Monogamy and biparental care in the cichlid fish, *Eretmodus cyanostictus*

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Acknowledgements
1. Mouthbrooding and Biparental care: an unusual combination

1.1. Abstract

Biparental care is expected to occur if (i) the costs of desertion for a parent are high due to greatly reduced survival prospects of offspring and/or (ii) the benefits of desertion are low, e.g. because of limited remating opportunities. Among mouthbrooding fishes, biparental care is rare because the mouth cavity provides a very safe brooding site, thus reducing the selective advantages of shared brood care. Exceptions occur when clutches are too large to fit into a single mouth cavity or when young have to be defended by both parents after release. Both factors cannot explain biparental care in *Eretmodus cyanostictus*, a monogamous mouthbrooding cichlid in which the entire clutch is first brooded by the female and then by the male. It has been suggested that *E. cyanostictus* parents may be forced to be monogamous due to limited desertion opportunities for both sexes. However, it has never been tested whether females alone could produce viable young.

In our study, single females prolonged their incubation period and released an equal number of young compared to pairs. However, they only partially compensated the duration of the male incubation. As a consequence, young were smaller and less developed at release. Unassisted females lost more weight during incubation and had a prolonged interspawning interval, but they produced similar egg numbers and weights in the successive clutch compared to pairs. These results suggest that the male’s brood care effort is an important cause for the maintenance of biparental care and monogamy in *E. cyanostictus*.

1.2 Introduction

The parental care pattern found in a species is likely to reflect the outcome of a contest played between the sexes over evolutionary time (Westneat & Sargent 1996). Often the pay-offs of the two alternatives – care giving or desertion of a brood – diverge substantially between males and females (Trivers 1972, Clutton-Brock 1991). Biparental care is likely to be the outcome if it is significantly more effective than
uniparental care and if the payoff of desertion is low for both parents. For example, in many altricial birds biparental care considerably increases the survival chances of young (Lack 1968, Oring 1982). The pay-off of desertion is low if the remating probability is low or search costs for a new mate are high (reviewed in Clutton-Brock 1991).

Cichlid fishes (Cichlidae) provide excellent opportunities to study sexual conflict and parental care decisions. They show a large variety of parental care patterns (substrate breeding, delayed or immediate mouthbrooding, biparental, female-only and male-only care; Keenleyside 1991). In most substrate breeding species both parents are needed for the defence of young and breeding site. Contrary to this, most mouthbrooders show female-care and sequential polygyny. Only in a few mouthbrooders parents share in brood care (Oppenheimer 1970, Keenleyside 1991, Kuwamura 1997). A common explanation for the rarity of biparental mouthbrooding is that the mouth of one parent provides a sufficiently safe incubation site (Oppenheimer 1970, Barlow 1984, Gross & Sargent 1985).

Biparental care in mouthbrooders would be expected only if (i) a large brood size requires both parents for incubation or (ii) if parents need to co-defend the free swimming fry after release (Perrone & Zaret 1979, Clutton-Brock 1991). While this appears to apply to most biparental mouthbrooders (e.g. Kuwamura 1986, Yanagisawa 1986, reviewed by Perrone & Zaret 1979, Clutton-Brock 1991), there are at least three known exceptions. In the Lake Tanganyika cichlids Eretmodus cyanostictus, Tanganicodus irsacae and Xenotilapia boulengeri the females take up the total clutch after spawning to incubate it for some time before the young are transferred to their partners who incubate them until independence. Young are not defended after release (Kuwamura 1986, Kuwamura et al. 1989, Morley & Balshine 2002).

We studied factors maintaining biparental care under these circumstances in Eretmodus cyanostictus. In this species, females incubate the young for about 8-12 days before males continue for another 10-16 days (Neat & Balshine-Earn 1999, Morley & Balshine 2002). Apparently, parents starve during incubation (Neat & Balshine-Earn 1999, Morley & Balshine 2002). Females may gain little from desertion because the time until they are able to spawn again is much longer than the incubation period of the males (Morley 2000). But why do males join in brood care?
Evidence from a field population suggests that both sexes are constrained to monogamy because there are little re-mating chances for males due to a male biased sex ratio and strong intrasexual competition for mates (Neat & Balshine-Earn 1999, Morley & Balshine 2002). Morley and Balshine (2002) further argued that biparental care is neither essential nor more efficient, because anecdotal observations in captivity suggested that females continue to care if males are removed. However, it is unknown whether and how absence of male care would affect the condition of offspring and brood caring females.

To test for this potential effect, we conducted an experiment where females were either assisted by males or forced to incubate alone. We tested whether male absence would influence female incubation duration, body condition and the duration of the interspawing intervals. Also, we compared developmental stage, size and weight of young at release from parental care between biparental and uniparental treatments. If male desertion reduces offspring viability, this should influence the male’s desertion decision. In addition, effects on the future reproduction of females were assessed, namely the quality of future clutches.

### 1.3 Methods

**Study species**

*E. cyanostictus* is endemic to Lake Tanganyika where it inhabits shallow rocky coastal zones around the lake (Kuwamura 1986, Kuwamura et al. 1989, Taylor et al. 2001). Pairs defend all-purpose territories, which they only leave to chase away conspecific intruders (Morley 2000). Like the other “goby-like cichlids” of the tribe Eretmodini, *E. cyanostictus* has a reduced swim bladder, which allows it to remain close to the substrate even in turbulent water conditions (Konings 1998). The fish feed by scraping epilithic algae from rocks with their teeth (Yamaoka 1997).

**Experimental conditions**

The experiment was conducted from March 2002 to March 2003. Experimental fish were taken from a stock of adult fish kept at the University of Berne, consisting of
fish imported from Lake Tanganyika, Zambia, and from their first generation offspring. Experimental pairs were held in 100-l compartments of a 200-l tank. A layer of sand covered the bottom of each compartment and nine clay flowerpot halves and two PVC tubes (5cm in diameter) were provided as shelters. Gravel (about 2 cm in diameter) covered the sand to provide shelter for the offspring after being released by the parents. Each compartment was equipped with an internal biological filter. Fish were kept at water temperatures of 26-27°C on a 13 : 11 hours light/darkness cycle.

