approximately 2.5L/min. A centrally positioned 25mm up-stand pipe with a stainless steel grid drained the tanks into a common 110mm drainpipe that led to the settlement tank. The water drained from the surface of the settlement tank (8,800L) into the gravel bed compartment of the biological filter (9,000L) from where it was drawn down through size-graded biological filter media

and pumped back to the tanks (Figure 2.2). In addition to solar radiation, the water was heated by two 3kW heater elements situated in a 350L asbestos tank. This tank was connected to the outflow of the biological filter and was thermostatically controlled with a probe located in the settlement tank at the point where the out-flowing water from the tanks entered the settlement tank. In addition to the sixteen fibreglass tanks, there were eighteen 50L plastic tanks and two 3,200L concrete tanks connected to the same recirculating system (Figure 2.2), which were either used for holding fish or for other experimental work. The entire system was located in an 8 by 30m horticultural-type tunnel covered by 500 μ m clear plastic.

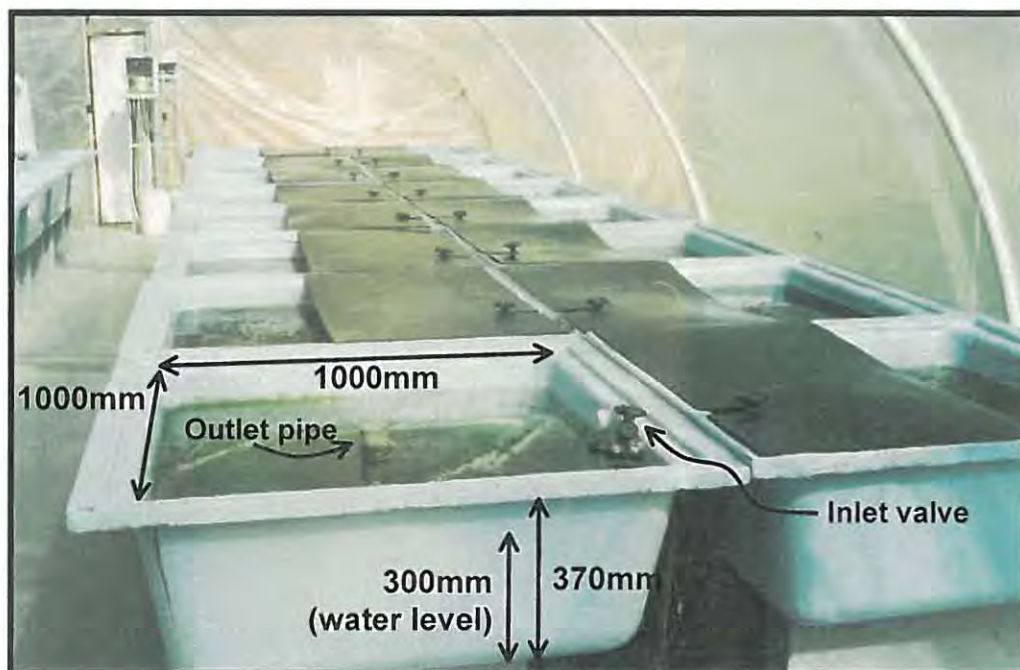


Figure 2.1. The sixteen 300L fibreglass tanks that form part of the closed recirculating system located in an 8 by 30 m horticultural greenhouse tunnel, at the Department of Ichthyology and Fisheries Science, Rhodes University.

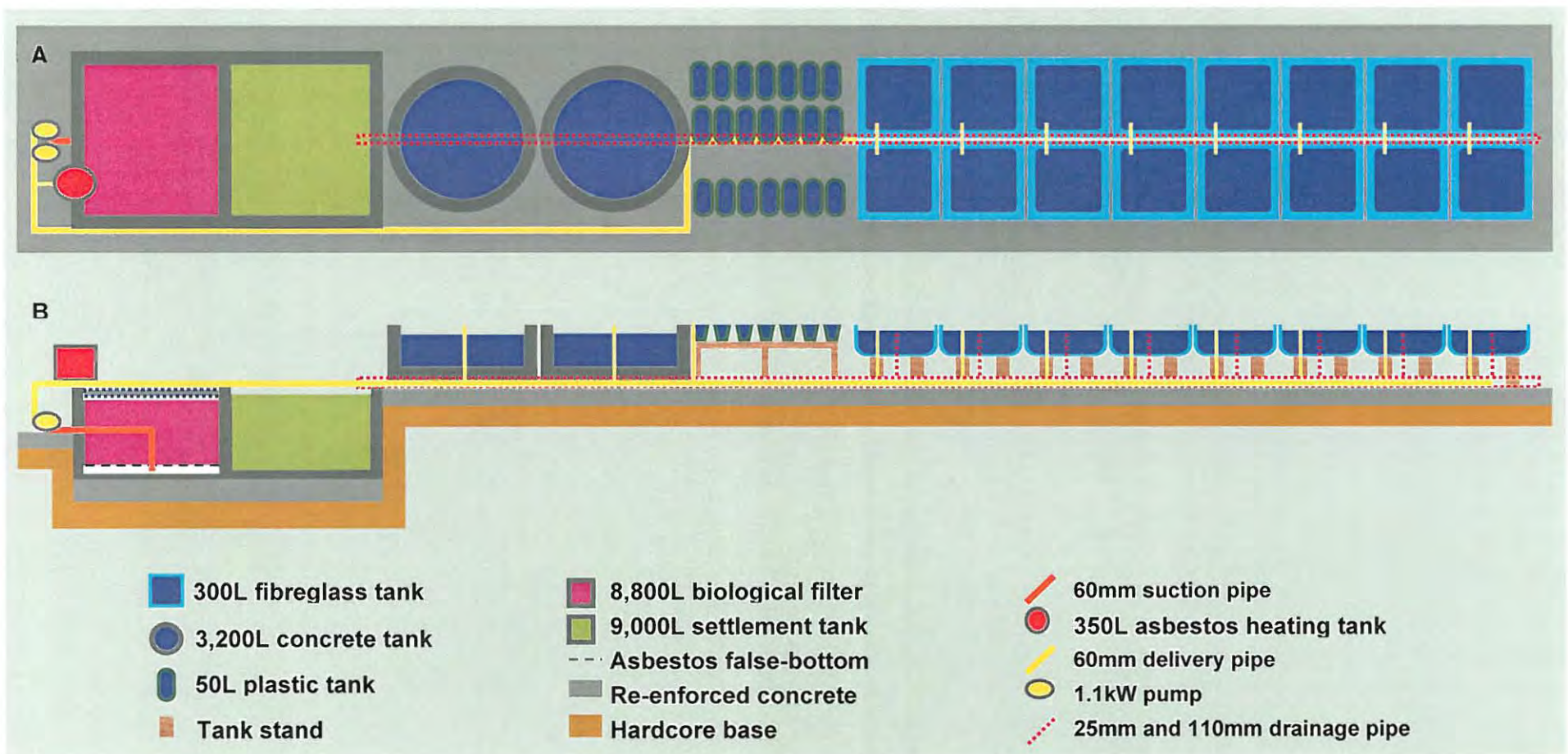


Figure 2.2. An aerial view (A) and a cross-sectional view (B) of the closed recirculating fish culture system that is housed in a greenhouse tunnel at the Department of Ichthyology and Fisheries Science, Rhodes University. An additional sixteen 300L fibreglass tanks from a second recirculating system, identical to the system presented here and housed in the same greenhouse tunnel, were used in other experiments that required more than 16 tanks (e.g. Chapter 6); in which case the systems were connected by continually exchanging water between the two to ensure that there was no difference in water chemistry.

Broodstock and broodstock conditioning

Adult *X. helleri* (Gold Victory strain) were randomly taken from a breeding population maintained at the Department of Ichthyology and Fisheries Science, Rhodes University. The broodstock used in this experiment were the offspring of fish obtained from Amatikulu Hatchery, South Africa. The adult fish were acclimated to their environment in fifteen of the 300L tanks (Figure 2.1 and Figure 2.2) for two weeks before the start of the experiments. The outflow of each tank was covered with 0.5mm mesh to ensure that juveniles were not lost during the study. A 300g bunch of shredded plastic ribbons was placed into each of the tanks to provide refuge for the juveniles. The fish were subjected to a natural photoperiod of approximately 13L:11D, and were acclimated to their respective experimental tanks at the same sex ratios and at the same population densities that they were subjected to during the experimental period. They were fed a commercially available flake diet (Amatikulu Hatchery, South Africa) with a crude protein and lipid content of 41.8% and 14.7%, respectively, on a dry-weight basis. Food was provided in excess of satiation twice daily, in the morning and in the afternoon, to ensure that insufficient feeding would not affect the results. Filamentous algae grew continuously on the tank walls, even though tanks were cleaned once weekly. Thus, algae and other live food items associated with them were available to the adult fish at all times.

A preliminary investigation to determine the effect of refuge on juvenile survival

This experiment was designed to lay a basis for the methods and materials of the subsequent trials. To test the effect of refuge availability on the rate of juvenile survival, eight of the 300L tanks (Figure 2.1 and Figure 2.2) were randomly chosen and filled with 600g, 300g, 100g or 0g of shredded 5mm-wide plastic strips. Each of these four treatments was represented in duplicate. The bunches of plastic refuge floated at the water surface to a depth of about 65% of the tank, and covered approximately 75%, 40%

and 15% of the water surface area of the tank, respectively. The tanks were each stocked with ten randomly chosen adult *X. helleri* at a sex ratio of 1:4 (male:female). All the juveniles were collected from the tanks and counted once daily in the morning, over a period of 14 days.

The effect of adult stocking density and adult sex ratio on juvenile survival

The effect of broodstock population density on juvenile survival was tested by stocking the 300L tanks with 4, 10 and 16 adult fish per tank, each with a male:female ratio of 1:1. Furthermore, to test the effect of sex ratio on juvenile survival, tanks were randomly chosen and stocked at male:female ratios of 1:1, 1:4, and 4:1, each with a constant stocking density of 10 fish per 300L tank. Juveniles were collected from each tank and counted every day in the morning approximately 3 hours after sunrise, just before the morning feed. The trials lasted 70 days.

The study comprised the following five groups (male:female): A (8:8), B (5:5), C (2:2), D (2:8), and E (8:2), at three replicates each. Treatments A, B and C constituted *Experiment 2.1* to test the effect of adult population density on juvenile survival, and treatments B, D and E constituted *Experiment 2.2* to test the effect of adult sex ratio on juvenile survival. Treatment B was common to both experiments. These treatments made it possible to determine apparent cannibalism, inter-brood period and production.

Apparent cannibalism, inter-brood period and production

Apparent cannibalism (*AC*) is the theoretical number of juveniles that were cannibalised by adults and was calculated using the expected and actual numbers of juveniles produced over the period of the experiment. The actual number of juveniles collected from each

tank over the duration of the trial was termed 'productivity' and the expected number of juveniles for the equivalent period was termed 'expected harvest.' The expected harvest per tank (EH) was estimated without taking cannibalism into account by using the number of reproductively active females in the tank and average fecundity and inter-brood period. Fecundity was determined at the end of the trial by dissecting and counting the number of developing embryos found in two randomly selected gravid females from each tank. Inter-brood period (IB), which is the average time in days between the parturition of two consecutive broods from an individual female, was determined by Equation 2.1 (Vondracek *et al.*, 1988):

$$IB(days) = \frac{7 * No. females in population}{No. broods per week} \quad \text{Equation 2.1}$$

The expected harvest (EH) was estimated using Equation 2.2:

$$EH = \frac{Fecundity * No. females * Trial duration(days)}{IB(days)} \quad \text{Equation 2.2}$$

Both productivity and expected harvest were used to calculate apparent cannibalism (AC):

$$AC = \left(\frac{EH - Productivity}{EH} \right) * 100 \quad \text{Equation 2.3}$$

At the beginning and end of the study the standard length of all adult fish was measured to the nearest mm and weighed to the nearest 0.1g. Gonadosomatic index (GSI) was calculated at the end of the trials by randomly selecting two gravid females from each

tank, removing the gonads and dividing the mass of the gonads by that of the eviscerated body.

Water-quality analysis

Water temperature was measured every second day with a mercury thermometer (n=37). Ammonia and nitrite concentrations were measured spectrophotometrically with the use of Nessler's reagent and the diazotization method approximately once every 7 days (Merck, 1974) (n=11 and n=7, respectively), and pH readings were made with a portable pH meter (Hanna Instruments, USA) (n=42). The pH readings were recorded twice per week at midmorning and twice per week at mid-afternoon. The mean pH was calculated by averaging the anti-log of all pH values, and logging the resultant average.

Statistical analysis

The data from both *Experiment 2.1* and *Experiment 2.2* were tested for normality using the Shapiro-Wilk's test at an error level of 5%. These data were analysed using a one-way analysis of variance (ANOVA), and treatment means were compared with a Tukey's multiple range test at $p < 0.05$ (Zar, 1984).

RESULTS

Water quality

The temperature of the water ranged between 21.2 and 26.5°C, and averaged 24.2°C (n=37). The pH ranged between 7.1 and 9.2, with an average of pH 7.9 (n=42). The ammonia ($\text{NH}_4^+\text{-N}$) concentration averaged 0.06 mgL^{-1} (range: 0.03 - 0.10 mgL^{-1} ; n=11) and nitrite ($\text{NO}_2^-\text{-N}$) concentration averaged 0.001 mgL^{-1} (range: 0.000 - 0.003 mgL^{-1} ; n=7).

Effect of refuge on juvenile survival

The availability of refuge had a significant effect on the survival of juveniles when compared to the treatment with no refuge (ANOVA; $p < 0.05$; Table 2.1). However, the quantity of refuge did not affect the number of juveniles that survived. For practical purposes 300g of shelter material was chosen for subsequent studies of this chapter.

Table 2.1. Average number ($\pm$ standard error) of juvenile *X. helleri* harvested over a period of 14 days from broodstock tanks with different amounts of refuge presented in the form of bunches of plastic strips. Means with different superscripts are significantly different from each other (ANOVA; $p < 0.05$).

Refuge (g)	Surface area covered (%)	Average number of juveniles
0	0	6 ± 1.4^a
100	15	65 ± 3.2^b
300	40	91 ± 8.1^b
600	75	99 ± 2.5^b

Inter-brood period, fecundity and GSI

The inter-brood period did not differ significantly between treatments (ANOVA; $p > 0.67$) and averaged 28 ± 4 days. The average expected fecundity per female for the 70-day period, equivalent to $2\frac{1}{2}$ brood-cycles, was 414 ± 22.7 embryos per female ($n=28$). This equates to 166 embryos per female per brood cycle. There was no significant difference in fecundity between treatments (ANOVA; $p > 0.75$). The average standard length (73.1 ± 0.9) of the females did not differ between treatments (ANOVA; $p > 0.25$ and $p > 0.5$, respectively). Average female mass (8.5 ± 0.3 g/fish) and gonadosomatic index

($16.1 \pm 1.01\%$) were also independent of population density (ANOVA; $p > 0.25$) and sex ratio (ANOVA; $p > 0.98$).

Experiment 2.1: The effect of adult population density on juvenile survival

At a constant sex ratio of 1:1, population density had a significant effect on both the number of juveniles collected per tank (ANOVA; $p < 0.01$; Table 2.2), and the estimated rate of cannibalism (ANOVA; $p < 0.01$; Table 2.2). The lowest population density of four fish per tank yielded the lowest number of juveniles and resulted in the lowest rate of estimated cannibalism (Table 2.2).

Table 2.2. Effect of adult population density on the number of juvenile *X. helleri* harvested over a 70-day period and the estimated rate of cannibalism, at a constant adult sex ratio of 1:1. All values are an average of three replicates (means $\pm$ standard errors). Different superscripts represent significant differences within each column (ANOVA; $p < 0.05$).

Treatment	Population density (adults/300L)	No. juveniles per female	No. of juveniles harvested	Cannibalism (%)
A	16	191.3	1530.7 ± 140.9^x	53.9 ± 4.2^a
B	10	259.3	1296.7 ± 57.8^x	37.5 ± 11.3^{ab}
C	4	392.2	784.3 ± 79.3^y	5.5 ± 9.6^b

Experiment 2.2: The effect of adult sex ratio on juvenile survival

At a constant population density of 10 adult fish per 300L tank, sex ratio significantly affected the number of juveniles collected (ANOVA; $p < 0.01$; Table 2.3), with all treatment means differing from each other. The highest yield was achieved at a sex ratio of 1:4

(male:female) (treatment D) and the lowest at the opposite sex ratio (treatment E). Estimated cannibalism, however, was not influenced by sex ratio (ANOVA; $p>0.44$; Table 2.3), and averaged 44.4% over all treatments.

Table 2.3. Effect of adult sex ratio on the number of juvenile *X. helleri* harvested over a 70-day period and the estimated rate of cannibalism, at a constant population density of 10 adults per 300L tank. All values are an average of three replicates (means $\pm$ standard errors). Different superscripts represent significant differences within each column (ANOVA; $p<0.05$).

Treatment	Sex ratio (Male:Female)	No. of juveniles per female	No. of juveniles harvested	Cannibalism (%)
B	1:1	259.3	1296.7 $\pm$ 57.8 ^x	37.5 $\pm$ 11.3 ^a
D	1:4	215.7	1725.7 $\pm$ 141.4 ^y	48.0 $\pm$ 4.3 ^a
E	4:1	217.4	434.7 $\pm$ 59.5 ^z	47.6 $\pm$ 7.2 ^a

DISCUSSION

The relationship between fecundity and estimated cannibalism

Apparent cannibalism was dependent on both the number of juveniles collected and the total fecundity of the females in each tank for the duration of the trial, and total fecundity was dependent on inter-brood period. Therefore, a miscalculation of the inter-brood period would affect apparent cannibalism. This would explain why the rate of cannibalism estimated by Jones *et al.* (1998a) differed from that estimated in this study. In this study, cannibalism was estimated to range from 5.5 to 53.9%, in contrast to values ranging from -19.2 to 41.8% reported previously (Jones *et al.*, 1998a). Jones *et al.* (1998a) assumed an inter-brood period of 35 days, based on work carried out by Kruger (1995). However,

in this study the inter-brood period was determined according to the method described by Vondracek *et al.* (1988), and it was found to be 28 ± 4 days. The differences in the estimated rate of cannibalism between the findings of Jones *et al.* (1998a) and those presented here can be explained by the difference in the assumed inter-brood period.

Population density appears not to affect the fecundity of *X. helleri* at the densities employed in the present study, as fecundity did not differ among the different stocking densities. However, population density has been found to be inversely proportional to fecundity for some poeciliid species (Rose, 1959; Warren, 1973; Dahlgren, 1979). Dahlgren (1979) found that *P. reticulata* kept at densities of 0.7 females/L had a significantly lower average fecundity than females kept at 0.18 females/L. Since the number of fish per tank differed between treatments in this study, it was assumed that the estimated rate of cannibalism would need to be corrected for the expected change in fecundity. However, as there was no difference in fecundity among the different stocking densities it was not necessary to make corrections. The difference in population density between the treatments in this study were not as great as the difference in population density between the treatments in the study described by Dahlgren (1979), and may have been too small to affect fecundity. Alternatively, the duration of the trial was possibly not long enough for a change in fecundity to occur, but this is unlikely as significant changes in the reproductive index in another poeciliid, the western mosquito fish (*Gambusia affinis*), were found to occur in periods as short as 50 days (Meffe and Crump, 1987). Nonetheless, the results suggest that unlike some of the other poeciliid species population density does not affect fecundity in *X. helleri*. To verify this conclusion, further investigations that focus specifically on the effect that a wider range of population densities have on *X. helleri* fecundity are required.

Estimated rate of cannibalism

Adult *X. helleri* cannibalise their young, which is consistent with observations on other poeciliid species (Rose, 1959; Thibault, 1974; Meffe, 1984; Loekle *et al.*, 1982; Meffe and Crump, 1987; Hubbs, 1991; Weeks and Gaggiotti, 1993). In all but two of the fifteen tanks, the expected number of juveniles was not harvested. The negative rate of cannibalism in two of the tanks belonging to treatment C may be explained by the statistics of the data set. The upper boundary of the 95% confidence interval for the fecundity data in treatment C would extend to 220 embryos per female. Thus, with 95% certainty the highest expected number of juveniles could have reached a maximum of 1,100 for the period equivalent to 2½ brood-cycles. This indicates that the estimated rate of cannibalism was within the 95% error range of the data set.

A significant decrease in the rate of cannibalism was observed with a decrease in broodstock population density. This is comparable to increased intercohort cannibalism of other poeciliids observed at higher stocking densities (Rose, 1959; Warren, 1973; Thibault, 1974; Meffe, 1984); however, researchers did not explain the increase. Hecht and Pienaar (1993) stated that intracohort cannibalism appears to be positively density dependent in all species in which it has been investigated. For example, the rate of sibling cannibalism in koi and common carp (*Cyprinus carpio*) and in juvenile African catfish (*Clarias gariepinus*) increased with an increase in stocking density (Pienaar, 1990). For *C. gariepinus*, this was ascribed to an increase in competition for niche space (Hecht and Pienaar, 1993), which explains density dependence of intracohort cannibalism, but it does not provide an explanation for intercohort cannibalism observed in *X. helleri*. As the number of available juveniles per brood did not increase with an increase in adult stocking density, and since there is no evidence of intercohort competition for space among *X. helleri*, the explanation for increased sibling cannibalism is unlikely to be applicable here.

The finding in the present study may not be a direct result of reduced population density, but rather the result of adults encountering juveniles less frequently over time. The frequency at which parturition occurs in the tank can only increase with an increase in the number of females in the tank. When adult fish are exposed to juveniles less frequently over time, reinforcement of cannibalistic behaviour may take place less frequently and thus explain why the adult fish were less cannibalistic at a reduced population density. This discussion will continue in Chapter 4 where changes in cannibalism and adult behaviour during parturition were observed over time.

Warren (1973) reported an increase in aggressive behaviour at increased stocking densities of adult *P. reticulata*. The present investigation was not designed to directly observe levels of aggression. An increase in the rate of juvenile cannibalism is not sufficient evidence to suggest that an increase in aggressive behaviour was the reason for the increase in cannibalism. Thibault (1974) reported "mobbing behaviour" where the attack response of one female *Poeciliopsis monacha* resulted in attack responses of other adult conspecifics. Similarly, an increase in the rate of cannibalism at higher stocking densities of this study could have been related to the greater number of adult conspecifics preying on juveniles from a single newly born batch. Detailed behavioural observations need to be conducted to further evaluate the behavioural reasons for differences in the rate of cannibalism. This is addressed in Chapters 3, 4 and 5.

Number of juveniles collected

An increase in broodstock population size above a particular density resulted in smaller harvests of juveniles relative to the number of adults in the tank. The decrease in broodstock population density from sixteen to four adults per tank (treatments A and C, respectively) resulted in a significant decrease in the total number of juveniles collected,

although the estimated rate of cannibalism was lower at reduced adult stocking densities. Therefore, the optimum adult stocking density would be the density that is high enough to maximise juvenile production without negatively influencing fecundity, which decreases with increasing population density in some poeciliids (Thibault, 1974; Dahlgren, 1979), and without increasing the rate of cannibalism to a point where total harvest is negatively affected. This can be further evaluated by a comparison of treatments A and B. Because there were eight females in treatment A and only five females in treatment B, a greater number of juveniles would be expected from treatment A. However, a comparison of these two treatments showed that the total number of juveniles collected from the five females in treatment B was similar to the total number collected in treatment A. Thus, the increase in the number of females did not lead to improved productivity. The additional adult fish in treatment A increased the number of cannibals available to attack juveniles after the moment of parturition.

The finding that the number of juveniles collected was related to the number of females in the tank was similar to data presented by Thibault (1974), who established that the intensity of cannibalism of juvenile *P. monacha* increased with an increase in the number of females in the population. However, the rate of cannibalism in *X. helleri* was independent of sex ratio. This suggested that male and female *X. helleri* were equally cannibalistic, which is not consistent with the work of Warren (1973), who found that female *P. reticulata* were more cannibalistic than males, and the work of Hubbs (1991) who reported similar results for the genus *Gambusia*. Thus, the results of this section of the study required verification by direct observation as described in Chapter 4.

Towards an explanation for filial cannibalism

Under the conditions of this study cannibalism does not appear to increase the immediate future reproductive success of the cannibal since GSI remained unaffected by cannibalism. Therefore, the findings reported here do not support the theory of parental investment as described by FitzGerald (1992a). Unlike the evidence presented by FitzGerald (1991 and 1992a), there was no evidence in the present study to suggest that the loss in fitness due to an individual eating its own young was counterbalanced by enhanced reproductive success in the future. The somatic and reproductive performance of *X. helleri* were not found to be enhanced due to cannibalism, as there were no differences in inter-brood period, fecundity, GSI and somatic weight between the fish in treatment C (4 fish per 300L tank) and the fish in treatment A (16 fish per 300L tank), where the rate of cannibalism was significantly different: $5.5 \pm 9.6\%$ compared to $53.9 \pm 4.2\%$, respectively. Similarly, cannibalism did not appear to influence the reproductive success of cannibals in later experiments (see Chapter 6). Filial cannibalism enhanced the future reproductive success of several parental care-giving species (DeMartini, 1987; Marconato and Bisazza, 1988; Belles-Isles and FitzGerald, 1990; FitzGerald, 1992a) and it appears that it also enhanced the reproductive success of at least one livebearer as Meffe and Crump (1987) found that a cannibalistic diet increased the somatic and reproductive performance of the western mosquito fish (*G. affinis*) in a period of 50 days. However, there is no evidence from the present data set to suggest that cannibalism increased the reproductive success of *X. helleri* during the 70-day period of this study.

The success of embryonic development is increased in fishes that exhibit parental care, such as the sticklebacks and many of the cichlids. Similarly, the success of embryonic development is enhanced in the livebearers because the embryos remain protected and in

some cases nourished by the female body (Constantz, 1989). The difference in reproductive investment between male and female *X. helleri* is difficult to identify as a denominator common to both sexes for the term “investment” has not yet been defined in this species. While the male invests energy in sperm production, caudal fin development, and courtship behaviour, the cytoplasmic investment of the female and her investment in embryo development are probably considerably greater than that of the male. FitzGerald (1992a) hypothesised that differences in the rate of filial cannibalism, between males and females in species that exhibit parental-care, should occur relative to the investment made by each parent. However, this theory does not appear to accommodate the behaviour of *X. helleri* since the results of this study suggest that cannibalistic behaviour may not differ between the parents. This result was confirmed in Chapter 4 of this thesis when a direct observation of male and female behaviour was made. The apparent similarity in cannibalistic behaviour between the two sexes, together with the assumption that energetic investment in reproduction is not the same for males and females, suggests that filial cannibalism in *X. helleri* is not directly related to energy invested in reproduction. Therefore, the proximate cause might be no more than opportunistic predation where the prey happens to be a conspecific. Although this suggestion differs with conclusions reached for parental care-giving species, it agrees with the suggestion by Smith and Reay (1991) that most cases of cannibalism in fishes are simply intraspecific predation, rather than an example of cannibalistic species actively seeking conspecific prey in preference to non-conspecific prey.

Cannibalism enhanced both the somatic and reproductive performance of the western mosquito fish (*G. affinis*) (Meffe and Crump, 1987), so filial cannibalistic behaviour in the mosquito fish can be explained by the improved reproductive success of the cannibal. This finding was a significant advancement on the “unclear” explanation for the adaptive

value of cannibalism in the genus *Poeciliopsis* (Thibault, 1974). However, the finding does not contribute to the explanation for cannibalism in *X. helleri*. The present study and the work of Meffe and Crump (1987) were carried out with fishes under captive conditions, thus, neither study is able to make predictions regarding the evolutionary status of cannibalism in the relevant species. However, assuming that the behaviour of these species under captive conditions does not differ considerably from that in their natural environment, a comparison of the two studies suggests that the ultimate benefit for cannibalism in *G. affinis* is not the same as that in *X. helleri*. It would be interesting to test if cannibalism has evolved independently more than once in the family Poeciliidae, as the ultimate benefit from cannibalism may differ among poeciliids.

Conclusion

Adult *X. helleri* cannibalise their young after parturition. Juvenile survival is a function of the number of broodstock in the tank, with a decrease in cannibalism at lower adult population densities. The evolutionary benefits of cannibalism in this species remains unclear and require further investigation, yet it appears that cannibalism of *X. helleri* may not enhance the reproductive success of the individual cannibals. Males and females appear to be equally responsible for cannibalism of juveniles (this will be dealt with further in Chapter 4), while the female's energetic investment in offspring development is assumed to be greater than that of the male. Thus, the proximate cause for the cannibalism of juvenile *X. helleri* by the adults may be no more than opportunistic predation where the prey happens to be a conspecific. There is insufficient evidence to conclude that cannibalism does not improve the overall physical fitness and viability of the population as a whole – a topic addressed in later sections of the work.

A better understanding of the behaviour of adult and juvenile *X. helleri* during the parturition period is required before production procedures and technologies, aimed at mitigating cannibalism under production conditions, can be developed.

CHAPTER 3

MATERNAL BEHAVIOUR AND CANNIBALISM DURING THE PARTURITION PERIOD

ABSTRACT

To better understand the parturition-associated behaviour of *Xiphophorus helleri*, the behaviour of eight individually kept females was recorded on videotape. Three levels of female activity (low, medium and high) were quantified by measuring the duration of each. Four additional behavioural events (non-cannibalistic feeding, birth, attack and cannibalism of offspring) were quantified by determining the frequency of each. Parturition occurred most frequently at a low level of activity at the top of the water column. Eighty-four percent of recorded attacks made by females on juveniles occurred in light. Most attacks and most cannibalistic events were observed at the bottom of the water column. Females spent more time at the top of the water column during light conditions, whereas in the dark they spent more time at the bottom. It is hypothesised that the rate of cannibalism may be reduced if parts of the tank are kept constantly dark and females are restricted from areas in the tank where the frequency of cannibalism appeared highest.

INTRODUCTION

Maternal behaviour in some fishes appears to reduce the opportunity for filial cannibalism at the time of parturition or spawning. For example, the male Cortez damselfish (*Stegastes rectifacens*) is a nest-guarder known to cannibalise eggs (Hoelzer, 1988). However, by spawning in nests that contain the eggs of other females the chance of a female's own eggs being cannibalised is reduced; the male consumes only enough eggs to maintain condition, so the probability of a female's eggs being cannibalised is reduced if eggs are laid in a nest that already contains eggs (FitzGerald and Whoriskey, 1992). This behaviour has also been observed in the threespine stickleback (*Gasterosteus aculeatus*) (FitzGerald and Whoriskey, 1992), another nest-guarder known for cannibalism (FitzGerald, 1991; FitzGerald, 1992a; FitzGerald, 1992b; FitzGerald and Whoriskey, 1992). Furthermore, there is evidence that *G. aculeatus* is able to recognise its own offspring and that individuals of this species preferentially cannibalise the progeny of other conspecifics over their own (FitzGerald and Whoriskey, 1992). Similar behaviour that effectively inhibits filial cannibalism has been observed in poeciliids such as the guppy (*Poecilia reticulata*) and the black molly (*Poecilia sphenops*) (Loekle *et al.*, 1982). Both species are cannibalistic and females preferentially cannibalised unrelated conspecifics and avoided cannibalising their own offspring (Loekle *et al.*, 1982).

Differences in the spatial distribution of adult fish and their eggs and/or juveniles appear to affect the rate of intercohort cannibalism in non-guarding species such as walleye pollock (*Theragra chalcogramma*) and whitefish (*Coregonus lavaretus*) (Bailey, 1989; Eckmann, 1991). For example, in summer adult walleye pollock inhabit deeper waters while the juveniles are found closer to the surface. This vertical separation results in a significant reduction in cannibalism of juveniles during summer, compared to the rate of cannibalism in autumn and winter when juveniles are not spatially separated from cannibalistic adults

(Bailey, 1989). Moreover, Eckmann (1991) found that the whitefish population of Lake Constance made a diurnal migration that only occurred during the spawning season. The descent of the entire population at dawn was thought to reduce the risk of cannibalism on freshly spawned eggs (Eckmann, 1991). The rate of encounter between individuals and the presence of refuge will also affect intracohort cannibalistic behaviour. For example, Hecht and Appelbaum (1988) showed conclusively that sibling cannibalism in larval and early juvenile African catfish (*Clarias gariepinus*) was positively density dependent because of the resultant increase in contact between fish at higher densities. The addition of refuge resulted in a reduced rate of cannibalism due to a decrease in encounter rate between fish, as they did not move from their hiding places in the refuge (Hecht and Appelbaum, 1988).

Behaviour that appears to minimise the effect of intercohort cannibalism takes place in some poeciliids (Loekle *et al.*, 1982) as well as some non-poeciliid care-giving species (FitzGerald and Whoriskey, 1992). Furthermore, certain environmental factors have been found to influence cannibalism and other aspects of behaviour in fishes, and include reduced light intensity, the availability of refuge, population density and the resultant encounter rate of juvenile and adult conspecifics, and the spatial distribution or separation of cohorts (Loadman *et al.*, 1986; Bailey, 1989; Eckmann, 1991; Britz and Pienaar, 1992; Hecht and Pienaar, 1993; Gardner and Maguire, 1998; Dou *et al.*, 2000; Baras and Jobling, 2002). For example, intracohort cannibalism in species that rely on visual orientation is usually reduced at a low light intensity (Baras and Jobling, 2002). Reduced light has a negative effect on the rate of cannibalism in species such as walleye (*Stizostedion vitreum*), African catfish (*Clarias gariepinus*), juvenile Japanese flounder (*Paralichthys olivaceus*) and Australian giant crab (*Pseudocaracinus gigas*) (Loadman *et*

al., 1986; Britz and Pienaar, 1992; Hecht and Pienaar, 1993; Gardner and Maguire, 1998; Dou *et al.*, 2000).

Data in Chapter 2 show that between approximately 6 and 54% of juvenile *X. helleri* produced in a 70-day period were cannibalised by adult fish under captive conditions. However, little is known about the general behaviour associated with parturition in the species. Of particular interest is whether or not the frequency of cannibalism can be affected by light intensity, the movement of adults in the water column or the vertical separation of adults and juveniles; and whether cannibalism is the result of chance encounter between adults and juveniles (opportunistic predation) or if the adults actively seek conspecific juveniles; and whether or not females adopt behaviour aimed at minimising filial cannibalism. Short of the certainty that cannibalism occurs, the behaviour surrounding *post-partum* cannibalism in *X. helleri* has not yet been described. The objectives of this section of the study are to describe and quantify parturition-associated behaviour of female *X. helleri*, develop a better general understanding of intercohort cannibalism through behavioural observations, and begin formulating possible explanations for filial cannibalism in the species.

The study was not designed to test a specific set of hypotheses, but aimed to record a general description of female behaviour during parturition. Thus, the work forms a basis for the hypotheses tested subsequently in the experiments of Chapter 6.

MATERIALS AND METHODS

Broodstock conditioning

Fifty *X. helleri* broodstock (Gold Victory strain) were reared in a closed, recirculating freshwater system housed in a greenhouse tunnel (Chapter 2: Figure 2.1 and Figure 2.2).

Prior to the study the fish were kept for 14 days in five 300L tanks (Chapter 2: Figure 2.1), at a sex ratio of 1:4 (male:female) and a stocking density of 10 fish/tank. They were exposed to a photoperiod of 14L:10D during this period and were fed to satiation twice daily on a diet of Aqua Nutro tropical fish food with 40% crude protein and 6% crude lipid on a dry weight basis.

Observation procedure

Eight gravid females (68 to 78 mm total length; mean 74.1 mm) were randomly taken from the five conditioning tanks and each placed into a 10L glass tanks (200 X 200 mm, with a water depth of 250 mm). These tanks formed part of a closed, recirculating system (Figure 3.1) located in an indoor temperature-controlled laboratory, with an artificial photoperiod of 14L:10D. Daylight was simulated with the use of fluorescent tubes, which resulted in significantly different (Kruskall-Wallis, $p < 0.05$) light intensities of 6.6×10^{15} , 3.6×10^{15} , and 3.15×10^{15} quanta $\text{sec}^{-1} \text{cm}^{-2}$ between the top, middle, and bottom of the tank, respectively. Far-red light, that produced an average light intensity of 0.54×10^{15} quanta $\text{sec}^{-1} \text{cm}^{-2}$ throughout the vertical gradient of the tank, was used to observe the fish at night. Fish were fed to satiation twice daily, except during the parturition period when they received no food. Debris that consisted mostly of uneaten food and faecal matter was siphoned from the bottom of the tanks every second day at 08h00. Fish were acclimated to the system for a period of 7 days.

A video camera (Sony; VM-PS12) was positioned in front of the tanks so that each tank was visible on a television screen (Figure 3.1). The video camera was connected to a video recorder (Sanyo; VHR-250 SA) with a long-play function, making it possible to film the fish continually until the entire parturition-period of eight females was recorded on film.