**Experimental design**

The day after spawning, 28 pairs were alternately assigned to one of two treatments; (i) both parents incubating a clutch (pair treatment) or (ii) females only incubating a clutch (single-female treatment). In the single-female treatment, a mesh was placed in the middle of the 100-l compartment 2-5 days after spawning. Thereby, the transfer of young from the female to the male was prevented. The mesh allowed visual contact and water exchange between the partners. All fish had access to an *ad libitum* food source (cubes containing Tetramin flake food mixed with agarose gel) for 30 min each day during the incubation period until young were released.

All fish were weighed one day after spawning and on the day after end of incubation. In the pair treatment, both fish were weighed on day 6, i.e. on average 2.1 ± 1.29 (SD) days before the shift of young and the female was weighed again the day after the shift of young. After release, young of both treatments were counted, weighed, measured (standard length, SL) and transferred to an empty holding tank.

After release of young, the mesh was removed in the single-female treatment and pair members of both treatments stayed together until they spawned a second time. In the period between the release of young and next spawning (non-incubation period) fish were fed with Tetra dry flaked food. They received a daily equivalent of 3.5 % of pair total body mass, which approaches *ad libitum* food availability. After the second spawning, females were coaxed towards releasing the eggs by gently moving the fish up and down in a container of water while they were held in a head down position. Then, both parents were weighed again and eggs were counted and weighed.

Ten of 15 pairs in the pair treatment completed incubation and released young. Two females aborted incubation due to continued male aggression, one female did not
transfer the young to the male and two females shifted their young only partly after 11 and 17 days, respectively. In the single-female treatment, 11 of 13 pairs completed incubation and released young. Two females aborted incubation and swallowed the eggs. Three pairs of the pair treatment and one pair of the single-female treatment divorced during the non-incubation period before the successive spawning. One female of the single-female treatment died for unknown reasons after the release of young. Eggs of successive clutches were measured in 6 cases of the pair treatment and in 8 cases of the single-female treatment.

Behavioural observations

The length of the interspawning interval may have been influenced by the period of separation in the single-female treatment, as separation might alter the strength of the pair bond. Therefore we checked for behavioural differences between pairs of both treatments in the non-incubation period. We observed each fish daily for 10 minutes, at least 6 times if possible. Observations took place between 13:00 and 16:00 h. As a measure of activity we recorded two behaviour durations, time swimming around (activity) or time under cover (hiding). Further, we recorded the frequencies of the following behavioural events in 10 min: feeding rate (number of bites on small food items in the sand or on surfaces), displays towards mate (the focal fish undulates its body; the intensity of this movement may vary considerably from bending to shaking of the body), aggressive behaviour (chasing or biting the mate).

All behavioural observations were recorded with the OBSERVER 3.0 program. Average values of all 10 min observations of individuals are given in the results.

Statistical analysis

Statistical data analysis was performed using SPSS 10.0. All tests were non-parametric because assumptions of parametric tests were not met. Test procedures are noted in the results. All tests are two-tailed. Descriptive statistics are given as medians and quartiles (in square brackets) throughout.
1.4 Results

In the pair treatment, the average incubation time of females (8; [7, 9] days, N=10) was shorter than in males (13; [12.75, 15] days, N=10, Wilcoxon matched-pairs signed-ranks test, z=-2.81, P=0.005). In the single-female treatment the females incubated about twice as long as in the pair treatment (single females: 18 days; [17, 20], pair females: 8 days; [7, 9]; Mann-Whitney U test, U=0, N=11,10, P<0.001) but still shorter than the total incubation time of a pair (Mann-Whitney U test, U=8.5, N=11,10, P<0.001, Fig. 1).

![Fig. 1 Total incubation duration of pairs and of single females in the single-female treatment. The whiskers and boxes represent the 10th, 25th, 50th, 75th and 90th percentiles.](image)

**Weight changes of females and males during incubation**

Initial body weight did not differ between females used in the pair treatment (6.73g; [6.07g, 8.11g], N=8) and in the single-female treatment (6.45g; [5.19g, 6.74g], N=9; Mann-Whitney U test, U=23, P=0.24). Females of both treatments lost weight during incubation (comparison before and after incubation; single-female treatment; Wilcoxon tests, z=-2.668, P=0.008; pair treatment; z=-2.524, P=0.012; Fig. 2). During male incubation, female weight did not change significantly (Wilcoxon Test,
Single females lost more weight during their incubation period than females in the pair treatment (i) until the shift of young (Mann-Whitney U test, \( U=7, N=9,8, P=0.004 \)), and (ii) until end of male incubation (Mann-Whitney U test, \( U=12, N=8,8, P=0.021 \); Fig.). As a consequence, at release of the young pair females were heavier than single females (Mann-Whitney U test, \( U=19, N=19,9, P=0.043 \))

![Graph showing weight change of females in % of initial body weight](image)

**Fig. 2** Weight change of females in % of initial body weight; grey plots: females in the pair treatment at shift of young (left) and at the end of male incubation (right); white plot: females at the end of incubation in the single-female treatment. Box plot as in Fig. 1.

There was no difference in initial body weight of males between treatments (single-female treatment: 9.2g; [7.4, 11.1], pair treatment: 10.3g; [9.5, 12.7]; Mann-Whitney U-test, \( U=21, N=10,7, P=0.19 \)). Neither the males of the single-female treatment (Wilcoxon Test, \( z=-0.204, N=10, P=0.838 \)) nor the males of the pair treatment (Wilcoxon Test, \( z=-0.339, N=7, P=0.735 \)) showed a significant weight change during incubation of their mates (see Fig. 3). During their own incubation, however, males of the pair treatment decreased in bodymass (Wilcoxon Test, \( z=-2.803, N=10, P=0.005 \); Fig. 3). There was no difference in body weight of males between treatments at release of the young (Mann-Whitney U test, \( U=33, N=10,10, P=0.22 \)).
At the point of release offspring of single females were smaller than offspring of females in the pair treatment (Mann-Whitney U test, U=15, N=11,10, \( P=0.004 \), Fig. 4). There was no difference in offspring number (pair treatment: 11.5; [9, 19], N=8, single-female treatment: 15.5; [9, 18], N=8; Mann-Whitney U test, U=32, N=8,8, \( P=1.0 \)) and offspring weight (pair treatment: 27.8mg; [24.9, 30.4], N=10, single-female treatment: 26.5mg; [24.9, 30.1], N=11; Mann-Whitney U test, U=52, N=10,10, \( P=0.86 \)). On average 62\% of the offspring of single-female clutches had not fully absorbed their yolk sac at time of release (such young were found in 6 of the 8 included clutches), while this happened in none of the young released by males in the pair treatment (ratio of young with yolk remains and young without yolk remains per brood were compared; Mann-Whitney U test, U=8, N=8,8, \( P=0.01 \)).
Fig. 4 Standard length (SL) of offspring after release. Box plot as in Fig. 1.

**Interspawning interval and egg sizes**

Interspawning intervals were 28% longer in the single-female treatment than in the pair treatment (Mann-Whitney $U$ test, $U=3$, $N=7,9$, $P=0.001$, Fig. 5). The time from the end of female incubation until laying of the next clutch did not differ between treatments (pair treatment: 20 days; [18, 22]; single-female treatment: 20 days; [16, 22.5] days; Mann-Whitney $U$ test, $U=29.5$, $N=7,9$, $P=0.837$). Female body weight at subsequent spawning did not differ between treatments (pair treatment: 6.19g; [5.58, 8.27]; single-female treatment: 6.0g; [5.29, 6.66]; Mann-Whitney $U$ test, $U=25$, $N=8,7$, $P=0.78$).
There was no treatment effect on egg number (pair treatment: 20; [17.5, 27.8], N=6; single-female treatment: 23; [18, 26.5], N=8; Mann-Whitney U test, U=20.5, N=6,8, \( P=0.66 \)) or egg weight (pair treatment: 15.7g; [14.5, 17.7], N=6; single-female treatment: 15g; [14.2, 16.3], N=8; Mann-Whitney U test, U=18, N=6,8, \( P=0.49 \)) of the clutches succeeding the experimental period.

**Behavioural observations**

To check potential effects of the treatments on the behaviour of pair members we compared the different feeding rates, display rates and activity levels during the non-incubation periods following experimental treatments. None of these behavioural components differed between pairs that had been previously exposed to different treatments (Table 1).
Table 1 Comparison of behaviours between the two treatments during the non-incubation period (Mann-Whitney U-tests)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair (N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single female (N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity in % (time not hiding)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>15.59 [6.4,34.9] (6)</td>
<td>11.85 [5.8,40.7] (7)</td>
</tr>
<tr>
<td>Females</td>
<td>15.49 [13.1,59.9] (6)</td>
<td>17.09 [0.9,59.2] (7)</td>
</tr>
<tr>
<td>Feeding (bite/10 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.5 [0.8,5] (6)</td>
<td>0 [0.3,5] (7)</td>
</tr>
<tr>
<td>Females</td>
<td>4.75 [0,5] (6)</td>
<td>5 [0.5,7.5] (7)</td>
</tr>
<tr>
<td>Displays/10 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.5 [0.4,2.3] (6)</td>
<td>2 [0.4] (7)</td>
</tr>
<tr>
<td>Females</td>
<td>4.5 [2.5,8.8] (6)</td>
<td>4 [0.5,11] (7)</td>
</tr>
</tbody>
</table>

1.5 Discussion

If the survival chances of offspring can greatly be raised significantly by shared parental care, biparental care is likely to evolve (Clutton-Brock 1991). In our experiment, offspring of unassisted *E. cyanostictus* females were smaller at release than offspring of females receiving help by their mate. Furthermore, 62% of young raised by females alone still had visible yolk sac remains at release and hence were in an earlier stage of development. Under natural conditions the survival prospects of smaller, less developed young may be greatly reduced for several reasons. It has been shown in fish that burst swimming speed increases with offspring size (Garenc et al. 1999). Probably this is partly responsible for the observation that predation risk and the spectrum of predators of offspring decreases quickly with the latter’s body size (Nagoshi 1987, Sogard 1997). Larger juveniles are thought to tolerate physical extremes better than their smaller conspecifics (reviewed in Sogard 1997). We found no difference in weight of offspring between treatments. Thus, the small offspring of females in the single-female treatment may have had more reserves in relation to body length compared to the offspring of females in the pair treatment. However, if these
reserves are mainly stored in the yolk sac, this may additionally handicap the swimming abilities of young.
Females substantially prolonged their incubation period when raising young without a male. However, they did not compensate fully for the missing incubation effort of their mate, probably because they were energetically limited. As the feeding activity of females during incubation was highly reduced (unpub. data), females without male help lost on average 14.9% of initial body weight during incubation. The female with the longest incubation time (21 days) lost 34.8% of initial body weight. In comparison, females with male help lost only 5.1% of their weight during incubation and they started to feed again immediately after the shift. As a consequence, females with male help were heavier at the release of young than unassisted females. The results may have been confounded by the different handlings of the females in the two treatments. Because the females in the pair treatment were weighed two more times, they may have been stressed more and as a consequence probably lost more weight than the single females. Then, the difference in weight loss would even be bigger than we estimated. Energy expenditure of single females may also be higher under more natural conditions as shown in the biparental substrate brooding *Cichlasoma nigrofasciatum*. After desertion of the male, females increased their attack rate against other adults to compensate for male absence (Keenleyside et al. 1990).
A decrease in body condition may impair the survival of females. A lowered body condition has been shown to increase the risk of starvation, predation or diseases in fish (reviewed in Smith & Wootton 1995a). An elongated starvation period has been shown to increase interspawning intervals in other cichlids (Smith & Wootton 1994, 1995b, Balshine-Earn 1995) and reduced subsequent fecundity in the Galilee St. Peter’s fish, *Sarotherodon galilaeus* (Balshine-Earn 1995).
We found that the interspawning interval was extended by 28% in the single-female treatment. There were no differences in the behaviour of pairs between treatments, so we conclude that the partial separation of partners in one treatment did not affect intra-pair behaviour and therefore is unlikely to be responsible for the difference in interspawning intervals. Rather, this difference could be a consequence of the elongated starvation period of single females that lowered their body condition by the end of incubation. However, the interval between the end of female incubation and laying of the successive clutch of about 20 days did not differ between treatments, despite the lowered body condition of singly caring females. Our lab data revealed
that females need about three weeks to spawn again if their eggs have been removed immediately after spawning. Thus, apparently this interval is not influenced by the length of female incubation or by female body condition after incubation. Smith & Haley (1988) found that oocyte growth in the mouthbrooding cichlid *Oreochromis mossambicus* was arrested after the first days of broodcare until mouthbrooding ended. During this period the increase of ovarian steroid hormones, including estradiol, testosterone and progesterone was delayed. The arrestment of oocyte growth was not due to starvation, so hormonal control mechanisms could override effects of a decreases food intake (Smith & Haley 1988). The prolongation of the female incubation period in one treatment leads to an increase in the time between two successive clutches but apparently does not affect the period between the end of female incubation and laying of the next clutch. Based on Smith & Haley (1988) and our own results we assume that every additional day of mouthbrooding would increase the interspawning interval by about one day.