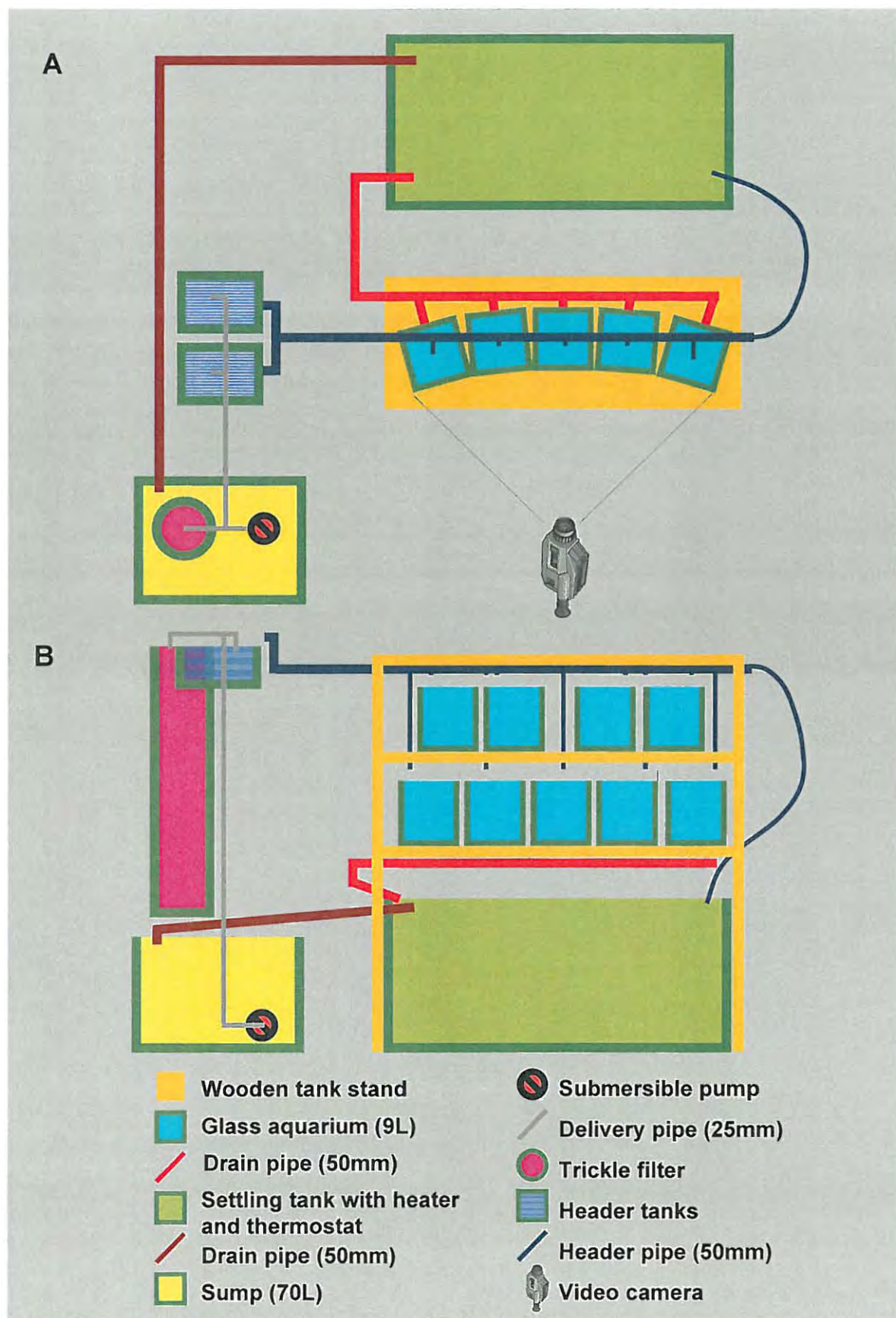


Figure 3.1. Diagrammatic aerial view (A) and side view (B) of the closed, recirculating system used for the observation of *post-partum* behaviour of female *X. helleri*.

Table 3.1. Terms and definitions used to describe the behavioural events of the birth-giving female, phases of parturition, and fish position in the tank during the parturition period. The frequency (F) and/or duration (D) of each event were recorded as observed on video.

Term	Definition	Frequency/Duration
Behavioural events		
Parturition	Appearance of a juvenile from the female genital pore	F
Attack	A sudden burst of speed by an adult directly towards a juvenile, beginning with the start of the caudal peduncle thrash that propelled the adult towards the juvenile, or, when the adult was in striking distance of the juvenile, the attack began with the movement of the adult's head directly towards the juvenile	F
Cannibalism	Successful predation of a live juvenile	F
Escape	Avoidance of cannibalism after being attacked	F
Settling	The juvenile remains inactive	F
Feeding	Ingestion of anything other than a live juvenile by an adult fish	F
Low activity	Zero to slow movement; no visible caudal peduncle movement	F & D
Medium activity	Slow movement; minimal caudal peduncle movement	F & D
High activity	Fast movement; rapid caudal peduncle & axial body movement	F & D
Phases of parturition period		
Pre-phase	Period before first parturition	D
Early-phase	First third of the time period between the first and last parturition	D
Mid-phase	Second third of the period between the first and last parturition	D
Late-phase	Last third of the time period between the first and last parturition	D
Post-phase	Period after the last parturition	D
Position in the tank		
Top	Top third of the water column	F & D
Middle	Middle third of the water column	F & D
Bottom	Bottom third of the water column	F & D

Observation analysis

The videotapes were viewed on a television monitor (Gold Star; CY-14A90Y) and behaviour was quantified by observing individual fish for periods of five minutes. These 5-minute observations were made at intervals during the parturition period. Since the length of the parturition-period varied between females (average: 318 minutes; range: 120 to 686 minutes), the time intervals between the 5-minute observations were adjusted to ensure that a similar number of observations were made per fish. The time between observations was chosen to be 15, 30 or 60 minutes for fish with a parturition time of either less than 150 minutes, more than 150 but less than 360 minutes, or more than 360 minutes, respectively. In addition to the observations made during the parturition-period, two 5-minute observations were made during the hour before the parturition-period and two additional observations were made during the first hour following the end of the parturition period of each fish. Approximately fifteen 5-minute observations were made per female.

Behavioural events, position of the female within the tank, level of activity, and phase of the parturition period were identified (Table 3.1). The level of activity and time spent at different positions in the water column were quantified by measuring the frequency and duration of each event observed during the 5-minute periods; the duration of an event started when the female either altered position in the water column or when a change in activity was observed, and ended with a change in either position or level of activity. The duration of the birth of an individual juvenile, attack, cannibalism and feeding events were too short to be measured accurately, so they were quantified by determining the frequency of each. The success of a female's attempt at cannibalising her young was calculated by determining capture efficiency (CE) (Equation 3.1):

$$CE = \left(\frac{\text{No. juveniles cannibalised}}{\text{No. juveniles attacked}} \right) * 100 \quad \text{Equation 3.1}$$

The rate of cannibalism (C) was calculated according to Equation 3.2:

$$C = \left(\frac{\text{No. juveniles cannibalised}}{\text{No. juveniles born}} \right) * 100 \quad \text{Equation 3.2}$$

Water-quality analysis

Water temperature was measured with the use of a mercury thermometer ($n=8$) at the end of the parturition period. Ammonia and nitrite concentrations were measured spectrophotometrically with the use of Nessler's reagent and the diazotization method (Merck, 1974) ($n=7$ and $n=7$, respectively) at the end of the parturition period; pH readings were taken using a portable pH meter (Hanna Instruments, USA) ($n=7$) at the end of each parturition period. The mean pH was calculated by averaging the anti-log of all pH values, then logging the average. Water quality analyses were not carried out at the end of parturition of one female, which accounts for an n -value of 7 for those readings.

Statistical analysis

Frequency distributions were analysed using χ^2 analysis (Zar, 1984). The null hypothesis stated that there was an even distribution of events, and was rejected at $p < 0.05$. If the expected frequency was less than 5, χ^2 analysis was adjusted ($\chi^2_{\text{adj.}}$) using Yates correction for continuity and contingency analysis was replaced by "Fishers exact" test. The duration of behavioural events was analysed using multifactor analysis of variance, with photophase, position within the water column, phase of parturition period, and level of the females' activity as main effects. Where no interactions were found at $p < 0.05$, a

Tukey's multiple-range analysis was used to compare the means of the main effects at $p < 0.05$ (Zar, 1984). Interactions between factors, or the lack thereof, were confirmed using forward stepwise regression at $p < 0.05$.

RESULTS

Water quality

The average water temperature was 27.7°C (range: 26.0 – 29.0°C; $n=8$). The pH ranged between 6.9 and 7.1 with an average pH of 7.0 ($n=7$). The ammonia ($\text{NH}_4^+\text{-N}$) and nitrite ($\text{NO}_2^-\text{-N}$) concentrations averaged 0.02 mgL^{-1} (range: 0.00 – 0.07 mgL^{-1} ; $n=7$) and 0.003 mgL^{-1} (range: 0.002 – 0.003 mgL^{-1} ; $n=7$), respectively.

Parturition behaviour

Sixty-five of the 112 observed births (58%) occurred while the female was at a low level of activity. Thirty-two and 15 births were recorded at medium and high levels of activity, respectively ($\chi^2=34.6$; $\nu=2$; $p < 0.001$; Figure 3.2). At a low level of activity, 38 of 65 observed juveniles (58%) were born at the top of the tank ($\chi^2=20.3$; $\nu=2$; $p < 0.001$; Figure 3.2). However, none of the 15 observed juveniles that were born at a high level of activity were born at the top of the tank ($\chi^2=24.4$; $\nu=2$; $p < 0.001$; Figure 3.2). Similar numbers of juveniles were born at the bottom of the tank at all three levels of activity ($\chi^2=1.08$; $\nu=2$; $p > 0.05$), with the same trend shown when females were positioned in the middle section of the water column ($\chi^2=6.12$; $\nu=2$; $p > 0.05$; Figure 3.2).

Of the births recorded in the dark only 5.7% were observed at the top of the tank ($\chi^2=15.49$; $\nu=2$; $p < 0.001$), while under light conditions, 7.7% of the observed births occurred in the middle section of the tank ($\chi^2=25.00$; $\nu=2$; $p < 0.001$; Figure 3.3).

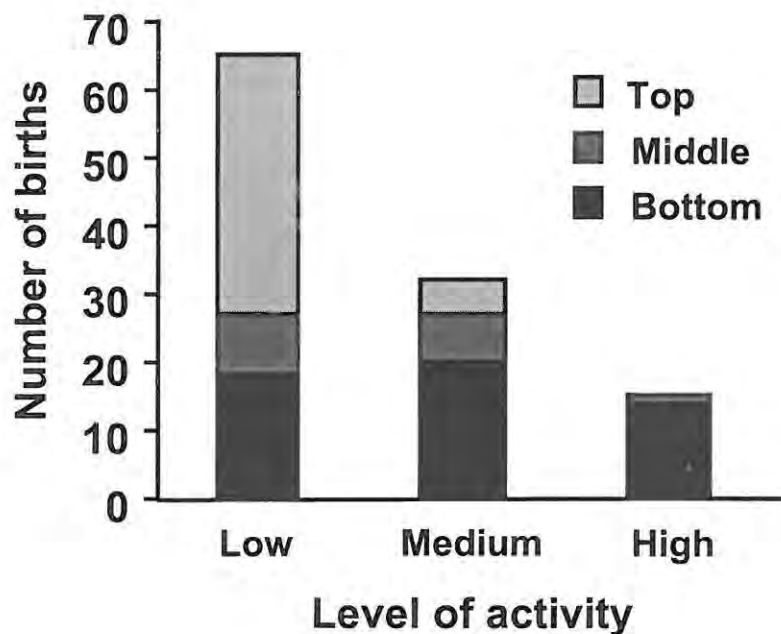


Figure 3.2. Number of births among eight female *X. helleri* observed during 107 five-minute observations at regular intervals during the parturition period in relation to level of activity and female positions in the water column. See Table 3.1 for definition of activity level and position in the tank.

Cannibalistic behaviour

Of the observed attacks by females on their young, 84.4% occurred in the light, with only 15.6% of the attacks recorded under dark conditions ($\chi^2=21.55$; $\nu=1$; $p<0.001$; Figure 3.3). Forty-three attacks in the light were observed at the bottom of the tank, and only 11 in the middle and 11 at the top of the tank ($\chi^2=31.51$; $\nu=2$; $p<0.001$; Figure 3.3). Under dark conditions, however, there was no significant difference in the frequency of attacks between the three positions ($\chi^2_{\text{adj.}}=2.19$; $\nu=2$; $p=0.391$; Figure 3.3).

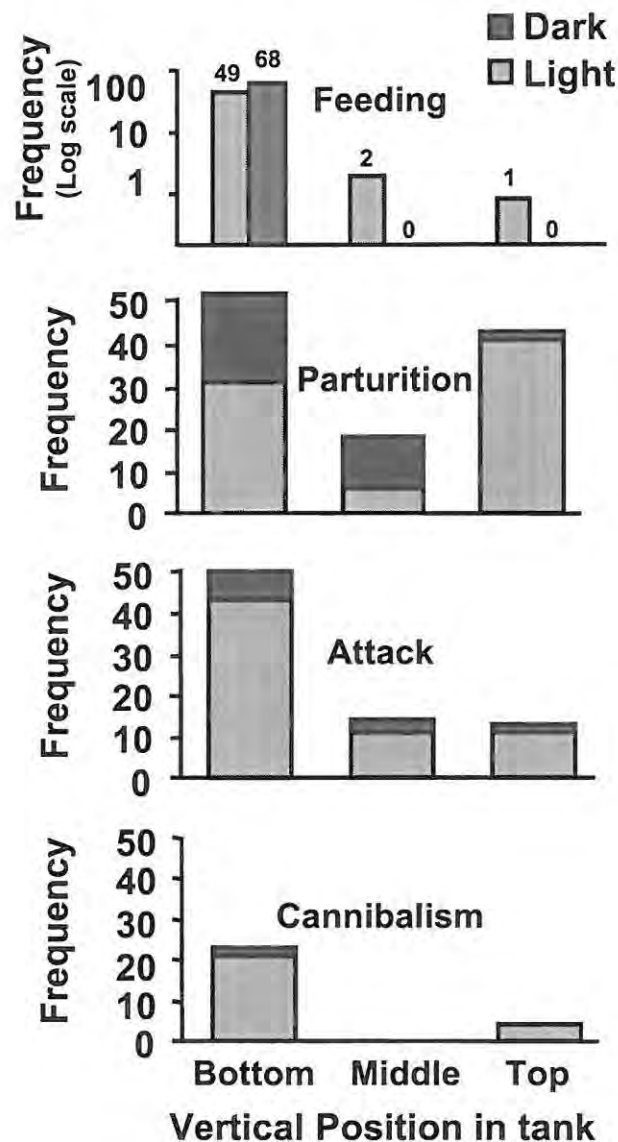


Figure 3.3. The frequency of feeding behaviour, parturition, attacks on newly born juveniles, and successful filial cannibalism, observed during 107 five-minute observations made at intervals during the parturition period. See Table 3.1 for definitions of behavioural events.

Seventy-three attacks and 27 cannibalistic events were observed. Under light conditions, 84.0% of cannibalism was observed at the bottom of the tank, 16.0% at the top, and no cannibalism was observed in the middle of the tank ($\chi^2=9.84$; $\nu=2$; $p<0.001$; Figure 3.3). Only 7.4% of all recorded cannibalistic events were observed in the dark ($\chi^2=19.58$; $\nu=2$;

$p < 0.001$; Figure 3.3). Of all the attacks that were observed at the bottom of the tank, the rate of capture efficiency was 46.0% (number of attacks = 50; number of successful attacks = 23). The rate of success was lower at the top of the tank with an efficiency rate of 30.8% (number of attacks = 13; number of successful attacks = 4), and in the middle of the tank there were no successful attacks.

Feeding behaviour

Almost all feeding activity (97.8%) was observed at the bottom of the tank ($\chi^2 = 276.13$; $p < 0.001$; Figure 3.3). Under light conditions the frequency of feeding was significantly lower at the top and the middle of the tank than at the bottom ($\chi^2 = 156.42$, $v = 2$; $p < 0.001$), and under dark conditions no feeding activity was observed at either the middle or the top ($\chi^2 = 136.00$; $v = 2$; $p < 0.001$; Figure 3.3).

Distribution and activity-related behaviour of females

At low levels of activity, females spent significantly shorter periods in the middle of the water column before changing to a different position or level of activity (ANOVA, $F_{0.05,2,932} = 3.00$; $p < 0.05$; Figure 3.4). At medium and high levels of activity, there were no differences in the average length of time spent at each position before changing to a different position or level of activity (ANOVA, $F_{0.05,2,1792}$ and $F_{0.05,2,410}$; $p > 0.05$; Figure 3.4). A medium level of activity was recorded most frequently in both the middle (64.8%; $\chi^2 = 436.52$; $v = 2$; $p < 0.001$) and bottom (57.0%; $\chi^2 = 509.56$; $v = 2$; $p < 0.001$) areas of the water column. At the top of the water column, both medium and low levels of activity were observed equally often, with a high level of activity recorded only 59 times out of 503 counts ($\chi^2 = 106.23$; $v = 2$; $p < 0.001$).

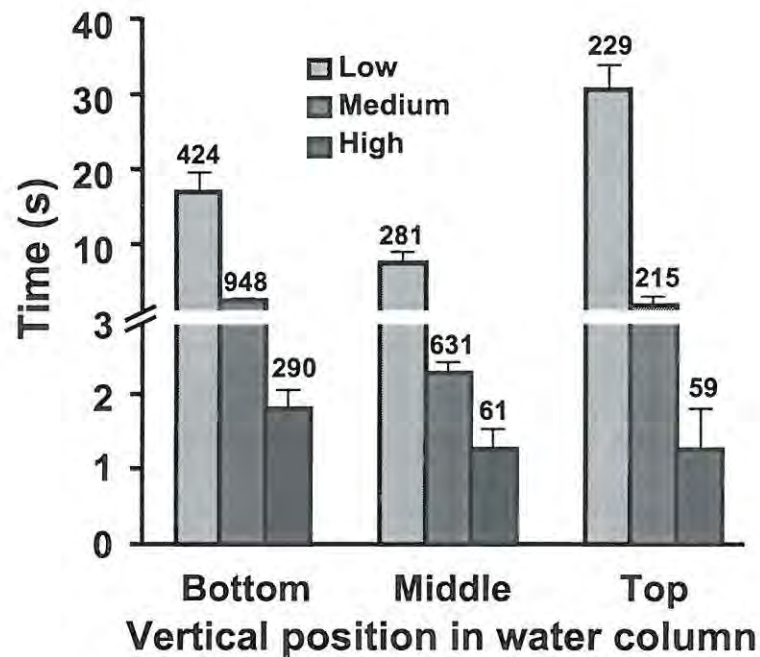


Figure 3.4. The average length of time (seconds) , per 5-minute observation, that female *X. helleri* spent at one of three levels of activity and at one of three positions in the water column, before changing to another position in the tank or level of activity. The frequency of occurrence is represented above each bar. Terms and definitions for behaviour patterns are presented in Table 3.1.

On average, a female spent 68.9% of the time during the parturition-period at a low level of activity, and only 5.4% at a high level of activity (Figure 3.5). There was a significant interaction between the phase of parturition and the three levels of activity (2-way ANOVA, $F_{0.05,8,3123}=1.94$; $p<0.01$). There was no difference in the average length of time spent at medium and high levels of activity in each phase of parturition, whereas significantly more time was spent at a low level of activity during the late- and post-birth phases (Figure 3.5).



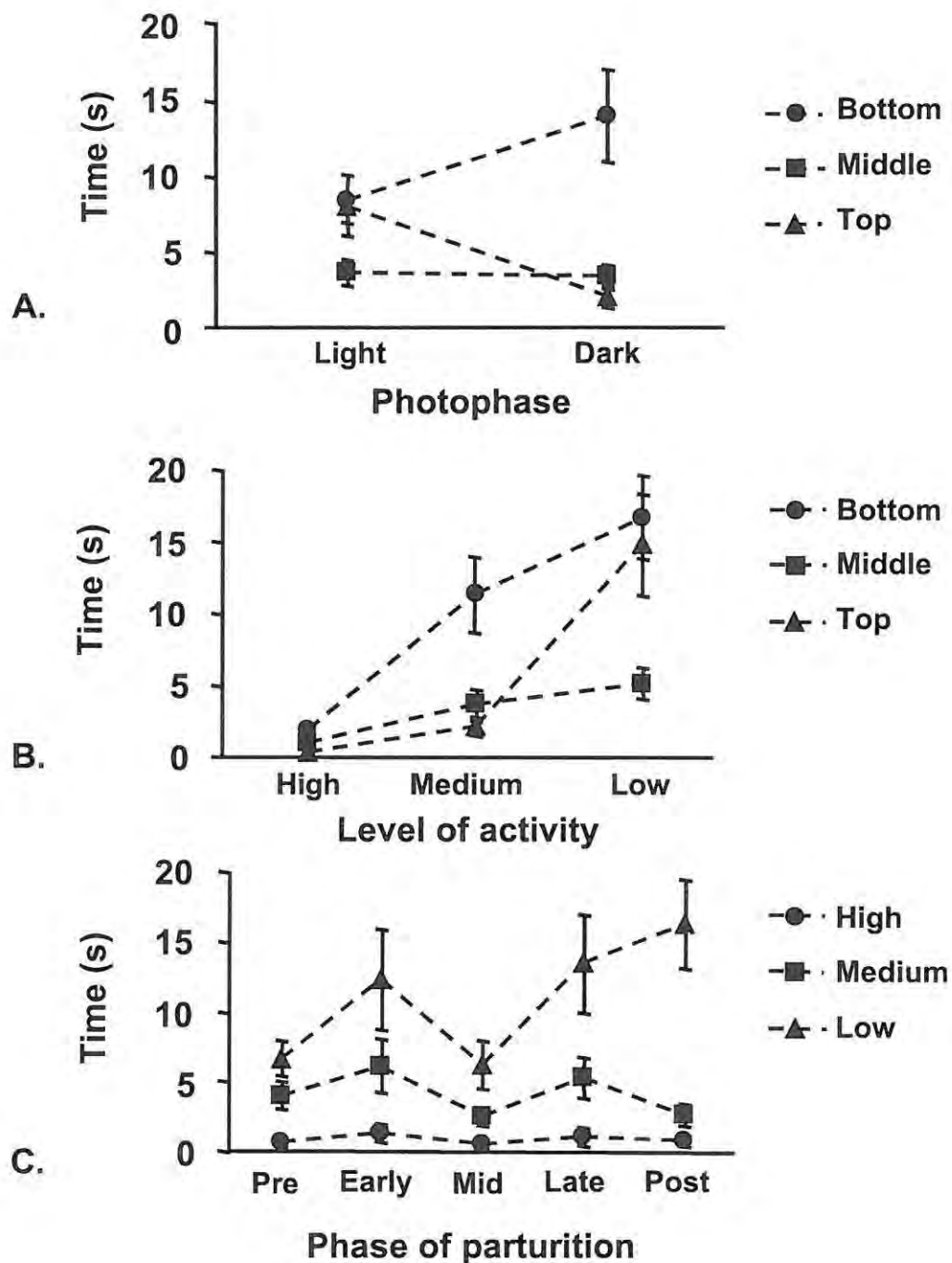


Figure 3.5. Average period (seconds) that female *X. helleri* were observed in each vertical position in the water column in light and dark conditions (A), at different levels of activity at each vertical position (B), and at either a high, medium or low level of female activity at each phase of the parturition-period (C). See Table 3.1 for terms and definitions.

There was also a significant interaction between the three levels of activity and the three positions within the tank (2-way ANOVA, $F_{0.05,4,3129}=2.37$; $p<0.01$). At low levels of activity the lengths of time spent at the bottom and top of the tank were equally long, whereas at both high and medium levels of activity the lengths of time spent at the top were less than those spent at the bottom (Figure 3.5).

Females spent significantly more time at the top of the tank under light conditions than they did in the dark; likewise, the period of time spent at the bottom under dark conditions was significantly longer than that spent at the bottom in the light (2-way ANOVA, $F_{0.05,3,3132}=2.61$; $p<0.05$; Figure 3.5). Equal periods were spent on average in the middle of the tank in both light and darkness (Figure 3.5).

All interactions presented in Figure 3.5 were confirmed using forward stepwise regression ($p<0.05$), and the same analysis found no female effect ($p<0.05$).

DISCUSSION

Filial cannibalism and capture efficiency

Female *X. helleri* were observed to cannibalise their young during and after parturition. 32.5% of the juveniles were cannibalised by the bearing female, which falls within the range ($5.5 \pm 9.6\%$ to $53.9 \pm 4.2\%$) estimated in Chapter 2 of this thesis. In Chapter 2 the fish were placed into tanks at densities of 0.03 females per litre and at a male:female sex ratio of 1:4; whereas the stocking density per unit volume was higher in this study and only the single adult female contained in the tank could be responsible for cannibalism. Furthermore, the estimated rate of cannibalism reported in Chapter 2 was calculated by subtracting the number of juveniles collected per tank from the expected fecundity of the

fish in that tank. Since the results presented in this chapter are independent of both the collection technique and the predicted fecundity, they are a direct estimation of maternal filial cannibalism as opposed to an estimated rate that comprises both filial and non-kin cannibalism. The data here definitively show that the maternal parent is a successful cannibal: on average 45% of all observed attacks resulted in filial cannibalism. Thibault (1974) found that maternal filial cannibalism in the *P. monacha*–*P. lucida* species complex was suppressed until parturition was complete. Unlike some species belonging to the genus *Poeciliopsis*, birth-giving *X. helleri* cannibalise their own young during the parturition-period. The practical implications of spatially separating the juveniles from the birth-giving female, and possibly the other adult fish, are further investigated in Chapter 6 of this work.

Photophase

Most juveniles were cannibalised during the day. Although parturition occurred in both darkness and light, it took place most frequently during the latter. This, together with the increased vulnerability of juveniles immediately after parturition, best explains why juvenile *X. helleri* were cannibalised most often during the day. Darkness had a negative effect on the rate of sibling cannibalism in larval walleye (*S. vitreum*) (Loadman and Moodie, 1986), larval African catfish (*C. gariepinus*) (Britz and Pienaar, 1992; Hecht and Pienaar, 1993) and Australian giant crab (*P. gigas*) (Gardner and Maguire, 1998). Sibling cannibalism in juvenile Japanese flounder (*P. olivaceus*) also declined significantly under dark conditions since vision is the major sensory receptor in prey capture for this species (Dou *et al.*, 2000). Dou *et al.* (2000) further suggested that the occurrence of cannibalism in the dark was a result of accidental encounters rather than the deliberate pursuit and attack. The reduction in the rate of cannibalism in the dark may also suggest that visual perception plays an important role in filial cannibalism of *X. helleri*. Although individuals were seen

pursuing juveniles under the dark conditions of this study (0.54×10^{15} quanta $\text{sec}^{-1} \text{cm}^{-2}$), a low light intensity may reduce the rate of filial cannibalism as it took place less frequently at a reduced light intensity. This hypothesis is addressed in Chapter 6.

Female position in the water column

Most adult attacks on juveniles and most cannibalism took place at the bottom of the tank. No cannibalism was observed in the middle of the water column, even though females did attack young in this area of the tank. Most attacks were followed by the female chasing the juvenile for a short distance. Attacks that occurred in the middle of the tank usually resulted in the female chasing the juvenile to either the top or the bottom of the water column, where it either escaped detection by the attacking parent or was cannibalised, which explains the zero incidents of cannibalism in the middle of the water column. In addition, females spent longer periods at the bottom, which contributes to the explanation for the increased frequency of attack and cannibalism in this part of the water column. Miraz *et al.* (2001) found that female *X. helleri*, attempting to escape potential predation, moved towards the bottom of the tank more often than in any other direction. Although the females in this study were not under the pressure of predation, the observations made by Miraz *et al.* (2001) that females favour the bottom of the tank, lends support to the present findings.

In summary, most attack and cannibalistic events occurred at the bottom of the tank and capture-efficiency was also highest there. Furthermore, various research has shown that refuge can have a positive effect on juvenile survival in livebearers, such as *X. helleri* (Jones *et al.*, 1998a; Chapter 2) and *G. affinis* (Benoit *et al.*, 2000), as well as other fishes, such as African catfish (*C. gariepinus*) (Hecht and Appelbaum, 1988; Pienaar, 1990; Hecht and Pienaar, 1993), walleye pollock (*Theragra chalcogramma*) (Sogard and Olla,

1993), perch (*Perca fluviatilis*) (Persson and Eklov, 1995), bicolor damselfish (*Stegastes partitus*) (Nemeth, 1998) and pink salmon (*Oncorhynchus gorbuscha*) (Willette *et al.*, 2001). Based on this information and data here that show the increased vulnerability of juvenile *X. helleri* to cannibalism immediately after birth, it is hypothesised that refuge in which juveniles can take cover at the bottom of the tank would reduce the rate of intercohort cannibalism in this species. This hypothesis is investigated in Chapter 6.

Female activity

Throughout all phases of parturition the females mostly maintained a low level of activity. However, when at the bottom of the tank, females frequently gave birth at a high level of activity. In accordance with the definition of attack and that of high activity (Table 3.1), a female would show a high level of activity when attacking a juvenile. Most attacks and most cannibalistic events took place at the bottom of the tank, which explains why the females attained a high level of activity at the bottom of the tank more frequently than in the middle or at the top of the water column. Considering this and that juveniles are more vulnerable in the seconds after birth (discussed in Chapter 5), it may be possible to reduce cannibalism if the adult fish are prevented from attaining a high level of activity at the bottom of the tank. This hypothesis is tested in Chapter 6.

Female behaviour during parturition enhances juvenile survival

Some aspects of female behaviour during parturition appear to reduce the chances of filial cannibalism at the time of birth. In the dark the females gave birth at the bottom more often than at the top of the water column, whereas in the light there was a shift towards giving birth at the top. This shift reduced the probability of the adult encountering newborn juveniles, which moved to the bottom immediately after birth (Chapter 5), thus reducing chances of attack and cannibalism. Adults attacked and cannibalised juveniles less

frequently at the top of the water column and also they fed less frequently at the top than at the bottom.

Furthermore, when a female attacks it must be at a high level of activity (see definition for attack and high activity in Table 3.1). Therefore, the probability of an attack increases when the female is highly active. However, females gave birth at a low level of activity more frequently than at a high level of activity, further reducing the chance of cannibalism at the time of parturition. Moreover, females spent significantly more time at a low level of activity at the top of the water column, and at the top they selected a low level of activity 75% more frequently than a high level of activity ($n=229$ and $n=59$, respectively). At the bottom of the water column the difference was considerably less with the frequency of high activity ($n=290$) being 68% that of low activity ($n=424$) (Figure 3.4). In other words, females were more active at the bottom and less active at the top, which further supports the benefit of a shift towards parturition at the top of the tank where juveniles are less likely to be cannibalised.

The results of Chapter 5 will further support this argument as they demonstrate that when juveniles are attacked by adult fish they tend to escape in a downwards direction and they tend to settle on the bottom of the tank more frequently which suggests a greater density of juveniles at the bottom of the water column. By giving birth at the top the chances of the birth-giving female encountering a juvenile and cannibalising it are reduced. Similarly, adult behaviour in other fishes in the wild is thought to reduce the rate of intercohort cannibalism. For example, adult and juvenile walleye pollock (*T. chalcogramma*) inhabit surface waters during winter, but in summer the adults move to the bottom over continental shelves while the juveniles remain close to the surface (Bailey, 1989). In summer the rate of intercohort cannibalism is reduced considerably due to the behaviour

of the adults and the resultant spatial separation of the cohorts (Bailey, 1989). Another example includes the whitefish (*C. lavaretus*) population of Lake Constance that make a diurnal vertical migration of up to 50 metres ascending at dusk and descending at dawn (Eckmann, 1991). This migration only takes place during the spawning season and it is thought that the migratory behaviour reduces the risk of cannibalism on freshly spawned eggs (Eckmann, 1991). Similarly, the behaviour of female *X. helleri* during the parturition period appears to reduce the likeliness of the females coming into contact with newborn juveniles and thus may ensure that filial cannibalism is kept to a minimum.

Some of the care-giving fishes are believed to have developed anti-cannibalism strategies (FitzGerald and Whoriskey, 1992). For example, the threespine stickleback (*G. aculeatus*) and the Cortez damselfish (*S. rectifacenum*) reduce cannibalism of an individual's own eggs by depositing them in nests that contain eggs from previous females, and the stickleback is able to recognise self-spawned eggs (FitzGerald and Whoriskey, 1992). Moreover, there is evidence of behaviour that reduces the chance of the maternal parent cannibalising its own young in poeciliids such as *P. reticulata*, *P. spheonops* and the *P. monacha*–*P. lucida* species complex (Thibault, 1974; Loekle *et al.*, 1982). The guppy (*P. reticulata*) and the black molly (*P. spheonops*) tend not to cannibalise their own offspring and are more likely to perform non-kin cannibalism than filial cannibalism. Furthermore, cannibalistic behaviour in the *P. monacha*–*P. lucida* species complex was temporarily suppressed in the birth-giving female until parturition was complete (Thibault, 1974). However, this behaviour has not yet been researched or documented in any other species of family Poeciliidae. Nonetheless, the findings of this study and the observations of Thibault (1974) and Loekle *et al.* (1982) suggest that poeciliid behaviour may be geared towards reducing the chance of a female cannibalising its own offspring during parturition. However, further studies are required to specifically

investigate if maternal behaviour during the parturition period serves to reduce cannibalism in *X. helleri* and other livebearing species. If behaviour of female *X. helleri* is geared towards reducing cannibalism during parturition, then the results further support the conclusion (Chapter 2) that cannibalism may be opportunistic.

Conclusion

This study has contributed towards an understanding of the maternal reproductive behaviour of *X. helleri*, as well as towards a more complete understanding of filial cannibalism in the species, by documenting and quantifying the way in which females cannibalise their young under captive conditions. These findings make it possible, for the first time, to predict a female's most likely level of activity or position in the water column during parturition, attack or cannibalism of young. Furthermore, the behaviour *X. helleri* was not dissimilar to other poeciliids such as *P. reticulata*, *P. spheonops* and females from the *P. monacha*–*P. lucida* species complex, as well as some non-poeciliid species such as whitefish (*C. lavaretus*), threespine stickleback (*G. aculeatus*) and walleye pollock (*T. chalcogramma*), as it appeared that female *X. helleri* behaviour ensured that cannibalism was less likely to take place during the parturition period.

The study has raised a number of questions. Firstly, it was not known whether the cannibalistic and general behaviour of the birth-giving female during the parturition period differed from that of other adult conspecifics, and whether or not this behaviour would alter in the presence of other adult fish. These questions are addressed in Chapter 4. It also became apparent that a better understanding of juvenile behaviour during the parturition-period was required in order to understand *post-partum* cannibalism in this species, and this topic is addressed in Chapter 5. Finally, since the results here suggest that cannibalism may be influenced by environmental manipulation such as reduced light

intensity or the presence of a false-bottom or refuge at the bottom of the tank, further investigation into the affect that manipulation of the environment can have on the rate of cannibalism and juvenile survival is presented in Chapter 6.

CHAPTER 4

PARTURITION-ASSOCIATED BEHAVIOUR OF CONSPECIFIC BROODSTOCK

ABSTRACT

To generate a description of adult *Xiphophorus helleri* behaviour during parturition, the parturition period of five females, each in the presence of a male and three other females that did not give birth during the observation period, was recorded on videotape. All parturition events occurred in the dark. When it occurred at the bottom of the water column it always took place in the open water, while at the top the female gave birth relatively more often in the artificial refuge; thus, although females are cannibalistic their behaviour might be geared towards increasing the chance of neonates survival. Most adults were found at the bottom of the water column during parturition and during the first 500 minutes after the first attack was observed, after which they were distributed equally in the water column. The movement of the concentration of adults away from the bottom coincided with a decrease in the rate of cannibalism, an increase in the rate of feeding on material other than live juveniles, and the onset of light conditions. Adults attacked juveniles most frequently during the first 200 minutes after the first attack was observed, and during this time, secondary attackers were observed frequently. A reduced attack rate may have been due the movement of adults away from the bottom of the tank or, alternatively, the result of operant conditioning or habituation since most attacks were unsuccessful (27.5% resulted in cannibalism). All adults were equally responsible for attacking and cannibalising offspring, so it is hypothesised that the removal of males from the breeding tank will decrease the rate of cannibalism. Since most attacks and most cannibalistic events occurred at the bottom of the water column it is also hypothesised that the rate of cannibalism can be reduced if adults are prevented from accessing the bottom.

INTRODUCTION

The behaviour of many fish species, such as walleye pollock (*Theragra chalcogramma*), whitefish (*Coregonus lavaretus*), scorpionfish (*Sebastiscus marmoratus*), mosquitofish (*Gambusia affinis*) and swordtail (*Xiphophorus helleri*), appears to increase the chance of egg or juvenile survival at the time of spawning or parturition (Bailey, 1989; Eckmann, 1991; Fujita and Kohda, 1998; Jones *et al.*, 1998b; Benoit *et al.*, 2000; Chapter 3). For example, adult *T. chalcogramma* undergo a seasonal migration and adult *C. lavaretus* undergo a diurnal migration during the spawning season, both of which cause a spatial separation of adults and juveniles or eggs and, consequently, reduced intercohort cannibalism (Bailey, 1989; Eckmann, 1991).

The timing of spawning also affects juvenile survival. For example, the viviparous scorpionfish (*S. marmoratus*) only gives birth at night when predators of both parent and offspring are inactive (Fujita and Kohda, 1998). Likewise, the time of spawning may change according to environmental conditions or else spawning may be geared to take place at particular times of day to assist larval survival (Shimura and Egusa, 1980; Schleyer *et al.*, 1997; Hirose and Kawaguchi, 1998; Tanaka, 1998; Stoner *et al.*, 1999). Examples include the winter flounder (*Pseudopleuronectes americanus*) that only spawns at night (Stoner *et al.*, 1999), the surf smelt (*Hypomesus pretiosus japonicus*) that spawns just after dark (Hirose and Kawaguchi, 1998) and damselfishes that do not receive protection from sea anemones that spawn predominantly under the cover of darkness (Tanaka, 1998). No controlled research has focused on the diurnal timing of parturition in poeciliids and the effect that the timing of birth has on juvenile survival. Hobbyists report that poeciliid parturition does not follow a diurnal rhythm but occurs at random times. In Chapter 3 it was shown that parturition occurred at night and during the day, which supports these informal observations.

Parturition and spawning behaviour that takes place in refuge has been shown to increase larval and juvenile survival in a range of species from broadcast spawners such as African catfish (*Clarias gariepinus*) (Hecht and Appelbaum, 1988; Pienaar, 1990; Smith and Reay, 1991; Hecht and Pienaar, 1993) to livebearers such as western mosquitofish (*G. affinis*) and swordtail (*X. helleri*) (Jones *et al.*, 1998a; Benoit *et al.*, 2000; Chapter 2). By inference, other adult behaviour at the time of spawning or parturition may increase the chance of egg or juvenile survival.

Parental care is defined as any form of parental behaviour following fertilization that is likely to increase offspring survival (Smith and Wootton, 1995). Livebearing falls within this definition and is considered an extreme form of parental care; guarding and mouth brooding are taken one step further as the young are cared for within the abdomen of the female parent. Livebearing, therefore, includes considerable energetic investment by the birth-giving female (Smith and Wootton, 1995). However, there are fitness costs to the bearing parent. For example, resources such as food that would have been available for somatic growth are redirected to cytoplasmic development or, in the case of viviparous animals, to the developing embryos. A reduction in growth is a fitness cost to a bearing female particularly where fecundity is a function of female size (Smith and Wootton, 1995), such as in *X. helleri* (Milton and Arthington, 1983; Kruger *et al.*, 2001a). Some parental care-giving species, such as the threespine stickleback (*Gasterosteus aculeatus*), have been known to either abandon or cannibalise their young if the cost of rearing them outweighs the benefit (FitzGerald, 1992a; Smith and Wootton, 1995). This is because filial cannibalism in care-giving species is sensitive to both the cost and the benefit of brood care and that the expected fitness benefits from a small clutch, for example, may never outweigh the cost of brood care (Lindstrom, 2000). However,

cannibalism may be more beneficial than abandonment as it allows the parent to recover some of the energy that was invested in the offspring (Smith and Wootton, 1995). Parents have been known to cannibalise young that are deformed, weak, sick, or otherwise unviable (Hrdy, 1979; Polis, 1981). Furthermore, the parent has nothing to lose by consuming a juvenile that is not going to contribute to its reproductive fitness. However, little is known about the adaptive advantage of recovering energy through cannibalism in the animal kingdom in general, and no conclusions have been drawn in this regard for fish of the family Poeciliidae.

Earlier findings have shown that male and female *X. helleri* are equally responsible for cannibalism of juveniles (Jones *et al.*, 1998a; Chapter 2). However, this is contrary to the findings of Warren (1973), Hubbs (1991) and Nesbit and Meffe (1993), who found females of other poeciliid species to be more cannibalistic than their male counterparts. Polis (1981) noted that although a sexual bias for cannibalism in the animal kingdom was usually neither reported nor investigated, females were reportedly more cannibalistic in 86% of the cases where sexual differences in cannibalism were noted. Further investigation into the behaviour of males and females is therefore required to contribute to this debate of sexually biased cannibalism in *X. helleri*.

Experiments have confirmed (Chapter 2) that cannibalism occurs under captive conditions and behavioural observations (Chapter 3) have increased our understanding of the behaviour of birth-giving females during the parturition period. However, it is not known how behaviour of the birth-giving female might change in the presence of other adult conspecifics, and whether or not her behaviour is comparable to that of males and females that did not give birth. Therefore, the aim of the present investigation is to develop a general description of parturition-associated behaviour in adult *X. helleri*. The

specific objectives are to describe, quantify and begin to explain the differences in feeding, parturition, attack, and cannibalistic behaviour of adult *X. helleri* in relation to time after parturition, light and dark conditions, and adult distribution in the water column, as well as to compare the behaviour of the birth-giving female to that of the male and females that did not give birth.

MATERIALS AND METHODS

Broodstock conditioning

Fifty *X. helleri* broodstock (Gold Victory strain) were reared in a closed recirculating system housed in a greenhouse tunnel as described in Chapter 2 (Figure 2.1). The fish were conditioned for 4 weeks in five 300L tanks (Figure 2.2), at a sex ratio of 1:4 (male:female) and a stocking density of 10 fish/tank. They were subjected to a photoperiod of 14L:10D, and fed to satiation daily at 08h30 and 16h30 on a diet of Aqua Nutro tropical fish food with 40% crude protein and 6% crude lipid on a dry-weight basis.

Observation procedure

A group of fish consisting of one male and four gravid females was randomly selected from each of the five conditioning tanks and placed into a glass aquarium that formed part of the closed recirculating laboratory system (see Chapter 3, Figure 3.1). The glass aquaria for this experiment held 35.5L of water and had internal dimensions of 290 by 490mm and a water depth of 250mm. A photoperiod of 14L:10D was maintained with the use of fluorescent tubes positioned directly above the tank, and far-red light was used to observe the fish at night. The light intensities at different depths in the water column did not differ from those described in Chapter 3.

A bundle of 15g of shredded plastic was placed in the tank as shelter for the newborn juveniles. Half of the shelter floated in the water column and covered about 15% of the water surface area to a depth of approximately 80mm, and two quartzite stones weighed down the other half that covered about 15% of the surface area of the tank bottom. The fish were fed to satiation once daily in the morning until the onset of parturition, where after they were not fed until parturition was complete. Debris consisting mostly of faecal matter and uneaten food was siphoned out of the tank every second day.

A video camera (Sony, VM-PS12) was positioned in front of the observation tank making the entire interior of the tank visible on a television monitor. The video camera was connected to a video recorder (Philips, VR456/93) with a long-play function that made it possible to film the fish continually until parturition occurred. The fish remained in the observation tank for a minimum of four days before one of the females produced young. The filming continued until the entire parturition period of one female in each group was recorded, after which the fish were removed from the observation tank and the process repeated using another group of fish. The parturition-associated behaviour of five different groups of *X. helleri* was recorded in this manner.

Observation analysis

The video tapes were viewed on a television monitor (Gold Star, CY-14A90Y) linked to a video player (Philips, VR456/93) that featured a slow-play function so that observations could be viewed at 16.7% the speed of normal viewing. The fish, their position in the water column and different behavioural events were recorded and evaluated according to the terms and definitions given in Table 4.1. All behavioural events were quantified by determining their frequency of occurrence. The time interval between attack of a juvenile and cannibalism of that juvenile was recorded, and capture efficiency by the adult fish was

Table 4.1. Terms and definitions used to describe various behavioural events that were identified while observing juvenile and neonate *X. helleri* during the parturition period, as well as the different positions within the observation tank.

Term	Definition
Age and description of fish	
Neonate	A fish born in the observation tank during an observation
Juvenile	A fish born in the observation tank before or during an observation (note that this term subsumes newborn juveniles)
Birth-giving female	Adult female that gave birth during observation
Non-birth-giving fml	Adult female that did not give birth during the observation period
Male	Adult male swordtail
Primary attacker	Adult fish that initiated an attack on a juvenile
Secondary attacker	Adult fish that joined in on an attack initiated by another adult
Behavioural events	
Parturition	Appearance of a juvenile from the female genital pore
Attack	A sudden burst of speed by an adult directly towards a juvenile, beginning with the start of the caudal peduncle thrash that propelled the adult towards the juvenile, or, when the adult was in striking distance of the juvenile, the attack began with the movement of the adult's head directly towards the juvenile
Cannibalism	Successful predation of a live juvenile
Escape	Avoidance of cannibalism after being attacked
Settling	The juvenile remains inactive
Feeding	Ingestion of anything other than a live juvenile by an adult fish
Phases of parturition period	
Pre-phase	Period before first parturition
Early-phase	First third of the period between the first and last parturition
Mid-phase	Second third of the period between the first and last parturition
Late-phase	Last third of the period between the first and last parturition
Post-phase	Period after the last parturition
Position in the tank	
Top	Top third of the water column
Middle	Middle third of the water column
Bottom	Bottom third of the water column
Artificial refuge	The area of the tank that contained shredded plastic
Open water	The area of the tank that did not contain shredded plastic

calculated according to Equation 3.1 (Chapter 3). The position of the adults in the tank was noted by dividing the tank into nine predetermined cells of equal size (i.e. the top, middle and bottom of the water column were each visually divided into three areas of equal volume). The number of times that adults were observed in each cell was recorded at 100-minute intervals. The average distance between individual adults was estimated by determining the length between the centres of the cells in which the adults were recorded.

The observation of each family group began when the adult fish began attacking the juveniles, which was soon after first parturition. Each observation began as an adult made the first attack on a juvenile and continued up to the point that the juvenile was eaten or until the juvenile escaped and settled elsewhere in the tank. The videotape was then forwarded to a point 30 minutes past the start of the previous observation, and then watched until the next attack was noted. This attack marked the start of the second observation, after which the tape was forwarded again. A minimum of 10 observations was made per group of adults. The observation of a group was terminated if no attacks were noted after 60 minutes of continuous viewing. The time at which observations were made was related back to the time of the first attack on a juvenile.

The number of times that adult *X. helleri* fed on material other than live juveniles was recorded during one-minute observations. These one-minute observations were made at 40-minute intervals from the time of the first attack for a maximum of 1000 minutes after the first attack. Filming time was limited to 1000 minutes due to number of videotapes available. Of the five groups of fish that were observed, two groups were not filmed for the full 1000 minutes; their feeding behaviour was observed for 560 minutes after the first

attack. The number of times that the fish in the observation tank fed during the one-minute observation periods was recorded and the feeding frequency was calculated.

Water-quality analysis

Water-quality readings were taken after the observation of each group of fish. Temperature was measured with a mercury thermometer ($n=5$). Ammonia and nitrite concentrations were measured spectrophotometrically with the use of Nessler's reagent and the diazotization method (Merck, 1974) ($n=5$ and $n=5$, respectively), and pH readings were taken using a portable pH meter (Hanna Instruments, USA) ($n=5$). The mean pH was calculated by averaging the anti-log of all pH values, and re-logging the resultant average.

Statistical analysis

A contingency analysis was used to analyse frequency distributions. Expected frequencies were adjusted to account for the difference in the number of non-birth-giving females as compared to males and birth-giving females. The null hypothesis (H_0) stated that the distribution of events was the same under all conditions, and H_0 was rejected at $p<0.05$. Frequency distributions for one variable were analysed with the use of a χ^2 analysis, where the null hypothesis (H_0) stated that there was an even distribution of events, and H_0 was rejected at $p<0.05$. The analysis was adjusted ($\chi^2_{adj.}$) using Yates correction for continuity if the expected frequency for χ^2 analyses was less than 5, and under similar circumstances Fishes Exact analysis replaced contingency analysis. The time interval between different behavioural events and the distances between fish were each tested for normality using Shapiro-Wilk's test at an error of 5%; if data were not normally distributed they were log-transformed to normalise the data. Data that could not

be normalised where analysed using the non-parametric Kruskal-Wallis test. Normally distributed data were analysed using one-way analysis of variance (ANOVA), and Tukey's multiple-range analysis was used to compare means between factors at $p < 0.05$. Least-square regression modelling was used to model the development of feeding and attack behaviour over time. The mean feeding rate in the dark was compared to that in the light using a Mann-Whitney U-test. This test was also used to compare the mean rate of attack in the dark to that in the light. Bartlett's test for equality of variance was used to determine the differences in variances of feeding under light and dark conditions, and to determine the difference in variance of rate of attack during the first 300 minutes after first attack (in the dark), and that during the following 700 minutes (in the light) (Ott, 1988).

RESULTS

Water quality

The mean water temperature of the observation system was 27.3°C (range: 26.5 to 28.5°C; $n=5$). The pH readings ranged between 6.9 and 7.2 with a mean of 7.1 ($n=5$). Ammonia ($\text{NH}_4^+\text{-N}$) levels ranged from 0.01 to 0.05 mgL^{-1} ($n=5$), while nitrite ($\text{NO}_2^-\text{-N}$) was not detected in the system using the chosen method.

Parturition behaviour

All females in this study gave birth under dark conditions. Artificial refuge affected the distribution of parturition events (birth of individual juveniles) across the vertical sections of the water column (contingency analysis; $\chi^2=8.94$; $v=2$; $p < 0.025$; Table 4.2). At the bottom of the water column parturition took place only in the open water and never in the refuge; at the top parturition took place equally often in the refuge and the open water.

Table 4.2. The frequency of parturition events in the artificial refuge or in the open water, at either the top, middle or bottom sections of the water column (contingency analysis; $\chi^2=8.94$; $\nu=2$; $p<0.025$).

	Top	Middle	Bottom	TOTAL
Artificial refuge	5	5	0	10
Open water	5	3	10	18
Total	10	8	10	28

Cannibalistic behaviour

The number of adults that attacked juveniles was highest during the first 100 minutes following the first attack on a juvenile, and decreased rapidly after 200 minutes (Figure 4.1) even though parturition continued. The birth-giving females, non-birth-giving females and males were all aggressive towards juveniles, and all exercised the role of primary attacker equally often ($\chi^2=1.33$; $\nu=2$; $p>0.50$; Table 4.3). The birth-giving female joined in as a secondary attacker on a juvenile more often than would be expected from an equal distribution, while the males joined in less frequently ($\chi^2=6.74$; $\nu=2$; $p<0.05$; Table 4.3). The fish stopped attacking as secondary attackers approximately 200 to 300 minutes after the adults had first attacked a juvenile, even though juveniles continued to be attacked by primary attackers (Figure 4.2).

The variation in the frequency of attacks during the first 300 minutes after the first attack was significantly greater than that during the following 700 minutes (Figure 4.3; Bartlett's test; $F_{0.05,40,9}=2.83$; $p<0.05$). There was no trend in the rate of attack with time ($r^2=2.59\%$; $p>0.25$), and there were no differences between the mean rates of attack under dark or light conditions (Mann-Whitney U-test; $p>0.05$).

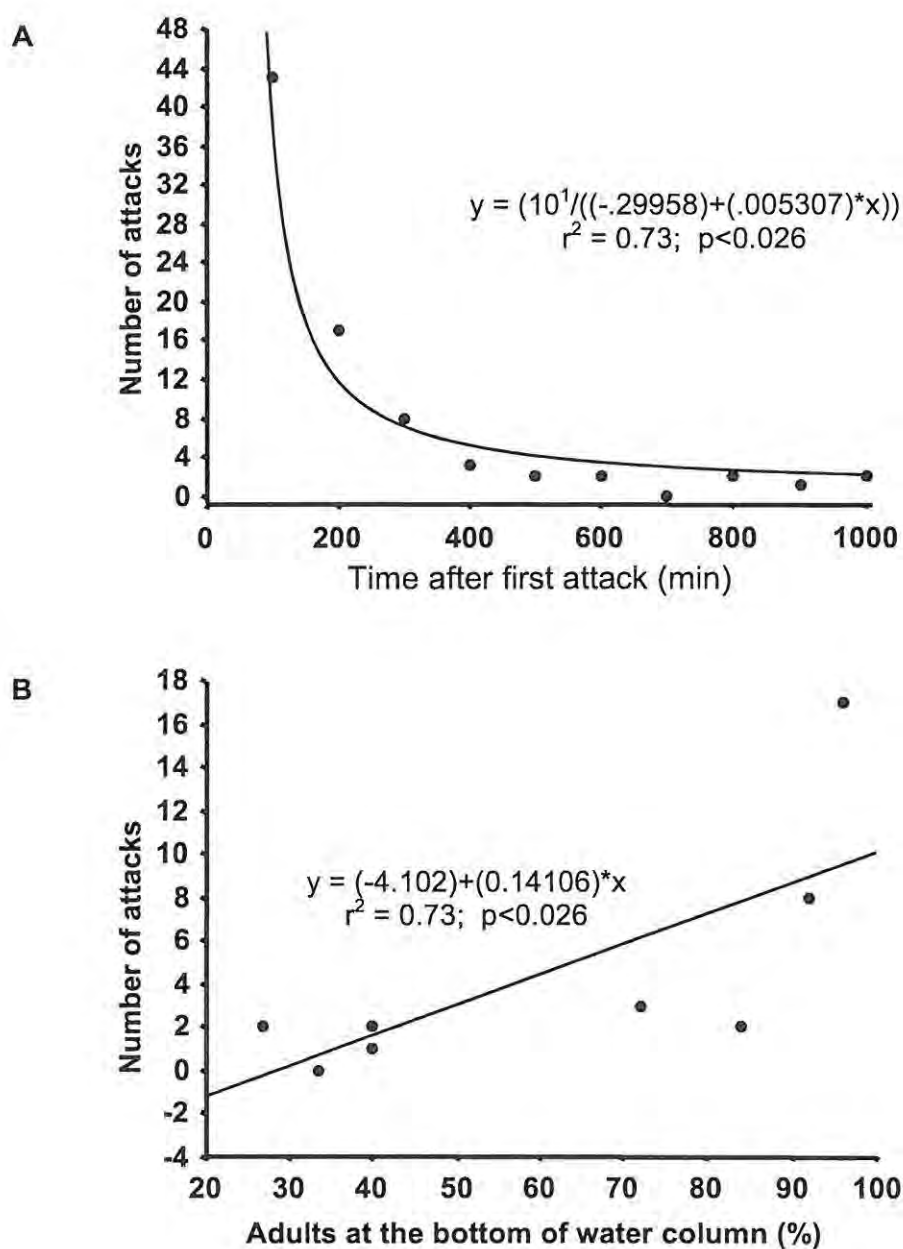


Figure 4.1. Percentage adult *X. helleri* population at the bottom of the water column in relation to: total number of attacks over time after first attack (A); and total number of attacks over percent of the adult population that was at the bottom of the water column following the first 100 minutes after first attack (B) together with line of best fit.

Table 4.3. The total number of attacks and total number of juveniles cannibalised by adult *X. helleri*, together with the corresponding rate of capture efficiency (%), and the number of times that the adult fish attacked juveniles as a primary (1°) or secondary (2°) attacker. Different superscripts represent an uneven distribution within each column (χ^2 ; $p < 0.05$). These are combined data from the five groups of fish that were observed.

	Attack	Cannibalism	Efficiency	1°attacker	2°attacker
Non-bearing female*	66 ^a	17 ^m	25.8	59 ^p	24 ^s
Bearing female	24 ^a	7 ^m	29.2	19 ^p	12 ^s
Male	12 ^a	4 ^m	33.3	14 ^p	2 ^t
Total (Average)	102	28	27.5	92	38

* Since each tank held three times more non-bearing females than males or bearing females, the expected value for the number of attacks and cannibalism by non-bearing females was adjusted to arrive at the correct expected value by increasing the expected value for non-bearing females by three.

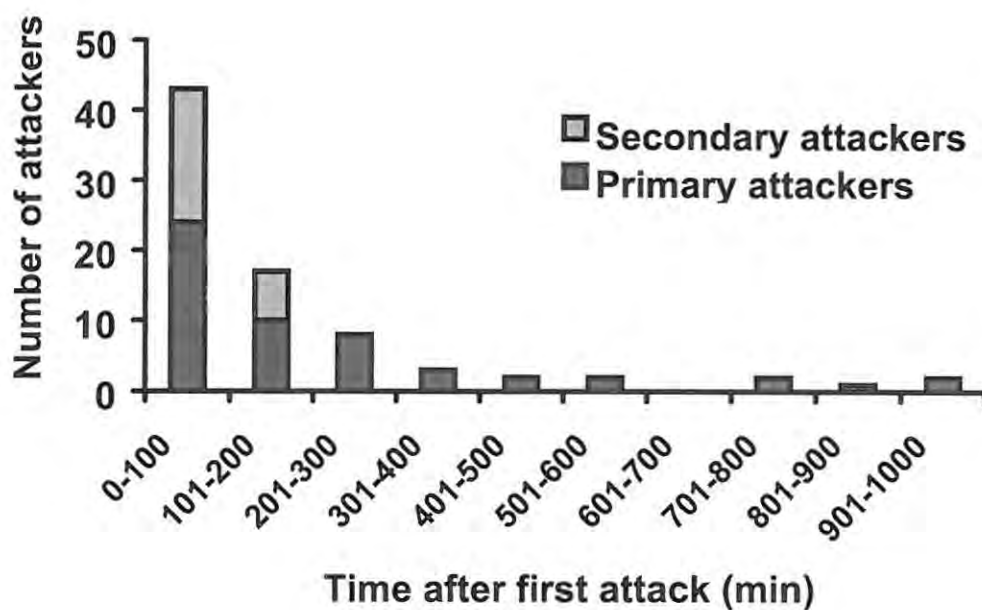


Figure 4.2. The number of primary and secondary attackers that attempted cannibalism during the observation period after first attack.

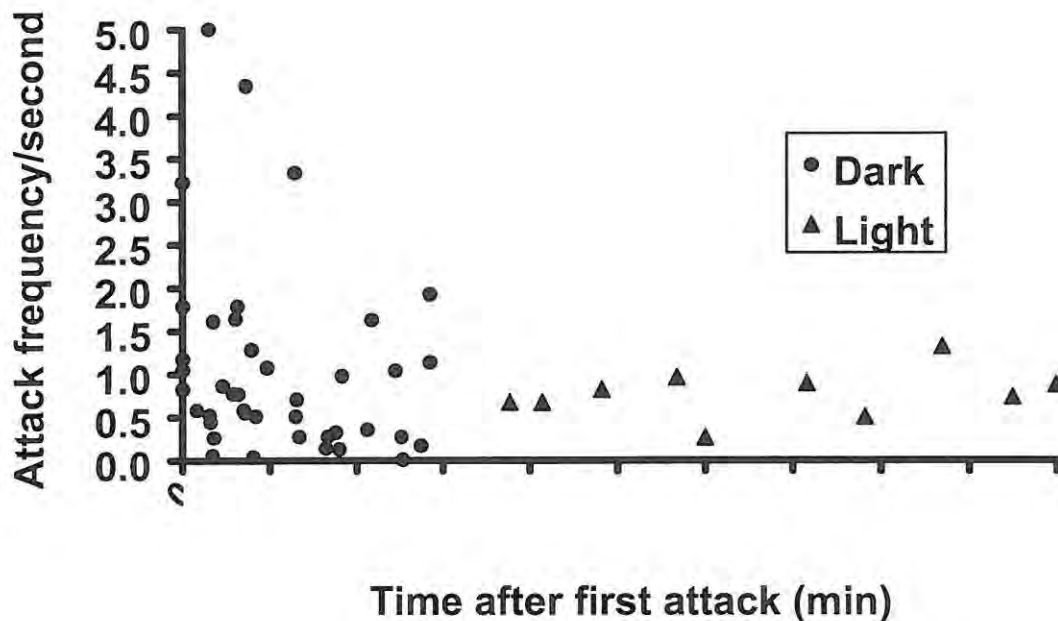


Figure 4.3. The frequency that adults *X. helleri* attacked juveniles during the observation period after first attack, under light and dark conditions. Each observation began with the first attack on a juvenile and ended when that juvenile was either cannibalised or, having escaped cannibalism, settled elsewhere in the tank.

There was no difference in the frequency of attack on juveniles by birth-giving females, non-birth-giving females and males ($\chi^2=4.47$; $v=2$; $p>0.10$; Table 4.3). Similarly, these fish were all equally cannibalistic ($\chi^2=0.81$; $v=2$; $p>0.50$; Table 4.3). The average time it took an adult to consume a juvenile after the juvenile was first attacked was 4.4 ± 0.2 s, and this did not differ between adults (ANOVA; $n=28$; $p>0.50$). The average time between final attack before the consumption of a juvenile was 1.4 ± 0.1 s, and this also did not differ between adults (ANOVA; $n=28$; $p>0.93$).

Adult distribution

Although non-birth-giving fish appeared to follow the birth-giving female around the tank, the average distance between the birth-giving female and the other fish in the tank remained at 142 ± 11.3 mm ($n=180$); this did not differ from the average distance between the other fish in the tank (Kruskal-Wallis; $H_{0.05,3,450}$; $p > 0.10$; Table 4.4), nor did this change over time (Kruskal-Wallis; $H_{0.05,10,450}$; $p > 0.05$). However, the vertical distribution of adult *X. helleri* in the water column did not remain constant over time (contingency analysis; $\chi^2 = 87.58$; $v=20$; $p < 0.001$; Figure 4.4). Adults were found at the bottom of the water column more often immediately after the first attack, but they became more evenly distributed in the water column with time (Figure 4.4).

Table 4.4. Average distance ($\pm$ standard error) between individual adult fish in the tanks. Different superscripts represent significant differences within the column (Kruskal-Wallis; $p < 0.05$).

Distance between:	Average distance (mm)	n
Male & birth-giving female	133 ± 18.6^a	45
Male & non-birth-giving female	118 ± 10.7^a	135
Birth-giving & non-birth-giving females	151 ± 10.7^a	135
Non-birth-giving & non-birth-giving females	153 ± 10.8^a	135
Birth-giving female & all other fish	142 ± 11.3	180
Average distance between all fish	139 ± 6.6	450

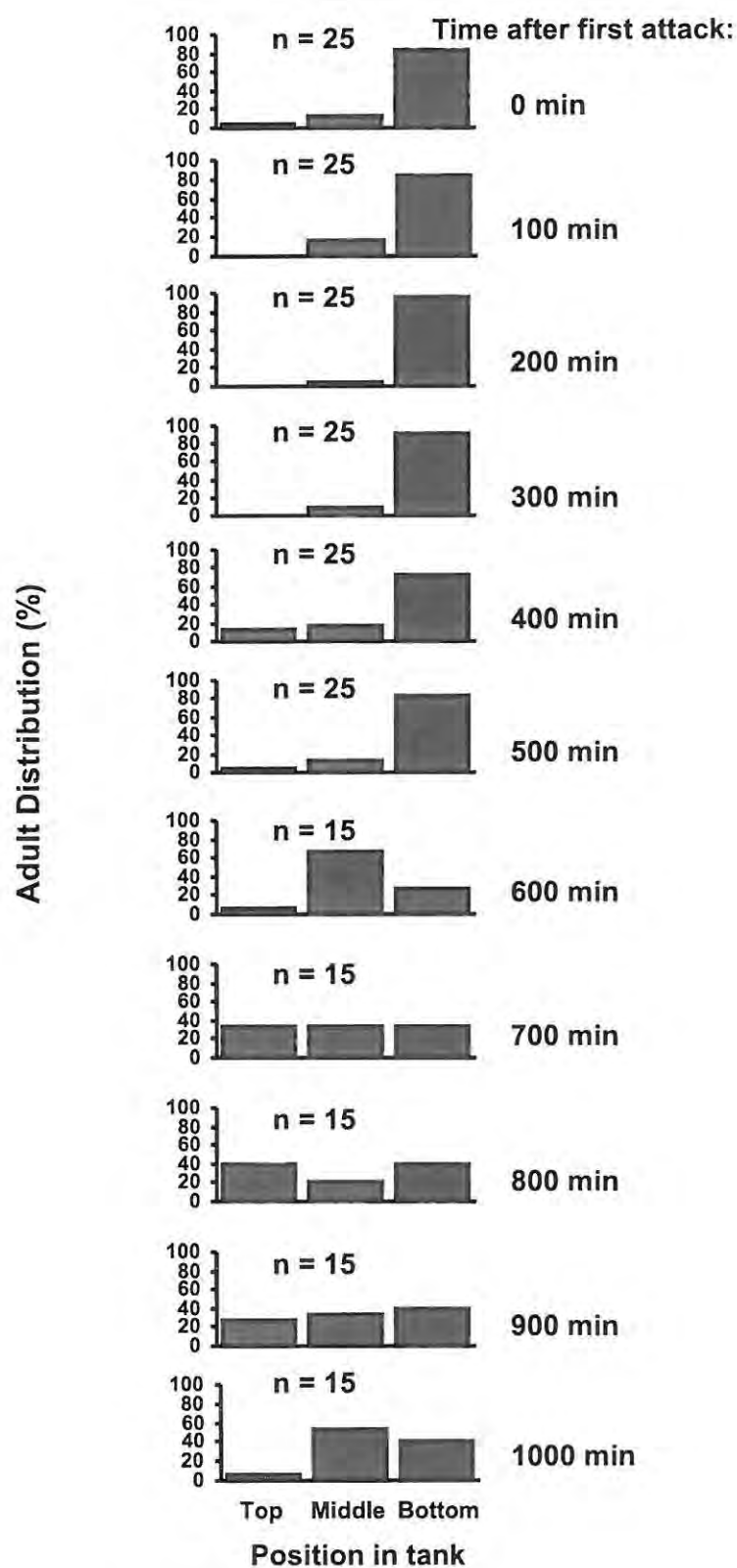
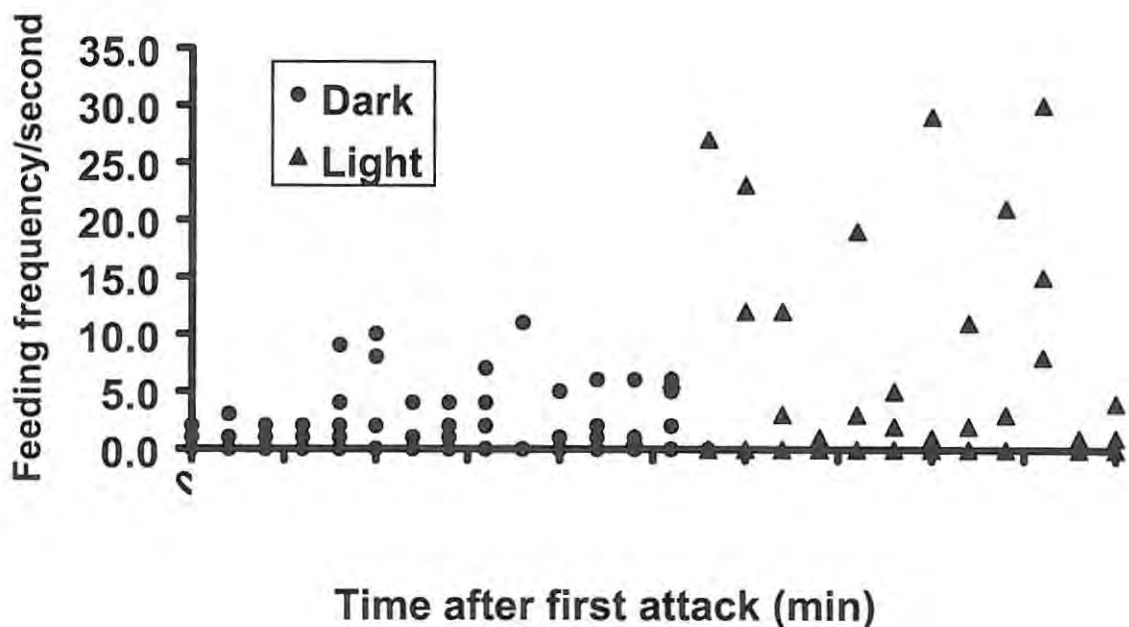


Figure 4.4. Distribution of adult *X. helleri* in the water column during time after first attack (contingency analysis; $\chi^2=87.58$; $v=12$; $p<0.001$).

Feeding behaviour

The number of times per minute that adults fed on material other than live juveniles never exceeded three feedings per minute for the first 160 minutes following the first attack, after which the feeding frequency increased progressively with time (Figure 4.5). The variance of feeding in the light was significantly greater than that in the dark (Bartlett's test; $F_{0.05,37,70} < 1.65$; $p < 0.001$). The feeding rate under light conditions did not change over time ($r^2 = 0.39$; $p > 0.71$) nor did it change in the dark ($r^2 = 0.34$; $p > 0.62$). The mean frequency of feeding in the light (9.5 feeding activities per minute) was significantly greater than in the dark (1.6 feeding activities per minute) (Mann-Whitney U-test; $p < 0.035$).



DISCUSSION

Parturition behaviour

All females in this study gave birth under dark conditions. This is contrary to the findings of Chapter 3 and Jones *et al.* (1998b) where the solitary females did not appear to show a preference for parturition in either the light or the dark. Together, the outcomes suggest that the timing of parturition may depend on the presence or absence of conspecific adults. Furthermore, prey capture efficiency was reduced in the dark (Chapter 3) and the rate of cannibalism was density dependent and increased with adult number (Chapter 2). Therefore, it is hypothesised that parturition occurs more frequently in the dark when it occurs in the presence of adults, and, secondly, that this behaviour is likely to increase juvenile survival because prey capture efficiency by adults is reduced in the dark. Similar behaviour has been observed in other viviparous species, such as the scorpionfish (*S. marmoratus*) that give birth at night when potential predators are less active (Fujita and Kohda, 1998). This was thought to be an adaptive advantage that increased juvenile survival (Fujita and Kohda, 1998). Similarly, there are numerous reports of a wide range of aquatic animals that give birth or spawn selectively under either light or dark conditions (Shimura and Egusa, 1980; Schleyer *et al.*, 1997; Fujita and Kohda, 1998; Hirose and Kawaguchi, 1998; Tanaka, 1998; Stoner *et al.*, 1999; Sakai and Kohda, 2001). However, no published data were available with which to compare the density-dependent changes in parturition behaviour that were observed here. Nonetheless, since cannibalism was positively density dependent and as prey capture efficiency was lower in the dark, juvenile *X. helleri* that are born into a population with a high adult density seem to have a greater chance of surviving at night. Although this study and the experiments of Chapter 3 were not designed to test the density-dependence of parturition behaviour, a comparison of the findings led to the hypothesis that stocking density affects the timing of parturition with regard to light and dark conditions.

The morphology of *X. helleri* may provide a possible explanation for the difference in parturition frequency at the top and bottom of the water column. Adults have a superior mouth that may enable them to better consume food and prey that is positioned above them. This assumption was supported by the observation that prey capture efficiency was highest at the top of the water column (see Chapter 5). Therefore, neonates may be more vulnerable to predation by conspecific adults if parturition takes place near the surface of the water, and less vulnerable if parturition takes place closer to the bottom. Parturition events occurred relatively more often in the artificial refuge positioned at the top, while at the bottom of the water column parturition occurred most often in the open water. Refuge availability significantly suppresses intracohort cannibalism in many fishes (Hecht and Appelbaum, 1988; Pienaar, 1990; Smith and Reay, 1991; Hecht and Pienaar, 1993) and it suppressed intercohort cannibalism in the poeciliids *G. affinis* and *X. helleri* (Jones *et al.*, 1989a; Benoit *et al.*, 2000; Chapter 2). Hence, when parturition takes place near the water surface, neonates have a greater chance of surviving cannibalism under the cover of refuge. The assumption here that juveniles are more vulnerable at the top, is not consistent with the finding of Chapter 3 that juveniles were attacked less frequently at the top and were therefore less vulnerable to cannibalism at the top. However, it will be shown in Chapter 5 that juveniles do not occur very often at the top of the water column, and, therefore, the result in Chapter 3 may not contradict the current finding if it had been possible to present it as rate of attack at the top rather than the frequency of attack. Yet, the current result supports the argument in Chapter 3 stating that female behaviour is likely to promote juvenile survival by reducing the chance of cannibalism immediately *post-partum*.

Adult distribution in the water column

Adults were observed at the bottom of the water column more often than at the middle or top for the first 500 minutes after the onset of parturition. Thereafter, the bottom, middle and top of the water column were occupied equally often. There was a change from dark to light conditions at this time, and this change is the most plausible explanation for this change in adult distribution. Furthermore, *X. helleri* appear to move to the bottom of the tank in the dark under production conditions (personal observation). The change in photophase from dark to light conditions occurred between 480 and 706 minutes after the first attack for all groups. Observation of birth-giving females (Chapter 3) showed that these fish spent significantly longer periods at the bottom of the tank under dark conditions. Since parturition occurred only in the dark when birth-giving females were in the presence of other adults, the juveniles would have been more prone to cannibalism because of the relative location of the adults in the tank (i.e. at the bottom) at the time of parturition (i.e. in the dark). It has been established that parturition takes place more frequently at the bottom of the tank under dark conditions (Chapter 3), and also that under dark conditions other adults tend to occupy the bottom of the tank more frequently. If the adults could be prevented from entering the bottom of the tank where parturition is most likely to occur, the juveniles may have a greater chance of surviving. The practicality of this suggestion is addressed in Chapter 6.

The decrease in the number of attacks after the first 100 minutes coincided with a general movement of adults away from the bottom of the tank. Experiments presented in Chapter 5 show that juveniles moved towards the bottom of the water column more frequently than to any other area. Sibling cannibalism in juvenile *C. gariepinus* was related to the number of encounters between the cannibal and the victim (Hecht and Appelbaum, 1988) and the spatial separation of the cannibal and victim had a significant negative effect on

intercohort cannibalism of juvenile walleye pollock (*T. chalcogramma*) and whitefish (*C. lavaretus*) (Bailey, 1989; Eckmann, 1991). The movement of *X. helleri* adults away from the bottom of the water column where juveniles were most abundant, and the corresponding drop in the rate of attack, suggests that frequency of cannibalism may be a result of random encounters between adults and juveniles. This further supports the theory that filial cannibalism in *X. helleri* is no more than opportunistic predation.

Attack and cannibalistic behaviour

An attack by an adult on a juvenile may stimulate other adults to join in the attack. Of the 92 observed attacks, 41% were made by secondary attackers that joined in after an attack on the juvenile had already been initiated. This was also observed by Thibault (1974), who termed it "mobbing behaviour" and described how single adult of the *Poeciliopsis monacha*-*P. lucidia* species complex attacked juveniles that were immediately attacked by a number of additional adults. Male and female *X. helleri* exercised the role of primary attacker equally often, which strengthens the argument made previously that male and female *X. helleri* are equally responsible for cannibalism on juveniles. Although it was found that males joined in the attack as a secondary attacker less frequently than expected, no significant difference was found in the overall rate of attack and cannibalism between male and female fish. This suggests that *X. helleri* are unlike some other poeciliid species where sexual differences in cannibalism have been observed (Warren, 1973; Hubbs, 1991). As male and female *X. helleri* were equally responsible for cannibalising juveniles, and as non-birth-giving females in the tank contributed significantly to the cannibalism of juveniles, it can be supposed that the removal of fish that are not about to bear young will have a positive effect on juvenile survival. Juvenile survival may be increased further if "mobbing behaviour" was reduced by the inclusion of

an obstruction in the tank, such as a strategically positioned screen or refuge. The application and practicality of these suggestions are addressed in Chapter 6 of this thesis.