Balshine-Earn (1995) showed that mouthbrooding decreased subsequent fecundity of females in the Galilee St. Peter’s fish. In our study, the period length of female incubation did not influence egg number or egg weight of the next clutch. Apparently, the reduced body condition of females in the single-female treatment at the moment when oocyte growth is thought to resume did not affect these variables. Egg weight and clutch size may be influenced by female body condition mainly closely before spawning, when we found no difference in body weight of females between treatments.

By deserting his mate and brood an *E. cyanostictus* male could feed and gain weight while a mouthbrooding male loses weight during incubation. In our study, males prevented from assisting their mates were slightly heavier at release of young than helping males, however, the difference was not significant. While benefits of desertion for males seem to be low due to a male biased sex ratio and high intrasexual competition for mates and territories in the field (Neat & Balshine-Earn 1999, Morley & Balshine 2002), our results revealed that the costs of desertion are high due to the decreased decreases survival prospects of young.

It has previously been suggested that in *E. cyanostictus* biparental care and monogamy are mainly maintained because benefits of desertion are low (Neat & Balshine-Earn 1999, Morley & Balshine 2002) but that biparental care per se is neither essential nor more effective. Contrary to this, we show that unassisted females
provided only partial compensation for the missing contribution of their partners. This
is also known from studies of biparental bird species. Moeller (2000) provided
comparative evidence that the degree of compensation of females is related to the
relative importance of the partner’s contribution. The importance of the male’s
contribution to offspring survival in *E. cyanostictus* needs further investigations,
especially under more natural conditions.

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2. The influence of sex ratio on different levels of sexual conflict about parental care in *Eretmodus cyanostictus*

2.1 Abstract

The pay-offs of caring for offspring or deserting them often differ between the sexes. Thus sexual conflicts about parental care are expected. The pay-offs of desertion mainly depend on the availability of alternative mating partners and are likely to be higher for males due to anisogamy. *Eretmodus cyanostictus* is a monogamous and biparantal mouthbrooding cichlid in which the clutch is first brooded by the female before it is incubated by the male. It has been suggested that parents are constrained to monogamy due to low remating probabilities for deserting individuals. By varying the sex ratio we intended to alter the sexual conflict between parents on two different levels. First, male desertion rate may depend on sex ratio. Males changed their mates in only 10.5% of all cases but lost their mate to a male intruder during their incubation in 26.7%. Females were most active and aggressive when additional females were present, while males where more active and tended to be more aggressive when additional males were present, but only during male incubation. Behavioural differences of a given sex between different sex ratios were strongest during incubation of that sex. Second, sex ratio is likely to influence the sexual conflict about the amount of parental care each parent provides. An, as yet undescribed display behaviour is clear evidence for a conflict about the timing of shift of young. Additionally, males took the offspring later when additional females where present. However, we found no difference in male or total incubation time between treatments.

2.2 Introduction

The decision of a parent to care for its offspring or not to care depends on the pay-offs of the two different decisions. Because these pay-offs often diverge substantially between the sexes (Trivers 1972, Clutton-Brock 1991) and also depend on the
decision of the partner (Maynard-Smith 1977) conflicts between males and females arise about the amount of parental care each parent should provide. Males are likely to gain more from leaving the current brood in order to find additional mating opportunities than females do due to anisogamy (Trivers 1972, Clutton-Brock). But the benefits of desertion or caring may also largely depend on ecological factors (Emlen-Oring 1977). Theoretical models have identified the availability of alternative mating opportunities as the factor that influences the benefits of desertion most (Maynard Smith 1977, Grafen & Sibly 1978, Balshine-Earn & Earn 1998). Empirical studies in fish (e.g. Keenleyside 1983, 1985, Balshine-Earn & Earn 1998) or birds (e.g. Székely & Cuthill) support the finding of these theoretical studies. However, if caring of both parents greatly increases the survival chances of the offspring, neither parent should desert (Maynard Smith 1977, Clutton-Brock 1991). Even in this case, a conflict may still arise about the amount of parental care each parent provides. The outcome of this conflict is likely to be influenced by the operational sex ratio (OSR). The lower the costs of replacing the current mate, the higher the probability that an individual will exploit the parental investment of its mate and reduce its own investment (Lessels 1998). These costs are influenced by the availability of additional mates. It has been shown, for example, that male fairy martins reduce participation in incubation when the availability of fertile females increased (Magrath & Elgar 1997).

*Ereptodus cyanostictus* is one of the few mouthbrooding species that show biparental care (Keenleyside 1991). Most mouthbrooders show female-care and polygyny (Oppenheimer 1970, Keenleyside 1991, Kuwamura 1997). A common explanation for the rarity of biparental mouthbrooding is that the mouth provides a safe incubation site for a small clutch to be protected by one parent alone (Oppenheimer 1970, Barlow 1984, Gross & Sargent 1985). Biparental care in mouthbrooders is expected to have few advantages unless (i) the size of the clutch is too large to fit into the mouth cavity of a single parent or (ii) if both parents are needed for co-defence of the free-swimming fry after release (Perrone & Zaret 1979, Clutton-Brock 1991). Indeed, these two conditions hold for most of the biparental mouthbrooding species (e.g. Kuwamura 1986, Yanagisawa 1986, reviewed by Perrone & Zaret 1979, Clutton-Brock 1991).
In *E. cyanostictus* the clutch is small enough to fit into a single mouth cavity and young are not defended after release (Kuwamura 1986, Kuwamura et al. 1989, Morley & Balshine 2002). Females incubate the young for about 8-12 days, then males incubate them for another 10-16 days (Morley & Balshine 2002, Grüter & Taborsky in prep.). The parent that incubates the clutch starves (Neat & Balshine-Earn 1999, Morley & Balshine 2002). Females would have few advantages by deserting in order to increase clutch frequency because they need at least 20 days to lay another clutch after the end of their own incubation (Grüter & Taborsky in prep.). It remains unclear, however, why males should not desert their mates. In an earlier study, we found that females without male assistance lost more weight and released smaller and less developed young. This suggests that the importance of male care for the survival prospects of young after release may be one important reason why males do not desert.