Adult *X. helleri* were most likely to attack juveniles soon after the onset of parturition as fewer adults joined in the attack with increasing time after parturition. Similarly, Thibault (1974) described a progressive reduction in the frequency that adults of the *P. monacha*–*P. lucida* species complex searched for juveniles during the hours after parturition. Thibault (1974) attributed this to a lack of shoaling behaviour by the juveniles, but the initial juvenile clusters that were observed may have been an artefact of the juveniles being introduced to the aquaria. A lack of shoaling behaviour cannot be used to explain the change of behaviour in the present study because adults both attacked and joined attacks as secondary attackers on juveniles that never formed shoals.

It is not likely that the progressive reduction in rate of attack can be explained by a reduction in juvenile density. Females produced young throughout the parturition period, which lasted for many hours, and the majority of these juveniles appeared to survive. Therefore, it is likely that the density of juveniles at the end of the parturition period was higher than that at the start. However, the precise density of juveniles was not monitored in this experiment, but it can be concluded that the reduction in the rate of attack was probably not due to a decrease in prey density.

The progressive reduction in rate of attack may be explained by operant conditioning. This is a form of learning, also referred to as trial-and-error learning, that takes place when an animal associates a specific behaviour as being either advantageous or disadvantageous and learns over time to either repeat or avoid that behaviour (Campbell, 1990). For example, Baras *et al.* (2000a) described a sharp decline in intracohort

cannibalism among dorada (*Brycon moorei*) after an increased frequency of injured fish were observed, and these injuries were attributed failed cannibalistic attempts. Similarly, more than 70% of all observed attacks on juvenile *X. helleri* were unsuccessful. Operant conditioning together with the shift in behaviour from cannibalism to feeding on material other than live juveniles may be used to explain the drop in the frequency of attack observed in this experiment. A reduced number of attacks over time might be a consequence of the adult learning, by trial-and-error, that attacking juveniles is futile due to the number of failed attacks.

However, adults were exposed to juveniles in the conditioning tanks prior to the observation study, thus they should have already experienced the advantage of foraging on alternative food items and the assumed disadvantage of unsuccessful attacks on conspecific juveniles. Therefore, if the reduced rate of attack was due to a "learning" experience, the initial high rate of attack should not have occurred. It has been shown that the prolonged absence of a stimulus can result in a reversion to the original response or lack of response to that stimulus (McFarland, 1981). For example, the appearance of a shadow released an escape response in adult guppies (*Poecilia reticulata*), but repeated exposure to a stimulus that had no actual consequence to the fish, resulted in a waning of the escape response (McFarland, 1981). When the appearance of the shadow was withheld for 24 hours before being reintroduced, it once again evoked an escape response (McFarland, 1981). Here, the adult swordtails had not been exposed to neonates or juveniles for a period of at least four days before the observations took place, which could account for the initial high frequency of attack behaviour. Operant conditioning and a shift in behaviour from attack to feeding cannot be excluded as a possible explanation for the decrease in attack frequency over time.

Alternatively, the reduced frequency of attack over time could be a result of a more rudimentary "learning" process called *habituation*. This is the waning of a response after repeated exposure to a stimulus that was inconsequential to the animal (McFarland, 1981). In this case, adults were repeatedly exposed to a stimulus (the juvenile or a primary attacker) that elicited an attack response from an adult. However, only 27.5% of the observed attacks resulted in cannibalism, which showed that adult *X. helleri* were apparently unsuccessful in about three out of every four attacks. The adults may have habituated to the stimulus that previously elicited an attack response because the stimulus proved to be inconsequential since the juveniles usually escaped when they were attacked.

Cannibalism occurred most frequently within the first 100 minutes after parturition; however, successful attacks on juveniles were occasionally observed after this time. Furthermore, if a juvenile was likely to fall victim to cannibalism, the event took place within a very short time (4.4 ± 0.18 s) of the initial attack. This, together with the finding that the frequency of attack declined with time after parturition, led to the hypothesis that the length of time that juveniles are exposed to the adult population has no bearing on the survival rate of juveniles, provided the juveniles are not cannibalised within the first 100 minutes after parturition. This hypothesis is tested in the exposure-time experiment described in Chapter 6.

A shift in behaviour from cannibalism to feeding on other material may also be used to explain the drop in the frequency of attack. The large number of attacks observed during the first 100 minutes after parturition corresponded with a low feeding rate on material other than live juveniles, over the same period. The change from dark to light conditions may be used to explain the increase in feeding activity on other food items. *X. helleri*

forage predominantly during the day and in the light. The chance of an adult fish encountering food items is increased in the light because they will encounter motionless food items as well as live ones. Under the dark conditions of this experiment motionless food items were consumed less frequently while moving juveniles were still detected by the adults. It has already been argued that cannibalism in some species including *X. helleri* is a form of opportunistic predation (Hrdy, 1979; Polis, 1981; Chapter 2 and this discussion), where the consumption of a conspecific is a by-product of foraging. Thus, the increase in feeding rate on other food items in the light and the relative decrease in adult encounters with juveniles in the dark could account for the decrease in the rate of attack. This further contributes to an argument supporting the theory of opportunistic predation.

Filial cannibalism – a strategy to recover energy

Although it is maintained that the most likely proximate explanation for filial cannibalism in *X. helleri* is opportunistic predation, a more innate reason for the behaviour of a parent cannibalising its young immediately after parturition could be the advantage of recovering energy. Birth-giving females will attack and sometimes cannibalise their offspring immediately (4.1 ± 0.1 s) after parturition (see Chapter 5). Furthermore, the average time between first attack and cannibalism was only 4.4 ± 0.2 s. Forty-five percent of all recorded attacks by the maternal parent ended in cannibalism and the rest ended with the juvenile escaping (Chapter 3). In the present study it was found that there were no differences in the rate of cannibalism between the birth-giving female and the other fish in the tank. With the birth-giving female being no more cannibalistic than the other adult fish, it stands to lose the investment already made if the juveniles fall victim to a predator other than herself. Physically stronger juveniles are more likely to escape an attack than weaker individuals. If the physically weaker juveniles are more likely to be preyed upon, either by conspecifics or other predators, the maternal parent may consume those juveniles that

are not likely to contribute to her fitness. Thus, she would recover the cytoplasmic investment in the egg and the energy invested in the development of the embryo (Smith and Wootton, 1995). Filial cannibalism is sensitive to the benefit as well as the cost of brood care (Lindstrom, 2000), and it is not uncommon for parents to eat offspring that are in some way deformed or weak and unlikely to survive (Hrdy, 1979; Polis, 1981). Moreover, cannibalism has been found to promote reproductive fitness in some fishes such as *Pseudocrenilabrus multicolor* (Cichlidae) (Smith and Wootton, 1995) and *G. affinis* (Poeciliidae) (Meffe and Crump, 1987), and improved reproduction would further increase the benefit derived from filial cannibalism (Smith and Wootton, 1995). Although, there is no evidence to definitively suggest increased reproductive success due to cannibalism in *X. helleri*, the recovery of spent energy is one possible adaptive reason for filial cannibalism in this species.

It must be borne in mind that the evidence upon which this conclusion is based was collected under artificial conditions on captive-bred fish that had been removed from their natural environment for an unknown number of generations. However, feral strains of poeciliid fishes also cannibalise their young under natural conditions (Meffe and Snelson, 1989). If it is assumed that the behaviour observed here does not differ from that of their feral relatives, it can be hypothesised that the recovery of energy is an evolutionary strategy favouring filial cannibalism in *X. helleri*. This hypothesis does not contradict the earlier conclusion that cannibalism is a form of opportunistic predation (Chapter 2) because it explains the behaviour from an evolutionary perspective, while opportunistic predation is a proximate cause.

Non-kin cannibalism may be a form of natural selection

Non-kin cannibalism of juvenile *X. helleri* by adult fish may be an example of natural selection since the fittest juveniles are less likely to be cannibalised. Cannibalism of less viable offspring by adults is not uncommon (Polis, 1981). However, a precise account of the physical strength or viability of juveniles that were cannibalised was not pursued during this study. The findings did show that juveniles fell victim to cannibalisation within a very short time after they were first attacked (4.4 ± 0.18 s) but they were less vulnerable to cannibalism with an increase in time after parturition. Therefore, if a juvenile was able to survive cannibalism immediately after parturition, there was a better chance that it would not be cannibalised. This would leave fitter survivors relative to siblings who were cannibalised in a matter of seconds after being attacked. It is hypothesised that cannibalism of juvenile *X. helleri* immediately after parturition is a process of natural selection with adaptive advantages from having physically weaker fish removed, and so improving the relative viability of the individuals that make up the population. The results presented in Chapter 6 give more insight into the physical strength and viability of juveniles that are cannibalised, in support of this hypothesis.

Conclusion

The proximate cause for cannibalism of *X. helleri* juveniles by adults immediately after parturition appears to be opportunistic predation. The apparent increase in availability of alternative food items under light conditions, and the reduced number of adult encounters with juveniles associated with the movement of adults away from the bottom of the tank, were both associated with the drop in number of attacks over the same period. This supports the conclusion that cannibalism of juveniles *X. helleri* is a by-product of predation.

The capacity of fish to "learn" is not well understood. However, there is evidence that poeciliids, such as *Girardinus falcatus* and *P. reticulata*, practice certain behaviours that are either repeated or avoided as a result of previous experience (McFarland, 1981; Goodey and Liley, 1986; Cantalupo *et al.*, 1995; Bisaza *et al.*, 1998). Learned behaviour is thought to be the result of interactions between an animal's genotype and the environment (Alcock, 1989). In other words, the capacity to perform a certain behaviour is probably genetically inherited, but the behaviour may be modified by experience and the modification may be remembered and either repeated or avoided in the future. In this study, the reduction in the number of attacks on juveniles during the time after the first post-parturition attack may have been a result of experience wherein the adults "learned" that there was little advantage in attacking a conspecific juvenile because most attacks after a certain point were not successful. The ability that *X. helleri* has to modify behaviour with experience will be discussed further in Chapter 5.

Males and females appear to be equally cannibalistic, which confirms earlier findings of this thesis, and the birth-giving female is no less cannibalistic than the other adults. Moreover, adults stimulate each other into attacking juveniles in what has been described as "mobbing behaviour" (Thibault, 1974), and these secondary attacks contribute significantly to the rate of cannibalism. However, by only giving birth in the dark and by giving birth under the cover of refuge when parturition occurs in areas of the tank where juveniles are more vulnerable to cannibalism, the birth-giving female appears to minimise the possibility of viable offspring being cannibalised. However, an advantage to intercohort cannibalism may be the removal of less viable juveniles from the population. Furthermore, females that cannibalise their offspring stand to recover energy that was invested in reproduction, and they stand to gain particularly if the juvenile that is cannibalised was unlikely to survive.

Based on the observation that adult *X. helleri* spend more time at the bottom of the water column during the parturition period and data that show most attacks and most cannibalism occurs at the bottom, it is hypothesised that juveniles are most likely to be cannibalised at the bottom of the water column and that juvenile survival will be significantly increased if adults are prevented from reaching the bottom area of the tank. Finally, since cannibalism in *X. helleri* was found to be density dependent and males and females appear equally cannibalistic it is hypothesised that the removal of males from the breeding tanks will increase juvenile survival at the time of parturition. The results of experiments designed to test each of the hypotheses suggested here are presented in Chapter 6.

CHAPTER 5

PARTURITION-ASSOCIATED BEHAVIOUR OF NEONATES AND JUVENILES

ABSTRACT

The behaviour of neonate and juvenile *Xiphophorus helleri* was recorded on videotape, both in the presence of the maternal parent only and in the presence of the maternal parent and other adult fish. The objective was to develop a general description of neonate and juvenile behaviour during and after parturition, upon which hypotheses could be formulated.

Neonates and juveniles settled on the bottom of the tank most frequently after parturition or attack, respectively. When they settled on the bottom they did so in the open area (62%) more often than in the artificial refuge (38%), while at the top they always settled in the refuge. Furthermore, neonates usually (49%) attempted to escape cannibalism in a downward direction. It was suggested that this apparent preference to move towards the bottom of the tank might be due to cerebral asymmetry where movement in one direction is favoured over movement in another. Alternatively, the downward escape response might be due to the development of anti-predator behaviour, as the adults appear to be better able to cannibalise offspring close to the water surface – capture efficiency was higher at the top (33.3%) than at the bottom (25.4%) of the water column.

The finding that juveniles moved to the bottom most often lead to the hypothesis that the rate of cannibalism will decline if refuge is placed at the bottom of the water column or if a false-bottom is included in the tank design. As 32% of the juveniles escaped in a sideways direction it is also hypothesised that rate of cannibalism will decline if false-sides are included in the tank design. It is also suggested that neonates are probably born with an innate ability to avoid predation, as cannibalism in some poeciliids is genetically inherent. Furthermore, neonates were able to avoid cannibalism with no prior experience of adult attack behaviour. However, a comparison of capture efficiency on neonates (67.0%) to that on older juveniles (27.5%) showed that juveniles were better able to avoid cannibalism than neonates, and led to the suggestion that juveniles may learn to better avoid cannibalism with increased experience of adult attack behaviour. It is also hypothesis that juvenile survival will not decrease with increased exposure time to cannibalistic adults.

INTRODUCTION

Intercohort cannibalism has been found to be genetically inherent (Thibault, 1974). Therefore, it is likely that selection in favour of anti-predator behaviour in offspring also exists. The genotype has been found responsible for anti-predator behaviour in many animals (Seghers, 1974; Giles 1984; Goodley and Liley, 1986; Magurran, 1990; Chivers and Smith, 1994; Miklósi *et al.*, 1997; Pongracz and Altbacker, 2000; Veen *et al.*, 2000). There is also evidence that this behaviour may be modified by experience and that its expression is subject to interactions with predators, conspecifics or the environment (Goodey and Liley, 1986; Chivers and Smith, 1994; Chivers and Smith, 1995; Healey and Reinhardt, 1995; Cantalupo *et al.*, 1995; Miklósi *et al.*, 1997; Bisazza *et al.*, 1998). The following background discussion focuses on the effect of social interactions on anti-predator behaviour as well as the inherent ability of animals to avoid predation at the time of parturition.

Population density reportedly affects the cannibalistic behaviour of adults in many poeciliid species, with an increased rate of cannibalism at higher population densities (Rose, 1959; Thibault, 1974; Meffe, 1984; Jones *et al.*, 1998a; Chapter 2). Furthermore, juveniles of the *Poeciliopsis monacha*–*P. lucida* species complex formed aggregations if their number exceeded ten individuals and these aggregations were thought to stimulate cannibalism in the adults (Thibault, 1974; Fox, 1975). However, it is plausible that such schooling behaviour was stimulated by the presence of predacious adults, rather than schools of juveniles stimulating cannibalism by adults as suggested by Thibault (1974). Thus, the presence of conspecific adults, other than the maternal parent, may possibly influence the behaviour of juvenile poeciliids. Furthermore, attacking behaviour and cannibalistic events may be influenced by the number of adults, as earlier investigations have indicated that juvenile *X. helleri* survival is affected by the number of adults in the tank (Jones *et al.*,

1998a). In this study, neonate and juvenile behaviour at the time of parturition are observed in the presence of the maternal parent only, and then compared to the behaviour of juveniles and neonates subjected to other adult conspecifics, with the objective of determining whether their anti-predator behaviour during the parturition period is density-dependent.

Unlike some higher vertebrates, the escape response in fishes is not a result of sophisticated evaluations (Bisazza *et al.*, 1998); in other words, there is no evidence of a thought process in their behaviour. A pair of giant reticulospinal neurons called the Mauthner (M-) cells mediates escape responses in fishes (Bisazza *et al.*, 1998). The axons of the M-cells synapse upon the motor neurons that are responsible for the C-shaped contraction of the fish's body which initiates the burst of speed that we refer to as an escape response. The neural commands for the "C-start reaction" are ballistic and need no sensory information from the stimulus once the movement has begun, but sensory information is required to coordinate the start of the reaction so that it results in the correct propulsion at the right time and in the most desired direction (Bisazza *et al.*, 1998). Cantalupo *et al.* (1995) investigated the ballistic reaction in the goldbelly topminnow (*Girardinus falcatus*) and found that it was initiated by a visual stimulus, which resulted in a predominantly rightward and then leftward escape response. Healey and Reinhardt (1995) suggested that the anti-predator behaviour of salmonid fishes is species specific, and this was substantiated by Laurila *et al.* (1998) who showed that brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) alevins escaped in a short swimming burst close to the bottom, while Arctic charr (*Salvelinus alpinus*) alevins escaped in an upward direction most often. The reasons for these differences were thought to be related to differences in predator regimes (Laurila *et al.*, 1998). Miraz *et al.* (2001) found a difference in the anti-predator behaviour of male and female *X. helleri*. Females usually

escaped in a downward direction when attempting to avoid predation, while males escaped more often towards the water surface. Miraz *et al.* (2001) attributed this to differences in morphology and reproductive behaviour between the sexes. Since there appear to be preferences in the direction in which adult *X. helleri* escape predation (Miraz *et al.*, 2001), particular attention will be given to the direction of juvenile escape behaviour. This may contribute to the development of technologies aimed at reducing cannibalism in *X. helleri* in aquaculture.

As mentioned, the genotype has been found to be responsible for anti-predator behaviour in a wide variety of animal species, ranging from higher vertebrates such the European rabbit (*Oryctolagus cuniculus*) (Pongracz and Altbacker, 2000) and the Seychelles warbler (*Acrocephalus sechellensis*) (Veen *et al.*, 2000) to lower vertebrates including fish (Seghers, 1974; Giles 1984; Goodley and Liley, 1986; Magurran, 1990; Chivers and Smith, 1994; Miklósi *et al.*, 1997). Seghers (1974) proposed that the anti-predator behaviour of the guppy (*Poecilia reticulata*) is determined by genetic inheritance, at least in part. The author found that geographically isolated populations of feral guppies in Trinidad performed different anti-predator behaviours that were subsequently found to persist over unselected generations under predator-free laboratory conditions (Seghers, 1974). Also, guppies that had been exposed to adult attack behaviour responded qualitatively in the same manner as guppies that had never experienced a predator (Goodley and Liley, 1986). Similarly, the escape response of the three-spined stickleback (*Gasterosteus aculeatus*) is thought to be under genetic control as it is expressed even when previous experience with a predator is lacking (Giles 1984) and the anti-predator behaviour of the European minnow (*Phoxinus phoxinus*) was also found to be inherited (Magurran, 1990). Although the anti-predatory behaviour of southeast Asian paradise fish (*Macropodus opercularis*) is under genetic control, the expression of the genotype is

thought to depend on a complex interplay between genetic and environmental factors that take place during the larval stage (Miklósi *et al.*, 1997). This suggests that both genetic inheritance and experience during early life may affect anti-predator behaviour in this species.

Other evidence indicates that anti-predator behaviour in a range of fishes may be learned by experience (Goodey and Liley, 1986; Chivers and Smith, 1994; Cantalupo *et al.*, 1995; Chivers and Smith, 1995; Healey and Reinhardt, 1995; Miklósi *et al.*, 1997; Bisazza *et al.*, 1998). For example, the goldbelly top minnow (*G. falcatus*) learned to modify anti-predator behaviour so as to actively avoid encounters with a dummy predator after repeated exposure to the stimulus; the same learning process occurred in adult and immature fish (Cantalupo *et al.*, 1995; Bisazza *et al.*, 1998). Furthermore, Goodey and Liley (1986) found that guppies (*P. reticulata*) with experience of adult attack behaviour during the first 48 hours of life were better able to avoid predation later than conspecifics that had not been attacked by adults. Similarly, Chivers and Smith (1994 and 1995) showed that predator avoidance in the fathead minnow (*Pimephales promelas*) was more a result of previous experience than genetic factors. This conclusion was based on a laboratory experiment where predator-naïve minnows failed to respond to the chemical stimulus of a natural predator, the northern pike (*Esox lucius*), while wild-caught minnows of the same age and size did elicit an escape response to the stimulus (Chivers and Smith, 1994). Modification of anti-predator behaviour in European minnow (*P. phoxinus*) was also found to take place when this fish experienced predators early in life (Magurran, 1990). Similarly, coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) are both able to modify their predator avoidance strategy after experience with predators (Healey and Reinhardt, 1995). However, the behavioural changes adopted by chinook salmon did not improve their ability to avoid predation,

whereas coho salmon learned to avoid predation more effectively after experience with a predator. This clearly demonstrates that predator avoidance strategies and the ability to learn are species-specific (Healey and Reinhardt, 1995).

There is little available information concerning the functional importance of experience and learning in the lower vertebrate taxa in general (Goodey and Liley, 1986). The inherent and/or learned ability of juvenile *X. helleri* to avoid cannibalism or predation in general has not yet been documented. An understanding of their inherent capacity to avoid predation at the time of birth and their ability or inability to modify escape behaviour with experience will facilitate the development of technologies and production procedures that mitigate intercohort cannibalism. Furthermore, such results will advance our general understanding of the functional importance of experience and learning in the lower vertebrates. Therefore, this study compares neonate behaviour at the time of birth to the behaviour of older juveniles with the aim of providing insight into the ability of *X. helleri* to modify behaviour by experience.

The specific objective of this study is to generate a general description of neonate and juvenile behaviour after parturition. Like the previous laboratory observation studies presented in this work (Chapters 3 and 4), the following study was designed to form a basis upon which further hypotheses could be built.

MATERIALS AND METHODS

Broodstock conditioning

Ault *X. helleri* (Gold Victory strain) were reared in a closed, recirculating freshwater system housed in a greenhouse tunnel (Chapter 2: Figure 2.1 and Figure 2.2). Prior to the laboratory observation studies the adults were held in five 300L tanks (Chapter 2:

Figure 2.1) at a sex ratio of 1:4 (male:female) and a stocking density of 10 fish/tank. They were subjected to a photoperiod of 14L:10D and were fed to satiation twice daily on a diet of Aqua Nutro tropical fish food with 40% crude protein and 6% crude lipid on a dry weight basis.

Neonate behaviour in the presence of the maternal female only

Nine gravid females were randomly chosen from the five conditioning tanks and each placed into one of nine 10L glass tanks that formed part of the system described earlier (see Chapter 3: Figure 3.1). Fish were fed to satiation twice daily, except during the parturition period when they received no food. Debris that consisted mostly of uneaten food and faecal matter was siphoned from the bottom of the tanks every second day at 08h00. Fish were acclimated to the system for a period of 7 days.

The behaviour of juveniles born from eight of the females was recorded on videotape using a video camera (Sony; VM-PS12) positioned in front of the tanks so that each tank was visible on a television screen (Chapter 3: Figure 3.1). The video camera was connected to a video recorder (Sanyo; VHR-250 SA) with a long-play function, making it possible to film the fish continually until the entire parturition period of eight females was recorded on film.

All neonate observations began when a neonate appeared from the female genital pore, and lasted until it was cannibalised or had settled in the tank. To ensure that equal numbers of observations were made per female, and since the periods of parturition ranged from 120 to 686 minutes, observations were made approximately every 15, 30, or 60 minutes for females that gave birth for less than 150 minutes, more than 150 but less than 360 minutes, or more than 360 minutes, respectively. If the females gave birth to

Table 5.1. Terms and definitions used to describe the behavioural events that were identified while observing juvenile and neonate *X. helleri* during the parturition period, as well as different positions within the observation tank.

Term	Definition
Age and description of fish	
Neonate	A fish born in the observation tank during an observation
Juvenile	A fish born in the observation tank before or during an observation (note that this term subsumes newborn juveniles)
Birth-giving female	Adult female that gave birth during observation
Non-birth-giving fml	Adult female that did not give birth during the observation period
Male	Adult male swordtail
Primary attacker	Adult fish that initiated an attack on a juvenile
Secondary attacker	Adult fish that joined in on an attack initiated by another adult
Behavioural events	
Parturition	Appearance of a juvenile from the female genital pore
Attack	A sudden burst of speed by an adult directly towards a juvenile, beginning with the start of the caudal peduncle thrash that propelled the adult towards the juvenile, or, when the adult was in striking distance of the juvenile, the attack began with the movement of the adult's head directly towards the juvenile
Cannibalism	Successful predation of a live juvenile
Escape	Avoidance of cannibalism after being attacked
Settling	The juvenile remains inactive
Feeding	Ingestion of anything other than a live juvenile by an adult fish
Phases of parturition period	
Pre-phase	Period before first parturition
Early-phase	First third of the period between the first and last parturition
Mid-phase	Second third of the period between the first and last parturition
Late-phase	Last third of the period between the first and last parturition
Post-phase	Period after the last parturition
Position in the tank	
Top	Top third of the water column
Middle	Middle third of the water column
Bottom	Bottom third of the water column
Artificial refuge	The area of the tank that contained shredded plastic
Open water	The area of the tank that did not contain shredded plastic

more than one neonate at any one moment the behaviour of all those neonates was recorded. The frequency of attack, cannibalism, escape (together with the direction of escape), and settling (together with the vertical position where the fish settled) were recorded according to their definitions (Table 5.1) and the occurrence of events under light or dark conditions was also noted. Time was recorded between parturition and the onset of neonate movement towards the bottom of the tank, between parturition and neonate settling, or between parturition and attack by an adult. Capture efficiency (*CE*) was calculated using Equation 5.1:

$$CE = \left(\frac{\text{No. juveniles cannibalised}}{\text{No. juveniles attacked}} \right) * 100 \quad \text{Equation 5.1}$$

Behaviour of neonates and juveniles in the presence of many adults

A group of fish consisting of one adult male and four gravid females was randomly selected from the conditioning tanks and placed into a 35.5L glass aquarium that formed part of the closed recirculating system (see Chapter 3, Figure 3.1). A bundle of 15g of shredded plastic was placed in the tank as shelter for the newborn juveniles. Half of the shelter floated in the water column and covered about 15% of the water surface area to a depth of approximately 80mm, and two quartzite stones weighed down the other half that covered about 15% of the area of the tank bottom. The fish were fed to satiation once daily in the morning until the onset of parturition, where after they were not fed until parturition was complete. Debris consisting mostly of faecal matter and uneaten food was siphoned out of the tank every second day.

The fish remained in the observation tank for a minimum of four days before one of the females produced young, at which time the behaviour of the juveniles was recorded using a video camera (Sony, VM-PS12) and recorder (Philips, VR456/93). After the parturition period of one female, the juvenile and adult fish were removed from the observation tank, and the process was repeated using another group of fish. Thus, parturition-associated behaviour of juvenile *X. helleri* from five different groups was recorded.

Juvenile observations began when an adult made the first attack on the juvenile. The behaviour of the juvenile was observed up to the point that cannibalism took place or until the juvenile escaped cannibalism and settled in the tank. The videotape was then forwarded to a point 30-minutes past the first attack, and then watched continuously until the next attack on a juvenile was observed. After this, the tape was forwarded again and the procedure repeated. A minimum of ten observations was made per group of fish. All behavioural terms are defined in Table 5.1. The frequency of attack and cannibalism in different vertical positions in the tank was recorded as well as the time that elapsed between attack and eventual cannibalism. When an individual juvenile was attacked more than once before being cannibalised, the time between final attack and cannibalism (i.e. the attack that resulted in the death of the juvenile) was also recorded. For those that were not cannibalised, the time between first attack and settling was recorded. The frequency of juveniles settling at the top, middle or bottom of the tank, either in the artificial refuge or in the open water was noted. Capture efficiency (*CE*) was calculated using Equation 5.1.

The observation of neonate behaviour in the presence of more than one adult conspecific began when a neonate appeared from the female genital pore and lasted until it was

cannibalised or had settled in the tank. These observations were made whenever parturition was observed. The frequency that neonates were attacked and cannibalised by the different adults in the tank, and the direction in which they attempted to escape were recorded, and the capture efficiency was calculated using Equation 5.1.

Water-quality analysis

Water temperature was measured with a mercury thermometer; ammonia (NH_4^+ -N) and nitrite (NO_2^- -N) concentrations were measured spectrophotometrically with the use of Nessler's reagent and the diazotization method (Merck 1974), respectively. pH readings were taken with a portable pH meter (Hanna Instruments, USA). These water-quality measurements were taken after the observation of each parturition period.

Statistical analysis

Frequency distributions were analysed either with a contingency table or a χ^2 analysis. If the expected frequency was less than 5, either the χ^2 analysis was adjusted ($\chi^2_{\text{adj.}}$) using Yates correction for continuity or the contingency table was replaced by "Fishers exact" test. The duration of behavioural events were analysed using a Student *t*-test, or else a one-way analysis of variance (ANOVA) and Tukey's multiple-range test was used to compare the means (Zar, 1984). Data that were not normally distributed (Shapiro-Wilk's test; $p > 0.05$) were log-transformed to normalise the data before being analysed (ANOVA); data that could not be normalised were analysed using the non-parametric Kruskal-Wallis test.

RESULTS

Water quality

Water temperature averaged 27.7°C (range: 26.0 to 29.0 °C; n=8), pH averaged 7.0 (range: 6.9 to 7.1; n=7), ammonia (NH₄⁺-N) averaged 0.02 mgL⁻¹ (range: 0.00 to 0.07 mgL⁻¹; n=7) and nitrite (NO₂⁻-N) averaged 0.003 mgL⁻¹ (range: 0.002 to 0.003 mgL⁻¹; n=7) during observations of neonates in the presence of the maternal female only.

Water temperature in the system averaged 27.3°C (range: 26.5 to 28.5°C; n=5), pH averaged 7.1 (range: 6.9 to 7.2; n=5), the ammonia (NH₄⁺-N) level ranged from 0.01 to 0.05 mgL⁻¹ (n=5) and nitrite (NO₂⁻-N) was not detected during the observation of neonates and juveniles in the presence of the maternal female and other adults.

Neonate behaviour in the presence of the maternal female only

Of 147 observed births, 54 neonates (36.7%) were attacked and 18 (12.2%) were cannibalised, thus 33% of all attacks ended in a maternal female cannibalising the young. Of the 155 observed occasions that neonates attempted to escape predation, 76 attempts occurred in a downward direction, 50 in a side-ways direction, and 29 in an upward direction ($\chi^2=21.46$; $v=2$; $n=155$; $p<0.001$) (Table 5.2). Of the 129 neonates that survived cannibalism, 128 settled on the bottom of the tank and only one settled at the top of the tank (Table 5.2).

The average time between parturition and the onset of a neonate's descent to the bottom of the tank was significantly shorter in the late-phase of parturition (2.1 ± 0.7 s; $n=40$) than in the early-phase (3.5 ± 0.8 s; $n=51$) (Mann-Whitney; $p<0.011$; Table 5.3). Similarly, the descent began later under dark conditions (4.0 ± 1.0 s; $n=49$) than in the light (1.3 ± 0.3 s;

Table 5.2. Direction in which neonates attempted to escape cannibalism and the position in the tank that neonates settled after escaping cannibalism in the presence of the birth-giving female only.

	Direction of escaped:			Settle position in tank:	
	Upward	Sideways	Downward	Top	Bottom
Escape	29	50	76	-	-
Settle	-	-	-	1	128

n=80) (Student's *t*-test; $p < 0.0007$; Table 5.3). The mean time that it took a neonate to settle on the bottom was the same for early, mid and late phases of parturition (ANOVA; $n=113$; $p > 0.10$; Table 5.3) under both light and dark conditions (Student's *t*-test; $n=113$; $p > 0.15$; Table 5.3).

The mean time between birth and attack was the same for all phases of parturition (ANOVA; $p > 0.13$; Table 5.3) and under light and dark conditions (Student's *t*-test; $p > 0.80$; Table 5.3), and averaged 4.1 ± 0.1 seconds.

Table 5.3. The average time in seconds ($\pm$ standard error) after parturition for neonates to (A) begin descending to the bottom of the water column, (B) settle on the bottom, or (C) be attacked by the maternal parent under light or dark conditions and during different phases of parturition. Different superscripts represent significant differences within a column (Student's *t*-test, ANOVA_‡, and Mann-Whitney_† at $p < 0.05$, $p < 0.05$ and $p < 0.017$, respectively).

Parturition/photophase	A	(n)	B	(n)	C	(n)
Early-parturition phase	$\pm 3.5 \pm 0.8^a$	51	$\pm 7.1 \pm 1.1^a$	41	$\pm 4.8 \pm 0.8^a$	17
Mid-parturition phase	$\pm 1.1 \pm 0.2^{ab}$	38	$\pm 4.5 \pm 0.5^a$	33	$\pm 2.7 \pm 0.4^a$	17
Late-parturition phase	$\pm 2.1 \pm 0.7^b$	40	$\pm 8.1 \pm 0.8^a$	39	$\pm 5.8 \pm 1.2^a$	8
Dark photophase	$\pm 4.0 \pm 1.0^x$	49	$\pm 7.3 \pm 1.4^x$	30	$\pm 4.4 \pm 0.8^x$	18
Light photophase	$\pm 1.3 \pm 0.3^y$	80	$\pm 6.4 \pm 0.5^x$	83	$\pm 4.0 \pm 0.6^x$	24

Behaviour of neonates and juveniles in the presence of many adults

Six of 28 observed neonates were attacked as they were born, two of which were attacked twice resulting in a total of eight attacks. Four of these neonates were cannibalised. All adult fish, including the birth-giving female, the other three females and the male, attacked ($\chi^2_{\text{adj.}}=0.03$; $\nu=2$; $n=8$; $p=0.86$) and cannibalised ($\chi^2_{\text{adj.}}=0.23$; $\nu=2$; $n=4$; $p=0.63$) neonates equally often. Of the six neonates that were attacked, four attempted to escape in a downward direction, two attempted to escape in an upward direction, and none attempted to escape in a side-ways direction. The remaining 24 neonates, which were either not attacked or were attacked but escaped cannibalism, settled on the bottom of the tank.

Table 5.4. The number of attacks (A) and the subsequent number of juveniles that were cannibalised by the adult broodstock (B), at different positions in the water column. The mean time between the first attack and cannibalism of the juvenile (C) and final attack (the attack that resulted in death of the juvenile) and cannibalism (D) was recorded. Different superscripts represent significant differences within each column (ANOVA; $p<0.05$). Different subscripts represent an unequal distribution within each column (χ^2 ; $p<0.05$).

Position in tank	A	B	C	D
Top	12 _a	4 _m	2.5±0.8 ^x	1.7±0.3 ^y
Middle	27 _a	8 _m	4.0±0.5 ^x	1.6±0.2 ^y
Bottom	63 _b	16 _n	3.3±0.3 ^x	1.3±0.1 ^y

Most of the attacks (61.8%) on juveniles that ranged in age from a few seconds to a number of hours took place at the bottom of the tank ($\chi^2=40.01$; $\nu=2$; $n=102$; $p<0.001$; Table 5.4). Most cannibalism also took place at the bottom of the tank (57.1%) ($\chi^2=8.00$; $\nu=2$; $n=28$; $p<0.025$; Table 5.4). The average time (4.4 ± 0.2 s) between first attack and

cannibalism did not differ with respect to position in the water column (ANOVA; $n=28$; $p>0.92$; Table 5.4). Likewise, the average time (1.4 ± 0.1 s) between final attack and cannibalism was the same at the top, middle and bottom of the water column (ANOVA; $n=28$; $p>0.71$; Table 5.4). Prey capture efficiency on juveniles (not just neonates) ranged from 25.4% success for adults that cannibalised at the bottom to 33.3% success for those at the top of the tank, with an overall average of 27.5% ($n=102$).

Juveniles settled more frequently on the bottom under both light and dark conditions (Fishers exact; $n=26$; $p>0.29$). There was no significant preference to settle in either the refuge or the open water when juveniles settled at the top or at the bottom of the water column (Fishers exact; $n=26$; $p>0.15$). Two juveniles were attacked after they had already settled, but escaped and settled for a second time. The average time taken for a juvenile to settle after being attacked was 10.3 ± 3.7 seconds, and did not differ among fish that settled at the top, middle or bottom sections of the tank (Kruskal-Wallis; $n=26$; $p>0.53$).

DISCUSSION

Cannibalism on neonates and juveniles

A comparison of neonate behaviour in the presence of the maternal female to that in the presence of more than one adult conspecific revealed that the rate of cannibalism on neonates appears to be independent of the number of adults in the tank. Four of the 28 neonates (14.3%) that were born under observation in the presence of additional adults were cannibalised, a portion similar to the 18 neonates that were cannibalised from 147 births (12.2%) when the only adult in the tank was the birth-giving female. At first glance, this observation would seem to contradict other findings that show cannibalism to be function of adult number (Thibault, 1974; Jones *et al.* 1998a; Chapter 2). However, there

may be no contradiction because previous conclusions were based on the behaviour of juvenile fish, whereas the results here refer only to neonate behaviour. A neonate is an individual that has been observed from the moment of birth, whereas a juvenile includes offspring of unknown age that were born before the start of an observation (Table 5.1). The most likely explanation for neonate survival being independent of the number of adults in the tank, is the short exposure period of the neonates to the adults relative to that of juveniles that have occupied the tank for longer periods. Whereas juvenile survival may be a function of adult number (Thibault, 1974; Jones *et al.*, 1998a; Chapter 2), neonate survival appears to be independent of adult number because they have yet to be exposed to the adult fish for a long enough period for the adult fish to affect their survival.

Capture efficiency on neonates and juveniles

Capture efficiency may be positively related to the number of adults in the tank. In the experiment that included the birth-giving female, three other females and a male, only eight attacks on neonates were observed, four of which ended in cannibalism. The small size of the data set makes it impossible to draw a decisive conclusion, but an initial comparison of this observation (i.e. prey capture efficiency of 50%) to the prey capture efficiency of 33.3% when the only adult in the tank at the time of parturition was the maternal parent, suggests that capture efficiency may increase with an increase in the number of adults. The reason for this might be explained by the added opportunity for secondary attacks. For almost all primary attacks made during the first 200 minutes of the observations (Chapter 4), at least one secondary attacker joined the attack (Figure 4.2). Thibault (1974) observed similar “mobbing” behaviour in the *Poeciliopsis monacha*–*P. lucida* species complex. With more than one attacker in the tank, a neonate is more likely to be cannibalised once an attack has been initiated. However, the small size of the data set requires that the observation made here should be verified in future studies.

The young were more vulnerable to cannibalism at parturition than they were in the minutes and hours following parturition. Capture efficiency on juvenile *X. helleri* was considerably lower than on neonates (27.5% and 67.0%, respectively), which suggests that juveniles are better able to avoid cannibalism than younger neonates. A decrease in the number of attacks with an increase in time after parturition (Chapter 4: Figure 4.1B) further supports this conclusion. Brooking *et al.* (1998) found that the ability of larval walleye (*Stizostedion vitreum*) to avoid predation increased with age. However, this was attributed to an increase in body size resulting in better ability to avoid predation (Brooking *et al.*, 1998). The change in behaviour of *X. helleri* with age is not likely to be a result of neuro-muscular maturation, as the difference in age between juveniles and neonates by definition (Table 5.1) does not allow time for substantial growth. Furthermore, in contrast to walleye, *X. helleri* are more K-selected since their life history includes no free-swimming larval stage and the young are born as competent juveniles (Constantz, 1989). The change in behaviour observed here might likely be a result of learning due to increased experience of adult attack behaviour rather than a process of neuro-muscular maturation, but remains to be tested.

Inherent and learned ability to avoid cannibalism

There is evidence of an inherent capacity to avoid predation in fishes such as three-spine stickleback (*G. aculeatus*) (Giles, 1984), European minnow (*P. phoxinus*) (Magurran, 1990), paradise fish (*M. opercularis*) (Miklósi *et al.*, 1997) and guppy (*P. reticulata*) (Seghers, 1974; Goodley and Liley, 1986). The ability of *X. helleri* neonates to respond when attacked by an adult for the first time is probably due to an inherent ability to avoid cannibalism or predation in general. There was only a short time between the time of parturition and the moment of first attack that ended with the neonate's escape (2.7 ± 0.4 to

5.8±1.2 seconds), hence it is suggested that neonates are predator-naïve when attacked for the first time. By comparison, juveniles are probably no longer predator-naïve as exemplified by the frequency that adults attacked juveniles (average 1.0±0.14 attack/second) (Chapter 4: Figure 4.2). In other trials as many as 42% of *X. helleri* juveniles were cannibalised within 24 hours after parturition (Jones *et al.*, 1998a), and a population of six to 14 adult guppies (*P. reticulata*) attacked their juveniles between 250 and 300 times a day (Goodey and Liley, 1986), which further confirm that juveniles are probably not predator-naïve.

As some neonates successfully escaped attacks by adult fish even though they had no previous experience of adult attack behaviour, it is hypothesised that the escape behaviour of *X. helleri* may be genetically inherent. This is further supported by the finding that intercohort cannibalism is genetically inherited (Thibault, 1974). For cannibalism to exist as an evolutionary stable strategy, where selection in favour of the behaviour does not jeopardise the fitness of the cannibal, one might expect the development of inherent and equally successful predator-avoidance behaviour in juveniles.

However, there is evidence that anti-predator behaviour is also learned in species such as fathead minnow (*P. promelas*) (Chivers and Smith, 1994; Chivers and Smith, 1995), European minnow (*P. phoxinus*) (Magurran, 1990) and at least two poeciliids, the guppy (*P. reticulata*) (Goodey and Liley, 1986) and goldbelly topminnow (*Girardinus falcatus*) (Cantalupo *et al.*, 1995; Bisazza *et al.*, 1998). The data show that juvenile *X. helleri* were better able to avoid cannibalism than neonates. This is explained by the hypothesis that neonates are predator-naïve, having had no previous experience of adult attack behaviour whereas juveniles are more likely to have been exposed to adult attack behaviour in the

past. This conclusion is consistent with data that show predator recognition and avoidance can improve due to previous experience (Goodey and Liley, 1986; Magurran, 1990; Chivers and Smith, 1994; Cantalupo *et al.*, 1995; Healey and Reinhardt, 1995; Bisazza *et al.*, 1998). Moreover, guppies that experienced adult attack behaviour during the first 48 hours of life were better able to avoid predation later than conspecifics that were never attacked when young (Goodey and Liley, 1986). Furthermore, adult *X. helleri* appear to habituate to stimuli that previously culminated in an attack (Chapter 4: Figure 4.2) and this habituation coincided with an increase in juvenile age, which further supports the suggestion that predator avoidance develops with experience in juvenile *X. helleri*.

Direction of neonate and juvenile escape and their position in the water column

Both neonates and older juveniles sought the bottom of the water column most often immediately after parturition and when attacked by adult fish. Neonate *X. helleri* attempted to escape in a downward direction most often when attacked by the maternal parent only and by groups of adults. Similarly, brown trout (*S. trutta*) and Atlantic salmon (*S. salar*) alevins responded to attack stimuli most often with short swimming bursts close to the bottom, whereas Arctic charr (*S. alpinus*) alevins responded most often with swimming bursts in an upward direction (Laurila *et al.*, 1998). There is evidence of cerebral asymmetry in fishes that can be used to explain why some behavioural patterns tend to occur in one direction more so than in another (Bisazza *et al.*, 1998). For example, evidence exists of lateralization of pectoral stridulation sounds in channel catfish (*Ictalurus punctatus*) as 50% of these fish showed preference for one fin over the other (Bisazza *et al.*, 1998). Furthermore, adult and immature goldbelly topminnow (*G. falcatius*) were found to turn initially in a rightward direction most often when escaping from a stimulus evoked by a simulated predator (Cantalupo *et al.*, 1995; Bisazza *et al.*, 1998). It may be plausible to suggest that a similar process is responsible for the downward

escape response of young *X. helleri*. However, a better understanding of this species nervous system is required to confirm the extent of cerebral asymmetry. Since young *X. helleri* moved towards the bottom of the water column most often after being attacked and since most unsuccessful attacks are followed by additional attacks (Chapter 4: Figure 4.2), it is hypothesised that refuge placed at the bottom of the water column could improve the survival rate of captive-bred *X. helleri*. This is tested in the work of Chapter 6.

The observation that juveniles and neonates settled on the bottom of the tank more frequently than in other sections of the water column may be partially explained by the feeding behaviour of this species. Under captive conditions the only predators that threaten young *X. helleri* are adult conspecifics; given this species' superior-placed mouth juveniles may be more vulnerable when positioned above the adults. Moreover, capture efficiency on juveniles was higher at the top of the tank than at the bottom, which supports the claim that adults are better equipped to capture prey positioned above them. However, since this finding was based on a very small data set, it should be accepted as a preliminary finding only, which requires further investigation in future studies. Nonetheless, their apparent increased vulnerability when positioned above the adults might explain the young's affinity for the bottom of the tank. Furthermore, when juveniles settled at the top, they all settled in the artificial refuge. Similarly, juvenile walleye pollock (*Theragra chalcogramma*) readily entered artificial seagrass refuge when they were vulnerable to predation but in the absence of predators they displayed a preference for the open water (Sogard and Olla, 1993). Since the presence of artificial refuge significantly increased juvenile *X. helleri* survival (Jones *et al.*, 1998a; Chapter 2), the present conclusion reinforces that of Chapter 4 that stated female behaviour probably enhanced offspring survival by giving birth in the artificial refuge when parturition took place at the top of the water column. Together, these results suggest that there may be benefit in

behaviour wherein individuals either attempt to escape in a downward direction or attempt to settle in the artificial refuge.

The time between parturition and the neonates descent to the bottom of the tank may be related to the behaviour of the adult fish at the time of parturition. Thibault (1974) found that juveniles of the *P. monacha*–*P. lucida* complex formed clusters near the surface of the tank in the presence of adult conspecifics, which suggests that the presence of adults may affect the behaviour of the juveniles. Similarly, the behaviour of juveniles in this study may have been affected by the behaviour of adult *X. helleri*. The significant time delay between parturition and neonate descent towards the bottom of the tank during the early-phase of the parturition period compared to the late-phase (Table 5.3) coincided with increased attacking behaviour (Chapter 4: Figure 4.2). As frequency of adult attack behaviour decreased with time after first parturition, the delay between the parturition of a neonate and that neonate's descent to the bottom of the tank also decreased. Furthermore, since juveniles are attacked less frequently in the dark than in the light (Chapter 3: Figure 3.3), and since the delay of neonate descent to the bottom of the tank was significantly longer under conditions of darkness (4.0 ± 1.0 s) as compared to light (1.3 ± 0.3 s), the combined evidence suggests that there may be a relationship between the time taken to start the descent to the bottom of the tank after parturition and the cannibalistic behaviour of the adult fish at the time of parturition. It has already been hypothesised that neonates are probably born with some inherent ability to avoid cannibalism immediately *post-partum*, but the causal factors and adaptive significance behind changes in neonate behaviour such as the delayed descent to the bottom under dark conditions remain unclear. The delay could be due to the absence of waterborne alarm substance, as there is evidence that such waterborne substances affect poeciliid behaviour (Warren, 1973; Thibault, 1974; Goodey and Liley, 1986; Miraz *et al.*, 2001).

For example, waterborne alarm substances have been found to stimulate anti-predator behaviour in adult *X. helleri* and *P. reticulata* (Goodey and Liley, 1986; Miraz *et al.*, 2001), and in non-poeciliid species such as fathead minnow (*P. promelas*) (Chivers and Smith, 1994) and goldfish (*Carassius auratus*) (Chivers and Smith, 1994). Alternatively, the change in neonate behaviour could be a response to reduced adult attack behaviour under dark conditions or a response to light intensity. Investigations into the reason for changes in neonate behaviour at the time of birth are suggested and should include the potential effect of waterborne substances, adult behaviour and light intensity.

Conclusion

The apparent preference that neonates and juveniles have to move towards the bottom of the water column more often than any other area after parturition and attack, and their preference for refuge when they are in the upper part of the water column can be explained in terms of anti-predator strategies. Therefore, it is hypothesised that juvenile survival will improve as a result of decreased cannibalism if the predators (adults) are prevented from following the juveniles to the bottom of the tank; this could involve a screen that selectively excludes the passage of adults or the inclusion of cover in the form of refuge at the bottom of the water column.

Since intercohort cannibalism may be genetically inherited (Thibault, 1974), juvenile *X. helleri* are probably born with an innate ability to avoid cannibalism. This hypothesis was supported by the finding that neonates, with no previous experience of adult attack behaviour, were able to avoid cannibalism. However, capture efficiency was higher on neonates than on juveniles, which suggests that neonates are more vulnerable to cannibalism at the time of parturition than they are in the hours and minutes after birth. Their improved ability to avoid cannibalism with age is unlikely to be a result of neuro-

muscular maturation; yet the present observations together with those of other researchers regarding other poeciliids (Goodey and Liley, 1986; Cantalupo *et al.*, 1995; Bisazza *et al.*, 1998) suggest that their improved ability to avoid predation is probably a process of learning through previous experience of adult attack behaviour. Therefore, concerning future work (see Chapter 6), it is hypothesised that if anti-predator behaviour of neonate *X. helleri* improves with age and juveniles are better able to avoid cannibalism than neonates, then the length of time that juveniles are exposed to adult fish will have no significant effect on their survival.

CHAPTER 6

TOWARDS THE PRACTICAL APPLICATION OF BEHAVIOURAL OBSERVATIONS

ABSTRACT

It is uncertain whether parturition-associated behaviour in *Xiphophorus helleri* under controlled laboratory conditions has any relevance in other environments, such as farming conditions. The overall objective of this study was to determine the applicability of the laboratory observations to practical farming conditions, by testing the hypotheses that the rate of cannibalism would be reduced under farming conditions if:

1. refuge was placed at the bottom of the tank;
2. a false-bottom was included in the tank design;
3. a false-side was included in tank design;
4. if part of the tank was kept under constantly dark conditions;
5. males were removed from the tank after fertilising the females.

Finally, it was also hypothesised that the rate of cannibalism was:

6. independent of time that juveniles spent in the broodstock tank before harvest.

The hypothesis that reduced light-intensity would reduce the rate of cannibalism was not accepted, while all the other hypotheses were accepted based on the results of this study. However, it became apparent that the method used to calculate the rate of cannibalism in this work included dead and deformed juveniles (dead and deformed juveniles were not observed in the laboratory studies). When the results were analysed to take dead and deformed juveniles into account (i.e. total productivity was compared with live harvest), it was concluded that a false-side and bottom-refuge, not only reduce the rate of cannibalism, but also promoted juvenile survival under farming conditions; a false-bottom did not increase juvenile survival. It was also concluded that laboratory observations of parturition-associated behaviour in *X. helleri* can contribute towards the development of hypotheses regarding behaviour under other environments, such as practical farming conditions. However, the results also highlight the need to test these hypotheses under different sets of environmental conditions to ensure their applicability under different conditions.

INTRODUCTION

Traits such as growth rate, age and size at sexual maturity, interbrood period, testis size and fecundity are reported to be plastic in poeciliid fishes and may change in accordance with changing environmental conditions (Trexler, 1989). It is therefore possible that poeciliid behaviour will also change under different conditions. The preceding behavioural observations were carried out in small glass tanks under controlled laboratory conditions. However, it remains uncertain whether or not the observed behaviour would remain the same under different conditions and whether or not the laboratory observations were applicable to other environments, such as practical farming conditions. Therefore, in this section of the work, *Xiphophorus helleri* behaviour was observed under a set of environmental conditions similar to farming conditions and comparisons are drawn to the observations made previously in the laboratory.

For example, it was shown in the laboratory that most juveniles attempted to escape towards the bottom of the tank when attacked, and neonates that were not cannibalised immediately after birth also settled on the bottom more often than at the top or in the middle of the water column (Chapter 5). These findings suggest that juvenile *X. helleri* prefer the bottom of the water column. However, in the laboratory juveniles were vulnerable to predation at the bottom of the water column, because the birth-giving female and other adult fish were usually observed near the bottom during parturition and in the minutes and hours after parturition (Jones *et al.*, 1998b; Chapters 3 and 4). In addition, the greater proportion of cannibalism of juveniles took place at the bottom of the water column (Jones *et al.*, 1998b; Chapters 3 and 4). Therefore, it is hypothesised, that a reduction in the rate of cannibalism could be accomplished under practical farming conditions if juveniles were protected by a false-bottom when they escape in a downward direction. Similarly, it is hypothesised that false-sides will reduce the rate of cannibalism

because they will provide added protection to juveniles that escape in a sideways direction. Furthermore, it has been shown that the availability of refuge significantly reduced the rate of cannibalism (Hecht and Appelbaum, 1988; Pienaar 1990; Hecht and Pienaar, 1993) and increased juvenile survival in a variety of species (Persson and Eklov, 1995; Nemeth, 1998; Willette *et al.*, 2001), including *X. helleri* (Jones *et al.*, 1998a; Chapter 2). Therefore, it is also hypothesised that under farming conditions, refuge may be more effective in reducing cannibalism when positioned at the bottom of the water column rather than near the water surface. These hypotheses are tested in Experiment 6.1 (bottom-refuge), Experiment 6.2 (false-bottom) and Experiment 6.3 (false-side).

Previous observations revealed that juvenile and mature guppies (*Poecilia reticulata*) and swordtails (*X. helleri*) were less likely to swim through an automatic size grader if the colour of the grader did not contrast that of the container into which the fish were being size sorted (unpublished personal observation). Thus, juvenile *X. helleri* are possibly less likely to move through a false-bottom if the false-bottom does not adequately contrast the colour of the tank. Therefore, before designing the false-bottom and false-side experiments, it was necessary to determine the most suitable false-bottom and tank colour contrast, that best promoted the movement of fish through the false-bottom or side. This was tested during a preliminary trial that preceded the other experiments of this study.

Males are reportedly less cannibalistic than females in some poeciliids (Warren, 1973; Hubbs, 1991; Hubbs 1995). For example, females of the genus *Gambusia* and female *P. reticulata* are more cannibalistic than males (Warren, 1973; Hubbs, 1991; Hubbs 1995). However, this was not observed in *X. helleri*, and it was hypothesised by Jones *et al.* (1998b) and in Chapters 2 and 4 of this work, that male and female *X. helleri* are equally

cannibalistic. Since predation has been found to be positively related to the number of adult fish in a tank (Thibault, 1974; Jones *et al.*, 1998a; Chapter 2), it is further hypothesised that if male *X. helleri* are removed after fertilisation, the rate of juvenile survival will increase as a result of a decrease in the number of cannibals in the tank. This is tested in Experiment 6.4.

In Chapter 5 it was concluded that juvenile *X. helleri* are better able to avoid cannibalism than younger neonates, probably due to a process of learning that takes place as a result of previous experience of adult attack behaviour. Improved ability to avoid predation after previous experience with a predator has also been reported for other poeciliids (Goodey and Liley, 1986; Cantalupo *et al.*, 1995; Bisazza *et al.*, 1998). In addition, it was suggested in Chapter 4 that adult *X. helleri* habituate to the stimulus that previously resulted in the attack of a juvenile, and this habituation occurred within about 3 to 5 hours after the first attack on a juvenile. Furthermore, Thibault (1947) found that *Poeciliopsis* juveniles were only prone to cannibalism during the first 24-hours after parturition. These findings suggest that juveniles are likely to survive in the presence of the adult fish once they have survived the first attack and the first 3 to 5 hours of life. Experiment 6.5 tests the hypothesis that the length of time that juveniles are exposed to adult fish will not affect the rate of intercohort cannibalism and juvenile survival.

Light intensity can affect intracohort cannibalism and aggression in fishes such as African catfish (*Clarias gariepinus*) (Pienaar, 1990; Britz and Pienaar, 1992; Hecht and Pienaar, 1993), vundu catfish (*Heterobranchus longifilis*) (Baras *et al.*, 1998) and Japanese flounder (*Paralichthys olivaceus*) (Dou *et al.*, 2000). Light intensity affected the rate of intercohort cannibalism in *X. helleri* as juveniles were attacked and cannibalised by the maternal parent significantly less often under low light-intensity (0.54×10^{15} quanta sec^{-1}

cm⁻²) than under high light-intensity (3.15×10^{15} to 6.6×10^{15} quanta sec⁻¹ cm⁻²) (Jones *et al.*, 1998b; Chapter 3). Hence, it is hypothesised that the rate of cannibalism will be reduced if part of the broodstock tank remains in the dark. This hypothesis is tested in Experiment 6.6.

Overall, Experiments 6.1 to 6.6 were designed to test production procedures and technologies aimed at reducing the rate of intercohort cannibalism in *X. helleri*. At the same time, they were also designed to test whether or not parturition-associated behaviour of *X. helleri* observed under the controlled laboratory conditions of previous experiments was similar to that under practical farming conditions.

MATERIALS AND METHODS

The preliminary study, designed to test the effect of contrasting false-bottom and tank colour on the movement of juveniles through a false bottom, was carried out under laboratory conditions, and the results were incorporated into the experimental design of the experiments that followed. Experiments 6.1 to 6.6 were carried out under practical farming conditions in a greenhouse tunnel system (Chapter 2: Figure 2.1), and were divided into two experimental series. This was done to account for the similar conditions of the experiments that were included in each of the two series (Table 6.1).

Table 6.1. Experiments and hypotheses tested in this chapter.

Experiment	Hypothesis/Objective
Preliminary Experiment	Determine optimum false-bottom and tank colour contrast that best facilitates movement through false-barriers
<i>Experimental Series 1</i>	
Exp. 6.1 - Bottom-refuge	Refuge at bottom will reduce rate of cannibalism
Exp. 6.2 - False-bottom	False-bottom will reduce rate of cannibalism
Exp. 6.3 - False-side	False-side will reduce rate of cannibalism
Exp. 6.4 - Remove-males	Rate of cannibalism will decrease when males are absent
<i>Experimental Series 2</i>	
Exp. 6.5 - Exposure to adults	Rate of cannibalism is independent of time that juveniles are exposed to adults
Exp. 6.6 - Light intensity	Rate of cannibalism will decrease if part of the tank is kept in the dark

Tank and false-bottom colour contrast experiment

A horizontal screen with 5mm slits was placed 100mm from the bottom of the observation tank (290 x 290mm, with a water depth of 200mm). This formed a false-bottom that allowed the passage of juveniles but prevented adults from following. Each quarter of the false-bottom was made of a different colour, i.e: red, black, light-blue, and white. This multi-coloured false bottom was positioned in either a red, black, light-blue or white tank. A cage was attached underneath each quarter to collect and count the number of juveniles that migrated through the different coloured false-bottoms. Two adult females and an adult male were acclimatised in the tank above the false-bottom for 60 minutes. Juveniles that were less than 12 hours old were placed into the centre of the tank with a hand net. The cages below each false-bottom quadrant were removed after 30 minutes

and the number of juveniles in each cage was counted. Juveniles that did not pass through the false-bottom were also counted. The procedure was repeated in triplicate for each tank colour. Different fish were used for each replicate of each tank colour.

Experimental Series 1

Conditions common to experiments in Series 1

Adult *X. helleri* (Gold Victory strain) were acclimated for four weeks in the tanks used for the experiments. All tanks formed part of the recirculating system described in Chapter 2 (Figure 2.1). The experiments were carried out in late summer to early winter. The fish were subjected to natural light during the day, however, the light-phase was extended with the use of fluorescent tubes attached to a timer switch to maintain a photoperiod of 14L:10D throughout the trial. The fish were fed a commercially available crumble diet (Aqua Nutro tropical fish food, Stellenbosch) containing 40% crude protein and a 6% lipid content on a dry-weight basis. Fish were fed once daily in the morning in excess of satiation. In addition, they had access to filamentous algae that grew continuously on the tank walls, even though the tanks were cleaned once a week. Thus, the algae and associated epiphytes were always available to the adult fish.

Experimental Series 1 consists of four experiments to test the hypotheses that bottom-refuge (Experiment 6.1), a false-bottom (Experiment 6.2), a false-side (Experiment 6.3), or the presence of males (Experiment 6.4) would reduce the rate of intercohort cannibalism. The four experiments were carried out simultaneously in the same recirculating system, and all experiments shared a common control treatment. The control had no bottom-refuge, no false-bottom, no false-side, and two males and eight females per tank.

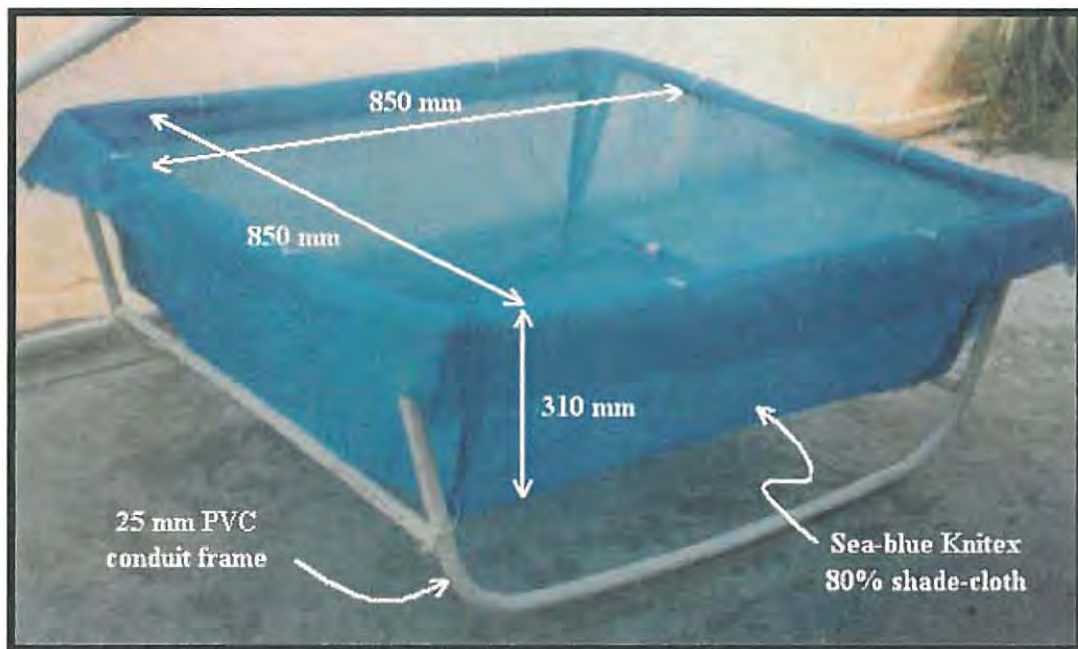


Figure 6.1. A control group cage constructed out of sea-blue Alnet shade-cloth with a mesh size of less than 1mm and a frame constructed out of 25mm PVC conduit.

Cages were constructed using 25mm PVC conduit and sea-blue Alnet shade-cloth with a mesh size of less than 1mm as illustrated in Figure 6.1. The dimensions of the cage were 850 X 850mm with a depth of 310mm. When placed into one of the fibreglass tanks illustrated in Figure 2.1 of Chapter 2, there was a 75mm space between the floor of the cage and the bottom of the fibreglass tank and a 75mm space between the sides of the cage and the walls of the tank. Water depth in the cage was 280mm resulting in an effective volume of 200L. The bottom and sides of the cages used in the false-bottom and false-side treatments, respectively, were replaced with black Alnet shade-cloth with a triangular mesh (base 4.1mm and height 6.3mm).

Shredded plastic (110g) was placed into each of the cages to provide refuge. The refuge was positively buoyant and floated at the water surface to 65% of the depth of the cage

and covered approximately 40% of the water surface area. The tanks were supplied with constantly flowing water at 2.5L/min. A 25mm outflow pipe, positioned in one corner of each tank, drained water back into the filter system. All outflow pipes were covered with 0.5mm stainless steel mesh to ensure that juveniles were not lost through the outflow. All tanks were covered by black Alnet shade-cloth with a triangular mesh size of 4.1mm and 6.3mm (base and height, respectively) to prevent adults from jumping out of the tank.

Ten adult fish were randomly stocked into each of the eighteen 200L cages at a male:female ratio of 2:8. Female total length averaged 71.6 ± 0.6 mm (mean $\pm$ standard error) and there was no difference in female length between any of the treatments (ANOVA; $p > 0.21$). The adult population density of 0.04 females per litre was maintained throughout the experiments by replacing fish in the event of mortality. Tanks were checked for juveniles every morning before feeding. When juveniles were present, the adults were removed from the tank with a hand net and placed into a 5L bucket of water taken from the same recirculating system. Juveniles were then harvested from either the top or the bottom of the tank: the top constituted the upper 75% of the volume of the cage (150L) (this incorporated the floating refuge), while the bottom of the tank constituted the lower 25% of the cage (50L) as well as the water in the space between the cage and the tank (100L). After the juveniles had been removed, the adults were returned to the cage.

Conditions unique to Experiment 6.1: Bottom-refuge

To test the hypothesis that artificial refuge positioned at the bottom of the tank will reduce the rate of cannibalism, a treatment with artificial refuge placed at the top of the water column was compared to a treatment where refuge was placed at the top and bottom of the water column.



Figure 6.2. (A) control cage in a 300L fibreglass tank, and (B) false-side cage and (C) false-bottom cage with the sides and floor, respectively, made out of 10% black Alnet shade cloth.

A 110g bunch of shredded plastic was divided in half for the treatment that included refuge at the top and bottom of the water column. One half was allowed to float and covered approximately 10 to 15% of the water surface of the cage, while the other half was held down with five ceramic weights to keep the refuge on the floor of the cage. The refuge at the bottom covered approximately 25% of the bottom surface of the cage. The control treatment did not have refuge at the bottom and the 110g bunch of shredded plastic floated at the water surface to approximately 65% of the depth of the cage and covered about 40% of the water surface area. The control was represented in triplicate and the test treatment was replicated four times. The experiment was carried out over a period of 84 days.

Conditions unique to Experiment 6.2: False-bottom

To test the hypothesis that a false-bottom will reduce the rate of cannibalism and the subsequent number of juveniles harvested, the numbers of juveniles harvested from tanks in which adults were kept in cages with a false-bottom were compared to those kept in cages where juvenile could not escape through the floor of the cage. A false-bottom was constructed out of black shade-cloth with a triangular mesh size of 4.1 X 6.3mm (base and height, respectively), and the cages were each placed into a light-blue 300L tank (Figure 6.2). The control treatment was represented in triplicate and the test treatment with a false-bottom was replicated four times. The experiment was carried out over a period of 84 days.

Conditions unique to Experiment 6.3: False-side

To test the hypothesis that false-sides incorporated in the tank design will reduce the rate of cannibalism and the subsequent number of juveniles harvested under farming conditions, harvest size and apparent cannibalism of adults kept in cages with false-sides

were compared to those with no false-sides. The false-sides in the cage were constructed out of the same shade-cloth described in Experiment 6.2 (Figure 6.2). The trial lasted for 84 days, and all treatments were represented in triplicate.

Conditions unique to Experiment 6.4: Male presence vs. male absence

It is hypothesised that the rate of cannibalism would decrease if males were removed from the tank after fertilisation. This was tested by comparing the number of juveniles harvested and the rate of cannibalism where males were either present or absent. In the first treatment, males were removed from the cages after the acclimation period, resulting in a male:female sex ratio of 0:8. However, the males were returned to each cage for a period of 12 hours, once every seven days, to ensure that poor fertility did not have a bearing on the results, and this took place in daylight when parturition was least likely to occur (see Chapter 4). Males were not removed from the second treatment; i.e. a male:female sex ratio of 2:8 per cage was maintained throughout the trial. The treatment with males was represented in triplicate and the treatment without males was replicated four times. The trial was carried out over 84 days.

Experimental Series 2

Conditions common to experiments of Series 2

Seventy-five adult *X. helleri* (Gold Victory strain) were stocked into fifteen 50L plastic tanks at five adults per tank, and a male:female ratio of 1:4. A population density of 0.08 females/L was maintained for the duration of the trial by replacing missing adults when necessary. The average length of females was 69.0 ± 1.0 mm total length, and there was no difference in length between treatments (ANOVA; $p > 0.70$). A 25g bunch of shredded plastic was placed into each tank to provide refuge for the juveniles. The refuge floated at

the water surface to a depth of approximately 65% of the depth of the tank, and covered about 40% of the water surface of the 50L tank.

The tanks were oval in shape, with a length of 600mm, a width of 450mm and a water depth of 250mm (effective volume of 50L) (Figure 6.3). Water flow into the tanks was approximately 0.5L/min, and a centrally positioned 25mm PVC up-stand pipe drained water back to the filter system. A 0.8mm plastic gauze mesh covered the outflow to ensure that no juveniles were lost. These tanks formed part of the same system as described in Chapter 2 (Figure 2.1). All tanks were covered with black 10% shade-cloth (made by Alnet) to prevent the adults from jumping out of the tanks. The fish were conditioned, fed and subjected to the same photoperiod as described for Experimental Series 1.

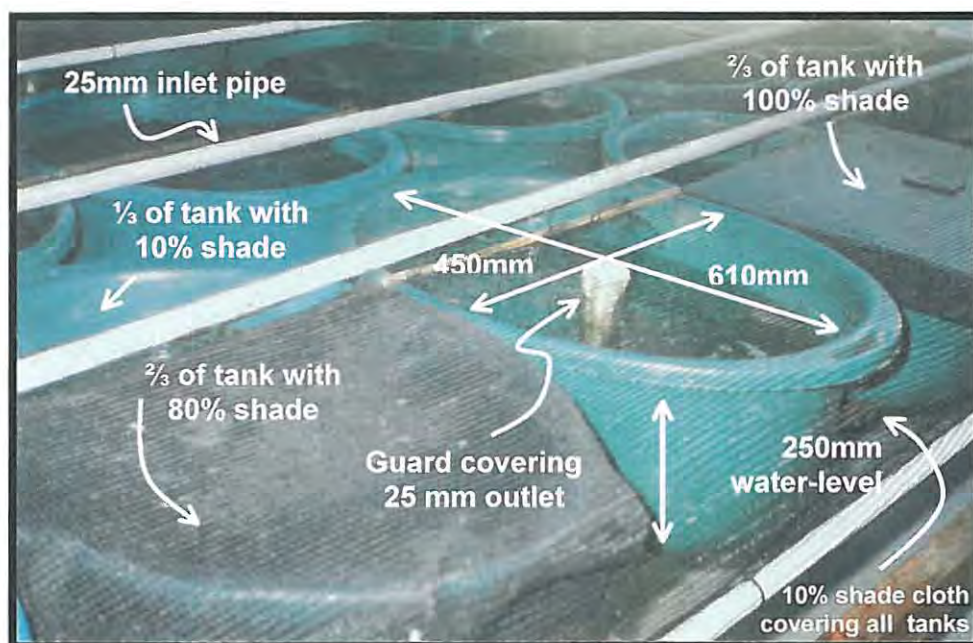


Figure 6.3. The 50L plastic tanks used for the exposure time and light-intensity experiments.

Conditions unique to Experiment 6.5: Exposure time of juveniles to adults

To test the hypothesis that the length of time juveniles are exposed to adult fish will not affect the rate of intercohort cannibalism and juvenile survival, juveniles were either collected once a day (H1), once every third day (H3) or once every seventh day (H7). All treatments were represented in triplicate. Collection took place in the morning before feeding, and all juveniles from each tank were counted to obtain an average harvest size per treatment. The experiment lasted 56 days.

Conditions unique to Experiment 6.6: Light intensity

It is hypothesised that cannibalism will be reduced if part of the broodstock tank remains in the dark. Therefore, a comparison of harvest size from shaded tanks was made to that from un-shaded tanks. The first treatment (L1) had no additional shade-cover over the tank other than the black 10% shade-cloth (made by Alnet) that covered the entire tank. The next treatment (L2) had 65% of the surface area of the tank covered with black 80% shade-cloth (made by Alnet) positioned approximately 100mm above the surface of the water (Figure 6.3). The next treatment (L3) had 65% of the tank surface area covered by a black plastic sheet that was impermeable to light (Figure 6.3). The remaining 35% of each tank was covered by black 10% shade-cloth, which allowed light into a third of the tank to ensure that the reproductive cycles of the fish were not affected by the lack of a photoperiod or a constantly low light intensity, as photoperiod and light-intensity have been found to influence reproduction in several poeciliids (Milton and Arthington, 1983; Hubbs, 1999).

Light-intensity was measured at midday, with a light meter (Biospherical Instruments Inc. QSL-100 [1345], USA), 50mm below the surface of the water directly in the centre of the

covered area of the treatments, and averaged 1.68×10^{16} quanta $\text{s}^{-1}\text{cm}^{-2}$ (range: 3.30×10^{15} to 7.20×10^{16} quanta $\text{s}^{-1}\text{cm}^{-2}$) and 2.17×10^{15} quanta $\text{s}^{-1}\text{cm}^{-2}$ (range: 9.80×10^{13} to 7.50×10^{15} quanta $\text{s}^{-1}\text{cm}^{-2}$) for treatments L1 and L2, respectively. No light was detected in treatment L3. All treatments were represented in triplicate. Juveniles were collected every morning before the broodstock were fed, and the trial lasted 56 days.

Productivity and apparent cannibalism

Apparent cannibalism (AC) is the theoretical rate at which juveniles are cannibalised by adults and was calculated using the expected and actual numbers of juveniles collected over the experimental period. The expected number of juveniles was termed expected harvest (EH) and was estimated without taking cannibalism into account using the number of reproductively active females in the tank, fecundity and inter-brood period. In the southern hemisphere between 35 and 65% of adult female *X. helleri* are reproductively active during the months of January to May (Milton and Arthington, 1983), which means that it is likely that not all females gave birth during the experimental period of this study. Since the expected harvest is dependent on the number of reproductively active females, the number of female fish that contributed to fecundity was estimated based on the total number of broods harvested during each trial and not on the number of females in each tank. The expected fecundity was determined at the end of the trial by dissecting and counting the number of developing embryos in one randomly selected gravid female from each tank ($n=18$ and $n=15$ for Experimental Series 1 and 2, respectively) and the total length of all females was measured to the nearest millimetre ($n=144$ and $n=60$ for Experimental Series 1 and 2, respectively).

Inter-brood period (*IB*), which is the average time in days between the parturition of two consecutive broods from an individual female, was determined by Equation 6.1 (Vondracek *et al.*, 1988) for all the females from Experimental Series 1 and Experimental Series 2 separately:

$$IB(days) = \frac{7 * No. females in population}{No. broods per week} \quad \text{Equation 6.1}$$

The expected harvest (*EH*) per tank for the duration of the experiments was determined by Equation 6.2:

$$EH = \frac{Fecundity * No. females * Trial duration(days)}{IB(days)} \quad \text{Equation 6.2}$$

Productivity and expected harvest (*EH*) were used to calculate apparent cannibalism (*AC*) for the different treatments of each experiment (Equation 6.3):

$$AC = \left(\frac{EH - Productivity}{EH} \right) * 100 \quad \text{Equation 6.3}$$

Productivity is the total number of juveniles collected from each tank over the duration of the experimental period (as defined in Chapter 2). However, during the course of these experiments dead and deformed juveniles were collected for the first time. Therefore, it became necessary to distinguish between productivity and live harvest. Productivity remained the total number of juveniles collected and included any deformed or dead

juveniles. Live harvest is defined as productivity minus all dead and deformed juveniles collected over the course of the experiment.