However, other studies suggested that males are constrained to biparental care and monogamy because there are little re-mating chances due to a male biased sex ratio (Neat & Balshine 1999, Morley & Balshine 2002). In addition, intrasexual competition for mates and territories appears to exist for both sexes, as pairs often show sex specific territory defence against members of the neighbouring pairs in the field (Morley 2000) and in the laboratory (personal observation).

To test the hypothesis, that parents may be constrained to biparental care and monogamy due to low mate availability, we varied the sex ratio experimentally. Experimental changes of the OSR have shown to induce or increase male and/or female desertion or polygyny in various cichlid species (Keenleyside 1983, 1985, Balshine-Earn & Earn 1998, but see Rogers 1987, Wisenden 1994). Furthermore, we looked for evidence of sex specific aggressive behaviour, which may serve mate guarding and could help to promote monogamy as shown in some fish (e.g. Reavis & Barlow 1998, Kokita 2002, Harding et al. 2003), shrimps (e.g. Rahman et al. 2003) and birds (reviewed in Slagsvold & Lifjeld 1994).

When neither parent deserted, we investigated whether there is a conflict over the relative amount of parental care each parent provides. Since in *E. cyanostictus* the amount of parental care is equivalent to the incubation duration, the timing of shift of young from the female to the male is of special interest. In this context we observed an as yet undescribed female display behaviour that appears to be a clear indication of a sexual conflict over the timing of the shift of young between mates. We measured
potential costs for either sex in terms of weight change during the two periods of mouthbrooding. Since it has been shown, that sexual conflict about parental care might impose costs on offspring (Royle et al. 2002) we also measured sizes and weights of offspring at the end of the incubation period of the pair.

2.3 Methods

*Study species*

*E. cyanostictus* (Cichlidae) is endemic to Lake Tanganyika. It inhabits the shallow rocky coasts of the lake (Kuwamura 1986, Kuwamura et al. 1989), where it feeds on epilithic algae (Yamaoka 1997). Due to a reduced swim bladder fish are able to stay close to the ground even in turbulent conditions (Konings 1998). Pairs aggressively defend all-purpose territories (Morley 2000). Males are larger, more active and more aggressive against intruders, while females feed and hide more (Morley 2000).

*Experimental conditions*

We conducted the experiment from March 2002 to March 2003. Experimental fish were taken from a stock of adult fish kept at the University of Berne, consisting of imported fish from Lake Tanganyika or from the first generation we bred in our lab. Experimental fish were held in 200 l tanks. A layer of sand covered the bottom of each aquarium and 16 flower pot halves, two PVC tubes (5 cm in diameter) and 10 PVC plates (40x20 cm) were provided as shelters. Several patches of gravel (about 20 pieces) were distributed over the bottom to provide shelter for the offspring after being released by the parents. The tank was equipped with an internal biological filter. Temperature varied between 26-27°C and the light regime was 13 h L : 11 h D. Fish were fed daily with flake food.

*Experimental design*

Thirtyseven different pairs were alternately assigned to one of three treatments. There was no difference in standard length of pair males or pair females between treatments (males: Kruskal-Wallis one-way ANOVA by ranks, N=12,12,13, $\chi^2=0.24$, P=0.89,
range: 5.9-7.4 cm; females: Kruskal-Wallis ANOVA, N=12,12,13, \( \chi^2 = 0.21, P = 0.9 \), range: 4.9-6.9 cm).

Fish were kept in the experimental tanks during one full reproductive cycle. Pairs and two additional unpaired fish were transferred from our stock to the experimental tank before spawning and stayed in the experimental tank until the pair finished incubation. (We shall use the simplified expression “intruders” to denote the two unpaired introduced fish other than the pair members from know on). For the three treatments, experimental tanks were stocked with the following combination of fish: Male treatment: a pair and two male intruders (3 males:1 female sex ratio), one of which was larger (in terms of body size) than the pair-male (big intruder), and one was smaller than the pair-male (small intruder). In 5 of 12 cases the big intruders were smaller than the pair male either because the big intruder became the pair male before spawning or because intruders bigger than the pair male were not available; equal treatment: a pair with a male and a female intruder (2:2 sex ratio), which were about the same size as the respective sex in the paired fish. The male intruder was larger than the pair male in 6 of 12 cases while the female intruder was 3 times larger, 5 times of equal size and 5 times smaller than the pair female; female treatment: a pair and two female intruders (1:3 sex ratio), one of which was larger (in terms of body size) than the pair female (big intruder) and one was smaller than the pair-female (small intruder). In 3 of 13 cases the big intruders were smaller than the pair female because intruders bigger than the pair female were not available.

All fish were weighed on day 1 after spawning and the day after end of total incubation. The pair was weighed again on day 6 before shift of young. The female and the two intruders were weighed the day after the shift of young. After release from incubation, young were weighed to the nearest 0.0001 g and their standard length (SL) was measured to the nearest 0.1 mm. The incubation periods of females and males as well as total incubation time of the pair was measured.

**Behavioural observations**

We observed all four fish at least four times after spawning and before shift of young from the female to the male and six times after shift of young until release of young whenever possible (before shift: 47.6 min \( \pm 13.9 \) (mean \( \pm \) sd); after shift: 61.7 min \( \pm \)
The observations were done between 13:00 and 16:00 and took 10 min each. All four fish were recorded simultaneously.