Water-quality analysis

Water temperature in all experiments was measured daily with a mercury thermometer. Ammonia and nitrite concentrations were measured using a commercially available test kit (Interpet Ltd., UK). pH was measured with a portable pH meter (Hanna Instruments, USA). Ammonia and nitrite concentrations and pH readings were taken at different times of day every two weeks ($n=6$ each). The mean pH was calculated by averaging the anti-log of all pH values, and re-logging the resultant average.

Statistical analysis

Although Experiments 6.1 to 6.4 were run simultaneously and shared a common control, the treatments of each experiment were not designed to be compared to each other. As there was always a difference in more than one variable between the test-treatments of the four experiments it was not possible to analyse them together. Thus, each treatment was compared to the control only as described below.

Mean harvest and percent cannibalism of the treatments in Experiments 6.1, 6.2, and 6.3 were compared to the control using a Student's *t*-test. The average numbers of dead and deformed juveniles of each treatment in Experiments 6.1 to 6.4 were compared to the control using a Student's *t*-test, while the differences in variance between the control and the treatment were compared using Bartlett's test for homogeneity of variances (Ott, 1988).

A one-tailed *t*-test was used to compare productivity, live harvest and the rate of cannibalism recorded in the treatment group from which males were removed, to those with males present.

Frequency distributions were analysed using a χ^2 test. The null hypothesis stated that the frequency of juveniles was evenly distributed between the top and the bottom of the tank at the time of harvest, and was rejected if $p < 0.05$. The volume at the top and the bottom of the tank was equal in the preliminary study, and in the false-bottom and false-side treatments; thus the same number of juveniles above and below the false-bottom/side was taken as the expected value. However, in treatments where juveniles did not have access to the area outside of the cage (i.e. control, bottom-refuge and male-absent), the volume of water available to the juveniles in the top portion (150L) of the cage was three times the volume available in the bottom portion (50L). Therefore, for the χ^2 test, the expected number of juveniles at the top was three times greater than that at the bottom, in the control, bottom-refuge and male-absent treatments.

Productivity and percent apparent cannibalism for all the treatments in Experiment 6.5 were tested for normality using the Shapiro-Wilk's test at an error level of 5%. These data were compared using a one-way analysis of variance (ANOVA), and Tukey's multiple-range test was used to compare means at $p < 0.05$ (Zar, 1984). The treatments of Experiment 6.6 were analysed using the same method.

RESULTS

Water quality

The average water temperature was 25.6°C (range: 22.5 to 29.0°C) during Experimental Series 1 and 24.4°C (range: 21.0 to 29.0°C) in Series 2. The pH ranged between 7.0 and 8.1 for both series of experiments, with means of 7.3 and 7.6 for the trials carried out in the 300L and 50L tanks, respectively. No ammonia (NH₄⁺-N) or nitrite (NO₂⁻-N) was detected in the system within the sensitivity of the test procedure.

Tank and false-bottom colour contrast experiment

The number of juveniles found above and below the false-bottom in the white and light-blue tanks were significantly different to those in the red or black tanks (Table 6.2; contingency analysis; $\chi^2=155.4$; $v=3$; $n=235$; $p<0.001$). In white and light-blue tanks the frequency of downward movement through the false-bottom was greater than expected by chance and more juveniles were counted beneath the false-bottom than above (Table 6.2). The opposite trend was found in the black and red tanks, where relatively more juveniles were found above the false-bottom (Table 6.2).

Table 6.2. The number of juvenile *X. helleri* that either remained above the false-bottom or moved through the false-bottom in the different coloured tanks. Treatments sharing a common subscript do not differ at $p<0.05$ (Contingency table analysis).

Tank colour	Juveniles above false-bottom	Juveniles below false-bottom
White _a	1	53
Black _b	17	38
Red _b	23	42
Light-blue _a	6	55

The number of juveniles that moved through the different coloured quarters of the false-bottom in the white and light-blue tanks differed significantly from those in the red and black tanks (Table 6.3; contingency analysis; $\chi^2=53.6$; $v=6$; $n=188$; $p<0.001$). In the white and light-blue tanks, a relatively higher number of juveniles passed through the black and the red quarters of the false-bottom, whereas the opposite was found in both the black and red tanks (Table 6.3).

It was concluded that most juveniles move through a false-bottom if it is black and placed in either a white or light-blue tank.

Table 6.3. The number of juvenile *X. helleri* that passed through the different coloured quarters of the false-bottom that was placed into each of four different coloured tanks. Treatments sharing a common subscript do not differ at $p<0.05$ (Contingency table analysis).

Tank colour	False-bottom Colour			
	White	Black	Red	Light-blue
White _a	7	30	13	3
Black _b	10	9	7	12
Red _b	10	10	8	14
Light-blue _a	9	19	16	11

Experimental Series 1 (Experiments 6.1 to 6.4)

The average interbrood period was 36 ± 3 days and there was no difference between treatment means (ANOVA; $p>0.05$). Average fecundity ($\pm$ standard error) was 108 ± 10 embryos per female per brood-cycle and did not differ between females in different

treatments (ANOVA; $n=20$; $p>0.11$). Thus, the expected fecundity per female for the duration of the 56-day trial (equivalent to 1.56 brood-cycles) was estimated at 169 ± 15 embryos ($\pm$ standard error).

Table 6.4. The rate of cannibalism (%), productivity and live harvest per tank ($\pm$ standard error) for treatments with bottom-refuge ($n=4$), a false-bottom ($n=4$) or a false-side ($n=3$) and were each compared with the control ($n=3$). Different superscripts within each column represent significant differences between the control and the treatment at $p<0.05$ (Student's t -test).

Treatment	Productivity	Live harvest	Cannibalism (%)
Control	254.7 ± 22.7^a	244.3 ± 24.0^m	62.2 ± 3.4^x
Bottom-refuge	459.0 ± 61.0^b	414.3 ± 40.2^n	31.9 ± 18.1^y
False-bottom	556.0 ± 88.2^b	487.8 ± 97.8^m	17.3 ± 13.1^y
False-side	441.7 ± 12.1^b	426.3 ± 19.6^n	34.5 ± 3.12^y

Productivity was significantly higher in the bottom-refuge, the false-bottom, and the false-side treatments as compared to the control (Student's t -test; $p<0.05$, $p<0.04$, and $p<0.002$, respectively; Table 6.4). However, there was no difference in live harvest (dead and deformed juveniles excluded) between the control and the false-bottom treatments (Student's t -test; $p>0.09$; Table 6.4), while the size of the live harvest in the bottom-refuge and false-side treatments was significantly larger than in the control (Student's t -test; $p<0.022$; $p<0.005$; Table 6.4).

Estimated cannibalism was significantly lower in the bottom-refuge, false-bottom, and false-side treatments, relative to the control group (Student's t -test; $p<0.04$, $p<0.03$, and $p<0.001$, respectively; Table 6.4).

When males were present in the tank, productivity and live harvest were both significantly lower (24.3% and 23.6%, respectively) than the treatment where males were absent (one tailed *t*-test; $p < 0.03$ and $p < 0.04$, respectively; Table 6.5). Apparent cannibalism was significantly greater in tanks with males than in tanks without males (one tailed *t*-test; $p < 0.03$; Table 6.5).

Table 6.5. Average rate of cannibalism (%), productivity and live harvest ($\pm$ standard error) in treatments where males were either absent ($n=4$) or present ($n=4$). Different superscripts within each column represent significant differences at $p < 0.05$ (one-tailed *t*-test).

Treatment	Productivity	Live harvest	Cannibalism (%)
Males present	254.7 $\pm$ 22.7 ^a	244.3 $\pm$ 24.0 ^m	62.2 $\pm$ 3.4 ^x
Males absent	336.5 $\pm$ 60.3 ^b	319.8 $\pm$ 68.2 ⁿ	50.1 $\pm$ 17.9 ^y

Of the 7,316 juveniles collected during the course of this series of experiments, 541 were dead or deformed. Most of the dead and deformed juveniles were fully developed dead juveniles or underdeveloped embryos, while some were "Siamese twins" that either shared a common yolk-sac, body or head. In total, 468 of these dead or deformed juveniles were collected from the bottom-refuge and false-bottom treatments, while the remaining 73 were collected from the false-side, treatment without males, and the control (Table 6.6). The average number of dead or deformed juveniles per collection from the control treatment (Student's *t*-test; 1.0 ± 0.4 ; $n=28$) did not differ from that of the bottom-refuge (Student's *t*-test; 4.5 ± 0.4 ; $n=40$; $p > 0.05$), false-bottom (Student's *t*-test; 6.0 ± 2.3 ; $n=48$; $p > 0.10$), false-side (Student's *t*-test; 1.0 ± 0.4 ; $n=27$; $p > 0.94$), and male-absent treatment (Student's *t*-test; 0.5 ± 0.2 ; $n=38$; $p > 0.1.8$). There was a significant difference

between the variance of dead and deformed juveniles in bottom-refuge compared to the control (Bartlett's test; $F_{0.05,39,27} = 1.88$; $p < 0.001$), false-bottom versus control (Bartlett's test; $F_{0.05,47,27} = 1.84$; $p < 0.001$), and male-absent versus control (Bartlett's test; $F_{0.05,27,37} = 1.74$; $p < 0.001$), while there was no difference between the variance of dead and deformed juveniles in the false-side treatment versus the control (Bartlett's test; $F_{0.05,27,26} = 1.79$; $p > 0.25$).

Table 6.6. Number of dead or deformed juveniles collected from the control (n=3), bottom-refuge (n=4), false-bottom (n=4), false-side (n=3) and male absent (n=4) treatments.

Treatment	Total no. of dead or deformed juveniles	Average no. dead or deformed/tank	Range of dead or deformed/collection
Control	28	9.3 (± 0.7)	0 – 7
Bottom-refuge	179	42.3 (± 21.2)	0 – 41
False-bottom	289	72.3 (± 29.4)	0 – 106
False-side	26	8.7 (± 1.7)	0 – 7
Male-absent	19	4.8 (± 1.3)	0 – 6

In the control treatment, more juveniles than expected by chance were harvested from the floating refuge at the top of the tank, and relatively few were harvested from the bottom ($\chi^2 = 241.34$; $v=1$; $p < 0.001$; Table 6.7). Similarly, in the male-absent treatment, more juveniles were found at the top than at the bottom ($\chi^2 = 324.01$; $v=1$; $p < 0.001$; Table 6.7). In the bottom-refuge, false-bottom, and false-side treatments, most of the juveniles were harvested from the bottom portion of the tank ($\chi^2 = 3314.32$, $\chi^2 = 1158.85$, and $\chi^2 = 1003.67$, respectively; $v=1$; $p < 0.001$; Table 6.7).

Table 6.7. The number of juveniles harvested from either the top or the bottom of the tank. The number collected from the top was significantly different from the number collected from the bottom, within each treatment (χ^2 ; $p < 0.05$).

Treatment	No. harvested from top of tank	No. harvest from bottom of tank
Control	731	1
Bottom-refuge	228	1,429
False-bottom	220	1,719
False-side	73	1,206
Males-absent	1,238	41

Mortality of female broodstock occurred in all treatments. A total of nine females died in the three control tanks over the 84-day period of the study. This did not differ significantly from the total of eight that died in the bottom-refuge treatment ($\chi^2=0.69$; $v=1$; $p>0.50$), 11 in the false-bottom treatment ($\chi^2=0.03$; $v=1$; $p>0.90$), seven in the false-side treatment ($\chi^2=0.25$; $v=1$; $p>0.75$), and seven in the male-absent treatment ($\chi^2=1.15$; $v=1$; $p>0.75$).

Experimental Series 2 (Experiments 6.5 and 6.6)

The average interbrood period in all treatments for both the light intensity and exposure time was estimated at 35 ± 4 days. There was no difference in interbrood period between fish kept at different light intensities (ANOVA; $p>0.43$), and no difference in fecundity between the treatments (ANOVA; $p>0.39$), with an average of 89 ± 7 embryos per female per brood-cycle. Thus, for the duration of the 56-day trial (equivalent to 1.6 brood-cycles) the average fecundity was 143 ± 11 embryos per female.

Table 6.8. Average number of juveniles harvested ($\pm$ standard error) over a period of 56 days and rate of cannibalism (%) for treatments with a collection frequency of once a day (H1), once every third day (H3) and once every seventh day (H7). Different superscripts represent significant differences within each column (ANOVA; $p < 0.05$).

Treatment	Exposure time	Harvest size	Cannibalism (%)
H1	One-day	135.7 $\pm$ 13.2 ^a	52.56 $\pm$ 4.6 ^x
H3	Three-day	153.3 $\pm$ 17.7 ^a	46.39 $\pm$ 6.2 ^x
H7	Seven-day	162.7 $\pm$ 50.5 ^a	43.12 $\pm$ 17.6 ^x

There were no significant differences in the number of juveniles harvested from the treatments where juveniles were exposed to adults for different periods (ANOVA; $p > 0.88$; Table 6.8). There was also no difference in apparent cannibalism between these treatments (ANOVA; $p > 0.88$; Table 6.8).

There were significant differences in both the number of juveniles harvested and in percent apparent cannibalism between treatments that were subjected to different light intensities. The lowest light-intensity treatment (L3) had a smaller average harvest and a higher apparent rate of cannibalism (ANOVA; $p < 0.05$ and $p < 0.05$, respectively; Table 6.9).

Table 6.9. Average number of juveniles harvested ($\pm$ standard error) over a period of 56 days and rate of cannibalism (%) for treatments with different light intensities. Different superscripts represent significant differences within each column (ANOVA; $p < 0.05$).

Treatment	Average light intensity (quanta s ⁻¹ cm ⁻²)	Harvest size	Cannibalism (%)
L1	1.68 $\times$ 10 ¹⁶	135.7 $\pm$ 13.2 ^a	52.6 $\pm$ 4.6 ^x
L2	2.17 $\times$ 10 ¹⁵	78.0 $\pm$ 15.6 ^{ab}	72.7 $\pm$ 6.6 ^{xy}
L3	No light detected	56.7 $\pm$ 7.5 ^b	80.2 $\pm$ 2.6 ^y

DISCUSSION

The discussion focuses primarily on the effect of different production procedures and technologies on the estimated rate of intercohort cannibalism and the subsequent rate of juvenile survival, with the overall objective of determining whether or not the hypotheses formulated in earlier chapters should be accepted when tested under practical farming conditions.

Interbrood period

The interbrood period calculated in Chapter 2 differs from that calculated here. At first glance, this might appear to cast doubt on the method of calculation used in these sections of the work. Since interbrood period affects the calculation of the rate of cannibalism (Equations 2.1 and 6.1; Vondracek *et al.*, 1988), it is worthwhile confirming that the method used to calculate interbrood period, in Chapter 2 and in these experiments, was correct.

The calculated interbrood periods in Experimental Series 1 and 2 (36 ± 3 and 35 ± 4 days, respectively) and that obtained in Chapter 2 (28 ± 4 days) were significantly different ($p < 0.001$ and $p < 0.001$, respectively). However, all estimates fell within the reported range of 26 to 35 days for members of the genus *Xiphophorus* (Tavolga and Rugh, 1947; Siciliano, 1972; Milton and Arthington, 1983; Kruger *et al.*, 2001a). Siciliano (1972) observed an interbrood period of 35.1 ± 0.7 days which was similar to the 35 day period reported by Kruger *et al.* (2001a), both of which match the findings in both experimental series here. However, Milton and Arthington (1983) and Tavolga and Rugh (1947) reported interbrood periods for *X. helleri* and *X. maculatus* of 29 and 28 days respectively, similar to the 28 ± 4 day interbrood period described in Chapter 2.

If not directly observed, interbrood period must be estimated and different methods may produce different results. However, it is unlikely that the methods were the cause of the disparity in interbrood period reported by the above authors and in this thesis. The method used to calculate interbrood period in this study and by Kruger *et al.* (2001a) was based on Equation 6.1 (Vondracek *et al.*, 1988), and was estimated to be approximately 35 days for both these studies. The same method was applied in Chapter 2, yet an interbrood period of 28 days was calculated. The method used by Siciliano (1972) and Tavalga and Rugh (1947) involved direct observation and timing of individual fish after they were inseminated. Siciliano (1972) and Tavalga and Rugh (1947) reported interbrood periods of about 35 and 28 days, respectively. Although they used the same method, these authors observed different interbrood periods. Furthermore, the different method (i.e. direct observation and timing after insemination) confirms the results obtained by Kruger *et al.* (2001a) and those of this study. Therefore, it can be concluded firstly, that the method of calculating interbrood period was probably not the reason for the differences reported here, and secondly, the interbrood periods calculated here and in Chapter 2 are probably reliable estimates of interbrood period.

An exact reason for the different interbrood periods remains unclear. However, there is evidence to suggest that interbrood period may follow a seasonal pattern, although there were no differences in water temperature between the experiments of Chapter 2 (28 day interbrood period) and those of Chapter 6 (35 day interbrood period). Nonetheless, seasonal changes in poeciliid reproduction have been recorded under natural conditions, even in the tropics where seasonal environmental changes are small (Winemiller, 1993). The current experiments and the investigation by Kruger *et al.* (2001a) (also undertaken at Rhodes University) were carried out between February and May (late summer to autumn)

and both yielded interbrood periods of approximately 35 days. The shorter interbrood period of 28 days, calculated in Chapter 2, was based on work undertaken between mid-July and late-September (mid winter to early spring). Milton and Arthington (1983) studied wild introduced populations of *X. helleri* in Queensland, Australia, and found a distinct seasonal pattern in their reproductive cycle, with a peak in October. The latitude of the Australian study area (27°30'S) is sufficiently similar to that of this study (33°20'S), to expect that the sites would experience similar seasonal effects. Therefore, it is suggested that *X. helleri* in South Africa may have followed a similar seasonal cycle under the experimental conditions. Although individual environmental cues associated with seasonal change, such as temperature, photoperiod and light intensity have been found to influence the reproductive cycle of poeciliid fishes, including *X. helleri* (Milton and Arthington, 1983; Meffe and Snelson, 1989; Hubbs, 1999), a full understanding of the combined effect of such environmental factors on the interbrood period of *X. helleri* and their reproduction in general, has not yet been achieved.

Therefore, further research specifically designed to better understand the reasons behind a fluctuation in interbrood period in *X. helleri* is required. Nevertheless, the different interbrood periods calculated for various studies throughout this work were probably good estimates, even though differences were found.

Effect of refuge on position of juvenile in the water column

The juveniles changed their position in the water according to the location of the refuge, and this behaviour appeared to reduce the likelihood of cannibalism. When an alternative to floating cover was included in the tank design, such as bottom-refuge, a false-bottom or a false-side, the juveniles were harvested mostly from the alternative refuge or from beneath the cage even though floating refuge was always available. This apparent

preference for the bottom of the water column is consistent with the observation that juveniles showed a tendency to move downwards most often immediately after parturition and when attacked (Chapter 5). However, when only floating cover was available, almost all juveniles were harvested from there.

It was suggested (Chapter 5) that a preference for downward movement might be due to neural lateralization, wherein movement in one direction is favoured over movement in another (Bisazza *et al.*, 1998). Neural lateralization has been used to explain the escape behaviour of another poeciliid, the goldbelly topminnow (*Girardinus falcatus*), as adults and juveniles were shown to favour a rightward escape response when first exposed to a dummy predator (Cantalupo *et al.*, 1995; Bisazza *et al.*, 1998). The observation that juveniles were located most often at the bottom of the water column, when refuge was provided there, is consistent with the observation made in Chapter 5 that juveniles favoured movement in a downward direction. The finding here that juveniles were not always located at the bottom of the water column (i.e. they were harvested from the floating refuge at the top of the water column when there was no cover at the bottom), does not contradict the observation in Chapter 5 that juveniles favour downward escape behaviour and the suggestion that this preference may be due to cerebral asymmetry. There is no evidence in the present study that juveniles did not first escape in a downward direction and then subsequently move towards the only cover in the tank, situated at the top of the water column. Rather, it may demonstrate that juveniles seek the cover of refuge once they survive an attack. Laurila *et al.* (1998) suggested that anti-predatory behaviour in salmonids is related to predator regimes. Similarly, the behaviour of juvenile *X. helleri* appears to be geared towards predator avoidance (they moved to the top of the water column if there was no alternative refuge at the bottom, and once positioned at the top they always took cover in the floating refuge). Juveniles sought the cover of refuge

when positioned close to the water surface where they are supposedly more vulnerable to predation (Chapter 5). Furthermore, data reported in Chapter 2 showed refuge to significantly increase juvenile survival. Overall, it appears that the location of refuge dictates the position of juveniles in the water column, and that this behaviour is geared towards increasing survival by reducing the chance of a successful attack by an adult. Finally, these data illustrate the importance of including refuge in the tank in order to decrease cannibalism under farming conditions.

Effect of male presence and absence on the rate of cannibalism

Male *X. helleri* contribute to the cannibalism of juveniles. It was correctly hypothesised that the removal of these potential cannibals from the tank would lead to a corresponding decrease in cannibalism (Chapter 4). Just as the presence of males in the tank increased the adult population size by 20%, so apparent cannibalism increased by 19.5% relative to the treatment where males were absent. This observation confirmed earlier findings that males are as cannibalistic as females (Chapter 2 and 4), and that additional adults in the tank contribute to the mortality of the juveniles of a given size brood (Thibault, 1974; Jones *et al.*, 1998a; Chapter 2). However, in contrast to the finding that male and female *X. helleri* are equally cannibalistic, Warren (1973) and Hubbs (1991 and 1995) found that female *P. reticulata* and female *Gambusia*, respectively, were more cannibalistic than males. Thus, sexual biases in the cannibalistic behaviour of adults are not consistent between poeciliid species.

The hypothesis that the rate of cannibalism will decrease if males are removed from the breeding tank is accepted. However, this conclusion is only relevant to populations of poeciliids where males and females are equally cannibalistic; also, it would only be practically possible to implement the removal of males under farming conditions in species

where females store sperm, as in *X. helleri* (Constantz, 1989). Nonetheless, the removal of males from breeding tanks can probably be recommended to reduce cannibalism in *X. helleri* under farming conditions, but the long term effects of their removal on productivity needs to be investigated.

Effect of light intensity on cannibalism

Based on the observation that the rate of cannibalism was significantly reduced at a low light intensity and that *X. helleri* is a visual predator (Jones *et al.*, 1998b; Chapter 3), it was hypothesised that the rate of cannibalism would be reduced at a low light intensity under farming conditions. Light intensity affects the reproductive cycle in several poeciliids that belong to the genus *Gambusia*, but the effect was not the same for all the species studied: *Gambusia affinis*, *G. geiseri*, and *G. heterochir* produced fewer broods at a low light intensity and *G. nobilis* produced fewer broods at a high light intensity (Hubbs, 1999). Therefore, to ensure that reduced light intensity did not influence the reproductive cycle of the adults used in the present study, one-third of the tank was left un-shaded. Instead of contributing to productivity by reducing the rate of cannibalism, it appears that a continuous low light intensity had a negative effect on productivity, since significantly fewer juveniles were harvested at a low light intensity (L3) in comparison to a high light intensity (L1). Reduced productivity was not attributed to reduced fecundity, fertility or interbrood period because these did not differ between treatments with a low or high light intensity. Moreover, significant changes in the fecundity of *P. reticulata* and the reproductive index of *G. affinis* were found to take place within 37 to 50 days, respectively (Dahlgren, 1979; Meffe and Crump, 1987). Apparently, *X. helleri* did not show a phenotypic response to the light intensities used in this study over the 56-day period of the experiment. Therefore, the only explanation for the reduced number of juveniles harvested at a low light intensity was an increase in the rate of cannibalism, which

conflicts with observations made in Chapter 3 that the rate of cannibalism was reduced in the dark and that *X. helleri* relies predominantly on vision to capture live prey.

Therefore, the hypothesis formulated by Jones *et al.* (1998b) and in Chapter 3 of this work, that reduced light intensity can be used to mitigate cannibalism under farming conditions, is not accepted. However, it is speculated that the reduced harvest size at a low light intensity might have been due to the parturition of fewer juveniles and not to an increased rate of cannibalism. Adult *X. helleri* were never observed in the un-shaded area in the reduced light intensity treatments, and were therefore exposed to a constantly low light intensity. A constantly low light intensity has been found to negatively influence reproduction in some poeciliids (Hubbs, 1999), which might explain the reduced number of juveniles produced here. However, fertility, fecundity and interbrood period of *X. helleri* were not influenced by reduced light intensity in this study. Embryo reabsorption might have affected the results since it has been reported in some poeciliids (Scrimshaw, 1944; Schultz, 1961; Meffe and Vrijenhoek, 1981). However, this possibility is disputed by some authors since no firm demonstration of embryo reabsorption has been made (Constance, 1989). Furthermore, there was no evidence of embryo reabsorption in fish that were dissected here. If the reduced rate of cannibalism calculated in this study was an artifact of fewer births, the reason for the reduced number of juveniles remains unclear. Future research is required to better explain the finding that fewer juveniles were produced at a constantly low light intensity. Until then, the hypothesis that reduced light intensity can be used to reduce the rate of cannibalism under farming conditions is rejected as cannibalism appears to increase under a constantly low light intensity.

Effect of exposure-time to adults on rate of cannibalism

Thibault (1974) found that *Poeciliopsis monacha*–*P. lucida* species complex juveniles were only subject to cannibalism during the first 24 hours after birth. A partial explanation for this is provided in Chapter 5, where older juvenile *X. helleri* were better able to avoid attacks by adults than neonates, and this ability was attributed to a learning process. It was also hypothesised that adult *X. helleri* habituate to a stimulus that previously resulted in the attack of a juvenile (Chapter 4), suggesting that juveniles are less vulnerable to cannibalism with an increase in time after parturition. It was thus hypothesised here, that the rate of cannibalism would not differ between treatments where juveniles were exposed to the adult fish for different lengths of time. Since this hypothesis was accepted, the result substantiates the finding of Thibault (1974) that juvenile *Poeciliopsis* are only subject to cannibalism during the first 24 hours of life, and it supports the combined observations reported in Chapters 4 and 5 that juveniles are less likely to be cannibalised with an increase in time after parturition.

Effect of bottom-refuge, a false-bottom and a false-side on the rate of cannibalism

The observation that most of the successful cannibalistic attacks took place at the bottom of the tank, under laboratory conditions, was confirmed in the experiments (under farming conditions) that included either refuge at the bottom, a false-bottom or a false-side. The rate of cannibalism in the control group, where adults had access to the juveniles at the bottom of the water column, was significantly higher than in all the treatments where females were prevented from following juveniles to the bottom of the tank. Juveniles were most susceptible to cannibalism immediately after birth, at which time most surviving neonates settled on the bottom or attempted to escape an attack most often in a downward direction (Chapter 5). Moreover, females spent significantly longer periods at the bottom of the tank during this period (Chapter 3), and 65% of observed attacks and

85% of observed cannibalistic events took place at the bottom of the water column (Chapter 3). The inclusion of refuge at the bottom of the water column or a false-bottom, both of which gave juveniles added protection when they escaped in a downward direction, reduced the rate of cannibalism by 49% and 72%, respectively (relative to the rate of cannibalism in the control). The similarity in behaviour between fish in the laboratory studies and under practical farming conditions implies that the cannibalistic behaviour of *X. helleri* in the laboratory studies reflects their expected behaviour under production conditions. The expectation that laboratory observations can be used as a tool to predict behaviour of poeciliid fishes under more extensive farming systems will be discussed in Chapter 7.

Similarly, the rate of cannibalism among fish in the false-side treatment was significantly lower than in the control group. According to behavioural observations described in Chapter 3, very little cannibalism took place in the middle of the tank, and the results of Chapter 5 show that between 29% and 38% of neonates attempted to escape in a side-ways direction when attacked by an adult. These observations help to explain why apparent cannibalism in the false-side treatments was 45% lower than that recorded in the control. Hence, the parturition-associated behaviour observed in the laboratory was an accurate reflection of behaviour under production conditions.

Dead and deformed juveniles were first observed when they were collected from the bottom-refuge and false-bottom treatments, where adults did not have access to them. In the laboratory studies the consumption of anything other than a live juvenile was termed feeding and the definition of cannibalism was restricted to the consumption of live juveniles only (see either Tables 3.1, 4.1 or 5.1 of Chapters 3, 4 and 5, respectively). Therefore, the incidence of dead and deformed juveniles requires consideration.

The implication of dead and deformed juveniles on interpretation of results

Dead and deformed juveniles were not considered when calculating the rate of cannibalism, where the actual number of juveniles harvested was subtracted from the expected harvest (Equation 2.1 and Equation 6.1). Expected harvest was estimated based on the assumption that it was equivalent to fecundity and that all the fertilised embryos would survive to parturition. However, during the practical farming experiments it became apparent that some embryos were not alive or viable at parturition. Of the 4,828 juveniles harvested during Experiment 6.1 (bottom-refuge) and Experiment 6.2 (false-bottom), 496 (10.3%) were either dead or deformed. In earlier experiments, they went unnoticed, because adults always had access to the bottom of the tank and all dead and deformed juveniles must have been immediately consumed.

The occurrence of dead and deformed embryos has been reported for several poeciliid fishes such as *P. monacha*, *P. prolifica* and *P. reticulata* (Meffe and Vrijenhoek, 1981). However, Meffe and Vrijenhoek (1981) observed only six of these abnormalities out of a total of 378 embryos examined *in vivo* before parturition (i.e. 1.6% were abnormal). In this study, more than 10% of juveniles collected from those treatments in which adults did not have full access to the bottom of the tank were dead or deformed. It is not uncommon among animals for parents to cannibalise newborn young that are deformed, weak or sick (Polis, 1981), and intracohort cannibalism of deformed fish larvae has also been reported (Baras *et al.*, 2000b). Of the 496 dead and deformed offspring that were collected during Experiments 6.1 and 6.2, 94.4% were taken from the bottom-refuge and false-bottom treatments (where adults did not have access to them) while only 5.6% were from the control tanks (where adults had access to dead and deformed juveniles at the bottom). From this it can be concluded that most abnormal juveniles are consumed if adults have

access to them, and that juvenile deformities and death at parturition are common features of captive-bred *X. helleri*.

The consumption of dead and deformed juveniles by adults affects the definition of the term cannibalism used earlier. It is important to bear in mind that the method of calculating the rate of cannibalism here and in Chapter 2, as well as that used by other authors (Vondracek *et al.*, 1988; Jones *et al.*, 1998a; Kruger *et al.*, 2001a), did not take the incidence of dead and deformed juveniles into account. Thus, the rate of cannibalism in these studies included the consumption of live juveniles as well as dead and deformed juveniles, whereas the definition of cannibalism in the laboratory studies of this work, excluded dead and deformed neonates (see Table 3.1 in Chapter 3). Therefore, an additional approach is required here that takes dead and deformed juveniles into account. It was possible to do this for treatments where the number of dead and deformed juveniles was known (i.e. false-bottom and bottom-refuge treatments). This was done by comparing productivity (i.e. number of juveniles harvested including dead and deformed juveniles) to live harvest (i.e. number of juveniles harvested, excluding dead and deformed juveniles).

The inclusion of bottom-refuge, a false-bottom and false-sides all significantly enhanced productivity under farming conditions. However, these interventions did not consistently result in a higher live harvest. The false-side and the bottom-refuge treatments had significantly larger live harvests in comparison to the control, indicating that a false-side or bottom-refuge promotes the survival of juveniles. Furthermore, their use under practical farming conditions is recommended as they promote the removal of dead and deformed juveniles. However, there was no significant difference in live harvest between the false-bottom treatment and the control. From this it can be concluded that the installation of a

false-bottom will not necessarily promote the survival of juveniles; rather, the high productivity of the false-bottom treatment was due to the inclusion of dead and deformed juveniles. Therefore, the use of a false-bottom in the breeding tanks is not recommended for farming *X. helleri*.

Conclusion

The consumption of dead and deformed juveniles in the earlier controlled laboratory studies was incorporated in the definition of feeding rather than cannibalism (see Table 3.1 in Chapter 3). This led to the realisation that some ethologists have defined the term cannibalism to include, not only the consumption of conspecific flesh, but also the killing of the conspecific (Fox, 1975; Polis, 1981; Smith and Reay, 1991; Hecht and Pienaar, 1993; Jones *et al.*, 1998b), and have therefore excluded the consumption of flesh that was not killed by the cannibal. McFarland (1981), in *The Oxford Companion to Animal Behaviour*, defines cannibalism as:

“... the eating of one's own species, alive or dead...”

and this definition concurs with other ethological dictionaries (Immelmann and Beer, 1989). Therefore, it is suggested that the act of killing a conspecific should be distinguished from the act of cannibalism in future ethological research, and the term cannibalism should not be used for both. Nonetheless, cannibalism of dead and deformed juveniles may not always carry the same weight to genetic fitness as that of healthy juveniles, and this work reveals the importance of distinguishing between the consumption of live (viable) individuals and dead or deformed (less viable) individuals, particularly to farming conditions. Therefore, the definition of cannibalism could encompass different levels, which range from the removal of potentially viable offspring

from the population to that where the act of cannibalism leads to a potential benefit as less viable offspring are removed. Future studies could place cannibalistic behaviour of fish or other animals on this presumed continuum, and thus weight cannibalism according to its positive or negative effect on the population.

The results highlight the importance of testing laboratory-based predictions under farming conditions, since some outcomes (for example the occurrence of large numbers of dead and deformed juveniles) were not observed or foreseen during the laboratory experiments. While the results of previous behavioural observations predicted that a false-bottom would significantly reduce cannibalism, the inclusion of a false-bottom did not enhance juvenile survival rate as the higher productivity in this treatment was due mainly to dead and deformed juveniles. Another example is the negative effect of a constantly low light intensity on *X. helleri* productivity under farming conditions. It was predicted earlier that juveniles would find greater protection under the cover of darkness and that this would lead to a reduction in the rate of cannibalism. However, it was not predicted that adults would also take cover in the dark. These findings illustrate the importance of verifying laboratory observations under different sets of environmental conditions to ensure the accuracy and completeness of the conclusions.

The results of this series of experiments confirm that parturition-associated behaviour of *X. helleri* under farming conditions is largely similar to that under highly controlled laboratory conditions, and behavioural observations can be used to develop practical technologies to improve farming production. For example, it was shown in the laboratory that most of the successful cannibalistic attacks took place at the bottom of the tank, while under production conditions it was found that the rate of cannibalism was reduced when adults were prevented from accessing the bottom of the water column. It was also

observed that most juveniles escaped an attack in a downward direction, while under production conditions most juveniles were collected from the bottom of the water column when bottom refuge was provided. Juveniles positioned near the water surface during laboratory observations usually sought the cover of refuge; similarly, under production conditions juveniles were almost always found in the floating refuge near the surface when there was no available refuge at the bottom. Furthermore, based on laboratory observations it was suggested that anti-predator behaviour in juveniles improved with increased exposure to adult attack behaviour. This was confirmed in the exposure time experiment where juvenile survival was independent of the length of time that they were exposed to adults.

To reiterate, controlled laboratory observations of fish behaviour can contribute towards the development of hypotheses regarding their behaviour in general. However, the results also highlight the need to test these hypotheses under different sets of environmental conditions, such as practical farming conditions, to ensure their applicability under different conditions.

CHAPTER 7

CONCLUDING DISCUSSION

Interpretation and extrapolation of cause and effect in ethology

Ethological studies require that behaviour is comprised of recognisable and repeatable units that can be identified and quantified (Noakes, 1992). Many facets of the biological sciences and particularly ethology depend largely on descriptions, which are often not easily quantified, and this makes them vulnerable to the criticism of being anecdotal. The description and definition of the behavioural units are the fundamental building blocks of ethology (Noakes, 1992). Ethologists need to determine and define these units so that they can be accurately measured, and then tested for consistency under other conditions. For that reason emphasis has to be placed on repeatability, which is dependent on definitions (Noakes, 1992). All recorded behaviours in this thesis were well defined, which makes it possible for them to be tested by other researchers.

When interpreting results, ethologists need to consider potential confounding factors. A confounding factor is the attribution of what may seem like an obvious cause or explanation to a particular false effect. Thus, in reality there may not be a relationship between the factors. Furthermore, the relationship between the unrelated factors may even be supported statistically, but the conclusions may be erroneous.

The studies by Thibault (1974), Britz and Pienaar (1992) and Appelbaum and Kamler (2000) will be discussed to show how the explanation of behavioural studies may be subject to the circumstances of the study and the author's interpretation. Thibault (1974) attributed an increase in the rate of cannibalism to an increase in the schooling behaviour of juveniles in a *Poeciliopsis* species complex. It was concluded that schooling behaviour

was related to the number of juveniles, since they formed a school if more than 10 juveniles were present in the tank. Furthermore, Thibault (1974) concluded that the schooling behaviour of the juveniles evoked cannibalistic responses from the adult fish in the tank. Personal observations of juvenile *Xiphophorus helleri* and juvenile *Poecilia reticulata* suggest that they only formed schools in the absence of refuge and in the presence of a predator. Thibault (1974) provided refuge in the tanks used in his study. However, although a control treatment was included, where juveniles were introduced to a tank with no adults to test that juvenile mortality would be zero in the absence of adults, no mention was made of the schooling behaviour of the juveniles in the absence of adults. The finding that juveniles did not form schools if 10 or fewer juveniles were placed into the tank will not be disputed here, but the schooling behaviour of young *Poeciliopsis* in the presence of adults as observed by Thibault (1974) may have been stimulated by the cannibalistic behaviour of the adults and not vice versa as suggested by Thibault (1974). The conclusion that schooling behaviour evoked cannibalism in this species, which was widely accepted at the time (Fox, 1975), may be interpreted differently under other conditions. If this hypothesis were to be tested under different conditions and widely accepted, it might be concluded that an increase in cannibalism stimulated the schooling behaviour of juvenile *Poeciliopsis*. Thus, the cause and effect of this behaviour may be different from that originally proposed by Thibault (1974).

Britz and Pienaar (1992) were the first authors to provide quantitative observations on the effects of light on the behaviour of larval *Clarias gariepinus*, and it was concluded that they were significantly less active under light conditions due to photophobic behaviour. Furthermore, the larvae took cover under light conditions, so Britz and Pienaar (1992) concluded that the cover-seeking behaviour in the light and a subsequent increase in encounters among conspecifics probably promoted cannibalism under light conditions.

However, Appelbaum and Kamler (2000) reported that juvenile *C. gariepinus* were probably more active under light conditions, and the increased activity and subsequent increase in encounters with conspecifics was used to explain the increase in cannibalism in the light. Britz and Pienaar (1992) and Appelbaum and Kamler (2000) appear to agree that cannibalism in *C. gariepinus* is a result of increased encounters between individuals, while their conclusion is supported by the observations of Hecht and Appelbaum (1988) that cannibalism in *C. gariepinus* was a response to tactile stimulation between individuals. Britz and Pienaar (1992) and Appelbaum and Kamler (2000) also appear to agree that light affected the behaviour of these fish, which ultimately increased the rate of cannibalism. However, their interpretations of how the cause (light) resulted in the effect (an increased rate of encounter between individuals) were entirely different. This illustrates how the conclusions of behavioural studies are subject to the author's interpretation.

The findings of Thibault (1974) and those of Britz and Pienaar (1992) and Appelbaum and Kamler (2000) are mentioned to highlight the importance of interpretations and their extrapolations onto different conditions. The same concern with extrapolations in experimental science and their validity could also be raised in reference to this study, where behaviour observations under controlled laboratory conditions were used to predict the behaviour of fish under culture conditions with different environmental variables.

The first objective of the concluding discussion is to compare behaviour of *X. helleri* observed in the laboratory to that observed in the greenhouse tunnel system to explore whether observations made under laboratory conditions in small glass aquaria are applicable to different conditions, such as a practical farming system. Furthermore, it is revealed that laboratory observations are potentially effective tools for predicting fish

behaviour under a variety of conditions, which could for example, be used to the benefit of ornamental fish farming industry. The second objective of the following discussion is to assess several conclusions drawn from observations of fish behaviour to anticipate potential shortfalls associated with extrapolations. In particular, it will be shown how the methods employed here allowed for the elimination of potentially confounding factors.

Various behavioural patterns of *X. helleri* under farm culture conditions were reflected in their behaviour in the laboratory. From the observations of fish in glass tanks it was predicted that juveniles would escape in a downward direction more often than in other directions. It was suggested that this might be a consequence of the juveniles' vulnerability to predation while near the water surface since other observations revealed that adults were more efficient at cannibalising juveniles in the upper part of the water column. The observations were supported statistically. However, it remains possible that juveniles may be more likely to escape downwards in glass aquaria due to the reflection of overhead light off the bottom of the aquarium, and that adults are more efficient at cannibalisation near the water surface because of the shape of the tank; thus, there may be no actual relationship between the downward movement of juveniles and the adult's improved capture efficiency at the top of the water column.

However, the prediction that juveniles will tend to escape in a downward direction and juveniles are more vulnerable to predation if they remain at the top of the water column was realised under production conditions, where the tank bottoms in the greenhouse tunnel did not reflect overhead light. Furthermore, the rate of juvenile survival significantly improved among fish raised in the greenhouse tunnel when juveniles were protected at the bottom of the tank, which reinforces the prediction that juveniles were less vulnerable to predation at the bottom. Thus, similar results were obtained under different conditions,

giving a higher degree of confidence that the observed behaviour was not influenced by unknown environmental factors particular to the experimental or practical farming conditions.

Many other observations made in the laboratory were reported under production conditions. For example, in the glass aquaria, juveniles most frequently escaped downwards, while attacks and successful cannibalistic events usually took place in the bottom section of the water column. Based on these observations it was predicted that the rate of cannibalism would be reduced if adults were denied access to the juveniles at the bottom of the tank. Under culture conditions the rate of cannibalism was reduced by a margin similar to that predicted for the laboratory studies by limiting access of adults to the juveniles at the bottom of the tank, which further confirms the similarity in *X. helleri* behaviour under different sets of environmental conditions.

Furthermore, it was observed that about one-third of neonates and about one-third of juveniles attempted to escape in a sideward direction when attacked by an adult. Based on this observation it was predicted that the rate of cannibalism under culture conditions could be reduced by approximately one-third if adults that attacked juveniles were prevented from following them. When a false-side was included in the design of the tank under culture conditions, the rate of cannibalism dropped by 45%.

Similarly, based on laboratory data indicating that male and female adults are equally cannibalistic it was predicted that if part of the adult population were removed the rate of cannibalism could be decreased by a similar margin. When 20% of the adult population was removed in farm culture experiments, the rate of cannibalism was reduced by an average of 19.5%. Thus, sex-related cannibalism did not appear to be affected by

differences in the conditions prevalent in the two studies.

All juveniles observed at the top of the water column in the glass aquaria chose refuge rather than open water, which led to the prediction that juveniles would exhibit the same preference under farming conditions. More than 99% of the juveniles harvested from the top of the water column in the greenhouse tunnel system were harvested from refuge.

Capture efficiency of older juveniles was lower than for neonates, suggesting that juveniles became better able to escape cannibalism with experience of attacks. It was also observed that adults appeared to habituate to a stimulus that previously released an attack response. There was no decrease in juvenile survival with a decrease in collection frequency even when juveniles were exposed to the adults for longer periods and under conditions different from those in aquaria. Thus, escape behaviour and predation efficiency were not influenced by the two sets of environmental conditions.

Between 57 and 98% of juveniles passed through the false-bottoms provided in glass aquaria under laboratory conditions. Thus, it was predicted that if a false-bottom or artificial refuge were placed at the bottom of large farm tanks, most juveniles would take advantage of this cover and settle at the bottom of the water column. In the greenhouse tunnel system, 86% of juveniles were harvested from bottom-refuge in that treatment, which left 14% positioned in cover at the top of the water column. Similarly, when juveniles had the option of a false-bottom 89% were harvested from beneath the false bottom, leaving 11% in the refuge at the top of the water column.

These examples show that observations of *X. helleri* behaviour in the laboratory (Chapters 3, 4 and 5) made it possible to predict their behaviour under different conditions. The

experiments described in Chapter 6 were designed using these predictions, and the results of that chapter confirm that many predictions were accurate forecasts of behaviour under farm culture conditions. It can be concluded that some *X. helleri* behaviour observed in the laboratory can be used to predict behaviour of that species under different conditions. However, certain findings from experiments in the greenhouse tunnel system were unexpected. For example, the occurrence of dead and deformed juveniles only became apparent during the experiments of Chapter 6, as the laboratory observations did not reveal the potential occurrence of unviable juveniles.

However, it has been shown that by testing the result under different conditions (for example in a small glass tanks and in a large greenhouse tunnel system) many predictions were meaningful and it became possible to obtain a complete understanding of their behaviour. Therefore, there is considerable value in confirming the behavioural observations under different conditions.

It is not possible to consider and test all factors that may potential influence an animal's behaviour. Therefore, the multitude of factors influencing behaviour can become a limiting feature of behavioural studies. The examples discussed above illustrate how factors can be isolated by verifying the findings under different conditions. If it is possible to reach similar conclusions under different conditions the potential that the conclusion was not founded on confounding factors is improved.

Predictions

The following discussion evaluates the potential for making meaningful predictions of behaviour. A qualitative comparison was drawn to determine whether there was a relationship between observations made in the laboratory and the change in the rate of

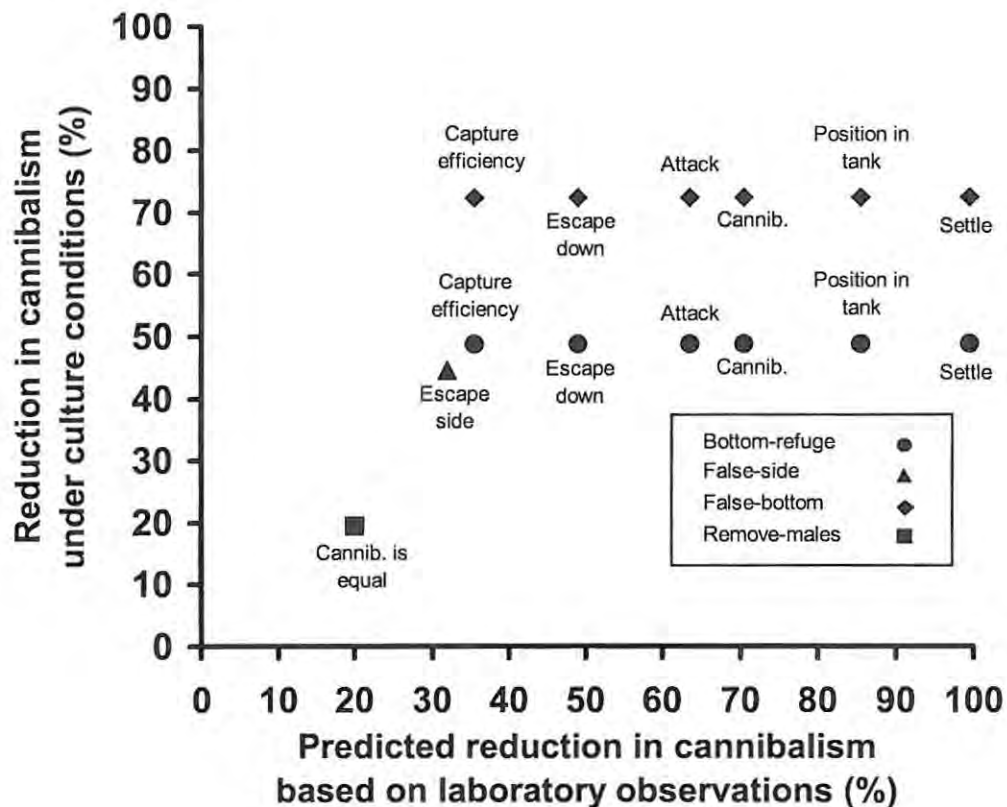


Figure 7.1. The relationship between behavioural observations made in the laboratory (Chapters 3, 4 and 5) and the reduction in cannibalism that occurred under the culture conditions (Chapter 6) when different production procedures or technologies were employed.

cannibalism under different farming conditions (Figure 7.1). Extrapolations were ranked based on the difference between predicted value (i.e. that based on observations made in the laboratory) and reduction in cannibalism realised under different culture conditions (Table 7.1). Observations such as the downward escape behaviour of juveniles and the position of attack and cannibalism, and the observation that males and females were equally cannibalistic, where all extrapolated with good accuracy (Table 7.1). However, some of the behaviours were better extrapolated than others. For example, the downward escape behaviour of juveniles predicted the reduction in cannibalism under culture conditions with provision of bottom-refuge with good accuracy, whereas the same behaviour explained the reduction in cannibalism in tanks with a false-bottom less

accurately. The position that juveniles settled in the tank, capture efficiency and the proportion of time that adults spent at different positions in the water column were extrapolated with less accuracy.

Table 7.1. The reduction in the rate of cannibalism that was realised under different culture conditions (B) was subtracted from the predicted reduction in cannibalism based on observation made in the laboratory (A). Extrapolations were ranked according to the difference between A and B.

Rank	Laboratory Behaviour	A (%)	Culture Condition	B (%)	A – B
1	Escape down	49.0	Bottom-refuge	48.7	0.3
2	Equal cannibalism	20.0	Remove-male	19.5	0.6
3	Cannib. bottom	70.5	False-bottom	72.2	-1.7
4	Attack bottom	63.5	False-bottom	72.2	-8.7
5	Escape side	32.0	False-side	44.5	-12.5
6	Capture efficiency	35.5	Bottom-refuge	48.7	-13.2
7	Adults at bottom	85.5	False-bottom	72.2	13.3
8	Attack bottom	63.5	Bottom-refuge	48.7	14.8
9	Cannib. bottom	70.5	Bottom-refuge	48.7	21.8
10	Escape down	49.0	False-bottom	72.2	-23.2
11	Settle on bottom	99.5	False-bottom	72.2	27.3
12	Capture efficiency	35.5	False-bottom	72.2	-36.7
13	Adults at bottom	85.5	Bottom-refuge	48.7	36.8
14	Settle on bottom	99.5	Bottom-refuge	48.7	50.8

Overall, the laboratory observations accounted for the behaviour of *X. helleri* under different culture conditions. Yet, this hypothesis should be tested using different populations of *X. helleri* and is offered here as a tool to predict behaviour of this species until future research develops other hypotheses or more refined models that better explain these findings. The application of these predictions will be considered further in the following discussions.

Application to industry

The production of tropical fishes in South Africa for the aquarium fish trade is largely restricted to small, intensive systems where environmental conditions such as temperature and photoperiod can be readily controlled (Kaiser *et al.*, 1997). For this reason the culture of ornamental species in many parts of the subcontinent are confined to indoor hatcheries or greenhouse tunnels where predominantly clear-water recirculating systems are used (Kaiser *et al.*, 1997), in contrast to extensive green-water pond culture systems commonly used by producers in the tropics (Singapore and Malaysia, 1999, personal observation). It is necessary to maximise production in intensive recirculating systems because space is limited, operating costs are high and infrastructure costs to establish them need to be recovered within a short time. Poeciliids make up a significant portion of the local and international ornamental fish trade (Kaiser *et al.*, 1997; Jones *et al.*, 1998a; Jones *et al.*, 1998b). Therefore, research has focused on many aspects of poeciliid biology, such as nutrition, mating behaviour and reproduction as well as their response to different social and abiotic environmental conditions such as sex ratio, stocking density and water flow rates (Snelson and Wetherington, 1980; Burns, 1985; Meffe and Snelson, 1989; Winemiller, 1993; Bisazza and Pilastro, 1997; Jones and Williams, 1997; Kaiser *et al.*, 1997; Kaiser and Olivier, 1997; Kaiser and Jones, 1997; Kolluru and Joyner, 1997; Marler *et al.*, 1997; Pilastro *et al.*, 1997; Trexler, 1997; Jones *et al.*, 1998a; Bisazza *et al.*, 2001; Kruger *et al.*, 2001a; Kruger *et al.*, 2001b). Cannibalism occurs in feral and captive-bred poeciliids (Thibault, 1974; Fox, 1975; Polis, 1981; Meffe and Snelson, 1989; Smith and Reay, 1991; Nesbit and Meffe, 1993) and various forms of cannibalism have been identified as debilitating factors in the commercial culture of many fish species (Folkvord, 1991; Smith and Reay, 1991; Hecht and Pienaar, 1993; Ottera and Folkvord, 1993; Baras and Jobling, 2002), including *X. helleri* (Jones *et al.*, 1998a;

Chapter 2). Prior to this thesis and related work (Jones, 1998; Jones *et al.*, 1998a; Jones *et al.*, 1998b; Jones and Kaiser, 1999; Jones and Kaiser, 2002; Jones *et al.*, 2002) no research has looked at the cannibalistic and general *post-partum* behaviour of the swordtail (*X. helleri*) under culture conditions. One of the objectives of this work was to ascertain if cannibalism poses a problem to industry production, and if so, to determine whether it is possible to mitigate cannibalism under farm culture conditions.

These objectives were achieved. Firstly, it was found that the rate of intercohort cannibalism in *X. helleri* under practical farming conditions was as high as 62% (Chapter 6); secondly, technologies were implemented (Chapter 6) that reduced the rate of cannibalism under farming conditions and these technologies were developed based on the observation studies of this thesis (Chapters 3, 4 and 5). For example, floating refuge had a positive effect on juvenile survival (Jones *et al.*, 1998a; Chapter 2). Furthermore, it was found that juveniles escaped in a downward direction more often than in any other direction (Chapter 5), and they were found at the bottom of the water column most often. It is a widespread practice for commercial producers of poeciliid fishes in South Africa and in other parts of the world such as Singapore and Malaysia to provide cover in which the juveniles are able to hide, however, such cover is commonly provided at the water surface (1999, personal observation). The findings of this thesis suggest that refuge placed at the bottom of the water column, or the inclusion of a false-bottom or a false-side, will significantly reduce the rate of intercohort cannibalism in *X. helleri*.

However, the relatively high numbers of dead and deformed juveniles that occurred in treatments where adults did not have access to the bottom of the tank and so could not cannibalise them, made questionable whether reduced cannibalism would actually result in enhanced poeciliid production since mitigating technologies may promote the survival of

less healthy juveniles. Smith and Reay (1991) suggested that the mitigation of cannibalism under culture conditions might have a negative impact on production in cases where the survival of smaller, slower growing or otherwise inferior fish is promoted by a reduction in the rate of cannibalism. Furthermore, Baras and Jobling (2002) end their discussion on the mitigation of cannibalism under culture conditions by suggesting that it might be disadvantageous to employ mitigating measures if the fish that are protected from cannibalism have lower resistance to stressors in the long-term. The occurrence of dead and deformed juveniles as observed in this study strongly supports the suggestions of Smith and Reay (1991) and Baras and Jobling (2002), but the long-term effect of increased survival of potentially unhealthy or less viable juveniles, due to a reduced rate of cannibalism, needs to be established.

Furthermore, a reduction in the rate of cannibalism may not be beneficial to the commercial production of species where intercohort cannibalism has been shown to enhance the reproductive success of the cannibal (Meffe and Crump, 1987; Fitzgerald, 1992a and 1992b). Fitzgerald (1991) used the behaviour of fish from the family Gasterosteidae to support the theory that filial cannibalism takes place to maximise lifetime reproductive success. Also, the consumption of conspecific flesh had a positive effect on both the somatic growth and the reproductive index of the mosquitofish (*Gambusia affinis*, Poeciliidae), with both being significantly greater after a period of 50 days (Meffe and Crump, 1987). Similar findings were not obtained in the present study, as no increase was found in fecundity, after a period of 84 days, in adults that were relatively more cannibalistic. Since cannibalism ensures the removal of deformed juveniles and possibly other unhealthy or less viable juveniles, and since there is evidence that cannibalism improves the fitness of some poeciliid fishes (Meffe and Crump, 1987), production tanks that combine a false-side with a solid bottom, and so allows cannibalism

of weaker individuals off the tank floor while still enhancing survival of non-deformed or potentially more viable juveniles, may be more beneficial to the commercial production of *X. helleri* than the use of tanks with a false-bottom. This supports the proposal that it may be possible to exploit cannibalism to the advantage of commercial aquaculture by not preventing it and thus promoting the removal of potentially less viable fish (Baras and Jobling, 2002).

Although tanks designed with a false-side and a solid bottom promoted the survival of healthy juveniles, the method used to harvest them from below such a cage requires further research; harvesting was labour-intensive and may have contributed to broodstock mortality. Harvesting juvenile *X. helleri* from below mesh cages involved removing the cage containing the adults so that the juveniles could be collected from beneath it. Broodstock mortality ranged from 1.8 ± 0.6 to 3.0 ± 0.6 per tank of eight females during the 84-day trial, whereas there were no adult mortalities in the experiments of Chapter 2, only a single reported mortality in a similar experiment carried out by Kruger *et al.* (2001a), and no broodstock mortalities reported by Jones *et al.* (1998a). The uncharacteristically high mortality in the present study is evidence that the collecting procedure may have been stressful to the adult fish. Thus, harvesting juveniles from below the cages had disadvantages, and the current harvesting technique requires further development. Coincidentally, the collection-frequency experiment of Chapter 6 showed that daily harvesting did not improve production. This, combined with the findings from the tank design-study of Chapter 6, leads to a recommendation for reduced harvest frequency.

Practical application of predictions

The following method was developed to determine the relationship between laboratory observations of fish behaviour and actual behaviour under culture conditions, and

attempts to make the extrapolations presented in Figure 7.1 and Table 7.1 available for practical application by fish farmers in the ornamental fish industry. A formula was developed to predict the cannibalistic behaviour of fish under culture conditions based on behavioural observations made in small aquaria. This formula is based on the assumption that the relationship between the behaviour observed in the laboratory and behaviour under culture conditions, as reported in this study, will remain the same for other populations. The utility of this formula must be tested with other populations and possibly other species; if successful the hypotheses from which the formula was derived can be accepted and retained until new factors are identified that better explain the findings.

Example

It is hypothesised that a reduction in the rate of cannibalism with the use of false-sides relates to the direction in which juveniles escape, and that the relationship will remain constant under different environmental conditions for different populations of *X. helleri*. Thirty-two percent of juvenile escape incidences took place in a sideways direction in the laboratory and it was thus predicted that cannibalism could be reduced by a similar margin if the juveniles could escape through a false-side. Under culture conditions, the rate of cannibalism was reduced by 45% with the inclusion of a false-side. Therefore, the relationship (ω) between sideways escape behaviour and the reduction of cannibalism as a result of a false-side would be:

$$\omega = \frac{45}{32} = 1.41$$

Equation 7.1

Table 7.2. The relationship (ω) between laboratory observations (p) and the reduction of cannibalism (k) when different technologies (t) were employed under farm culture conditions.

Prediction based on laboratory observation	p (%)	Culture technology (t)	k (%)	ω -value
All adult fish are equally cannibalistic (Ch 2&4)	20.0	Remove-males	19.5	0.97
Juveniles escape in sideways direction (Ch 5)	32.0	False-side	44.5	1.39
Offspring escape in a downward direction (Ch 5)	49.0	False-bottom	72.2	1.47
Attacks occur at the bottom of water column (Ch 3&5)	63.5	False-bottom	72.2	1.14
Cannibalism occurs at bottom of water column (Ch 3&5)	70.5	False-bottom	72.2	1.02
Prey efficiency at bottom of water column (Ch 3&5)	35.5	False-bottom	72.2	2.03
Offspring settle at the bottom (Ch 5)	99.5	False-bottom	73.2	0.74
Adults positioned at bottom of water column (Ch 4)	85.5	False-bottom	72.2	0.84
Offspring escape in a downward direction (Ch 5)	49.0	Bottom-refuge	48.7	0.99
Attacks occur at the bottom of water column (Ch 3&5)	63.5	Bottom-refuge	48.7	0.77
Cannibalism occurs at bottom of water column (Ch 3&5)	70.5	Bottom-refuge	48.7	0.69
Prey efficiency at bottom of water column (Ch 3&5)	35.5	Bottom-refuge	48.7	1.37
Offspring settle at the bottom (Ch 5)	99.5	Bottom-refuge	48.7	0.49
Adults positioned at bottom of water column (Ch 4)	85.5	Bottom-refuge	48.7	0.57

The constant $\omega = 1.41$ can be used to predict the relationship between the sideways escape behaviour of juveniles in other populations and the reduction in cannibalism that can be expected if a false-side is applied to production conditions. It is hypothesised that if behaviour observed in the laboratory is a consequence of an unknown or hidden factor, then the constant (ω) will not successfully predict or describe the behaviour of *X. helleri* in other populations. The alternative hypothesis assumes that Equation 7.2 will successfully predict cannibalism in other populations indicating that the parturition-associated behaviour observed throughout this study is applicable to *X. helleri* under different conditions. Under these terms, a reduction in the rate of cannibalism (k) may be predicted according to Equation 7.2:

$$k_t = p * \omega$$

Equation 7.2

where t represents the technology employed to reduce cannibalism, p is the laboratory observation used to make the prediction and ω is the relationship between the reduction in cannibalism and the behaviour observed in the laboratory studies. The ω -value for the relationship between sideways escape behaviour and the reduction of cannibalism due to use of a false-side, as determined by Equation 7.1, is one example. By using the same method that was applied to calculate the relationship (ω) in Equation 7.1, it was possible to calculate the relationship (ω) between each behaviour and the reduction in cannibalism due to each technology that was employed under the farm culture conditions (Table 7.2).

By way of example, Equation 7.2 is used to predict the reduction in the rate of cannibalism when refuge is placed on the bottom of the tank, and for this example, adult attack behaviour at the bottom of the tank is used as the predictor. The ω -value obtained from

Table 7.2 is $\omega = 0.77$, while it was observed in the laboratory that 63.5% of attacks took place at the bottom of the tank, so $p = 63.5$:

$$k_{\text{bottom-refuge}} = 63.5 * 0.77 = 49\%$$

Equation 7.3

If the ω -value established in Table 7.2 and a single behaviour under one set of environmental conditions together have the capacity to predict cannibalism under a second set of environmental conditions, then Equation 7.2 can be used to predict or describe the degree of cannibalism in other populations of *X. helleri*. Likewise, the capability of the equation to describe changes in cannibalism should be tested for other poeciliid species with cannibalistic behaviours similar to *X. helleri*. Future research is invited to test these hypotheses; if accepted, then Equation 7.2 and the ω -values established in Table 7.2 can be used to predict and describe cannibalism in other populations of *X. helleri* until new factors are identified that may better explain the findings of this thesis.

The selection of technology used to reduce cannibalism under farming conditions

The production procedures and technologies employed here may not be limited to culture of *X. helleri*. These concepts might enhance the commercial production of other poeciliids and even fishes of other families. The key in Table 7.3 was formulated to determine which technologies or production procedures were most effective at reducing cannibalism; it is hypothesised that this key will help predict the most suitable production technology for any fish species with similar cannibalistic behaviour.

Table 7.3. Key to determine the most suitable production procedures and technologies that may be used to reduce intercohort cannibalism in the Poeciliidae. The most suitable suite of production procedures and technologies can be determined by either accepting or rejecting the different observations.

Couplet	Observation	Go to Couplet ...
1	No photoperiod and a constant light intensity of 0.54×10^{15} quanta/s/cm ² or less, has a negative effect on fecundity or fertility	Accept ... 4 / Reject ... 2
2	Adults cannibalise significantly fewer juveniles at a light intensity of 0.54×10^{15} quanta/s/cm ² or less, compared to higher intensities	Accept... 3 / Reject... 4
3	A constant light intensity of 0.54×10^{15} quanta/s/cm² or less may be used as a tool to reduce cannib.	... 5
4	A constant light intensity of 0.54×10^{15} quanta/s/cm² or less can not be used as a tool to reduce cannib.	... 5
5	Refuge significantly reduces the occurrence of cannib.	Accept... 6 / Reject... 13
6	More than 50% of cannibalism takes place close to the surface	Accept... 9 / Reject... 7
7	More than 50% of cannibalism occurs in middle of water column	Accept... 10 / Reject... 8
8	More than 50% of cannibalism takes place close to the bottom	Accept... 11 / Reject... 12
9	Refuge at the top of the water column can reduce cannib.	... 14
10	Refuge in the middle of the water column can reduce cannib.	... 14
11	Refuge at the bottom of the water column can reduce cannib.	... 14
12	Refuge is needed in all areas of the tank to reduce cannib.	... 14
13	Refuge cannot be used as a tool to reduce cannib.	... 14
14	Adults spend more than half their time at the bottom	Accept... 15 / Reject ... 17
15	Juveniles escape downwards most often when attacked	Accept... 16 / Reject... 17
16	A false-bottom can be used as a tool to reduce cannib.	... 18
17	A false-bottom can not be used as a tool to reduce cannib.	... 18
18	At least 30% of juveniles escape in a sideward direction	Accept... 19 / Reject... 20
19	A false-side can probably be used as a tool to reduce	... 21
20	A false-side can not be used as a tool to reduce cannib.	... 21
21	Sperm can be stored by the female	Accept... 22 / Reject... 26
22	Males and females are equally cannibalistic	Accept... 24 / Reject... 23
23	Males are more cannibalistic than females	Accept... 24 / Reject... 25
24	The introduction of males to the female's tank for short periods of time only, to allow fertilisation to take place, can probably be used as a tool to reduce cannib.	... 27
25	The removal of ungravid females from the population may be used as a tool to reduce cannib.	... 27
26	Removal of males is not a practical method of reducing cannib.	... 27
27	Juveniles do not become less vulnerable to cannib. with age	Accept... 28 / Reject... 29
28	Increased harvest frequency will reduce cannib.	
29	Increased harvest frequency will not reduce cannib.	

The lack of behavioural data on different fish species is the most apparent limitation to this key. However, the protocol discussed above was developed with the aim of aiding the process of collecting the most suitable information required to understand cannibalistic behaviour in poeciliid fishes. Furthermore, as more behavioural data becomes available the key could be expanded and modified and, therefore, become increasingly more useful to the ornamental fish industry. Another limitation may be the key's applicability to other species. However, the extent of this limitation is unknown and requires investigation. Nonetheless, as additional data from different species becomes available it may be possible to further develop the hypotheses presented here and thus improve the key by making it more universally applicable to ornamental fishes that exhibit intercohort cannibalism.

The adaptive significance of cannibalism

There is evidence that cannibalism is at least partly under genetic control (Thibault, 1974; Fox, 1975; Polis, 1981; Pienaar, 1990; FitzGerald and Whoriskey, 1992; Hecht and Pienaar, 1993). This was first shown by Thibault (1974) when two closely related poeciliids (*Poeciliopsis monacha* and *P. lucida*) were hybridised. Thibault (1974) found that 74% of the *P. monacha*-*P. lucida* hybrids grew up to cannibalise their own progeny, while 95% of the parent *P. monacha* were shown to be cannibalistic and *P. lucida* were never observed to cannibalise their young. Furthermore, when the hybrids were backcrossed to each of the parent species the proportion of the offspring that grew up to cannibalise their young was 88% and 12% for hybrid-*P. monacha* and hybrid-*P. lucida*, respectively (Thibault, 1974). The differences in phenotype between the parent species, the hybrids and the backcrosses indicated polygenetic inheritance (Fox, 1975).

If cannibalism is under genetic control, then it is likely to be responsive to selection pressures (Bobisud, 1976; Polis, 1981; Hecht and Pienaar, 1993). In addition to the obvious nutritional advantages of cannibalism (Fox, 1975; Jones, 1982; Meffe and Crump, 1987; Hecht and Appelbaum, 1988; Folkvord, 1991; FitzGerald, 1992b; FitzGerald and Whoriskey, 1992; Folkvord and Ottera, 1993; Baras, 1999; Baras *et al.*, 2000a; Baras *et al.*, 2000b; Baras and Jobling, 2002) and the elimination of potential conspecific competitors for resources such as food, potential mates or living space (Fox, 1975; Polis, 1981; Jones, 1982; FitzGerald and Whoriskey, 1992), there are three hypotheses that may explain the adaptive advantage of cannibalism among closely related individuals. They are (1) *individual fitness*, (2) *inclusive fitness*, and (3) *parental manipulation* (Polis, 1981). According to all theories, the cannibal benefits as it increases its chances of making a genetic contribution to future generations. The difference between the hypotheses lies in the genotypic gain or loss experienced by the relative that is cannibalised: the *individual fitness* theory assumes that the victim experiences a genetic loss; *inclusive fitness* means that some of the victim's genes will be passed on because closely related kin will have a greater chance of expressing their own genotype, which includes variable proportions of the victim's genotype; and *parental manipulation* assumes that the fitness of the victim's parent is enhanced through the survival of offspring that were not cannibalised.

Noakes (1992) defined the ultimate factors affecting the behaviour of a fish as the long-term or evolutionary consequence of behaviour, while the proximate causes for behaviour refer to the immediate or short-term physiological mechanisms of behaviour. The adaptive significance of cannibalism among some of the care-giving species has been successfully explained in terms of *parental manipulation*, also described as the parental investment; here the lifetime reproductive success of the parent is enhanced by

cannibalising some of its own young (DeMatini, 1987; Marcondalo and Bisazza, 1988; Belles-Isles and FitzGerald, 1990; FitzGerald, 1991). The *parental manipulation* theory requires that the parent experiences reproductive advantage as a result of their cannibalistic behaviour, which ultimately increases their genetic fitness. The reproductive performance and survival of *X. helleri* that were more cannibalistic did not appear to differ from that of less cannibalistic fish (Jones *et al.*, 1998a; Chapter 2 and Chapter 6). With no direct benefit to the parent's reproductive performance and survival due to its cannibalistic behaviour, the theory of *parental manipulation* does not appear to explain filial cannibalism in *X. helleri* from the data accumulated in this work. There was no evidence that cannibalism increased the fitness of any of the other cannibals in the population, because the gonadosomatic-index, fecundity and somatic weight of individuals from populations where cannibalism was high were no different from those where the rate of cannibalism had been significantly reduced (Jones *et al.*, 1998a; Chapter 2 and Chapter 6). Therefore, the *individual fitness* theory (Polis, 1981) cannot fully explain filial cannibalism in *X. helleri*. The fish in this study were fed in excess of satiation and always had access to algae and associated food, which might have masked the effect of cannibalism on reproductive performance of *X. helleri*. However, cannibalism is independent of alternative food availability in poeciliids such as *P. monacha* (Thibault, 1974) and a cannibalistic diet positively affected the somatic and reproductive performance of the mosquitofish (*G. affinis*) over alternative artificial food (Meffe and Crump, 1987), so it is unlikely that the artificial diet influenced the effect of cannibalism on reproductive performance in the present study. However, as reproductive performance of the mosquitofish was enhanced by cannibalism, the theories of *individual fitness* and *parental investment* may contribute to an explanation for cannibalism in at least that poeciliid. Nonetheless, there is no evidence that cannibalism in *X. helleri* enhances genotypic expression of the cannibal through improved reproductive success.

Although there is no evidence to suggest that the cannibal experiences an increase in fitness due to its behaviour (i.e. no change in the reproductive output of more cannibalistic fish compared to less cannibalistic fish), the adaptive advantage of cannibalism in *X. helleri* may be accounted for, at least partly, in terms of the genetic benefit experienced by the victim of cannibalism. Polis (1981) suggested that *inclusive fitness* explains the evolution of cannibalism between relatives because the genotype of the victim is partly expressed in future generations through closely related kin who may have benefited from the death of the victim. Although the selection for cannibalism does not necessarily require large increases in fitness (Fox, 1975), the genotypic gain experienced by the victim's kin must outweigh any loss experienced by the victim. Deformed juveniles were harvested from the treatments that prevented the adult fish from accessing the bottom of the tank. However, when there was no false-bottom or refuge at the bottom of the water column these juveniles must have been consumed. It is hypothesised that if a deformed juvenile could survive to a reproductive age, the cannibalism of that juvenile would instead ensure that such individuals with potentially less viable genes are unable to express their genotype in future generations.

Therefore, the presence of a false-bottom or bottom refuge might allow the survival of potentially inferior fish that will negatively effect the genotype of future generations. This supports the proposal that the mitigation of cannibalism under culture conditions might in the long-term result in a population of individuals that are less resistant to stressors (Baras and Jobling, 2002). It could be further hypothesised that cannibalism results in selection pressures that prevent potentially undesirable genes from being included in the next generation. These hypotheses could be tested by comparing the physical viability of the individuals in successive generations in populations where cannibalism of weaker

individuals is allowed to take place, to that of individuals from populations in which the adults are largely prevented from cannibalising the weakest juveniles.

An animal's genotype is responsible for the expression of its phenotype, and part of the phenotype may enhance the physical fitness of the individual while other parts may be detrimental to its survival. Possibly, not all genes of a deformed fish are inferior or detrimental to its survival. Yet, if the deformed fish were to survive and pass its genes onto the next generation a greater percentage of the following generation would carry the inferior gene to the potential detriment of viable genes. The incidence of dead and deformed juvenile *X. helleri* went largely unnoticed before the experiments under practical farming conditions (Chapter 6); this may exemplify why the adaptive significance of cannibalism in this species, as relates to the occurrence of dead and deformed juveniles, has not been previously discussed in the literature. Nonetheless, it can now be hypothesised that the genotype of the victim might benefit to some extent if the victim were cannibalised so that only the victim's viable genes are passed onto the next generation through the genes of closely related kin. Therefore, it is suggested that the theory of inclusive fitness as discussed by Polis (1981), could be further developed to include, not only the expression of the victim's genes through the expression of the genotype of closely related kin, but the victim's own genotype is enhanced as inferior genes are not expressed in future generations. In other words, the victim's genotype is expressed in future generations through the enhancement of survival of kin, but the genes responsible for its inferior health are not passed onto future generations. The exclusion of inferior or less viable genes is as much an advantage to the survival of the victim's genotype in future generations as the expression of viable genes through the survival of the victim's kin. It is hypothesised that the exclusion of certain genes from future generations is advantageous to a victim of cannibalism when the victim was deformed and

it is suggested that this *gene exclusion* theory, presented here for the first time, may contribute to the explanation for the adaptive significance of cannibalism of closely related kin in *X. helleri* and possibly other species. This hypothesis remains to be evaluated by future research.

While the theories of *inclusive fitness* and *gene exclusion* might describe the long-term consequence of cannibalism in *X. helleri*, the most likely proximate benefit for the cannibalism of juvenile *X. helleri* is nutritional, which is probably a by-product of opportunistic predation. Smith and Reay (1991) suggest that in most cases cannibalism is probably only a result of intraspecific predation and Hrdy (1974) stated that "...cannibalism appears to be an end in itself not clearly distinguished from predation." There was no evidence to suggest that adult *X. helleri* actively sought conspecific juveniles over other food items. On the contrary, the rate of cannibalism in *X. helleri* decreased when the availability of food items other than live juveniles became available and cannibalism decreased when adults moved out of the area of the tank where juveniles were most abundant. In addition, in situations where capture efficiency dropped there did not seem to be any compensatory behaviour to increase the success of cannibalistic attacks. These results suggest that the cannibalism of juveniles is a result of chance encounter with an adult, which is not very different from the encounter-related intracohort cannibalism described by Hecht and Appelbaum (1988) and further supports the theory that the proximate cause of intercohort cannibalism in *X. helleri* is opportunistic predation where the prey happens to be a conspecific.