We recorded two states, time swimming around (active) or time under cover (hiding). Additionally, we recorded frequencies of behavioural events per 10 min: feeding rate (number of bites on small food items on surfaces or in the sand), courtship display (the focal fish undulates its whole body with varying intensity from bending to shaking of the body), aggressive behaviours: bite rate (aggressive biting of another fish), chase rate (chasing another fish), weak aggression rate (swimming towards another fish until it escapes, but not chasing it). It was recorded towards which other fish the behaviour was addressed. For more detailed description of the behaviours see Morley 2000, Appendix C.

Aggressive displays and courtship display of pair members towards conspecifics look almost identical to the observer (Morley 2000, Appendix C). However, these two behavioural categories can be distinguished from each other when taking behavioural sequences into account. Aggressive displays mostly appear in combination with other aggressive behaviours as bites, weak aggressions, chases or mouth fights. So all displays that were followed by another aggressive behaviour were excluded from the category “courtship display”.

Between fish a clear dominance relation existed. A “dominant” fish could initiate aggression towards a subdominant fish and chase it, but never the other way round. In some cases, pair males lost their dominance status to a male intruder. In these occasions, intruder males switched to become aggressive towards pair males and to chase them after they won a fight. All behavioural observations were done with the OBSERVER 3.0 program.

*The female-to-male shift display*

About on day 4 after spawning, females started to show a display behaviour that we interpret as an attempt of the female to transfer the young to the male (“female-to-male-shift display” or FMS-display). We measured frequency and number of the FMS-display with video recording. We recorded the tank every hour for 5 min 45 sec starting at 9:30 in the mourning until 19:30 in the evening (11 x 5 min 45 sec; total 63 min 15 sec per day). Video recording started on day 4 after spawning and ended after the shift of young from the female to the male. To estimate the total number of FMS-
displays of a given day, we used the following formula: $FMS_d \times \frac{780}{63:15}$, where $FMS_d$ represents the total number of FMS-displays recorded during that day, 780 represents the number of minutes of the light regime (13 h) and 63:15 represents the total recording time during a day.

Statistical analysis

All tests were non-parametric because assumptions of parametric tests were not met. All tests were two-tailed. Descriptive statistics are given as medians and quartiles (in square brackets) unless otherwise stated. Statistical analysis of data was performed using SPSS 10.0.

2.4 Results

Stability of pairs

Nine of 12 females in the male treatment, 9 of 12 females in the equal treatment and 8 of 13 females in the female treatment finished their incubation until the shift of young. Of the 11 cases were females did not shift the young to the male correctly, 2 females swallowed the eggs after strong aggression coming from the pair male. A third female died through these aggressions. Five females swallowed the eggs for unknown reason. In one case the female only shifted partly to the male. In two cases (one in the female treatment, one in the equal treatment), the pair male expelled his mate and formed a new pair. In both cases the new mate was bigger than the abandoned mate. Thus, in 19 cases (the five cases where females stopped incubation for unknown reason were excluded) where males had access to additional females, they changed their mate twice. In one of the two cases, the new pair female spawned soon after but was expelled as well by the male on day 9 after spawning and replaced by the male’s first mate.

Seven of 9 males in the male treatment, 9 of 9 males in the equal treatment and 7 of 8 males in the female treatment finished their incubation to release independent young. In one case of the male treatment it was not clear whether the male finished or stopped incubation because no offspring were found (intruders could have eaten them).
and incubation duration was very short. In two other cases males stopped incubation and swallowed the young for unknown reason. In one of these cases, the male lost the dominance status to the big intruder, which showed continuous aggression towards the former pair male. Males lost dominance status in two additional cases. In these three cases the pair female became the mate of the dominant intruder. In a fourth case the dominant pair was expelled by the male and female intruders, which formed a pair and spawned soon after. The male intruders who mated successfully were larger than the pair male in 3 of the 4 cases. Thus, during their incubation pair males lost the dominant status in the tank in 4 (twice in the male treatment, twice in the equal treatment) of 15 cases where male intruders where present in the tank.

**Behavioural observations of pair males and females**

Pair females were overall less aggressive against intruders, showed more courtship displays towards the mate than pair males and had a higher feeding activity (Table 1). Overall, there was no difference in activity between pair fish. However, incubating females were less active than males before the shift of young. Females were also much more active after the shift of young than before shift of young while in males there was no difference between these two phases (Table 1).
Table 1 Comparison of behaviours between males and females (Wilcoxon signed rank tests)

<table>
<thead>
<tr>
<th></th>
<th>Pair male</th>
<th>Pair female</th>
<th>z</th>
<th>P (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aggressive behaviours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>/10 min</td>
<td>2.5; [1.25,6]</td>
<td>0; [0,1]</td>
<td>-3.3</td>
<td>0.001 (25)</td>
</tr>
<tr>
<td><strong>Activity in %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40.4; [25.4,61]</td>
<td>26.3; [13.5,48.7]</td>
<td>-1.59</td>
<td>0.118 (26)</td>
</tr>
<tr>
<td>Before shift</td>
<td>37.6; [23.1,53]</td>
<td>13.4; [4.4,37.8]</td>
<td>-2.7</td>
<td>0.007 (26)</td>
</tr>
<tr>
<td><strong>Courtship/10min</strong></td>
<td>2.75; [1,4]</td>
<td>4; [3,6]</td>
<td>-3.93</td>
<td>0.007(24)</td>
</tr>
<tr>
<td><strong>Feeding rate</strong></td>
<td>3.5; [1.5,10.1]</td>
<td>16; [10.6,28.9]</td>
<td>-3.39</td>
<td>0.001 (24)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>before shift</th>
<th>after shift</th>
<th>z</th>
<th>P (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activity in %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>37.6; [23.1,53]</td>
<td>49.5; [24.9,69.2]</td>
<td>-1.49</td>
<td>0.137 (24)</td>
</tr>
<tr>
<td>Females</td>
<td>13.4; [4.4,37.8]</td>
<td>53.4; [26,80.1]</td>
<td>-4.2</td>
<td>&lt;0.001 (24)</td>
</tr>
</tbody>
</table>

**Behavioural comparisons between treatments**

Activity of males differed between treatments (Kruskal-Wallis ANOVA, $\chi^2$=11.465, N=8,8,8, P=0.003; Fig. 1a), which appears to be due to differential activity after the shift. During this period males were more active (Mann-Whitney U-test, U=13, N=16,8, P=0.001; Fig. 1a) and tended to be more aggressive against intruders (in the treatments where male intruders were present (Mann-Whitney U-test, U=35, N=16,8, P=0.081; Fig. 1b) in those treatments where at least one male intruder was present as compared to the treatment with females only present.
Fig. 1 (a) Activity and (b) aggressive behaviours of males after the shift of young from females to males; mm=male treatment, mf=equal treatment, ff=female treatment. The whiskers and boxes represent the 10th, 25th, 50th, 75th and 90th percentiles.