The recovery of spent energy is a potential benefit of the proximate nutritional advantage of cannibalism to the birth-giving female. Some poeciliids such as *P. reticulata*, *G. affinis*, *P. monacha* and *X. helleri* invest considerably in egg development and produce relatively

large yolk-filled eggs of approximately 2 mm in diameter, while females of others such as *Heterandria formosa* and some of the *Poeciliopsis* species that produce smaller eggs of 0.4 to 0.8 mm in diameter continue to contribute nutrients to the embryo during development (Constantz, 1989). Whether the investment in reproduction is made pre- or post-fertilization, all female poeciliids seem to experience a high energetic cost in reproduction with the benefit of an increase in offspring survival. However, it was found that a significant percentage of juvenile *X. helleri* that were cannibalised were physically inferior and so were unlikely to contribute to the fitness of the female. Since the female had already made energetic investments in these weak offspring, the female parent may have more to lose if the juvenile should fall victim to cannibalism by another adult. If the female were to cannibalise her own juvenile she would recover part of her investment (Smith and Wootton, 1995), and if the juvenile were physically inferior, her genetic fitness would not be compromised. Although this study presents no evidence that cannibalism increases the fitness of the cannibal, it is hypothesised that the recovery of energy is a proximate reason for filial cannibalism in *X. helleri*.

Towards an explanation for filial cannibalism

Bertram (1976) concluded that it would be "extremely dubious" to identify a selection pressure as a single cause for a behaviour. The following discussion does not attempt to describe or identify the pressures responsible for or against the selection of cannibalism, rather evidence is presented to show that selection against filial cannibalism in *X. helleri* is not necessarily expected. Filial cannibalism is the cannibalism of closely related offspring (Rohwer, 1978) and for the purpose of this discussion the term is limited to the consumption of juveniles by parental adults only.

Cannibalism appears genetically driven in some species of the Poeciliidae (Thibault, 1974) and selection advantages probably exist for cannibalism to persist (Bobisud, 1976; Polis, 1981; Hecht and Pienaar, 1993). Furthermore, it has been demonstrated that cannibalism can exist as an evolutionary stable strategy (Bobisud, 1976; Stenseth, 1985; Smith and Reay, 1991; Lindstrom, 2000). However, the adaptive advantage or benefit of cannibalism may be offset by detrimental factors such as the cannibalism of closely related kin, which would result in decreased fitness (Smith and Reay, 1991). If the genetic loss experienced by a filial cannibal as a result of its own cannibalistic behaviour were greater than the genetic value of the offspring that survive, selection against filial cannibalism can be expected. Fox (1975) suggested that cannibalism becomes disadvantageous when the cannibal destroys its own offspring and subsequent genotype faster than that of its conspecific competitors, and under such circumstances selection against the cannibalistic trait would be expected. Therefore, where the risk of cannibalising close kin is high some kind of inhibitory mechanism would be expected, such as those associated with parental care and kin recognition (Smith and Reay, 1991). For example, Loekle *et al.* (1982) found that female guppies (*P. reticulata*) and black mollies (*Poecilia sphenops*) were less likely to cannibalise their own juveniles than non-related conspecific juveniles. However, there is no evidence to suggest that such kin recognition takes place in *X. helleri*. Observations in this work indicate that kin recognition is not likely to occur, because adults that were most closely related to the juveniles (i.e. the birth-giving female and the male parent) were as cannibalistic as unrelated or more distantly related adults in the population.

In the following discussion a value is assigned to the genetic loss caused by filial cannibalism. Similarly, a genetic value is given to those juveniles that were not

cannibalised. A comparison of these values will show the extent of genetic loss due to filial cannibalism.

In order to make this comparison it was necessary to establish a unit of measure able to quantify genetic contribution or loss. Genetic fitness is measured firstly in terms of an animal's viability (i.e. whether or not it can survive), and secondly by its reproductive ability, wherein the fittest individual has the highest reproductive output (Falconer, 1989). Therefore, an animal's fitness can be measured by the number of juveniles produced. Provided it is possible to establish the genetic relatedness of a juvenile to other individuals in the population, the survival or loss of juveniles can be used as a unit to measure the genetic success or loss relevant to other individuals in the population.

The *degree of relatedness* (τ) between individuals in a population is the probability that a gene in one individual is a replica of a gene in the other (Bertram, 1976). It is possible to predict the probability that one individual in a population is related to another. Falconer (1989) proposed that the approximate degree of relatedness (τ) (or *rate of inbreeding*) in a population is related to the effective number of reproductively active males (N_m) and females (N_f), and can be estimated for small populations as follows (Falconer, 1989):

$$\tau = \frac{1}{8 * N_m} + \frac{1}{8 * N_f} \quad \text{Equation 7.4}$$

Equation 7.4 can be used to estimate the probability that the genotype of one individual in the population is the same as another individual in the population, where τ is not already known. For example, it is possible to calculate the average degree of relatedness of the adults used in this study because all the fish were drawn from a single breeding

population of *X. helleri* maintained in the Rhodes University greenhouse tunnel. The population comprised approximately 3,000 adults at the time of the study, with an even sex-ratio. The population originated from multiple broods over an unknown number of generations. The average degree of relatedness (τ) between the breeding individuals in this particular population may be calculated as:

$$\tau = \frac{1}{8 * 1500} + \frac{1}{8 * 1500} = 0.000167 \quad \text{Equation 7.5}$$

The process of meiotic division that takes place during gamete formation in diploid species ensures that a parent contributes half of its genes to each gamete, which means that on average $\tau = 0.5$ between a parent and its offspring (Bertram, 1976). The τ -value of 0.000167 (Equation 7.5) for individuals of the breeding population in this study will not be included in the following calculations (i.e. it is assumed to be negligible).

An equation to determine the *degree of genetic loss* (γ) as a result of a parent's own cannibalistic behaviour can be formulated using the control treatment in the first experiment of Chapter 6 as an example. An average of 62% of the juveniles were cannibalised in this treatment. This calculation will later be extended to other treatments. The total number of juveniles born per brood was based on fecundity, which averaged 108, and the average number that were cannibalised per brood was 67 juveniles. Since all adult fish in the experimental population contributed equally to the cannibalism of the offspring, it can be deduced that the parent's contribution to the cannibalism of its own offspring is probably the same as that of other adults in the population. Thus, a parent's contribution is equivalent to total cannibalism divided by the number of adults in the

population. The following equation can be used to determine the *degree of genetic loss* (y) as a result of a parent's cannibalistic behaviour:

$$y = \frac{\tau * c}{b} \quad \text{Equation 7.6}$$

where:

τ = genetic contribution or *degree of relatedness*;

c = number of juveniles cannibalised in a single brood;

b = number of cannibalistic adults in the population.

The results of Chapter 6 showed that some of the cannibalised juveniles from the control treatment were dead and deformed and therefore genetically unviable. Since the number of dead and deformed juveniles could be counted, it was possible to estimate the female's contribution to the cannibalism of her genes that did not include dead and deformed juveniles. A parent's contribution to the cannibalism of its own potentially viable genes may be estimated using the following equation:

$$y_2 = \frac{\tau * (c - d)}{b} \quad \text{Equation 7.7}$$

where τ , c and b are used as in Equation 7.6, and d = number of juveniles that were born dead or deformed.

The average number of juveniles cannibalised per brood had already been estimated at 67 and the genetic input is already known, while the probability of finding dead and

deformed juveniles was found to be between at least 0.15 but probably closer to 0.32 (i.e. 34.6 of 108 juveniles) (Chapter 6). Thus, it becomes possible to determine the degree of genetic loss experienced by a parent by applying the data to Equation 7.7:

$$y_2 = \frac{0.5 * (67.0 - 34.6)}{10} = 1.6 \text{ juveniles} \quad \text{Equation 7.8}$$

Hence, the probability that a female will cannibalise her own genes is $1.6/108 = 0.015$, after her genetic loss due to dead and deformed juveniles has been taken into account. However, the probability that a male will cannibalise his own genes would not be the same in this instance because the sex-ratio in the study population was skewed in favour of females. For every one male there were four females. Thus, per breeding cycle a male would have had four times as many offspring carrying his genes than a female. To make an estimate of a parent's contribution to the loss of its own genes due to his or her cannibalistic behaviour the total number of broods (e) to which he or she made a genetic contribution must also be taken into account:

$$y_3 = \frac{\tau * (c - d) * e}{b} \quad \text{Equation 7.9}$$

If a female gave birth once in a given period the degree of loss of viable genes would not differ from that calculated in Equation 7.8. However, that of a male over the same period in a population with a sex ratio of 1:4 (male:female) would be:

$$y_3 = \frac{0.5 * (67.0 - 34.6) * 4}{10} = 6.5 \text{ juveniles} \quad \text{Equation 7.10}$$

Although the male appears to cannibalise more potentially viable genes, he also produced more. Thus, in this population structure the probability that the male will cannibalise his own viable genes is no different to that of the female: $6.5/(108*4) = 0.015$. The similar genetic loss between males and females suggests that the selection pressures placed on them due to cannibalism would be similar.

The genetic value to the parent fish of the juveniles that survived (s) can be determined by subtracting the sum of the genetic value of the dead and deformed juveniles and the genetic value of the viable genes that were cannibalised, from the total genotype. Therefore, in order to determine the genetic value of the juveniles that survived (s), it is first necessary to estimate the genetic value of the dead and deformed juveniles that were cannibalised (z) and the total value of the genotype (t). The loss of genotype experienced by either the male or a female parent can be estimated using the following equation:

$$z = \tau * d * e \quad \text{Equation 7.11}$$

where z represents the genetic loss due to dead or deformed juveniles, τ is the parent's genetic contribution, d represents the number of dead and deformed juveniles per brood, and e represents the number of broods parented over a given period. For a single brood-cycle, this would equate to the following for females:

$$z = 0.5 * 34.6 * 1 = 17.6 \text{ juveniles} \quad \text{Equation 7.12}$$

Over the same period with the same assumptions as the previous examples, Equation 7.11 would amount to the following for males:

$$z = 0.5 * 34.6 * 4 = 69.1 \text{ juveniles} \quad \text{Equation 7.13}$$

The total value of the genotype (g) to a parent can be calculated using the average degree of relatedness between the parent and its offspring (τ), the total number of juveniles produced in a single brood (f), which is equivalent to fecundity, and the number of broods to which the parent has made a genetic contribution (e):

$$g = \tau * f * e \quad \text{Equation 7.14}$$

For a female parent from the control group of Chapter 6 this would equate to:

$$g = 0.5 * 108 * 1 = 54 \text{ juveniles} \quad \text{Equation 7.15}$$

while for the male parent from the same population over the same period this would amount to:

$$g = 0.5 * 108 * 4 = 216 \text{ juveniles} \quad \text{Equation 7.16}$$

The genetic value to the parent fish of the juveniles that survived (s) can be determined by subtracting the sum of the genetic value of the dead and deformed juveniles (z) and genetic value of the viable genes that were cannibalised (y), from the total genotype (g). As the y -value, the z -value and the g -value have been established for both males and

females (Equations 7.6 to 7.16), the genetic value of the juveniles that survive (s) to the parent can be determined as follows:

$$s = g - (z + y) \quad \text{Equation 7.17}$$

For females in the above example the value of y , z , and g for a period equivalent to one brood-cycle were determined using Equation 7.8, Equation 7.12 and Equation 7.15, respectively. Therefore, the genetic value of the surviving juveniles (s) to the female parent would equate to:

$$s = 54 - (17.6 + 1.6) = 35.1 \text{ juveniles} \quad \text{Equation 7.18}$$

For males the value of y , z , and g for a period equivalent to one brood-cycle were determined using Equation 7.10, Equation 7.13 and Equation 7.16, respectively. Therefore, Equation 7.17 can be used to determine the genetic value of the surviving juveniles (s) to the male parent over a period equivalent to one brood-cycle:

$$s = 216 - (69.1 + 6.5) = 140.4 \text{ juveniles} \quad \text{Equation 7.19}$$

A certain percentage of potentially viable genes were lost to cannibalism. When the estimated genetic loss due to cannibalism is compared to the genetic value of the survivors, it would appear that a relatively small percentage of viable genes are destroyed. The genetic values to the female and male parent of the juveniles that survive were equivalent to 35.1 and 140.4 juveniles, respectively (Equations 7.18 and 7.19). This was greater than the genetic loss experienced by either parent as a result of its cannibalistic

behaviour, which was equivalent to 1.6 juveniles for females (Equation 7.8) and 6.4 juveniles for males (Equation 7.10). The destruction of viable genes comprising up to 4.6% of the parent's viable genes (in the form of surviving juveniles) is the lower limit of cannibalism that is not yet selected against. That is, selection against cannibalism would not be expected if 4.6% or less of the viable genes were cannibalised. The method and equations proposed here could be used in future studies to develop a better understanding of the degree of viable gene destruction required before fitness is affected negatively, which would probably result in selection pressure against cannibalism. The calculations made here would not have been possible without an estimation of dead and deformed juveniles, presented here for the first time. Since the thesis provides knowledge of the contribution of each parent to cannibalism, fecundity values, and behaviour-related factors, the findings contribute to our understanding of factors that may determine cannibalism in *X. helleri*. Although the exact data is probably species-specific, the procedure used to make these estimates could be applied to other species provided there is a reliable method to determine the viability of the offspring.

Towards an explanation for non-kin cannibalism

Non-kin intercohort cannibalism is the term used to describe the cannibalism of offspring by unrelated adults (Smith and Reay, 1991). Rohwer (1978) referred to this form of cannibalism as heterocannibalism. FitzGerald and Whoriskey (1992) indicated that it is not known how distant the relationship between fish has to be before they can be considered non-kin. In this section of the discussion any adult that was not identified as a parent will be considered non-kin and its cannibalistic behaviour defined as non-kin cannibalism.

Cannibalism only becomes disadvantageous to the genotype if it affects it negatively at a rate greater than its success; under such circumstances, selection against cannibalism would be expected. The following discussion aims to show to what degree the genetic loss experienced by a victim of cannibalism is smaller than the genetic success realised by the victim as a result of inclusive fitness and the subsequent expression of the victim's genes through its surviving siblings. It will be shown that in some cases, selection against cannibalism is likely not to be expected; this may further help to explain why cannibalism persists in *X. helleri*.

Because the average degree of relatedness of the adults in the population was very low (Equation 7.5), a non-parent adult, on average, would experience very little loss in fitness as a result of its own cannibalistic behaviour. Thus, the average relatedness between a non-parent adult and the offspring has not been included in the following calculations. It is further assumed that full siblings were related to each other with a probability of $\tau = 0.5$ while the value for half siblings is $\tau = 0.25$ (Bertram, 1976). For the purpose of the following example it is assumed that each male contributed equally to the fertilisation of eggs in the broods. Bertram (1976) showed that the average genetic relatedness of the chicks in a hypothetical polyandrous bird species, where the two males were assumed to be unrelated, was an average of $\tau = 0.5$ and $\tau = 0.25$, i.e. $\tau = 0.38$. Therefore, average relatedness between the siblings of a single brood in this example, where two male *X. helleri* contributed equally to fertilising the eggs, is assumed to be $\tau = 0.38$.

Because all juveniles in a brood are related to each other, they have similar genotypes (on average $\tau = 0.38$); thus, if any of the juveniles were to die, the genetic fitness of the surviving juveniles in the brood would be negatively affected. An extreme example is the

death of the entire brood. In that case, a juvenile would not only lose its own genes ($\tau = 1$), but it would lose the potential of its own genotype being expressed by its siblings at a rate of $\tau = 0.38$. The genetic value of the entire brood (j) to a single juvenile in that brood can be calculated:

$$j = 1 + \tau * (f - 1) \quad \text{Equation 7.20}$$

where j is genetic value equivalent to the number of juveniles, 1 represents the juvenile's own genes, τ is the average degree of relatedness between the juveniles, and f is the number of juveniles born in a brood (equivalent to fecundity). For a juvenile born in the control group of Chapter 6 where fecundity averaged 108, this equates to:

$$j = 1 + 0.38 * (108 - 1) = 41.1 \text{ juveniles} \quad \text{Equation 7.21}$$

Therefore, if the entire brood of 108 juveniles were killed the genetic loss experienced by any one of the juveniles would be equivalent to the entire genetic value of the brood to that juvenile: i.e. its own genotype plus the degree to which it was related to its siblings through inclusive fitness, the total of which was estimated to be equivalent to 41.1 juveniles (Equation 7.21). Similarly, it was possible to calculate the genetic value of the juveniles of any sub-group within a brood (j_{subgroup}) to any one juvenile. If only X juveniles of the brood were cannibalised, leaving Y survivors, it is possible to calculate the genetic value of X and Y to any one of the juveniles using Equation 7.22:

$$j_{\text{subgroup}} = \tau_1 * (a - n) + \tau_2 \quad \text{Equation 7.22}$$

where τ_1 remains the genetic relatedness of the juveniles in the brood, a is the number of juveniles that belong to the subgroup (in the case of the above example, either Y or X juveniles), n would equal one if the juvenile for whom the genetic value was being calculated was included in the subgroup or 0 if this juvenile was not part of the subgroup, and τ_2 is the genetic contribution to the sub-group made by the juvenile for whom the genetic value is being calculated (i.e. $\tau_2 = 1$ if the juvenile is included in the subgroup or $\tau_2 = 0$ if the juvenile is not included in the group). In the control group of Chapter 6 the average rate of cannibalism was found to be 0.62, so 41 survivors in a brood of 108 juveniles would be expected. The genetic value of this subgroup to any one of the survivors of the brood would equate to:

$$j_{subgroup} = 0.38 * (41 - 1) + 1 = 16 \text{ juveniles} \quad \text{Equation 7.23}$$

The genetic value of the same subgroup (i.e. the group that survived) to one of the juveniles that was cannibalised would equate to:

$$j_{subgroup} = 0.38 * (41 - 0) + 0 = 15.4 \text{ juveniles} \quad \text{Equation 7.24}$$

Furthermore, it is possible to calculate the genetic loss experienced by a single juvenile (y_4) as a result of cannibalism (Equation 7.25). This equation also takes into account the loss experienced as a result of dead and deformed juveniles, so y_4 is the genetic loss of viable genes (defined as genes not carried by dead and deformed juveniles) experienced by a surviving juvenile of a brood that was subject to cannibalism:

$$y_4 = \tau_1 * [(c - d) - n] + \tau_2 \quad \text{Equation 7.25}$$

where τ_1 remains the average degree of relatedness between the juveniles, c is the number of juveniles cannibalised, d is the expected number of juveniles found dead or deformed at birth, and τ_2 is the genetic contribution to the sub-group made by the juvenile for whom the genetic value is being calculated (i.e. $\tau_2 = 1 = n$ if this juvenile is included in the subgroup, or $\tau_2 = 0 = n$ if the juvenile is not included in the group). It is possible that the genetic relatedness between deformed and non-deformed juveniles is not homogenous and that genetic differences exist between these groups of juveniles. However, for the purposes of this discussion, it will be assumed that the genetic relatedness between all siblings is, on average, homogenous. A surviving juvenile born to a female in a control tank of Chapter 2 would have experienced the following genetic loss as a result of its siblings being cannibalised:

$$y_4 = 0.38 * [(67.0 - 34.6) - 0] + 0 = 12.2 \text{ juveniles} \quad \text{Equation 7.26}$$

while any one of the victims from the same brood would have experienced the following genetic loss:

$$y_4 = 0.38 * [(67.0 - 34.6) - 1] + 1 = 12.8 \text{ juveniles} \quad \text{Equation 7.27}$$

There is very little difference in average genetic loss between a victim ($y_4 = 12.8$) and a survivor ($y_4 = 12.2$). Therefore, in terms of survival of the genotype, it makes little difference if the juvenile is a victim to cannibalism or else a survivor.

The genetic loss experienced by a victim of cannibalism ($y_4 = 12.8$) is smaller than the genetic success experienced by the same victim as a result of inclusive fitness and the subsequent expression of its genes through its surviving siblings ($y_4 = 15.4$). The potential of a net decrease in fitness is a disadvantage that may select against cannibalism (Polis, 1981). In other words, cannibalism becomes disadvantageous when the rate of destruction of an animal's genotype as a result of cannibalism is greater than the rate of success of the genotype. As the success of the genotype ($y_4 = 15.4$) was greater than the loss experienced due to cannibalism ($y_4 = 12.8$), it would appear that selection pressure against cannibalism does not occur when the genetic loss due to cannibalism is less than the success of the genotype. However, this model needs to be developed further in future studies so as to take into account the multi-specific context to which *X. helleri* was likely to have been subjected, in particular, the effect that non-specific predation might have had on the evolution of cannibalistic behaviour should also be considered.

Explanations for cannibalism are applicable to other populations

The equations developed here help to explain filial and non-kin cannibalism in terms of genetic investment and loss due to cannibalism. It was possible to conclude that selection against filial and non-kin cannibalism is not expected at the rate of cannibalism observed here. These equations were developed and applied to a population of ten adult *X. helleri* consisting of eight females and two males and it was assumed that both males contributed equally to the fertilisation of each brood. It is possible that these assumptions will limit the predictability of these formulae to other population structures. For example, the average genetic relatedness between the juveniles in one brood was calculated as $\tau = 0.38$ and this was based on the assumption that there were two males in the tank. However, this τ -value would need to be corrected if the number of males in the tank

changed or if the males were not equally responsible for fertilising each brood. Similarly, the degree of genetic loss experienced by the parents was subject to the total number of adults in the population (b), the sex-ratio, and the number of broods to which each male was related (e) (Equation 7.9). Therefore, the genetic loss experienced by a parent as a result of its cannibalistic behaviour is likely to change under different population structures. The next section of the discussion demonstrates that the estimations arrived at here may be applied to other population structures.

A critical assumption made when determining the genetic value of cannibalism to the juveniles was that the two males in the population were equally responsible for fertilising the eggs of every brood. There was a chance that a single brood could have been fathered by more than the two males because females can store sperm from a single mating and use it to fertilise more than one successive brood (Constanz, 1989). This would have affected the average relatedness of the juveniles in the brood. Furthermore, the possibility exists that one male might fertilise all the embryos of a single brood, which would make all the juveniles full siblings. The following discussion will show that the argument regarding the genetic value and loss to the juveniles due to cannibalism may hold true under different conditions, and is therefore one appropriate explanation for the lack of selection against cannibalism in *X. helleri*, based on the available data.

If all the juveniles in a brood had a single father they would all be full siblings ($\tau = 0.5$; Bertram, 1976) and if every egg in the brood was fertilised by a different male they would all be half siblings ($\tau = 0.25$; Bertram, 1976). For both cases, the average genetic loss due to cannibalism (γ) and the average genetic value of the survivors to a victim of cannibalism (j) were determined using Equations 7.20 and Equation 7.22 respectively

(Table 7.4; Columns 1 and 5). Under all circumstances it was assumed that $f = 108$ juveniles (total number of juveniles in the brood), $c = 67$ juveniles (i.e. 62% of the juveniles were cannibalised; $a = 41$ juveniles (i.e. 38% of the juveniles survived cannibalism), and $d = 34.6$ juveniles (i.e. 32% of the juveniles were expected to be dead or deformed). Similarly, the value of y and j for three intermediate τ -values were also calculated. The intermediate τ -values assumed either that: one male was responsible for fertilising 75% of a brood and 25% of the brood eggs were fertilised by other males ($\tau = 0.44$; Table 7.4); one male was responsible for fertilising 50% of a brood and the remaining 50% of the embryos were fertilised by other males ($\tau = 0.38$; Table 7.4); or, one male was responsible for fertilising 25% of a brood and 75% of the embryos were fertilised by other males ($\tau = 0.31$; Table 7.4).

Table 7.4. The genetic loss of cannibalism (y) experienced by survivors and victims of cannibalism, and the genetic value of the surviving juveniles to the victims (j) through inclusive fitness for juveniles of a single brood where relatedness ranged from full siblings (1) to half siblings (5), and for all the juveniles in the population (6).

	Full sibs ← → Half sibs < Half					
	1	2	3	4	5	6
Average relatedness between juveniles (τ)	0.50	0.44	0.38	0.31	0.25	0.16
Genetic loss from cannib. (y) to survivor	16.2	14.3	12.3	10.0	8.1	5.2
Genetic loss from cannib. (y) to victim	16.7	14.8	12.9	10.7	8.9	6.0
Genetic value of survivors (j) to victim	20.5	18.0	15.6	12.7	10.3	6.7

The genetic loss from cannibalism (y) and the genetic value of the survivors experienced by the victim (j) were also calculated using the average genetic relatedness of all the juveniles in the population. In order to calculate average relatedness it was assumed that

the population consisted of eight females and two males, all the females gave birth in a given period to an equal number of juveniles, the males were equally responsible for fertilising the eggs, and relatedness between the adults was negligible (Equation 7.5). It was therefore possible to determine the average relatedness between all juveniles in the tank at any given time as $\tau = 0.16$ (Table 7.4; Column 6).

The degree of relatedness between the juveniles of a single brood will decrease with an increase in the number of males that mate with the female. Nonetheless, even when the average degree of relatedness was as low as possible between the juveniles in a brood ($\tau = 0.25$), the genetic loss experienced by the victim was similar to the genetic loss experienced by the survivor, and the genetic value of the survivors to the victim was greater than the genetic loss experienced by the victim due to cannibalism (Table 7.4).

The estimates remained consistent even when the calculations were made based on the relatedness of all the juveniles in the population, where average relatedness was less than half siblings (Table 7.4; Column 6). Therefore, the conclusion that the genetic loss experienced by a victim of cannibalism was smaller than the genetic success experienced by the same victim as a result of inclusive fitness and the subsequent expression of its genes through its surviving siblings, appears to be applicable under the assumptions described here.

The average genetic loss experienced by the male and the female parent (y) and the genetic value of the surviving offspring to both parents (s) were calculated using Equation 7.9 and Equation 7.17 for different populations A to F (Table 7.5). Population structures A to E resemble those described in Chapter 2, while population D was similar to the

example used to derive Equations 7.9 and 7.17; F and G were hypothetical populations. For all the calculations used to determine y and s (Table 7.5) it was assumed that the average degree of relatedness between parent and juvenile was $\tau = 0.5$, the average fecundity (f) was 108 juveniles, and 32% of the juveniles on average were dead or deformed ($d = 34.6$). The number of adults (b) and number of broods to which the parent made a genetic contribution (e) varied according to the population structure. The rate of cannibalism was assumed to be that estimated for each population structure as described in the relevant chapters. Cannibalism in the hypothetical population structures (F and G) was assumed to be 62%, equivalent to that used to determine the equations in this discussion.

Table 7.5. Number of juveniles cannibalised (c), adults number (b) and the number of broods to which an adult is related (e) were used in Equation 7.9 and Equation 7.17 to determine the genetic loss (y) and the genetic value of the juveniles that survived cannibalism (s) for parents belonging to population structures A to G.

Population Structure	A	B	C	D	E	F	G
Total number adults (b)	16	10	4	10	10	300	3,000
Total number females	8	5	2	8	2	240	2,400
Total number males	8	5	2	2	8	60	600
Number of broods male is related to (e)	1.0	1.0	1.0	4.0	0.3	4.0	4.0
Number of broods female related to (e)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Estimated cannibalism (%)	53.9	37.5	5.5	62.0	47.6	62.0	62.0
Number juveniles cannibalised/brood (c)	58	41	6	67	51	67	67
Genetic loss of viable juveniles							
1. ... experienced by male parent (y)	0.7	0.3	-3.6	6.5	0.2	0.2	0.0
2. ... experienced by female parent (y)	0.7	0.3	-3.6	1.6	0.8	0.1	0.0
Genetic value of surviving juveniles							
1. ... to the male parent (s)	36.0	36.4	40.3	140.4	9.0	146.6	146.8
2. ... to the female parent (s)	36.0	36.4	40.3	35.1	35.9	36.6	36.7

The degree of genetic loss (y) for both males and females was in every case less than the genetic value of the juveniles that survived cannibalism (s), irrespective of population size and sex ratio (Table 7.5). A net decrease in fitness due to cannibalism is a disadvantage that may select against cannibalism (Polis, 1981). However, as the losses in cannibalism for all the population structures tested in Table 7.5 were always less than the genetic value of the juveniles that survived, there was always a net gain in fitness to the cannibalistic parent. Therefore, selection against filial cannibalism would not be expected in any of the population structures tested at the rates of cannibalism observed here. Thus, the theory developed in the above discussion to explain filial cannibalism in *X. helleri* is not only applicable to the population for which the theory was first developed, but it also appears to apply to a wide range of population structures.

Cannibalism may account for population structure

Bertram (1976) demonstrated that it was possible to estimate the average relatedness among the individuals of a pride of lions (*Panthera leo*), provided that certain information regarding the species' social system and reproduction was available. Furthermore, the relatedness among the lions was used to explain the behaviour and population structure of the pride. For example, male lions in the Serengeti often allow cubs to feed from food in their possession while females are less tolerant of this behaviour (Schaller, 1972). Bertram (1976) accounted for this behaviour by comparing the average relatedness between the lion cubs, the lions and the lionesses. The males were on average more closely related to the cubs while the lionesses were on average less closely related to the cubs, so it was suggested that males would generally experience greater benefit through improved cub survival as a result of this tolerance (Bertram, 1976). Bertram was thus

able to conclude that there was probably a selection pressure that favoured male tolerance of lion cubs, which accounts for this sexual difference in behaviour. Similarly, the genetic relatedness of individuals in a population of *X. helleri* may be used to explain the behaviour and subsequent population structure. The following arguments will evaluate the theory that there is selection advantage to an individual that belongs to a large population with a sex-ratio skewed in favour of females. The structure of feral poeciliid populations is also considered against this prediction.

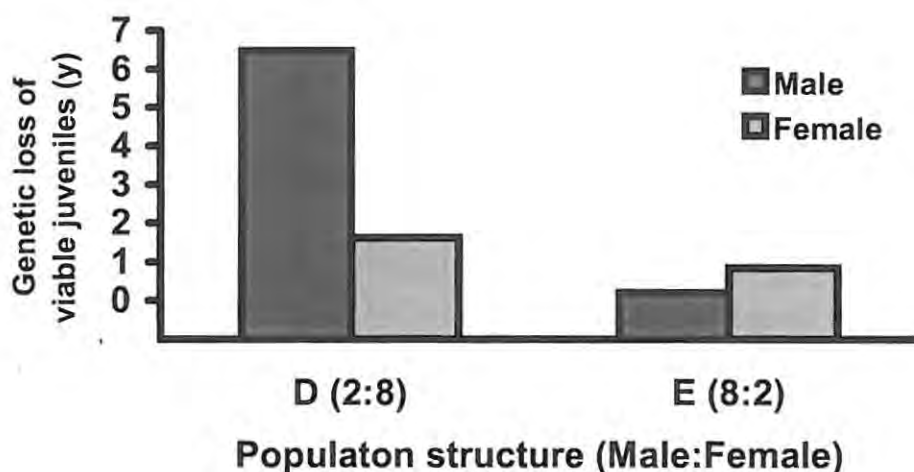


Figure 7.2. Genetic loss of viable juveniles experienced by male and female *X. helleri*, from different populations, because of their own cannibalistic behaviour. The y-values here summarise those presented in Table 7.5 for populations D and E.

The sex-ratio of a population affects the genetic loss of viable juveniles (y) in regard to the male but not the female parent (Figure 7.2). The female is always related to the brood but on average a male will fertilise fewer embryos if the overall number of males in the population increases. This was shown by a comparison of $e = 4.0$ broods per cycle and e

= 0.3 broods per cycle for males in a population with a sex ratio of 2:8 and 8:2 (male:female), respectively (populations D and E; Table 7.5 and Figure 7.2). The respective value of y and s for the males in these populations illustrates that the model has the capacity to fit different population structures and is, therefore, probably applicable to other *X. helleri* populations. Furthermore, this data provides evidence that male *X. helleri* were at a genetic advantage when the sex-ratio was skewed in favour of females. Therefore, it is likely that selection in favour of fewer males occurs in *X. helleri*. This might explain high the levels of aggression among male *X. helleri* (2000, personal observation).

The genetic value of the surviving juveniles to the parents increased with an increase in population size. However, this increase was marginal as there was almost no difference between the s -values for males and females in a population of 10, 300 or 3,000 adults (D, F and G, respectively; Table 7.5). However, the genetic loss of viable juveniles to the parent due to their own cannibalistic behaviour was dependent on population size: the y -value decreased with an increase in the size of the population (Table 7.5). This is because the parent's proportional contribution to cannibalism of its brood decreased as the total number of cannibals in the population increased. Therefore, the probability of selection pressures acting against filial cannibalism would be even more unlikely in a larger population.

Furthermore, the difference between genetic loss to a juvenile that survived cannibalism and the genetic loss experienced by a victim was least when the average relatedness of the siblings was high, $\tau = 0.5$ (Table 7.4). Similarly, the difference between the genetic value of the survivors to a victim of cannibalism and the genetic loss experienced by the victim increased with an increase in average relatedness between the siblings. Therefore,

the findings show that a victim of cannibalism experienced less of a genetic loss when it was on average more closely related to its siblings. This not only confirms that cannibalism in *X. helleri* can probably be explained by the theory of *inclusive fitness*, but it also suggests that selection in favour of single mating is likely to occur, which in turn supports the previous suggestion that selection probably exists in favour of a female-biased sex-ratio.

Although the study was not specifically designed to establish reasons for *X. helleri* population structure, the evidence presented here leads to the hypothesis that selection pressures probably operate in favour of a large population with a sex-ratio skewed in favour of females. Although no known work has investigated the sex-ratio of *X. helleri* at birth, a female-biased adult sex-ratio is a common characteristic in the Poeciliidae (Snelson and Wetherington, 1980; Snelson, 1989), including feral populations of *X. helleri* and *X. maculatus* (Milton and Arthington, 1983). In his review of the social and environmental control of life-history traits in poeciliid fishes, Snelson (1989) supplied a number of possible explanations for the biased sex-ratio in poeciliids, such as differential predation on males, that males succumb more readily to stressors, and evidence of seasonal recruitment of males in some species. However, Snelson (1989) encapsulated the discussion with the following statement, "... skewed adult sex ratios [in the poeciliids] beg for an explanation...." The findings of this thesis suggest that there might be selection pressures that favour a female-biased sex ratio. Furthermore, there is evidence that sex determination is genetically controlled in the genus *Xiphophorus* as autosomal genes interact with the sex chromosomes to determine sex, and furthermore, some crosses produce a consistently biased sex ratio (Snelson, 1989). Although the reports of Milton and Arthington (1983) and Snelson (1989) support the hypothesis presented here, further investigation is required to test its potential.

Conclusion

Cannibalism of juvenile *X. helleri* by parent and non-parent adults took place under varied conditions that ranged from small glass aquaria to a large recirculating farm culture system. The behaviours associated with cannibalism were identified and described and it was found that swordtail behaviour generally did not differ under different environmental conditions. However, there were certain inconsistencies between some of the results obtained under the different conditions, which led to the recognition that such potentially confounding factors might limit the reliable interpretation of behavioural studies. Nonetheless, as the tests and results were largely repeatable and similar under different environmental conditions the probability that the data were biased by the conditions under which the experiments were carried out or that findings were subject to subjective interpretation was low.

Furthermore, observations of fish in the laboratory had potential for predicting behaviour under other conditions and it was possible to measure the predictive potential of the behaviours. Although some behaviour had a better prediction capacity, so to speak, than others, all the hypotheses presented in this thesis remain to be tested under other conditions with different populations. If these hypotheses receive wide acceptance, the models and protocols based on them can be applied to creating a framework for future research as well as commercial production of poeciliid fishes.

Past research has inferred that cannibalism may be a debilitating factor to the commercial production of fish (Folkvord, 1991; Smith and Reay, 1991; Hecht and Pienaar, 1993; Ottera and Folkvord, 1993; Baras and Jobling, 2002). The initial findings of this thesis (Chapter 2) appeared to concur with this since cannibalism of seemingly healthy juveniles

occurred in proportions in excess of 50% of the total brood. However, as a result of changes in production technology it became apparent that on average half of the juveniles that were being cannibalised were dead or deformed at birth, and probably less viable and unlikely to survive had they not been consumed. This agreed with the suggestion that cannibalism under culture conditions might be beneficial to the commercial production of some fishes (Smith and Reay, 1991; Baras and Jobling, 2002). Nonetheless, a tank design with a false-side and a solid bottom was suggested as the most appropriate method of mitigating cannibalism of commercially produced *X. helleri* because it promoted the survival of viable juveniles yet allowed the adults to cannibalise less viable offspring off the bottom.

The nutritional benefit was the most likely proximate reason for cannibalism and there was evidence in support of cannibalism being a by-product of predation, as was alleged by Hrdy (1974) and Smith and Reay (1991); possibly it is a means of recovery of nutritional energy. However, there is sufficient evidence to suggest that cannibalism is under genetic control in some of the poeciliids (Thibault, 1974). It appears that the selection pressures responsible for cannibalism in poeciliids are not the same for all species in the family as the reproductive efficiency of *G. affinis* improved as a direct result of cannibalism (Meffe and Crump, 1987) while that of *X. helleri* remained unchanged. The genetic models developed here showed that cannibalism in *X. helleri* could be accounted for at least partly by inclusive fitness. However, based on the incidence of dead and deformed juveniles it was inferred that the inclusive fitness theory should be further developed to include the genetic benefit experienced by the victim of cannibalism through the expression of only its viable genotype, by closely related kin, without the potentially negative effect of its less viable genes that might have been expressed if the deformed juvenile had survived. This was described as the *gene exclusion* theory.

The proposed models to account for cannibalism are the most feasible given the findings of this thesis and the literature that was available. Preliminary comparisons of these models to different population structures demonstrated that they were likely to be applicable to other populations and furthermore were able to help explain the population structure of feral *X. helleri*. If they are found to be an appropriate account of *X. helleri* behaviour when tested under different conditions on other populations, it will confirm that the explanations here were applicable and can account for filial and non-kin cannibalism in *X. helleri*, until future research develops other hypotheses that better explain the behaviour that was observed here.

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