To the contrary, activity in females differed between treatments only before shift (Kruskal-Wallis ANOVA, $\chi^2=9.214$, N=9,9,8, P=0.01; Fig. 2a). When additional females were present, females were more active (Mann-Whitney U-Test, U=24, N=9,17, P=0.004; Fig. 2a). We also found differences in overall aggression of females towards intruders between treatments (Kruskal-Wallis ANOVA, $\chi^2=8.872$, N=9,8,8, P=0.012). This difference was especially strong before shift (Kruskal-Wallis ANOVA, $\chi^2=11.31$, N=9,9,8, P=0.003; Fig. 2b), when females showed more
aggression if at least one additional female intruder was present (Mann-Whitney U-test, $U=36$; $N=9,17$, $P=0.01$; Fig. 2b)

![Box plot showing activity percentage of females in different treatments](a)

![Box plot showing aggressive behaviors per 10 minutes of females in different treatments](b)

**Fig. 2** (a) Activity and (b) aggressive behaviours of females before the shift of young from females to males.

Aggression towards the big intruder did not differ from aggression towards the small intruder neither in pair males (Wilcoxon signed-rank test, $z=-1.875$, $N=25$, $P=0.061$; Fig. 3) nor pair females (Wilcoxon signed-rank test, $z=-0.55$, $N=25$, $P=0.58$; Fig. 3).
Behaviours of intruders

The behaviour of the intruders is likely to influence the behaviour of the pair. In the experimental situation, pair fish were highly aggressive towards. As a consequence, activity times, feeding rates, courtship rates and aggression rates were zero or close to zero for big as well as small intruders (Table 2). Our observation time of 10 minutes was too short to detect possible differences of intruder behaviour between treatments.

Fig. 3 Aggressive behaviours of pair males and females towards big and small intruders during total incubation; big=big intruder, sm=small intruder.
Table 2 Behaviour of big and small intruders; medians [quartiles]

<table>
<thead>
<tr>
<th></th>
<th>Big intruder</th>
<th>Small intruder</th>
<th>(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aggressive behaviours</strong></td>
<td>0; [0,0]</td>
<td>0; [0,0]</td>
<td>(26)</td>
</tr>
<tr>
<td><strong>Activity in %</strong></td>
<td>0.09; [0,1.5]</td>
<td>0; [0,1.3]</td>
<td>(26)</td>
</tr>
<tr>
<td><strong>Courtship/10min</strong></td>
<td>0; [0,0]</td>
<td>0; [0,0]</td>
<td>(26)</td>
</tr>
<tr>
<td><strong>Feeding rate</strong></td>
<td>0; [0,0]</td>
<td>0; [0,0]</td>
<td>(26)</td>
</tr>
</tbody>
</table>

The female-to-male shift

Females started to show the FMS-display at the earliest on day four. If the male approaches the incubating female, she takes a head-down position, opens her mouth and starts to shake her body with various intensities. The female may remain in this position from a short moment to several seconds. The intensity of shaking seems to depend partly on male distance. The closer he is the more intense she shakes her body. In most cases, the male ignores her, swims away or tries to hide. If the male is very close, the female sometimes drops a young while she is in this position (Fig. 4). If the male does not take it up, the female tries to catch it as fast as possible, mostly before the young touches the bottom. When the shift of young finally takes place, males suddenly catch the young when dropped by the female. If the male is not fast enough, she tries to catch it quickly. It was not obvious to the observer what determines the behavioural switch when the male suddenly takes the young.
Fig. 4 FMS-display. A female in a head down position shakes her body until a young drops out of her mouth. The male who does not take it turns away.

We estimated that females showed the FMS-display between 0 and 1067 times before the shift until males took the offspring, but on average 111 times [46, 250]. There was no difference of estimated FMS-display number from day 4 to shift between treatments (Kruskal-Wallis ANOVA, $\chi^2=1.196$, N=7,7,8, P=0.55; Fig. 5).

![Graph showing total displays of females towards their mates until shift of young from female to male.](image)

Fig. 5 Total number of displays of females towards their mates until shift of young from female to male.
Incubation duration

Incubation duration of females differed between treatments (Kruskal-Wallis, $\chi^2=6.8$, $N=9, 9, 8$, $P=0.033$; Fig. 6), with female incubation being two days longer when additional females were present than when no female intruder was present (Mann-Whitney U-test: $U=29.5$, $N=9, 17$, $P=0.009$; Fig. 6). Male incubation time was on average 13 days [11, 14.5] and did not differ between treatments (Kruskal-Wallis ANOVA, $\chi^2=3.79$, $N=7, 7, 7$, $P=0.15$). Total incubation time was on average 21 days [20, 23.5] and we found no difference between treatments as well (Kruskal-Wallis ANOVA, $\chi^2=3.519$, $N=7, 7, 7$, $P=0.17$).

![Box plot showing incubation duration of females in days.](image)

**Fig. 6** Incubation duration of females in days.

Offspring measures and weight change of pair members

We measured the weight development of pair fish during the incubation to assess whether differences in incubation duration influence weight change differently. Such differences between treatments could reflect costs and benefits of conflict about the moment of shift. Weight gain of males before shift differed between treatments...
Males gained more weight in the two treatments where additional females were present (Mann-Whitney U-test: U=18, N=7, 15, P=0.014). In this context we found a positive relationship between the duration of female incubation and the increase in male body mass (Pearson correlation, r=0.69, P<0.001; Fig. 7). Females lost on average 7.5% (=median; [-9.7, -4.8]) of body mass during incubation, but weight loss did not differ between treatments (Kruskal-Wallis ANOVA, $\chi^2=1.1$, N=8,8,7, P=0.58). We then tested whether the amount of weight loss relates to the number of FMS-displays and found that females showed the FMS-display more often when they lost less % of initial body mass during incubation (Spearman’s $\rho=0.46$, N=20, P=0.041).

After the shift, males lost on average 8.4% (=median; [-12.2, -3]) while females gained 11.1% (=median; [4.4, 18.6]). But neither in males nor in females we found differences between treatments (Kruskal-Wallis ANOVAs; males: $\chi^2=2.37$, N=7,6,6, P=0.31; females: $\chi^2=1.23$, N=6,6,7, P=0.54).

Standard lengths of offspring after release (Kruskal-Wallis ANOVA, $\chi^2=2.44$, N=6,7,6, P=0.296) as well as offspring weight (Kruskal-Wallis, $\chi^2=2.364$, N=6,7,6, P=0.31) did not differ between treatments.
2.5 Discussion

Because the pay-offs of caring for young or deserting them often differ between parents, a sexual conflict about the parental care form or the relative amount of parental care a parent provides is expected (Trivers 1972, Clutton-Brock 1991). Usually males are expected to gain more from deserting their mate and current brood in order to find additional mating opportunities because of a higher potential reproductive rate due to anisogamy (Trivers 1972, Clutton-Brock 1991).

In *E. cyanostictus*, it has been suggested that male desertion in the field is unlikely due to intra-sexual aggression and low availability of alternative mating partners and (Neat & Balshine-Earn 1999, Morley & Balshine 2002). We investigated how variation in the availability of potential alternative mates influenced the probability of mate switching and readiness to care for the offspring. Experimental changes in mate availability have been shown to favour polygyny or desertion by either sex in other cichlids (Keenleyside 1983,1985, Balshine-Earn & Earn 1998).

In our study, males changed mates during female incubation in only 10.5% of all cases, even when female intruders where bigger than the mate and closer to spawning condition. One male that abandoned his current incubating female and mated with a small female intruder switched back to the original mate during incubation of the new mate. Several reasons could explain why males did not change mates more often.

Grüter & Taborsky (in prep.) showed that consequences of male absence can be severe for offspring and female condition. Thus, strong selection pressures probably have imposed monogamy and biparental care on *E. cyanostictus* and decreased the flexibility of the mating system (see also Rogers 1987, Wisenden 1994).

Another reason for rare desertions seems to be sex-specific aggression. Sex-specific aggression, especially in females, has been shown to stabilize or increase monogamy in other fish (e.g. Reavis & Barlow 1998, Kokita 2002, Harding et al. 2003), shrimps (e.g. Rahman et al. 2003) and birds (reviewed in Slagsvold & Lifjeld 1994). We found that females were more active and aggressive when at least one additional female was present as compared to the male treatment. On the other hand, males were more active and tended to be more aggressive in the treatments with male intruders present, but only when incubating themselves. In general, differences in aggressive behaviour and activity between treatments were apparent or strongest during a parent’s own incubation period. During female incubation, females are more in danger of being
deserted or expelled, while during male incubation, male risk of losing its mate and territory to a strong male intruder, should be highest. Indeed, in 26.7% of the cases where at least one additional male intruder was present. Males with a clutch in their mouths are likely to have difficulties in mouth fights and aggressive biting, and thus have a reduced ability to defend their mate and territory. Behavioural shifts during incubation seem to be a reaction to these increased risks. Neither males nor females showed intruder-size-dependent aggressive behaviour, so we reject the possibility that sex specific aggression observed in the field is the result of size assortative aggression between pair females and pair males.

When pairs remained stable over the total incubation period, we found an apparent sign of sexual conflict about the timing of the shift of young from females to males, which determines the amount of parental care performed by females. We interpret the display behaviour, described here for the first time, as a clear and explicit sign of conflict between males and females. In rare cases, a female had to display more than 1000 times until the male took over the young. This behaviour may be costly in terms of energetics and predator attraction. Contrary to our expectation we found that females who showed the FMS-display more often lost less body mass during incubation. This suggests that the display is not very costly in terms of energy expenditure. Alternatively females that loose less body mass for some other reason could be able to afford an increased energy expenditure to FMS-displays. We also found no differences in the total number of displays between treatments, probably due to the small sample size of our data. In future studies this display may be used to quantify the conflict about the timing of shift.

Males took over the offspring two days later when at least one female other than the pair female was present as compared to the females in the male treatment. This corresponds to an extension of 28.6% of female incubation time as compared to the male treatment. During this period males probably attempt to build up reserves before the starvation period during male incubation. This would explain the positive relationship between female incubation time and the increase in male body mass during this period. Because males could forage for two more days in the equal and female treatments they showed a higher increase in body mass. These results support the idea that males are more likely to exploit female investment if costs of replacing the current mate are reduced (Lessels 1998). Surprisingly, males did not seem to reduce their own incubation period when females incubated longer. One possibility is
that males just did not behave optimally. Alternatively, males may have adjusted their parental investment but this effect was not apparent due to a reduction of incubation time in the male treatment for other reasons. The risk to lose their territory and their mate during male incubation is strongly enhanced when large male intruders are present. Incubating shorter may reduce this risk. This possibility could be tested experimentally.

Females lose weight during incubation. If female incubation periods differ due to sex ratio, we expect differences between treatments in body mass loss as a cost of sexual conflict. The reasons why we found no differences between treatments probably are a small sample size and a large individual variation. Additionally we found no indication that offspring suffer from the conflict between the sexes. Bodysize and mass did not differ between treatments, probably because also the total incubation times were not different between the treatments.

This study suggests that lack of alternative mating partners is not the reason for the maintenance of monogamy and biparental care in *E. cyanostictus*. The main factors explaining the stability of this breeding system seem to be the males contribution to offspring survival, thus the need for biparental care (Grüter & Taborsky in prep.) in conjunction with mechanisms reducing the opportunity for re-pairing for each sex such as sex specific aggression.

To understand how the conflict about the timing of shift is solved between the sexes, further experiments are needed. The role of internal and external factors such as the body condition of both sexes, predation risks for the female and the young when dropped by the female and which sex is responsible for the decision about timing of shift need to be investigated.

The conflict about the timing of shift of young from female to male needs further investigation. Other factors such as body condition of males and/or females or predation risk may be important.

### 2.6 References


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